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Driving Plasticity in Human Adult Motor Cortex Is Associated with Improved Motor Function after Brain Injury

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Summary

Changes in somatosensory input can remodel human cortical motor organization, yet the input characteristics that promote reorganization and their functional significance have not been explored. Here we show with transcranial magnetic stimulation that sensory-driven reorganization of human motor cortex is highly dependent upon the frequency, intensity, and duration of stimulus applied. Those patterns of input associated with enhanced excitability (5 Hz, 75% maximal tolerated intensity for 10 min) induce stronger cortical activation to fMRI. When applied to acutely dysphagic stroke patients, swallowing corticobulbar excitability is increased mainly in the undamaged hemisphere, being strongly correlated with an improvement in swallowing function. Thus, input to the human adult brain can be programmed to promote beneficial changes in neuroplasticity and function after cerebral injury.

Introduction

Sensorimotor reorganization within the human cortex occurs dynamically throughout life, for example during central nervous system development in early childhood, following training, or after brain damage (cf. Benecke et al., 1991; Classen et al., 1998; Cohen et al., 1997; Thulborn et al., 1999; Weiller et al., 1995). The reorganization of neuronal connections, and the associated changes in their excitability, is a characteristic of "cortical plasticity," defined as any enduring change in cortical properties such as strength of internal connections, altered representational patterns, or neuronal territories, either morphological or functional (cf. Donoghue et al., 1996). Furthermore, while plasticity may be beneficial for normal function and for compensatory recovery after injury (cf. Buonomano and Merzenich, 1998; Cohen et al., 1998; Hallett, 2000), plasticity may also be deleterious being linked to a number of pathological conditions (cf. Cohen et al., 1998).

More recently, there has been great interest in the clinical relevance of modulating plasticity in the adult human brain, either through driving or restricting change in order to achieve an optimal behavioral outcome. The rationale is the belief that harnessing plasticity may be one method to improve recovery of human function following brain injury. Although work on primates has suggested that rehabilitative training can influence cortical plasticity (cf. Nudo et al., 1996), there has been no direct demonstration in man that any of the forms of experimentally derived stimulus-induced plasticity are functionally relevant to clinical rehabilitation.

In previous studies with transcranial magnetic stimulation (TMS), we have described the normal pattern of projections to swallowing musculature in human motor cortex. Pharyngeal and esophageal motor cortices are organized bilaterally, but display interhemispheric asymmetry with functional dominance to either hemisphere, independent of handedness (cf. Hamdy et al., 1996). Following unilateral hemispheric stroke, one third of patients develop oro-pharyngeal dysphagia (cf. Gordon et al., 1987), almost certainly a consequence of damage to the dominant swallowing hemisphere, putting them at risk of aspiration pneumonia and malnutrition (cf. Cook and Kahrilas, 1999; Kidd et al., 1995). Swallowing in most patients usually recovers slowly over several weeks, and is functionally associated with reorganization of swallowing motor areas in the cortex of the undamaged, previously nondominant hemisphere (cf. Hamdy et al., 1998a).

We have also demonstrated that the organization of healthy human swallowing motor cortex can be altered in a sustained manner after electrical sensory stimulation of the pharynx: a 10 Hz train of stimuli for 10 min resulted in increased excitability of the pharyngeal corticobulbar projection for 30 min (cf. Hamdy et al., 1998b). However, it remains unclear what characteristics of the stimuli are important for driving these changes, or whether these changes bear any functional relevance to swallowing performance, either in health or following cerebral injury, such as dysphagic stroke.

The aim of this study was to explore the possible link between physiologic cortical reorganization and its practical application in clinical rehabilitation; our objectives were to: (1) identify the optimal stimulus parameters promoting swallowing motor cortex reorganization in health; (2) determine its functional significance to swallowing; (3) examine its effects on swallowing motor cortex organization in acute cerebral injury; and (4) determine its functional relevance in driving swallowing recovery after acute cerebral injury.

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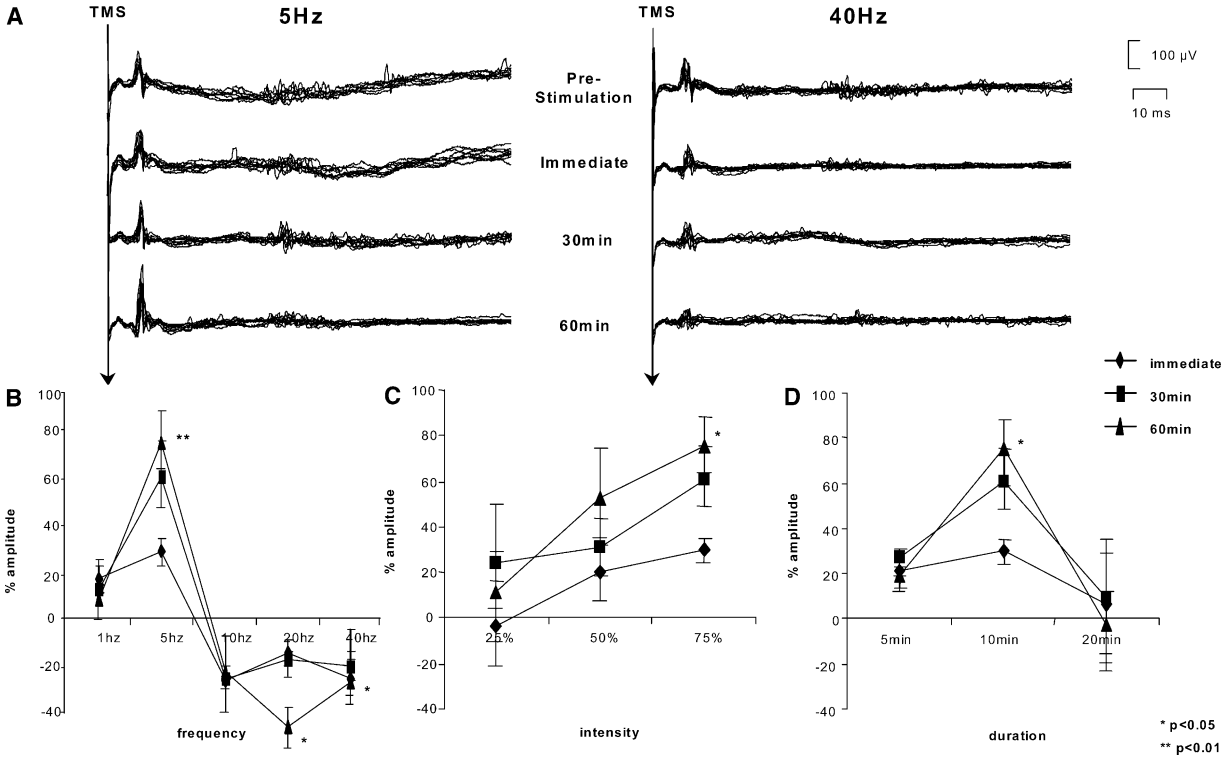

