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Functional MRI evidence for adult motor cortex plasticity during motor skill learning

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PERFORMANCE of complex motor tasks, such as rapid sequences of finger movements, can be improved in terms of speed and accuracy over several weeks by daily practice sessions. This improvement does not generalize to a matched sequence of identical component movements, nor to the contralateral hand. Here we report a study of the neural changes underlying this learning using functional magnetic resonance imaging (MRI) of local blood oxygenation level-dependent (BOLD)[1–4] signals evoked in primary motor cortex (M1). Before training, a comparable extent of M1 was activated by both sequences. However, two ordering effects were observed: repeating a sequence within a brief time window initially resulted in a smaller area of activation (habituation), but later in a larger area of activation (enhancement), suggesting a switch in M1 processing mode within the first session (fast learning). By week 4 of training, concurrent with asymptotic performance, the extent of cortex activated by the practised sequence enlarged compared with the unpractised sequence, irrespective of order (slow learning). These changes persisted for several months. The results suggest a slowly evolving, long-term, experience-dependent reorganization of the adult M1, which may underlie the acquisition and retention of the motor skill.

In the motor task used, the fingers of the non-dominant hand were opposed to the thumb in two specified sequences, A and B (Fig. 1*a*). Subjects were instructed to tap each sequence, without looking at their hand, as accurately and rapidly as possible.

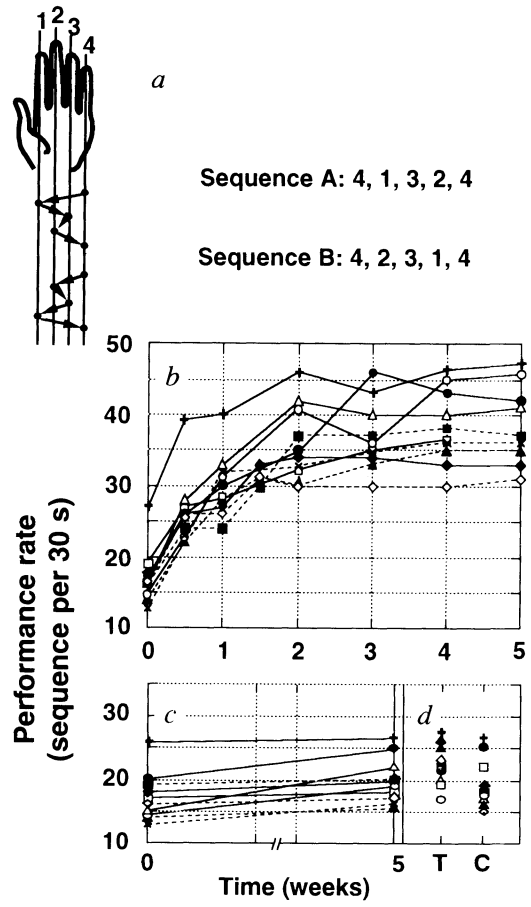

FIG. 1 *a*, The sequence of finger-to-thumb opposition movements. In Sequence A the order of finger movements was 4,1,3,2,4 (numbering the fingers from index to little), and in Sequence B the order was 4,2,3,1,4 as indicated by the arrows (matched, mirror-reversed sequences). Practice consisted of tapping the designated training sequence as fast and accurately as possible for 10–20 min per day, using the non-dominant hand only, light touch of finger pad to thumb pad, and without looking at the hand. Performance was measured in terms of speed and accuracy by two observers for a 30 s interval: one observer counted the number of sequences completed, the other the number of errors. *b*, Learning curves for the trained sequence. Each curve (symbol) depicts the performance of a single subject as a function of time. Pretraining (time point 0), day 3 and 10 of training, and performance on the day of the subsequent weekly imaging sessions is shown for 10 subjects: the 6 who underwent fMRI scanning, and 4 additional subjects (3 females, 1 male; 26–46 years old) who were tested in the behavioural task but were not scanned (broken lines). The number of complete sequences performed in a 30-s test interval (rate) increased from 17.4 ± 3.9 to 38.4 ± 5.8 (mean ± s.d.; week 0 and 5 weeks of training, respectively; paired *t*-test, $P < 0.001$). Accuracy also improved, with the number of sequences that contained errors decreasing from a mean of 2.4 ± 0.9 to 0.5 ± 0.5 (paired *t*-test, $P < 0.001$). (Although some subjects could perform the task at a rate of up to 7 component movements per second at the later stages of training, there was good inter-observer agreement in error counting (mean difference of 0.7 ± 0.7, 2 observers, 10 measurements).) *c*, There was no significant improvement for the control sequence (performance rate 18.1 ± 3.7 to 19.4 ± 4.2; 0 and 5 weeks of training, respectively; paired *t*-test, n.s.). *d*, There was little or no transfer of the learning effect to the contralateral (dominant) hand. Trained (T) versus control (C) sequence performance rates, at week 5, were 22.3 ± 2.9 and 19.8 ± 4.0, respectively (paired *t*-test, $P = 0.097$).

