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Oscillatory activity in forelimb muscles of behaving monkeys evoked by microstimulation in the cerebellar nuclei☆

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Abstract

Coherent 20–35 Hz (β) oscillations are a prominent feature of activity in primary motor cortex and muscles of monkeys and humans performing voluntary movements. We found that coherent β oscillations are also present in the cerebellar nuclei (CN). Two monkeys were operantly conditioned to perform a wrist flexion/extension step-tracking task while we recorded neuronal activity or microstimulated in CN and recorded EMG activity from forelimb muscles. Coherent β oscillations were found between discharges of some CN neurons and tonically active shoulder, elbow and wrist/finger flexion and extension muscles. Similarly, localized microstimulation pulses in CN evoked transient β oscillations in widespread forelimb muscles. We conclude that coherent motor system β oscillations are present in CN and that CN may be an important nodal point for the generation and/or propagation of β oscillations throughout the motor system.

Keywords: Cerebellar nuclei; β oscillations; Motor control; Monkey; Muscle; Limb movement

Synchronous periodic discharges, or oscillations, are commonly observed in networks of neurons and have interesting implications for neural mechanisms of information processing. For example, synchronization of 40-Hz activities in separate areas of visual cortex has been suggested as a mechanism to 'bind' together different features of a coherent visual percept (e.g. orientation, shape, color and motion) [18]. In the motor system, coherent 20–35-Hz or β oscillations are a prominent feature of activity in primary motor cortex and muscles of monkeys and humans performing voluntary movements [1,4,7,10,13]. Coherent β oscillations have also been observed in other motor control nuclei, such as sub-thalamic nucleus [9], motor thalamus [8], supplementary motor area (SMA) [11] and cerebellar cortex [12]. The aim of the present study was to determine whether coherent β oscillations are present in another important motor control center, the cerebellar nuclei (CN).

Recordings were obtained in two adult *Macaca nemestrina* (monkeys A and B) performing a wrist flexion/extension step-tracking task with their right forelimb. Each monkey placed its hand with digits extended into a wedge-shaped manipulandum and tracked a displayed target with a cursor whose position was linearly proportional to flexion/extension about the wrist joint. Wrist movement was opposed by an elastic load proportional to displacement. During task performance we recorded single unit activity from the right cerebellar nuclei (CN) and EMG activity from 10–15 muscles acting about the shoulder, elbow, wrist and finger joints of the right forelimb. Histological reconstruction of electrode tracks in monkey A enabled identification of interpositus (IP) and dentate (D) recording sites.

Transient periods of oscillation in the activity of CN neurons were often seen during task performance. The IP neuron illustrated in Fig. 1 discharged one or more action potentials in periodic fashion during the wrist flexion and extension hold periods (gray shaded areas in the single trial records in Fig. 1Ac and Dc). Autocorrelograms of unit activity compiled during the flexion and extension hold periods confirmed this periodic activity to occur at ~25 Hz (black histograms in Fig. 1Ad and Dd). Moreover, spike-triggered averages (SpTAs) of rectified agonist muscle activity also revealed coherent oscillations at ~25 Hz, as illustrated for a wrist flexor muscle (palmaris longus) during flexion holds (blue histogram in Fig. 1Ad) and a wrist/finger extensor muscle (extensor digitorum 4,5) during extension holds (Fig. 1Dd). Coherent β oscillations were also present

☆ This paper is dedicated to Professor Manfred Zimmermann on the occasion of his 70th birthday and in recognition of his outstanding work as Editor-in-Chief of Neuroscience Letters.

