

The dynamic regulation of cortical excitability is altered in episodic ataxia type 2

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Episodic ataxia type 2 and familial hemiplegic migraine are two rare hereditary disorders that are linked to dysfunctional ion channels and are characterized clinically by paroxysmal neurological symptoms. Impaired regulation of cerebral excitability is thought to play a role in the occurrence of these paroxysms, but the underlying mechanisms are poorly understood. Normal ion channels are crucial for coordinating neuronal firing in response to facilitatory input. Thus, we hypothesized that channel dysfunction in episodic ataxia type 2 and familial hemiplegic migraine may impair the ability to adjust cerebral excitability after facilitatory events. We tested this hypothesis in patients with episodic ataxia type 2 ($n=6$), patients with familial hemiplegic migraine ($n=7$) and healthy controls ($n=13$). All subjects received a high-frequency burst (10 pulses at 20 Hz) of transcranial magnetic stimulation to transiently increase the excitability of the motor cortex. Acute burst-induced excitability changes were probed at 50, 250, 500 and 1000 ms after the end of the burst. This was done using single-pulse transcranial magnetic stimulation to assess corticospinal excitability, and paired-pulse transcranial magnetic stimulation at an interstimulus interval of 2 and 10 ms to assess intracortical inhibition and facilitation, respectively. The time course of burst-induced excitability changes differed between groups. Healthy controls showed a short-lived increase in excitability that was only present 50 ms after the burst. In contrast, patients with episodic ataxia type 2 showed an abnormally prolonged increase in corticospinal excitability that was still present 250 ms after the transcranial magnetic stimulation burst. Furthermore, while controls showed a decrease in intracortical facilitation during the 1 s period following the transcranial magnetic stimulation burst, patients with episodic ataxia type 2 had increased intracortical facilitation 1000 ms after the burst. Intracortical inhibition was unaltered between groups. Patients with familial hemiplegic migraine were not significantly different from either controls or patients with episodic ataxia type 2. Together, these findings indicate that patients with episodic ataxia type 2 have an excessive increase in motor cortex excitability following a strong facilitatory input. We argue that this deficient control of cortical excitability may set the stage for the emergence of paroxysmal neural dysfunction in this disorder.

Keywords: Familial hemiplegic migraine; episodic ataxia; transcranial magnetic stimulation; cortical excitability; calcium channel

Abbreviations: EA2 = episodic ataxia type 2; TMS = transcranial magnetic stimulation

Introduction

Familial hemiplegic migraine and episodic ataxia type 2 (EA2) are two rare, hereditary paroxysmal disorders that are caused by dysfunctional ion channels. Both the disorders are thought to result from impaired regulation of neuronal excitability, but the underlying mechanisms are unknown. Understanding these mechanisms could provide important insights into the physiological role of these ion channels, and in the neural changes mediating the occurrence of paroxysmal attacks in both disorders (Catterall *et al.*, 2008). Familial hemiplegic migraine is a subtype of migraine with aura, where the aura consists of motor weakness (hemiparesis) (International Headache Society, 2004). EA2 is characterized by spontaneous episodes of ataxia, with many patients also developing interictal nystagmus and progressive ataxia (Jen *et al.*, 2004). Despite these apparent clinical differences, there is also considerable overlap in the symptoms of familial hemiplegic migraine and EA2. For example, many patients with familial hemiplegic migraine show permanent cerebellar signs (Wessman *et al.*, 2007), whereas more than half of the patients with EA2 fulfil the International Headache Society criteria for migraine and some patients with EA2 experience episodes of paresis (Jen *et al.*, 2004).

The pathophysiology of familial hemiplegic migraine and EA2 has been related to specific mutations that give rise to dysfunctional neuronal ion channels. Familial hemiplegic migraine has been linked to three different mutations affecting the function of presynaptic voltage-gated calcium channels (FHM1) (Ophoff *et al.*, 1996), the sodium–potassium pump (FHM2) (Ducros *et al.*, 1997; Gardner *et al.*, 1997) or voltage-gated sodium channels (FHM3) (Dichgans *et al.*, 2005). EA2 is allelic with FHM1 and this mutation also causes dysfunctional voltage-gated calcium channels (Vahedi *et al.*, 1995). Although the exact site of the mutation differs between FHM1 and EA2, an identical mutation in the *CACNA1A* gene can give rise to both EA2 and hemiplegic migraine within one pedigree (Jen *et al.*, 1999). These findings suggest that EA2 and familial hemiplegic migraine share a similar pathophysiological pathway.

To test for *in vivo* effects of ion channel dysfunction on cerebral excitability in patients with EA2 and familial hemiplegic migraine, we used transcranial magnetic stimulation (TMS) to measure corticomotor excitability. TMS is a well recognized method to measure the excitability of corticospinal output neurons and is sensitive to trans-synaptic and intrinsic changes in corticospinal excitability (Kobayashi and Pascual-Leone, 2003). In familial hemiplegic migraine, two previous TMS studies have given conflicting results; no changes were found in one study (Werhahn *et al.*, 2000), whereas the other found increased motor thresholds in patients with familial hemiplegic migraine (van der Kamp *et al.*, 1997). To date there have been no TMS studies that investigated cortical excitability in EA2, so the net effect of this *CACNA1A* mutation on cortical excitability is still unclear.