Initial performance was measured for both sequences immediately before the first scanning session. Thereafter, each subject was randomly assigned one sequence (trained) to be practised for 10–20 minutes each day for several weeks, whereas the other sequence (control) was to be performed only during scanning.

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Initial performance of the two sequences did not differ (Fig. 1*b*). However, practice had a large effect on performance: the speed at which the trained sequence could be performed increased across several consecutive sessions, reaching asymptote after approximately 3 weeks of training in all subjects, with more than doubling of the initial rate and a concurrent gain in accuracy. This improvement was specific to the trained hand, with little or no transfer of the learning effect to the contralateral hand (Fig. 1*d*). Moreover, learning did not generalize to the performance of the control sequence, although both sequences were made up of identical component movements (Fig. 1*c*). These results suggest that a lateralized, discrete representation of the learned sequence undergoes experience-dependent changes.

We investigated whether these changes in performance would be reflected in the evoked BOLD signal in M1. Six adult male subjects, 24–44 years old (five right-handed, one left-handed) were scanned once a week for 4–6 consecutive weeks in a 4 T MRI system equipped with echo planar imaging capabilities[5]. Each session consisted of 6–10 experimental sets. The alternation of experimental conditions over time, within a set, is shown in Fig. 2. The general model was rest, first activation (X1), rest, second activation (X2), rest, where X1 and X2 were, respectively: sequence A then B; B then A; B then B; or A then A. Throughout the study, during scanning, both the trained and the control sequence were performed at a fixed, slow rate of two component movements per second, paced by the loud magnetic field gradient switch noise. Thus both rate and component movements were matched, and the only difference between sequences during imaging sessions was the difference in practice histories. Data analysis consisted of determining those pixels in which signal intensity changed, relative to the level at rest, during interval X1 and X2 in each set, and then comparing the extent of the two statistical maps generated from each set.

In session 1, before training, a comparable extent of M1 was activated by the execution of both A and B (41.8 ± 12.2 and 42.3 ± 10.8; number of pixels summed over slices and averaged over sets for six subjects, respectively; mean ± s.d.). However, in the early sets of session 1 a strong ordering effect was found. Either sequence consistently resulted in a larger area of evoked response when executed first rather than second within the set, that is, the area of the evoked BOLD signal, irrespective of sequence type, was greater for X1 than X2. By the latter part

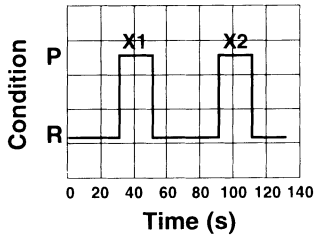

FIG. 2 The alternation of experimental conditions over time within an imaging set (R, rest; P, sequence performance). Each set was 128 s long: 28 s of rest, then 20 s of motor performance (X1), 40 s of rest, second interval of 20 s of motor performance (X2), and a final 20 s rest interval. Images were collected every 2 s.

