Behavioral/Systems/Cognitive

Neuronal Basis of the Slow (≤1 Hz) Oscillation in Neurons of the Nucleus Reticularis Thalami In Vitro

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During deep sleep and anesthesia, the EEG of humans and animals exhibits a distinctive slow (≤1 Hz) rhythm. In inhibitory neurons of the nucleus reticularis thalami (NRT), this rhythm is reflected as a slow (≤1 Hz) oscillation of the membrane potential comprising stereotypical, recurring “up” and “down” states. Here we show that reducing the leak current through the activation of group I metabotropic glutamate receptors (mGluRs) with either trans-ACPD [(+/-)-1-aminoacycloptane-trans-1,3-dicarboxylic acid] (50–100 μM) or DHPG [(S)-3,5-dihydroxyphenylglycine] (100 μM) instates an intrinsic slow oscillation in NRT neurons in vitro that is qualitatively equivalent to that observed in vivo. A slow oscillation could also be evoked by synaptically activating mGluRs on NRT neurons via the tetanic stimulation of corticothalamic fibers. Through a combination of experiments and computational modeling we show that the up state of the slow oscillation is predominantly generated by the “window” component of the T-type Ca2+ current, with an additional supportive role for a Ca2+-activated nonselective cation current. The slow oscillation is also fundamentally reliant on an Ileak current and is extensively shaped by both Ca2+- and Na+ -activated K+ currents. In combination with previous work in thalamocortical neurons, this study suggests that the thalamus plays an important and active role in shaping the slow (≤1 Hz) rhythm during deep sleep.

Key words: sleep; EEG; rhythm; thalamus; calcium current; T-type; mGluR; CAN current

Introduction

During deep sleep and under certain types of anesthesia, the EEG of humans and animals is characterized by the slow (1 Hz) rhythm (Steriade et al., 1993a,b; Simon et al., 2000, 2003). In cortical neurons, this rhythm is correlated with alternating episodes of intense synaptic barrages and disfacilitation leading to distinct “up” and “down” membrane potential states (Steriade et al., 1993a, 2001; Contreras and Steriade, 1995; Sanchez-Vives and McCormick, 2000). In the thalamus, the cellular counterpart of the slow rhythm in both glutamatergic thalamocortical (TC) neurons and GABAergic neurons of the nucleus reticularis thalami (NRT) is also characterized by recurring up and down states (Steriade et al., 1993c; Contreras and Steriade, 1995; Timofeev and Steriade, 1996). However, in these cell types, up and down states appear to be formed more by a stereotypical slow (≤1 Hz) oscillation than simple variations in synaptic activity (Contreras and Steriade, 1995; Crunelli et al., 2002). Consistent with this, we recently showed that after activation of the metabotropic glutamate receptor (mGluR) that is post synaptic to cortical input, mGluR1a, TC neurons in vitro exhibit an intrinsic slow oscillation with identical properties to those observed in vivo (Hughes et al., 2002a). This result therefore challenged the notion that the slow rhythm in vivo is exclusively generated by cortical network operations (Steriade et al., 1993b; Sanchez-Vives and McCormick, 2000).

In TC neurons, the intrinsic slow oscillation arises when mGluR1a activation reduces the leak K+ current, Ileak, below a certain threshold where it can interact with the “window” component of the T-type Ca2+ current, ITwindow to produce a form of intrinsic bistability (Williams et al., 1997; Tóth et al., 1998; Hughes et al., 1999, 2002a). The slow oscillation in TC neurons also relies on a Ca2+-activated, nonselective cation (CAN) current (Hughes et al., 2002a; Crunelli et al., 2005). Because neurons in the NRT (1) exhibit a substantial IT window current (Huguenard and Prince, 1992; Klockner et al., 1999; Talley et al., 1999; Perez-Reyes, 2003), (2) are subject to a reduction in Ileak after mGluR activation (Lee and McCormick, 1997; Cox and Sherman, 1999), and (3) possess a prominent CAN current (Bal and McCormick, 1993), we recently predicted that activation of mGluRs could also bring about an intrinsic slow oscillation in this cell type (Crunelli et al., 2005).

In this study, we confirm that mGluR activation is a reliable pathway for inducing an intrinsic slow oscillation in NRT neurons in vitro with properties that are qualitatively equivalent to those observed in vivo (Steriade et al., 1986, 1993c; Contreras and Steriade, 1995; Timofeev and Steriade, 1996). We also show that this oscillation requires the specific stimulation of mGluR1a and that it depends primarily on ITwindow. This activity also involves a number of additional intrinsic conductances, which combine to shape its unique properties. This study, therefore, further endorses the idea that the thalamus actively influences the slow (≤1 Hz) rhythm in the whole brain.
Materials and Methods

Experiments were performed in accordance with local ethical committee guidelines and the United Kingdom Animals (Scientific Procedure) Act, 1986. All efforts were made to minimize the suffering and number of animals used in each experiment.