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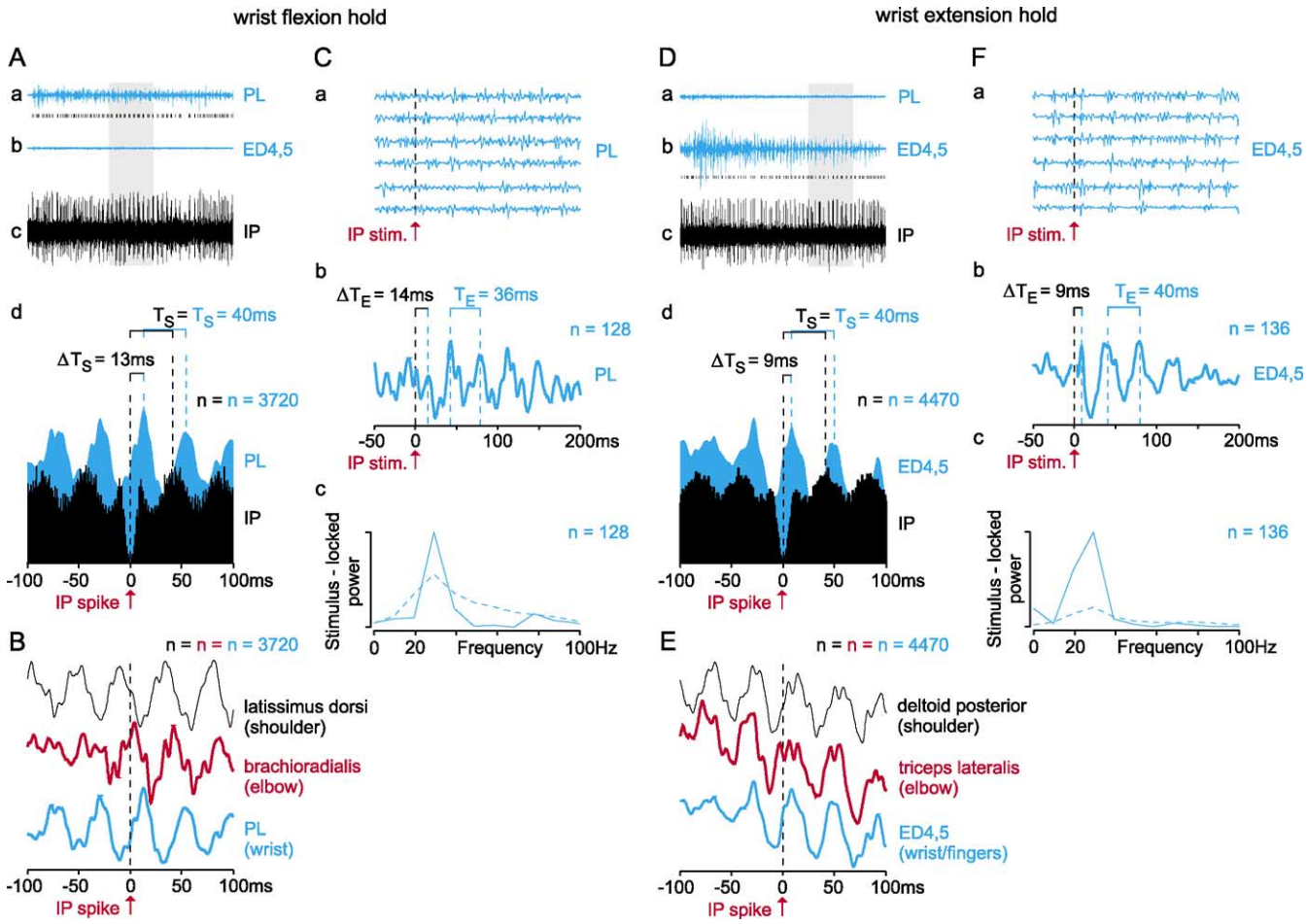