METHODS. Data were processed by three separate routines, all software written in Interactive Data Language (Research Systems, Inc.). First, pixel-by-pixel Z-maps for each of the two motor performance intervals (X1 and X2) were constructed; this consists of dividing the difference between the interval of task performance and its immediately preceding and following rest periods by the noise value calculated from the first rest period[23]. Second, a pixel-by-pixel percentage change map of signal intensity was calculated, again for each motor performance interval relative to the average of the rest intervals. As a final control, sample data sets were analysed using zero-mean, unit-variance, cross correlation of the detrended intensity changes for each pixel and a square-wave function corresponding to the alternation of conditions. As BOLD responses lag relative to the initiation and termination of movements[1,24] (Fig. 4*b*, *c*), 3 scans were discarded in each transition between conditions in the set. Comparisons were then made, always within the set, and for each slice, between the number of pixels exceeding threshold in M1 during intervals X1 and X2, at arbitrary thresholds of $Z > 2.0$, $\Delta\% > 2.0$ and $r > 0.7$. A balanced randomized design was used. Pairs of sets, one made of X1 being A and X2 being B, and the other with A and B in reversed order, were run. The order of these sets within a pair was randomized. Control sets in which a given sequence was repeated twice within the set (at intervals X1 and X2) were run at the beginning and end of each session. Performance during scanning sessions was monitored for accuracy and rate by two observers. A set was repeated during the same session if more than a single error in the performance of the sequence was noted: this occurred only once. Having all comparisons made within sets avoids problems of head motion and drifts in absolute signal intensities between sets and between sessions. Sets showing evidence of within-set head motion were discarded and replaced by data sets acquired within one week. All 3 types of analysis yielded statistically significant effects. Only the Z-score map results are shown here.

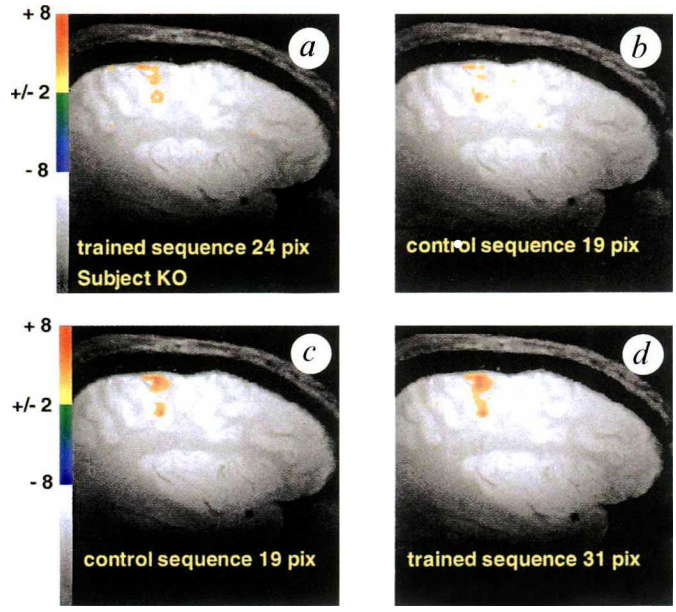

FIG. 3 Differential evoked responses to the trained versus control sequence following 3 weeks of daily practice by subject KO on the designated training sequence, using his left hand; session 4. A parasagittal plane of section through the right hemisphere centred 35 mm from midline is shown; right, anterior; top, dorsal aspect of the brain. Activation maps from two sets (top and bottom) are shown superimposed on the corresponding structural MR image. Top, trained sequence performed first in the set (*a*) versus the untrained sequence performed second (*b*). Bottom, untrained sequence performed first (*c*) versus the trained sequence performed second (*d*). Z-score values are indicated by the pseudo-colour scale. The area of evoked response in M1 for the trained sequence was larger in extent irrespective of the order in which the sequences were performed. It did not, however, extend beyond the hand representation itself, as indicated by control experiments conducted in the initial and fifth session in 2 subjects, in which we mapped the evoked signal for each digit's flexion–extension movements, all four digits-to-thumb opposition, wrist extension–flexion, and eye (orbicularis oculi) closure (results not shown).

METHODS. Images were collected by a 4 T MRI system (Oxford magnet, Omega console), equipped with echo planar imaging (EPI) capabilities and a radio frequency (RF) surface coil. Sequence parameters: gradient-echo EPI, repetition time (TR) = 2 s, echo time (TE) = 26 ms, field of view (FOV) = 160 mm, resolution = 64 by 64. Four contiguous parasagittal slices centred about 35 mm from midline with slice thickness of 5 mm each were acquired, ensuring that all of the contralateral hand representation area was scanned[2]. Subjects' heads were immobilized using foam pads. The EPI images were superimposed (using a two-dimensional warping algorithm) on structural MR images collected at the end of each session in the same plane of section. These were used to identify the central sulcus. Analysis was restricted to M1–anterior bank of the central sulcus and the precentral gyrus[25].