Here we used TMS to test a novel hypothesis about altered corticomotor excitability in EA2 and familial hemiplegic migraine. In contrast to previous work, we focused on changes in the temporal dynamics of cortical excitability in EA2 and familial hemiplegic migraine. We hypothesized that EA2 and familial hemiplegic migraine are associated with altered responses of cortical neurons to transient excitatory events. This hypothesis is based on evidence that intact neuronal ion channels are essential for dynamically coordinating neuronal firing. Specifically, voltage-gated calcium channels allow calcium to enter the cell, which triggers the release of glutamate into the synaptic cleft, but it also rapidly terminates synaptic transmission, e.g. by acting on calcium-gated potassium channels (Stocker, 2004; Faber and Sah, 2007). Furthermore, calcium channels are important for adapting synaptic strength to the previous history of stimulation (short-term synaptic plasticity), e.g. by inhibiting synaptic transmission after repetitive firing (Xu and Wu, 2005; Kim *et al.*, 2007; Xu *et al.*, 2007; Catterall and Few, 2008). Accordingly, it has been suggested that ion channel dysfunction disturbs the temporal coordination of synaptic transmission in EA2 (Walter *et al.*, 2006) and familial hemiplegic migraine (Pietrobon, 2010). Finally, altered kinetics of mutated ion channels may further contribute to these alterations. For example, slower inactivation of calcium currents after repetitive firing has been observed in cultured neurons with the EA2 (Spacey *et al.*, 2004) and familial hemiplegic migraine mutation (Tottene *et al.*, 2005), which may produce abnormally prolonged synaptic transmission. Together, this leads to our hypothesis that ion channel dysfunction in familial hemiplegic migraine and EA2 may produce altered dynamics of corticomotor excitability after facilitatory input, which could trigger paroxysmal attacks (Wessman *et al.*, 2007). To test this, we applied high-frequency bursts of TMS pulses to transiently increase the excitability of the motor cortex. By probing the time course of burst-induced excitability changes between 50 and 1000 ms after the end of the burst, we could measure and compare the temporal dynamics of cortical excitability across groups. This was done with single and paired-pulse TMS using the same coil through which burst TMS had been applied.

Materials and methods

Subjects

We studied seven patients with familial hemiplegic migraine [one male; age 43.0 ± 10.2 years (mean \pm SD)], six patients with EA2 (three males; age 40.3 ± 17.7 years) and 13 healthy subjects (seven males; mean age 42.2 ± 16.5 years; Table 1). All subjects were right-handed. Age and gender did not significantly differ between groups [age: $F(2,27) = 0.05$; $P = 0.95$; gender: $\chi^2 = 3.1$; $P = 0.21$]. The experiments were performed with the approval of the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery.

Table 1 Clinical characteristics

Code	Age	Gender	Medication	Symptoms						
				Provocation	Major symptom	Other symptoms	Migraine	Site of attacks	Duration	Interictal
EA-01	27	M	FL	SP, EX, ST	AT	DI, VO	Yes, after attack	L>R	>6h	DY, TR
EA-02	22	M	None	SP, EX, ST	AT	DI	No, only headache	B	5–20 min	None
EA-03	57	F	BB, AS	SP, ST	AT	DY, NA, VO, PH, SM	Yes (separate attacks)	B	45 min	None
EA-04	67	M	None	EX	PA	No	Yes (separate attacks)	B	30 s	AT, TR
EA-05	34	F	FL	SP	AT	Coma, VO	Yes, after attack	B	12–13 h	TR
EA-06	35	F	AC	ST	AT, PA	DY	No	L>R	?	AT, NY
FHM-01	50	F	OP, BB, AS	SP, ST	PA	SE, VO	Yes, during attack	R	24–30 h	None
FHM-02	36	M	TRi, AS, AM	SP, ST	PA	SE, AP, VO	Yes, during attack	B	1–4 days	None
FHM-03	61	F	OP, AS	SP	PA	SE, NA, VO, PH, AP	Yes, during attack	R	2–3 days	None
FHM-04	35	F	None	SP, ST	PA	SE, DY, PH	Yes, during attack	R	1–2 days	None
FHM-05	39	F	FL, AC, TRi	SP	PA	SE, AP	Yes (separate attacks)	R	3 days	None
FHM-06	47	F	TO	SP	PA	PH, AP	Yes, after attack	R	<1 week	None
FHM-07	33	F	None	SL	PA	DY, PH, SE	Yes, after attack	L	2 days	None

The clinical features of six patients with episodic ataxia type 2 (EA2) and seven patients with familial hemiplegic migraine (FHM) are listed.

AC = acetazolamide; AP = aphasia; AS = aspirin; AT = ataxia; AM = acetaminophen; BB = beta-blocker; DI = dizziness; DY = dysarthria; EM = eye movement disorder; EX = exercise; FL = flunarizine; HE = head trauma; L = left side of the body; MI = migraine; NA = nausea; NY = nystagmus; OP = opioids; PA = paresis; PH = photophobia; R = right side of the body; SE = sensory symptoms; SL = sleep deprivation; SM = smells; SP = spontaneous; ST = stress; TR = tremor; TRi = triptans; TO = topiramate; VO = vomiting; ? = not available.

Patients with episodic ataxia type 2

All patients with EA2 had at least one first- or second-degree family member with similar attacks. Five patients had ataxic attacks; one patient (EA-04; Table 1) experienced predominantly paretic attacks without headache. Four patients had interictal symptoms, mostly ataxia and tremor. Four patients also experienced migraine, associated with the attack ($n=2$) or present between the attacks ($n=2$). None of the patients had symptoms of myokymia, which is characteristic of EA type 1 (Jen *et al.*, 2007). First onset of symptoms was at an early age of 12.9 ± 11.6 years (mean \pm SD). Two patients did not use any medication. The other patients were measured while on their normal medication (Table 1). Four of the six patients belonged to one family (EA-01 to EA-04; Table 1) which has been described previously (Graves *et al.*, 2008). These patients have a C to G substitution at nucleotide position 5562 in alternatively spliced exon 37A in the gene coding the Cav2.1 α 1-subunit. This mutation results in a premature stop (Y1854X) and as a consequence deletion of the entire intracellular C-terminus of this variant of the Cav2.1 α 1-subunit. The remaining two patients (EA-05 and EA-06) belonged to two different families that were not genetically tested.

Patients with familial hemiplegic migraine

All patients were diagnosed with familial hemiplegic migraine according to the criteria of the International Headache Society (2004). All patients experienced migraine attacks with fully reversible motor weakness (lateralized to one side in six patients) and similar attacks occurred in at least one first- or second-degree family member. Two patients did not use any medication. The other patients were measured while on their normal medication (Table 1). Patients FHM-01 and FHM-02 were sister and brother, patients FHM-03 and FHM-04 were mother and daughter. All patients had other aura symptoms, mostly sensory disturbances. None of the patients had interictal

symptoms. Age at onset was 16.3 ± 8.7 years (mean \pm SD). Genetic testing was not performed.

