Tremor entrainment by patterned low-frequency stimulation

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High-frequency test stimulation for tremor suppression is a standard procedure for functional target localization during deep brain stimulation. This method does not work in cases where tremor vanishes intraoperatively, for example, due to general anaesthesia or due to an insertional effect. To overcome this difficulty, we developed a stimulation technique that effectively evokes tremor in a well-defined and quantifiable manner. For this, we used patterned low-frequency stimulation (PLFS), i.e. brief high-frequency pulse trains administered at pulse rates similar to neurons’ preferred burst frequency. Unlike periodic single-pulse stimulation, PLFS enables one to convey effective and considerably greater integral charge densities without violation of safety requirements. In a computational investigation of an oscillatory neuronal network temporarily rendered inactive, we found that PLFS evokes synchronized activity, phase locked to the stimulus. While a stronger increase in the amount of synchrony in the neuronal population requires higher stimulus intensities, the portion of synchronously active neurons nevertheless becomes strongly phase locked to PLFS already at weak stimulus intensities. The phase entrainment effect of PLFS turned out to be robust against variations in the stimulation frequency, whereas enhancement of synchrony required precisely tuned stimulation frequencies. We applied PLFS to a patient with spinocerebellar ataxia type 2 (SCA2) with pronounced tremor that disappeared intraoperatively under general anaesthesia. In accordance with our computational results, PLFS evoked tremor, phase locked to the stimulus. In particular, weak PLFS caused low-amplitude, but strongly phase-locked tremor. PLFS test stimulations provided the only functional information about target localization. Optimal target point selection was confirmed by excellent post-operative tremor suppression.

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1. Introduction

Within the past decade, deep brain stimulation (DBS) has become the standard therapy for medically refractory movement disorders such as Parkinson’s disease or essential tremor (Benabid et al. 1991; Blond et al. 1992; Hubble et al. 1996; Factor 1999; Gross & Lozano 2000; Pillon et al. 2000; Ondo et al. 2001; Vesper et al. 2002; Volkmann 2004). Crucial for the therapeutic effect is a proper electrode placement (Starr et al. 1998; Hariz 2000). For optimal target localization, CT and MRI provide anatomical information, whereas functional information is derived from presumed target sites by stimulation and recording techniques (Hutchison et al. 1998; Lemaire et al. 1999; Yoon & Munz 1999). High-frequency stimulation (HFS) at more than 100 Hz is administered to achieve optimal tremor suppression and to evoke characteristic side effects indicating positional errors (Hassler et al. 1960; Gross et al. 2006). Microelectrode recordings allow a more detailed functional mapping by relating single-cell activity monitored in target structures to movements of different body parts or sensory stimulation (Lenz et al. 1988, 1994; Hutchison et al. 1998; Guridi et al. 2000; Rodriguez-Oroz et al. 2001; Abosch et al. 2002; Benazzouz et al. 2002; Hamel et al. 2003; MacMillan et al. 2004; Romanelli et al. 2004; Hurtado et al. 2005; Gross et al. 2006). However, the intraoperative microelectrode recordings lead to an increase in surgical risk of intracranial haemorrhage, whereas the motor outcome (the unified Parkinson’s disease rating scale) is not improved (Hariz & Fodstad 1999; Gross et al. 2006).

HFS and microrecordings can only be used in awake patients. The quality of microelectrode recordings is affected by sedative medication, even in small doses (Hutchison & Lozano 2000). Furthermore, under general anaesthesia, tremor vanishes, so that no test stimulations can be performed (Maltête et al. 2004; see also Krauss et al. 1996; Böhmdorfer et al. 2003; Burton et al. 2004). Accordingly, the precision of depth electrode implantation under general anaesthesia compared with local anaesthesia is worse (Maltête et al. 2004). However, even in awake patients, intraoperative high-frequency test stimulation for tremor suppression may be hindered or rendered impossible in cases where tremor temporarily decreases or vanishes after insertion of the (chronically implanted) macroelectrode. The latter may cause an oedema, so that an insertional effect may rapidly appear within minutes and last for days (Yousif & Liu 2007).

Tremor induction via electrical low-frequency stimulation was discovered some decades ago. During stereotaxic interventions in awake patients, Hassler et al. (1960) observed that tremor could be reinforced by periodically delivering single pulses at 4–8 Hz in the pallidum and the thalamus. However, the stimulation effect strongly depended on the initial motor situation and required the use of rather strong stimuli, which at least partially violated present safety requirements (see below). Anodal cortical stimulation of a Parkinsonian patient resulted in tremor induction if applied with a frequency close to the normal tremor frequency, whereas thalamic stimulation in patients without motor dysfunctions induced tremor of the contralateral arm (Alberts 1972). Repetitive transcranial magnetic
stimulation of the motor cortex at frequencies between 10 and 30 Hz has the potential to induce tremor very similar to cerebellar tremor (Topka et al. 1999). Electrical low-frequency stimulation of the thalamic sensory nucleus resulted in a pronounced tremor induction in two patients suffering from a complex regional facial pain syndrome (Constantoyannis et al. 2004). Bejjani et al. (2000) studied the effects of low-frequency stimulation in the ventral intermediate (VIM) nucleus of the thalamus in five patients. A stimulation frequency of 5 Hz left the amplitude of the ongoing tremor mostly unaffected, while a tendency for the stimulation to pace the tremor frequency was reported (Bejjani et al. 2000). Higher frequencies (10–70 Hz) mostly caused a more irregular tremor pattern with decreased tremor amplitude (Bejjani et al. 2000). Recently, electrical low-frequency stimulation was used to investigate the role of oscillatory bursting in the MPTP primate model of Parkinsonism (Rivlin-Etzion et al. 2008).

Based on these observations, the purpose of the present study was to develop a stimulation technique that improves target localization under general anaesthesia, by evoking tremor in a reliable and well-defined manner. We hypothesized that evoking tremor under general anaesthesia might require stronger stimuli compared with tremor reinforcement in awake patients. Therefore, it was necessary to use stimuli which in comparison with single pulses, if necessary, enable the delivery of greater charge densities, but nevertheless fulfil safety requirements (Brummer & Turner 1975; Brummer et al. 1983; Harnack et al. 2004), to prevent tissue damage. Accordingly, we replaced the periodic administration of a long single pulse, as used by Hassler et al. (1960), by a patterned low-frequency stimulation (PLFS), where we periodically delivered a brief high-frequency pulse train, each pulse train consisting of a sequence of brief charge-balanced single pulses. A brief high-frequency pulse train is able to safely convey greater amounts of the integral charge density (see below). Also, findings from animal studies (Brozek et al. 1996) indicate that brief high-frequency pulse trains may cause a phase reset of neural oscillations. Furthermore, in theoretical studies of oscillatory networks (Tass 2001, 2003), brief high-frequency pulse trains turned out to have a strong phase resetting effect, comparable with strong single pulses. However, a greater integral charge density does not necessarily imply an improved entrainment effect (i.e. an improved synchronization between stimulus and neuronal activity). Accordingly, first, in a computational study, we analysed the effects of PLFS on the dynamics of a population of mainly inactive FitzHugh–Nagumo (FHN) neurons, the latter serving as a simple model for a network of oscillatory neurons temporarily rendered inactive due to general anaesthesia. The FHN neuron model is derived from the Hodgkin–Huxley equations and retains the properties of physiological interest, i.e. stimulus-induced firing (FitzHugh 1961; Nagumo et al. 1962). At low stimulation intensities, PLFS induced weak synchrony (i.e. coincident firing), which was nevertheless phase locked to PLFS. Only at high stimulation intensities, and if the frequency of PLFS was in the range of the neurons’ preferred frequency, the whole population became strongly synchronized to PLFS. Importantly, PLFS that induced only low-amplitude local field potential (LFP) oscillations nevertheless caused a solid phase entrainment.

