Procedures

Tactile discrimination tasks. Plastic, steel-backed gratings measuring about 20 mm^2 and with equal groove and ridge widths were manufactured as described[18]. An electromechanical device was used to apply the gratings (for 63-ms duration) to the index fingerpad of the right hand, oriented either along or across the long axis of the finger (Fig. 1). The ability to discriminate orientation increases monotonically as grating groove width (GW) increases up to 3 mm (ref. 19). Subjects were first tested psychophysically on each task. For the orientation task, the discrimination threshold was taken as the GW yielding 75% correct performance[20]. Threshold determinations were based on at least 20 trials for each grating. A grating yielding performance that was above threshold but below ceiling was then selected for each subject. This grating had the maximal GW of 3 mm for most subjects and 2 mm for others. For the spacing task, a similar procedure was followed, resulting in selection of a pair of gratings for each subject (GW 3 mm and 2, 1.5 or 1.2 mm). Gratings were applied in either orientation in the spacing task, at random. Both tasks used two-alternative forced-choices, subjects responding whether the grating was oriented along or across the finger (in the orientation task) or whether the grating grooves/ridges were wide or narrow (in the spacing task). Performance was expressed as the percentage of correct responses.

Electrical stimulation. Electrical stimulation was delivered to the right index fingerpad of five subjects in Experiment 1. A suprathreshold stimulus intensity was chosen for each subject.

TMS. TMS was applied using a Cadwell MES-10 (Cadwell Laboratories) stimulator with a custom high-efficiency iron-core coil that measured 12 cm × 7.5 cm. This coil induces an electric field similar to a figure-of-eight coil, with its maximum directly below the centre[21]. Stimuli were cosine pulses of 200 μs duration, at 150% of the relaxed motor threshold[22]. The inter-stimulus interval was at least 1 s, to avoid seizures. An electronic circuit incorporating a variable delay was used to deliver TMS at particular delays following the onset of the tactile stimulus, registered electrically in the case of gratings with the help of silver gel. On the basis of pilot studies indicating that the peak of the TMS interference effect over occipital cortex occurred at 150–200 ms, the 180-ms delay was chosen as representative of the peak effect. For TMS in air, the coil was held close to but not in contact with the scalp and rotated to direct the magnetic field away from the head.

In Experiment 1, 11 subjects were tested with TMS applied at the M4 (occipital midline, 4 cm above the inion), L3 and R3 sites (both 3 cm above and 4 cm lateral to the inion on the left and right, respectively), except for one subject who dropped out after testing at M4 at the 10- and 180-ms delays. TMS was applied at the M2 site (2 cm above the inion in the midline) in 5 of the 11 subjects. Six subjects (including three who had taken part in Experiment 1) were tested with occipital TMS in Experiment 2, at L6, R6 (both 6 cm above and 2 cm lateral to the inion on the left and right, respectively) and M4. For stimulation over primary somatosensory cortex, the site over the left hemisphere from which a motor response in the right thumb and/or index finger could just be evoked was located. The coil was then moved posteriorly until the motor response just disappeared, and this site was chosen as the somatosensory cortical site. In two out of three subjects, this localization was aided by reports of somatic sensations in the hand—warmth in one subject and numbness in another. As the early peak (presumably from primary somatosensory cortex) in the evoked potential response to stimulation with the grating occurred at 30–50 ms (data not shown), a delay of 30 ms was chosen for TMS at this site. Owing to the discomfort resulting from stimulation at this site (due to muscle contraction), only a single delay was used and only three subjects were tested. The stimulus intensity at this site had to be reduced to the motor threshold for two subjects as they could not tolerate stimulation at higher intensities. Trials were presented in interleaved blocks of 10 with a minimum of 20 trials at each delay and location.

To determine the relationship between the sites at which effects of occipital TMS were obtained and the previously reported PET activation locus[1], we used the averaged resting motor threshold[22] in our subjects to scale the TMS-induced electric fields in a model head[13,23]. The approximate boundaries for physiological effects of TMS (taken as 90% of the resting motor threshold[24]) at selected sites are displayed in Fig. 3.

Statistical testing. Paired *t*-tests (two-tailed: $\alpha = 0.05$) were used to assess the statistical significance of differences between conditions.

