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LETTERS

Direct control of paralysed muscles by cortical neurons

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A potential treatment for paralysis resulting from spinal cord injury is to route control signals from the brain around the injury by artificial connections. Such signals could then control electrical stimulation of muscles, thereby restoring volitional movement to paralysed limbs[1–3]. In previously separate experiments, activity of motor cortex neurons related to actual or imagined movements has been used to control computer cursors and robotic arms[4–10], and paralysed muscles have been activated by functional electrical stimulation[11–13]. Here we show that *Macaca nemestrina* monkeys can directly control stimulation of muscles using the activity of neurons in the motor cortex, thereby restoring goal-directed movements to a transiently paralysed arm. Moreover, neurons could control functional stimulation equally well regardless of any previous association to movement, a finding that considerably expands the source of control signals for brain-machine interfaces. Monkeys learned to use these artificial connections from cortical cells to muscles to generate bidirectional wrist torques, and controlled multiple neuron–muscle pairs simultaneously. Such direct transforms from cortical activity to muscle stimulation could be implemented by autonomous electronic circuitry, creating a relatively natural neuroprosthesis. These results are the first demonstration that direct artificial connections between cortical cells and muscles can compensate for interrupted physiological pathways and restore volitional control of movement to paralysed limbs.

Spinal cord injury impairs neural pathways between the brain and limbs, but spares both the motor cortex and muscles. Recent studies have shown that quadriplegic patients could volitionally modulate activity of neurons in the hand area of the motor cortex, even after several years of paralysis[6], and that monkeys could use cortical activity to control a robotic arm to acquire targets[4] and feed themselves[5]. These and other brain–machine interface studies used sophisticated algorithms to decode task-related activity of neural populations and to calculate requisite control parameters for external devices[4–6,8–10]. An alternative strategy to restore limb function is to directly connect cortical cell activity to control the stimulation of a patient's paralysed muscles (Fig. 1a). Here we show that monkeys can learn to use direct artificial connections from arbitrary motor cortex cells to grade stimulation delivered to multiple muscles and restore goal-directed movement to a paralysed arm.

In previous biofeedback studies monkeys rapidly learned to control the discharge rates of newly isolated neurons in the motor cortex to obtain rewards[14,15]. We used similar operant conditioning techniques for single neurons in the hand and wrist area of the motor cortex of two monkeys (see Methods and Supplementary Information). We tested volitional control of cell activity by displaying smoothed discharge rate as cursor position on a monitor and rewarding the monkeys for maintaining activity within randomly presented high- or low-rate targets. The directional tuning of most cells was also characterized in an isometric two-dimensional wrist target-tracking task. Our experiment, however, used all sufficiently well-isolated cells encountered, with no selection bias for possible association to movement or directional tuning.

Monkeys demonstrated volitional control of the discharge rates of nearly all cells tested within the first 10-min practice session. Although cell activity controlled the cursor directly, monkeys often continued to produce wrist torques during these initial sessions (Supplementary Fig. 1). We then blocked peripheral nerves innervating the wrist muscles with a local anaesthetic (see Methods). Despite loss of motor function and sensory feedback from the innervated forearm, monkeys continued to control the cursor with cell

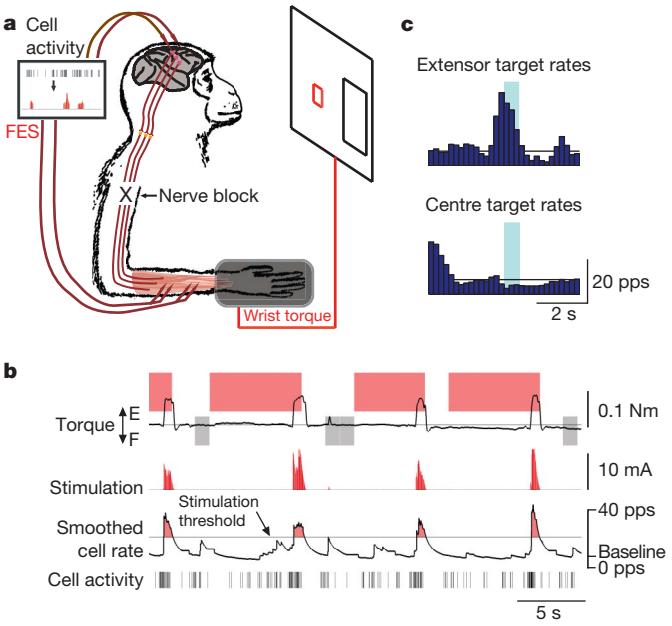

Figure 1 | Brain-controlled functional electrical stimulation (FES) of muscle. **a**, Schematic shows cortical cell activity converted to FES during peripheral nerve block. **b**, Example of motor cortex cell activity controlling FES of paralysed wrist extensors. Extensor (red shading) and centre (grey shading) wrist torque targets were randomly presented. Monkeys learned to modulate smoothed cell rate to control proportional muscle stimulation. FES was delivered to muscles EDC and ED4,5 at 50 s^{-1}, with current proportional to cell rate above a stimulation threshold (0.4 mA pps^{-1} × [cell rate − 16 pps]; ≤10 mA). Here pps indicates pulses per second. **c**, Histograms of cell rates while acquiring the extensor and centre targets, illustrating cell activity used to successfully control FES. Shading indicates target hold period and horizontal line denotes baseline cell rate.

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activity for 45 out of 46 cells after the nerve block. Supplementary Fig. 1 shows the loss of flexor and extensor torques after injections of local anaesthetic, while the monkey continued to volitionally control the cell activity. The nerve block was confirmed by the inability of the monkeys to perform the two-dimensional torque-tracking task.

We then converted cell activity into proportional stimuli delivered to paralysed muscles, generating functional electrical stimulation (FES). The cursor was now controlled by wrist torque, and the monkeys were rewarded for maintaining FES-evoked torque within peripheral and centre (that is, zero-torque) targets for 0.5–1.0 s. To allow the monkeys to grade contraction force, stimulation current was made linearly proportional to cell rate when the cell discharged above a threshold.

The example in Fig. 1b, c shows a monkey modulating cell activity to generate appropriate torques by controlling FES of paralysed wrist extensor muscles. The monkey learned to increase cell activity to activate the stimulator and acquire the extensor targets, and to maintain activity below the stimulation threshold to relax the muscle and acquire the centre targets. Both monkeys were able to control muscle FES during nerve block and acquire torque targets with 44 out of the 45 cells tested (5 cells from monkey I and 39 from monkey L).