Next, we used PLFS as the only functional means that enabled us to verify a target point derived from purely anatomical information (from CT and MRI) in a spinocerebellar ataxia type 2 (SCA2) patient with pronounced tremor, who had to be operated on under general anaesthesia. Intriguingly, although our computational model network was certainly simpler than the patient’s
tremor-generating network, nevertheless in the patient, we observed the same basic qualitative dynamical entrainment features caused by PLFS as in the computational model: weak PLFS evoked low-amplitude tremor phase locked to the stimulus, whereas a pronounced tremor reinforcement required strong PLFS, with integral charge densities exceeding those feasible with periodic single-pulse stimulation. The selected target point was approved by the patient’s unusual clinical benefit (Freund et al. 2007). Our results indicate that PLFS may provide a novel means for target point evaluation under general anaesthesia.

2. Material and methods: theoretical part

(a) Stimulation protocol

Owing to the clinical safety requirements (see below), we used brief high-frequency pulse trains. In oscillatory networks, it has been shown that brief high-frequency pulse trains may cause a reset, which is comparable with that caused by a single strong pulse (Tass 2001). For this, the frequency of the brief high-frequency pulse train has to exceed the principal frequency of the collective oscillation by a factor of 20 or more (Tass 2001). Accordingly, in the theoretical as well as the experimental investigations presented here, we delivered HFS at a low frequency (figure 1a). The term low frequency refers to a frequency in the range of the principal frequency of the pathological dynamics, i.e. the tremor frequency, which is at approximately 5 Hz. By contrast, the term high frequency refers to the rates of the standard DBS above 100 Hz (Benabid et al. 1991). As an abbreviation, HFS administered at a low frequency will be denoted as PLFS.

For our investigations, we introduced the phase of the PLFS. The latter was determined by standard linear interpolation, so that between consecutive onsets of HFS it increases linearly by 2π (figure 1b; Rosenblum et al. 2000). N identical bursts were periodically delivered at onset times \( \tau_1, \tau_2, \ldots, \tau_N \), with constant period \( T=\tau_{k+1}-\tau_k \) (\( T=200 \) ms in figure 1a). Accordingly, the phase of the PLFS reads (figure 1b): \( \phi_{\text{PLFS}}(t)=2\pi(t-\tau_1)/T \).

Figure 1. Schematic and phase of PLFS. (a) PLFS is a stimulation protocol where HFS is delivered at a frequency in the tremor frequency range (here 5 Hz). The frequency of the HFS is greater than 100 Hz (here 130 Hz). Each HFS is a pulse train containing six single pulses. In the schematic, a vertical bar stands for a single cathodic first charge-balanced pulse of 60 μs duration. (b) The phase \( \phi_{\text{PLFS}} \) of the PLFS increases linearly by 2π between consecutive onsets of the HFS.
(b) Mathematical model for a population of FHN neurons

We used the two-dimensional model proposed by FitzHugh and Nagumo (FitzHugh 1961; Nagumo et al. 1962) to model the active membrane properties at the axon initial segment:

\[
\frac{dv}{dt} = \frac{c}{\alpha} \left( w + v - \frac{1}{3} v^3 \right) + \frac{I}{\alpha}, \quad \frac{dw}{dt} = \frac{a - v - bw}{c\alpha},
\]

where \( a, b \) and \( c \) are appropriate positive constants \((a, b, c>0)\); \( t \) denotes time; and \( \alpha \) is a positive time-scaling parameter. The first equation describes the dynamics of the membrane voltage \( v \), whereas the second equation describes the dynamics of a recovery variable \( w \). \( I \) is the current generated by external and synaptic sources. If the accumulated activities of the nearby neurons are large enough, the neuron produces an action potential, which propagates along the axon. The underlying physiological properties are modelled in a more detailed form by the well-known Hodgkin–Huxley equations (Hodgkin & Huxley 1952). Therein, the dynamical properties of the axon hillock are controlled by the sodium and potassium currents and by an additional leakage current. The FHN model is derived from the Hodgkin–Huxley equations \((V, m, h, n)\) by an elimination of one dimension from each of the planes \((V, m)\) and \((h, n)\) by linear projection, where the variable \( V \) denotes the membrane potential of the Hodgkin–Huxley equation and \( m, h, n \) denote the gating variables of the sodium and potassium currents (FitzHugh 1961; Nagumo et al. 1962). The latter variables are a function of the time-varying membrane potential \( V \). The resulting \((v, w)\) plane represents a physiological state diagram for the Hodgkin–Huxley system, and retains the properties of physiological interest (FitzHugh 1961; Nagumo et al. 1962). Thereby, the FHN model is a standard model, which approximates the Hodgkin–Huxley model (Murray 1989).

In the simulations, we used the following parameters: \( a=0.9; b=0.9; \) and \( \alpha=20 \). With this choice, there is a subcritical Hopf bifurcation of the FHN model at \( I^{(1)} = -2.6505 \) and \( I^{(2)} = -1.3495 \). For \( I^{(1)} < I < I^{(2)} \), there exists a stable periodic solution and an unstable stationary solution. For \( I < I^{(1)} \) and \( I^{(2)} < I \), there exists only one stable stationary solution. For \( I^{(1)} < I < I^{(1)} \) and \( I^{(2)} < I < I^{(2)} \), the system is bistable. At \( I = I^{(1)} \) and \( I = I^{(2)} \), a saddle-node bifurcation of periodic orbits takes place. From numerical calculations, we obtain \( I^{(1)} = -2.6969 \) and \( I^{(2)} = -1.3031 \). Hence, the oscillation interval \( I_{osc} \) reads \( I_{osc} = [I^{(1)}, I^{(2)}] \). In other words, for weak external and synaptic inputs, the neurons are silent and rarely show noise-induced single spikes. If the inputs are increased, a narrow bistable region is reached and short transient inputs can induce a switch of the neuronal dynamics between the silent and the tonically firing mode. A further increase in the synaptic or external input results in tonic firing, while too large inputs are reflected by a saturation of the membrane potential without tonic spiking. For an illustration and more detailed discussion of the bifurcation properties of the FHN model, we refer to Giannakopoulos et al. (2001), especially fig. B1 therein.

For the modelling of the synaptic coupling between the neurons, we used a simple representation of the synaptic transmission. The onset and the decay time of the synaptic input were assumed to be identical: \( \tau (du(t)/dt) = -(u(t)/\alpha) + \sum_{\text{connected neurons}} (g/\alpha) g[v(t)] \), where \( u(t) \) denotes the total post-synaptic potential.
of the neuron and \( v(t) \) is the membrane potential of a connected neuron. \( \tau = 40 \text{ ms} \) is a time constant characterizing the dynamical properties of the synapse of interest. The quantity \( q = 1 \) reflects the connection strength between coupled neurons. For positive (negative) \( q \), the synapse is excitatory (inhibitory), respectively. The function \( g \) is increasing, non-negative and bounded and describes the relationship between pre-synaptic potential and post-synaptic reaction. In the simulations, we used \( g[v(t)] = 1/(1 + e^{-4v(t)}) \).

The actual state of the neurons is controlled by the external current \( I \), which is composed of several parts: \( I = e_{\text{individual}} + s(t) + cu(t) + \zeta(t) \), where \( c = 0.05 \) represents a weak synaptic connectivity that does not cause a synchronization in the network and \( e_{\text{individual}} \) stands for constant external currents adjusting the FHN model with respect to the lower bifurcation point. To approximate a situation under general anesthesia in the simulations, the values of \( e_{\text{individual}} \) were Gaussian distributed with mean \(-2.7\) and deviation of \( 1 \) per cent. In the absence of PLFS, this results in a mostly passive network, where only \( 17 \) per cent of the neurons were permanently active (with at least 1000 spikes in 520 s), and 11 per cent of the neurons were temporarily active (with 100–999 spikes in 520 s), whereas the rest showed little or no activity at all.

