Benign essential blepharospasm (BEB) is a focal cranial dystonia affecting eye closure. Here, we tested the hypothesis that BEB is associated with abnormal plasticity of the neuronal circuits mediating reflex blinks. In patients with BEB and healthy age-matched controls, we used the conditioning protocol introduced by Mao and Evinger (2001) to induce long-term potentiation (LTP)-like plasticity in trigeminal wide dynamic range neurons of the blink reflex circuit. High-frequency trains of electrical stimuli were repeatedly given over the right supraorbital nerve (SO) and timed to coincide with the R2 response elicited by a preceding SO stimulus. High-frequency stimulation (HFS) resulted in a long-lasting and input-specific potentiation of the R2 response in both groups, yet the facilitation of the R2 response was markedly increased in patients relative to controls. Botulinum toxin (BTX) injections in both orbicularis oculi muscles normalized the previously enhanced LTP-like plasticity of the R2 response. The increased responsiveness to HFS provides first-time evidence that LTP-like plasticity is increased in the trigeminal reflex circuit of patients affected by BEB. The results also show that the enhanced modifiability is not fixed in BEB, because BTX injections can transiently restore normal LTP-like plasticity. We propose that an abnormal corneal input induced by excessive blinking exacerbates increased LTP-like plasticity in BEB. BTX treatment removes the latter and restores plasticity toward normal values. Our results support the concept that maladaptive reorganization contributes to the pathophysiology of focal dystonias.

Key words: blepharospasm; blink reflex; focal dystonia; long-term potentiation; LTP; maladaptive plasticity; basal ganglia

Introduction

Benign essential blepharospasm (BEB) is a focal dystonia characterized by sustained, involuntary blinking and closure of the eyelids typically caused by spasms of the orbicularis oculi (OO) muscles (Fahn, 1988). Although the pathophysiology of BEB is still unknown, several recent studies of the blink reflex have given some important clues about the nature of this condition (Hallett, 2002; Rothwell and Huang, 2003). Affected individuals show a normal R1 and R2 response to single-pulse stimulation of the supraorbital nerve (SO), but paired-pulse stimulation reveals a shortening of the recovery cycle of the R2 response (Berardelli et al., 1985; Tolosa et al., 1988; Pauletti et al., 1993). Schicatano et al. (1997) introduced an animal model of BEB in which symptoms occurred after coupling subclinical depletion of dopamine (DA) in the basal ganglia with weakening of the OO muscles. They proposed that DA loss exaggerated the usual adaptive increase in the gain of the blink reflex that follows weakening of the OO. This results in excessive blinking and eventual spontaneous eyelid closure typical of BEB. It has been proposed that a similar pre-existing abnormality in plasticity of the blink reflex circuits explains why some patients develop blepharospasm-like symptoms on the side contralateral to facial nerve palsy (Chuke et al., 1996). Supporting this view, paired-pulse inhibition of the blink reflex was shown to be attenuated in patients with residual facial weakness but not in patients who fully recovered facial strength (Syed et al., 1999).

In healthy individuals, Mao and Evinger (2001) demonstrated that repetitive bursts of high-frequency stimulation (HFS) to the SO can facilitate or attenuate the R2 response of the blink reflex, if HFS is appropriately paired with movement feedback from the eyelid (Mao and Evinger, 2001). A long-lasting facilitation of reflex blinks was observed if HFS coincided with the reflex blink (Mao and Evinger, 2001). Conversely, HFS provoked a long-lasting suppression of subsequent reflex blinks when HFS preceded the reflex blink. No consistent modulation of the blink reflex occurred when HFS was given immediately after the reflex blink. The conditioning effect was always restricted to the trigeminal afferents conditioned by HFS. The characteristics of the after effects suggest that supraorbital HFS induced long-term potentiation (LTP) and long-term depression-like associative plasticity in the wide dynamic range neurons belonging to the blink reflex circuit (Mao and Evinger, 2001). These adaptive changes in the size of the blink reflex allow for dynamic adjustment of the gain of...
reflex blinks and thus help to maintain an appropriate relationship between sensory input (i.e., feedback from the eyelid movement) and motor output (i.e., blink amplitude) (Mao and Evinger, 2001).

The paradigm introduced by Mao and Evinger (2001) provides a unique opportunity to study plasticity-related phenomena in cranial dystonias. Here, we used the facilitatory HFS protocol to induce LTP-like plasticity in the blink reflex circuit in patients with BEB. Our prediction was that the blink reflex circuits would be more responsive to the facilitatory HFS protocol in patients with BEB compared with healthy controls.