For each cell the monkeys' control improved with practice, as evidenced by more rapid acquisition of targets and fewer errors. Monkeys began using cell activity to control the stimulator almost immediately, and improved substantially during the relatively brief practice sessions with each cell (mean duration 66 min). To quantify this improvement we compared performance during the initial two minutes of practice and during the two-minute period with the highest performance, typically just before task difficulty was increased to probe the limits of FES control. The rate of target acquisition with FES control was over three times greater during peak performance (14.1 ± 5.3 torque targets acquired per min; mean ± s.d.) compared to the beginning of practice (4.0 ± 4.3 targets per min; $P < 0.001$; Supplementary Fig. 2). Peak target acquisition rates during brain-controlled FES were similar to those seen when cell activity controlled the cursor directly before nerve block (13.2 ± 5.5 targets per min; $P = 0.66$).

With continued practice monkeys also learned to control the torque more precisely with cell activity, making fewer target acquisition errors and often acquiring targets on the first attempt. A target acquisition error was defined as triggering the stimulator to acquire the peripheral target when the centre target was displayed. Monkeys made target errors on only 0.8 ± 5.1% of targets during peak performance for each cell compared to 20.7 ± 28.9% of targets at the beginning of practice ($P < 0.001$; Supplementary Fig. 3). They also made 81% fewer failed attempts to acquire the target during peak performance (0.10 ± 0.31 failed attempts per target) compared to the beginning of practice (0.52 ± 0.93; $P < 0.001$).

To test whether FES could also be controlled by decreases in cell activity, we set stimulation current to be inversely proportional to cell rate below a threshold for 11 cells. Monkey L learned to control stimulation with this inverse relation just as well as with a positive relation between cell rate and stimulus current (38 cells, some tested in both groups; $P > 0.46$), acquiring 13.4 ± 3.9 targets per minute and making no errors during peak performance.

The activity of a single cell could also be used to control stimulation of antagonist muscle groups and restore bidirectional movements. Figure 2 shows an example of one cell that controlled stimulation of flexor muscles with high discharge rates and extensor muscles with low rates. The monkey learned to control cell activity and grade contraction force to rapidly satisfy targets at five different torque levels. The nerve blocks remained very effective, as evidenced by negligible torques produced in either direction when the stimulators were turned off during target presentation (Fig. 2b). Seven cells tested with such bidirectional control performed similarly to cells that controlled only one muscle group, although target acquisition rates were marginally slower (9.8 ± 3.7 targets per min; $P = 0.06$).

The assumptions underlying common neural decoding schemes would predict that monkeys should be able to control FES torque better with cells that are strongly related to wrist movements than with unrelated cells. To investigate this, we documented cell activity during a two-dimensional wrist target-tracking task before the nerve

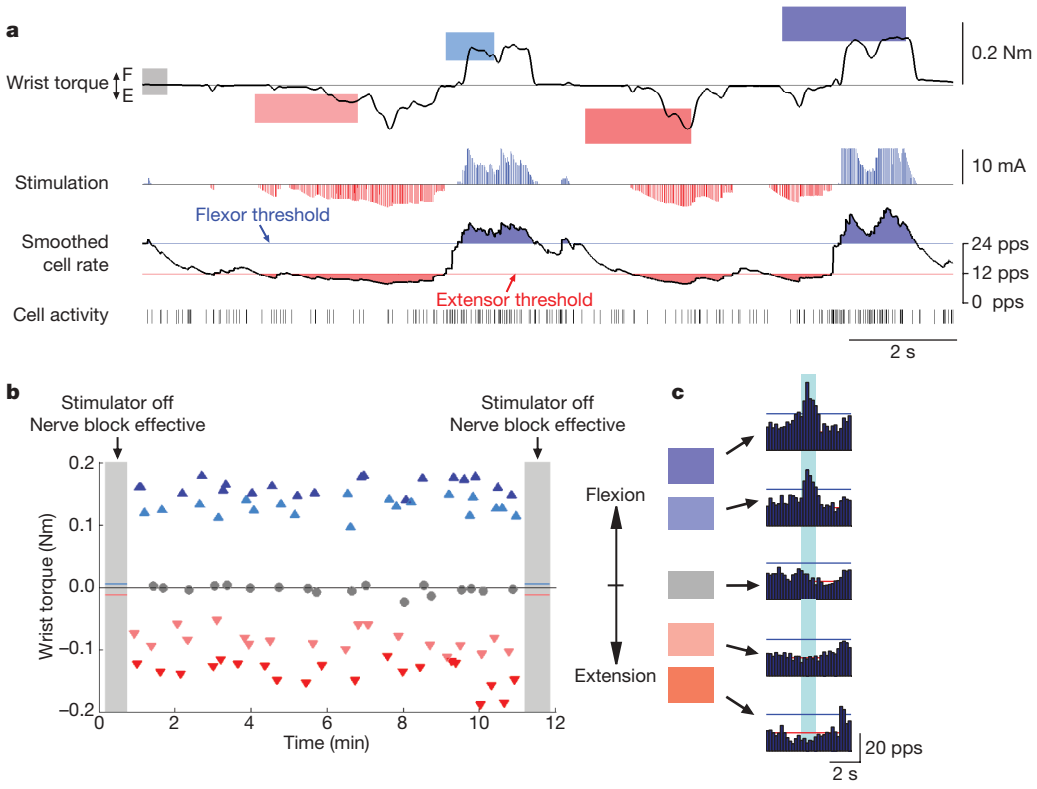

Figure 2 | Brain-controlled FES of multiple muscles restores graded torque in two directions. **a**, The monkey acquired targets at five levels of flexion-extension (F-E) torque using the activity of a single cell to grade FES delivered to both flexor (FCU) and extensor (ECU and ED4,5) muscles. Flexor FES was proportional to the rate above a threshold (0.8 mA pps^{-1} × [cell rate − 24 pps]; ≤10 mA); extensor FES was inversely proportional to the cell rate below a second threshold (0.6 mA pps^{-1} × [12 pps − cell rate]; ≤10 mA). **b**, Average torques produced to satisfy the five targets during 12 min of practice. With the stimulator off (shaded periods) the monkey could not produce torques greater than 10% of magnitudes used to acquire the targets (blue and red lines), confirming the efficacy of nerve block. **c**, Histograms of cell rate used to acquire five target levels (coloured boxes at left). Horizontal lines indicate FES thresholds for flexor (blue) and extensor (red) stimulation.