The stimulation \( s(t) \) was a PLFS (figure 1a). The single pulses were biphasic, charge-balanced, asymmetric pulses with amplitudes between 20 and 80 and length 0.4 ms (length of negative part 1.2 ms). We have chosen rather long pulses in order to prevent the numerical integration from getting stiff. However, also for smaller pulse duration, we obtained the same results. The stimulation strength was dependent on the position of the neuron in the two-dimensional network. The amplitude decayed proportionally to \( e^{-2d} \), where \( d \) is the distance of a neuron from the stimulation electrode that was located in the centre of the quadratic network. \( \zeta(t) \) denotes weak exponentially correlated individual noise: the temporal correlation time of the noise was 20 ms and the amplitude was 0.001. The topological details of the connections within the populations on which most of the current studies of PD focus, basal ganglia and subthalamic nucleus (STN), are poorly understood (e.g. Terman et al. 2002). Accordingly, in a first step, we adopt the simplest case of global homogeneous synaptic strength.

(c) Calculation of the LFP of the population of FHN neurons

In our mathematical model, the LFP was represented by the averaged neuronal activity given by LFP\( (t) = N^{-1} \sum_{j=1}^{N} [v_j(t)]. \)

(d) Calculation of the synchrony of the population of FHN neurons

To assess the amount of synchrony (i.e. in-phase synchronization) and the mean phase of the FHN population, we calculated its order parameter \( R(t) \exp[i \Theta(t)] = N_{\text{active}}^{-1} \sum_{j=1}^{N_{\text{active}}} \exp[i \psi_j(t)] \) (Kuramoto 1984), which is the centroid, i.e. the circular mean of the phases \( \psi_1(t), \psi_2(t), ..., \psi_{N_{\text{active}}}(t) \), of all \( N_{\text{active}} \) active neurons (Batschelet 1981), where the phase of each neuron was determined by the onset of its firing activity. Note that only active neurons are considered for the calculation of the extent of synchrony. We defined the phase of the individual neuron by a standard interpolation (Pinsky & Rinzel 1995; Neiman et al. 1999). The number of neurons was \( N = 100 \). \( R(t) \) and \( \Theta(t) \) are the time-dependent amplitude and phase of the FHN population, respectively.

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In physics, \( R(t) \) is a standard measure for in-phase synchronization: \( R(t) = 1 \) if all oscillators of the population are perfectly synchronized in phase, whereas \( R(t) = 0 \) if there is no in-phase synchronization at all (Kuramoto 1984).

(c) Calculation of the phase entrainment of the population of FHN neurons

To assess the effect of PLFS on the phase dynamics of the FHN population, we quantified the extent of phase entrainment by means of an entrainment index \( E \). The term phase entrainment stands for phase synchronization between periodic force (i.e. PLFS) and driven system in the presence of noise. The entrainment index detects how strongly the phase of the entrained signal gets adapted to the phase of the PLFS. The phase difference between entraining PLFS and entrained signal reads \( \Delta \Phi(t) = \phi_{\text{PLFS}} - \Theta(t) \). To quantify the extent of entrainment, we applied the concept of phase synchronization (Stratonovich 1963; Rosenblum et al. 1996). Based on the basic work of Stratonovich (1963), phase synchronization between two oscillators in the presence of random forces is defined as the appearance of one or more prominent peaks in the distribution of the cyclic phase difference (i.e. the phase difference modulo \( 2\pi \); Tass et al. 1998). The definition of phase synchronization between two oscillators can also directly be applied to quantify the extent of entrainment between a periodic driving force and a noisy and/or chaotic oscillator (Pikovsky 1985; Stone 1992). Accordingly, phase entrainment with PLFS is characterized by the appearance of one or more prominent peaks in the distribution of the cyclic phase difference \( \Delta \Psi(t) = [\Delta \Phi(t)] \mod 2\pi = [\phi_{\text{PLFS}} - \Theta(t)] \mod 2\pi \), where mod stands for modulo (see also Tass et al. 1998). Since we observed only unimodal distributions (i.e. distributions with only one pronounced peak), we used a particularly suitable first Fourier mode-based phase entrainment index, defined by \( E = |M^{-1} \sum_{k=1}^{M} \exp[i \Delta \Psi(t_k)]| \), where \( M \) is the number of data points in the time window considered for analysis and \( t_1, t_2, \ldots, t_M \) are the time points at which the signals are sampled (Batschelet 1981). Perfect phase entrainment in terms of a constant value of the phase difference corresponds to \( E = 1 \), whereas \( E = 0 \) in the absence of phase entrainment. Phase entrainment means that one HF burst of the PLFS is tightly phase locked to one cycle of the LFP.

3. Material and methods: experimental part

(a) Safety requirements for pulsatile stimulation

When pulse trains or single pulses are administered to a patient’s brain, tissue damage may be caused by unbalanced charge density accumulating in close vicinity of the electrodes. In our clinical stimulation protocol, we used only charge-balanced pulses, where each square pulse was charge balanced by a secondary pulse of opposite polarity, administered directly after the primary square pulse. Hence, the unbalanced charge density was maximal at the end of the primary square pulse. The charge density is influenced not only by the amplitude and the duration of a pulse but also by the resistance and the electrode contact surface (Harnak et al. 2004; Kuncel & Grill 2004). To guarantee a safe stimulation, it is highly advisable to fall below the mandatory charge density limit of \( p_q = 30 \mu \text{C cm}^{-2} \), since recent studies show that a spatially non-uniform charge density distribution
might result in a potentially damaging stimulation even if the charge density averaged across the electrode stays below the critical limit \( \rho_q = 30 \mu \text{C cm}^{-2} \) \citep{Kuncel2004, Gimsa2006} and references therein). For this reason, in accordance with Kuncel & Grill (2004), in our study, we have chosen charge densities that fall short of the limit of \( \rho_q = 10 \mu \text{C cm}^{-2} \).

In our SCA2 patient, we used stimulation amplitudes of \( U = 2, 3 \) and \( 4 \) V. The width of the pulses was set to \( d = 60 \mu \text{s} \) (same as in Harnak et al. 2004). In DBS target areas, the resistance \( R \) usually ranges from 500 to 2000 \( \Omega \) \citep{Kuncel2004}. The DBS electrode implanted in our SCA2 patient (Medtronic DBS Lead 3387) has a contact surface of \( F = 0.06 \text{ cm}^2 \). The charge deposited by one pulse is given by the formula \( \rho_q = U d / (R F) \) \citep{Harnak2004}. With \( U = 4 \) V, \( d = 60 \mu \text{s} \), \( R = 500 \Omega \) and \( F = 0.06 \text{ cm}^2 \), we got \( \rho_q = 8 \mu \text{C cm}^{-2} \). Consequently, with these parameters, a single charge-balanced pulse is safe, even under difficult conditions, i.e. at low resistance. By the same token, this holds for a pulse train of consecutive charge-balanced pulses. By contrast, if we replaced \( n \) consecutive charge-balanced pulses, each with duration \( d = 60 \mu \text{s} \), by one long charge-balanced pulse with duration \( n d \), we exceeded the critical charge density limit already for \( n = 4 \), i.e. in the case of a four times longer charge-balanced pulse (critical pulse duration \( d = 225 \mu \text{s} \)). Analogously, for weaker stimulation, at \( U = 3 \) V or \( U = 2 \) V, the pulse duration got critical at 300 \( \mu \text{s} \) or 450 \( \mu \text{s} \). Hence, replacing \( n \) consecutive charge-balanced pulses with duration \( d = 60 \mu \text{s} \) by one long charge-balanced pulse with duration \( n d \), for \( U = 3 \) V or \( U = 2 \) V, the critical charge density limit was exceeded already for \( n = 5 \) or \( n = 8 \), respectively. Note that in our SCA2 patient, we used brief high-frequency pulse trains with \( n = 6 \) single pulses. Hence, periodic single-pulse stimulation with pulse duration \( d = 360 \mu \text{s} \) would have been possible only for a stimulation amplitude of \( U = 2 \) V. By contrast, stimulation at 3 or 4 V would have caused a violation of the safety requirements.