Fig. 1. Single unit recording and microstimulation in interpositus (IP) cerebellar nucleus of monkey A performing wrist flexion (A–C) and extension (D–F) holds against elastic loads. (A) Recordings during a flexion hold trial from (a) palmaris longus (PL) (wrist flexor), (b) extensor digitorum 4,5 (ED4,5) (wrist/finger extensor) and (c) an IP neuron. A raster plot of the times of IP neuron discharge is displayed below the PL muscle activity. During the period indicated by gray shading the IP neuron discharged one or more action potentials periodically at ~25 Hz. (d) Autocorrelogram of IP neuron discharges during all flexion hold periods (black histogram) superimposed on the corresponding spike-triggered average (SpTA) of rectified and smoothed PL muscle activity (blue analog average). The center bin of the autocorrelogram (time = 0) was removed. Vertical black and blue dashed lines signify one cycle of oscillation of neuron and muscle activity, respectively. T_S = spontaneous oscillation period and ΔT_S = temporal phase shift between neuron and muscle activity. (B) SpTAs of rectified and smoothed EMG activity during all flexion hold periods recorded from latissimus dorsi (shoulder extensor and adductor) (black), brachioradialis (elbow flexor) (red) and PL (blue). (C) PL responses to single microstimuli (60 μA) delivered at the same IP site during wrist flexion hold periods: (a) six selected trials, (b) stimulus-triggered average of rectified and smoothed EMG for all trials, (c) average stimulus-locked FFT power of rectified and smoothed EMG for all trials; dashed line = 95% confidence interval. In (b), vertical blue dashed lines signify one cycle of oscillation of evoked muscle activity. T_E = evoked oscillation period and ΔT_E = temporal phase shift between CN microstimulation and short-latency muscle activity. (D–F) Exact same format as A–C with the same IP neuron and microstimulation site as A–C but during wrist extension hold periods with different active muscles.

in other co-activated muscles acting about the shoulder, elbow and wrist/finger joints during wrist flexion (Fig. 1B) and extension (Fig. 1E).

We analyzed 43 well-isolated CN neurons (29 in monkey A and 14 in monkey B) whose discharge rate was consistently modulated with forelimb movements, and whose activity was recorded during ≥ 7 of each of the wrist flexion/extension hold periods. Auto-correlograms of CN neuron discharges compiled during the hold periods showed clear periodic features in 5/29 CN neurons in monkey A (all at ~25 Hz) and 2/15 in monkey B (one at ~45 Hz and the other at ~77 Hz). In monkey A, periodically active CN neurons were found in both IP ($n = 3$) and in D ($n = 2$). Spike-triggered averages of rectified EMG compiled during the hold periods revealed coherent oscillations between IP neuron discharge and at least one forelimb muscle whenever oscillations were apparent in the unit autocorrelogram. Of the three IP neurons in monkey A that discharged periodically during periods of muscle activity, two exhibited coherent oscillations in SpTAs of both wrist flexor muscles (during flexion holds) and wrist extensor muscles (during extension holds). Two of the IP neurons in monkey A also displayed coherence with muscles acting across two forelimb joints (either shoulder and wrist or elbow and wrist) during at least one of the hold periods.

To further test the role of the CN in generating oscillatory activity we investigated the effects of delivering single pulse

microstimuli at CN sites. Biphasic stimulus pulses (−0.2 ms/+0.2 ms; 5 Hz) were delivered at 32 and 25 CN sites in monkeys A and B, respectively, during task performance. Stimulus currents were subthreshold for evoking any muscle responses in the absence of background EMG activity. Stimulus intensities averaged 39.5 ± 22.2 μA (mean ± S.D.; range: 5–60 μA) in monkey A and 43.8 ± 12.9 μA (10–50 μA) in monkey B. At the site of the IP cell in Fig. 1, single-pulse microstimuli evoked 2–3 cycles of periodic muscle activity (Fig. 1Ca,Fa) which appeared in stimulus-triggered averages of rectified EMG as β-frequency oscillations (Fig. 1Cb,Fb). CN stimulus-evoked β oscillations were most prominent during the wrist flexion/extension hold periods of the step-tracking task, when muscles were tonically active against an opposing load. For both flexion and extension, the periods of the evoked oscillations (T_E in Fig. 1Cb and Fb) closely matched the periods in the oscillatory activity occurring spontaneously (T_S in Fig. 1Ad and Dd). Moreover, the latencies of the initial evoked muscle responses (ΔT_E in Fig. 1Cb and Fb) corresponded to the respective temporal phase shifts of the spontaneous oscillations (ΔT_S in Fig. 1Ad and Dd). This suggests a close relationship between the activities of the CN unit and muscles that can be probed with microstimulation.