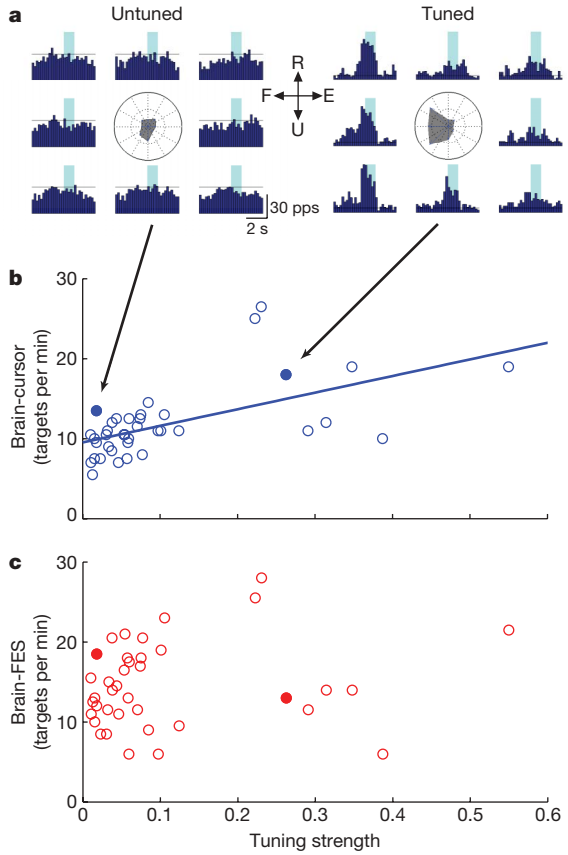

Figure 3 | Cell directional tuning is unrelated to FES control. **a**, Responses of an untuned and a strongly tuned cell (solid symbols in **b** and **c**). The surrounding peri-event histograms show cell activity while matching (shading) each of eight peripheral torque targets in the flexion-extension (F-E) and radial-ulnar (R-U) plane during the unparalysed tracking task (horizontal lines denote baseline cell rates). The radial plot at the centre summarizes cell activity while matching each peripheral target. **b**, **c**, Maximum target acquisition rates during direct brain control of cursor (**b**) and brain-controlled FES (**c**) plotted as a function of directional tuning strength for cells recorded during the torque-tracking task ($n = 38$). Performance controlling a cursor directly with cell activity was significantly correlated with cell tuning (**b**; $r^2 = 0.33$, $P < 0.001$). Subsequent brain-controlled FES performance was uncorrelated with cell tuning (**c**; $r^2 = 0.03$, $P = 0.33$).

block, and calculated the directional tuning for each cell (Fig. 3a). The magnitude of directional tuning did correlate significantly with the ability of the monkeys to bring the cursor into the optimally placed targets with cell activity during the initial 10-min practice period ($r^2 = 0.33$, $P < 0.001$; Fig. 3b). However, cell tuning was not a good predictor of the peak target acquisition rates during subsequent brain-controlled FES ($r^2 = 0.03$, $P = 0.33$; Fig. 3c). For example, with the untuned cell on the left in Fig. 3a the monkey acquired 18.5 targets per minute. The tuned ($n = 9$) and untuned ($n = 29$) cells showed no differences in any measure of FES control (target acquisition rates, errors or failed attempts; $P > 0.51$).

Extending the strategy of direct neural control to more complex movements will require further control signals. As a first step towards this goal, we tested a monkey's ability to simultaneously control two cell–muscle pairs. Figure 4 shows monkey L using high discharge rates of one cell to control FES of flexor muscles and high rates of a second cell to control extensor muscles. The monkey learned to independently modulate the activity of five cell pairs in order to control antagonist muscles and rapidly acquire bidirectional torque targets at rates similar to single cells (11.6 ± 3.8 targets per min, $P = 0.32$).

These findings have several implications for future approaches to neuroprosthetic control. In contrast to the conventional strategy of deriving control signals from the combined activity of a neural population[4–6,8–10], it may prove efficacious to maintain separate signal pathways from cells to muscles. Using direct channels from single cells to specific muscles may provide the brain with more distinguishable outcomes of the cell activity[16] and allow innate motor learning mechanisms to help optimize control of the new connections. The ability of the brain to adapt to new but consistent sensorimotor contingencies has been amply documented[17,18], and motor cortex can adapt rapidly to learn new motor skills[19,20]. Motor circuitry can compensate for drastic changes in connectivity, such as surgically

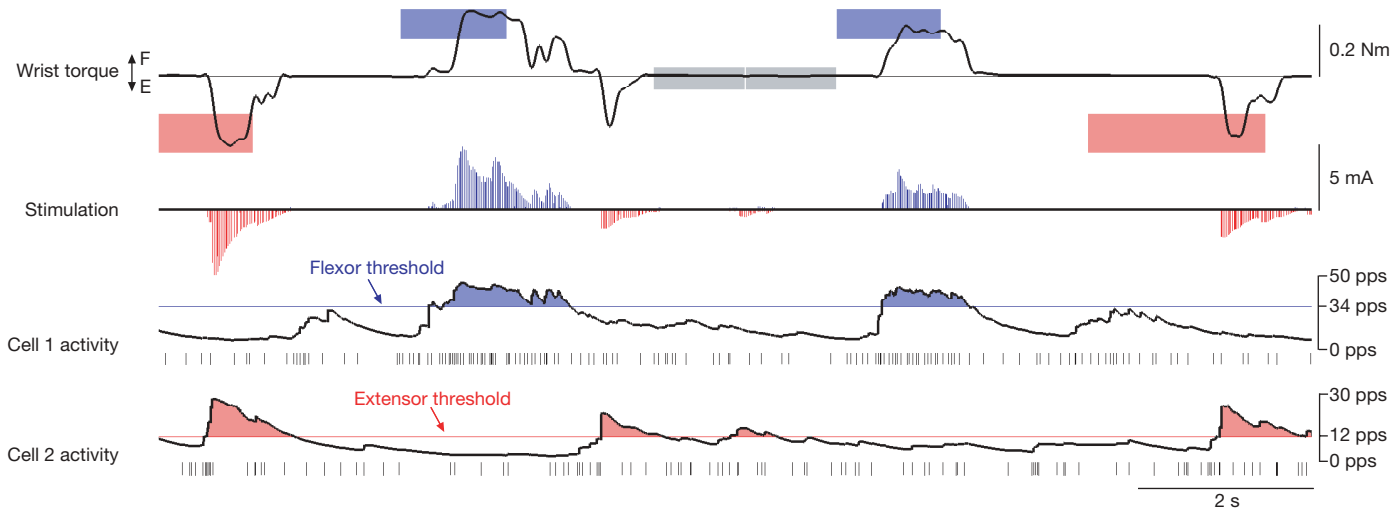

Figure 4 | Two neurons control FES. Monkey L simultaneously modulated activity of two neurons, each controlling proportional stimulation of a different muscle group when above a threshold. Monkey L acquired randomly presented flexor (blue), extensor (red) and centre (grey) targets by using cell 1 to stimulate a flexor muscle (FCU; 0.2 mA pps^{-1} × [cell rate − 34 pps]) and cell 2 to stimulate extensor muscles (ECU and ED4,5; 0.4 mA pps^{-1} × [cell rate − 12 pps]).

cross-connected nerves controlling wrist flexor and extensor muscles[21], or targeted reinnervation for control of prosthetic limbs[22].