In vitro slice preparation and maintenance. Young adult cats (1–1.5 kg) were deeply anesthetized with a mixture of O2 and NO2 (2:1) and 2.5% halothane, wide craniotomies were performed, and the brains removed. Sagittal slices (450–500 μm) of the thalamus containing either the lateral geniculate nucleus (LGN), ventrolateral (VL) nucleus, or ventrobasal (VB) nucleus and the associated sectors of the NRT [i.e., perigeniculate nucleus (PGN), peri-VL sector, or peri-VB sector, respectively] were prepared and maintained as described previously (Hughes et al., 2002a). For recording, slices were perfused with a warmed [35 ± 2°C] continuously oxygenated (95% O2, 5% CO2) artificial CSF (ACSF) containing the following (in mM): 134 NaCl, 2 KCl, 1.25 KH2PO4, 1 MgSO4, 2 CaCl2, 16 NaHCO3, 10 glucose. For experiments involving NiCl2 or CdCl2, MgSO4 was replaced with MgCl2 and KH2PO4 was omitted. All drugs were dissolved directly in ACSF. Drugs were obtained from the following sources: DL-2-amino-5-phosphonovaleric acid (a-APS) (NMDA receptor antagonist), (+)-2-methyl-4-carboxyphenylglycine (LY367385) (mGlur1a selective antagonist), [S-(R*,R*)]-[3-[[1-(3,4-dichlorophenyl)[ethyl][amino]-2-hydroxypropyl](cyclohexylmethyl)phosphonic acid (CGP54626) (GABAa receptor antagonist), 6-cyano-7-nitroquinoline-2,3-dione (CNQX) (AMPA/kainate receptor antagonist), (S)-3,5-dihydroxyphenylglycine (DHPG) (group I selective mGluR agonist), 6-mono-3-(4-methoxyphenyl)-1(6H)-pyridazinethanobutenoic acid (SR95531) (GABAa receptor antagonist), (+/-)-1-amino cyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) (nonselective group II mGluR agonist), tetrodotoxin (TTX) (Na+ channel blocker), and 4-(N-ethyl-N-phenylamino)-1,2 dimethyl-6-(methylyamo)-pyrimidinium chloride (ZD7288) (h-channel blocker) from Tocris Cookson (Bristol, UK); apamin [small conductance KCa (SK) channel blocker] from Sigma (Poole, UK).

In vitro electrophysiology. Extracellular single-unit recordings were performed using glass pipettes filled with 0.5 M NaCl (resistance, 1–5 MΩ) connected to a Neurolog 104 differential amplifier (Digitimer, Welwyn Garden City, UK), with the resulting signals being filtered at 0.2–20 kHz. Intracellular recordings, using the current-clamp technique, were performed with standard or thin wall glass microelectrodes filled with 1 M potassium acetate (resistance, 80–120 MΩ or 30–60 MΩ, respectively), and in some cases 2% bicytion, and connected to an Axoclamp-2A amplifier (Molecular Devices, Foster City, CA) operating in bridge mode. Impaled cells were identified as NRT neurons using established electrophysiological and morphological criteria (Uhlrich et al., 1991; Bal and McCormick, 1993; Contreras et al., 1993). The apparent input resistance (Ri) was estimated from voltage responses evoked at −60 mV by small (20–50 pA) hyperpolarizing current steps. Only neurons with overshooting action potentials and an Ri>50 MΩ were selected for further experimentation and analysis. In slices where neurons had been filled with biocytin, visualization of the dye was performed as described previously (Hughes et al., 2002b). Voltage and current records were stored on a Biologic DAT recorder (IntraCel, Royston, UK) and later analyzed using Clampfit (Molecular Devices). The effect of apamin on extracellular recordings was assessed after the slices had been exposed to this drug for at least 30 min. In all experiments, statistical significance was assessed using Student’s t test.

Stimulation of corticothalamic fibers was performed in sagittal slices selected for further experimentation and analysis. In slices where neurons had been filled with biocytin, visualization of the dye was performed as described previously (Hughes et al., 2002b). Voltage and current records were stored on a Biologic DAT recorder (IntraCel, Royston, UK) and later analyzed using Clampfit (Molecular Devices). The effect of apamin on extracellular recordings was assessed after the slices had been exposed to this drug for at least 30 min. In all experiments, statistical significance was assessed using Student’s t test.
of the LGN-PGN using a bipolar tungsten electrode placed in the optic radiation (50 Hz for 500 ms, with each stimulus being a 0.2 ms pulse of 300 µA). Thalamic stimulation was also performed in sagittal LGN-PGN slices, using identical parameters, with the bipolar electrode positioned in lamina A of the LGN. All stimulation experiments were performed in the presence of CNQX (10 µM), APV (50 µM), SR95531 (20 µM), and CGP54626 (20 µM).