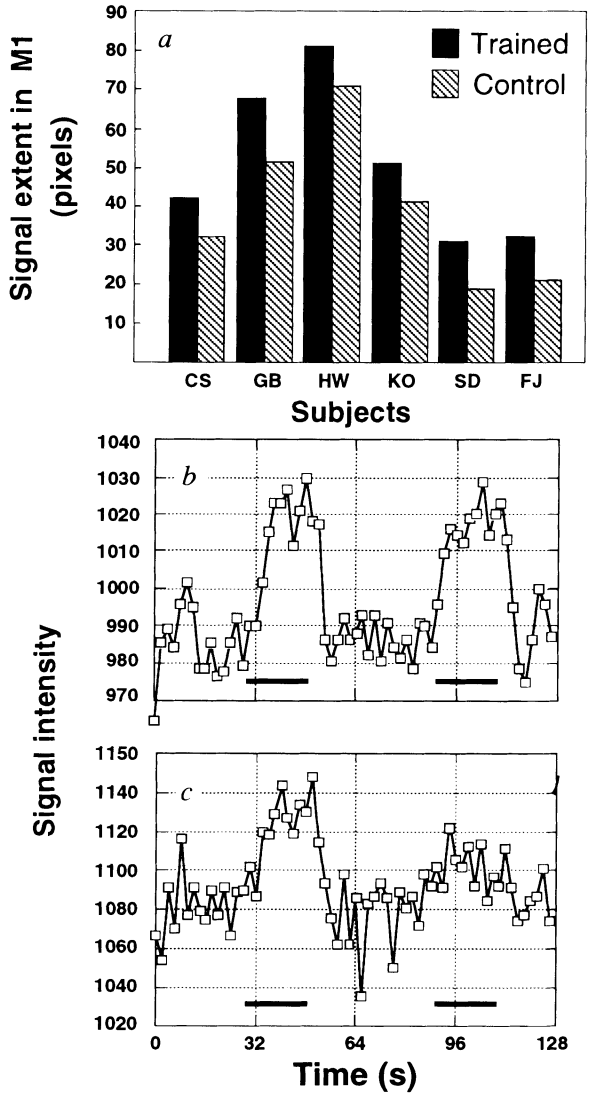

FIG. 4 *a*, The extent of M1 cortex responding for the trained versus untrained sequences in session 4, for 6 subjects (48.7 ± 21.9 and 39.7 ± 19.4 pixels, respectively; mean, s.d.; paired *t*-test, $P < 0.001$). For each subject the number of pixels was summed over slices and averaged over sets in the session to yield the total number of pixels responding for each sequence throughout the volume studied. *b*, Time-course data from a single pixel (2.5×2.5 mm) in M1, recorded during session 4, showing signal intensity changes within a set in which the subject executed the trained sequence during interval X1 and the control sequence during X2. Horizontal bars, intervals of motor performance. The activity-dependent change in intensity was of a similar magnitude for both sequences. *c*, Time-course data from a different pixel in M1, taken from the same set as above. The trained, but not the control, sequence evoked a significant intensity change. No significant differences were found in the mean signal intensity levels, relative to baseline, evoked in M1 by the performance of the trained versus the untrained sequence.

of session 1, however, by which time each subject had typically performed six two-minute experimental sets over the course of approximately 30 minutes, the ordering effect was reversed. A larger extent of M1 was activated by a given sequence when executed second rather than first in the set (greater for X2 than X1). This reversal of the ordering effect was specific to the sequences that were repeatedly performed during the session; when a new, third, motor sequence was introduced, again consisting of the same component movements (4,3,1,2,4; Fig. 1*a*), the initial habituation-like response[6,7] to repetition returned. This specificity suggests that the switch in ordering effects may reflect learning on a short time scale (fast learning). The conjecture is supported by the finding that the paced, repetitive execution of a sequence, as during the initial scanning session, resulted in a significant improvement in both speed and accuracy (correct sequences per 30 s increasing from 17.8 ± 3.3 to 22.5 ± 4.0; paired *t*-test, $P < 0.001$; number of errors decreasing from 2.6 ± 0.8 to 1.6 ± 0.5; paired *t*-test, $P < 0.05$; 6 subjects).