Figure 1. Stimulus Frequency, Intensity, and Duration Effects on Corticobulbar Excitability

(A) Cortically evoked pharyngeal EMG responses in one individual are shown before and after pharyngeal stimulation at 5 Hz and 40 Hz. Ten responses are superimposed for each time interval. (B) Group mean % change in cortically evoked pharyngeal response amplitudes before and after stimulation at 1, 5, 10, 20, and 40 Hz. Note the increase in excitability produced by 5 Hz stimulation compared with the suppression by 20 and 40 Hz stimulation. Pharyngeal latencies remained unaffected. (C) 25% and 50% (maximally tolerated) stimulation intensities induced less excitation post-stimulation than 75%. (D) Stimuli train lengths of 5 min and 20 min induced less excitation compared to 10 min. The greatest increase in mean cortically evoked pharyngeal response amplitudes occurred after stimulation at 5 Hz and 75% maximum tolerated intensity for 10 min. All data are plotted as group mean ± SEM, for immediate (closed diamond), 30 min (closed square), and 60 minute (closed triangle) intervals. (*$p < 0.05$, **$p < 0.01$).

Results

Changes in Excitability of the Corticobulbar Projection to Pharynx Are Stimulus Dependent

Eight healthy subjects received electrical stimuli at different frequencies, intensities, and durations through a bipolar platinum ring pharyngeal electrode built into an intraluminal catheter inserted trans-orally or nasally, in order to determine which pattern produced the largest effect on corticobulbar excitability. Electromyographic (EMG) responses evoked in the pharynx by TMS were tested before stimulation and then immediately, 30 min afterwards, and 60 min afterwards.

Frequency

Electrical stimuli were given through the pharyngeal electrode for 10 min at frequencies of 1, 5, 10, 20, or 40 Hz. For each frequency, the intensity was set at 75% of the maximum tolerated intensity so that the sensation was approximately equal. The Δ mean currents for each stimulus frequency were 38 ± 8, 20 ± 4, 16 ± 4, 14 ± 4, and 10 ± 3 mA for 1, 5, 10, 20, and 40 Hz, respectively. Figure 1A shows some typical EMG responses before and after stimulation at 5 and 40 Hz; Figure 1B plots the average post-stimulus effect normalized to the prestimulus values in all eight subjects. Changing the frequency of stimulation had a dramatic effect on the response to TMS (two-factor ANOVA, main effect of freq: $F(2.7, 16) = 9.8$, $p < 0.001$). Stimulation at 1 or 5 Hz increased excitability as determined by greater response amplitude, without altering latency, whereas stimulation at 10, 20, and 40 Hz decreased excitability. Five hertz stimulation had the largest effect, and this was maximum 30 and 60 min after the end of stimulation. Subsequent studies were performed with 5 Hz stimulation.

Intensity

Figure 1C plots data from the same eight subjects after stimulation (5 Hz, 10 min) at different intensities. The higher the intensity of pharyngeal stimulation, the larger the effect on corticobulbar excitability (two-factor ANOVA, main effect of intensity: $F(1.9, 13.5) = 8.5$, $p < 0.005$). At these higher intensities, direct laryngoscopy in two subjects showed that stimulation also induced occasional minute twitch contractions in the pharynx. There was a marginally significant main effect of time after the end of stimulation ($F(1.6, 11.3) = 3.8$, $p = 0.06$), with larger responses at 60 min compared with immediately after stimulation.

Duration of Sensory Stimulation

There was a significant main effect of the duration of pharyngeal sensory stimulation (5 Hz, 75% maximum tolerated intensity, mean 16 ± 2 mA) ($F(1.7, 12.2) = 4.6$, $p < 0.05$). Stimulation for 5 or 20 min facilitated motor-