Dynamic clamp and computational modeling. The dynamic clamp system was implemented as described previously (Hughes et al., 1998, 1999, 2002a). The model NRT neuron was of the single-compartment Hodgkin and Huxley (1952) type and possessed the following voltage- and intracellular ion-gated currents: a fast inactivating Na⁺ current, \( I_{Na} \); a delayed rectifier K⁺ current, \( I_{Kdr} \); a low-threshold, T-type Ca²⁺ current, \( I_{CaT} \) (Huguenard and Prince, 1992); a hyperpolarization-activated, h-type current, \( I_{h} \) (Brunton and Charpak, 1997); a Ca²⁺-activated K⁺ current, \( I_{CaK} \) (Bal and McCormick, 1993); an Na⁺-activated K⁺ current, \( I_{NaK} \) (Kim and McCormick, 1998); and a Ca²⁺-activated, nonselective cation current, \( I_{CaNa} \) (Bal and McCormick, 1993; Destexhe et al., 1994). The descriptions of \( I_{CaK} \) and \( I_{h} \) were based on corresponding currents in TC neurons (Tóth and Crusnelli, 2001). The description of \( I_{CaNa} \) was constructed by adapting the experimental results of Huguenard and Prince (1992). The description of \( I_{NaK} \) was based on the experimental findings of McCormick and Pape (1990) in TC neurons with parameters modified to reflect the behavior of NRT neurons. \( I_{CaNa} \) was modeled by using the experimental results of Huguenard and Prince (1992) and assuming a linear dependence of the conductance on the local intracellular Ca²⁺ concentration. For \( I_{CaNa} \), we adopted the model given in Dale (1998). The description of \( I_{CaNa} \) was based on that constructed for TC neurons (Hughes et al., 2002a) but with modified parameter values, which take into account the differences between the two neuron types. Finally, the model also included a leak current, \( I_{leak} \), the conductance of which was voltage-independent. The membrane capacitance \( C_m \) and reversal potentials \( E_{rev} \) were the conductances of the NRT neurons were directly estimated from experimental data using the method described in Tóth and Crusnelli (2001) and are as follows:

\[
C_m = 63.3 \text{ pF}; E_{Kdr} = -95 \text{ mV}, E_{KNa} = 180 \text{ mV}; E_{Ca} = 55 \text{ mV}; E_{Na} = 964 \text{ mV}; E_{CaNa} = 180 \text{ mV}, g_{Ca} = 17 \text{ nS}; g_{Na} = -33 \text{ mV}, g_{Na} = 8 \text{ nS}; E_{CaNa} = 10 \text{ mV}, g_{Ca} = 20 \text{ nS}; E_{leak} = -65.3 \text{ mV}, g_{leak} = 1.5 \text{ nS}; g_{CaNa} = 3 \text{ nS}; g_{CaNa} = 100 \text{ nS}.
\]

The processing of the local intracellular ion concentrations was modeled according to

\[
dc/dt = pl - \gamma(c - c_{eq}),
\]

where \( c \) is the local intracellular concentration of the ions, \( I_{leak} \) is the current carrying the ions into the cell, and \( \rho = 9 \times 10^{-5} \text{ nA} \cdot \text{cm}^{-2} \cdot \text{s}^{-1} \) for Ca²⁺ and \( 5 \times 10^{-5} \text{ nA} \cdot \text{cm}^{-2} \cdot \text{s}^{-1} \) for Na⁺. The mean \( R_{ion} \) of these cells was 130.7 ± 13.6 MΩ (n = 61). All neurons exhibited electrophysiological (Fig. 1A–1C) and morphological (Fig. 1A) characteristics consistent with previous descriptions of this cell type (Uhlrich et al., 1991; Bal and McCormick, 1993; Contreras et al., 1993). In particular, at the offset of sufficiently large hyperpolarizing current steps, 87% (n = 53 of 61) of NRT neurons generated one or more low-threshold Ca²⁺ potentials (LTCPs), which gave rise to bursts of action potentials that displayed a distinctive accelerating-decelerating pattern (Fig. 1A,2A–2B) (Domich et al., 1986). In addition, these LTCPs were often ensued by a transient period of rhythmic single-spike activity (Domich et al., 1986; Bal and McCormick, 1993; Brunton and Charpak, 1997). Application of the nonspecific group I/II mGluR agonist trans-ACPD (100 µM) caused a depolarization of NRT neurons such that, in the absence

**Results**

Activation of mGluR1a instates an intrinsic slow (<1 Hz) oscillation in neurons of the NRT

In control conditions, NRT neurons recorded intracellularly exhibited either a quiescent, stable resting membrane potential (−63.4 ± 2.4 mV; n = 50 of 61; 82%) (Fig. 1A1) or spontaneous tonic action potential firing at low frequencies (5.8 ± 1.7 Hz; n = 11 of 61; 18%). The mean R_{ion} of these cells was 130.7 ± 13.6 MΩ (n = 61). All neurons exhibited electrophysiological (Fig. 1A1) and morphological (Fig. 1A) characteristics consistent with previous descriptions of this cell type (Uhlrich et al., 1991; Bal and McCormick, 1993; Contreras et al., 1993). In particular, at the offset of sufficiently large hyperpolarizing current steps, 87% (n = 53 of 61) of NRT neurons generated one or more low-threshold Ca²⁺ potentials (LTCPs), which gave rise to bursts of action potentials that displayed a distinctive accelerating-decelerating pattern (Fig. 1A2,A3) (Domich et al., 1986). In addition, these LTCPs were often ensued by a transient period of rhythmic single-spike activity (Domich et al., 1986; Bal and McCormick, 1993; Brunton and Charpak, 1997). Application of the nonspecific group I/II mGluR agonist trans-ACPD (100 µM) caused a depolarization of NRT neurons such that, in the absence

