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Chapter 4

Interaction of high and low frequency stimulation in thalamocortical relay cells in-vitro

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Abstract

In this experimental study, we use an in-vitro rat thalamic slice preparation to investigate how low frequency (LF, 2-6 Hz) and high frequency (HF, 30-130 Hz) sinusoidal current injections modulate the membrane dynamics of thalamocortical relay (TCR) neurons. We observe that LF sinusoidal current injection induces phase related firing. Amplitude of the LF input determines phase of TCR spiking. Superimposing a HF sinusoidal input, initially delays spiking activity phase locked to the LF component. Increasing the amplitude of the HF component gives rise to action potential generation at sub-harmonics of the LF input and further increasing the amplitude of the HF component, completely suppresses spiking activity. Application of a pulse-train, phase locked to the LF sinusoidal input, modulates the response of TCR neurons in a similar fashion. Overall effect of the phase-locked pulse-train is dependent on at which phase of the LF sinusoidal input the pulse train is applied and amplitude of the LF input.
4.1 Introduction

Recent experimental and computational studies point out that the basal ganglia-thalamocortical loop is a non-linear dynamical system both during normal and pathological states (Obeso et al. 2000). During Parkinson’s disease (PD), loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) leads to changes in the firing rate, firing pattern and synchrony of Basal Ganglia (BG) neurons (Alexander and Crutcher 1990; Alexander et al. 1986; Brown 2003; Delong 1990; Obeso et al. 2000; Raz et al. 2000). These changes are reflected on to the entire basal ganglia-thalamocortical loop via the GABAergic projection from the BG output nucleus, globus pallidus internum (GPI), to the thalamocortical relay (TCR) neurons (Alexander and Crutcher 1990; Alexander et al. 1986; Smith et al. 1998).

Modulation of the TCR neurons by the GABAergic GPI projection is proposed to be one of the key factors in sustaining synchronous oscillations in the theta (3.5-7 Hz) and beta (13-30 Hz) frequency bands during PD in the basal ganglia-thalamocortical loop (Rubin and Terman 2004). Deep Brain Stimulation (DBS) is an invasive therapy used as a last resort in the management of PD. High frequency stimulation (HFS) of the subthalamic nucleus (STN) or the GPI results in alleviation of PD motor symptoms. Clinically effective stimulation frequency exhibits an inverse relationship with required intensity for suppression of PD motor symptoms (Benabid et al. 1991; Moro et al. 2002).

The mechanism of action of DBS is still unclear and the fact that lesioning the nucleus and stimulating at high frequencies produce a comparable clinical outcome has puzzled researchers. Early experimental studies showed that spontaneous firing in the stimulated nucleus decreases drastically during DBS (Benazzouz et al. 2000; Boraud et al. 1996; Dostrovsky and Lozano 2002). In addition, experimental and computational studies demonstrated that DBS has an effect on downstream nuclei (Hashimoto et al. 2003; Holsheimer et al. 2000; McIntyre et al. 2004a; McIntyre et al. 2004b). For instance, STN-DBS modulates the firing patterns and increases the firing rate of GPI neurons (Hashimoto et al. 2003). Despite a somatic block of activity at the stimulated nucleus, passing fibers, apparently activated by HFS, lead to downstream changes (Holsheimer et al. 2000; McIntyre et al. 2004a; McIntyre et al. 2004b).
Given their integrative role in the basal ganglia-thalamocortical loop, TCR neurons have played a central role in computational studies looking into mechanisms behind the efficacy of DBS (Cagnan et al. 2009; Guo et al. 2008; McIntyre et al. 2004a; Rubin and Terman 2004). The TCR neurons receive GABAergic inputs from the GPi and glutamatergic inputs from the cortex (Smith et al. 1998). They project back to the cortex, thus closing the thalamocortical loop (Alexander and Crutcher 1990; Delong 1990). Rubin and Terman (2004) have shown that STN-DBS restores thalamocortical relay by replacing the phasic inhibition applied to the TCR neurons during PD with constant inhibition during DBS (Rubin and Terman 2004). In another modeling study, Cagnan et al. (2009) observed that applying HFS to the STN restores the relay capability of the TCR neuron and that the specific relation between stimulation frequency and required stimulus amplitude followed the experimental curve that was determined in patients (Benabid et al. 1991). This computational study also suggested that HFS was effective when applied at certain periods of the phasic inhibition (Cagnan et al. 2009).