Experimental design

The experiment was designed to measure the temporal evolution of acute changes in cortical excitability after a short burst of high frequency repetitive TMS (Fig. 1). To do this, corticomotor excitability of the hand area of the contralateral primary motor cortex (M1-HAND) was measured, manipulated with repetitive TMS and then measured again on a trial-by-trial basis. This was done using a figure-of-eight coil that was attached to three TMS stimulators via a specifically designed tri-stim module (Magstim Co., Whitland, Dyfed, UK). The tri-stim module enabled connection to a Magstim Rapid stimulator and two Magstim 200 stimulators to the same figure-of-eight shaped coil placed over the M1-HAND. The Magstim Rapid stimulator was used to generate 10 high-frequency biphasic pulses (pulse width of $\sim 300 \mu$ s) at an inter-pulse interval of 50 ms (i.e. 10-pulse burst at 20 Hz) and intensity of 80% of individual active motor threshold. Active motor threshold was defined as the lowest stimulus intensity at which motor evoked potentials of 150μ V amplitude were elicited in the tonically contracting first dorsal interosseus muscle (10% of maximum voluntary contraction). The two Magstim 200 stimulators were used to probe regional cortical excitability in the stimulated M1-HAND before and after the application of a single high-frequency burst (Fig. 1). The experimental session consisted of 120 trials (separated by 10 s), each trial consisting of one pre-repetitive TMS measurement, one repetitive TMS burst and one post-repetitive TMS measurement. There were two experimental factors. First, we measured changes in burst-induced excitability in three different ways: (i) single pulse TMS to assess corticospinal excitability; (ii) paired pulse TMS with an interstimulus interval of 2 ms (PP-2) to assess short-latency intracortical inhibition and; (iii) paired pulse TMS with an interstimulus interval of 10 ms (PP-10) to assess intracortical facilitation. Short-latency intracortical inhibition and intracortical facilitation were calculated by normalizing the conditioned motor evoked

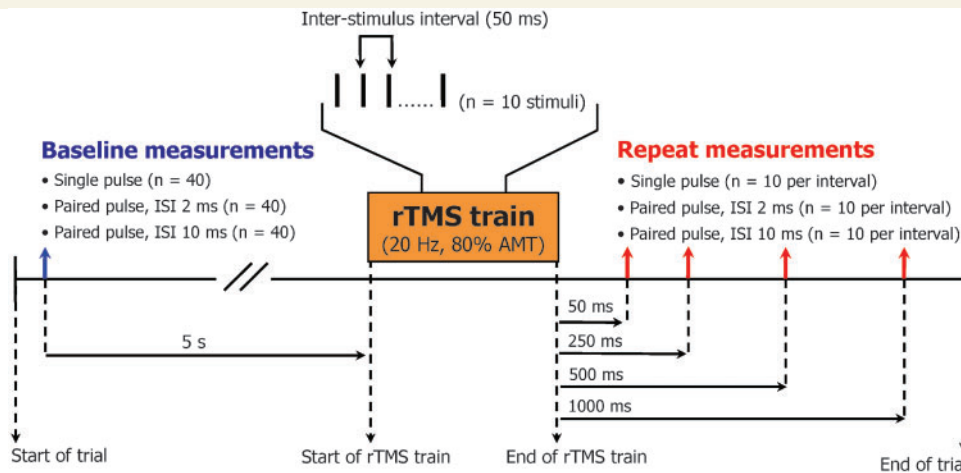


Figure 1 Experimental design. Each trial consisted of one baseline measurement (*left*, in blue), followed by a repetitive TMS (rTMS) train (*centre*, in orange; duration 500 ms) and one post-repetitive TMS measurement (*right*, in red). The baseline measurement consisted of one of the three possible conditions: single pulse TMS, paired-pulse TMS with an inter-stimulus interval (ISI) of 2 ms (PP-2) or 10 ms (PP-10). The post-repetitive TMS measurement consisted of one of the three conditions (single pulse, PP-2 or PP-10) and of one of four delays (50, 250, 500 or 1000 ms). Then there was a delay of 10 s before the next trial started. There were 120 trials in total, which were randomized over conditions for each subject. AMT = active motor threshold.

potential (PP-2 or PP-10) to the single pulse motor evoked potential, as described by Kujirai *et al.* (1993). The intensity of the conditioning stimulus was set to 80% of active motor threshold to avoid floor or ceiling effects. The intensity of the test stimulus was set at a level that, when given alone, would evoke an EMG response of ~ 1 mV peak to peak. Second, we assessed cortical excitability at baseline (i.e. pre-repetitive TMS measurement) and at four different intervals (50, 250, 500 and 1000 ms) after the end of the repetitive TMS burst (i.e. post-repetitive TMS measurement). The pre-repetitive TMS measurement was always performed 5 s before the onset of the repetitive TMS burst. This gave rise to 15 experimental conditions (Fig. 1). The pre- and post-repetitive TMS measurements within one trial always belonged to the same condition, but the order of conditions was randomized across trials.

Experimental procedures

TMS was applied through a standard figure-of-eight shaped coil over M1-HAND. The coil was placed tangentially to the scalp, with the handle pointing backward and laterally at a 45° angle away from the midline, approximately perpendicular to the central sulcus. This orientation was chosen because the lowest motor threshold is achieved when the induced electrical current in the M1-HAND flows approximately perpendicular to the central sulcus (Brasil-Neto *et al.*, 1992; Mills *et al.*, 1992). In controls and patients with EA2, repetitive TMS was always applied over the motor hotspot of the left M1-HAND. In patients with familial hemiplegic migraine, repetitive TMS was applied over the motor hotspot of the most affected hemisphere (i.e. the M1 contralateral to the side with predominant hemiparesis; Table 1). Hence, six patients with familial hemiplegic migraine received TMS over the left M1-HAND and one patient was stimulated over the right M1-HAND. We determined the optimal position for TMS by moving the coil in 0.5 cm steps around the presumed M1-HAND starting from a position with the coil centre 5 cm lateral and 1 cm anterior to the vertex. The site at which stimuli of slightly suprathreshold intensity consistently produced the largest motor

evoked potentials in the contralateral first dorsal interosseus muscle was marked as the 'hotspot'. The coil was held by hand in relation to marks made on the scalp indicating each subject's motor hotspot. After defining the motor hotspot, we determined the individual motor threshold, expressed as a percentage of maximum stimulator output. Resting motor threshold was defined as the minimum stimulus intensity that produced a motor evoked potential of $>50 \mu\text{V}$ in 5 out of 10 consecutive trials.