Evoked potentials. Evoked potential recordings were carried out in five subjects in response to tactile stimulation of the right index fingerpad with a grating, as described above. The evoked potentials were averaged with band-pass filters of 0.1–200 Hz. In one session, the active scalp electrodes were positioned on the left side of the head over frontal (F_3), central (C_3), parietal (P_3) and occipital (O_1) sites. In another session, recordings were made with the active scalp electrodes in the corresponding positions on the right. A midline frontal electrode (F_z) was used as the reference. In each session, two runs of 100–120 trials were performed for each condition (discrimination of grating orientation and counting stimuli). The two conditions alternated with each other and the order was counterbalanced across subjects. To ensure that the posterior location of the observed peak in Fig. 4 was not an artefact of the frontal reference electrode used, evoked potentials were also recorded in two subjects using a linked earlobe reference electrode. The potential thus obtained (data not shown) was similar in scalp distribution and time course to that in Fig. 4.

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Primate spinal interneurons show pre-movement instructed delay activity

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Preparatory changes in neural activity before the execution of a movement have been documented in tasks that involve an instructed delay period (an interval between a transient instruction cue and a subsequently triggered movement). Such preparatory activity occurs in many motor centres in the brain, including the primary motor cortex[1–6], premotor cortex[7–9], supplementary motor area[6,10,11] and basal ganglia[6,12,13]. Activity during the instructed delay period reflects movement planning, as it correlates with parameters of the cue and the subsequent movement (such as direction and extent[5,6,9]), although it occurs well before muscle activity. How such delay-period activity shapes the ensu-

ing motor action remains unknown. Here we show that spinal interneurons also exhibit early pre-movement delay activity that often differs from their responses during the subsequent muscle activity. This delay activity resembles the set-related activity found in various supraspinal areas, indicating that movement preparation may occur simultaneously over widely distributed regions, including spinal levels. Our results also suggest that two processes occur in the spinal circuitry during this delay period: the motor network is primed with rate changes in the same direction as subsequent movement-related activity; and a superimposed global inhibition suppresses the expression of this activity in muscles.

The generation of voluntary movements is commonly thought to develop sequentially in the motor system[3,9], beginning with preparatory activity in higher motor areas and propagating through the premotor and primary motor cortex (M1) to the spinal cord. Alternatively, these motor areas may operate in a predominantly parallel mode[6,12], in which movement-related activity would effectively appear simultaneously in many motor areas, regardless of their relative synaptic 'distance' from muscles[6]. Indeed, indirect evidence has indicated that early stages of motor preparation may involve circuits as peripheral as the spinal cord. Spinal reflexes, both mono- and polysynaptic, can be modulated during an instructed delay period[15–18]. This modulation is not mediated through the γ-motor-neuronal system[19,20] and has been partially attributed to presynaptic inhibition[15]. Another contribution to reflex modulation may arise from spinal interneurons (INs), which receive direct inputs from M1 and higher premotor areas[14,21–24]. The role of segmental INs in preparatory changes occurring before voluntary movement is unknown.