Here we use a rat in-vitro thalamic slice preparation and patch-clamp the TCR neuron either in voltage or in current-clamp to experimentally investigate their response to low frequency (LF) and high frequency (HF) phasic inputs. TCR neurons can easily be recognized based on their location, their specific stellate morphology, by a tendency to burst fire under appropriate conditions and by the presence of a Low-Voltage-Activated (LVA) Ca\(^{2+}\) current (Destexhe et al. 1996; Destexhe et al. 1998; Sherman 2001; Soltesz and Crunelli 1992). To simplify the quantifications, we use direct current injection through the pipette instead of synaptic activation, which most likely transfers the oscillations under physiological conditions. This configuration is not identical to synaptically driven activation, but it is experimentally well controlled and the current injection mimics the interference of HF and LF oscillations. We used a "slow" clamp system to guarantee that current-clamp protocols always started from the same membrane voltage (-70 mV). Superimposed on this level we then could apply one or two sine waves (LF: 2-6 Hz, HF: 30-130 Hz) and/or an alpha function. Injecting hyperpolarizing LF oscillatory current into the TCR neuron around resting membrane potential (-70 mV) resulted in phase related firing and/or bursting. Increasing the input
amplitude at a fixed frequency, phase advanced the spiking. Superimposing an additional HF oscillatory input of sufficient amplitude prevented the phase-locked spiking of the TCR neuron. The relation between frequency and current amplitude required to silence the cell followed the inverse relationship that was predicted by Cagnan et al. (2009) and experimentally observed by Benabid et al. (1991).
4.2 Material and Methods

Slice preparation: Electrophysiological experiments were performed using 300 µm thick coronal brain slices from wistar rats (Harlan, Zeist, Netherlands; postnatal days 12-16) that were killed by decapitation under anesthesia (i.e. isoflurane). Slices contained thalamic nuclei at the level of the hippocampus. They were cut with a vibroslicer (Leica VT1000S) in ice-cold solution containing in mM: NaCl (120), KCl (3.5), CaCl$_2$ (0.5), MgSO$_4$ (6), NaH$_2$PO$_4$ (1.25), NaHCO$_3$ (25), and glucose (25); continuously bubbled with 95% O$_2$-5% CO$_2$ (pH=7.4). Slices were incubated at 32 °C for 1 hour in ACSF containing (in mM): NaCl (120), KCl (3.5), CaCl$_2$ (2.5), MgSO$_4$ (1.3), NaH$_2$PO$_4$ (1.25), NaHCO$_3$ (25), and glucose (25); continuously bubbled with 95% O$_2$-5% CO$_2$ (pH=7.4).

Cell labeling: During recordings, cells were filled with biocytin (4 mg/ml; Sigma) for identification of their morphology. Slices were fixed overnight in 0.1 M phosphate-buffered saline (PBS, pH=7.4) containing 4% paraformaldehyde. After 30 min permeabilization in 0.3% Triton X-100-PBS, slices were incubated for 60 min in avidin-biotin-peroxidase complex (Vectastain ABC Elite kit, Vector Laboratories; Burlingame, CA, USA). Biocytin was visualized as a dark brown substrate using DAB (3,3’-diaminobenzidine-4 HCl, Sigma Chemical Co., St. Louis, MO, USA) reaction (Hancock 1986).

Recording: Experiments were approved by the animal welfare committee of the University of Amsterdam. During recording, slices were kept submerged at room temperature (20-22 °C) and were continuously superfused with ACSF. Patch pipettes were pulled from borosilicate glass and had a resistance of 2-3 MΩ when filled with solution containing (in mM): K-gluconate (130), KCl (10), EGTA (5), HEPES (10), Mg-ATP (4), Na-GTP (0.4) at pH=7.3. Whole-cell voltage and current-clamp recordings were made using an EPC9 patch-clamp amplifier controlled by either PULSE software (HEKA Electronic GmbH, Germany) or an in-house software package running under MATLAB (MathWorks, Cambridge, UK). Signals were filtered at 5-10 kHz and sampled at 10-20 kHz. Series resistance ranged from 5-10 MΩ, and in voltage-clamp mode, was compensated for about 70%. Membrane potentials and command potentials were corrected for a 10 mV liquid junction potential. In voltage-clamp, classical step protocols
were employed. In current-clamp, either step depolarizations were used in order to quantify firing properties or signals that consisted of LF (2-6 Hz) or summated LF and HF (30-130Hz) sine waves were used to provide oscillatory input to the TCR cell. A phase locked pulse train (PLPT) consisted of periodic alpha functions. Frequency of the PLPT was set equivalent to the frequency of the LF sine wave driving the TCR cell (i.e. 2 Hz). Time constant for each alpha function was set to 20 ms. Membrane voltage was then recorded following liquid junction potential correction. An automatic drift compensation system guaranteed a stable level in between protocols.