Electromyography recordings

Motor evoked potentials were recorded with surface EMG using 1-cm-diameter silver chloride disk electrodes placed over the contralateral first dorsal interosseus muscle. The EMG signal was amplified, analogue filtered (32 Hz to 1 kHz) by a Digitimer D150 amplifier (Digitimer Ltd., Welwyn Garden City, Herts, UK), and acquired at a sampling rate of 5 kHz. Data were stored on a personal computer for off-line analysis (Signal software; Cambridge Electronic Devices, Cambridge, UK). During the experiments, EMG activity was continuously monitored with visual (oscilloscope) and auditory (speakers) feedback. Subjects were seated comfortably in a reclining chair and were instructed to relax but to keep their eyes open and fixed on a target directly in front of them.

Statistical analysis: dynamic regulation of repetitive transcranial magnetic stimulation-induced excitability

We tested for alterations in the time course of corticomotor excitability induced by high-frequency repetitive TMS bursts (dynamic regulation of corticomotor excitability). This was achieved in two different ways. First, we compared the time course of corticospinal excitability between groups. Thus, for the single pulse condition, we normalized the different post-repetitive TMS measurements to the pre-repetitive TMS measurement (amplitude ratio of post-repetitive TMS motor

evoked potential to pre-repetitive TMS motor evoked potential). This normalization procedure facilitates a comparison between groups and it was justified by the absence of significant pre-repetitive TMS differences (see below). Using these normalized motor evoked potential values as the dependent variable, we assessed the effects of factors Group (three levels: controls, EA2 and familial hemiplegic migraine) and Time (four levels: 50, 250, 500 and 1000 ms post-repetitive TMS) in a two-way ANOVA. Second, we compared the time course of intracortical facilitation and short-latency intracortical inhibition between groups. Thus, for each paired pulse condition (PP-10 and PP-2), we normalized the paired pulse motor evoked potential to the single pulse motor evoked potential (amplitude ratio), separately for each time point. Using these normalized motor evoked potential values as the dependent variable, for each condition we assessed the effects of factors Group (three levels: controls, EA2 and familial hemiplegic migraine) and Time (five levels: pre-repetitive TMS and 50, 250, 500 and 1000 ms post-repetitive TMS) in a two-way ANOVA. The Greenhouse-Geisser correction was used to correct for non-sphericity when applicable. A $P < 0.05$ was considered significant for all statistical analyses. Conditional upon a significant Group \times Time interaction, we performed *post hoc* two-samples *t*-tests to test for group differences at specific time points.

Statistical analysis: static regulation of corticomotor excitability

We also tested for altered corticomotor excitability without conditioning repetitive TMS bursts (static regulation of corticomotor excitability). This was done in three different ways. First, we compared the size of the resting motor threshold and active motor threshold between groups by performing a two-way ANOVA with factors Group (controls, EA2 and familial hemiplegic migraine) and Threshold (active motor threshold, resting motor threshold). Second, we compared the amount of pre-repetitive TMS intracortical facilitation and short-latency intracortical inhibition (motor evoked potential size normalized to the single pulse condition) between groups by performing a two-way ANOVA with factors Group (three levels: controls, EA2, familial hemiplegic migraine) and TMS condition (two levels: intracortical facilitation and short-latency intracortical inhibition). Third, we compared the size of the absolute pre-repetitive TMS motor evoked potential to single pulse TMS, to PP-10 and to PP-2 between groups by using a two-way ANOVA with factors Group (three levels: controls, EA2 and familial hemiplegic migraine) and TMS condition (three levels: single pulse, PP-10 and PP-2).

Statistical analysis: changes in corticomotor excitability over trials

Finally, we tested for changes in pre-repetitive TMS excitability during the time of measuring. This was done to rule out the possibility that the transient burst-induced effects were driven by gradual changes in baseline excitability. For each condition (single pulse, intracortical facilitation and short-latency intracortical inhibition), we divided the 40 pre-repetitive TMS trials into four bins (i.e. trial 1–10, 11–20, 21–30 and 31–40) and we calculated the average response for each time bin. Then we used these values as the dependent measure in an ANOVA with factors Group (controls, EA2 and familial hemiplegic migraine) and Time (Bin 1, 2, 3 and 4).

Results

Alterations in the dynamic regulation of repetitive TMS-induced corticomotor excitability

We started by assessing the effects of high-frequency repetitive TMS bursts on the time course of corticomotor excitability in healthy controls, patients with EA2 and patients with familial hemiplegic migraine. A two-way ANOVA on single pulse motor evoked potentials revealed a significantly altered time course between the three groups [Group \times Time interaction: $F(6,69) = 2.6$, $P = 0.025$; Fig. 2A]. This effect was driven by prolonged hyperexcitability in patients with EA2, as compared to controls [Group \times Time interaction: $F(3,51) = 3.3$, $P = 0.027$]. *Post hoc t*-tests revealed that patients with EA2 had a significantly higher corticomotor excitability at 250 ms than controls [$t(17) = 2.3$, $P = 0.035$, two-samples *t*-test; Fig. 2A]. Patients with familial hemiplegic migraine were not significantly different from either controls [no Group \times Time interaction: $F(3,54) = 1.8$, $P = 0.16$] or from patients with EA2 [no Group \times Time interaction: $F(3,33) = 2.3$, $P = 0.093$].

