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Research report

Activity-dependent changes in the electrophysiological properties of regular spiking neurons in the sensorimotor cortex of the rat *in vitro*

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ABSTRACT

Sensorimotor performance is highly dependent on the level of physical activity. For instance, a period of disuse induces an impairment of motor performance, which is the result of combined muscular, spinal and supraspinal mechanisms. Concerning this latter origin, our hypothesis was that intrinsic properties and input/output coupling of cells within the sensorimotor cortex might participate to the alteration in cortical motor control. The aim of the present study was thus to examine the basic electrophysiological characteristics of cortical cells in control rats and in animals submitted to 14 days of hindlimb unloading, a model of sensorimotor deprivation. Intracellular recordings were obtained *in vitro* from coronal slices from cortical hindpaw representation area. We have also made an attempt to determine the morphological characteristics as well as the location of the investigated neurons by biocytin labelling. Passive properties of neurons were affected by hindlimb unloading: input resistance and time constant were decreased (−20%), the rheobase was increased (+34%), whereas the resting potential was unchanged. The frequency–current relationships were also modified, the curve being shifted towards right. The size of body area of recorded neurons was unchanged in unloaded rats. Taken together, these data reflect a decrease in excitability of cortical cells in response to a decreased cortical activation.

1. Introduction

Sensorimotor performance is highly dependent on the level of physical activity. In particular, a low physical activity (for instance in elderly people, or during bed rest or immobilization) is known to induce an impairment in motor performance (lack of equilibrium, limited walking speeds...). This behavioural alteration is due in part to an alteration in muscle function. In fact, disuse is related to plastic changes of skeletal muscles (atrophy, loss of force, decrease in twitch contraction time...) [30,34]. However, neural mechanisms should not be excluded [14]. In particular, it has been shown recently that short-term disuse induces alterations in interjoint coordination and trajectory formation, which are potentially related to a cortical synaptic depression [27]. To sustain this hypothesis, several studies have provided evidence for a reorganisation of motor and somatosensory cortices during immobilization [23,24,38].

Prolonged bed rest, immobilization or even spaceflight in humans can be mimicked in rats using the hindlimb unloading model [29]. In this situation, the plantar sole is not in contact with the ground, the motor activity is reduced, and the charge imposed to hindlimbs is suppressed. In consequence, the sensory (proprioceptive and tactile) input is reduced. Hindlimb unloading can thus be considered as a model of sensorimotor deprivation. As a matter of fact, it has been shown that hindlimb unloading alters the motor behaviour in rats [3,4] and that supraspinal structures are involved in the alteration of motor performance [1,2]. Previous studies have also shown that a 14-day period of hindlimb unloading produces a remapping of the somatosensory cortical maps: a shrinkage of the hindpaw representation area and an enlargement of receptive fields were observed [13,20].

Several studies also indicate a higher excitation of somatosensory cortical cells in response to peripheral stimulation: the number of Fos-immunoreactive neurons was increased in rats submitted to 14 days of hindlimb unloading [21], and extracellular recordings performed in the somatosensory cortex of anesthetized rats have shown a reduction of threshold and an increase in cell activity in response to low tactile stimulation [12]. The origin of the higher activity of cortical neurons is not fully understood. Previous works indicate that it might be the result of a change in the balance of excitation and inhibition, since the level of inhibitory neurotransmitter GABA was decreased in unloaded rats [6,9] without significant changes in the excitatory amino acid glutamate [6]. The cortex might also adapt to altered sensorimotor activity through

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changes in intrinsic properties and input/output coupling of cortical cells. This form of cortical plasticity has been demonstrated during learning tasks or various forms of activity-dependent plasticity (for review: see Ref. [10,39]).

The aim of the present study was thus to examine the basic electrophysiological characteristics of cortical cells in rats submitted to 14 days of hindlimb unloading. We compared the intrinsic properties of neurons located within the hindpaw representation area, whose activity is altered in response to peripheral stimulation after hindlimb unloading [12,21], and in the surrounding areas. We have also made an attempt to determine the morphological characteristics as well as the location of the investigated neurons.

2. Materials and methods

2.1. Animals

The experiments were conducted in 38 male Wistar rats weighing approximately 250–350 g purchased from a commercial breeder (Harlan, France). Animals were randomly assigned to one group: Control ($n = 19$) or Hindlimb unloaded ($n = 19$). Animals were housed with a regular light/dark cycle (lights on 07:00 to 19:00 h) and constant temperature (23 °C). They had free access to water and food. All efforts were made to minimize the number of animals used and their suffering. All procedures described below were approved by both the Agricultural and Forest Ministry and the National Education Ministry (veterinary service of health and animal protection, authorization 59–00999).

2.2. Hindlimb unloading procedure

Hindlimb unloading was obtained using the model adapted from Morey-Holton and Globus [29]. The tail of each rat was cleaned, dried, and wrapped in antiallergenic adhesive plaster. This cast was secured to an overhead swivel that permitted 360° rotation and allowed the rats to walk freely on their forelimbs and have free access to food and water. The rats were unloaded by the tail at a ∼30° head-down angle in order to avoid a contact of the hindlimbs with the ground.

2.3. Slice preparation

Rats were deeply anesthetized with pentobarbital sodium (60 mg/kg, IP) and perfused with ice-cold artificial cerebrospinal fluid (ACSF) oxygenated with carbogen (95% O_2–5% CO_2). Normal ACSF consisted of 117 mM NaCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 4.7 mM KCl, 23 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 10 mM glucose.

The animal was mounted in a stereotaxic apparatus and a coronal block of tissue was rapidly dissected from a zone corresponding to the putative hindpaw representation area of the rat sensorimotor cortex. The block was then glued on the platform of a vibratome (Vibroslice NVSL, WPI), submerged in ice-cold ACSF and four coronal slices (400 μm thick) were cut.