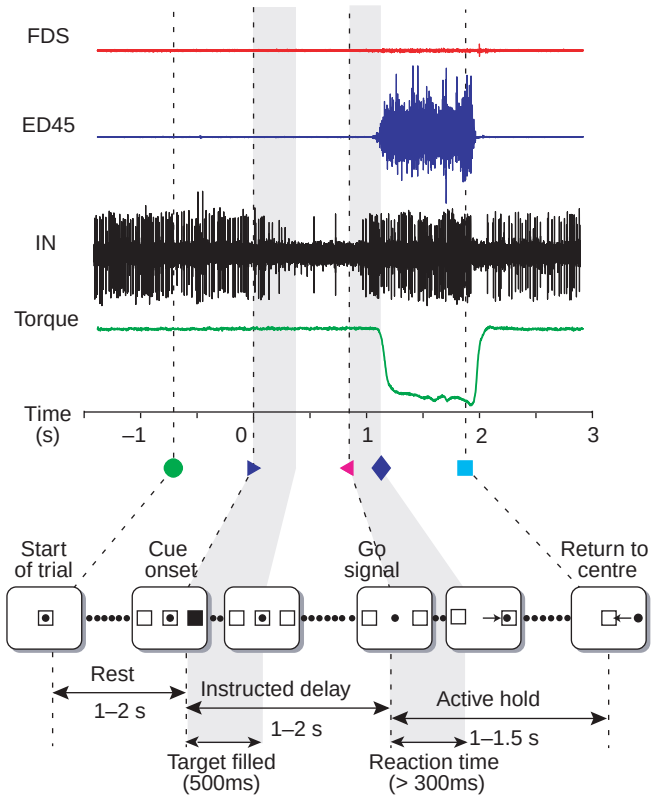

Figure 1 Example of a single instructed delay trial. Traces (from top) show activity of two muscles (flexor digitorum sublimis (FDS, red) and extensor digitorum 4 and 5 (ED45, blue)), activity of IN (black) and torque signal (green). Bottom, sequence and timing of events during a single trial (see Methods for details). During the delay period, the firing rate of this unit decreases, with no accompanying torque deflection or EMG activity of either muscle.

Table 1 Proportions of spinal INs showing significant delay modulation

	T-test		Sign rank	
	No.	%	No.	%
Total SDM	133	33.5*	149	37.5*
SDM in pre-extension only	59	44.4†	62	41.6†
SDM in pre-flexion only	40	30.1†	45	30.2†
SDM in both pre-extension and pre-flexion‡	34***	25.6†	42***	28.2†

*Per cent of the total number of valid cells ($n = 397$).
† Per cent of the number of SDM cells ($n = 133$).
‡ For cells with SDM in both flexion and extension, the expected number of cases was computed assuming independence of SDM occurrence in flexion and extension trials. In both cases the counts were significantly higher than expected (Poisson, $P \ll 0.001$***).

To address this issue we investigated the activity of cervical INs in monkeys performing an isometric flexion–extension instructed delay task (Fig. 1). Such a task separates motor preparation from execution of movement. Most INs in awake monkeys are active in the absence of active torque, unlike motor neurons[25]. We searched for cells that modulated their activity during a post-instruction delay period, relative to their activity during rest.

Figure 2 shows the activity of three INs whose firing rate was modulated during the instructed delay period. They show increased activity during one or both delay periods (Fig. 2b, d) and suppression during both delay periods (Fig. 2c). For comparison, Fig. 2a shows an IN that is activated during active torque but not during the delay period. Of 450 cells recorded from two monkeys, 397 had background activity at rest and were analysed for the existence of delay-related changes in activity (Table 1). About one-third of these INs showed significant delay modulation (SDM) in flexion and/or extension trials (paired *t*-test, $P < 0.05$). The median onset time of SDM after the onset of the cue was 190 ms (mean 227.3 ms). Inhibitory SDMs tended to begin earlier than excitatory SDMs (median, 170 and 230 ms, respectively). The proportions of set-related and movement-related cells in the spinal cord were similar to the proportions found in other motor areas (Table 2). The main difference was the relatively small number of 'pure' set-related spinal INs, namely INs with no movement-related but only set-related activity.

An obvious possibility is that the SDM could have been associated with low levels of muscle activity. Electromyogram (EMG) activity of forearm muscles was routinely recorded in parallel with INs and revealed no muscle activity in either flexors or extensors during the delay (Fig. 1). Detailed analysis of data from 17 recording sites (from which 69 cells were recorded) showed no evidence of significant muscle activity in the delay periods.

Delay activity in spinal INs could also represent the early onset of subthreshold movement-related rate modulation. In this case, the change in firing during the delay period would have the same polarity (excitation or inhibition) as the subsequent modulation during active torque. This, however, was not the general case. For the 167 cases of SDM (pre-flexion or pre-extension), Fig. 3 shows the average change in rate during the delay period relative to the preceding rest period (abscissa) plotted against the corresponding rate modulation during the hold period (ordinate). Sixty-four per cent of the points lie in the two quadrants of the graph corresponding to the 'congruent' case, in which the SDM changes were in the same direction as the change of rate during the active torque period (lower-left and upper-right quadrants). Thirty-four per cent of the points fall in the upper-left quadrant, representing a decrease in rate during the delay followed by a rate increase during the active torque. INs tended to be inhibited more than excited during the delay period compared with the active torque period. The proportions of cells with inhibition versus excitation in the delay period were significantly different from the proportions found for the hold-period activity (McNemar's test, $P < 0.01$). Suppression is also common when inhibition is seen during the delay period

in other motor structures including M1 (ref. 4), basal ganglia[13] and premotor cortex[26]. In particular, changes in primary motor cortex neurons were similar to those in spinal INs, although fewer M1 cells had opposite responses during delay and movement (16% compared with 34% in our study). Most of these reversals in M1 involved inhibition during the delay and activation during movement, similar to our INs.