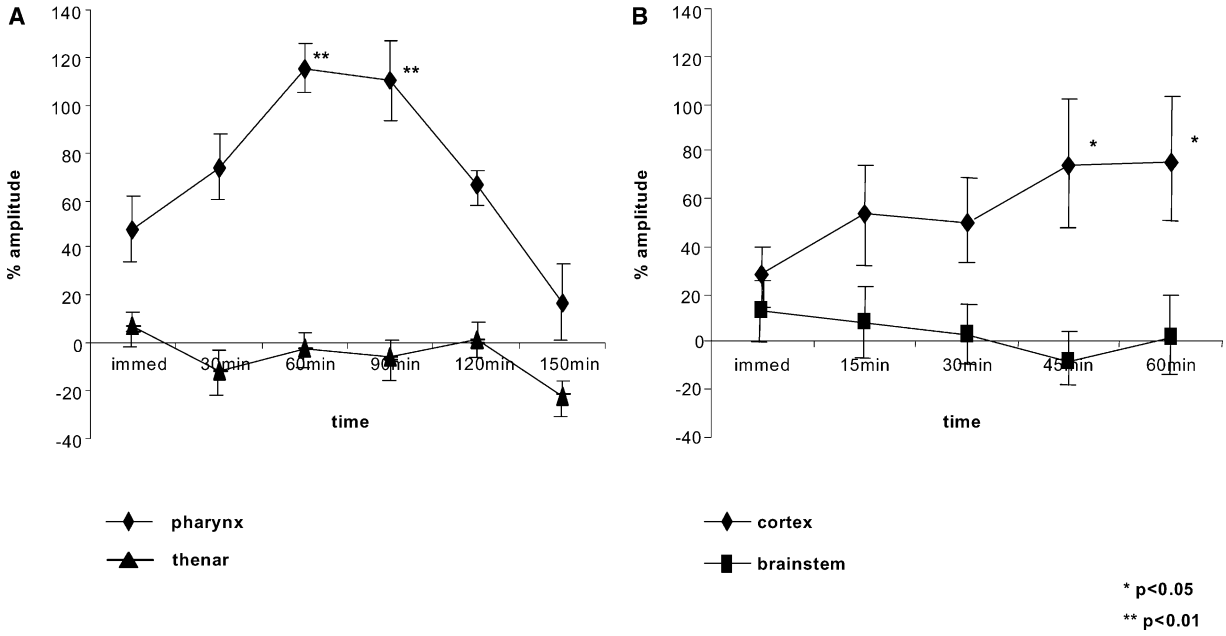

Figure 2. Duration of Stimulation Effect on Corticobulbar Excitability

Length of time of stimulation-induced effect (A) and effect of stimulation on brainstem swallowing reflexes (B) are shown. (A) Maximal increase in corticobulbar excitability for pharynx (closed diamond) occurs at 1 hr and persists for more than 150 min. Thenar excitability (closed triangle) remains unaffected. (b) Brainstem reflexes (closed square) remain unaffected, compared to cortically evoked (closed diamond) pharyngeal responses at 1 hr. Data plotted as group mean ± SEM. (*$p < 0.05$, **$p < 0.01$).

evoked potentials (MEPs) less than stimulation for 10 min (paired t test 10 min versus mean of 5 + 20 min, $p < 0.01$) (Figure 1D).

These initial experiments suggested that pharyngeal stimulation at 5 Hz and 75% maximum tolerated intensity for 10 min produced the largest excitatory effect on corticobulbar excitability. There was also a suggestion that the rise in excitability might take some time to build up to maximum. The study was therefore repeated using this pattern of pharyngeal stimulation with additional recordings of MEP amplitudes 90 min, 120 min, and 150 min after the end of stimulation. To test whether the effect was specific for the projection to bulbar muscles, thenar muscle excitability was also monitored prior to and after stimulation. A two-factor ANOVA on these data in Figure 2A, with muslce and time as main factors revealed a significant muscle × time interaction ($F(5, 35) = 2.8$, $p < 0.05$). There was a significant effect of time on responses in the pharynx, whereas thenar responses were unaffected. Maximal facilitation of the pharynx occurred at 60 and 90 min after the end of pharyngeal stimulation.

Brainstem Excitability

Trans-cutaneous electrical stimulation of brainstem resulted in large artifacts, which obscured evoked pharyngeal EMG responses, making interpretation of pyramidal excitability impossible. Thus, in six of the eight subjects, we tested whether pharyngeal stimulation affected the size of brainstem reflexes evoked in bulbar muscles by TMS (via a small figure-of-8 coil) of the right supraorbital nerves. Figure 2B shows that although MEPs to cortical stimulation were enhanced by a period of pharyngeal stimulation (5 Hz, 10 min, 75% max tolerated intensity), there was no effect on the amplitude of brainstem reflexes (two-factor ANOVA, main effect of response type ($F(1, 6) = 9.6$, $p < 0.001$).

Sensory Stimulation Induces Changes in the Topography of the Corticobulbar Projection to Pharynx

Three subjects (two male, age range 22–34 years) then underwent a detailed bi-hemispheric topographic mapping study of the pharyngeal and thenar corticobulbar projection before and after pharyngeal electrical stimulation. TMS was applied to individual points on a 70-point scalp grid matrix, positioned 2 cm lateral and posterior to the vertex, before and 1 hr after pharyngeal stimulation. Three stimuli were delivered to each scalp grid point and the responses recorded. Individual scalp maps representing the areas of response for pharynx and thenar were then generated for each subject. An increase in the size of the pharyngeal area of response occurred in all subjects studied, the number of scalp sites evoking a response increasing bilaterally (left hemisphere: 15 ± 3 to 26 ± 2 sites; right hemisphere: 12 ± 4 to 15 ± 4 sites), with the effect appearing greatest in the pharyngeal dominant hemisphere. Thenar motor maps, acting as a control for the pharynx, were unaffected by pharyngeal stimulation (left hemisphere: 16 ± 5 to 14 ± 2 sites; right hemisphere: 18 ± 5 to 14 ± 1 sites).

Sensory Stimulation of the Pharynx Promotes Functional Increases in Sensorimotor Cortex Activation during Healthy Volitional Swallowing

Eight healthy subjects, six of whom had previously participated in the TMS protocols, were studied by fMRI to determine the extent of activation of cortical regions

Table 1. fMRI Study of Water Swallowing with and without Pharyngeal Stimulation

	Brodmann Area	Number of Activated Voxels		Talairach Coordinates		
		Without Stimulation	With Stimulation	X	Y	Z
R SI/MI	1–4	24	118	54	−7	21
L SI/MI	1–4	20	111	−54	−7	21
R + L anterior cingulate/SMA	6, 32	73	61	1	−9	56
R + L posterior cingulate	31	54	78	0	−35	40
R + L pre-frontal	10/11	82	114	0	47	−12
R temporal lobe	22	65	113	58	2	−3
L temporal lobe	22	62	86	−50	2	−2
R + L thalamus		24	11	0	−20	3
Cerebellum		115	116			

The Brodmann areas, Talairach coordinates, and number of activated voxels in the group mean activation map are shown.
SI = Primary sensory cortex
MI = Primary motor cortex
SMA = Supplementary motor cortex

associated with swallowing. Each subject was studied twice, once 1 hr after electrical pharyngeal stimulation, and once after sham. The order of the studies was randomized. Cortical areas activated by swallowing are shown in Table 1. These are the areas in which a significant change in blood oxygenation (BOLD signal) was associated with swallowing. The number and intensity of activated pixels are tabulated both with and without pharyngeal stimulation. During the task of swallowing in the grouped analysis, pharyngeal stimulation was associated with a bilateral increase in the area of voxel activation within lateral sensorimotor (SI/MI) cortex (229 versus 44 voxels) and a greater Z score (4.47 versus 3.81, $p = 0.047$) compared to the no-stimulation control (Table 1, Figure 3).