Analysis: Currents evoked by step protocols were corrected for leak. Voltage dependent activation was determined using peak current amplitude. Voltage dependent permeability was fitted using a Boltzmann function. Voltage dependent steady-state inactivation was determined using step protocols from a variable level followed by a fixed depolarization; normalized current was also fitted with a Boltzmann function.

In current-clamp the current signal was decomposed into its frequency components and a mean value. Phases were computed with respect to the LF sine wave. Action potentials were detected using a level crossing paradigm.

Statistical analysis: Data are given as mean ± SEM. When not mentioned otherwise comparisons were done by Students t-test. p < 0.05 was assumed to indicate a significant difference.
4.3 Results

4.3.1 Thalamocortical relay neurons

Figure 1: Morphological and electrophysiological characterization of TCR cells. A: TCR cell filled with biocytin. B: whole-cell current-clamp recordings illustrating the voltage response of a TCR cell to depolarizing current injections (B1: 25 pA, B2: 100 pA). Note the burst firing mode characteristic of TCR cells. C1: Whole-cell voltage-clamp recording of Ca$^{2+}$ currents evoked by step depolarizations from a pre-pulse potential of -130 mV to depolarizing potentials ranging between -100 and -20 mV. C2: Voltage-dependant activation function of the Ca$^{2+}$ current determined by the protocol of C1. Normalized permeability $P/P_{max}$ given by Goldman-Hodgkin-Katz current equation was fitted to a Boltzmann equation (see below). C3: Voltage dependant steady state inactivation of Ca$^{2+}$ current was
evaluated using a 200 ms depolarization to -60 mV preceded by a 500 ms hyperpolarization to levels between -130 to -60 mV. Data (n=7) for voltage-dependent activation and inactivation were fitted with a Boltzmann equation \( P = \frac{P_{\text{max}}}{1 + \exp((V - V_h)/V_c)} \) where \( V_h \) is the potential of half maximal activation (inactivation) and \( V_c \) is a slope factor. Results are given as mean ± SEM. Activation: \( V_h = -64.2 \pm 1.1 \) mV, \( V_c = 1.6 \pm 0.3 \) mV; Inactivation: \( V_h = -79.7 \pm 1.3 \) mV, \( V_c = -6.5 \pm 1.2 \) mV

In the thalamic slice, TCR neurons can be recognized based on their location and their specific stellate morphology (Fig. 1A). Most of the neurons that we recorded were filled with biocytin so that this specific morphology could afterwards be confirmed. Once a putative TCR neuron was patched, current-clamp recordings demonstrated a tendency to fire bursts under the appropriate conditions (Fig. 1B). In voltage-clamp, the presence of a Low-Voltage-Activated (LVA) \( \text{Ca}^{2+} \) current, not blocked by TTX, could be demonstrated using classical step protocols (Fig. 1C). Step protocols that depolarize to increasing voltage levels from a long hyperpolarizing potential at -130 mV reveal the voltage-dependent activation function (Fig. 1C2), while step protocols from various hyperpolarizing levels to a fixed depolarizing potential of -60 mV allow to construct the steady-state inactivation of the LVA current (Fig. 1C3). The in-situ recording conditions in the slice and the dendritic tree compromise a precise and detailed analysis of the current kinetics due to space-clamp problems.

After characterization in voltage-clamp, the recording was switched to current-clamp mode for the remaining part of the experiment. Stepwise depolarization evoked repetitive firing and often induced burst events characteristic for TCR cells (Fig. 1B1 and B2). Under current-clamp, the membrane was kept at a mean value of -70 mV with a slow automatic current injection to compensate for drift; the control loop was set on hold during the time that the stimulation protocols ran.