Parametric studies showed that cortical delay activity could be related to several parameters of the subsequent movement, including the direction[1,5,6,26] and extent[5,9]. We tested the coding of these parameters by spinal INs. The directional selectivity of each IN during the instructed delay period was evaluated by comparing its firing rate during the delay period in flexion trials with its rate during the same period in extension trials. Out of 450 cells, 43 (10%) exhibited directionality in firing during the delay period (analysis of variance (ANOVA), $P < 0.05$), 166 (37%) during the pre-active period (from go signal until torque onset) and 207 (46%) during the active torque period. Seventeen of the 43 cells (40%) were directional during the delay but not during the active torque period. Most INs had the same polarity of delay activity in both flexion and extension trials, as opposed to reciprocal activation. Similar results were obtained for the relation between torque magnitude and delay period activity of INs.

The functional identity of the cells that showed SDM is of considerable interest. Chronic recording conditions limit our ability to identify the types of IN recorded in terms of reflex properties[25]. However, INs with functional linkages to the recorded forearm muscles could be identified by spike-related changes in EMG activity[25]. Of 300 tested INs, 46 had functional linkages with muscle activity. Those INs were significantly ($P < 0.01$) more likely to exhibit SDM (27/46, 59%) than INs without such links (94/254, 37%). In both groups SDM was most often inhibitory.

A comparison of activity changes during the instructed delay with activity during the subsequent movement period indicates that there may be two types of delay-period activity in the spinal cord. Some INs showed a 'congruent' pattern in which the delay activity