Sensory Stimulation of the Pharynx Enhances Corticobulbar Excitability, Topographic Representation, and Swallowing Function in Dysphagic Hemispheric Stroke Patients

Sixteen dysphagic, acute hemispheric stroke patients were recruited to the study (mean recruitment 4 days ± 12 hr of stroke). Stroke patient details are shown in Table 2. All patients were intubated with the pharyngeal electrode, which recorded pharyngeal EMG responses (evoked by TMS); ten of them were randomized to a treatment group that received 10 min electrical stimulation (using parameters defined by data in healthy subjects) at 5 Hz and 75% maximum tolerated intensity (16 ± 2 mA), the remainder simply kept the catheter in place for the same amount of time. The corticobulbar projections from both hemispheres were mapped using a stimulus intensity of 110% cortical threshold for the undamaged hemisphere before and 1 hr afterwards. These data were analyzed either as the number of points from which responses of 40 μV or more could be evoked (map area), or the mean amplitude of the responses evoked from the five sites giving the largest responses (excitability) (Figure 4A). Both sets of measurements were combined and analyzed using a three-way ANOVA with hemisphere, treatment, and time after stimulation as main factors. There was a significant hemisphere × time interaction ($F(1, 14) = 7.8$, $p < 0.05$), which was due to the fact that pharyngeal stimulation had more effect on the excitability of responses evoked from the unaffected hemisphere than those recorded from the affected hemisphere. Despite the fact that real stimulation appeared to have a larger effect on response amplitudes and map areas (Figure 4A), there was no statistical difference between the effects of pharyngeal stimulation in the two treatment groups (real versus no stimulation: no significant interaction terms involving treatment).

All stroke patients underwent extubated videofluoroscopic assessment before and 1 hr after receiving pharyngeal stimulation or with the catheter placed in situ without stimulation as previously described. In contrast to healthy subjects, where pharyngeal stimulation had no enhancing effect on swallowing performance (swallow response time; Δ −7%, Wilcoxon test; $p = 0.4$, pharyngeal transit time: Δ 1%, $p = 0.7$), pharyngeal stimulation resulted in a reduction in pharyngeal transit time ($p < 0.01$), swallowing response time ($p < 0.01$), and most clinically relevant, the aspiration score ($p < 0.01$) compared to pre-stimulation values. Presence of the catheter alone without electrical stimulation had no effect on any of the swallowing parameters (Figure 4B).

Finally, we found that there was a highly significant correlation within patients between the total change in excitability (Spearman's rank correlation coefficient $(r) = 0.7$, $p = 0.01$) or map size ($r = 0.6$, $p = 0.03$) measured over both hemispheres, and the change in aspiration before and after pharyngeal stimulation (Figure 5). This would be compatible with a causal relationship between increased cortical excitability and improvement in swallowing function across individuals in the group.

Discussion

Driven Plasticity of the Swallowing Corticobulbar Projection Is Stimulus Dependent and Functionally Relevant

Our data demonstrate that stimulation-driven reorganization of the cortical projection to swallowing muscles depends on the nature of the stimulus. Excitability can be increased or decreased according to the frequency, duration, and intensity of electrical sensory stimulation

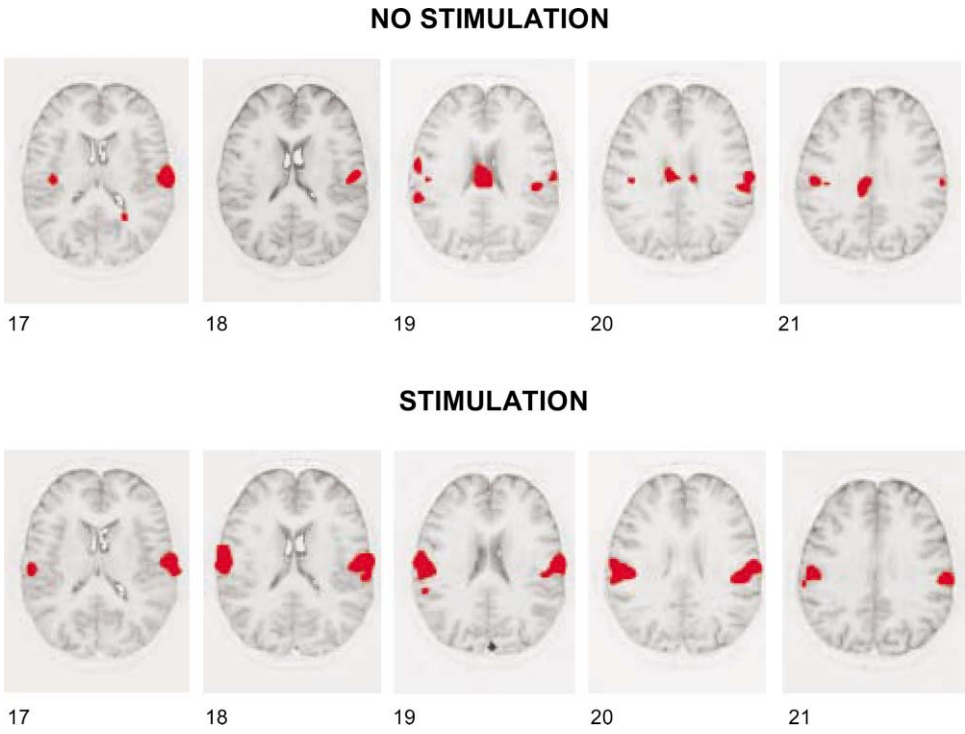

Figure 3. Effect of Pharyngeal Stimulation on Cortical BOLD (Blood Oxygenation Level Dependent) fMRI Signal in Healthy Subjects during Normal Swallowing

Group mean activation data are show as a series of normalized transverse brain slices, with no stimulation paradigm across the top, and the stimulation paradigm across the bottom. Activated pixels in red, and brain slice numbers indicated below. Greater bilateral functional activation occurs within sensorimotor cortex (BA 3,4), most overt in slices 17–19, after pharyngeal stimulation compared to no stimulation.

of the pharynx. In addition, we have shown that these changes in excitability are functionally relevant to voluntary swallowing movements. Our fMRI studies on healthy subjects show that periods of pharyngeal electrical stimulation can change the pattern of brain activation seen during normal wet swallows. More importantly, we show that the same paradigms applied to dysphagic hemiplegic patients are associated with faster initiation of each swallow and a reduction in the frequency of aspiration for at least 1 hr after pharyngeal stimulation.