Alterations in the dynamic regulation of repetitive TMS-induced intracortical facilitation and short-latency intra-cortical inhibition

We assessed the effect of high-frequency repetitive TMS bursts on intracortical facilitation and short-latency intracortical inhibition. This was done by testing for changes in conditioned motor evoked potentials relative to single pulse motor evoked potentials (amplitude ratio). First, a two-way ANOVA on the amount of intracortical facilitation revealed a significantly altered time course between groups [Group \times Time interaction: $F(8,92) = 2.1$, $P = 0.047$; Fig. 2B]. This effect was mainly driven by a prolonged hyperexcitability in patients with EA2, as compared to controls [Group \times Time interaction: $F(4,68) = 3.4$, $P = 0.013$]. *Post hoc t*-tests revealed that patients with EA2 had a significantly higher intracortical facilitation at 1000 ms post-repetitive TMS, as compared to healthy controls [$t(17) = 2.2$, $P = 0.040$, two-samples *t*-test; Fig. 2B]. Patients with EA2 also had lower intracortical facilitation at 50 ms post-repetitive TMS than controls [$t(14.7) = -2.4$, $P = 0.029$, two-samples *t*-test]. However, since intracortical facilitation is expressed as the amplitude ratio of motor evoked potentials to conditioned versus single pulse TMS, the early (< 500 ms) repetitive TMS-induced changes in EA2 are most likely driven by the increased corticomotor excitability to single pulse TMS at these time points (Fig. 2A). Importantly, these effects did not account for the increased intracortical facilitation at 1000 ms, because at that time point corticomotor excitability to single pulse TMS was unaltered [EA2 versus control patients, $t(17) = 0.40$, $P = 0.70$, two-samples *t*-test; Fig. 2A]. The familial hemiplegic migraine group was not different from either controls [no Group \times Time interaction: $F(4,72) < 1$, $P = 0.68$], or from patients with EA2 [no Group \times Time

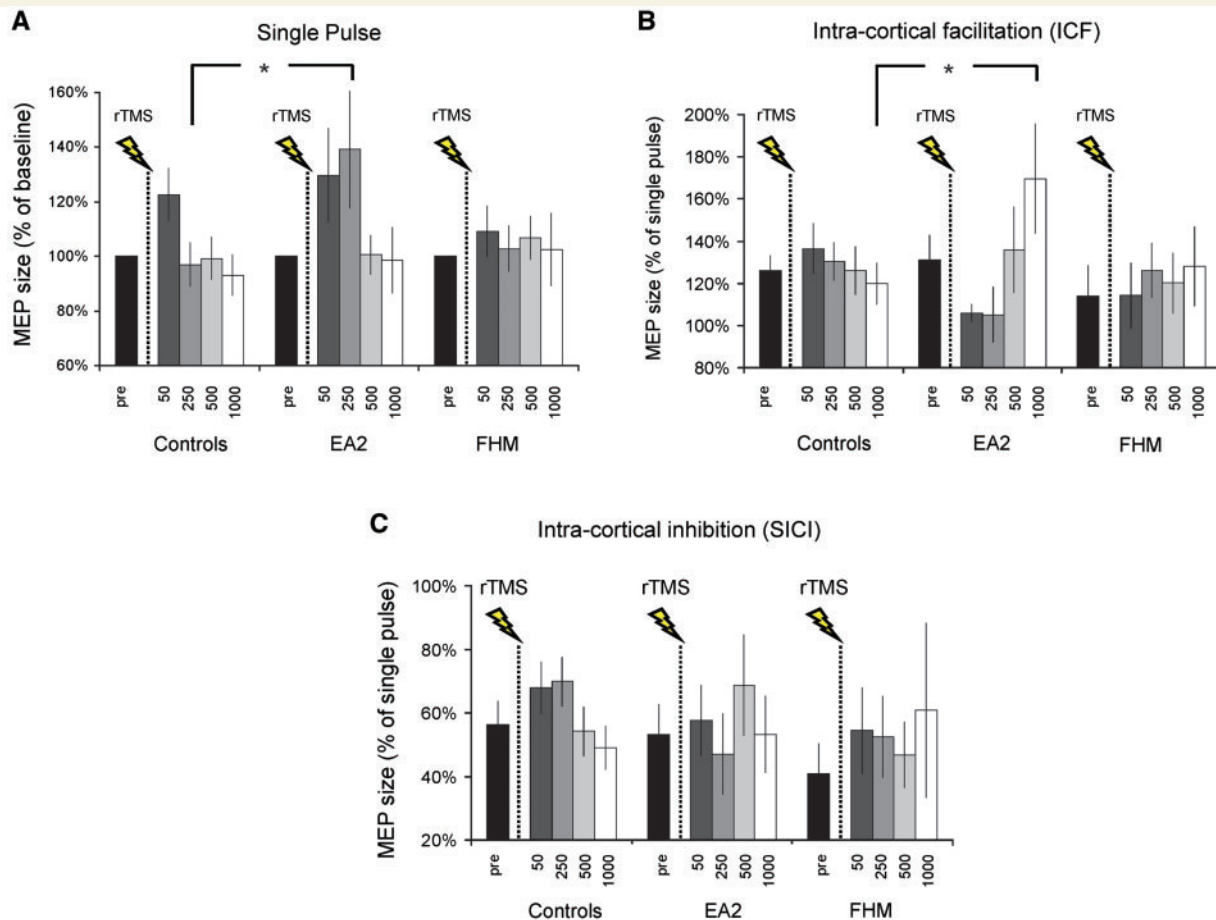


Figure 2 Effect of repetitive TMS conditioning on corticomotor excitability, intra-cortical facilitation and short-latency intra-cortical inhibition. **(A)** The change in motor cortex excitability induced by the repetitive TMS bursts, as measured with single pulse motor evoked potentials (MEP). Motor evoked potential size (on the y-axis) is normalized to the pre-repetitive TMS condition, which is set at 100%. The x-axis shows the time delay since the end of the repetitive TMS (rTMS) burst (in ms), separately for each group. Healthy controls ($n=13$) showed a transient facilitatory effect at 50 ms, which was present for all three conditions (Table 2). Patients with EA2 ($n=6$) showed a significantly prolonged hyperexcitability to single pulse TMS at 250 ms, whereas patients with familial hemiplegic migraine (FHM) ($n=7$) were not significantly different from controls. **(B)** The change in intracortical facilitation (ICF) induced by the repetitive TMS bursts. Motor evoked potential size (on the y-axis) represents the ratio of paired pulse motor evoked potential to single pulse motor evoked potential (which is set at 100%), separately for each time point. Patients with EA2 showed a significantly altered time course of intracortical facilitation, with relatively increased intracortical facilitation at 1000 m post-repetitive TMS (* in panel B indicates a $P < 0.05$ for a two-samples t -test between patients with EA2 and controls). Patients with familial hemiplegic migraine did not show significant changes in intracortical facilitation. **(C)** The change in short-latency intracortical inhibition (SICI) induced by the repetitive TMS bursts. Motor evoked potential size (on the y-axis) represents the ratio of paired pulse motor evoked potential to single pulse motor evoked potential (which is set at 100%), separately for each time point. There were no group differences in short-latency intracortical inhibition.

interaction: $F(4,44)=1.9$, $P=0.13$]. Second, a two-way ANOVA on the amount of short-latency intracortical inhibition showed no significant changes over time [no main effect of Time: $F(4,92) < 1$, $P=0.58$] and there were no differences between groups [no Group \times Time interaction: $F(8,92)=1.6$, $P=0.14$].