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**Figure 2.** The slow (<1 Hz) oscillation forms part of a continuum of activity in NRT neurons. A. Intracellular recording of an NRT neuron in the PGN in the presence of trans-ACPD (100 µM) displaying a continuum of oscillatory activity at different levels of DC current. Sections marked above are expanded in B. Injection of sufficient hyperpolarizing DC current abolished all oscillatory activity (−650 pA). As this negative DC current was gradually removed, a diverse range of oscillatory activity became evident. Initially, rhythmic LTCP bursts were present (−520 pA). As the negative DC current was further removed, a slow oscillation was present in isolation (−480 pA). Removal of further DC current abolished the slow oscillation and led to continuous tonic firing (−400 pA). B. Expanded sections as indicated in A. CNQX (10 µM), APV (50 µM), SR95531 (20 µM), and CGP54626 (20 µM) were present in the recording medium for the experiment depicted in this figure.
of any DC current, all cells displayed continuous tonic firing (Fig. 1B1, top trace, 0 pA) (37.8 ± 4.3 Hz; n = 42). trans-ACPD also led to a 25% rise in Rm (162.7 ± 10.4 MΩ; n = 42) (Fig. 1B2).

In addition to responding to trans-ACPD with a robust depolarization and increase in Rm, a substantial proportion of NRT neurons also became able to generate a slow (<1 Hz) membrane potential oscillation (n = 27 of 61; 44%) (Fig. 1B3), middle trace, −410 pA (Crunelli et al., 2005) when they were hyperpolarized via the injection of DC current. This slow oscillation was manifested as alternating up and down states of the membrane potential that were separated by ~10–15 mV. During the up state, NRT neurons displayed sustained tonic firing, whereas during the down state, these cells were quiescent and often exhibited a slow sag-like depolarization (Fig. 1B3). The transition from up to down state was typically characterized by a clear inflection point in the membrane potential, whereas the switch from down to up state was punctuated by the presence of one or more LTCPs and accompanying bursts of action potentials (see below) (Fig. 1B3). The frequency of the slow oscillation ranged from 0.02–0.7 Hz with a mean minimum frequency across a population of cells of 0.16 ± 0.04 Hz (n = 20). A higher Rm was associated with the ability to display a slow oscillation (oscillating neurons: Rm = 156.7 ± 14.3 MΩ, n = 16; nonoscillating neurons: Rm = 132.0 ± 24.9 MΩ, n = 21), although this was not found to be statistically significant (p = 0.26). No major differences in the incidence of the slow oscillation were observed between different sectors of the NRT (PGN: 18 of 37, 49%; peri-VB sector: n = 7 of 18, 39%; peri-VL sector: n = 2 of 6, 33%).

**Figure 3.** The capacity to generate a slow (<1 Hz) oscillation is blocked by the selective mGluR1 antagonist LY367385. A, Intracellular recording of an NRT neuron in the peri-VB sector exhibiting a slow oscillation in the presence of trans-ACPD (100 μM) (A1). Addition of LY367385 (250 μM) (A2). The capacity to generate a slow oscillation (A3). In this condition, depolarization of the neuron from a state of hyperpolarized quiescence to continuous firing through the injection of DC current (see arrows for specific values) does not reveal a slow oscillation. After washout of LY367385, the neuron spontaneously depolarizes again and recovers its capacity to exhibit a slow oscillation (A3). The sections marked by continuous bars are expanded on the right. B, Effect of tetanically stimulating CT fibers in an NRT neuron from the PGN at levels of initial membrane potential close to ~75 mV (B1). At ~74 mV, the poststimulus response is an isolated, long-lasting self-sustained up state (top trace). At ~76 mV, this initial up state is shorter and is followed by a second spontaneous up state, thus leading to a brief slow oscillation (second trace from the top). A similar effect is observed at ~77 mV, where a transient slow oscillation comprising three consecutive up states is apparent (third trace from the top). At ~80 mV, CT stimulation leads to a long lasting EPSP (bottom trace) (compare Hughes et al. (2002a), their Fig. 5). The generation of stimulus-dependent up states and the slow EPSP are both abolished by LY367385 (250 μM) (B2). Note, in the absence of CT stimulation, this neuron was unable to generate a slow oscillation or any type of spontaneous self-sustained up states. C, Two consecutive responses (left and right traces) of an NRT neuron in the PGN to tetanic CT and thalamic stimulation show that the mGluR1-dependent slow EPSP is a specific response to activating the CT pathway. D, Histograms summarizing the properties of the poststimulus response at ~80 mV after CT stimulation in control conditions (Con) and in the presence of LY367385 (LY), and after thalamic stimulation (Thal) (**p < 0.01; n = 5). In both B and C, the stimulus protocol consisted of 25 pulses of 300 μA lasting 0.2 ms delivered at 50 Hz. CNQX (10 μM), APV (50 μM), SN95531 (20 μM), and CGP54626 (20 μM) were present in the recording medium for the experiments depicted in B–D. Error bars indicate SE.
NRT neurons while closely scrutinizing the effect of trans-ACPD on their mode of activity. In control conditions, the majority (n = 27 of 46; 59%) of extracellular recordings displayed a lack of spontaneous activity (supplemental Fig. 1A, available at www.jneurosci.org as supplemental material). The remainder comprised either rhythmic LTCP-mediated bursts at δ frequencies (1.3 ± 0.1 Hz; n = 10), low-frequency tonic firing (4.1 ± 1.0 Hz; n = 5), or a spontaneous slow oscillation (0.14 ± 0.05 Hz; n = 4). When recording from sites that initially lacked any firing, application of trans-ACPD led to a progressive increase in activity, which always culminated in continuous tonic firing (supplemental Fig. 1A, available at www.jneurosci.org as supplemental material).