Our finding that monkeys could learn to use virtually any motor cortex cell to control muscle stimulation—regardless of the cell's original relation to wrist movement (Fig. 3c)—suggests another advantage of directly tapping single cell activity. Strategies based on decoding the activity of neural ensembles to obtain movement parameters or muscle activity depend on finding cells that modulate sufficiently with the output variables during actual or imagined movements[4–6,8–10]. Instead, arbitrary cells available on recording arrays could be brought under volitional control using biofeedback, substantially expanding the source of control signals for brain–machine interfaces. This and previous biofeedback studies[14,15] have shown that even cells with no discernable relation to muscles can be volitionally modulated after brief practice sessions. Issues concerning the use of individual cells and neural populations for prosthetic control are further discussed in Supplementary Information.

The degree of FES control demonstrated here was limited by the relatively brief training time provided by the transient nerve block. Implanted electronic circuitry will enable adaptive learning over much longer times and under more varied conditions[1]. For example, the autonomous 'Neurochip' system can discriminate single cell activity and deliver stimulation through days of free behaviour[23,24]. In several preliminary FES sessions, we confirmed that this system would allow a monkey to trigger stimulation of a paralysed muscle with cell activity and acquire torque targets (Supplementary Fig. 4). Such autonomous low-power circuits could permit subjects to practice continuously with an artificial connection from brain to muscles or the spinal cord[1,25–27], without requiring complex decoding algorithms or robotic arms. Further development of such direct-control strategies may lead to implantable devices that could help restore volitional movements to individuals living with paralysis.

METHODS SUMMARY

Subjects. Two male *Macaca nemestrina* monkeys participated in the experiments (4–5-yr-old, weight 4.5–6.5 kg). All procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Recording and paralysis. The activity of single motor cortex cells was recorded using either acute (Monkey I and L) or chronic (Monkey L) electrodes. Each session began by quantifying the response of cells during an isometric, eight-target wrist torque-tracking task. Volitional control of cell activity was confirmed by operantly rewarding acquisition of targets with a cursor controlled by cell rates. Wrist muscles were then paralysed by injecting anaesthetic (3% chloroprocaine or 2% lidocaine, each with 1:100,000 adrenaline) into catheters or cuffs surrounding the median, ulnar and/or radial nerves.

Brain-controlled FES. Cell activity controlled the intensity of stimuli delivered by means of bipolar electrodes implanted in one or more paralysed wrist muscles. When cell rate (smoothed over a 0.5-s sliding window) crossed a threshold, biphasic constant-current stimuli (cathode leading; 0.75–1.0 ms pulse width) were delivered at 50 s^{-1}. For most cells, stimulus current was made proportional to cell rate above a threshold to allow the monkey to grade contraction force (for example, stimulus current = 0.1 mA × [cell rate − threshold]; to a maximum of 10 mA). Some cells controlled stimulation in inverse proportion to cell rate below a threshold.

Analysis. The strength of directional tuning was calculated for cells during the initial torque-tracking task using the vector method[28] (see Supplementary Information). Peak performance was quantified by the maximum number of targets acquired during a two-min period. Peak performance was compared among conditions and to performance during the initial two minutes of practice using the non-parametric rank sum test. Regression analysis determined correlations between directional tuning and peak performance during brain control of a cursor or FES.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Cortical recording. Sterile surgeries were performed with isoflurane anaesthesia (1–1.5% in 50:50 O_2:N_2O). All surgeries were followed by a program of analgesics (buprenorphine 0.15 mg kg^{-1} and ketoprofen 5 mg kg^{-1}) and antibiotics (cephalexin 25 mg kg^{-1}). Each animal was implanted with a cranial recording chamber over the left hand and wrist area of the motor cortex using stereotaxic coordinates (anterior: 13 mm, lateral: 18 mm) to permit cortical recordings[29,30]. To obtain longer duration cell recordings, monkey L was re-implanted with a chronic electrode array over the left motor cortex. The array of 12 independently movable microwires is fully described elsewhere[31]. In brief, 50 μm tungsten wires were threaded through individual polyamide guide-tubes in a 2×6 array that was anchored to the skull. This array provided stable recordings from the same isolated cell for the duration of an experimental session, and across multiple days for ten cells[24,31,32].

Nerve block implant. Reversible paralysis of the right wrist was achieved with one of two nerve block methods. First, catheters were implanted in the brachial plexus, near cords giving rise to the radial, ulnar and median nerves. Epidural catheters (19 gauge, Arrow International) were inserted into the epineurium surrounding each nerve and anchored in place with cyanoacrylate. Second, nerve cuffs with catheters[33] were implanted around the median and ulnar nerves in the upper arm. Catheters terminating in the lumen of each Silastic cuff (4 mm inner diameter, 30 mm long) permitted the nerves to be bathed in anaesthetic. Nerves were identified by electrical stimulation, and catheters were tunnelled subcutaneously to exit the skin on the upper back and sealed with an injection port. Thirty-one cells controlled FES during nerve blocks induced by the catheter method, and the remaining 13 cells during blocks induced by cuffs.

Experimental procedures. The monkey sat with his right elbow and hand immobilized by padded splints while a transducer measured the flexion-extension (F-E) and radial-ulnar (R-U) torques produced about the wrist (see Fig. 1a). To receive an applesauce reward, the monkey maintained wrist torque within a centre target (zero torque) followed by one of eight peripheral targets specifying different combinations of F-E and R-U torque (average magnitude 0.13 ± 0.01 nM). Isolated cell activity was discriminated online using template-matching software (Alpha Omega MSD). Subsequently, cell activity controlled cursor movement in one dimension. Interspike intervals were averaged over a 0.5-s sliding window to create a continuous signal for cursor position (and later FES control). If the cell was directionally tuned, targets were aligned with its preferred direction. For untuned cells or cells without tuning information (that is, cells isolated after nerve block began), either the left or right screen position was arbitrarily chosen to represent high discharge rates for visual feedback. Monkeys practiced cell control for 10 min, maintaining discharge rates within each target for 0.5–1.0 s to receive a reward.

Nerve block. We blocked nerves leading to wrist muscles with local anaesthetic to create temporary motor paralysis. Block onset typically occurred after 5–60 min, depending on anaesthetic and block method. During this time the monkeys continued to perform the cell-controlled target-tracking task. Further doses were given regularly to maintain paralysis during FES control.

Brain-controlled FES. After onset of paralysis and an average of 36 ± 22 min of cell-controlled target tracking, the cell activity was converted to stimuli delivered to one or more paralysed muscles. Wrist torque controlled the position of the cursor, and targets were randomly displayed on the monitor in one dimension. Monkeys were required to maintain torque within each target for 0.5–1.0 s (mean 0.56 s) to receive a reward. Targets remained on the screen until satisfied, followed by presentation of the next target either immediately or after a variable reward period (1.5–1.7 s). Forty-two cells controlled stimulation current in proportion to cell rate, permitting the monkey to grade contraction force. Two of these cells also controlled stimulation via the autonomous 'Neurochip'[23,24] to deliver a 1-s train of stimuli (2.5 mA, 50 s^{-1}) when smoothed cell rate exceeded a threshold (Supplementary Fig. 4). Similarly, the first two cells in monkey I also triggered a 1-s train of 50 s^{-1} stimuli at 5 mA.

