Sensory motor mismatch within the supplementary motor area in the dystonic monkey

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Dystonia, a movement disorder characterized by abnormal postures, is associated in primary forms of the disease with subtle proprioceptive troubles and aberrant somatotopic representation in the somatosensory cortex (SC). However, it is unclear whether these sensory features are a causal phenomenon or a consequence of dystonia. The supplementary motor area proper (SMAp), a premotor cortical region, receives strong inputs from both the SC and basal ganglia. We hypothesized that disruption in sensory-motor integration within the SMAp may play a part in the pathophysiology of dystonia. Using a model of secondary dystonia obtained by 3-nitropropionic acid intoxication in rhesus monkeys, we first provide evidence that the SMAp was over-excitable in dystonic animals. Second, we show that proprioceptive inputs processed by SMAp neurons were dramatically increased with wider sensory receptive fields and a mismatch between sensory inputs and motor outputs. These findings suggest that abnormal sensory inputs impinging upon SMAp neurons play a critical role in the pathophysiology of dystonia.

Introduction

Dystonia is characterized by sustained muscle contractions causing twisting and repetitive movements or abnormal postures (Fahn and Eldridge, 1976). During voluntary movements, there is a lack of selectivity of motor command with an overflow of activity in muscles normally inactive in movement execution (Rothwell et al., 1983; Cohen and Hallett, 1988). However, several lines of evidence suggest that the disorders of movement in dystonia could be associated with disturbances in sensorimotor integration (Byl et al., 1996; Bara-Jimenez et al., 1998; Elbert et al., 1998; Jahanshahi, 2000; Murase et al., 2000; Meunier et al., 2001; Muller et al., 2001; Lerner et al., 2004).

Imaging studies have demonstrated overactivity in the lateral prefrontal and premotor cortices during motor tasks in primary dystonia (Ceballos-Baumann et al., 1995b) but contradictory data have been reported concerning activation of the caudal supplementary motor area (SMAp) and primary sensorimotor cortex, depending on the clinical expression of dystonia at the time of scanning (Ceballos-Baumann et al., 1995a; Ibanez et al., 1999; Pujol et al., 2000; Lerner et al., 2004). These cortical changes are thought to result from an increased inhibition of the medial globus pallidus leading to an increased thalamic drive to premotor cortices (Berardelli et al., 1998). However, the correlation between clinical status, metabolic and neuronal changes is still elusive. Single-unit recording techniques allow a direct and detailed approach to neuronal activity but limit investigation to one or a few brain regions. We decided to focus on the SMAp for several reasons: 1) within the motor loop, this cortical area is the main target of basal ganglia output projections (Alexander and Crutcher, 1990); 2) it is involved in postural control through its direct projections to regions of the primary motor cortex (Muakkassa and Strick, 1979) and spinal *cord* (Hutchins et al., 1988; Dum and Strick, 1996; Picard and Strick, 1996) controlling the proximal musculature; and 3) the SMAp receives dense sensory inputs from S1 (Rizzolatti and Fadiga, 1998).

Several questions remain unanswered concerning the role of the SMAp in the pathophysiology of dystonia. The first is whether this part of the mesial premotor cortex is overactive. To test this hypothesis we studied SMAp spontaneous single neuronal activity and microstimulation in normal and dystonic primates. The second question is whether the processing of sensory inputs is modified within SMAp. To date, enlarged somesthetic receptive fields have been reported in the sensory thalamus (Lenz et al., 1999; Blake et al., 2002) and primary somesthetic cortex (Bara-Jimenez et al., 1998; Elbert et al., 1998; Meunier et al., 2001) of patients with dystonia, but they have never been documented within the premotor cortical areas. Therefore, we extensively studied the response of SMAp neurons to passive limb movements in normal

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and dystonic sub-human primates in a model of secondary dystonia obtained by intramuscular chronic treatment with 3-nitropropionic acid (NP), a mitochondrial toxin that induces large and specific lesions of the striatum (Palfi et al., 1996, 2000; Brouillet et al., 1999; Ghorayeb et al., 2002).

Materials and methods

Animals

Two female rhesus macaques (*Macaca mulatta*) served as subjects in these experiments. The animals weighing 5 and 6 kg were housed in individual primate cages. They had *ad libitum* access to water and food and received daily a supplementation with various fruits. Their care was supervised by veterinarians skilled in the health care and maintenance of non-human primates, in strict accordance with the European Community Council Directive for experimental procedures in animals. The light–dark cycle (lights on from 7 a.m. to 7 p.m.), temperature (22 °C) and humidity (60%) were kept constant in the animal room. For several weeks before surgery, monkeys were trained daily to sit in a primate chair and to remain quiet during palpation of various parts of the body. Monkeys were regularly videotaped in their home cage or primate chair. These videos were used to assess the severity of movement disorder using the Burke–Fahn–Marsden scale (Burke et al., 1985). Electromyographic recordings were abandoned after several attempts because they were impossible to perform in good conditions in freely moving monkeys.