However, while passing between a state of no activity and tonic firing, the majority (n = 16 of 27; 59%) of NRT neurons transiently displayed a slow oscillation (supplemental Fig. 1A, available at www.jneurosci.org as supplemental material). In some cases (n = 4), the slow oscillation was preceded by rhythmic LTCP-mediated bursts at δ frequencies (supplemental Fig. 1A, available at www.jneurosci.org as supplemental material) so that trans-ACPD application was able to elicit the full repertoire of NRT behavior observed with intracellular recordings (Fig. 2). In circumstances in which extracellular NRT recordings did not exhibit a slow oscillation during trans-ACPD application, we observed a direct transition from quiescence to tonic firing (n = 11 of 27; 41%) (data not shown). Thus, at appropriate intensities of mGluR activation, a slow oscillation constitutes the normal activity of a large proportion of NRT neurons. Indeed, application of a lower concentration of trans-ACPD (50 μM) led to a sustained slow oscillation being present in 59% (n = 16 of 27) of extracellular recordings (see Figs. 4 D, E, 6 B).

The action of trans-ACPD in bringing about a slow oscillation in NRT neurons was mimicked by the group I selective mGluR agonist, DHPG (100 μM). When assessed with intracellular recordings, addition of DHPG was associated with a tonic depolarization beyond the action potential threshold (mean frequency of tonic firing, 13.7 ± 5.5 Hz; n = 6) and a pronounced increase in R_C (control, 110.1 ± 16.4 MΩ; DHPG, 142 ± 12.2 MΩ; n = 6), with a slow oscillation being evident after steady hyperpolarization in 50% of neurons (n = 3 of 6). During extracellular recordings, DHPG application induced a change from quiescence to tonic firing in all cases (n = 10), with this transition occurring via a slow oscillation in 80% of recordings (n = 8 of 10) (supplemental Fig. 2A, available at www.jneurosci.org as supplemental material). The effects of trans-ACPD and DHPG observed with extracellular recordings were prevented when the mGluR1-specific antagonist, LY367385 (250 μM), was included in the recording medium (n = 4) (data not shown), suggesting that the ability of NRT neurons to generate a slow oscillation was dependent on the specific activation of mGluR1a. This was confirmed by intracellular recordings showing that the capacity to produce a slow oscillation after trans-ACPD application was consistently and reversibly blocked by LY367385 (250 μM) (Fig. 3A) (n = 3 of 3).

To test whether the synaptic activation of mGluRs could also induce a slow oscillation, we examined the response of NRT neurons in the PGN to the tetanic stimulation of corticothalamic (CT) fibers. When neurons were held at an initial membrane potential close to −75 mV, a substantial proportion (n = 4 of 9; 44%) exhibited self-sustained up states after the offset of the stimulus epoch that could last for several seconds (5.7 ± 2.8 s; n = 4) (Fig. 3B) and which were similar to those observed spontaneously during the agonist-induced slow oscillation. Furthermore, in some cases these initial stimulus-induced up states could be closely enshey by one or two additional up states, thus constituting a slow oscillation (mean frequency, 0.14 ± 0.03 Hz; n = 4). When neurons were hyperpolarized to approximately −80 mV, the generation of up states no longer occurred in response to CT
stimulation. Instead, in this condition the poststimulus response was a long-lasting, slow EPSP (peak amplitude, 5.0 ± 0.5 mV; duration, 12.1 ± 2.5 s; n = 4) (Fig. 3B1, bottom trace, D). Both, the stimulus-induced up states and the slow EPSP were suppressed by LY367385 (250 μM) (n = 3) (Fig. 3B2, D). Thus, the effects of exogenously activating mGluR1a in bringing about a membrane depolarization and slow oscillation can also be achieved via a physiological release of glutamate after the stimulation of CT fibers. Notably, however, a similar effect was not attainable after tetanic stimulation of the thalamocortical pathway after placement of a stimulating electrode in lamina A of the LGN (Fig. 3C, D).

