

cholinergic receptors enhances the endogenous release of brain acetylcholine by blocking the presynaptic cholinergic receptors that are subjected to feedback inhibition by acetylcholine<sup>12</sup>. Therefore, it is likely that the hypothermia induced by d-tubocurarine was due to the enhanced release of acetylcholine in the brain.

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### Reaching-related potentials in caudate nucleus and cerebellum of rats

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**Summary.** Electrical correlates of the lateralized reaching reaction were studied in rats with implanted electrodes. Averaged event-related potentials (ERPs) in the cerebellar dentate nucleus consisted of a negative wave coinciding with reach onset and followed 70 msec later by a positive deflection. The most prominent component of the more variable caudate ERPs was a negative wave culminating 60–120 msec after reach onset. The positive and negative ERP deflections corresponded to inhibitory and excitatory reactions respectively, at the single neuron level.

Rats trained to recover food pellets from a narrow tubular feeder perform the reaching movement consistently with either left or the right forepaw<sup>3</sup>. The lateralized reaction has features of a ballistic movement<sup>4</sup> and is therefore well suited for electrophysiological analysis. Reaching is accompanied by unit activity changes in the motor cortex and caudate nucleus<sup>5</sup>, and in the cerebellum<sup>6</sup>. Reaching-related EEG potentials are most pronounced in the motor cortex of the hemisphere contralateral to the preferred forepaw<sup>7</sup> and resemble event-related potentials (ERP) or motor potentials accompanying trained forelimb movements in the cat<sup>8</sup> or monkey<sup>9,10</sup>. The aim of the present study is to extend the ERP analysis of reaching in rats to basal ganglia and cerebellum.

15 male hooded rats were used. Under pentobarbital anesthesia (40 mg/kg), animals were bilaterally implanted with stainless steel electrodes (200  $\mu$ m in diameter) placed in the heads of the caudate nuclei (AP 2, L 2, V 5) or in the dentate nuclei of cerebellum (AP 12, L 3, V 3) according to the stereotaxic atlas of Fiková and Maršala<sup>11</sup>. A silver screw (2 mm in diameter) in the nasal bone 7 mm rostral to the bregma served as the reference electrode. 2 silver electrodes were used to pick up the surface activity of the cerebellar hemispheres (AP 12, L 4). The leads were connected to a miniature 5-pin transistor socket and the whole implant was fixed to the skull bones with anchoring bolts and acrylate. Recording started 1 week after surgery. Rats were trained to reach for 50-mg pellets of Larsen's diet placed 10–15 mm from the entrance of a narrow (11 mm in diameter) horizontal tube. Reaching was monitored by a photoelectric sensor, the output of which was used as the synchronization signal for a computer. The animal was connected through a counterbalanced 5-lead cable with the input of a conventional EEG apparatus with filters set to pass 1.5–200 Hz without attenuation. A LINC-8 computer was used for peri-event averaging ( $n=32$ ) of monopolar or bipolar records in a 768-msec interval starting 256 msec before reach detection. The position of the electrodes was histologically verified.

Cerebellar ERPs were obtained for 7 rats. Characteristic monopolar recordings are shown in figure 1. ERPs from the cerebellar cortex consisted of a protracted negative wave starting about 70 msec before reach onset and lasting approximately 200 msec. More complex ERPs were recorded in the dentate nucleus: a negative wave starting almost 100 msec before reach onset culminated during forelimb extension ( $-54 \pm 8 \mu$ V,  $n=7$ ) and abruptly changed to a positive deflection ( $+51 \pm 15 \mu$ V,  $n=7$ ) with the maximum at 70 msec after reach onset. This waveform was sometimes followed by another sequence of negative-positive deflections. Bipolar recordings between the dentate nucleus and the cerebellar cortex stressed the components with opposite polarities which were most pronounced shortly after reach

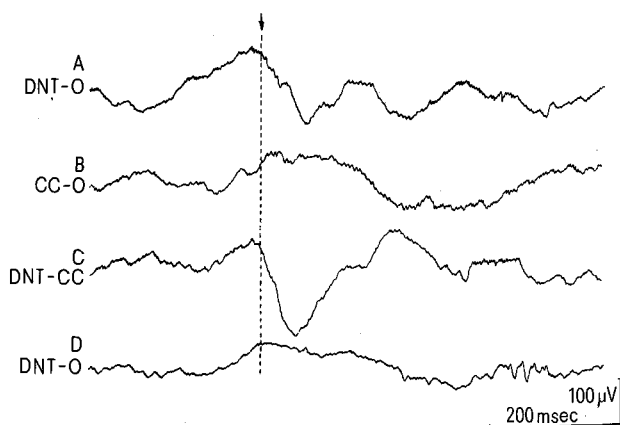


Figure 1. Averaged ERPs from the dentate nucleus (DNT) and cerebellar cortex (CC) ipsilateral to the reaching forepaw. Reach detection indicated by arrow. A, B Monopolar recordings; C bipolar recording; D ERP taken 24 h after electrolytic lesion of the recording site. The ERPs are averages of 32 responses. Calibration: 200 msec, 100  $\mu$ V. Negativity upwards.

onset (fig. 1C). The ERP amplitudes and latencies were similar in the hemisphere ipsilateral to the preferred forepaw. The local character of the dentate ERPs was confirmed by coagulation of the area of recording (anodal current, 1 mA, 20 sec). Dentate ERPs recorded 24 h after the electrolytic lesion lost the movement-related phasic components and resembled the attenuated cortical ERPs (fig. 1D).