### 4.3.2 Sinusoidal current input at 2-6 Hz

Following this elementary characterization, each TCR neuron was subjected to hyperpolarizing sinusoidal current injection at 2 Hz (Fig. 2A) that lasted for 5 seconds.
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Figure 2: In response to low amplitude sinusoidal current injection (A - grey line), a TCR neuron exhibited sub-threshold oscillations (B - grey line) locked to the frequency of the sinusoidal current injection (i.e. 2 Hz). Increasing the amplitude of the sinusoidal current injection (A - black line) gave rise to spiking at every cycle of the sinusoidal input (B - black line). C: Distribution of the TCR spike-phase as a function of the amplitude of the sinusoidal current injection at 2 Hz. The amplitude of the sinusoidal current injection was varied between 60 pA and 120 pA, in steps of 10 pA. As the amplitude of the current injection was increased from 60 pA to 120 pA, spikes locked to the sinusoidal input were phase-advanced. The distribution is obtained from 15 TCR neurons and the scale bar indicates the total number of spikes observed at a specific phase of the sinusoidal current injection due to a specific current injection amplitude. D: shows the average spike-phase observed during the 5 second recording, as a function of the amplitude of the current injection, averaged over 15 neurons. The error bars indicate the standard error of the mean observed during the 5 second recordings (15 neurons). As the amplitude of the current injection increases, the variation in the spike-phases observed during the 5 second recording reduces.
Amplitude variation of the sine wave was programmed in hyperpolarizing direction assuring that the maximum of the injected sine wave was always at 0 pA. The sinusoidal current was added to the current necessary to keep the cell at a "resting" potential of -70 mV (Fig. 2A, compare grey and black lines). Low amplitude current injection resulted in sub-threshold oscillations phase-locked to the injected sinusoid (Fig. 2B, grey line compare with grey line in 2A). As the amplitude of the current injection was increased, we observed a transition from sub-threshold oscillation to spiking again strictly phase-locked to the sine wave and capable of following each cycle as soon as the amplitude was sufficiently above threshold (see below) (Fig. 2B, black line, to be compared with black line input in 2A). Spike-phase, in degrees, is calculated using the peak of the first action potential and its time difference relative to the phase of the injected sine wave (reference taken to be the point at which phase of the LF sine wave was zero). Further increasing the amplitude of the current injection at a fixed frequency gradually advanced the phase at which firing occurred. Figure 2C gives a population summary of 15 TCR neurons, showing the distribution of the spike-phase as a function of the injected current amplitude ranging between 60 pA (threshold) and 120 pA; absolute spiking density is calculated as a function of phase and sine wave amplitude using all cycles of all experiments in all neurons. The mean spike-phase as a function of the LF sine wave amplitude advances about 25 degrees from threshold to saturation (15 TCR neurons analyzed) (Fig. 2D). As the amplitude of the injected current increased, the phase lock of the spiking to the sine wave became tighter, as can be concluded from the decrease of variance with amplitude as shown in figure 2C. Next, we repeated these experiments for frequencies of 4 Hz and 6 Hz with essentially the same results. However, when increasing the sine wave amplitude above threshold at these frequencies we more often observed a sub-harmonic response, where spiking did not occur at each cycle of the input (see Supplementary Material).

4.3.3 Superimposing high frequency sinusoidal input onto the 2 Hz current injection

Next we investigated the effect of superimposing a HF (HF: 30 Hz, variable amplitude) sine wave onto the existing LF
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current injection (LF: for this experiment fixed at frequency: 2 Hz, and amplitude: 100 pA). The amplitude of the HF component was modulated; as before in such a way that the maximum of the combined signal was kept at 0 pA (to be added to the value that held the TCR at its desired "resting" potential of -70 mV). Amplitude as well as frequency dependency of the response to the HF component were investigated.

Figure 3: As the amplitude of the HF component was varied from 0 pA (A) to 20 pA (B), while keeping the frequency fixed at 30 Hz; we observed that spiking locked to the LF component was phase delayed (B) with respect to spike-phase observed in the absence of the HF component (A). When the amplitude of the HF component was further increased to 50 pA, spiking did not occur at every cycle of the LF component (C). For HF component amplitudes greater than or equal to 60 pA, spiking was completely suppressed (D).