**Analysis of the different manifestations of the slow (<1 Hz) oscillation in NRT neurons and their modification by membrane polarization**

As already indicated, the essential appearance of the slow oscillation comprised the rhythmic alternation of distinct up and down membrane potential states with the up state supporting intense action potential firing in every case (see Figs. 1–7; supplemental Figs. 1, 2, 4, available at www.jneurosci.org as supplemental material). This firing generally decelerated as the up state progressed from a maximum frequency of 50.1 ± 8.5 Hz (range, 6.5–125 Hz; n = 16) at the start of the up state to a minimum of 9.4 ± 1.4 Hz (range, 2.3–18.1 Hz; n = 16) at its end. In 70% of intracellular and 62% of extracellular (n = 19 of 27 and n = 20 of 32, respectively) recordings, the switch from down to up state was always marked by the generation of a single LTCP-mediated burst of action potentials (“basic” slow oscillation) (Figs. 2, 4A, D; supplemental Fig. 2, available at www.jneurosci.org as supplemental material). This was true regardless of the DC current level or oscillation frequency. However, the remainder of cells (intracellular, n = 8 of 27, 30%; extracellular, n = 12 of 32, 38%) could exhibit a group of rhythmic LTCP-mediated bursts at this transition point (“grouped LTCP” slow oscillation) (Figs. 4B, E, 5B, 6C; supplemental Fig. 1, available at www.jneurosci.org as supplemental material). In some cases, these burst sequences were so pervasive that they essentially replaced the down state of the slow oscillation (Figs. 4E1, 5B; supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Rhythmic burst sequences occurred at a range of frequencies (1.3–8.2 Hz; n = 10) that encroaches on both the δ (1–4 Hz) and sleep spindle (7–14 Hz) frequency bands, with the precise interburst frequency usually (n = 18 of 20; 90%) increasing as the sequence progressed (Fig. 4C, F). In some neurons, this increase was subtle (Fig. 4B1), whereas in other cells, the increase was more conspicuous and associated with an obvious, underlying depolarizing envelope in intracellular recordings (Fig. 4B2).

The overall frequency of the basic manifestation of the slow oscillation in NRT neurons decreased with depolarization (Figs. 2, 5). This occurred through a gradual increase in the duration of the up state and the consequent steady decline in the incidence of a down state, the properties of which were largely insensitive to membrane polarization (Fig. 5A). For slow oscillations involving grouped LTCP episodes, depolarization also caused an increase in the duration of the up state. However, in these oscillations, the down state could also be dynamic. This was especially true in the initial stages of depolarization, where a reduction in the number of LTCP-mediated bursts and an increase in their interburst frequency were often observed (Fig. 5B). The sensitivity of the properties of rhythmic LTCP burst sequences to membrane polarization meant that the relationship between DC current and slow oscillation frequency for grouped LTCP slow oscillations was not straightforward, often exhibiting an initial increase followed by a clear decline (Fig. 5B2).

**The slow (<1 Hz) oscillation is resistant to blockade of Na⁺ channels**

The slow oscillation in NRT neurons is dependent on intrinsic ionic mechanisms because it was routinely observed in the presence of blockers of ionotropic glutamate, GABA_A, and GABA_A.
receptors (CNQX, 10 μm; APV, 50 μm; SR95531, 20 μm; CGP54626, 20 μm; n = 12) (Figs. 4 D, E; supplemental Figs. 1, 2, available at www.jneurosci.org as supplemental material). The slow oscillation was also resistant to the application of TTX (1 μm) (n = 4 of 4) (Fig. 6A), suggesting that (1) it arises as a direct activation of postsynaptic mGluR1a receptors, (2) it is not reliant on Na⁺ channels, and (3) it does not require an Na⁺-activated K⁺ current (Iₓ(Na)) (Kim and McCormick, 1998; Bhattacharjee and Kaczmarek, 2005). TTX did, however, cause a clear decrease in the duration of the down state (15.8 ± 6.6 vs 5.3 ± 0.3 s; n = 4; p < 0.1) (Fig. 6A), an effect that might be related to a secondary inhibition of Iₓ(Na) (Fig. 9B). In TC neurons, the instatement of a slow oscillation only occurs when Iₓ leak is reduced below a specific threshold (Williams et al., 1997; Tóth et al., 1998; Hughes et al., 1999). To test whether some NRT neurons lack a slow oscillation because mGluR1a activation in these cells does not sufficiently reduce Iₓ leak, we artificially induced a further suppression of this current using a dynamic-clamp system in non-oscillating NRT neurons that had been subjected to TTX (Hughes et al., 1999, 2002a) (supplemental Fig. 3A, available at www.jneurosci.org as supplemental material). In all cases (n = 3 of 3), for a certain level of additional artificial Iₓ leak reduction (−5.2 ± 0.5 nA), we were able to induce a slow oscillation (Fig. 3B). Thus it appears that the ability to generate a slow oscillation is a general property of NRT neurons, which becomes evident when Iₓ leak is reduced below a particular threshold.