As in our previous experiments, we have used electrical stimulation of the pharynx as our source of sensory input. Such stimulation preferentially activates sensory afferents from the pharynx, and at higher intensities may evoke small twitches of the pharyngeal muscles. The latter would produce additional natural sensory stimulation that might add to the electrically induced activity. The main advantage that electrical stimulation has over natural sensory inputs (e.g., tactile faucial pillar stimulation) is that the parameters of stimulation are easily

Table 2. The Clinical Details, Stroke Location, Barthel Index, and Motor Scores of All Stroke Patients at Presentation

Patient	Age	Sex	Hand	Stroke	Barthel Index	Motor Score
1	85	M	R	L basal ganglia	61	3.5
2	83	F	R	R temporoparietal	27	3.5
3	76	M	R	R frontoparietal	0	0
4	77	F	L	R temporoparietal	39	4
5	66	M	L	Multi-infarct	78	4
6	71	M	L	L temporal	72	4
7	93	F	R	R frontoparietal	30	1.5
8	65	M	R	L temporoparietal	35	2
9	78	F	R	R internal capsule	49	2
10	84	M	R	L internal capsule	69	2.5
11*	68	F	R	R internal capsule	60	3
12*	68	M	R	R internal capsule	82	4
13*	78	M	R	L basal ganglia	42	3
14*	69	F	R	L internal capsule	71	3
15*	56	M	R	L internal capsule	74	3.5
16*	70	M	R	L basal ganglia	56	4

Patients were randomized to either electrical stimulation of pharynx or catheter alone (indicated by *).

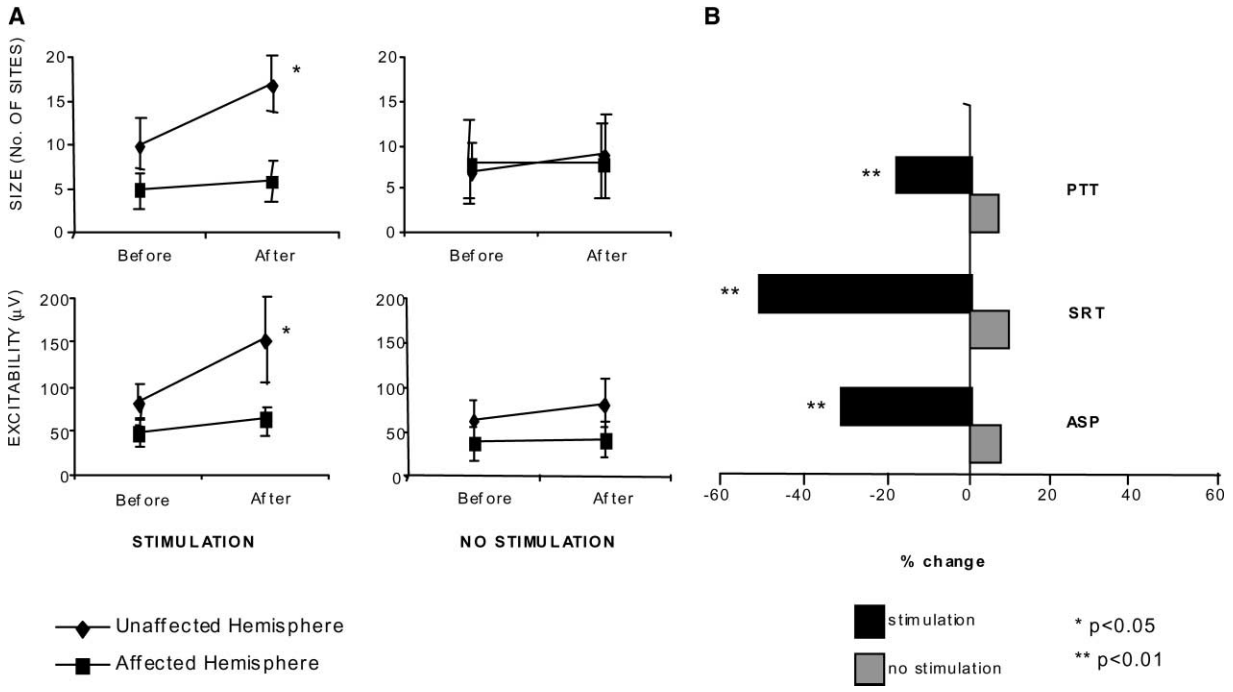

Figure 4. Changes in the Organization of the Cortical Projection to Swallowing Muscles and Swallowing Function after Pharyngeal or No Stimulation in 16 Acutely Dysphagic Stroke Patients

A marked increase in pharyngeal corticobulbar excitability and topographic representation occurs in the undamaged hemisphere (closed diamond) compared to the affected hemisphere (closed square) in patients receiving pharyngeal stimulation (A). This is mirrored by the changes to swallowing (with stimulation [black] versus without stimulation [gray] with a functionally beneficial reduction in PTT, SRT, and aspiration [B]). PTT = pharyngeal transit time, SRT = swallowing response time, ASP = aspiration. (*$p < 0.05$, **$p < 0.01$).

controlled and replicated across subjects. The present experiments explored only a subset of the total possible range of parameters, but even within those we found that it was possible to reverse the effect from facilitation to inhibition by, for example, changes in frequency. The implication is that the pattern of stimulation is an important parameter in producing plastic changes in corticobulbar excitability, and must be borne in mind when

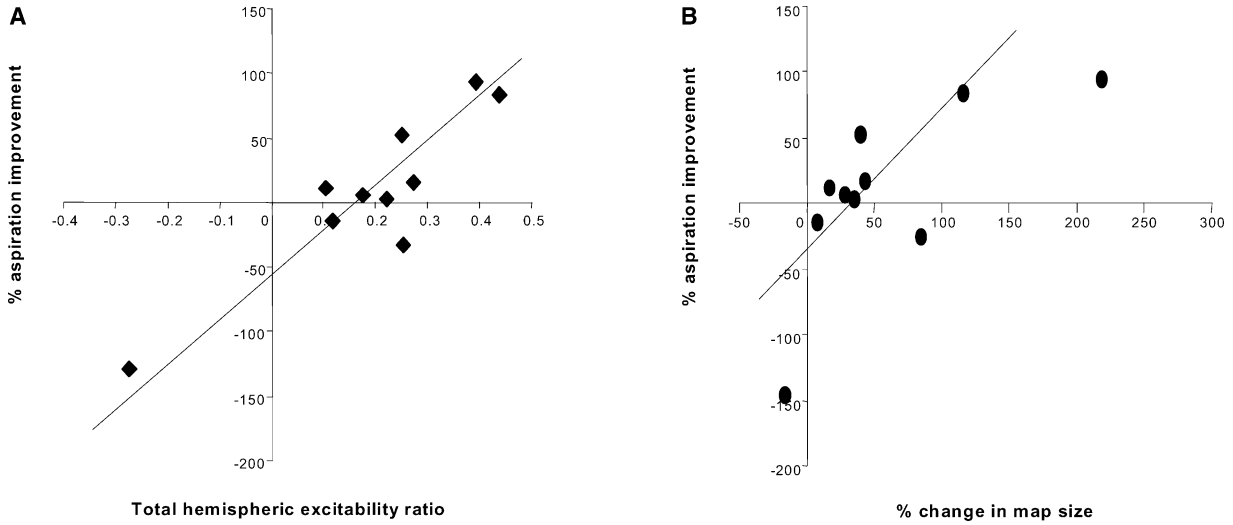

Figure 5. Changes in Total Cortical Excitability and Map Size with Aspiration in Dysphagic Stroke Patients after Pharyngeal Stimulation

