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CONTROL OF ORO-FACIO-LINGUAL MOVEMENTS BY THE SUBSTANTIA NIGRA PARS RETICULATA: HIGH-FREQUENCY ELECTRICAL MICROSTIMULATION AND GABA MICROINJECTION FINDINGS IN RATS

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Abstract—To provide direct evidence for substantia nigra pars reticulata (SNr) control of oro-facio-lingual muscle activity, high-frequency electrical microstimulation (mainly trains of 20, 333-Hz cathodal pulses at 40–60 μA) and GABA microinjection (1–5 μl of 10 mM GABA in saline) were carried out using a three-barreled microelectrode at the same SNr site in lightly anesthetized, chronically decorticated rats (n=39). Decortication eliminated the possibility that SNr microstimulation might activate corticofugal fibers descending in the adjoining cerebral peduncle. When the most ventral layer of the SNr was approached, high-amplitude electromyographic (EMG) activity of up to 6 mV with a distinctive waveform appeared synchronously with electrical stimuli in the anterior digastric, masseter, genioglossus, and levator labii superioris muscles. This EMG activity was evoked bilaterally, with an ipsilateral predominance. Eye movements, mostly rotation of the eyeball vertically down in the orbit, were noted. Infrequent blinking was also noted. Histologic examination localized the effector site to the middle third of the mediolateral extent of the caudal SNr corresponding to between 5.8 mm (level of the oculomotor nerve) and 6.5 mm (caudal end of the SNr) caudal to bregma; and to the ventralmost peripeduncular region of the SNr corresponding to 7.7 mm to 8.0 mm beneath the cortical surface. We referred to this site as the substantia nigra pars reticulata oro-facio-lingual (SNr-ofl) region. GABA injection produced tonic EMG discharge with consistent amplitude in all of the four muscles studied. The GABA effect was negated by a preceding microinjection of the GABA-A receptor antagonist bicuculline, whereas saline control injection had no effect. Changes in amplitude of evoked EMG activity according to location of the stimulating microelectrode reflected somatotopic organization of the SNr-ofl region. This extremely localized electrical and receptor microstimulation in the SNr produced synchronized powerful contraction of jaw, tongue, and facial muscles with different neural innervation. These findings advance our understanding of the mechanisms of the SNr concerning oro-facio-lingual movements.

Key words: basal ganglia, jaw muscle, tongue muscle, electromyography, GABA microinjection, decortication.

The projection fibers from the substantia nigra pars reticulata (SNr) of the rat are known to be distributed mainly in the thalamus, the superior colliculus, and the mesencephalic reticular formation (RF) (Deniau and Chevalier, 1992). A number of studies have indicated, however, that fibers from the SNr project further down to the RF of the pons and medulla oblongata. Tract tracing studies (von Krosigk and Smith, 1991; Yasui et al., 1992) of nigrobulbar projections have demonstrated in the rat that a considerable number of SNr neurons send projection fibers to the lateral parts of the pontine and medullary RF, which contain the bulk of orofacial premotor neurons (Travers and Norgren, 1983).

The SNr participates in control of oro-facio-lingual movements. Tremulous jaw movements induced in rats by i.p. injection of tacrine (an anticholinesterase) are blocked by bilateral injection of muscimol, a potent agonist at GABA-A receptors, into the SNr (Finn et al., 1997). Neuronal activity in the dorsolateral portion of the rat SNr is modulated by electrical stimulation of the orofacial sensorimotor cortex (Nishimuta et al., 2002). On the other hand, spontaneous activity of rat SNr neurons has been reported to be fully suppressed by iontophoretic application of GABA to the SNr (Martin and Waszczak, 1994), while high-frequency electrical microstimulation of the human SNr often inhibits spontaneous firing of SNr neurons after termination of the pulse train (Dostrovsky and Lozano, 2002).

In light of these recent findings, we sought to obtain further direct evidence for SNr control of oro-facio-lingual muscle activity. In the present study we developed a new microelectrode which permits both electrical and chemical microstimulations of an identical SNr locus, followed by placement of a minute electrolytic lesion in the stimulated locus for histologic identification of the site. Thus, we identified a small SNr focus where high-frequency microstimulation and GABA microinjection evoked augmented electromyographic (EMG) activity in the jaw, tongue, and facial

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Abbreviations: ACh, acetylcholine; BIC, bicuculline methiodide; DIG, anterior digastric muscle; EMG, electromyogram/electromyographic; GG, genioglossus muscle; Glu, glutamate; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; LEV, levator labii superioris muscle; MAS, anterior superficial masseter muscle; RF, reticular formation; S.E.M., standard error of the mean; SJR, striatal jaw region; SNr, substantia nigra pars reticulata; SNr-ofl, substantia nigra pars reticulata oro-facio-lingual; STN, subthalamic nucleus; VLS, ventrolateral striatum.

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muscles. Some of these results have been published in an abstract (Amano et al., 2004).

EXPERIMENTAL PROCEDURES

The experiments were conducted on 39 male Sprague–Dawley rats (Kudo, Saga, Japan) weighing 350–500 g. All procedures of the experiments were carried out in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. During experiments all effort was made to minimize pain as well as the number of rats used.