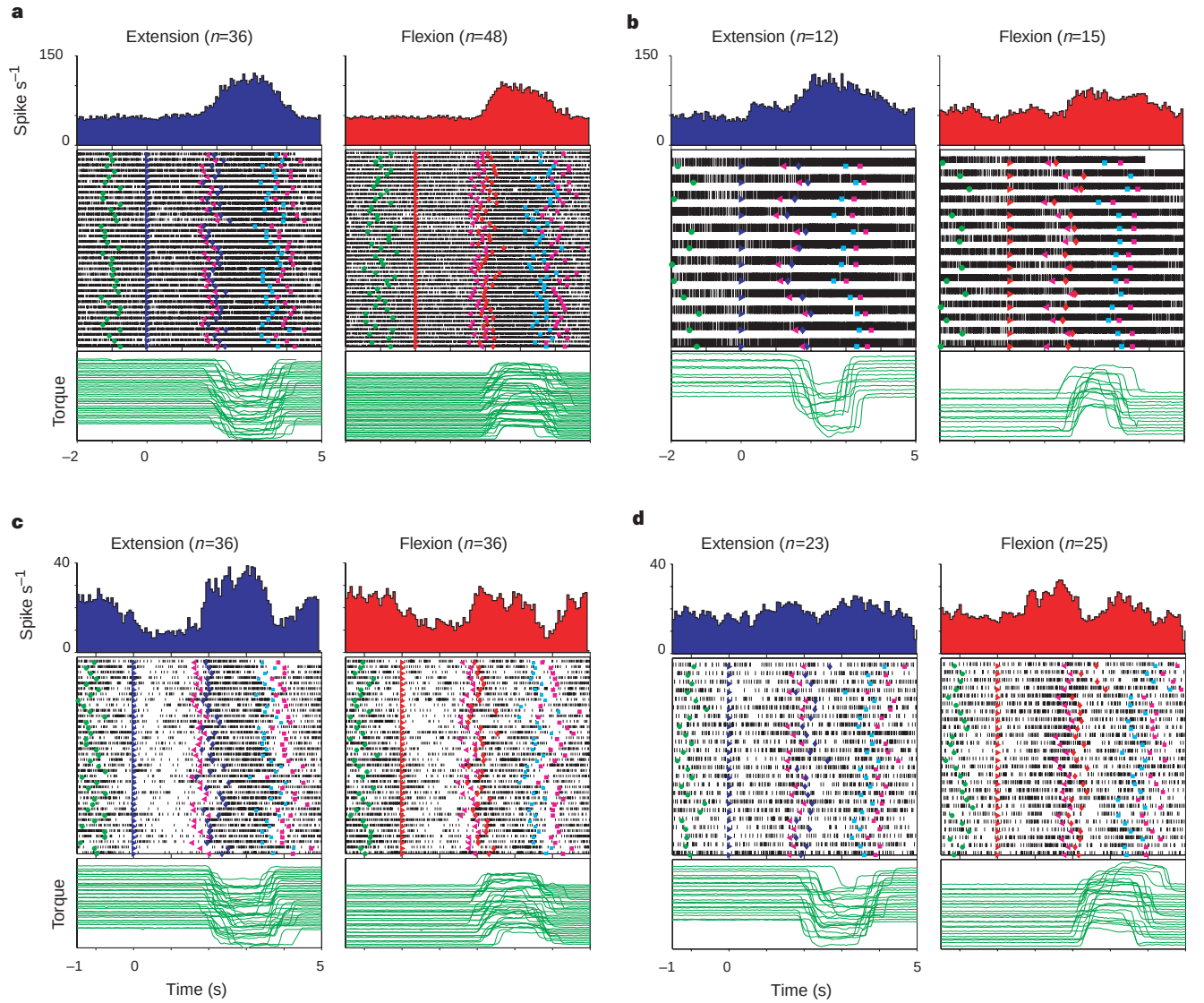

Figure 2 Response properties of spinal INs. Trials are aligned with cue onset (blue and red triangles). Lowest traces (green) show the single trial torques. The raster plots present the unit activity. The symbols code trial onset (green circles), go signal (pink triangles), movement onset (red and blue diamonds), return to centre signal (cyan squares) and movement offset (pink squares). The peri-stimulus time histograms (top) quantify the IN's stimulus-locked rate modulations. **a**, IN with no rate modulation during delay but increased rate during active torque periods. **b**, IN with a pre-flexion excitatory delay modulation. **c**, IN with inhibitory delay modulations in flexion and extension trials. **d**, IN with excitatory delay modulations in flexion trials.

Table 2 Response properties of neurons in different motor areas

Area	Spinal cord	Primary motor (M1)	Pre-motor	SMA	Putamen	
Ref. no.		6	10	6	6	12
Set only (%)	2.5	10.9	20.9	32.5	24.3	20.3
Movement only (%)	67.4	62.9	50.6	45.5	66.9	76.7
Set + Movement (%)	30.1	26.2	28.6	22.5	8.8	3.0
n	405	202	91*	222	317	232

Relative proportions of set-related, movement-related, and set + movement-related cells found in different regions of the motor system. Note that different studies used different statistical methods to determine response properties of the cells.
* From these data we omitted cells with a pure signal-related response (8 cells).

had the same polarity (albeit with smaller amplitude) as the modulation during active torque (lower-left and upper-right quadrants of Fig. 3). This type of SDM activity appeared to be locked in time to cue onset, and had a relatively late onset time. The second type of delay-period activity was largely inhibitory during the delay, irrespective of the subsequent active-torque rate modulation (left quadrants of Fig. 3). This inhibitory activity seemed to be more loosely locked to the cue onset and to begin earlier than excitatory SDMs. The two quadrants that contain the 'purest' examples of these two groups (upper-left and upper-right quadrants) had the greatest difference in onset times: median 250 ms (mean 257.2 ms) versus 110 ms (mean 172.3 ms). This difference was significant ($P < 0.01$, Mann–Whitney test). The inhibitory cases in the lower left quadrant could represent either mechanism and had SDMs with onset times between the two extreme values. The congruent pattern can be understood as a subthreshold preparation for movement involving the priming of cells in the direction in which they must fire during movement execution. The inhibitory delay patterns could reflected a superimposed general 'braking' mechanism, which suppresses the tendency to initiate movement. This inhibition is released at the onset of the go signal, when movement starts. The fact that many cells with functional linkages to muscle activity show inhibitory SDM supports this interpretation.