**Blocking Ca²⁺-activated K⁺ channels with apamin dramatically lengthens the period of the slow (<1 Hz) oscillation**

NRT neurons possess a prominent Ca²⁺-activated K⁺ current (Iₓ(Ca)), which profoundly influences the electroresponsiveness of these cells (Bal and McCormick, 1993). To investigate whether Iₓ(Ca) plays a role in determining the properties of the slow oscillation, we assessed the effect of the SK channel blocker apamin on NRT neurons exhibiting this activity. When apamin (200 nM) was applied during extracellular recordings of spontaneous slow oscillations that had been induced by 50 μM trans-ACPD (see above), we consistently observed a dramatic decrease in the frequency of the oscillation (0.02 vs 0.006 Hz; n = 6). This decrease was brought about by a progressive lengthening of both the up (18.9 ± 4.2 vs 141.3 ± 40.5 s; n = 6; p < 0.01) and down states (15.6 ± 1.1 vs 28.3 ± 3.3 s; n = 6; p < 0.001) (Fig. 6B). Apamin also blocked the generation of grouped LTPC epsodes, when they were present, such that after its application, all up states commenced with a single LTPC-mediated burst (n = 2) (data not shown). Intracellular recordings revealed a similar pattern to that observed extracellularly and showed that the lengthening of the oscillation period was also accompanied by a significant increase in the level of hyperpolarization reached during the down state (−76.7 ± 0.9 vs −86.3 ± 0.9 mV; n = 4; p < 0.001) (Fig. 6C).

**The slow (<1 Hz) oscillation requires the activation of Iₖ**

In most, although not all, instances of the slow oscillation, the down state was characterized by a slow or sag-like depolarization of the membrane potential (Figs. 1, 2, 5A, 4B, 5, 6A, 7A, top trace) (but see Figs. 4A, B, 6G, preapamin trace). A similar slow depolarization could also be observed in the voltage responses to large hyperpolarizing current steps illustrating that it is a fundamental property of NRT neurons (Fig. 7A, control in bottom traces) (Brunton and Charpak, 1997). The slow depolarization observed in response to large current steps was abolished by the potent and selective h-channel blocker ZD7288 (30 μM) (Bo-Smith et al., 1993) (n = 8) (Fig. 7A, ZD7288 in bottom traces). Predictably, block of the slow depolarization also led to neurons being unable to generate a slow oscillation (n = 5) (Fig. 7B). Importantly, this was true regardless of whether the down state of the slow oscillation exhibited an overt slow depolarization (n =
TTX to NRT neurons exhibiting a slow oscillation. In this condition, NRT neurons were still able to generate clear equivalent up states when challenged with small depolarizing current steps \((n = 2)\) (Fig. 7C). Interestingly, these equivalent up states were commonly characterized by a low amplitude (\(\sim 3-6\) mV from peak to peak) oscillatory activity (mean frequency, \(8.4 \pm 1.9\) Hz; \(n = 2\)) (Fig. 7C).

**The up state of the slow (<1 Hz) oscillation is dependent on T-type Ca\(^{2+}\) channels**

In TC neurons, the mechanism underlying the generation of the up state in the slow oscillation relies critically on the window component of the T-type Ca\(^{2+}\) current (i.e., \(I_{T\text{window}}\)) (Crunelli et al., 2005). To test whether a similar mechanism might also be involved in the generation of up states in NRT neurons, we compared the effect of equal concentrations of Ni\(^{2+}\) and Cd\(^{2+}\) ions on the activity of slow oscillating NRT neurons that had been subjected to ZD7288. In doing so we were able to show that whereas application of Cd\(^{2+}\) (250 \(\mu\)M) \((n = 3)\) was unable to block the generation of equivalent up states (Fig. 8C), Ni\(^{2+}\) (250 \(\mu\)M) caused a reversible abolition of these events in all cases \((n = 3\) of 3) (Fig. 8A–C) (Beurrier et al., 1999). Because Ni\(^{2+}\) is much more effective at blocking T-type Ca\(^{2+}\) channels than Cd\(^{2+}\) (Crunelli et al., 1989) this result strongly suggested that \(I_{T\text{window}}\) might also be fundamental to the generation of the up state of the slow oscillation in NRT neurons. However, an alternative explanation is that the up state is generated purely by the action of a CAN current, which is activated by Ca\(^{2+}\) entry through T-type channels (Bal and McCormick, 1993; Destexhe et al., 1994), and which would, therefore, also be preferentially inhibited by Ni\(^{2+}\) rather than Cd\(^{2+}\).

**Computer simulations**

To fully understand the mechanism of the slow oscillation, we constructed a biophysically realistic model of an NRT neuron. This model reliably recreated the main features of the slow oscillation including (1) a clear membrane potential inflection at the transition from up to down state, (2) a slow depolarization during the down state, (3) the presence of rhythmic LTCP burst sequences at the switch from down to up state for certain levels of DC current, and (4) decelerating tonic firing during the up state (Fig. 9A). Consistent with experimental results, the slow oscillation in the model did not require Na\(^{+}\) channels (Fig. 9B1–B3). However, in similarity to the effect of TTX, a suppression of the transient Na\(^{+}\) current led to a clear shortening of the down phase (8.0 s with \(g_{\text{Na}} = 964\) nS vs 2.4 s with \(g_{\text{Na}} = 0\) nS for a DC current of \(-50\) pA) (compare Figs. 6A, 9A, 10B2,B3). Importantly, further excluding \(I_{\text{CAN}}\) from the model did not prevent a slow oscillation, although in this condition, the oscillation displayed a greatly reduced amplitude (12 vs 21 mV from peak to peak) (Fig. 9B3). With \(I_{\text{Na}}\) and \(I_{\text{CAN}}\) reinstated to the model, specific inhibition of \(I_{K(\text{Na})}\) caused a decrease in the duration of the down state (9.6 s with \(g_{\text{Na}} = 0\) nS vs 5.6 s for a DC current of \(-50\) pA) (compare Figs. 6A, 9A, 10B3,B3). This suggests that the reduction in the length of the down state observed after TTX application in experiments is indeed caused by a secondary inhibition of \(I_{K(\text{Na})}\) (Fig. 6A). As in experiments, an inhibition of \(I_{K(\text{Ca})}\) (i.e., \(g_{K(\text{Ca})} = 0\) nS) caused both a lengthening of the slow oscillation period (15.0 vs 25.2 s for a DC current of \(-50\) pA) and an augmentation of the level of hyperpolarization reached during the down state (\(-90\) vs \(-78\) mV) (Fig. 9C; compare Fig. 6C).