Preparation of decorticated rats

Accurate electrical microstimulation in the most ventral layer of the rat SNr requires the complete absence of electrical current spreading to adjoining structures, especially the cerebral peduncle. To prevent such current spread, we performed the stimulation in chronically decorticated rats whose corticobulbar fibers descending to brainstem orofacial motor nuclei had degenerated. Impulse conduction is well known to cease completely at 72 h after transection of a nerve fiber (Csillik and Toth, 1987). All rats to be used in the present study underwent an operation to aseptically remove the sensorimotor cortex at least 4 days prior to the electrophysiologic experiment. Each rat was anesthetized with a combination of ketamine (95 mg/kg, i.m.) and xylazine (15 mg/kg, i.m.), and then placed in a stereotaxic apparatus. Rectal temperature was maintained at 36–37 °C with a thermostatically controlled heating pad.

A total of 24 burr holes 2 mm in diameter were drilled bilaterally through the frontal and parietal bones in a honeycomb pattern extending from 7 mm anterior to 5 mm posterior to the bregma. Drilling was performed with a round diamond bur (diameter, 1.7 mm) in a dental air-turbine handpiece running at 450,000 revolutions per minute, without cooling. The depth of drilling included underlying right and left neocortical tissue. In addition, curettage was performed using a microspatula inserted through the individual bur holes to reach layers V and VI of the neocortex. This procedure resulted in severe mechanical damage and thermal injury to most of the sensorimotor cortex, excepting the most lateral portions. Next, a long, slim, curved microspatula was inserted into the right intracranial cavity through a long slit drilled into the lateral ridge of the right calvarium to perform limited curettage. This curettage reached layers V and VI of the frontal and insular cortices including the A- and P-areas shown to be capable of inducing rhythmic jaw movements by Zhang and Sasamoto (1990). Then the skin was sutured. Antibiotics and antiinflammatory agents were administered postoperatively, and liquid food was given using a gavage feeding needle when necessary.

Surgery for the electrophysiologic experiment

After an uneventful recovery, each decorticated rat was studied electrophysiologically at a single session 4–14 days postoperatively. On the day of the electrophysiologic experiment each animal was tracheotomized under ether anesthesia and mounted in a stereotaxic apparatus. A surgical level of anesthesia was induced with 1% to 2% halothane in a mixture of 50% N_2O and 50% O_2. Rectal temperature was maintained at 36–37 °C using a heating pad, and the heartbeat was monitored continuously through a speaker. The spinal cord was transected at C-2 level to restrict evoked movements to the head and face. For access to the right SNr, a circular hole 2 mm in diameter was drilled in the right skull at a point 5.5 mm posterior and 2.0 mm lateral to the bregma.

EMG recording

For bipolar EMG recording, two fine enamel-insulated copper wires (diameter, 0.28 mm) were sewn to the muscle belly at an interpolar distance of 2–3 mm. Muscles studied included the anterior digastric (DIG) as a jaw opener, the anterior superficial masseter (MAS) as a jaw closer, the genioglossus (GG) as a tongue protruder, and the levator labii superioris (LEV) as a facial muscle. All EMG responses were monitored continuously with a memory oscilloscope and an audio monitor before they were stored on magnetic tape. Selected EMG data were processed with a computer-based averager (QC-111J, Nihon Kohden, Fukuoka, Japan).

Electrical microstimulation and chemical microinjection

We developed a three-barreled microelectrode to perform electrical microstimulation and chemical microinjection at the same SNr site, following which we made a minute electrolytic lesion for histologic site verification. Three-barrel glass tubing with internal filaments (outside diameter, 1.2 mm; inside diameter, 0.68 mm; 3B120F-4, WPI, New Haven, CT, USA) was pulled and beveled to a total tip diameter of 80–100 μm. One of the three barrels contained an etched and insulated Elgiloy filament; one of the remaining barrels contained GABA (10 mM) and another contained either the GABA-A receptor antagonist bicuculline (BIC; 10 mM bicuculline methiodide) or physiological saline as a control. Three-barreled microelectrodes with a thicker tip of 150 μm were also produced for the experiment in which the effect of a large amount of GABA was tested. In this experiment, both barrels were filled with GABA solution.

The Elgiloy filament of three-barreled microelectrode was connected to an optically coupled stimulus isolator (SS-402J, Nihon Kohden). The respective barrels containing the solutions were connected with a pneumatic PicoPump (PV800, WPI) using hard polyethylene tubes. Both the stimulus isolator and the PicoPump were operated by an electronic stimulator (SEN-7203, Nihon Kohden).

Chemicals

GABA and BIC were obtained from Sigma-Aldrich Japan (Tokyo, Japan). All chemicals were dissolved in physiological saline.

Ejection apparatus and volume calibration

Compressed nitrogen gas was used as the pressure source for the PicoPump. The airway between PicoPump and micropipettes of the three-barreled microelectrode was switched manually using the trigonal cocks. Control over the volume of ejected solutions, which was less than 1 μl or more than 1 μl, was attained by using the three-barreled microelectrode with the appropriate kind of tip diameter, and adjusting pressure and duration of the pressure pulse, using the stimulator. A vacuum pump was connected to the vacuum input of the PicoPump to produce mild suction when pressure was not being applied. The volume of ejected solution less than 1 μl was determined by measuring the diameter of the droplet on the tip of the micropipette under visual inspection in air, using a binocular surgical microscope. The formula for the volume of a sphere was used. The volume more than 1 μl was measured with a micropipette collecting the ejected solution.

As the extent of spread when 1 μl of solution is injected into the brain has been estimated to be about 1 mm in diameter (Sawaguchi and Goldman-Rakic, 1991), we considered 1 μl of 10 mM GABA solution to be a sufficient volume for inhibition of a small cluster of GABAergic SNr neurons. Using a three-barreled microelectrode with a tip of 80–100 μm, under the pressure of 8 psi and the pulse width of 50–80 ms, four continuous-pressure