Subgroup analysis

Genetic information was available for four patients with EA2, who all had a loss-of-function mutation in the *CACNA1A* gene [these subjects are described in more detail by Graves *et al.* (2008)]. To test whether these patients showed the same

effects as described earlier, we compared this subgroup with the 13 healthy controls. We found that this homogeneous and genetically characterized family of patients with EA2 had the same altered time course of single pulse motor evoked potentials [Group \times Time interaction: $F(3,45)=4.3$, $P=0.009$], the same altered time course of paired pulse motor evoked potentials [PP-10; $F(3,45)=4.1$; $P=0.010$], and the same altered time course of intracortical facilitation [Group \times Time interaction: $F(4,60)=3.0$, $P=0.024$]. This provides more direct evidence for the involvement of deficient voltage-gated P/Q type calcium channels in the dynamic regulation of corticomotor excitability.

Static regulation of corticomotor excitability, intracortical facilitation and short-latency intracortical inhibition

Comparison of the motor thresholds between groups showed that the mean resting motor threshold was consistently higher than mean active motor threshold [main effect of Threshold: $F(1,21)=117.04$, $P < 0.001$], but there was no main effect of Group and no Group \times Threshold interaction (Fig. 3A). Furthermore, comparison of pre-repetitive TMS intracortical facilitation and short-latency intracortical inhibition (expressed as the amplitude ratio of conditioned motor evoked potential to single pulse motor evoked potential) between groups revealed a prominent main effect of TMS condition [$F(1,23)=73.6$, $P < 0.001$; Fig. 3B], but no effect of Group ($P=0.25$) and no interaction between Group and TMS condition ($P=0.92$). The same effect was

observed when we performed statistics on absolute motor evoked potential size (pre-repetitive TMS motor evoked potential to single pulse TMS, PP-2, and PP-10). Specifically, there was a significant effect of TMS condition [$F(2,46)=38.1$, $P < 0.001$; Table 2], but no effect of Group ($P=0.36$) and no interaction between Group and TMS condition ($P=0.37$). *Post hoc t*-tests revealed that motor evoked potentials to PP-2 were significantly smaller than motor evoked potentials to single pulse TMS [$t(25)=7.1$, $P < 0.001$; paired-samples *t*-test], that motor evoked potentials to PP-10 were significantly larger than motor evoked potentials to single pulse TMS [$t(25)=-3.4$, $P=0.002$; paired-samples *t*-test] and that motor evoked potentials to PP-10 were significantly larger than motor evoked potentials to PP-2 [$t(25)=-7.4$, $P < 0.001$; paired-samples *t*-test]. Overall, these findings indicate that the static excitability of intracortical inhibitory and facilitatory circuits was similar between groups.

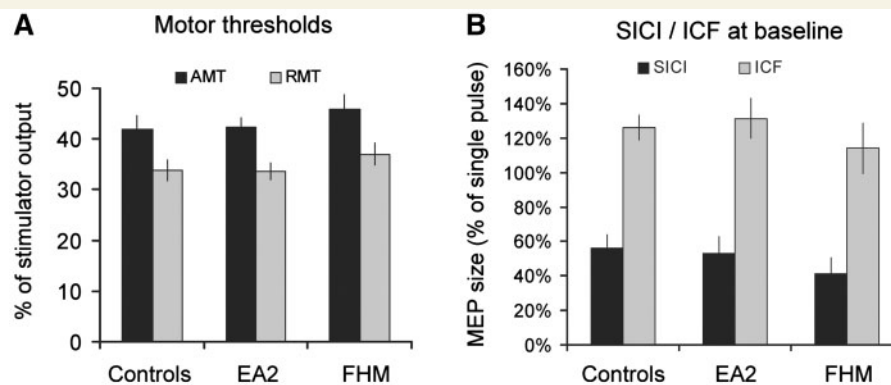


Figure 3 Baseline measurements. (A) Baseline measurements consisted of the active (AMT) and resting (RMT) motor thresholds. Resting motor threshold was always higher than active motor threshold, but there were no differences between groups. (B) Paired-pulse measurements before the repetitive TMS trains showed clear intracortical inhibition (SICI, in black) and intracortical facilitation (ICF, in grey) with respect to single pulse TMS, dependent on the interstimulus interval between conditioning pulse and test pulse (2 ms for short-latency intracortical inhibition, 10 ms for intracortical facilitation). These effects were similar between groups. MEP = motor evoked potential.

Table 2 Absolute motor evoked potential values

Group	Condition	Motor evoked potential size (mV) (mean \pm SEM)				
		Baseline	50	250	500	1000
Controls ($n=13$)	SP	0.98 (0.14)	1.21 (0.20)*	0.97 (0.17)	0.94 (0.14)	0.93 (0.16)
	PP-2	0.55 (0.11)	0.75 (0.13)**	0.64 (0.12)	0.49 (0.09)	0.41 (0.07)
	PP-10	1.25 (0.20)	1.54 (0.24)**	1.21 (0.20)	1.22 (0.22)	1.05 (0.18)
EA2 ($n=6$)	SP	0.68 (0.09)	0.89 (0.18)	0.91 (0.15)	0.68 (0.11)	0.68 (0.14)
	PP-2	0.37 (0.10)	0.44 (0.06)	0.40 (0.11)	0.46 (0.14)	0.35 (0.10)
	PP-10	0.86 (0.09)	0.93 (0.18)	0.93 (0.17)	0.94 (0.21)	1.01 (0.12)
FHM ($n=7$)	SP	1.10 (0.17)	1.24 (0.25)	1.15 (0.23)	1.19 (0.23)	1.11 (0.22)
	PP-2	0.42 (0.13)	0.55 (0.12)*	0.49 (0.11)	0.50 (0.13)	0.50 (0.16)
	PP-10	1.19 (0.18)	1.29 (0.23)	1.41 (0.25)	1.31 (0.19)	1.29 (0.20)

The absolute motor evoked potential values (in mV, mean \pm standard error of the mean; SEM) for all 15 experimental conditions, separately for each group.