The reversed ordering effect was, however, gradually overridden by the effects of continued training (slow learning). By session 4, which corresponds to three weeks of daily practice on the designated training sequence, and for all subsequent sessions, the activation map evoked by the trained sequence was consistently larger in extent than the map evoked by the control sequence, irrespective of the order in which the sequences were executed (Fig. 3). The emergence of this consistent difference in the evoked MRI signal corresponded in time to the attainment of maximal asymptotic performance on the trained sequence. Figure 4*a* depicts this differential response for all six subjects after three weeks of daily practice.

Does the differential signal in M1 reflect an increase in signal amplitude or an expansion in the cortical representation? In analysing pixels that showed a response to both the trained and control sequences, we found that the activity-dependent change in signal intensity was similar for both sequences (Fig. 4*b*). However, there was a subpopulation of pixels that exceeded threshold in the trained sequence map but showed little or no response during the execution of the control sequence (Fig. 4*c*). This result indicates that, with practice, the extent of the trained sequence representation in M1 does indeed expand. We conjecture that motor practice induces the recruitment of additional M1 units into a network specifically representing the trained motor sequence. This interpretation is in agreement with findings in the monkey of cortical changes associated with motor, as well as perceptual skill, learning[8–10]. One substrate for the enlargement of cortical representations may be the unmasking of pre-existing lateral connections between populations of neurons whose outputs result in different sets of movements[11]. This can occur on a short time scale, and may subserve fast learning. However, to account for the gradual evolution of asymptotic performance as well as the differential cortical responses, we suggest that improved synaptic connections between task-relevant units subserving the execution of the acquired skill are formed as the result of long-term practice[12,13]. Indeed, results from two subjects have shown the persistence of the differential evoked signal in M1, as well as superior performance, over intervals of 10 and 21 weeks of no additional training (results not shown).

This study was designed to examine local cortical changes in the adult motor cortex that result from long-term practice. Several positron emission tomography (PET) studies have examined short-term changes occurring as a consequence of practice in motor[14–17] and sensorimotor[18,19] tasks within a single session. Although some studies have suggested that, as learning proceeded within the session, blood flow in M1[14,17–19] (and the supplementary motor area[16,18]) increased, no significant M1 changes have been found when the rate of movements in the trained and untrained conditions were controlled[15,16]. A consistent decrease in activation in the cerebellum[14–16] and prefrontal[16] cortex (with conflicting observations concerning premotor cortex[16,17,19]) was, however, found to occur with practice within a session even independently of performance rate. Because the decrease in activation in areas projecting to M1 occurred over a similar time scale as the switch in ordering effects on repetition that we observed in M1 within the first session, we suggest that the switch reflects changes in inputs to M1. Moreover, the switch in processing mode, dependent on a critical amount of practice, may constitute an important step in initiating ('gating') subsequent experience-dependent changes in M1. This interpretation may resolve some apparently contradictory transcranial magnetic stimulation studies[20,21]; although sequence performance induced

a decreasing area from which motor responses could be evoked, once a sequence of movements was made explicit[21], the area from which a response could be evoked expanded across five consecutive daily sessions[20]. Our results suggest that motor skill learning has two distinct phases, analogous to those subserving perceptual skill learning[12]. First, a within-first-session switch in processing mode is induced, which may be thought of as the acquisition of a task-relevant routine or set (perhaps an indication that a specific sequence of movements constitutes a special entity of behavioural significance). Second, there follows a much slower between-session phase of learning, which may reflect the gradual evolution of a specific representation of the skilled movements requiring long-term practice over several weeks to be completed[10,12,22]. The final outcome of training is a new, more extensive representation of the trained sequence in primary motor cortex, which may constitute a site for the long-term memory of the skill in the adult human brain. □

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Changes in retinal cell fate induced by overexpression of EGF receptor