Starting from a condition where the LF current injection induced phase-locked spiking at 2 Hz, we observed that increasing the amplitude of the HF component delayed firing in a gradual and systematic way. The left column of figure 3A illustrates the current injection for increasing HF amplitudes, the middle column gives the corresponding voltage response of the TCR neuron and the right column gives one cycle at an
expanded time scale to better appreciate the phase relation. Increasing the amplitude of the HF component delayed the phase of the spiking activity that was locked to the 2 Hz sinusoidal input. Figure 4 gives the population summary of all 7 TCR cells for all cycles, relating spike density to phase delay and HF input amplitude; the figure was constructed in a similar fashion to figure 2C. When the amplitude of the HF component reached a certain value (Fig. 3C) spiking switched to a sub-harmonic regime and above an even higher level (Fig. 3D) it ceased completely. The same can be concluded from figure 4: spike phase delay correlates well with current amplitude and the absolute spike density decreases with current amplitude and becomes zero above a critical value.

The last experiment was then repeated with HF input ranging in frequency between 30 Hz and 130 Hz (Fig. 4). The behavior of spike density as a function of phase-delay and HF input amplitude was well comparable to the one described above for 30 Hz. After a gradual increase in phase delay, the TCR cell always switched to sub-harmonic firing at a critical HF amplitude and at a slightly higher amplitude firing ceased altogether. The value where firing ceased was called the threshold amplitude and it was determined as a function of the sinusoid stimulation frequency. To compare individual cells with different conductances, frequency dependence was normalized over the frequency range from 30 - 130 Hz before averaging (Fig. 5). Threshold amplitude is inversely proportional to the frequency of the HF component (Fig. 5) reaching a minimal value for frequencies higher than 100 Hz.
Figure 4: Distribution of the TCR spike-phase delays as a function of the HF current injection amplitude. Prior to the transition from spiking at 2 Hz to sub-threshold oscillation at 2 Hz, spiking activity locked to the 2 Hz sinusoidal input was phase delayed. As the amplitude of the HF current injection was varied between 10 pA to 100 pA (in steps of 10 pA), spike-phase was delayed proportional to the amplitude of the HF current injection. Consecutive panels indicate the distribution of spike phase delays due to HF current injection at frequencies between 30 and 110 Hz in steps of 20 Hz. Values are obtained from 7 TCR neurons and the scale bar indicates the total number of spikes observed at a specific phase of the LF input cycle for a given HF current injection amplitude.
4.3.4 Superimposing phase locked pulses onto the LF sinusoidal current input

We reasoned that if HF sinusoidal stimulation manipulates spike phase delay, it is well possible that rightly timed pulse stimulation could do the same. To that end we applied periodic alpha pulses (step pulses that decay with a time constant of 20 ms) at various phases of the LF input and measured the response of the TCR neuron. As the phase of the pulse train was varied in steps of 15 degrees starting from 240 degrees, phase at which spiking occurred was delayed within the cycle and application of a PLPT at 330 degrees completely suppressed the spiking activity locked to the 2 Hz
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sinusoidal input (Fig. 6). Phase of the PLPT was defined in exactly the same way as we defined spike phase.

Figure 6: Response of a TCR neuron to the application of PLPT: Application of a PLPT at 330 degrees of the 2Hz sinusoidal current injection lead to suppression of spiking activity locked to the sinusoidal input. LF input parameters were kept constant; frequency at 2 Hz and amplitude at 50 pA. Pulse amplitude was kept at 250 pA and pulse time constant was set at 20 ms. A: Phase of the PLPT set at 270 degrees. B: phase at 300 degrees C: phase at 330 degrees. Left column shows current injection and right column indicates voltage response.

We recorded the TCR membrane potential while the amplitude of the LF current injection was varied between 25 pA to 125 pA and the amplitude of the PLPT was kept constant at 250 pA. As the phase of the PLPT was varied from 240 degrees to 360 degrees, spikes locked to the LF current injection were delayed. When the phase of the PLPT was greater than 330 degrees, phase of the spiking activity was reset. The amount of spike-phase delay and suppression of
spiking activity induced by PLPT were dependent on the amplitude of the LF current. For 25 pA LF current injection, spiking activity was extensively suppressed when the PLPT was applied at phases greater than 300 degrees. The spike-phase delay induced by the PLPT, when the LF current injection amplitude was 25 pA, was greater than the delay observed with higher LF current injection amplitudes (i.e. 50-125 pA). For higher amplitude LF current injections, the phase delays induced by the PLPTs were more uniform (Fig. 7). The main difference observed among spike-phase delays for different LF current injection amplitudes (e.g. 50-125 pA) was the dependency of the spike-phase on the strength of the LF current injection amplitude (Fig. 2B and 2C). Using a stronger PLPT gives qualitatively similar results (Fig. 8).