FHM = familial hemiplegic migraine; PP-2 = paired pulse TMS with an interstimulus interval of 2 ms (inhibitory); PP-10 = paired pulse TMS with an interstimulus interval of 10 ms (facilitatory); SP = single pulse TMS. The baseline column refers to the pre-repetitive TMS condition; the other columns refer to the time delay (in ms) since the end of the repetitive TMS burst.

*Indicates a significant difference between the baseline motor evoked potential and the post-repetitive TMS motor evoked potential, where * = $P < 0.05$ and ** = $P < 0.01$ (paired-samples *t*-test, two-tailed).

Changes in corticomotor excitability over trials

By separating the trials by 10s, we purposely aimed to minimize gradual changes in corticomotor excitability over trials. It might be argued that the prolonged excitability in patients with familial hemiplegic migraine and EA2 could nevertheless cause a gradual increase in corticomotor excitability during the experiment, which may influence the repetitive TMS effects. We found that the amplitude of motor evoked potentials to single pulse TMS at baseline did not change over the course of four time bins [no effect of Time; $F(3,66)=1.3$, $P=0.28$; no Group \times Time interaction: $F(6,66)=1.1$, $P=0.39$]. There were also no effects for intracortical facilitation [no effect of Time: $F(3,66) < 1$; no Group \times Time interaction: $F(6,66) > 1$] or for short-latency intracortical inhibition [no effect of Time: $F(3,66)=1.2$, $P=0.32$; no Group \times Time interaction: $F(6,66)=1.3$, $P=0.25$]. This indicates that the short-term effects reported here are not confounded by gradual changes in corticomotor excitability over trials.

Discussion

In this study, we tested the hypothesis that EA2 and familial hemiplegic migraine have altered time-varying responses to transient excitatory events. We investigated this by applying high-frequency bursts of TMS pulses to increase transiently the excitability of the motor cortex, and probed the time course of burst-induced excitability changes between 50 and 1000 ms after the end of the burst. There are three main findings. First, patients with EA2 showed a different time course of burst-induced excitability than controls, which was evident as: (i) prolonged corticomotor excitability to single pulse TMS, lasting up to 250 ms after the burst; and (ii) increased intracortical facilitation at 1000 ms after the burst. Second, patients with familial hemiplegic migraine were not significantly different from either controls or patients with EA2. Third, patients with EA2 and familial hemiplegic migraine showed normal excitability at baseline (intracortical facilitation, short-latency intracortical inhibition and motor thresholds), and there were no differences in the time course of short-latency intracortical inhibition. These findings suggest that patients with EA2 are impaired in tuning down facilitatory responses to strong excitatory input. We relate this finding to dysfunctional voltage-gated calcium channels, and propose that our results may explain the occurrence of paroxysmal events in these patients.

Regulation of cortical excitability in healthy subjects

Previous studies in healthy subjects showed that brief repetitive TMS trains can evoke both cortical facilitation (Berardelli *et al.*, 1998) and inhibition (Modugno *et al.*, 2001; Arai *et al.*, 2009), depending on the intensity and duration of stimulation (Modugno *et al.*, 2001), as well as the time of measurement after the repetitive TMS train (Huang and Rothwell, 2004). The effects of longer repetitive TMS trains on cortical excitability have been related to long-term potentiation and depression (Hoffman and Cavus,

2002), possibly mediated by post-synaptic calcium channels (Wankerl *et al.*, 2010). In contrast, the transient (i.e. < 1 s) effects of short repetitive TMS trains follow the same time scale as short-term synaptic plasticity that occurs in the order of milliseconds to minutes (Xu *et al.*, 2007; Catterall and Few, 2008). These effects are most likely mediated in the cortex, given that (i) spinal excitability (measured with H-reflexes) remained unaltered in most studies (Modugno *et al.*, 2001; Huang and Rothwell, 2004; Huang *et al.*, 2005); and (ii) the repetitive TMS bursts were applied at a very low stimulation intensity (80% active motor threshold) that does not directly activate the corticospinal tract. Further support for this idea comes from animal work, where the effect of TMS on single-unit activity in the cat visual cortex was investigated (Moliadze *et al.*, 2003; Allen *et al.*, 2007). Interestingly, the authors found TMS-induced facilitation up to 200 ms after the end of the pulse that was followed by more prolonged cortical inhibition (Moliadze *et al.*, 2003). This temporal pattern is consistent with our findings in healthy subjects.

Regulation of cortical excitability in patients with episodic ataxia type 2

Patients with EA2 showed clear differences in the dynamic regulation of excitability compared to controls, whereas both groups had comparable static regulation of excitability. Whereas controls had only transient increases in burst-induced excitability, patients with EA2 showed abnormally prolonged corticomotor hyperexcitability up to 250 ms (to single pulse TMS), and increased intracortical facilitation at 1000 ms after the burst. We suspect that early changes in intracortical facilitation may have been masked by the early (< 500 ms) and pronounced increase in corticomotor excitability observed in EA2 (Fig. 2A). For this reason, we are careful to make specific inferences about the timing of intracortical facilitation changes. Still, it should be noted that the increased intracortical facilitation at 1000 ms occurred in the context of unaltered responses to single pulse TMS (no differences with respect to baseline at that time point; Fig. 2A). This indicates that the altered intracortical facilitation is not an artefact of increased excitability to single pulse TMS (Florian *et al.*, 2008; Alle *et al.*, 2009).

Although patients with EA2 showed significant changes in intracortical facilitation, there were no group differences in the dynamic regulation of short-latency intracortical inhibition (Fig. 2C). If short-latency intracortical inhibition and intracortical facilitation are mediated by distinct populations of cortical interneurons (Reis *et al.*, 2008), then this implies that EA2 preferentially affects facilitatory intracortical circuits. In a mouse model of FHM1, which is based on a mutation in the same *CACNA1A* gene as EA2, such population-specific effects have been shown. For example, the affected $Ca_v2.1$ Ca^{2+} channel functioned normally in some types of neurons but abnormally in others, depending on the duration of the action potential in the neuron (Inchauspe *et al.*, 2010). Interestingly, another study showed that the FHM1 mutation severely affected excitatory cortical neurotransmission, but left inhibitory neurotransmission at fast spiking interneurons intact (Tottene *et al.*, 2009). If similar mechanisms apply to EA2, then this might explain why alterations were present for

intracortical facilitation, but not for short-latency intracortical inhibition.