(A) Correlation between fractional change in total hemispheric excitability (calculated as the sum of the change in mean excitability (response amplitude) of each hemisphere divided by the sum of the post-stimulation maximal response amplitude of each hemisphere) and % improvement in aspiration across individuals showing that an increase in excitability is strongly associated to an improvement in swallowing behavior ($r = 0.7$, $p = 0.01$). (B) Correlation between % change in cortical map size and % improvement in aspiration across individuals showing that an increase in map size is also strongly associated with an improvement in swallowing behavior ($r = 0.6$, $p = 0.03$).

developing strategies to promote recovery after injury. Our experiments did not address the mechanism of the "sensory" driven effects and why they were sensitive to the pattern of stimulation. Other workers have suggested that "after-effects" on motor cortical excitability produced by a variety of methods may be related to phenomena such as long-term potentiation (LTP) and depression (LTD). Indeed, the time course of the effect, with maximum facilitation being reached some minutes after the end of the sensory stimulation, is similar to that seen in other studies on human motor cortex (cf. Stefan et al., 2000; Ridding et al., 2000). The fact that LTP and LTD are evoked respectively by fast and slow frequencies of repeated stimulation may therefore be related to our finding that the pattern of sensory input has an influence on the excitability that we measured. However, such arguments are speculative. The precise mechanisms driving the changes we have observed cannot be addressed in experiments on human cortex, and will have to be addressed in further work in animal preparations.

Sensorimotor Cortex Is the Main Region of Change after Sensory Stimulation

Changes in the size of motor-evoked potentials in the pharynx at rest were our measure of corticobulbar excitability. In the corticobulbar system, it is technically difficult to test in human subjects whether these changes are due to effects on cortical or bulbar excitability. However, we did not find any evidence that pharyngeal stimulation increased the excitability of brainstem reflexes in the pharynx. This, together with the changes in cortical function revealed by the fMRI experiments, leads us to favor the idea that the cerebral cortex was the main site of the changes we observed. A cortical origin for these effects would also be consistent with the finding that electrical stimulation had the largest effect on excitability of projections from the "dominant" swallowing hemisphere when mapped with TMS. A change in brainstem excitability might have been expected to have some effect on projections from both hemispheres.

An important aspect of the present study was that we tried to relate changes in the excitability of corticobulbar projections to changes in performance of volitional swallows. This has rarely been done in other studies of motor cortex plasticity in humans, and in some cases, no functional changes have been seen (cf. Muellbacher et al., 2000). Nevertheless, in our study of healthy subjects, we observed distinct changes in the recruitment of brain activations during voluntary swallows in fMRI. The main effect was within the sensorimotor cortex, which is recognized to play a major role in the functional neuroanatomy of volitional swallowing (cf. Hamdy et al., 1999).

Sensory-Driven Plasticity Correlates with an Improvement in Functional Performance after Brain Injury

The two most important practical aspects of our study were (1) that a pattern of electrical stimulation, which produced effects in healthy subjects, increased corticobulbar excitability in patients after acute stroke and (2) that the effect on corticobulbar excitability correlated strongly with an improvement in swallowing. Although the effect was seen within the first hour after stimulation, the total duration was not monitored in this study. The data in Figure 2 on cortical excitability in healthy subjects would suggest a duration of 2–3 hr, but this would need to be verified in future patient work. Interestingly, much of the TMS-measured change in excitability occurred within the uninjured rather than the injured hemisphere. This observation is in keeping with that seen naturally after stroke in which the slow spontaneous recovery of swallowing appears more closely associated with topographic changes in representation within the undamaged hemisphere with little change in the damaged hemisphere (cf. Hamdy et al., 1998a). The effect of real pharyngeal stimulation on corticobulbar excitability did not differ significantly from the presence of the catheter alone, although the latter appeared much weaker. This may imply that the catheter itself elicits either some "placebo" or sensory effects, which in susceptible patients (i.e., cerebral injury) may alter the measured size of the cortical response. It does appear, however, that to induce functionally relevant effects, a certain threshold of excitability and/or recruitment of swallowing neurons is required. This was only achievable when greater strengthens of input (i.e., electrical stimulation) were delivered. Although the correlation between changes in corticobulbar excitability and swallowing recovery before and after pharyngeal stimulation is not proof of a causal connection, these data provide unequivocal support for the hypothesis that changes in human cortical plasticity have behavioral relevance.

In conclusion, we demonstrate that changes in sensory input can increase cortical excitability in a sustained manner with associated improvement in end organ performance. While previous studies have shown that the cortical topography of limb muscles can be altered when intensive training is applied to a stroke patient over days or weeks (cf. Liepert et al., 2000), it is difficult to distinguish conclusively between the effects of training driven plasticity and those of spontaneous recovery or changes in muscle strength. Our model is not subject to these confounding variables since the changes that we described occur within the first hour following stimulation. Thus, sensory-induced changes in corticobulbar excitability provide the strongest evidence yet for functionally relevant driven plasticity and may prove to be a useful adjunct to therapies designed to promote recovery of function after brain injury such as for dysphagia after hemispheric stroke.