The source of the SDM is probably supraspinal, as afferent input to the cord is the same during rest and the delay period[19,20]. An obvious candidate is the cerebral cortex, because arm-related premotor areas exhibit robust delay activity and project to the spinal cord[14,24] (mostly to the intermediate grey zone–layers V–VII

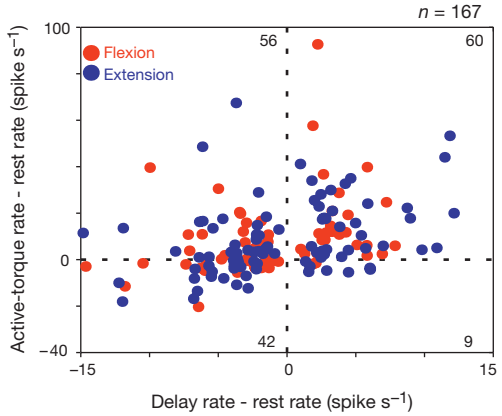

Figure 3 Relations between the magnitude of the SDM and the modulation in firing during active torque. *x* axis plots the rate modulation during delay period relative to rest. The values were computed by averaging for each cell the single-trial difference in firing rate during the delay period and the rest period. *y* axis values are the rate modulations relative to rest during active torque period. Each dot represents a cell with an SDM during pre-flexion (black) and/or pre-extension (grey) periods (34 cells contributed values for both flexion and extension). The numbers of points in each quadrant are given in the corners.

(ref. 21). Indeed, it has been suggested that the premotor cortex inhibits motor pathways[27,28], as has been shown during epileptic activity[29].

Previous evidence indicated that supraspinal motor centres operate in a distributed parallel mode[3,6,14]. Our results support and extend this view to spinal levels, where segmental INs exhibit instructed delay activity whose timing and properties are similar to those observed in supraspinal centres. This contrasts with the traditional view that the spinal cord is simply an output relay, executing motor plans that are pre-generated in supraspinal structures. It indicates that motor cortical areas may interact continuously with spinal networks during the earliest stages of preparation for movement. The modulation of spinal INs during the instructed delay could not only prepare for the subsequent motor actions but also modulate transmission of afferent information from the somatic periphery to spinal[1] and supraspinal levels. □

Methods

Animals and behavioural task.

Two monkeys (*Macaca nemestrina*) performed a flexion–extension task with a delay. The monkey controlled a cursor on a computer screen (black circle in Fig. 1) by isometric torque. A trial was initiated by the appearance of a central box. The monkey positioned the cursor inside the box by generating zero torque for a rest period of 1–2 s. Then two symmetric targets appeared in the flexion and extension positions with one target filled for 500 ms (cue), defining the onset of a delay period. The disappearance of the central box, 1–2 s after cue onset, served as the go signal. The monkey then had to acquire the previously filled target by generating an isometric torque in the appropriate direction, and to keep the cursor within the target box for an active-torque period of 1–1.5 s. After this period the two target boxes disappeared and the central box reappeared. The monkey returned to the rest position and received a reward, after which the screen went blank for 500 ms and a new trial started.

Recording sessions.

Details of the recording technique are described elsewhere[25]. A chamber was implanted above the cervical spinal cord (C_6–T_1). We recorded single-unit activity with tungsten electrodes and EMG activity from 6–12 forearm muscles.

Data analysis.

We selected cells with a firing rate above a minimal level (median of 1.5 spikes per s) in the rest and/or delay period. Rest period was the time from trial onset to cue onset. The delay period was defined as the time from cue onset until either onset of the go signal or 300 ms before movement onset, whichever occurred first. SDM was measured by comparing the single trial rates during the delay period with the corresponding rates during the rest period. This test identifies consistent rate differences across trials. The results were then confirmed using a nonparametric sign-rank (Wilcoxon) paired test.

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Polyamine-dependent facilitation of postsynaptic AMPA receptors counteracts paired-pulse depression