For comparison, Hassler et al. (1960) periodically delivered rather long and strong pulses with duration 500 \( \mu \text{s} \) and amplitude \( U = 4.5 \) V via electrodes with contact surfaces of \( F = 0.0867 \text{ cm}^2 \) or \( F = 0.1621 \text{ cm}^2 \). For a resistance of \( R = 500 \Omega \), this corresponded to a charge density of \( \rho_q = 54.9 \mu \text{C cm}^{-2} \) (small contact) or \( \rho_q = 27.7 \mu \text{C cm}^{-2} \) (large contact) per single pulse. For more details on the study by Hassler et al. (1960), see §5.

(b) SCA2 patient

The 22-year-old patient suffered from an unusually severe truncal, head and extremity tremor that had rapidly progressed during the last half year. The severe intention and postural tremor affected all extremities and was only arrested during sleep. Accordingly, sedation was built up over the last months. Before, a moderate ataxia, dysmetria and dysarthria had developed slowly during the 6 years since disease onset. The diagnosis of SCA2 was established by molecular genetic testing. The patient is described in detail elsewhere \citep{Freund2007}.

Informed consent was given by the parents of the patient who was unable to communicate in any way due to the necessary deep sedation. According to German law, the operation was performed as an individual attempt at healing (‘individueller Heilversuch‘). The study was performed in accordance with the Declaration of Helsinki.
(c) Tremor recording and frequency analysis

To detect whether PLFS induced a peripheral tremor in the SCA2 patient during depth electrode implantation, we monitored the effect of PLFS with accelerometers (ACCs) attached to the left hand, left foot and right hand. To determine whether PLFS and induced tremor were of the same frequency, we determined the time-varying frequency spectrum of the ACC signals with the periodogram technique.

(d) Phase calculation of tremor recordings

We applied our analysis to broadband ACC signals with a frequency range from 0 to 100 Hz. We determined the phase of the broadband ACC signal as well as the phases of its fundamental rhythmic components.

Phase of rhythmic components of ACC signal. To determine the phase of the basic rhythmic components of a broadband ACC signal, denoted as $x(t)$, we first extracted the fundamental frequency components by means of bandpass filters. For this, we used a REMEZ Parks–McClellan optimal equiripple FIR filter with passband edges of $nF \pm 1.5$ Hz and stopband edges of $nF \pm 2.5$ Hz, where $F$ is the (low) frequency of the PLFS and $n$ is an integer ($n=1, 2, ..., 10$). The peak frequencies in the frequency spectrum of $x(t)$ are located at $F$ and integer multiples of it (see below). The band-pass-filtered ACC signal, denoted as $x_{BP}(t)$, was then decomposed into its instantaneous phase $q(t)$ and its instantaneous amplitude $a_{BP}(t)$ by means of the Hilbert transform (Panter 1965), which yields $x_{BP}(t) = a_{BP}(t)\cos[q(t)]$ (for further details, see Rosenblum et al. 1996, 2000; Tass et al. 1998). Note that filtering results in a phase shift of the frequency component of interest. However, we are interested in the strength of phase synchronization rather than the value of the phase difference. We use the phase difference to calculate the phase entrainment index, which is sufficiently robust against such phase shifts.

Phase of broadband ACC signal. In this case, we proceeded without bandpass filtering. Rather we directly applied the Hilbert transform (Panter 1965) to the broadband ACC signal $x(t)$ and obtain $x(t) = a(t)\cos[\varphi(t)]$. Phase detection with the Hilbert transform is well defined for narrow-band signals (Panter 1965). Nevertheless, we here applied this approach also to the broadband ACC signal, in order to demonstrate the robustness of the phenomenon.

(e) Phase entrainment of tremor

The ACC signal of the evoked tremor had frequency peaks at the frequency of PLFS and at higher harmonics. To account for the excitation of higher harmonics, we detected not only 1 : 1 entrainment, but also $n : 1$ phase entrainment. For this, we introduced the $n : 1$ phase difference between entraining PLFS and entrained band-pass-filtered narrow-band ACC signal by $\Delta \Phi_{n,1}(t) = (n\phi_{PLFS} - \theta(t))/(0.5(n+1))$ as well as between entraining PLFS and the original broadband ACC signal by $\Delta \Phi_{n,1}(t) = (n\phi_{PLFS} - \varphi(t))/(0.5(n+1))$. As explained for the numerical signals above, we introduced the corresponding cyclic $n : 1$ phase differences for the narrow-band and broadband signals by $\Delta \Psi_{n,1}(t) = [\Delta \Phi_{n,1}(t)]\mod 2\pi = [(n\phi_{PLFS} - \theta(t))/(0.5(n+1))]\mod 2\pi$ and $\Delta \Psi_{n,1}(t) = [\Delta \Phi_{n,1}(t)]\mod 2\pi = [(n\phi_{PLFS} - \varphi(t))/(0.5(n+1))]\mod 2\pi$. In order not to overload

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the notation, we denote both cyclic $n:1$ phase differences by $\Delta \Psi_{n,1}(t)$ and refer to their narrow-band or broadband origin in the corresponding text. We introduced the $n:1$ phase entrainment index by $E_{n,1} = \left| M^{-1} \times \sum_{k=1}^{M} \exp[i\Delta \Psi_{n,1}(t_k)] \right|$, where $M$ is the number of data points in the time window considered for analysis and $t_1, t_2, \ldots, t_M$ are the time points at which the signals are sampled. Perfect $n:1$ phase entrainment corresponds to $E_{n,1}=1$, whereas $E_{n,1}=0$ in the absence of any $n:1$ phase entrainment. $n:1$ phase entrainment means that one HF burst of the PLFS is tightly phase locked to $n$ cycles of the LFP.

(f) Baseline for phase entrainment

To estimate whether the amount of the $n:1$ phase entrainment of the tremor signal was significant, we determined the prestimulus baseline of the $n:1$ phase entrainment $E_{n,1}^{pre}$ by applying the $n:1$ phase entrainment analysis described above to the ACC signal in a prestimulus window of 10 s length. During the prestimulus baseline epoch, no PLFS was administered, but we performed our analysis by using a phase of the PLFS as if PLFS were delivered at the same
frequency as during the subsequent stimulation epoch. The baseline was used to compensate for effects of bandpass filtering and finite data length.

Quantification of PLFS-induced tremor

To investigate the effect of PLFS on the amplitude of the broadband ACC signal $x(t)$, we calculated its Hilbert transform $x_H(t)$, composed a complex signal $X(t) = x(t) + i z_H(t)$ (Panter 1965), and then determined the average Hilbert amplitude of the ACC signal over the whole period of stimulation with PLFS according to $A = 1/(t_{\text{end}} - t_{\text{begin}}) \int_{t_{\text{begin}}}^{t_{\text{end}}} |X(t)| \, dt$. In addition, to determine a pre-stimulus amplitude, we calculated the average amplitude of the ACC signal in a prestimulus window of length 10 s according to $A_{\text{baseline}} = 1/(t_2 - t_1) \int_{t_1}^{t_2} |X(t)| \, dt$, where $t_2 - t_1 = 10$ s.

Single sweep and mean steady-state ACC responses

We detected the mean steady-state responses according to $\bar{x}(t) = N^{-1} \sum_{k=1}^{N} x(t + \tau_k)$, where $x(t)$ is the ACC signal; $N$ is the number of PLFS epochs; and $\tau_1$, $\tau_2$, ..., $\tau_N$ are the timing points of the PLFS onsets. For illustration, we plotted the single-sweep broadband ACC responses. Furthermore, we determined the minima and maxima of the single-sweep broadband ACC responses, so that we came up with a distribution of the minima and maxima across single sweeps (in addition to simply considering the minima and maxima of the mean responses). For the detection of the extrema, we used a smoothing running average over the five nearest neighbours.