To confirm continued nerve block during the practice session, the stimulator was turned off after every 10 min of FES to assure that the monkey could not acquire the peripheral target through volitional muscle contractions. Figure 2b and Supplementary Fig. 4 illustrate the torques produced with the stimulator active compared to periods when the stimulator was turned off for 30 s. With the stimulator off, the monkey repeatedly attempted to satisfy the target but produced $\leq$10% of the torque used to acquire the active target. For all such test periods, with each cell the monkeys produced an average maximum of only 18.0 ± 21.3% of the torque used to satisfy the targets.

Data sampling. Signals were digitized and stored to disk for offline analysis. Raw recording from motor cortex was band-passed from 1–10 kHz and sampled at 25 kHz, along with spike times from the online discrimination. Wrist torques (flexion-extension and radial-ulnar) were sampled at 5 kHz, and smoothed and down-sampled to 500 Hz during offline analysis. We also recorded behavioural parameters (for example, target on screen), and muscle stimulation amplitude and timing (5 kHz).

Data analysis. Task difficulty was increased incrementally by raising levels of torque targets and increasing hold times. This complicated the quantification of skill learning. Improvements were evident as higher performance levels before increments in task requirements (see, for example, Supplementary Fig. 3), and these times were compared with performance at the beginning of a practice session. Specifically, the two-minute period with the peak performance was compared to the initial two minutes of practice (for example, targets per minute). Control precision was measured by target errors and the number of failed attempts to reach a target. A target acquisition error was counted when the monkey activated the stimulator while the centre target was on the screen, resulting in sufficient torque to satisfy any peripheral target had it been presented. Target errors are reported as the percentage of centre targets presented. A failed attempt was counted whenever the monkey briefly acquired a peripheral torque target but did not satisfy the required hold time. A Student's *t*-test was used to compare average torques during graded FES control. Otherwise, the non-parametric rank sum test was used for all comparisons as at least one data set for each remaining comparison failed the Lilliefors test for normality. All reported values are means $\pm$ s.d.

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SUPPLEMENTARY INFORMATION

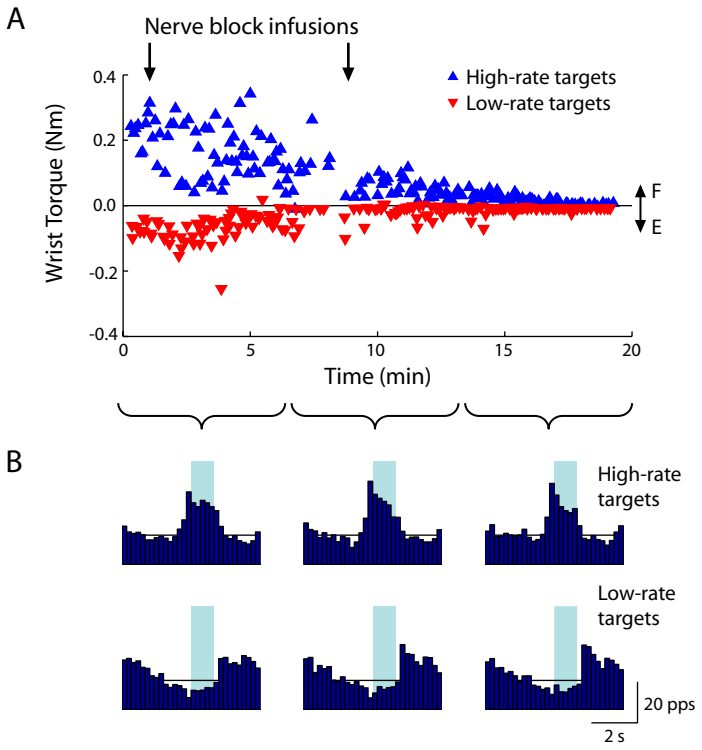

Figure S1: The effect of nerve block on ability to generate wrist torques. **(A)** Infusion of local anesthetic near the median, ulnar and radial nerves (arrows) eliminated wrist torques as the monkey controlled a cursor using cortical cell activity. **(B)** Cell activity while acquiring high (top row) and low (bottom row) rate targets was unchanged during nerve block onset shown in A. Shading indicates target hold period and horizontal line denotes baseline cell rate.

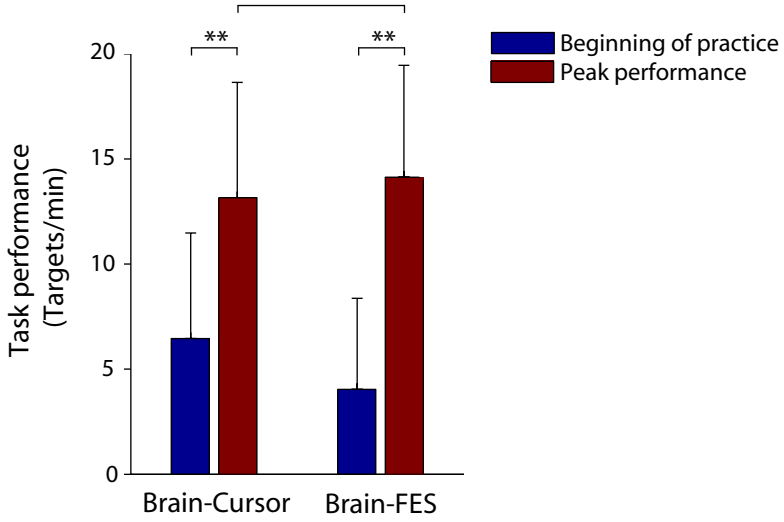

Figure S2: The number of targets acquired at the beginning of practice and during peak performance as brain cells control a cursor directly or control muscle FES (n = 42 cells). Monkeys improved with practice for both conditions (** $p < 0.001$); control during peak performance for each condition was not different ($p = 0.66$). Error bars are standard deviation.