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At many glutamatergic synapses in the brain, calcium-permeable α - amino - 3 - hydro - 5 - methyl - 4 - isoxazolepropionate receptor (AMPAR) channels mediate fast excitatory transmission[1–6]. These channels are blocked by endogenous intracellular polyamines[7–9], which are found in virtually every type of cell[10,11]. In excised patches, use-dependent relief of polyamine block enhances glutamate-evoked currents through recombinant and native calcium-permeable, polyamine-sensitive AMPAR channels[12]. The contribution of polyamine unblock to synaptic currents during high-frequency stimulation may be to facilitate currents and maintain current amplitudes in the face of a slow recovery from desensitization or presynaptic depression[12,13]. Here we show, on pairs and triples of synaptically connected neurons in slices, that this mechanism contributes to short-term plasticity in local circuits formed by presynaptic pyramidal neurons and postsynaptic multipolar interneurons in layer 2/3 of rat neocortex. Activity-dependent relief from polyamine block of postsynaptic calcium-permeable AMPARs in the interneurons either reduces the rate of paired-pulse depression in a frequency-dependent manner or, at a given stimulation frequency, induces facilitation of a synaptic response that would otherwise depress. This mechanism for the enhancement of synaptic gain appears to be entirely postsynaptic.

To determine the contribution of polyamine (PA)-dependent facilitation to short-term plasticity of excitatory synaptic transmission, we selected synapses of pyramidal cells on multipolar interneurons in layer 2/3 of rat neocortex[14]. This excitatory connection is characterized by a high release probability[14], and somatic AMPAR channels in multipolar interneurons are Ca^{2+}-permeable (unpublished observations). Hence, this connection is a probable site for PA-mediated effects.

We first tested whether AMPAR channels that are expressed in the soma of multipolar interneurons undergo PA-dependent facilitation. When two brief (1 ms) glutamate (1 mM) pulses at 40 Hz were applied to outside-out patches pulled from the soma of multipolar interneurons with standard PA-containing intracellular solution (see Methods), the ratio of the second current amplitude to the first one (I_2/I_1) was 0.6 ± 0.07 (mean ± s.d., $n = 3$). The current–voltage (I–V) relation for the glutamate-activated currents was doubly rectifying. With PA-free intracellular solution (see Methods), the ratio was significantly smaller (0.37 ± 0.05, $n = 3$), and the I–V relation was linear (Fig. 1a). In contrast, in patches pulled from layer 2/3 pyramidal cells, neither the shape of I–V relation nor the degree of current reduction under the same experimental conditions was affected by the presence ($I_2/I_1 = 0.81 \pm 0.04$, $n = 3$) or absence ($I_2/I_1 = 0.79 \pm 0.06$, $n = 3$) of spermine in the recording pipette (Fig. 1b). Thus, AMPAR channels in the soma of multipolar interneurons, but not in pyramidal neurons, are sensitive to intracellular PAs and undergo facilitation similar to that reported for Ca^{2+}-permeable recombinant and native AMPAR channels[12].

We then tested whether synaptic AMPAR channels in multipolar interneurons were PA-sensitive. Simultaneous whole-cell recordings were made from pairs of synaptically connected layer 2/3 pyramidal and multipolar interneurons. Excitatory postsynaptic potentials (EPSPs), evoked by action potentials in presynaptic

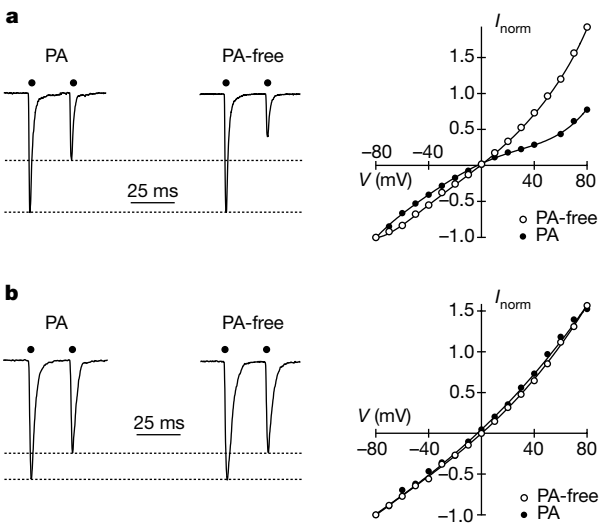

Figure 1 Polyamine-dependent facilitation counteracts AMPAR-mediated current desensitization in outside-out patches. **a**, Glutamate-activated current traces recorded from outside-out patches excised from multipolar interneurons with standard PA-containing and PA-free intracellular solutions. Membrane potential, –60 mV. Currents were normalized to the amplitude of the first resonse. Right, corresponding I–V relations for the first currents. For comparison, current amplitudes were normalized to the values at –80 mV. **b**, As in **a** except that outside-out patches were excised from pyramidal neurons.