(g) Quantification of PLFS-induced tremor

To investigate the effect of PLFS on the amplitude of the broadband ACC signal $x(t)$, we calculated its Hilbert transform $x_H(t)$, composed a complex signal $X(t) = x(t) + i z_H(t)$ (Panter 1965), and then determined the average Hilbert amplitude of the ACC signal over the whole period of stimulation with PLFS according to $A = 1/(t_{\text{end}} - t_{\text{begin}}) \int_{t_{\text{begin}}}^{t_{\text{end}}} |X(t)| \, dt$. In addition, to determine a pre-stimulus amplitude, we calculated the average amplitude of the ACC signal in a prestimulus window of length 10 s according to $A_{\text{baseline}} = 1/(t_2 - t_1) \int_{t_1}^{t_2} |X(t)| \, dt$, where $t_2 - t_1 = 10$ s.

(h) Single sweep and mean steady-state ACC responses

We detected the mean steady-state responses according to $\bar{x}(t) = N^{-1} \sum_{k=1}^{N} x(t + \tau_k)$, where $x(t)$ is the ACC signal; $N$ is the number of PLFS epochs; and $\tau_1$, $\tau_2$, ..., $\tau_N$ are the timing points of the PLFS onsets. For illustration, we plotted the single-sweep broadband ACC responses. Furthermore, we determined the minima and maxima of the single-sweep broadband ACC responses, so that we came up with a distribution of the minima and maxima across single sweeps (in addition to simply considering the minima and maxima of the mean responses). For the detection of the extrema, we used a smoothing running average over the five nearest neighbours.
4. Results

(a) Effects of PLFS on the LFP of the population of FHN neurons

To mimic a situation under general anaesthesia, we have chosen a neuronal population which showed little activity and only weak synaptic interactions of the FHN neurons, where only a small portion of the FHN population fires at a frequency around 4.7 Hz. Hence, in the absence of stimulation, there was no pronounced synchrony, and the LFP fluctuates close to zero (figure 2a). Accordingly, the network of FHN neurons spontaneously generated only little oscillatory activity around 4.7 Hz, as revealed by the time-varying frequency spectrum of the LFP (figure 2b).

PLFS caused oscillations of the LFP of the neuronal population (figure 2a,b), where the frequency of the LFP oscillations corresponded to the frequency of the PLFS that means the frequency at which the bursts were delivered (figure 1). The amplitude of the LFP oscillations depended on the strength of the stimulation and on the mismatch between the frequency of the PLFS and the neurons’ preferred frequency. For intermediate and high stimulation amplitudes, PLFS at a frequency close to the neurons’ preferred frequency caused high-amplitude oscillations of the LFP (see PLFS epochs at 5 Hz and amplitudes greater than 40; figure 2a,b). By contrast, LFP oscillations caused by PLFS at a frequency that differed from the neurons’ preferred frequency by approximately 25 per cent were considerably weaker (see PLFS epochs at 3.5 Hz, figure 2a,b). The neurons’ weak spontaneous rhythmic activity at approximately 4.7 Hz strongly decreased during PLFS at 5 Hz at weak, intermediate as well as strong stimulation amplitude (figure 2b).

PLFS at a frequency close to the neurons’ preferred frequency resulted in a nearly perfect phase entrainment of the LFP, characterized by a nearly horizontal time course of the phase difference between PLFS and LFP (see PLFS epochs at 5 Hz; figure 2c). The greater the strength of PLFS, the stronger was the entrainment. PLFS at a frequency substantially different from the neurons’ preferred frequency induced a weaker, but still clearly developed phase entrainment, although the amplitude of the induced LFP oscillation was small (see PLFS epochs at 3.5 Hz and amplitude greater than 40; figure 2c). Note the differential effects of PLFS on LFP amplitude and phase entrainment. PLFS that induced only low-amplitude LFP oscillations nevertheless caused a solid phase entrainment.

(b) Phase entrainment and synchrony boosting effects of PLFS

To study how synchrony and phase entrainment of the network of FHN neurons depend on the strength of PLFS, we varied the number of pulses contained in each burst, where the frequency of PLFS was chosen to be 5.5 Hz, i.e. in the vicinity of the neurons’ preferred frequency (figure 3). We have chosen a non-ideal match of PLFS frequency and neuronal frequency, since this is what is to be expected under realistic conditions. We delivered PLFS to the FHN network during 400 s (corresponding to 1869 periods) and assessed the amount of synchrony and phase entrainment during the last 390 s (corresponding to 1833 periods). We skipped the first 36 periods in order to avoid transients. We performed these simulations for different values of the stimulation amplitude, Stimulus-induced synchrony was determined with the synchrony measure $R$ (figure 3a), whereas phase entrainment of the FHN network was calculated with the entrainment index $E$ (figure 3b; see §§2 and 3).
As the number of pulses per burst increases, the synchrony measure \( R \) as well as the entrainment index \( E \) increase and finally saturate. However, the entrainment index approaches its saturation levels already at smaller number of pulses compared with the synchrony measure. Furthermore, strong phase entrainment can already be achieved with weak stimuli, i.e. with brief pulse trains and intermediate stimulation amplitudes, whereas a strong synchrony boosting effect requires strong stimuli, with longer pulse trains and greater stimulation amplitudes (compare, for example, red and green curves in figure 3). In fact, PLFS at low stimulation amplitudes causes a phase entrainment, but no boost of synchrony (figure 3, dark blue and light blue curves).

By analysing the populational discharge pattern of our FHN population (with raster plots) and the corresponding firing frequencies, we found that only FHN neurons were synchronized, which were phase locked to PLFS. In particular, we did not find additional clusters of neurons, which were mutually synchronized, but not phase locked to the stimulus.

In addition, to investigate the frequency dependence on the stimulation effects, we varied the frequency of PLFS (i.e. the frequency at which the HF bursts were delivered; see figure 1) and determined the extent of synchrony \( (R) \) and of phase entrainment \( (E) \). While the stimulation amplitude was kept constant and equal to 80, simulations were performed for different numbers of pulses per burst. PLFS caused an excitation of synchrony only within a narrow range of frequencies, particularly around the neurons’ preferred frequency at approximately 4.7 Hz, where with increasing number of pulses per burst (i.e. with increasing strength) the peak was slightly shifted towards higher frequencies (figure 4a). For weak PLFS, we observed only a minor increase in synchrony, within a particularly small frequency range.

Figure 4. Frequency dependence of the effects of PLFS. We show the extent of (a) synchrony \( (R) \) and of (b) phase entrainment \( (E) \) for different frequencies of PLFS. The stimulation amplitude was kept constant and equal to 80. Simulations were performed for different numbers of pulses per burst (asterisk, 15; circle, 10; diamond, 7; cross, 3). Note that phase entrainment occurred already at low stimulation intensities (i.e. with brief bursts) and also at stimulation frequencies that considerably deviated from the neurons’ preferred frequency (approx. 4.7 Hz), whereas an increase in synchrony could only be achieved at higher stimulation intensities and with stimulation frequencies close to the neurons’ preferred frequency.
range (figure 4a, cross curve). By contrast, strong phase entrainment was achieved within a wide range of stimulation frequencies, provided PLFS was strong enough, i.e. for more than three pulses per burst. By contrast, weak PLFS caused a strong phase entrainment only around the neurons’ preferred frequency and, again, at higher stimulation frequencies (figure 4b, cross curve). We restricted our analysis to the frequency range from 3 to 8 Hz, since in our patient (due to time constraints during operation), we performed PLFS only at frequencies close to the preoperative tremor frequency.

In summary, in our neuronal FHN network, phase entrainment turned out to be the considerably more robust effect of PLFS, which was strongly induced already at low stimulation intensities and even at stimulation frequencies that
deviated from the neurons’ preferred frequency by 60 per cent and more. By contrast, a stronger increase in synchrony could only be achieved at higher stimulation intensities and with stimulation frequencies close to the neurons’ preferred frequency. In particular, a strong phase entrainment does not require the whole population to be strongly synchronized.