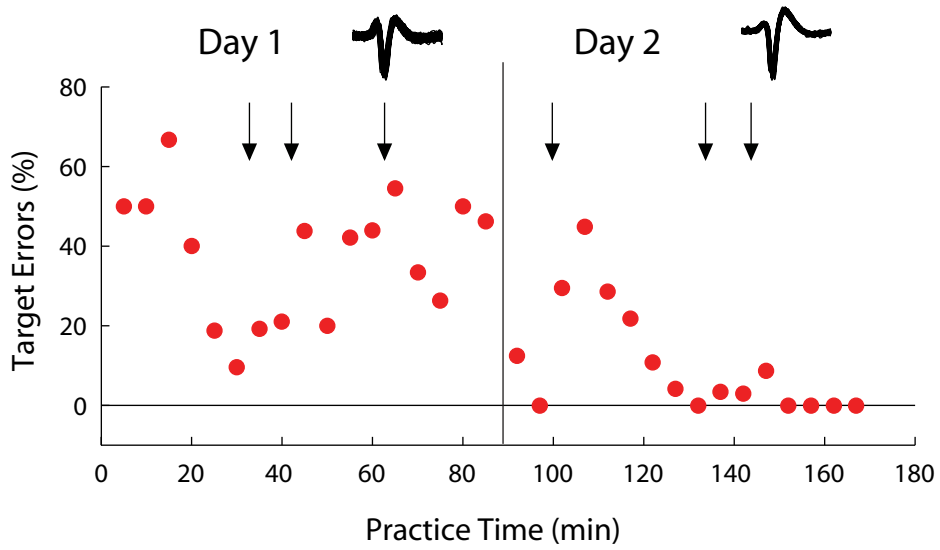

Figure S3: Target acquisition errors gradually decrease with practice. For the same cell shown in Figure 1B-C, the percentage of center targets during which the monkey activated the stimulator in error over two days of practice. Task difficulty was increased at each arrow, and was equal at the beginning of day 1 and 2. Note the absence of errors by the end of the second day of practice. Inset waveforms show overlaid cell action potentials during 30 s of FES control on day 1 and 2.

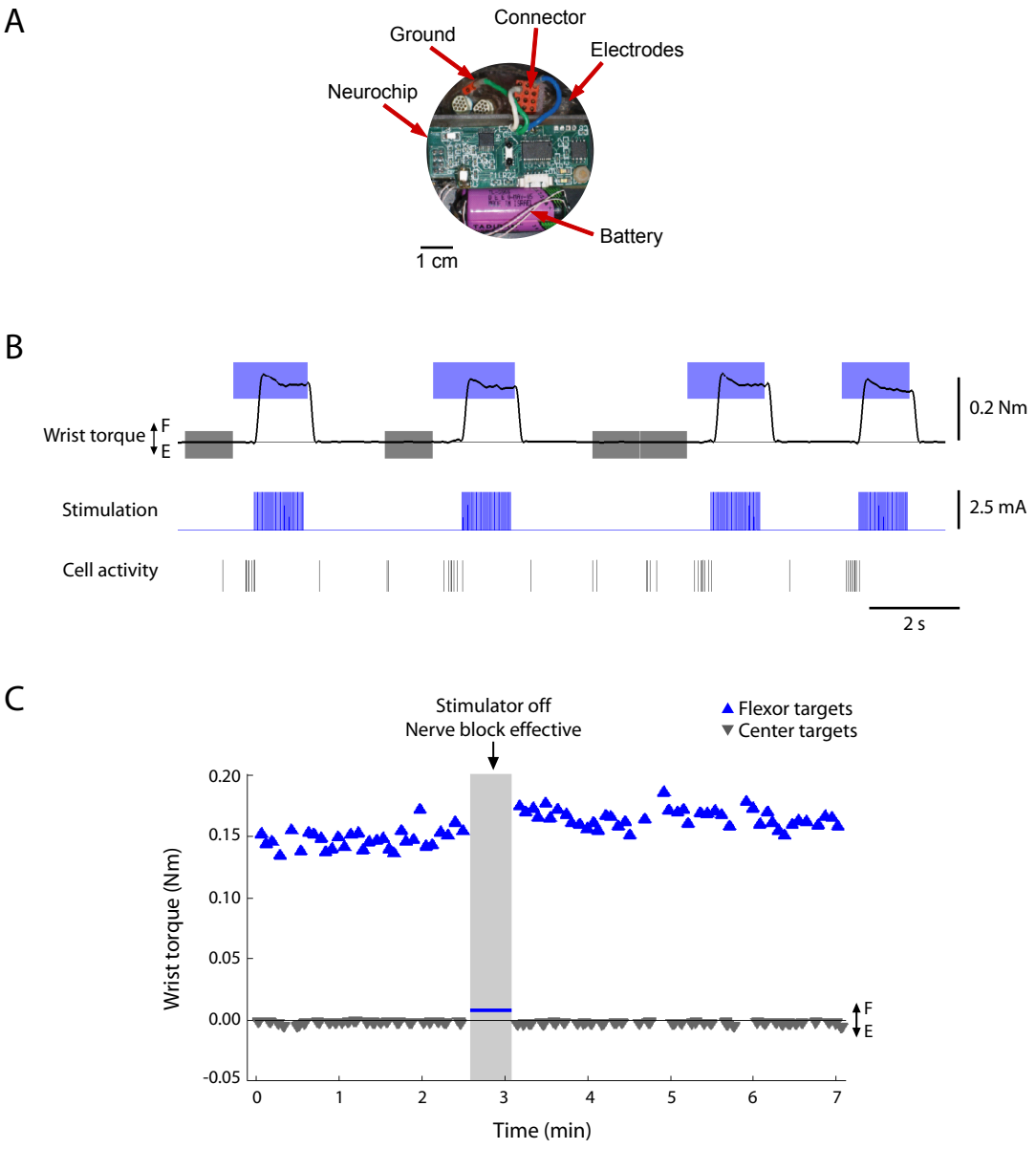

Figure S4: The autonomous Neurochip system **(A)** was used to convert cortical activity to FES of a paralyzed muscle (FCU). Monkey L modulated cell activity to trigger a 1 s train of 2.5 mA pulses at 50/s each time the cell rate exceeded 8 pps **(B)**. Monkey L rapidly acquired flexor (blue) torque targets and produced less than 5% of this torque (blue line) with the stimulator off (grey shading; **C**).

Supplementary methods

Cortical recording. These studies employed all well-isolated active neurons encountered by the recording electrodes, without any selection bias for cells related to wrist movement. The consequent proportion of significantly tuned cells recorded (9/38; 24%) agrees approximately with Evart's original report that ≤31% of pyramidal tract neurons in motor cortex were clearly correlated with hand movement[29]. Location of sites in primary motor cortex was confirmed by intra-cortical microstimulation (ICMS) during preliminary mapping with acute electrodes to identify sites for low-threshold (10-50 μA) activation of forearm muscles. ICMS was not used during experimental sessions because stimulation prior to recording tended to suppress neural activity for long periods, and stimulus effects after the experiments were potentially compromised by residual nerve block. Postmortem examination of monkey I confirmed recording sites were in primary motor cortex; monkey L is ongoing.

