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Motor cortex excitability in seizure-free *STX1B* mutation carriers with a history of epilepsy and febrile seizures

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HIGHLIGHTS

- TMS testing of motor cortical excitability in adult *STX1B* mutation carriers with seizure history.
- Resting motor threshold, and MEP, SICI and ICF input-output curves were normal.
- *STX1B* mutation carriers have normal $GABA_A$ergic and glutamatergic motor cortical excitability.

ABSTRACT

Objective: Mutations in *STX1B* encoding the presynaptic protein syntaxin-1B are associated with febrile seizures with or without epilepsy. It is unclear to what extent these mutations are linked to abnormalities of cortical glutamatergic or GABAergic neurotransmission. We explored this question using single- and paired-pulse transcranial magnetic stimulation (TMS) excitability markers.
Methods: We studied nine currently asymptomatic adult *STX1B* mutation carriers with history of epilepsy and febrile seizures, who had been seizure-free for at least eight years without antiepileptic drug treatment, and ten healthy age-matched controls. Resting motor threshold (RMT), and input-output curves of motor evoked potential (MEP) amplitude, short-interval intracortical inhibition (SICI, marker of $GABA_A$ergic excitability) and intracortical facilitation (ICF, marker of glutamatergic excitability) were tested.
Results: RMT, and input-output curves of MEP amplitude, SICI and ICF revealed no significant differences between *STX1B* mutation carriers and healthy controls.
Conclusions: Findings suggest normal motor cortical $GABA_A$ergic and glutamatergic excitability in currently asymptomatic *STX1B* mutation carriers.
Significance: TMS measures of motor cortical excitability show utility in demonstrating normal excitability in adult *STX1B* mutation carriers with history of seizures.

1. Introduction

Febrile seizures affect 2–4% of all children and have been related to strong genetic predisposition (Berg et al., 2013). Recently, mutations of the *STX1B* gene have been identified as a shared genetic mechanism implicated in the pathogenesis of febrile seizures with or without epilepsy (Schubert et al., 2014). *STX1B* encodes the presynaptic protein syntaxin-1B, a significant component of the soluble-N-ethylmaleimide sensitive factor attachment receptor (SNARE) complex, which tethers synaptic vesicles in the presynaptic membrane and mediates the vesicle exocytosis and release of neurotransmitters at the synapse (Kearney, 2015). In a genetic study of families and sporadic patients with fever-associated epilepsy syndromes, whole-exome or targeted sequencing revealed nonsense or missense mutations in *STX1B* predicting either truncated, presumably non-functional, or dysfunctional syntaxin-1B proteins (Schubert et al., 2014). *STX1B* mutations have been associated to a broad spectrum of clinical phenotypes in patients, ranging from simple febrile seizures, afebrile myoclonic-astatic, absence and generalized tonic-clonic seizures with onset in early childhood, to severe epileptiform encephalopathies with

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global developmental delay (Schubert et al., 2014; Vlaskamp et al., 2016). Excitability abnormalities at brain level in individuals with *STX1B* haploinsufficiency have not been investigated so far.

Transcranial magnetic stimulation (TMS) represents a non-invasive technique, which allows investigation of cortical excitability *in vivo*, and has been broadly employed in epilepsy research, as a tool to study underlying pathophysiological mechanisms of cortical hyperexcitability in epilepsy, as well as a way to study the mode of action of antiepileptic drug treatments (Badawy et al., 2014; de Goede et al., 2016; Ziemann et al., 2015, 1998b). Two classical paired-pulse TMS paradigms have been particularly used as indexes of intracortical inhibitory or facilitatory mechanisms, known as short-interval cortical inhibition (SICI) and intracortical facilitation (ICF), respectively (Kujirai et al., 1993; Ziemann et al., 1996c). SICI occurs when a subthreshold conditioning stimulus (CS) precedes a suprathreshold test stimulus (TS) at an interstimulus interval (ISI) of 1–5 ms, and is reflected by an attenuation of the conditioned motor-evoked potentials (MEPs) compared to the unconditioned test MEPs (Kujirai et al., 1993; Ziemann et al., 1996c). The putative mechanism underlying SICI is that the CS activates low-threshold inhibitory interneurons, thereby leading to an attenuation of the generated action potentials in target corticospinal neurons (Di Lazzaro et al., 1998; Ilic et al., 2002). At the synapse level, SICI is thought to reflect γ-aminobutyric acid type A receptor ($GABA_AR$)-mediated cortical inhibition (Di Lazzaro et al., 2000; Ziemann et al., 1996a). Positive modulators of the $GABA_AR$, such as benzodiazepines, increase SICI (Ziemann et al., 1996a). On the other hand, ICF occurs when a subthreshold CS is applied 10–30 ms before a suprathreshold TS, thereby inducing a potentiation of the conditioned MEPs (Kujirai et al., 1993; Ziemann et al., 1996c). ICF is considered to be the net effect of strong excitatory postsynaptic potentials, mediated by N-methyl-D-aspartate (NMDA) glutamate receptors (Ziemann et al., 1998a), and the weaker tail of $GABA_AR$-mediated inhibitory postsynaptic potentials (Hanajima et al., 1998). Importantly, SICI and ICF can also be affected by alterations of presynaptic neurotransmitter release as indicated by pharmacological studies (Werhahn et al., 1999). Importantly, paired-pulse TMS protocols cannot differentiate between post- and presynaptic mechanisms. This would be possible by triple-pulse TMS protocols that test, for example, presynaptic $GABA_B$ergic autoinhibition of SICI by long-interval intracortical inhibition (for review, (Ni et al., 2011)).