(c) Phase entrainment of tremor recordings

Based on anatomical MR information, the subthalamic electrode leads stimulated an area including the cerebello-thalamic projection. Intraoperatively, there was no tremor to be recorded because the patient was on general anaesthesia.
Therefore, to functionally reveal the optimal electrode position in the cerebellotthalmic projection of the right hemisphere, PLFS was used to evoke rather than to suppress tremor. We performed a bipolar PLFS between contacts 0 and 1 of the Medtronic electrode (i.e. the pair of contacts of the Medtronic DBS Lead 3387 closest to the cerebellar projection) to affect only a reduced volume of tissue and, hence, to avoid stimulation of neighbouring structures (McIntyre et al. 2004).

PLFS of sufficient strength, with amplitude greater than 2 V, strongly and reproducibly evoked tremor contralateral to the side of stimulation (figure 5a). Remarkably, although less pronounced, PLFS also evoked ipsilateral tremor (figure 5a, d). The evoked tremor had frequency peaks at the frequency of PLFS and higher harmonics, i.e. integer multiples of the PLFS frequency (figure 5b–d). In several PLFS epochs, the higher harmonics were more pronounced than the PLFS frequency peak (figure 5b–d). For instance, for the evoked tremor of the contralateral hand, the third harmonic was most pronounced for PLFS at 3.5 Hz, whereas the second harmonic was most strongly excited by PLFS at 5 Hz (figure 5b).

We performed an $n:1$ phase entrainment analysis for the basic PLFS frequency peak and higher order harmonics of the ACC signal for $n=1, 2, \ldots, 10$ (see §3). The $n:1$ relationship that yielded the greatest value of the $n:1$ phase entrainment index $E_{n,1}$ was selected for display in figures 6 and 7. During epochs with pronounced evoked tremor, we observed a strong 1 : 1, 2 : 1 or 3 : 1 phase entrainment, where the dominant frequency peak was associated with the strongest $n:1$ phase entrainment: a dominating basic PLFS frequency peak corresponded to 1 : 1 phase entrainment, whereas a dominating second or third harmonic was associated with 2 : 1 or 3 : 1 phase entrainment, respectively (compare figure 5b–d with figure 7). During epochs with pronounced tremor, the $n:1$ phase difference between PLFS and bandpass-filtered ACC had a nearly perfect horizontal time course (figure 6).

Intriguingly, although tremor evoked by weak PLFS (at 2 V) was hardly detectable by both ACC signal and frequency spectrum (figure 5), we nevertheless observed a clear $n:1$ phase entrainment associated (figure 7b) with a nearly horizontal $n:1$ phase difference between PLFS and ACC (figure 6a). By comparing the $n:1$ phase entrainment index $E_{n,1}$ with the prestimulus baseline $E_{n,1}^{\text{pre}}$ (figure 7), we observed this dissociation between the effect of weak PLFS stimulation (at 2 V) on the amplitude and on the phase of the ACC signal of the contralateral hand for all three epochs and for the contralateral foot as well as for the ipsilateral hand for two out of three epochs (figures 5 and 7). For intermediate (3 V) or strong (4 V) PLFS in all epochs, we found significant $n:1$ phase entrainment. Owing to time constraints during electrode implantation, it was not possible to perform as many PLFS epochs at 5 Hz as at 3.5 Hz. Accordingly, it was difficult to compare effects of PLFS at 5 Hz with those of PLFS at 3.5 Hz. Given this restriction and our findings (figure 7b), we could not derive any significant differences of the phase entraining effect of PLFS at 3.5 Hz and PLFS at 5 Hz.

The robustness of the phase entrainment is further demonstrated by our phase entrainment analysis of the broadband ACC signal (figure 7a). Since the Hilbert transform is well defined for narrow-band signals only (see §3), but we are nevertheless able to detect significant $n:1$ phase entrainment even for the broadband ACC signals, we may conclude that the ACC signals basically represent $n:1$ phase entrained motion without any further substantial non-entrained dynamics.
Analogously, we quantified the effect of PLFS on the amplitude of the ACC signal of the left hand, left foot and right hand by calculating the average amplitude of the ACC signal (figure 7c; see §3). Compared with the phase entrainment effect of PLFS (figure 7a), the amplitude boosting effect of PLFS was considerably weaker (figure 7c). Weak PLFS, at 2 V, did not cause a relevant increase in the tremor amplitude above prestimulus baseline (figure 7c). On the left, contralateral side PLFS at amplitudes greater than 2 V caused an increase in the tremor amplitude.
amplitude above prestimulus baseline (figure 7c). By contrast, PLFS caused hardly any increase in the tremor amplitude above prestimulus baseline of the ipsilateral, right hand (figure 7c). Only PLFS at 4 V and especially at 5 Hz evoked tremor with an amplitude that exceeded the prestimulus baseline amplitude (figure 7c). All in all, PLFS at 5 Hz caused the strongest tremor amplitude increase at all three limbs. Owing to time constraints during electrode implantation, it was not possible to perform as many PLFS epochs at 5 Hz as at 3.5 Hz. Accordingly, it was difficult to compare effects of PLFS at 5 Hz with those of PLFS at 3.5 Hz. Nevertheless, our findings might indicate that PLFS at 5 Hz is more favourable for tremor reinforcement than PLFS at 3.5 Hz.

Owing to time constraints during electrode implantation, it was not possible to perform PLFS via the Medtronic electrode implanted in the cerebello-thalamic projection of the left hemisphere.

Figure 8. Cross-trial analysis of the single-sweep ACC responses of the (i) left hand and (ii) left foot for PLFS epochs (a) no. 2 (3.5 Hz, 4 V) and (b) no. 3 (3.5 Hz, 4 V) from figure 5. Extrema density: for each stimulation epoch, the upper panel shows the distribution density of the local maxima (upward boxes) and local minima (downward boxes) of the single-sweep ACC responses relative to PLFS onset (at time zero in the upper and middle panels). Cross-trials: for the left hand and the left foot, all single-sweep ACC responses were plotted (in red and blue, respectively) along with their corresponding mean ACC response averaged across trials (the mean steady-state ACC response). ACC signal: examples of single-sweep ACC responses are shown along with the corresponding PLFS onsets (indicated by vertical green lines).
In our modelling, we have assumed that the evoked tremor is due to an entrainment of a damped oscillator. The dissociation between effects of PLFS tremor amplitude and phase is in accordance with this assumption. However, an alternative mechanism for the generation of tremor-like activity induced by stimulation still had to be ruled out. If the depth electrode was misplaced or stimulation was administered at rather high amplitudes, a direct stimulation of the pyramidal tract might have been possible. ACC responses to direct pyramidal tract stimulation are stereotypical, tightly time locked to the stimulus and occur exclusively at the contralateral side at short latencies of approximately 20 ms for the hand (corresponding to the transmission time). A close proximity of

**Figure 9.** (a) 1:1 phase difference between (i) broadband ACC signal of the right hand and PLFS and (ii) narrow-band ACC signal of the right hand and PLFS. Stimulation parameters are 5 Hz and 4 V (PLFS epoch no. 7 from figure 5). A bandpass filter (ii) was used as described in §§2 and 3. 1:1 phase difference during prestimulus interval (sham PLFS, in black) and during PLFS epoch (violet) and corresponding distribution of the 1:1 phase difference (y-axis, n:1 phase difference: x-axis, density). Same format as in figure 6. (b) Cross-trial analysis of the single-sweep broadband ACC responses during the PLFS epoch no. 7 from figure 5: (i) extrema density, (ii) single-sweep ACC responses and corresponding mean ACC response averaged across trials and (iii) single-sweep ACC responses with PLFS onsets (green vertical lines). Same format as in figure 8.