Muscle stimulation. Bipolar stimulating wires were implanted surgically or transcutaneously in eight forearm muscles. Pairs of multi-stranded stainless steel wires (AS631 or AS637, Cooner Wire), bared of insulation for 0.2-0.5 cm, were inserted into the muscle belly ~2 cm apart and parallel to muscles fibers. During surgical implantation (monkey I), wires were sutured to the epimysium. Wires inserted transcutaneously (monkey L) were secured to the skin with tissue glue (Vetbond, 3M) and porous tape (Transpore, 3M). Implantation in the following muscles was confirmed by responses to brief trains of stimulation: flexor muscles (digitorum superficialis, FDS; carpi radialis, FCR; carpi ulnaris, FCU; palmaris longus, PL); extensor muscles (digitorum communis, EDC; carpi radialis, ECR; carpi ulnaris, ECU; digitorum 4&5, ED4,5).

During experimental sessions, each cortical cell typically controlled simultaneous stimulation of two synergist muscles (e.g., wrist flexors or extensors). Four (of 44) cells

controlled only a single muscle due to technical difficulties with the second stimulator. Seven cells also controlled stimulation of antagonist muscle groups, activating two synergist muscles (with high rates) and one (2 cells) or two (3 cells) antagonist muscles (with low rates) to produce bi-directional torques.

Recording sessions. Data are reported for 44 cells recorded during nerve blocks performed during 47 separate sessions (1 session/day). Nine cells were recorded during two consecutive sessions, and one cell was recorded during five sessions. In addition, during ten sessions, two cells were used to control FES either successively (2 cells) or simultaneously (5 cell pairs, with 3 pairs recorded over 2 sessions each).

Nerve block. During most sessions, both flexor and extensor wrist muscles were blocked. In some sessions, however, nerve block targeted flexor muscles (median and ulnar nerves) or extensor muscles (radial nerve), depending on the direction of cell control. For example, when a cell discharged high rates for targets in the extensor direction, the radial nerve was blocked. Focusing on flexors or extensors permitted the duration of nerve blocks to be extended with additional doses, while avoiding systemic reactions to the anesthetics (maximum total dose was 1 ml/kg). Comparing cells with complete nerve blocks of both flexors and extensors ($n = 25$) to cells with only flexors ($n = 15$) or extensors paralyzed ($n = 4$) showed no difference in the monkeys' ability to use cell-controlled FES ($p > 0.30$). During cell-controlled FES nerves were blocked by chloroprocaine for 33 cells and lidocaine for 11 cells.

Conversion of cell activity to FES. One of our goals was to test relatively direct transforms between cell activity and muscle stimulation. The most direct transformation is triggering a stimulus pulse from every cell discharge, but this proved problematic for several reasons. First, changing stimulus rate did not grade contraction force as effectively as changing pulse width or

amplitude, which presumably changed the number of motor units recruited. Second, cell rates in excess of 40 pps are required to achieve fused tetanus when stimulating monkey forearm muscles directly, and few of the cells tested discharged above this rate for the duration of the target hold period. Finally, most of the cells had a non-zero baseline rate that would generate sustained low-frequency stimulation and produce muscle fatigue. These issues were resolved by imposing a discharge rate threshold to gate stimulation. We then delivered 50/s stimuli with intensities proportional to discharge rate exceeding (standard case), or falling below (inverse case), this threshold.

Unit discrimination amidst artifacts. The stimulation parameters permitted reliable discrimination of cortical cell activity despite stimulus artifacts present in the recordings. Stimulus artifacts were sufficiently brief to preserve ≥ 80% of simultaneous neural signals for spike discrimination, and their shapes were reliably separable from spike events. To minimize artifacts, stimuli delivered to two synergistic muscles were simultaneous.

Directional Tuning. To quantify directional tuning in the isometric flexor-extensor and radial-ulnar plane, vectors (V_i) were constructed in each of the eight peripheral target directions (θ_i) with magnitudes proportional to the average cell rate (r_i) during the target hold period[28,34,35]. These vectors were then summed and normalized to produce a resultant vector (V) with magnitude between 0 and 1 (equation 1).

$$\mathrm{V} = \frac{\sum V_i}{\sum \| V_i \|} \qquad (1)$$

Larger resultant magnitudes indicate more sharply tuned cells. A bootstrap test was used to determine significant ($p < 0.05$) directional tuning by assigning measured spike rates to random target positions 4,000 times[35]. Based on this test, cells were considered tuned (n = 9) or untuned

(n = 29). Regressions of tuning strength vs. performance were calculated for 38 of the 44 cells for which sufficient data were available.

Baseline activity. Baseline cell rates were obtained during one-minute periods in which the monkey viewed a blank screen immediately following the first 10-minute period of cell control of the cursor. Baseline rates were computed from average cell activity only over periods when the monkey produced no wrist torque.

Task performance. Quantification of performance during operant conditioning is complicated by the fact that task requirements were incrementally increased as skill improved over time for most cells. When the monkey learned to regularly acquire targets with a new cell, hold times were increased, target magnitudes were increased and/or target width reduced to make the task more challenging. Performance measures would not be expected to increase monotonically throughout the conditioning period (e.g., Figure S3). There were periods of time, however, when performance reached a high level before the task was made more challenging. We used these times to quantify peak performance and improvement compared to the beginning of a practice session. In addition, stimulus current gain (current/spike rate) was sometimes adjusted to offset muscle fatigue and maintain a consistent relation between wrist torque and cell spike rate. Improvements in target error rates were not due to changing task difficulty or stimulus gain, as target parameters and gain were not significantly different between the initial and peak performance ($p > 0.25$).

To probe the limits of FES control, the task was made more challenging for 27/44 cells in one or more of the following ways. Target hold times were gradually raised by an average of 0.34 ± 0.31 s above a baseline value of 0.50 s (17 cells). Torque magnitude required to satisfy a target was increased by an average of 25% (0.03 ± 0.01 nM; 17 cells), and target width was

decreased by an average of 19% (0.07 ± 0.04 nM; 17 cells). Task difficulty was not changed for cells recorded from monkey I (although target hold times of 1.0 s were used), or for the initial 12 cells recorded from monkey L (7 of which were recorded from acute electrodes permitting limited practice durations).

Supplementary results

Data from 44 cells (5 from monkey I and 39 from monkey L) were pooled across monkeys and recording techniques, since there were no differences in performance or directional tuning among groups. Specifically, there was no difference in FES control (i.e., target acquisition rates, failed attempts or target acquisition errors) achieved by the two monkeys ($p > 0.26$). All cells in monkey I and 7/39 cells in Monkey L were recorded using acute electrodes. The remaining 32 cells in monkey L were recorded via a chronic electrode array. There was no difference in FES control via cells recorded on acute and chronic electrodes ($p > 0.43$).

Practice times controlling the stimulator with each cell ranged from 2 to 317 minutes (mean 66 min; $n = 44$) and continued until cell recordings were lost, the monkey became satiated, or the nerve block began to wear off. There was no difference in the strength of directional tuning among cells used to control stimulus currents proportionally or inversely to rate ($p = 0.29$). In addition, the degree of modulation in cell activity to acquire the FES torque targets was similar for both types of stimulator control. The cells controlling inverse stimulation were modulated by 10.2 ± 3.4 pps, while the cells controlling direct stimulation were modulated by 14.7 ± 10.8 pps ($p = 0.14$). Overall, cells modulated over a slightly greater range (14.3 ± 10.3 pps) during FES control compared to activity during the unparalyzed torque-tracking task (12.5

± 9.3; p = 0.04). Cell modulation was greatest during brain control of a cursor before nerve block (19.2 ± 11.4 pps; p < 0.002).