Materials and Methods

Participants. Sixteen patients (nine females; 64.1 ± 8.6 years of age) with BEB and 11 normal subjects (five females; 59.2 ± 12.3 years of age) participated in the study. All participants gave their written informed consent, and experimental procedures were approved by the local Ethics Committee. The clinical details of the patients are summarized in Table 1. The severity of dystonia was assessed using the Marsden–Schachter Scale, severity factors, items for BEB before the experiment (Burke et al., 1981). Last treatment with botulinum toxin Before BTX After BTX

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Duration of disease (years)</th>
<th>Marsten–Schachter scale (1981)</th>
<th>Last treatment with botulinum toxin</th>
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</table>

f, female; M, male.

Table 1. Clinical details of patients with BEB

The paradigm introduced by Mao and Evinger (2001) provides a unique opportunity to study plasticity-related phenomena in cranial dystonias. Here, we used the facilitatory HFS protocol to induce LTP-like plasticity in the blink reflex circuit in patients with BEB. Our prediction was that the blink reflex circuits would be more responsive to the facilitatory HFS protocol in patients with BEB compared with healthy controls.

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and left OO muscles. Using the area of the R2 response as a dependent variable, the effects of HFS conditioning were evaluated using ANOVA for repeated measurements. We computed an ANOVA with time (four levels, B, T0, T30, and T60) and muscle (two levels, right vs left OO muscle) as within-subject factor and group (two levels, patients vs controls) as between-subjects factor.

In patients, the effects of botulinum toxin treatment on blink reflex potentiation was tested performing a separate ANOVA with time (four levels, B, T0, T30, and T60), treatment (two levels, before and after BTX), and muscle (two levels, right vs left OO muscle) as within-subject factors.

In healthy controls, the effects of stimulus intensity and muscular preactivation on HFS conditioning were assessed in separate ANOVA using the relative change in R2 amplitude as the dependent variable. To test the effects of stimulus intensity, we performed an ANOVA, which tested the intensity of SO stimulation (two levels, 2 TR2 or 3 TR2, threshold intensity for SO stimulation) and muscle (two levels, right vs left OO muscle) as the within-subject factor. The effects of background activity in the OO muscle on the amount of R2 facilitation were assessed computing an ANOVA with type of preactivation (three levels, no preactivation, continuous preactivation, and intermittent preactivation) and muscle (two levels, right vs left OO muscle) as the within-subject factor.

The Greenhouse–Geisser method was used if necessary to correct for nonphericity. Conditional on a significant F value, post hoc paired-sample t tests were used to explore the strength of main effects and the patterns of interaction between experimental factors. A post hoc Tukey’s honest significant difference test was performed to explore the strength of main effects and the patterns of interaction between experimental factors. A p value of < 0.05 was considered significant. All data are given as mean ± SEM.

Results

Subjects did not report any adverse side effects during the course of the study. Moreover, patients did not notice any significant effect of HFS in blepharospasm intensity and frequency.

The mean intensity used for stimulation of the right SO nerve did not differ between patients (12.5 ± 6.3 mA) and controls (11.5 ± 5.3 mA). There was also no between-groups difference in the size of the R2 response at baseline (right OO muscle: patients, 84.9 ± 21 μV/s; controls, 119 ± 29.8 μV/s; p = 0.1; left OO muscle: patients, 60.3 ± 7.5 μV/s; controls, 80 ± 12 μV/s; p = 0.08).

In both groups, HFS conditioning induced a bilateral increase in area of the R2 response when the blink reflex was evoked by electrical stimulation of the treated (right) SO nerve (Fig. 1). This was reflected by a main effect of time (F(3,78) = 17.3; p < 0.001) in the ANOVA. However, HFS conditioning induced a stronger increase in R2 area in patients relative to healthy controls. In healthy controls, there was a mean increase in R2 area of ~ 20% (range, 5–50%), whereas patients showed a mean R2 facilitation of ~ 60% (range, 15–400%) relative to baseline values (Fig. 1). This differential effect of HFS conditioning was confirmed by a time by group interaction (F(3,78) = 5.37; p = 0.002). ANOVA revealed no main effect of side and no interaction between side and the other factors, indicating that HFS produced a symmetrical potentiation of the R2 area in both OO muscles.