SICI and ICF have been repeatedly assessed as measures of cortical excitability and are conceived as promising diagnostic markers in epilepsy, where hyperexcitability of cortical networks and hypersynchronicity are common pathophysiological mechanisms (Badawy et al., 2014; Bauer et al., 2014; de Goede et al., 2016).

In this study, we aimed to investigate SICI and ICF in a group of individuals with a history of epilepsy or febrile seizures related to *STX1B* mutations, who had been seizure-free for years without antiepileptic drug treatment and presented no relevant comorbidities. The aim of this study was, to assess, by means of TMS, motor cortical excitability in *STX1B* mutation carriers compared to age-matched healthy controls. We hypothesized a decrease in SICI and/or increase in ICF due to possible abnormalities in their presynaptic GABA and/or glutamate release machinery.

2. Methods

2.1. Patients

Nine individuals (all right-handed, mean age 27.1 ± 14.6 years, 7 women, 2 men) with identified *STX1B* mutations participated in this study, along with ten healthy right-handed volunteers (all right-handed, mean age 28.3 ± 6.6 years, 5 women, 5 men). Six of the investigated mutation carriers (1–6 in Table 1) belonged to the same family pedigree (Fig. 1a), carrying the same *STX1B* in-frame insertion-deletion mutation (c.133_134insGGATGTGCATTG; p.Lys45delinsArgMetCysIleGlu and c.135_136AC>GA; p.Leu46Met) (Schubert et al., 2014), and five of them had a history of infantile or early childhood onset fever-associated epilepsy (Weber et al., 2008). Three mutation carriers (7–9 in Table 1) belonged to a second pedigree (Fig. 1b), with a history of febrile and afebrile seizures, and harbored the same co-segregated *STX1B* mutation c.166C > T (p.Gln56*) (Schubert et al., 2014). In all investigated subjects with history of epileptic seizures, the epilepsy resolved after childhood (while one mutation carrier had never experienced seizures), and all patients remained seizure-free also after withdrawal of antiepileptic drug treatment (Table 1). Thus, according to the new diagnostic criteria for epilepsy (Fisher et al., 2014), eight of the nine studied mutation carriers had no diagnosis of epilepsy at the time of investigation (>10 years seizure-free without medication), and one individual had been seizure-free for eight years and off-medication for six years. None of the *STX1B* carriers presented with a clinically recognizable cognitive decline.

All individuals provided written informed consent, which was obtained according to the Declaration of Helsinki, and the study was approved by the local ethics committee of the University of Tübingen. For the healthy controls, exclusion criteria involved a history of comorbid central nervous system (CNS) disorders and treatment with CNS active drugs. Also, the *STX1B* mutation carriers had no other comorbid medical conditions and were not on any CNS active medication. Details regarding demographic features and clinical data of the *STX1B* group are presented in Table 1.

2.2. Transcranial magnetic stimulation (TMS)

Paired-pulse TMS was applied using a Bistim^2 unit (Magstim Company Ltd., Whitland, Carmarthenshire, UK) with a figure-of-eight magnetic coil (external diameter of each wing, 90 mm). The optimal coil position over the hand area of the left primary motor cortex for eliciting MEPs in the first dorsal interosseous (FDI) or in the abductor pollicis brevis (APB) muscles of the right hand was determined as the site where TMS at a slightly suprathreshold intensity consistently produced the largest MEP amplitudes. Surface electromyogram (EMG) was recorded using Ag-AgCl cup electrodes in a belly-tendon montage. The EMG raw signal was amplified and band-pass filtered (20 Hz to 2 kHz; D360 amplifier, Digitimer, Hertfordshire, UK), and digitized at an A/D rate of 10 kHz per channel (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK). The coil was held tangential to the scalp with the handle pointing backwards and 45° away from the midline, to activate the corticospinal system preferentially transsynaptically via horizontal cortico-cortical connections (Di Lazzaro et al., 2008). The optimal coil position for activating the right-hand FDI or APB muscle was marked with a felt pen on the scalp. Subjects were comfortably seated in a chair and maintained a relaxed state throughout the experiment.

Single-pulse TMS was performed to estimate the resting motor threshold (RMT). RMT was determined as the minimum intensity to the nearest 1% of maximum stimulator output (MSO) that elicited MEPs larger than 50 μV peak-to-peak amplitude in at least five out of ten consecutive trials in the relaxed muscle (Rossini et al., 2015). The primary target muscle was the right APB. The right FDI was only selected as target, when RMT for the APB was significantly higher (>5% MSO) compared to FDI (this was the case in 2/10 healthy controls and in none of the *STX1B* mutation carriers) to study the target hand muscle with highest excitability at the APB hot spot. In addition, the intensity to elicit MEPs of approximately 1 mV peak-to-peak amplitude was determined. Furthermore, MEP input-output curves were obtained, based on the