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THE differentiation of multipotential progenitor cells in the vertebrate retina into photoreceptors, neurons and glial cells is regulated in part by cell–cell signalling[1–11]. Transforming growth factor (TGF)-α is one of the extracellular signals implicated in the control of several aspects of retinal development, including proliferation and cell fate[5,6,11–13]. The way cells interpret pleiotropic signals such as TGF-α is influenced by the level of expression of epidermal growth factor receptor (EGF-R) in some cell lines[14,15]. To address the influence of receptor level on responses of retinal progenitor cells to TGF-α, additional copies of EGF-Rs were introduced *in vitro* and *in vivo* with a retrovirus. Normally *in vitro*, low concentrations of TGF-α stimulated proliferation whereas high concentrations biased choice of cell fate, inhibiting differentiation into rod photoreceptors while promoting differentiation into Müller glial cells. We report here that introduction of extra EGF-Rs into progenitor cells *in vitro* reduced the concentration of TGF-α required for changes in rod and Müller cell differentiation but did not enhance proliferation. Introduction of extra EGF-Rs *in vivo* increased the proportion of clones that contained Müller glial cells, suggesting that receptor level is normally limiting. These findings demonstrate that responsiveness to extracellular signals during development can be modulated by the introduction of additional receptors, and suggest that the level of expression of receptors for these signals contributes to the regulation of cell fate.

Recent observations *in vitro* suggest that differences in both the availability of extracellular signals[7–11] and the responsiveness of progenitor cells[6,8,16] contribute to the sequential development of 7 different types of cells in the vertebrate retina. Although some of the differences among progenitor cells may reflect the presence or absence of appropriate receptors for extracellular signals, recent studies with several cell lines suggest that the level of receptor expression may also play an important role in determining how cells interpret extracellular signals. For example, the introduction of additional copies of wild-type forms of EGF receptors was reported to increase 3T3 cell proliferation and frequency of transformation in response to EGF[15], and change the response of PC12 cells to EGF from proliferation to differentiation[14]. To determine whether limitations in the level of EGF receptor expression influence the way retinal progenitor cells respond to TGF-α or related ligands, additional copies of wild-type EGF-Rs were introduced into progenitor cells in newborn rat retinas *in vitro* and *in vivo*. At this age, retinal progenitor cells express endogenous EGF receptors (Fig. 1*a*), and several ligands that activate EGF-Rs, including TGF-α, are expressed in the developing retina[5,6,17]. To follow the fate of progenitor cells, a replication-incompetent retrovirus vector expressing a histochemical marker (B-geo; 'control virus', Fig. 1*b*) was used to label these cells. To assess the effects of extra EGF receptors on progenitor cell proliferation and differentiation, a virus that coexpresses the marker and wild-type human EGF receptors was produced ('EGF-R virus', Fig. 1*b*, *c*). The level of expression of EGF-Rs by cells infected with this virus was at least double the level of endogenous EGF-R expressed by neighbouring uninfected cells 4 days after infection (Fig. 2*a*).

Initial studies were performed *in vitro* with explants of newborn retina. Progenitor cells in explants divide and differentiate in parallel with progenitor cells *in vivo*[18], providing a physiological system in which the concentration of ligand could also be modulated. The fate of progenitor cells was followed by infecting them when explants were prepared and analysing their proliferation and differentiation over a 2–3 week period. At this stage *in vivo*, multipotential progenitor cells generate rod photoreceptors, bipolar and amacrine neurons, and Müller glial cells[1].

Progenitor cells infected with control virus in explants developed predominantly into rod photoreceptors, the normal fate of most progenitor cells in newborn rat retina *in vivo* (Fig. 3*a*)[1]. Exogenous TGF-α (1–100 ng ml^{-1}) antagonized development into rods (Fig. 3*a*)[11]. In some cells, rod development appeared to be delayed rather than inhibited: the proportion of cells expressing rod markers rose from 26% of control (untreated) levels to 54% between 2 and 3 weeks *in vitro* in 10 ng ml^{-1} TGF-α. In other cells, TGF-α inhibited development into rods, promoting development into Müller glial cells instead (Fig. 3*b*–*d*). Müller cells were identified by their larger size and expression of vimentin, glutamine synthetase (GS), and glial fibrillary acidic protein (GFAP)[19,20]. During the first week *in vitro*, TGF-α stimulated proliferation in explants (Fig. 3*e*), as previously reported[6]; however, optimal effects on proliferation were