Figure 7: As the phase of the PLPT was varied between 240-360 degrees, spiking activity locked to the 2 Hz LF current injection was phase delayed (top panels) and the likelihood that a sinusoidal cycle produced a spike was reduced (bottom panels). Amplitude of the LF current was varied between 25-125 pA while the amplitude of the
PLPT was kept at 250 pA (A-E). Spike-phase distribution and spike induction success rate were calculated based on recordings of 5 seconds (11 neurons analyzed). The dotted lines in the top panels indicate the mean spike-phase observed over 11 neurons as a function of the pulse phase. The scale bar indicates the total number of observed spikes as a function of spike phase and pulse phase.

Figure 8: Amplitude of the LF current injection was kept at 75 pA while the amplitude of the PLPT varied between 100-300 pA (A-E). As the phase of the PLPT varied between 240-360 degrees, spiking activity locked to the LF 2 Hz current was phase delayed (top panels) and the spike induction success rate declined (bottom panels) (6 neurons analyzed). Other parameters were as indicated for figure 7.
4.4 Discussion

Thalamic neurons receive two types of synaptic input; driver and modulator (Sherman 2001). GABAergic inputs to thalamic neurons are less likely to be drivers since the information transfer that can be achieved by the GABAergic input is inferior to the information transfer that can be achieved by the excitatory inputs (Sherman 2001). On the other hand, TCR neurons express T-type Ca\(^{2+}\) channels, which allows the detection and relay of the GABAergic input over certain ranges (Cagnan et al. 2009; Guo et al. 2008; Rubin and Terman 2004). Therefore, depending on the dynamic range of the TCR neuron (i.e. resting membrane potential and activity level) and frequency of the input, the GABAergic input from the GPi can act as either a driver or a modulator (Sherman 2001).

Depending on the resting membrane potential, TCR neurons, can respond either in tonic firing mode or in burst mode (Sherman 2001). TCR neurons respond in the tonic mode when T-type Ca\(^{2+}\) channels are inactivated and in burst mode when the inhibitory input is capable of de-inactivating T-type Ca\(^{2+}\) channels. Depending on the response mode of the TCR neurons, encoding of the input pattern differs. For instance when the TCR neuron is in the tonic mode and driven by a slow sinusoidal input, the spontaneous activity of the neuron is high and the mean response is also sinusoidal (Sherman 2001). On the other, when the neuron is in the burst mode, spontaneous activity is low and the neuron only fires at specific phases of the sinusoidal input. In this study, response of the TCR neuron when driven by the slow oscillatory input at 2 Hz matches the previously described response of the TCR neurons in burst mode (Sherman 2001; Smith et al. 2000). Moreover in agreement with Smith et al. (2000) we observe that when the input frequency is high, the TCR neuron does not spike at each cycle of the input but rather at sub-harmonics of the input (see also Supplementary Material). As the amplitude of the LF current is increased, the response shifts from sub-harmonic encoding of the inhibitory input to one-to-one encoding of the input frequency.

Several computational studies have investigated the BG network and its projection onto TCR neurons in the context of DBS and PD (Cagnan et al. 2009; Guo et al. 2008; Rubin and Terman 2004). Local field potential and micro electrode
recordings obtained from different DBS target nuclei (such as the STN or the GPi) have shown that during PD, BG nuclei exhibit continuous synchronized activity patterns in the beta frequency band and these activity patterns are suppressed following Levodopa administration or surgical interventions (i.e. lesioning or DBS) (Brown 2003; Kuhn et al. 2008; Kuhn et al. 2006). Rubin and Terman (2004) have noted that phasic inhibition of TCR neurons impairs relay capabilities of these neurons. Moreover, they have shown that HF stimulation of the STN replaces phasic inhibition of the TCR neurons and restores their relay capability. Based on a computational model, Cagnan et al. (2009) observed that during PD, TCR neurons are predominantly driven by the inhibitory input they receive from the BG output nucleus: GPi. Moreover, it was shown that there exists an inverse relationship between DBS frequency and stimulus intensity; required to restore the excitatory input from the cortex as the driver of the thalamocortical activity (Cagnan et al. 2009). The inverse relationship outlined in Cagnan et al. (2009) is a direct consequence of the BG network properties and membrane dynamics of the TCR neurons. This relationship is in line with experimental studies which have indicated that there exists an inverse relationship between DBS frequency and stimulus intensity required for suppression of PD motor symptoms (Benabid et al. 1991; Moro et al. 2002).