In separate follow-up ANOVAs, we explored the effects of HFS conditioning for each group. The ANOVA model included the factors time (four levels; baseline and T0, T30, and T60) and side (two levels; right vs left OO muscle) as within-subject factors. For both groups, ANOVA demonstrated a main effect of time (patients: F(3,45) = 16.42; p < 0.00001; controls: F(3,33) = 6.01; p = 0.002), but there was neither a main effect of side nor a time by side interaction. Pair-wise comparisons showed that in both groups, the R2 response was consistently increased at T0, T30 and T60 relative to baseline (p < 0.01).

In eight patients and eight healthy controls, we additionally assessed the size of bilateral R2 responses elicited by electrical stimulation of the untreated (left) SO nerve before and after HFS conditioning of the right SO nerve. There was no main effect of time, side, and group and no interaction between these factors, indicating that HFS of the right SO nerve did not modify R2 responses after the stimulation of the untreated side (Fig. 2).

Eight patients received the same conditioning HFS 4 weeks after BTX treatment. BTX injections produced no significant change in R2 area (right OO muscle: before BTX injections, 99 ± 24 μV/s; after BTX injections, 75 ± 7 μV/s; p = 0.3; left OO muscle: before BTX injections, 58 ± 10 μV/s; after BTX injections, 55 ± 21 μV/s; p = 0.78), but BTX treatment resulted in a marked attenuation of HFS-induced facilitation of the R2 area that was paralleled by significant clinical improvement. Indeed, BEB patients showed significant improvement after BTX injection (Marsden and Schachter Scale score before BTX, median 2; after BTX, median 1; Wilcoxon test, p < 0.002) (Fig. 3, Table 1).

This was confirmed by the ANOVA, which showed a significant time by treatment interaction (F(3,21) = 4.02; p = 0.02). We computed an additional ANOVA to compare R2 measurements...
after BTX injections in patients with R2 measurements of eight age-matched healthy controls. This ANOVA showed no time by group interaction (F(3,42) = 0.43; p = 0.7), indicating a normal amount of R2 facilitation in BTX-treated patients.

In five healthy controls, electrical stimulation of the right SO nerve was used to elicit an R2 response before and immediately after HFS conditioning. HFS conditioning at an intensity of 2 TR2 or 3 TR2, whereas an intensity of 2 TR2 was always used for preconditioning and postconditioning measurements of the R2 response. Response size was measured bilaterally. An SO stimulus at 3 TR2 resulted in a stronger R2 response compared with an SO stimulus at 2 TR2 (mean R2 amplitude at 2 TR2, 0.15 ± 0.05; mean R2 amplitude at 3 TR2, 0.28 ± 0.01; t(1,8) = -3.3; p = 0.01). An increase in stimulus intensity to 3 TR2 did not enhance the conditioning effect of HFS in healthy controls (Fig. 4). Accordingly, ANOVA showed no main effect of stimulus intensity and no interaction between stimulus intensity and muscle.

In five healthy controls, we determined the effect of continuous or intermittent preactivation on the amount of R2 facilitation induced by HFS. Preactivation of the OO muscles during HFS conditioning did not increase the conditioning effect of HFS (Fig. 5). A two-factorial ANOVA showed no effect of preactivation and no interaction between preactivation and muscle.

Discussion

Confirming the study by Mao and Evinger (2001), HFS conditioning of the SO nerve produced long-lasting facilitation of the trigeminal blink reflex if HFS was appropriately timed to coincide with an electrically induced R2 response. The facilitatory effect on reflex blinks evolved rapidly and was stable for a minimum of 1 h. The after effect was topographically specific, because only eye blinks evoked by supraorbital stimulation on the treated side were facilitated. These features are compatible with LTP-like plasticity as suggested by Mao and Evinger (2001).

In patients with BEB and healthy controls, HFS conditioning of the right SO produced similar conditioning effects in terms of the direction, time course, and input specificity. The critical new finding was that untreated patients with BEB showed a stronger potentiation of the blink reflex than healthy controls. This is, to our knowledge, the first demonstration that BEB is associated with an increased modifiability of trigeminally evoked reflex blinks.

The increased response to HFS conditioning cannot be as-
cribed to stronger reflex blinks in BEB patients relative to healthy subjects. First, the area of the R2 response did not differ between patients and healthy controls. This implies that there was no difference in movement feedback between groups at the time of HFS conditioning. Second, in five healthy volunteers, HFS conditioning at an intensity of 3 T$_{R2}$ did not increase the amount of blink potentiation compared with HFS at an intensity of 2 T$_{R2}$.