The presence of CN-stimulus-evoked β oscillations in muscles was quantified by calculating stimulus-locked power for stimuli delivered during the flexion/extension hold periods of the step-tracking task. In the frequency domain, microstimulation-locked power was calculated using a modification of the fast Fourier transform (FFT) algorithm on Hanning-windowed data segments 102.4 ms long, digitized at 5 kHz, aligned to each stimulus (stimulus artifact excluded). The Fourier coefficient $F_n(f)$ for the nth stimulus ($n = 1, 2, \ldots, N$) is a complex number representing the amplitude and phase at frequency f:

$$F_n(f) = \frac{L}{2} a_n(f) e^{i\phi_n(f)} \quad (1)$$

The power spectrum $P(f)$ is the squared magnitude of each Fourier coefficient averaged across all stimuli:

$$P(f) = \frac{1}{N}\left|\frac{2}{L}F_n(f)\right|^2 = \frac{1}{N}\sum_{n=1}^{N} a_n(f)^2 \quad (2)$$

Stimulus-locked power is calculated by averaging the coefficients before taking the squared magnitude:

$$P_{s-l}(f) = \left|\frac{1}{N}\sum_{n=1}^{N}\frac{2}{L}F_n(f)\right|^2 \quad (3)$$

In this method, the phase component of each Fourier coefficient is included in the average, and frequency components without a constant phase relative to the stimulus are averaged out. The significance of stimulus-locked power was determined by randomly shuffling the phase components of these same segments (L) of data 1000 times and calculating the 95th centiles. Phase-locked power values greater than this were considered significant at the corresponding confidence level. This FFT analysis had a resolution of ~10 Hz, so power at ~20 Hz and ~30 Hz was analyzed and is presented throughout this report.

Significant (>95% confidence interval) β activity was evoked in at least one active forelimb muscle from most CN sites (25/32 in monkey A (e.g. Fig. 1Cc,Fc) and 16/25 in monkey B). Averaging across all sites, CN stimulation evoked significant 20 Hz activity in 23% (range 0–89%), and 30 Hz activity in 30% (0–100%) of recorded muscles in monkey A (with 5–6 muscles active in flexion and 9–10 muscles active in extension). In monkey B the corresponding proportions were 11% (0–100%) and 12% (0–80%) (five muscles active in flexion and five muscles active in extension).

Stimulation at a given CN site generally evoked transient β activity in multiple muscles at proximal and distal joints that were tonically active during the wrist flexion/extension holds. For example in monkey A, stimulation at 7/32 CN sites evoked significant β oscillations in at least one shoulder, elbow and wrist/finger extensor muscle during wrist extension holds. Stimulation at a given CN site could also evoke significant β oscillations in antagonist muscles acting about the same joint. Stimulation at 14/32 and 5/25 CN sites in monkeys A and B, respectively, evoked β oscillations in wrist/finger extensor muscles during extension holds and wrist/finger flexor muscles during flexion holds.

Stimulation in interpositus (IP) was more effective in evoking β activity in muscles than stimulation in dentate (D). Averaging across all IP sites in monkey A ($n = 11$), stimulation evoked significant 20 Hz activity in 38% (range 0–89%) and 30 Hz activity in 44% (0–100%) of recorded muscles. The corresponding proportions for all D sites in this monkey ($n = 21$) were 16% (0–89%) and 23% (0–100%). This difference was significant ($P = 0.002$ (20 Hz) and $P = 0.015$ (30 Hz), t-tests, IP vs. D) and was not due to stimulating with higher currents in IP (IP current = 36.4 ± 21.0 μA, D current = 41.2 ± 23.1 μA, $P = 0.57$, t-test).