(e) **Entrainment of a damped central oscillator**

In our modelling, we have assumed that the evoked tremor is due to an entrainment of a damped oscillator. The dissociation between effects of PLFS tremor amplitude and phase is in accordance with this assumption. However, an alternative mechanism for the generation of tremor-like activity induced by stimulation still had to be ruled out. If the depth electrode was misplaced or stimulation was administered at rather high amplitudes, a direct stimulation of the pyramidal tract might have been possible. ACC responses to direct pyramidal tract stimulation are stereotypical, tightly time locked to the stimulus and occur exclusively at the contralateral side at short latencies of approximately 20 ms for the hand (corresponding to the transmission time). A close proximity of
the implanted depth electrode to the pyramidal tract was ruled out by intra-operative X-ray controls (Treuer et al. 2005). In addition, to functionally rule out a direct stimulation of the pyramidal tract, we considered the PLFS induced the single-sweep ACC responses as well as the mean steady-state ACC response (figure 8). Our main findings ruling out pyramidal tract stimulation, but supporting the entrainment of a damped central oscillator are as follows.

(i) PLFS did not cause stereotypical ACC responses. By contrast, PLFS with the same parameters caused different responses with different $n:1$ phase relationships (see ACC of the left foot in figure 8a,b).

(ii) PLFS responses were not restricted to the contralateral side. By contrast, we observed both amplitude and phase responses of the ipsilateral hand (figure 9).

(iii) We assessed the latencies of the ACC responses by determining the time difference between PLFS onset and occurrence of the first maximum or minimum of the ACC response. In the case of a pyramidal tract stimulation due to the stimulus-induced transmission of the action potentials to the spinal cord, the latencies of the hand responses should be much shorter than those of the foot responses. However, for instance, in figure 8a, the first deflexion (minimum) of the ACC response of the left foot occurs considerably earlier than the first minimum of the ACC responses of the left hand. Furthermore, in a certain portion of trials, the ACC response of the left foot advances the stimulus onset.

5. Discussion

(a) Effective entrainment by PLFS

In our study, we computationally developed a stimulation technique for effective entrainment of neuronal populations. We experimentally verified our approach by evoking tremor phase locked to the stimulus at a proper electrode location in an SCA2 patient who had to be operated on under general anaesthesia.

Computationally, we demonstrated that patterned stimuli administered at a frequency in the range of the neurons’ preferred frequency (figure 1) provide a particularly effective means for entrainment of the neuronal population. Phase locking between the phase of the collective oscillation and the stimulus can robustly be achieved already at low stimulation intensities (figure 3) and at stimulation frequencies that deviate from the preferred neuronal frequency by 60 per cent and more (figure 4).

Our computational findings refer to dynamical features on the level of the collective dynamics of a population of oscillatory neurons and correspond to what is known from single oscillators. In a single oscillator, especially in a limit cycle oscillator, a perturbation of the oscillator’s amplitude typically requires considerably more energy compared with a perturbation of its phase (Winfree 1980; Haken 1983; Glass & Mackey 1988). We here showed that PLFS enables one to control the phase of the collective oscillation (i.e. the phase of the order parameter) already at low stimulation intensities, whereas an increase in the amplitude of the collective oscillation (i.e. the synchrony) requires higher stimulation intensities (figure 3). In contrast to periodic stimulation with long single pulses, PLFS reliably fulfils
safety requirements even at high stimulation intensities (see §§2 and 3) and, hence, makes an effective phase control and, in particular, a strong amplitude reinforcement available for clinical use.

Entrainment of oscillators by periodic stimuli has been studied extensively (Winfree 1980; Haken 1983; Ermentrout & Rinzel 1984; Glass & Mackey 1988). Sakaguchi (1988) studied the effect of an external sinusoidal periodic stimulus on a population of active phase oscillators. Apart from the group of desynchronized oscillators and the group of oscillators phase locked to the external periodic force, for particular parameters he observed the emergence of clusters of oscillators consisting of mutually synchronized oscillators, which were not phase locked to the stimulus. In our study, we did not find such additional non-stimulus-locked clusters. This might be due to the fact that in the absence of stimulation the majority of the neurons in our model were inactive. Also, our model differed from Sakaguchi’s model in several other respects, including the nature of the single oscillatory unit (FHN neuron versus phase oscillator), the interaction (synaptic interaction versus smooth coupling) and the periodic force (PLFS versus smooth sinusoidal force).

(b) PLFS for target localization

Target localization under general anaesthesia is difficult for several reasons: (i) macrostimulation and microrecordings are not feasible (see §1), (ii) the patient is not able to consciously perceive and report non-motor side effects such as dysesthesia, visual hallucinations, etc. and (iii) stimulation-induced dyskinesia cannot be observed (Venkatraghavan et al. 2006).

In an SCA2 patient under general anaesthesia, we showed that at a proper electrode location PLFS evokes tremor, strongly phase locked to the stimulus. PLFS of weak (at 2 V) or intermediate intensity (at 3 V) evoked low-amplitude tremor phase locked to the stimulus. By contrast, a pronounced tremor reinforcement required strong PLFS at 4 V (figures 5 and 6). The precision of the electrode location was approved by the patient’s optimal clinical benefit.

Note that PLFS enabled us to periodically deliver stimuli (i.e. brief high-frequency pulse trains), which carried effective amounts of the integral charge density, thereby remaining well below the safety limit. This is because the charge density of each single pulse contained in a brief high-frequency pulse train was far below the safety limit. Hence, the high-frequency pulse train fulfilled the safety requirements, although its integral charge density, being the sum over the charge densities of all associated single pulses, exceeded the safety limit of a corresponding long single pulse (see §§2 and 3). By contrast, replacing PLFS by a periodic stimulation with long single pulses with equivalent charge density would have violated the safety requirements already at a stimulation amplitude of 3 V. In other words, only weak periodic single-pulse stimulation (at 2 V) would have been feasible (see §§2 and 3). This remarkable fact justifies our effort to elaborate a stimulation technique that enables one to periodically deliver stimuli with effective amounts of the integral charge density, which nevertheless reliably satisfies safety requirements.

Another promising application of PLFS might be target localization in akinetic patients without tremor or where tremor vanished after macroelectrode insertion. An insertional effect may rapidly appear and make tremor suppression impossible. Also, in awake patients with active tremor and without insertional effect, tremor entrainment with PLFS at low stimulation intensities might provide additional
valuable information and, thus, improve target localization. Note that, to model a neuronal network affected by general anaesthesia, we have chosen a network that displayed little spontaneous activity. However, the stimulation effects of PLFS are not restricted to the network being mainly passive. By contrast, pronounced stimulation effects of PLFS, both robust phase entrainment and an increase in synchrony, can be obtained at even lower stimulation intensities if the network is more or entirely active, that is, if in the simulations the values of $e_{\text{individual}}$ were chosen to be Gaussian distributed with mean greater than $-2.7$.

In general, in awake patients, high-frequency DBS suppresses Parkinsonian or essential tremor, whereas periodic single-pulse DBS at low frequencies in the tremor range reinforces tremor (Hassler et al. 1960; Benabid et al. 1991, 1993, 1996; Blond et al. 1992; Caparros-Lefebvre et al. 1994). However, in awake patients, the initial motor situation, e.g. limb positions and ongoing pathological activity, strongly influences the effects of low-frequency DBS (Hassler et al. 1960). Furthermore, atypical effects of low-frequency DBS have been reported, for example, by Liu et al. (2002). In two patients with Parkinson’s disease during bilateral STN depth electrode implantation, Liu et al. (2002) observed that unilateral high-frequency DBS at 100 Hz of the STN induced tremor at 4 Hz in both forearms and oscillations of the LFP of the contralateral STN at 4 Hz. Conversely, periodic single-pulse low-frequency DBS at 5 Hz induced contralateral arrhythmic involuntary movements at 3 Hz, while the contralateral STN activity remained unchanged (Liu et al. 2002). Hence, it might be interesting to see whether in awake patients PLFS reproducibly induces tremor phase locked to the stimulus.

Another procedure for target localization, developed by Klostermann et al. (2003), can also be used under general anaesthesia. They have shown that somatosensory evoked potentials can be used for the functional identification of VIM, but not for STN (Klostermann et al. 2003). In a forthcoming study, we shall demonstrate that PLFS works equally well in both VIM and STN.