Another possible explanation relates to the fact that patients with cranial dystonia have an increased background contraction associated with involuntary movements in facial muscles. Thus, it is conceivable that the greater plasticity of the blink reflex after HFS in BEB was caused by the increased background contraction of the OO muscle. However, this is unlikely because in an additional control experiment performed in healthy subjects, we showed that preactivation of the OO muscles did not affect the degree of R2 facilitation after HFS conditioning.

The enhanced modifiability of the R2 response in patients with BEB is consistent with an increasing body of research suggesting that focal task-specific dystonias of the hand result from aberrant sensorimotor plasticity (Berardelli et al., 1998; Hallett, 1998; Quartarone et al., 2003, 2005). In monkeys, rapid, repetitive, stereotypical movements in a learning context can actively degrade the cortical representations of sensory information that guide fine hand movements (Byl et al., 1996). In patients with writer’s cramp, a focal hand dystonia affecting handwriting, several recent studies have shown an enhanced responsiveness of the motor cortex to the conditioning effects of transcranial cortex stimulation (Hallett, 2002; Rothwell and Huang, 2003; Siebner et al., 2003). The recent study by Quartarone et al. (2003) on patients with writer’s cramp has particular parallels with the present work. They used a sensorimotor conditioning protocol in which low-frequency stimulation of the right median nerve was followed by suprathreshold transcranial magnetic stimulation of the contralateral hand area at an interstimulus interval of 25 ms [paired associative stimulation (PAS)]. In healthy volunteers, this PAS protocol induces LTP-like increases in the amplitude of the motor-evoked potential elicited by transcranial magnetic stimulation (Stefan et al., 2002; Quartarone et al., 2003; Wolters et al., 2003). In an analogy to the present study, Quartarone et al. (2003) reported that facilitation was stronger in patients with writer’s cramp (Quartarone et al., 2003). We speculate that enhanced modifiability of sensorimotor circuits may be characteristic of many types focal dystonia, perhaps at many different levels of the CNS.

It remains to be clarified which mechanism drives the enhanced response to HFS conditioning in patients with BEB. As initially proposed by Schicatano et al. (1997) in their animal model of BEB, endogenous factors such as a latent dysfunction of the basal ganglia may create a permissive environment for mal-adaptive plasticity to occur (Hallett, 1998). Small adjustments of the blink to changes in sensory input that occur in healthy subjects could become magnified in this environment and trigger symptoms of increased blinking. Indeed, if excess blinking induces additional irritation of the cornea, this could lead to a vicious circle of additional increases in blink gain and eventual appearance of spontaneous eyelid closure.

BTX treatment normalized the modifiability of the blink reflex in BEB, such that HFS conditioning potentiated the blink reflex to the same extent as in healthy subjects. At first sight, this normalization of blink “plasticity” seems at odds with previous work showing that BTX fails to reduce the heightened excitability of the blink in BEB as reflected in reduced prepulse inhibition and the enhanced recovery cycle of the R2 component (Girlanda et al., 1996). However, this may be related to the fact that two types of adaptation occur in the blink reflex circuit. Schicatano et al. (2002) examined the adaptive changes of reflex blinks during and shortly after a 2 h period of unilateral restraint of the upper lid in healthy subjects and showed that there were two distinct types of motor adaptation driven by two distinct error signals. In one, the difference between the actual and the planned blink magnitude created a proprioceptive error signal that drove an adaptive increase in excitability of ipsilateral motoneurons controlling the restrained eyelid. This led to increased blinks in the ipsilateral eye after stimulation of ipsilateral or contralateral SO nerve. In the second, a reduced blink amplitude caused corneal irritation. The corneal error signal produced an increased excitability of trigeminal reflex blink circuits and bilaterally enhanced blinks after stimulation of the ipsilateral but not contralateral SO nerve.

Mao and Evinger (2001) proposed that the modification of the blink reflex by HFS was caused by long-term changes in the responsiveness to inputs from the SO nerve of wide dynamic range (WDR) neurons in the trigeminal complex. We suggest that there is an endogenous increase in the plasticity of this circuit in BEB. However, increased blinking in BEB may itself reinforce this tendency by irritating the cornea and causing increased corneal input to the WDR neurons, making them more susceptible to conditioning with HFS. Treatment with BTX reduces blinking, reduces the corneal error signal and hence restores plasticity of the trigeminal complex toward normal values. However, at the same time, muscle weakness leads to an increase in the proprioceptive error signal, because each blink causes less movement than expected from a given motor output. This increased error feeds into the ipsilateral motoneurons and maintains the amplitude of the blink reflex after BTX despite the reduced plasticity of the circuit as demonstrated by HFS.

References