Experimental Procedures

Subjects and Patients

Transcranial Magnetic Stimulation (TMS)

Eight healthy subjects (6 male, 2 female, age range 23–34 years, mean age 26 years) and 16 first-time stroke patients recruited over an 18 month period (10 male, age range 56–93 years, mean age 74 years) were studied. All satisfied the inclusion criteria of (1) being capable of giving informed consent, (2) having no other neurological disease or serious intercurrent illness, (3) taking no neuromuscular modulating drugs, and (4) having no contraindications to undergoing TMS. In patients, the site and type of stroke as well as the severity of dysphagia were identified before each study by computerized tomography (CT) and videofluoroscopy (VFS), respectively. Hand preference was also determined, in all healthy subjects and patients,

prior to the stroke. All stroke patients were acutely dysphagic having had a unilateral hemiplegic stroke within 10 days of their study. Assessment of stroke severity was performed in all patients using the MRC power rating scale (cf. MRC, 1976) and a modified Barthel Index (cf. Mahoney and Barthel, 1965). Where more than one study was performed on an individual, interval between studies was at least 24 hr (median 5 ± 2 days). All participants gave informed written consent before the study, which was approved by the Salford & Trafford Health Authority Ethics Committee.

Assessment of Swallowing and Dysphagia

All stroke patients underwent a detailed digitally acquired VFS assessment to examine their swallowing function. Patients were classified as being dysphagic when a disruption in the normal flow of the bolus resulted in barium appearing in the airway during VFS (cf. Rosenbek et al., 1996).

Functional Magnetic Resonance Imaging (fMRI)

Eight healthy subjects (7 male, 1 female, age range 23–34 years, mean age 26 years), six of whom participated in the TMS studies above, were studied using fMRI. None reported any swallowing problems and all gave informed written consent before the study, which was approved by the Salford Health Authority Ethics Committee.

Electrophysiological and Imaging Techniques

TMS

Cortical Stimulation. Focal transcranial stimulation of the cerebral cortex was achieved using a magnetic stimulator (Magstim 200, MAGSTIM Company Limited, Whitland, Wales) connected to a 70 mm outer diameter figure-of-8 coil which resulted in a maximal output of 2.2 Tesla (T).

Cranial Nerve Stimulation. To study brainstem reflexes, trigeminal nerve stimulation was performed using TMS, as previously described (cf. Hamdy et al., 1997), with a smaller 50 mm outer diameter figure-of-8 coil, sited over the right supraorbital region of the face.

Electromyographic (EMG) Recordings

EMG responses were recorded from the pharynx and thenar muscles. Pharyngeal responses were detected using a pair of bipolar platinum ring electrodes, built into a 3 mm diameter, intraluminal catheter (Gaeltec, Dunvegan, Scotland). A solid-state strain-gauge transducer was also incorporated into the catheter, between the electrode pair for motility recording. This enabled the position of the pharyngeal electrodes to be maintained in position by adjustment with reference to the motility pattern. Thenar responses were detected using two skin electrodes applied 1 cm apart on each thenar eminence. Response signals were passed through a preamplifier (CED 1902, Cambridge Electronic Design, Cambridge, England, United Kingdom) with filter settings of 5 Hz–2 kHz and then collected through a laboratory interface (CED 1401 plus) at a sampling rate of 4–8 kHz. Data were displayed on a 486 SX PC, which allowed immediate visualization, averaging, and archiving of traces to file for later analysis.

Pharyngeal Stimulation

Electrical stimulation of the pharynx was performed using the pharyngeal electrodes connected to an electrical stimulator (Model DS7; Digitimer, Welwyn-Garden City, Herts, United Kingdom) via a trigger generator (Neurolog System, Digitimer), which delivered stimuli (0.2 ms pulses, 280V) at a set frequency, intensity (current), and duration.

Videofluoroscopy (VFS)

Videofluoroscopic assessment was performed using a Siemens Fluorospot® H Sireskop SX Unit (Siemens Aktiengesellschaft Medical Engineering, Germany). X-ray images were recorded via digital video recorder (Sony DHR 1000, Sony UK Ltd., United Kingdom).

fMRI

fMRI was performed on a 1.5 Tesla Philips ACS-NT MRI scanner (Imaging Science and Biomedical Engineering, Manchester University, Manchester, United Kingdom).

Characterization of the Effect of Electrical Sensory Stimulation on Excitability of the Corticobulbar Projection to Pharynx in Health

Eight healthy subjects participated. For each study, the subject sat comfortably in a chair, the cranial vertex was marked on the scalp, and the pharyngeal catheter inserted trans-nasally or orally depending on subject preference. To determine the effects of pharyngeal stimulation on excitability of the corticobulbar projection to swallowing muscles, the optimal site for cortical magnetic stimulation was first determined by discharging the figure-of-8 coil over both hemispheres, using 100% stimulator output. Hemispheric sites evoking the greatest EMG response from pharynx and thenar muscles were identified and these two positions marked on the scalp. Next, a series of cortical stimulations were performed over these positions, commencing at subthreshold intensity and increasing by 5% steps until threshold intensity was found that evoked EMG responses in the pharynx greater than 20 μV and thenar EMG responses greater than 100 μV, on at least 5 of 10 consecutive stimulations. These scalp sites were then stimulated repeatedly, in a randomized order, at intensities of 90%, 95%, 100%, 105%, and 110% threshold before and after electrical pharyngeal stimulation. Ten stimuli were delivered to each site at each intensity with an interval of 5 s between stimuli. To avoid any inadvertent facilitation of the cortically evoked responses, all subjects kept as relaxed as possible and minimized swallowing, coughing, or speaking during the stimulation procedure. If any of these activities did inadvertently occur during the recording of an evoked response, that response was discarded and the stimulus repeated.

Stimulus Frequency, Intensity, and Duration

To determine the effects of stimulus frequency on swallowing corticobulbar excitability, electrical stimulation of the pharynx was performed in eight healthy subjects for 10 min at frequencies of 1, 5, 10, 20, or 40 Hz. Subjects described the sensation as nonpainful and pulsing or tingling in nature, although high-frequency stimulation (20–40 Hz) tended to be regarded as less comfortable. Direct laryngoscopy was performed in two subjects and demonstrated occasional twitch contractions of the pharyngeal musculature at the higher intensities. The effect of stimulus intensity was then established using 5 Hz (the frequency shown with a stimulation intensity of 75% maximum tolerated, to induce maximal post-stimulation excitability of the pharyngeal corticobulbar projection) at 25% and 50% of the maximum tolerated intensity. The effect of stimulus length was then established using 5 Hz at 75% of maximum tolerated intensity (the frequency and intensity shown for a train length of 10 min, to induce maximal post-stimulation excitability) with 5 and 20 min periods. For all studies, transcranial magnetic stimulation was performed prior to and immediately, 30 min, and 60 min after pharyngeal stimulation.