Caudate ERPs were recorded in 8 rats. The average responses were more variable than in the cerebellum. Figure 2 shows the 4 most frequently encountered waveforms. The most prominent and consistently present component of the ERP was a negative wave ( $-67 \pm 8 \mu\text{V}$ ,  $n=15$ ) culminating between 60 and 120 msec after reach onset. This was preceded by smaller and less regular positivity, ( $+34 \pm 12 \mu\text{V}$ ,  $n=14$ ), coinciding with forepaw extension. A small negative wave (about  $30 \mu\text{V}$ ) appeared at half of the recording sites 100–50 msec before reach detection. Later ERP components were well expressed in some rats but their irregular latencies reflected individual

variability of movement duration. There were no systematic differences in the ERPs in the hemisphere ipsilateral and contralateral to the preferred forepaw.

In comparison with the reaching-triggered ERPs in the motor cortex of rats<sup>4</sup> the caudate ERPs were of opposite polarity and lower amplitude. At the single cell level, caudate neurons are inhibited rather than excited during reaching<sup>5</sup>. The positive wave corresponds to the maximum incidence of inhibitory reactions in the caudate nucleus, the subsequent negative wave to fast decay of this inhibition.

Dentate neurons react to reaching predominantly with excitation<sup>6</sup>, the peak of which corresponds to ERP negativity. The subsequent ERP positivity coincides with termination of the excitatory reaction in most neurons. Comparison of the neuronal population response with the ERP agrees with the conventional assumption that excitation is reflected by negativity and inhibition by positivity in the monopolar macroelectrode recordings.

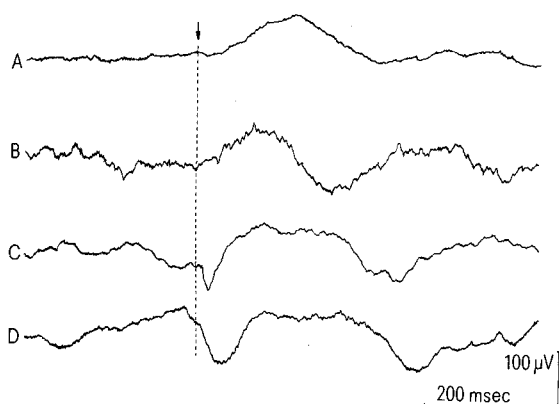


Figure 2. Examples of reach-related potentials from the contralateral caudate nucleus. Reach detection indicated by arrow. Averages of 32 response. Calibration: 200 msec, 100  $\mu\text{V}$ . Negativity upward.

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## Response of briefly glycerinated smooth muscle to $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$

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**Summary.** Smooth muscle, treated with 50% glycerol solution at  $27^\circ\text{C}$  for 20 min, contracted on the application of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ . The briefly glycerinated smooth muscle can be used as a model system of smooth muscle contraction.

Recently, it has been suggested by many investigators that the  $\text{Ca}^{2+}$ -regulatory contractile mechanism in smooth muscle is different from that in skeletal muscle<sup>1–6</sup>. We have shown that glycerinated smooth muscle contracted almost maximally when exposed to 15 mM  $\text{Mg}^{2+}$  and 5 mM ATP in the absence of  $\text{Ca}^{2+}$ , while glycerinated skeletal muscle needed  $\text{Ca}^{2+}$  with 15 mM  $\text{Mg}^{2+}$  and 5 mM ATP to contract<sup>7</sup>. The contraction of the smooth muscle without  $\text{Ca}^{2+}$  appeared slowly and was  $\text{Mg}^{2+}$ -dependent<sup>8</sup>. These data were obtained from muscle glycerinated in the usual way by treatment with 50% glycerol solution containing 60 mM KCl, 4 mM  $\text{MgCl}_2$  and 15 mM Tris-HCl (pH 6.8) under  $-20^\circ\text{C}$  for 1–2 weeks. It is probable that the responses of these glycerinated muscles cannot be exactly

compared to those of intact muscles. In the present study, we describe a new method of glycerol treatment, in which the period of glycerination is shortened and the temperature is kept constant at  $27^\circ\text{C}$ .

**Materials and methods.** Canine intestinal smooth muscle (jejunal portion), free from mucosa, was separated into longitudinal and circular muscles. The circular muscle (about 0.5 mm diameter and about 5 mm length) was suspended in an organ bath (1 ml) containing Tyrode's solution at  $27^\circ\text{C}$ , bubbled with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . After 1 h the bathing medium was changed to the relaxing solution, the composition of which was: 80 mM KCl, 4 mM ethylene glycol bis (2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 5 mM adenosine tri-