Model of dystonia

The model of dystonia was obtained by chronic intoxication with a mitochondrial toxin, 3-nitropropionic acid (3-NP, Sigma, Lyon, France), using a protocol previously described (Palfi et al., 2000). Briefly, the monkeys received daily intramuscular injections of 3-NP (twice daily, 10:00 a.m. and 7:00 p.m. for 7 days). The total daily starting dose was 10.0 mg/kg/day for both monkeys). Thereafter they received a weekly increment of 2.0 mg/kg until obvious neurologic signs were detected, at which point 3-NP intoxication was stopped.

Surgery and electrophysiological recordings

A stainless steel recording chamber (diameter 19 mm, Narishige, Tokyo, Japan) was implanted in the skull under general anesthesia (ketamine 10 mg/kg, PanPharma, Fougères, France; xylazine 2 mg/kg, Bayer Pharma, Puteaux, France; diazepam 0.5 mg/kg, Sigma, Lyon, France; atropine sulfate 0.2 mg/kg, Aguettant, Lyon, France). Supplemental doses of ketamine were given every hour to maintain a state of deep anesthesia. The central axis of the cylinder was stereotaxically positioned at A24 and L0 in both monkeys. A head-holder was embedded with dental cement around the chamber for immobilization during neuronal recording. Antibiotic (ampicillin, 100 mg/kg, DUPHAMOX L.A®, subcutaneously, Fort Dodge Santé Animale, Agen, France) and analgesic (paracetamol, 30 mg/kg, per os, UPSA, Agen, France) treatments were given for 1 week after surgery.

Animals were left to recover for 2 weeks before the start of the experiments. Then, they were placed daily for the 3 h of experimental sessions in a primate chair with their head restrained. Extracellular single-unit activity was recorded using tungsten microelectrodes insulated with epoxy (impedance 0.5–1.0 Mohms at

1 kHz) piloted with a microdrive (Narishige, MO-95, Tokyo, Japan). Neuronal activity was amplified (10–20 K), filtered (300 Hz–3 KHz) and displayed on an oscilloscope. A window discriminator was used to select spikes from background activity. These were then processed through an analogic-digital interface and stored on-line on a microcomputer. Neuronal activity was stored for 3 min of recording as the monkey sat quietly and then analyzed using the chart system soft (Chart 5.0, ADI instruments, USA).

Proprioceptive receptive fields (PRF) were defined as the pattern of neuronal responses to various passive limb movements. To this end, the different joints of the upper and lower contralateral limbs were gently displaced in various directions. Monkeys were trained to remain quiet during this procedure and were often rewarded with fruit juice. Accelerometers were fixed with straps on the mobile part of the explored limb. The latter was also manually maintained in contact with a capacitive proximity detector. These two systems were used to assess the onset and end of passive movements. Four joints of the upper (shoulder, elbow, wrist, fingers) and lower (hip, knee, ankle, toes) limbs were studied on both sides of the body. The effect of passive movements was tested for each joint in the following directions: shoulder (antepulsion, retropulsion, abduction, adduction, rotation), elbow (flexion, extension, pronation, supination), wrist (flexion, extension, cubital inclination, radial inclination), fingers (extension, flexion), hip (extension, flexion, abduction, adduction), knee (extension, flexion), ankle (extension, flexion) and fingers (extension, flexion). The effect of trunk and tail movement was also assessed. Neuronal activity was stored for 10 to 25 passive movements.

Intracortical microstimulation (ICMS) was performed at the end of each neuronal recording session at sites where PRF were found. A train of cathodal pulses (width 0.2 ms, train duration 150 ms at 300 Hz, intensity between 5 μA and 200 μA) was applied through a constant-current stimulator with the same electrode used for extracellular recordings. The threshold of current intensity giving observable movements was systematically recorded. By this method we mapped the motor-evoked response (MER) at each site of stimulation.

Neuronal recordings and ICMS were performed exactly at the same sites before and after the lesion in each animal.

Statistical analysis

Spontaneous firing frequencies between the normal and dystonic monkey were compared with a Mann–Whitney U-test. To characterize the pattern of discharge, we used a variability index (VI) corresponding to the rate of spike interval SD/spike interval mean calculated for each neuron (Escola et al., 2003). Neurons with a regular pattern had a low VI and those with an irregular pattern a high VI. A mean value of VI was calculated for neurons recorded in the normal and dystonic monkey, respectively, and compared with a Mann–Whitney U-test.

For each type of passive movement, a peri-stimulus histogram with respect to the onset of joint displacement was obtained by aligning neuronal activity on the signal delivered by the proximity detector or the accelerometer (Fig. 1). The mean discharge frequency was calculated during two epochs of 250 ms before and after passive limb movement. These two values were compared with a Wilcoxon signed rank test. Neuronal changes in relation with limb displacement were considered to be significant for $P < 0.05$.

During ICMS, movements were recorded if they were evoked repeatedly and clearly identified by two investigators. Their features