In-vitro studies exploring effects of DBS on TCR neurons made use of extra-cellular stimulation in order to investigate mechanisms underlying the efficacy of thalamic DBS (Anderson et al. 2006; Anderson et al. 2004; Kiss et al. 2002). Main focus of these studies has been identification of different pathways which are activated as a result of extra-cellular stimulation and whether these pathways have excitatory or inhibitory effects on TCR neurons (Anderson et al. 2006; Anderson et al. 2004; Kiss et al. 2002). In this study, down-stream effects of DBS and changes in thalamocortical membrane dynamics are investigated using current injection. Using current injection as input model enables us to look in detail to interactions between different components of an input; such as the response of the TCR neuron to an oscillatory input with two distinct frequency components.

Here, we explore the modulatory effect of HF input on a TCR neuron around its resting membrane potential. In the absence of HF input, the TCR neuron exhibits spiking locked to the LF
sinusoidal input, at 2 Hz. Superimposing the HF input on to the LF sinusoidal input, first delays the spiking activity of the TCR neuron and for higher amplitude HF current injection, completely suppresses the spiking activity locked to the LF sinusoidal input. There exists an inverse relationship between the frequency of the HF component and the threshold amplitude marking the transition from spiking activity locked to the LF sinusoidal input to sub-threshold oscillation at the frequency of the LF sinusoidal input. This result experimentally validates that cellular dynamics of the TCR neurons contribute to the inverse relationship observed previously in Cagnan et al. (2009)

HF component of the current injection modulates the response of the TCR neuron by interfering with the recovery from inactivation of the T-type Ca\(^{2+}\) current. As the amplitude of the HF current injection is increased, while keeping the frequency of the HF current injection constant, T-type Ca\(^{2+}\) current recovers more slowly from inactivation, phase delaying the spiking. If the amplitude of the HF current is increased even further, T-type Ca\(^{2+}\) current does not recover completely from inactivation and burst firing is suppressed. Frequency of the HF component directly influences the membrane potential which in turn is reflected in the membrane dynamics of the TCR neuron. For instance 30 Hz HF stimulation modulates membrane potential stronger than 130 Hz.

Soltesz and Crunelli (1992) investigated the role played by synaptic inputs in generating thalamocortical oscillations together with how frequency and phase of these oscillations could be modulated and if a single pulse could reset the response of a thalamocortical neuron away from exhibiting oscillatory activity patterns. They observed that intracellular depolarizing pulses applied at specific phases of the pacemaker oscillation gave rise to phase-resetting of spiking activity (Soltesz and Crunelli 1992). Moreover, application of a single pulse of critical amplitude and duration abolished pacemaker oscillations (Soltesz and Crunelli 1992).

In Cagnan et al. (2009) it was observed that suppression of the spiking activity driven by the inhibitory input from the GPi due to DBS occurred at specific phases of the phasic inhibition from the GPi. Building upon this observation, we studied the modulatory effect of pulses applied at specific phases of the LF sinusoidal input. Such pulses modulate the
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TCR neuron’s response to LF current injection in the same manner as HF current injection. When the PLPT is applied at specific phases of the LF current injection spiking locked to the slow oscillation are phase delayed. Parameters which determine the amount of phase delay are amplitude of the 2 Hz oscillation and PLPT amplitude. Also PLPT at 2 Hz can prevent spiking locked to the slow oscillation when applied at the “right time”. As the phase of the PLPT is altered, T-type Ca\(^{2+}\) current recovers more slowly from inactivation, giving rise to a delay of spiking activity locked to the slow oscillation. Prevention of spiking due to application of PLPT is due to the fact that the T-type of Ca\(^{2+}\) current does not completely recover from inactivation. These results indicate that, response of TCR neurons driven by slow oscillations can be modulated by train of pulses and lead to delay and eventually prevention of spiking locked to slow oscillations.

In summary, we investigated how HF oscillations modulate the spiking pattern exhibited by TCR neurons. Based on the observation that HF oscillations are effective in modulating TCR response at specific phases of the LF phasic input, we investigated whether activity patterns of the TCR neuron can be modulated using a PLPT. We observed that depending on the phase of the pulse train with respect to the LF oscillatory input, well timed pulses can mimic the modulatory effect HF oscillations have on TCR neurons.
4.5 Supplementary Materials

Figure SM: LF current injection at A: 4 Hz and at B: 6 Hz gave rise to spiking activity at sub-harmonics of the LF input.
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