The abnormal regulation of excitability in EA2 is probably related to dysfunctional $\text{Ca}_v2.1 \text{Ca}^{2+}$ channels, which is the pathophysiological hallmark of the disease (Pietrobon, 2010). Most previous studies investigated the functional consequences of EA2 mutations at the level of a single channel in a single cell, whereas we focused on net excitability changes in human patients with EA2. Although it is difficult to translate single-cell characteristics into *in vivo* excitability changes, the following mechanisms may account for our results. First, whole cell patch-clamp recordings have shown altered kinetics of the affected $\text{Ca}_v2.1 \text{Ca}^{2+}$ channel in EA2, such that the current decay after repetitive stimulation (at 1 Hz) was slower than for normal $\text{Ca}_v2.1 \text{Ca}^{2+}$ channels. This could lead to prolonged synaptic transmission, which would fit with our findings. Second, the mutated $\text{Ca}_v2.1 \text{Ca}^{2+}$ channel of patients with EA2 is thought to be important for normal short-term synaptic plasticity (Catterall and Few, 2008). Short-term synaptic plasticity shapes the postsynaptic response to bursts of impulses and is crucial for encoding information in neurons (Mochida *et al.*, 2008). For example, calcium-dependent inactivation of the presynaptic calcium current causes rapid synaptic depression for stimuli ranging from 2 to 30 Hz (Xu and Wu 2005). Thus, impairments in short-term synaptic depression may prevent inhibitory responses after a burst of stimuli, which would be consistent with the prolonged hyperexcitability that we found in EA2. Third, the reduced calcium current in EA2 may have downstream effects on ion channels that are activated by intracellular Ca^{2+} , for example calcium-activated potassium channels (K_{Ca}). In a mouse model of EA2, reduced activation of K_{Ca} channels has been linked to irregular firing of cerebellar Purkinje cells, which can produce ataxic attacks (Walter *et al.*, 2006). In the healthy state, activation of K_{Ca} channels limits the firing frequency of neurons by regulating after hyperpolarization following a train of action potentials. This endows the neuron with the ability to self-regulate its activity and to curb excessive excitability (Faber and Sah, 2007). Impaired activation of K_{Ca} channels may thus lead to pathologically prolonged neural activity after facilitatory events. Finally, compensatory reactions to the loss of functional $\text{Ca}_v2.1 \text{Ca}^{2+}$ channels may play a role. For example, single-cell recordings in mouse models of EA2 have shown increased Ca^{2+} sensitivity of the intracellular release machinery (Piedras-Renteria *et al.*, 2004) and increased expression of other Ca^{2+} channel subtypes (i.e. N-type calcium channels; Inchauspe *et al.*, 2004). We propose that these alterations in neurotransmission interfere with the ability of patients with EA2 to regulate the dynamic response to facilitatory input. This could lead to abnormally prolonged neuronal excitability following transient facilitatory events, resulting in paroxysmal attacks that are characteristic for EA2.

Regulation of cortical excitability in patients with familial hemiplegic migraine

In patients with familial hemiplegic migraine, the time course of burst-induced excitability changes was not significantly different

from healthy controls. We attribute this finding to increased variability in the familial hemiplegic migraine group, because this disorder is linked to mutations in three different genes, whereas EA2 is monogenetic (Pietrobon, 2010). Future research may test whether subgroups of patients with FHM1, FHM2 and FHM3 have different dynamic responses to brief facilitatory events. Similar to EA2, the familial hemiplegic migraine group had normal baseline measures of excitability. This finding is consistent with a previous report (Werhahn *et al.*, 2000), but not with another study (van der Kamp *et al.*, 1997). Differences between studies may be explained by different underlying mutations, or by different disease characteristics (e.g. age, disease severity or use of prophylactic medication).

Interpretational issues

A few points are important for interpreting our results. First, on the basis of our results, we cannot draw solid conclusions about the causal relationship between altered cortical excitability and the occurrence of attacks. For instance, it is known that the attacks in familial hemiplegic migraine can leave interictal neurological abnormalities (Wessman *et al.*, 2007), which may be related to structural brain damage caused during the attacks (Hayashi *et al.*, 1998). However, this would predict that patients should also show alterations on baseline measures of corticomotor excitability, which is not what we found. Second, it might be argued that the effects reported here are related to the use of medication by the majority of patients. However, the results of two patients with EA2 and two patients with familial hemiplegic migraine without treatment showed similar changes as the whole group (data not shown). Therefore, we infer that the altered corticomotor excitability in EA2 and familial hemiplegic migraine is not a by-product of medication. Third, different inhibitory mechanisms are thought to be responsible for the intracortical inhibition occurring at different interstimulus intervals (Fisher *et al.*, 2002; Hanajima *et al.*, 2003). Since we tested inhibition only at an interstimulus interval of 2 ms, we may have missed changes in inhibitory circuits mediating intracortical inhibition at other intervals. This could be tested in future research. Fourth, one might argue that the small number of subjects in this study limits our findings. While a larger sample size would certainly be preferable, it is important to note that familial hemiplegic migraine and EA2 are extremely rare disorders. For instance, EA2 has an incidence of less than 1/100 000 based on cases seen in expert centres (Jen *et al.*, 2007), and familial hemiplegic migraine has an estimated prevalence of 1/50 000 based on a population-wide search in Denmark (Lykke *et al.*, 2002). This may explain why to date, no TMS studies have been performed in EA2, and why only two previous TMS studies have been performed in familial hemiplegic migraine (van der Kamp *et al.*, 1997; Werhahn *et al.*, 2000).

Conclusion

Our results support the hypothesis that patients with EA2 have altered time-varying responses to transient increases in corticomotor excitability. We relate these findings to the primary deficit of this

disorder, i.e. dysfunction of voltage-gated calcium channels that regulate synaptic excitability and plasticity in the central nervous system. The abnormally prolonged hyperexcitability observed in EA2 could be a neurophysiological prelude to the occurrence of clinically visible paroxysmal attacks in these patients.

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