Performance during the torque-tracking task without paralysis can be compared to brain-controlled FES performance. Before paralysis, monkeys acquired 30.6 ± 1.9 targets per minute by producing 0.16 ± 0.01 nM of flexion-extension torque. After paralysis, monkeys used single cells to control FES and acquired up to 14.1 ± 5.3 targets per minute by producing 0.14 ± 0.04 nM of flexion-extension torque. Thus, FES-evoked torques were similar to the unparalyzed case, although targets were acquired about half as fast and with 4-times greater torque variability.

Despite greater torque variability overall, monkeys were able to grade FES-evoked torque using cell activity to acquire multiple targets in one direction. For example, monkey L produced 0.08 ± 0.02 nM to acquire the near extension target and 0.14 ± 0.02 nM to acquire the far extension target using the cell illustrated in Figure 2. For all 5 cells tested, the torques produced to acquire graded torque targets in the same direction were significantly different (p < 0.001). These cells acquired 11.3 ± 5.0 graded torque targets per minute, comparable to the standard FES task (p = 0.28), and made no target acquisition errors or extra attempts during peak performance.

During sessions in which monkey L simultaneously controlled two cell-muscle pairs to acquire bi-directional targets (5 pairs of cells), all performance measures were similar to single cell control (p > 0.26). Both cells of each pair showed comparable performance measures. This monkey also improved with practice when using two cells simultaneously, acquiring 11.6 ± 3.8 targets per minute during peak performance (compared to 3.8 ± 1.4 targets per min at the beginning of practice; p = 0.003; n = 5), and requiring no additional attempts to reach each target (compared to extra attempts on 53% of targets at the beginning; p = 0.008). Practice times with each cell pair ranged from 30 to 99 minutes (mean 56 min).

Supplementary discussion

Several factors may have limited tracking performance while the monkeys controlled FES via neural activity, including muscle fatigue, recording stability and training time. During some of the prolonged practice sessions muscle responses to electrical stimulation fatigued, as evidenced by a decrease in wrist torque evoked by a given stimulus intensity. This may have occurred because muscle stimulation preferentially activated large, fast-fatiguing motor units. Better methods to preserve a natural recruitment order of motor units and avoid fatigue could be employed in future studies, including stimulating over the motor point[36], via nerve cuffs[37], or in the spinal cord[25,27], which produces more natural recruitment order and fatigue-resistant contractions[38].

Given the numerous sensory projections to primary motor cortex[39], it was quite possible *a priori* that acute peripheral nerve block would disrupt volitional control of cells. Anesthetic doses sufficient to block both large and small diameter motor fibers also eliminate most sensory feedback from innervated areas[40]. Nonetheless, 44 of 45 cells tested could be controlled after peripheral nerve block with similar or slightly greater modulation compared to the "unparalyzed" task. This is consistent with reports that tetraplegic patients with chronic spinal cord injury can modulate cell activity in motor cortex despite both a lack of sensory feedback[6], and the remodeling in cortical activity known to accompany chronic paralysis[41].

Our finding that cells could be used equally well to control FES, regardless of their original association to wrist movements, illustrates the flexibility of the cortical motor system. While strong correlations can be found between cortical activity and muscle force[29], for example, correlations can also be found with any number of movement parameters[42]. Moreover, these

correlations change, depending on the experimental conditions, and new correlations can emerge with brain control of cursors[4,10] as well as FES.

The relatively direct control of FES from single neurons demonstrated here invites comparisons with the more conventional strategy of decoding movement-related activity from neural populations for prosthetic control. Given the rapid and robust operant control demonstrated for individual cells[14,16] it seems remarkable that populations of cells used simultaneously have not provided more accurate control than they do. Several explanations are possible[16]. First, demonstrating control of only one or two cells, with the rest of the brain free to optimize that control, is inherently simpler than controlling large numbers of neurons simultaneously. Second, prosthetic control functions defined on a population of neurons with stochastic variance may obscure the contribution of individual neurons and limit the brain's ability to learn to optimize those contributions. Third, the strategies that are typically used to optimize the transform from ensemble activity may undermine motor learning, by producing a 'moving target' through adaptive and frequently re-calibrated decoding methods. Instead, it may turn out that multiple direct connections from cortical cells to muscles will provide more explicit and intuitive functional connectivity that can be learned through innate motor learning mechanisms, particularly under conditions of long-term training.

An important next question for this approach is determining the degree to which multiple direct links from arbitrary cells can be used to control additional muscle patterns. Adding more channels could initially impose a greater cognitive load on the subject. Indeed, one rationale for the decoding approach is that tapping the natural activity patterns of appropriate cells would make control relatively intuitive[6]. This decoding method assumes that the cells' correlation to behavior remains fixed; however most cells have volitionally controllable inputs that modulate

their activity significantly to meet behavioral requirements[16]. Consequently, the need to search for decodable cells may become less compelling if new but consistent sensorimotor contingencies can be learned with sufficient training time. The remarkable adaptability of cognitive motor control has been amply demonstrated[17,18], including the learning of BCI control: subjects that initially controlled a cursor through motor imagery soon began to imagine controlling the cursor directly[43].

Maintaining long-term, stable recordings from isolated neurons is considered necessary for their clinical use in neuroprosthetics, so achieving reliable isolation of the same units is a major goal of neural engineering[44]. Using direct connections from single cells to control muscles would seem to be particularly vulnerable to loss of cell isolation. However, long-term isolation of the same units may not be a critical prerequisite, since humans and monkeys can rapidly learn to control external devices with newly acquired neural activity[4,6-10,14,15]. In the present study monkeys improved control significantly despite an average of only 66 minutes of practice with each cell controlling FES. This suggests that occasionally substituting newly recorded cells or multi-unit activity need not preclude effective control of neuroprosthetics.

A clinically useful prosthetic control system of this type would probably employ multiple cells directly connected to many muscles. This would engage the inherent redundancy of the cortico-muscular system and help protect against loss of any single neuron. Single cells could also control functional muscle synergies, such as a coordinated contraction of many muscles to produce grasp[12,45]. Such coordinated activation could be produced by programmed patterns of stimuli delivered to multiple muscles or to spinal sites activating synergistic muscles[1,25-27,46]. Given the likely development of improved implantable circuitry enabling long-term motor adaptation[23,47,48], the strategy of implementing relatively direct and separate connections from

cortical cells to motor outputs offers a promising alternative to the current approach of intermingling signals from decoded neurons.

Supplementary references

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