Previous studies performed *in vivo* have shown that, although some individual variations exist, the hindpaw representation extends from anterior 0 to −2 and lateral 2.5–3.5 with Bregma as bone reference point in both control and unloaded rats, with a functional centre at coordinates anterior −1 and lateral 3 [4]. We considered that cells of the first two slices (and located laterally from 2.5–3.5) had a high probability to be within the hindpaw area (anterior $-0.8 \rightarrow -1.6$). Cells recorded in the forth slice (more posterior than −2.0) and/or outside the lateral limits were likely outside the hindpaw area.

The slices were immediately placed in a fluid–gas interface recording chamber continuously perfused with carbogenated ACSF and maintained at room temperature for one hour. The humidified atmosphere over the slices was saturated with carbogen. The ACSF was then warmed (32 °C) and the slices were allowed to incubate for at least 30 min before recording.

2.4. Electrophysiological recording

Glass microelectrodes (70–150 MΩ resistance) were pulled (PUL100, WPI) from borosilicate glass capillaries (1 mm outer diameter × 0.58 mm inner diameter, Harvard Apparatus). Electrodes were filled with 3 M K-acetate. Sometimes, 0.3% biocytin was added to the solution to achieve labelling of recorded neurons. The signals were amplified with an in-house high impedance amplifier with active bridge circuitry, filtered and digitised using a digidata 1322A interface (Axon Instruments). Data were recorded, stored and analysed using a pClamp software (Axon Instruments).

2.5. Data analyses

Resting potential was measured after subtracting the junction potential at the end of the recording session after the electrode was withdrawn from the neuron. The rheobase was measured as the lowest current intensity leading to spike discharge from resting potential (pulses of 300 ms). The threshold for spike initiation corresponded to the slope breakpoint. The amplitude of action potentials was calculated as the potential difference between their voltage threshold and their peak (Fig. 1A). The spike duration was estimated by measuring the width of the action potentials determined at half peak amplitude. For the determination of input resistance, hyperpolarizing and depolarizing current pulses (100 ms duration, from −0.2 to 0.8 nA, with 0.05 nA steps) were applied. Current–voltage (*I–V*) curves were constructed by plotting the amplitude of the membrane potential against the intensity of the applied current step. Input resistance was measured from *I–V* curves. Membrane time constant (τ) was determined by fitting one decaying exponential function to the cells' voltage response to small negative current steps (−0.2 nA, 100 ms). The hyperpolarization-actived sag was measured as the difference of the mean membrane potential for the last 10 ms of the hyperpolarizing current pulse (100 ms duration) and the mean value found in the 80 ms following the beginning of the pulse. Only cells with a resting potential more negative than −50 mV, input resistance above 20 MΩ and overshooting action potentials were analysed.

The amplitude of the after-hyperpolarization (AHP) corresponds to the difference between the min value determined by the software in a given time scale following action potential (<10 ms for fast AHP; 10–100 ms for medium AHP; 100–500 ms for slow AHP), and the resting potential (mean value).

2.6. Tissue processing and histology

Following the recording session, an intracellular injection of biocytin was performed by passing a depolarizing current (100 ms, 0.3 nA, 1 Hz) over 5–15 min. The slices were fixed overnight in 4% paraformaldehyde at 4 °C, rinsed three times in phosphate buffer (0.1 M, pH 7.5) and stored in phosphate-buffered sucrose (30%) at 4 °C. The slices were sectioned at a thickness of 150 μm using a freezing microtome. They were then rinsed three times with phosphate buffer saline (PBS), and incubated with ABC solution (Vector Laboratories) in the presence of 0.1% Triton X-100 for 5 h. After 3 rinsing in PBS, they were incubated with DAB in nickel ammonium sulphate with 30% H_2O_2 for 10 min. The slices were rinsed in PBS 3 times 10 min. After that, the slices were dehydrated in graded alcohols and toluene and coversliped.

2.7. Statistics

Values are presented as mean ± SD. Comparison between the three groups was established by a one-way analysis of variance followed by a Tukey's post hoc comparison in case of significant effect. The slope of *PA–I* curves were analysed by linear regression (Prism 4 software). The threshold difference was assessed by comparison of the *y*-intercept of the opposite *I–PA* linear regression [31]. Morphological characteristics were compared with a two-way analysis of variance. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Localisation

Data were obtained from 92 neurons (48 cells in control rats and 44 in hindlimb unloaded ones). All neurons were classified as regular spiking cells based on their firing properties [7,11]. Cells were located in layers II–VI of the sensorimotor cortex (Fig. 1B). They were supposed to be located in the area of interest, i.e., the hindpaw representation area, when their stereotaxic coordinates were anterior −0.5 to −1.5 and lateral 2.5 to 3.5 with Bregma as reference point [5]. These cells were classified as CON^+ ($n = 31$) and HU^+ ($n = 31$). Cells located outside these limits were classified as CON^- ($n = 17$) and HU^- ($n = 13$). No significant differences were detected for the various parameters between CON^+ and CON^- cells. Thus, they have been pooled in one CON group. We compared the characteristics of neurons of the different cortical layers (II–III vs. IV vs. V–VI) but we did not detect any difference.

3.2. Cell characteristics

Table 1 presents the properties of the neurons. Concerning passive electrical properties, input resistance (R_N) and time constant (τ) were decreased by 20% ($P < 0.05$) in HU^+ cells, whereas the resting potential was unchanged. The rheobase was increased (+34%, $P < 0.05$).

The characteristics of action potentials (amplitude, duration and threshold) were not affected by hindlimb unloading. When cells were activated by depolarizing current near threshold,