To determine whether the frequency of CN stimulus-evoked muscle oscillations was specific to the location of CN stimulation or to particular muscles we assigned a dominant β frequency (either 20 or 30 Hz) to each muscle according to the frequency that had the greater significant stimulus-locked power (e.g. ED4,5 in Fig. 1Fc was assigned 30 Hz). There was no tendency for stimulation at particular CN sites in monkeys A or B to evoke muscle oscillations of a particular dominant β frequency (either 20 or 30 Hz); nor was there any tendency for IP sites to evoke a different dominant frequency of β oscillation than D sites in monkey A. For example, stimulation at most CN sites (11/16 in monkey A and 3/4 in monkey B) evoked 20-Hz dominant oscillations in some muscles and 30-Hz dominant oscillations in others (of the sites eliciting significant β

Table 1
Dominant CN microstimulation-evoked β (20 or 30 Hz) frequencies in forelimb muscles during wrist flexion and extension holds

	Flexion						Extension									
	Brachio-radialis	Biceps longus	**FDS2,3**	**FDS4**	**FDP**	**PL**	Deltoid posterior	Brachio-radialis	Triceps lateralis	Triceps longus	**EDC**	**ED2,3**	**ED4,5**	**ECR**	**ECU**	Pronator teres
Monkey A																
20 Hz	3	2	**3**	**2**	**3**	**6**	3	0	4	3	**2**	**1**	**2**	**2**	**2**	3
30 Hz	12	7	**10**	**6**	**4**	**5**	7	10	7	0	**8**	**9**	**12**	**11**	**10**	10
Monkey B		Deltoid posterior	**FCU**	**FDS**	**FDP**	**PL**					**APL**	**ED2,3**	**ED4,5**	**ECR**	**ECU**	
20 Hz		2	**5**	**1**	**3**	**2**					**0**	**1**	**2**	**0**	**0**	
30 Hz		1	**6**	**2**	**5**	**0**					**1**	**1**	**2**	**3**	**4**	

See text for further explanation. Wrist/finger flexor and extensor muscles are highlighted in bold.

oscillations in ≥ 4 muscles during flexion and extension holds). In contrast, some muscles tended to exhibit the same frequency of oscillation irrespective of the CN stimulus site. For example, wrist/finger extensor muscles were activated preferentially at 30 Hz from most CN sites during wrist extension, as was brachioradialis during wrist flexion and extension (Table 1). Oscillations at predominantly 20 Hz were evoked more often in wrist/finger flexor muscles and triceps longus (Table 1).

Taken together, these results suggest that CN may be an important nodal point in the generation and/or propagation of β oscillations throughout the motor system. Coherent β oscillations were seen between the discharges of single IP neurons and widely distributed forelimb muscles in SpTAs. Although this has also been seen for motor cortical neurons during selected periods of oscillatory activity [4,10], it is not typical of SpTAs from cortical cells [10]. Moreover, CN microstimulation evoked or reset β-band rhythms in tonically active forelimb muscles of behaving monkeys. Stimulation of the entire pyramidal tract can reset cortical β rhythm and evoke repetitive EMG activity [6], but repetitive responses have not been evoked by single intracortical microstimuli [5]. In contrast, single-pulse microstimuli at sites in CN could evoke β oscillations in widespread muscle groups, including antagonist muscles acting about a single joint, and muscles acting about the shoulder, elbow and wrist/finger joints.

These effects could be mediated by the highly divergent pathway between CN and primary motor cortex (via motor thalamus) [2,3,14,15,17]. Indeed, extensive divergence of single axonal arbors has been demonstrated anatomically in both the cerebello-thalamic and thalamo-cortical components of this pathway in cat and rat [2,3,14,17]. Moreover, cerebello-thalamic synapses are remarkably potent, with unitary excitatory postsynaptic potentials of 1.5 mV on average [15]. Such potent and divergent output suggests that oscillations at a localized CN site might entrain oscillations over widespread areas of M1 [19] and thereby in different muscles. Other subcortical motor centers affected by CN output might also be entrained by localized CN oscillations, since the same CN output neurons can project axon collaterals to both red nucleus and motor thalamus [16].

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