(c) Effects of periodic stimulation

Already in the 1950s, neurophysiological observations were obtained in human patients during stereotaxic interventions (Hassler et al. 1960). At that time, for the therapy of extrapyramidal motor disturbances, stereotaxic lesions of certain subcortical structures were achieved by using high-frequency coagulation (e.g. Hassler et al. 1960). Apart from a pneumoencephalographic X-ray check-up, electrical stimulation of target structures was used to verify the accurate location of the electrode tip (Hassler & Riechert 1954). During these operations, patients were fully awake, so that they could report about the effects caused by stimulation and coagulation. In Parkinson patients, coagulation of pallidal structures effectively diminished rigidity, whereas the beneficial effect on tremor was less complete and less regular (Hassler et al. 1960). The best therapeutic effect on tremor was achieved by coagulation of the posterior portion of the oral ventral thalamic nucleus (ventral oral posterior, VOP; Hassler et al. 1960). Both targets were identified with stimulation. In the pallidum and especially in VOP, it was possible to reinforce or (if the tremor was temporarily absent) to evoke tremor with low-frequency stimulation (4–8 Hz; Hassler et al. 1960). Furthermore, Hassler et al. (1960) mentioned that tremor could be brought into step with low-
frequency stimulation (4–8 Hz) in the pallidum. By contrast, stimulation in the pallidum at higher frequencies (25–100 Hz) mostly reduced the tremor amplitude. Hassler et al. (1960) did not use stimulation frequencies greater than 100 Hz. In the awake patient, the effect of stimulation strongly depended on the initial motor situation, including limb position and ongoing pathological movement patterns (Hassler et al. 1960). Note that Hassler et al. (1960) used no PLFS, but simple periodic low-frequency stimulation, where single pulses were periodically delivered at 4–8 Hz.

(d) Differential effect of PLFS on tremor amplitude and phase

To assess the effect of PLFS on the tremor amplitude, we calculated the mean amplitude $A = 1/(t_{\text{end}} - t_{\text{begin}}) \int_{t_{\text{begin}}}^{t_{\text{end}}} |X(t)| \, dt$ of the broadband ACC signal $x(t)$ over the whole period of stimulation with PLFS and compared it with the prestimulus baseline $A_{\text{baseline}} = \int_{t_{\text{begin}}}^{t_{\text{end}}} |X(t)| \, dt$. For this, the complex signal $X(t)$ is obtained from the ACC signal $x(t)$ with the Hilbert transform $x_H(t)$ according to $X(t) = x(t) + ix_H(t)$ (see §§2 and 3). Weak PLFS induced only low-amplitude tremor, which was nevertheless significantly and, for particular parameters, nearly perfectly entrained by the stimulation (see the effects of PLFS at 5 Hz and 2 V on left foot and left hand; figures 5 and 6). This is because weak PLFS caused a weak narrow-band excitation in the frequency spectrum at the stimulus frequency (plus at higher harmonics), which did not strongly alter the mean broadband amplitude $A$. By bandpass filtering and consecutive Hilbert transform, we were able to extract the phase of this weak narrow-band excitation (see §§2 and 3). Irrespective of its low amplitude, the phase of this narrow-band excitation was well defined via the Hilbert transform formalism (Panter 1965) and significantly or even perfectly phase locked to the stimulus (see PLFS at 5 Hz and 2 V; figure 5).

Our computational results show that in our model neuronal network strong phase entrainment is less dependent on a proper choice of the stimulation frequency, whereas strong tremor reinforcement requires the stimulation frequency to be sufficiently close to the neurons’ preferred frequency (figure 4). In our model, we assumed a narrow frequency distribution (see §§2 and 3). Since we have to expect that the tremor-generating network in the patient is more complex, we cannot expect that the computational findings hold for the patient in detail, too. However, our experimental results indicate that the tremor reinforcement was more pronounced with PLFS at 5 Hz, whereas we could not detect a relevant difference between PLFS at 5 and 3.5 Hz concerning the strength of the $n:1$ phase entrainment (figure 7). However, the patient’s preoperative tremor frequency was at approximately 3 Hz. The discrepancy between the two frequencies might be due to the complex nonlinear properties of the patient’s network and/or the fact that the dynamical network properties were altered due to anaesthesia. A more detailed investigation with a larger number of stimulation frequencies was not feasible owing to time constraints during depth electrode implantation.

In our simple model of a population of synaptically interacting neurons, we found the differential PLFS effect on the amplitude and phase of the evoked tremor. These effects were reproduced in our patient and enabled an accurate electrode placement. In addition, the patient’s ACC signal showed an excitation of higher harmonics (figure 5). This effect will be taken into account in an ongoing development of a physiologically realistic model of the affected neuronal
target population. The experimentally revealed differential effects of PLFS on amplitude and phase of the evoked tremor were in accordance with PLFS acting on a damped oscillator (as in our mathematical model), whereas our experimental data are in contradiction to a simple stimulation of the pyramidal tract, which causes stereotypical and tightly stimulus-locked ACC responses.

\[(e)\] **Patterned stimulation for modulation of rhythms and synaptic efficacy**

The impact of stimuli on oscillations of single neurons as well as neuronal populations has been studied extensively (Steriade et al. 1990; Timme et al. 2002). For instance, it has been shown that single pulses may reset membrane potential oscillations (Pedroarena & Llinás 1997). Another established tool for modulating the phase dynamics of neural rhythms is the administration of brief high-frequency pulse trains. For instance, lick-synchronized stimulation (a 100 ms train of 0.1 ms wide rectangular pulses at 100 Hz and 25–150 μA) delivered at the oral part of the nucleus reticularis gigantocellularis, applied during continuous licking (after eight regular consecutive licks), has been used to cause a phase shift of licks emitted after stimulus delivery, presumably by resetting the central rhythm generator of spontaneous consummatory licking (Brozek et al. 1996).

As shown in a different context, patterned stimulation may enhance the efficacy of synapses. HFS delivered at a frequency that mimics naturally occurring hippocampal theta optimizes long-term potentiation (Larson et al. 1986; Diamond et al. 1988). This is in agreement with computational results showing that low-frequency administration of brief high-frequency pulse trains may shift a neuronal network with synaptic plasticity from a stable desynchronized attractor state to a stable synchronized attractor state, by increasing the mean synaptic weight due to an increase in the amount of coincident firing (Tass & Majtanik 2006).

The impact of DBS on neuronal plasticity may contribute to the patient’s remarkable clinical benefit. In computational studies, it has been shown that desynchronizing brain stimulation can induce an anti-kindling, i.e. it can shift a neuronal network from a pathological synchronized attractor state to a physiological desynchronized attractor state, by decreasing the mean synaptic weight caused by a decrease in the extent of coincident firing (Tass & Hauptmann 2006, 2007; Tass & Majtanik 2006; Hauptmann & Tass 2007). According to computational studies, anti-kindling can best be achieved with stimulation techniques which selectively counteract synchrony (Tass & Hauptmann 2006, 2007; Tass & Majtanik 2006; Hauptmann & Tass 2007), such as coordinated reset stimulation (Tass 2003). However, also standard high-frequency DBS may cause a desynchronization and, hence, induce an anti-kindling, provided it affects predominantly excitatory structures and only few or no inhibitory structures (Hauptmann & Tass 2007). In our SCA2 patient, a combined subthalamic–thalamic approach was chosen. To this end, the upper two electrode leads were placed within the ventral portions of the VOP–VIM nuclei, whereas the lower two leads were located in the underlying white matter including the zona incerta and the cerebello-thalamic projection (Freund et al. 2007). The cerebello-thalamic projection is exclusively excitatory. Accordingly, after implantation of the generator, the standard high-frequency DBS might have exerted a strong

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excitatory impact on the VIM via the two lower leads in the cerebello-thalamic projection and, hence, caused an anti-kindling, which led to the unusual and long lasting clinical benefit.

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