Duration of Effect

The duration of effect of stimulation was also determined in the same subjects ($n = 8$) using the parameters defined above to provide optimal excitation (5 Hz, 75% maximum tolerated intensity, 10 min). The corticobulbar excitability of the thenar muscle projection was recorded after stimulation as a control for the pharynx.

Effect of Stimulation on Brainstem Excitability

To determine the effect of pharyngeal stimulation on brainstem excitability, brainstem reflexes in 6 of the 8 subjects were simultaneously recorded with motor cortex responses in random order, prior to and at 15 min intervals post-pharyngeal stimulation for 60 min, using the parameters established above.

Effect of Sensory-Driven Plasticity on Brain Activation during Healthy Volitional Swallowing

To determine the effect of electrical sensory stimulation on the areas of brain activated during healthy swallowing, eight healthy subjects were studied using fMRI on two separate occasions 1 hr after randomization to pharyngeal stimulation or sham. On each occasion, subjects underwent two 8 min functional scans consisting of a 1 min "on-period" of 12 water swallows (5 ml at 5 s intervals) alternating with a 1 min "off-period" without swallowing. A visual cue was used to indicate when each swallow should occur during the on- and off-periods. Each scan consisted of a series of 96 T2*-weighted single shot gradient echo, multi-slice echo planar image sets containing 40 contiguous slices with whole head coverage (TR 5000 ms, TE 50 ms, voxel size 3.5 × 3.5 × 3.5 mm). A T1-weighted inversion recovery image set (TR 6850 ms, TE 18 ms, TI 300 ms) was also obtained from each subject as an anatomical reference.

Effect of Electrical Sensory Stimulation of the Pharynx on Corticobulbar Excitability and Topographic Representation in Dysphagic Hemispheric Stroke Patients

To determine the effect of pharyngeal stimulation on the excitability of the corticobulbar projection to the swallowing muscles after acute cerebral injury, 16 acutely dysphagic hemispheric stroke patients underwent a bi-hemispheric mapping study, performed as described in study 1, both before and 1 hr after randomization to pharyngeal stimulation (as determined by data in healthy subjects) or sham.

Effect of Electrical Sensory Stimulation of the Pharynx on Swallowing Function in Dysphagic Hemispheric Stroke Patients

To determine the effect of pharyngeal stimulation on swallowing function after acute cerebral injury, all 16 acutely dysphagic patients underwent further videofluoroscopic assessment before and 1 hr after randomization to either pharyngeal stimulation or sham. In addition, swallowing studies were performed before and after pharyngeal stimulation in the eight healthy subjects previously studied with TMS. For each study, the patient was seated and images taken from the lateral position. Each received six boluses of 5 ml thin barium (40% w/v). Anatomical markers were: (1) the lips anteriorly; (2) the cervical spine posteriorly; (3) the nasopharynx superiorly, and (4) the upper margin of the thoracic esophagus inferiorly. Quantitative measurements of oropharyngeal bolus flow and aspiration were made on six consecutive swallows, before stimulation or sham and at 60 min. Screening time was below 80 s (range 42–73 s) with a radiation dose of less than 0.3 mSv in all cases.

Data Analysis

TMS

For stimulus/response plots of corticobulbar excitability, the individual value of each of the ten EMG responses was calculated for each intensity and muscle group (cf. Ridding and Rothwell, 1997).

For topographic mapping, the mean value of the three EMG responses was calculated for each of the muscle groups of interest. When no response to stimulation was obtained, a zero amplitude value was given. Cortical maps were measured as number of sites that evoked a response of greater than 40 μV for pharynx and 100 μV for thenar. In the stroke patients, an additional analysis of cortical excitability for pharynx was used, calculated as the mean of the five largest responses for each hemisphere, before and after stimulation.

fMRI

The images were transferred to a Sun Workstation for analysis using the TINA software package as previously described (cf. Hobday et al., 2001). The steps involved automated rigid body realignment of the images (cf. Thacker et al., 1999a), followed by re-slicing and global normalization to enable all subjects to be compared in the same stereotactic space (cf. Vokurka et al., 1999, cf. Thacker et al., 1999b). Individual activation maps were calculated using a cross-correlation measure equivalent to that employed by other groups (cf. Friston et al., 1994). The functional MRI scans and activation maps were then aligned to a standard brain in Talairach space (cf. Hobday et al., 2001) and the activation maps averaged to form a group mean activation map. It should be recognized that because of the spatial smoothing applied to individual brains to generate the aligned group data, activation strength and volume of activation are not independent.

VFS

Measurements of bolus movement were made by slow time frame by frame digital analysis of recorded VFS. These were: duration of bolus transit through the pharynx (pharyngeal transit time: PTT); swallow response time (SRT), defined as the interval between the presentation of the bolus at the hypopharynx and laryngeal elevation; and aspiration score (ASP) (cf. Rosenbek et al., 1996).

Statistical Methods

TMS

With the stimulus/response plots, individual mean values of the cortically evoked EMG responses across all intensities for each interval after pharyngeal stimulation were compared with those evoked before pharyngeal stimulation, using the GLM repeated measures ANOVA in SPSS. A Greenhouse-Giesser correction for degrees of freedom was applied when required. Values were expressed in terms of percentage change compared to baseline and shown (data and figures) as mean $\pm$ standard error of the mean (SEM) unless otherwise stated. Only significant main effects and interactions are reported.

fMRI

To decrease the likelihood of detecting spurious activations in the group mean activation maps, a threshold of $p = 0.001$ (uncorrected for multiple comparisons) was used, with a cluster size threshold of 4 voxels (cf. Forman et al., 1995). The peak Z score and the number of voxels with a Z score above 3 after pharyngeal stimulation were then compared to the sham-stimulation control for each subject using the paired student t test.

VFS

The individual mean responses for the swallowing measures (PTT, SRT, and ASP) were compared before and 60 minutes after pharyngeal stimulation using the Wilcoxon signed ranks test. Values were expressed in terms of percentage change compared to baseline and shown as mean $\pm$ SEM. Finally, the percent aspiration improvement was correlated with change total hemispheric excitability and map size using a Spearman's rank correlation coefficient.

For all studies, a p value of <0.05 was used to indicate statistical significance.

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