Regardless of the values of \(g_{\text{Na}}, g_{K(\text{Na})}\), and \(g_{K(\text{Ca})}\), and as--
pected from experiments, the model was unable to reproduce a slow oscillation when either \( I_\text{T} \) or \( I_\text{Ca} \) were absent. Also in line with our experimental results, during a simulated block of \( I_\text{Na} \), self-sustained equivalent up states could still be generated through the injection of brief depolarizing current pulses (Fig. 10A). In further accordance with experiments, these equivalent up states were resistant to the removal of \( I_\text{Na} \) (Fig. 10B) but abolished when \( I_\text{T} \) was absent (Fig. 10C). To test whether or not \( I_\text{Ca} \) is crucial for the generation of equivalent up states, the behavior of the model was scrutinized in the absence of this current (i.e., \( E_\text{Ca} = 0 \text{ mV} \)). In this condition, equivalent up states could still be produced for certain values of DC current, albeit with a reduced duration (Fig. 10D). This is consistent with the finding that a slow oscillation can be still be generated in the absence of \( I_\text{Ca} \) (Fig. 10B), and further supports the hypothesis that equivalent up states are dependent on \( I_\text{Twindow} \).

To confirm this, we reinstated \( I_\text{Ca} \) to the model and specifically reduced \( I_\text{Twindow} \) by shifting the steady-state inactivation curve of \( I_\text{T} \) by 3 mV in the negative direction (Fig. 10E, top plot). By performing this shift we ensured that the transient activation of \( I_\text{T} \) was essentially unchanged but that \( I_\text{Twindow} \) was virtually eliminated (Hutcheon et al., 1994; Williams et al., 1997) (Fig. 10E, bottom plot). Under these conditions, the model was unable to generate equivalent up states (Fig. 10E). Importantly, for this set of simulations, we adjusted the equation governing the local intracellular \( Ca^{2+} \) processing \((\rho = 1.08 \times 10^{-4} \text{ mV} \cdot \text{pA}^{-1} \cdot \text{s}^{-1})\) so that its time course (i.e., peak value and time to decay) after transient \( I_\text{T} \) activation matched that apparent for the control scenario (comparison not shown). This guaranteed that the time course of \( I_\text{Ca} \) also matched that occurring in control simulations and enabled us to be fully confident that the effect on voltage responses of shifting the steady-state inactivation curve of \( I_\text{T} \) was a consequence of reducing \( I_\text{Twindow} \).

Finally, when \( I_\text{T} \) was reintroduced to the model, we found that the 3 mV hyperpolarizing shift in the steady-state inactivation curve of \( I_\text{T} \) prevented a slow oscillation from being generated (data not shown). Thus, equivalent up states, and therefore the slow oscillation, are critically reliant on the presence of \( I_\text{Twindow} \). A detailed summary of the ionic mechanisms of the slow oscillation in NRT neurons is given in supplemental Figure 5, available at www.jneurosci.org as supplemental material.

**Discussion**

The main findings of this study are (1) reduction of \( I_\text{Ca} \) via exogenous or synaptic activation of mGluR1a in NRT neurons in vitro leads to the instatement of an intrinsic slow (<1 Hz) oscillation with similar properties to those observed in vivo during deep sleep (Domich et al., 1986; Steriade et al., 1986) and certain types of anesthesia (Steriade et al., 1993c; Contreras and Steriade, 1995; Steriade et al., 1996; Timofeev and Steriade, 1996), (2) the slow oscillation is predominantly reliant on \( I_\text{Ca} \) and \( I_\text{Na} \), and (3) the properties of the slow oscillation are also influenced by the activity of a CAN current (\( I_\text{Ca} \)), a \( Ca^{2+} \)-activated K\(^+\) current (\( I_{\text{KCa}} \)) and an Na\(^+\)-activated K\(^+\) current (\( I_{\text{KNa}} \)). This study is the first to expound the mechanisms of the slow oscillation in NRT neurons and endorses the idea that the slow (<1 Hz) sleep rhythm is actively shaped by the thalamus (Hughes et al., 2002a).

**Comparison with the slow (<1 Hz) oscillation observed in NRT neurons in vivo**

The slow oscillation in NRT neurons in vitro is qualitatively similar in several respects with that observed in vivo (Steriade et al., 1993c; Contreras and Steriade, 1995; Steriade et al., 1996; Timofeev and Steriade, 1996); thus, the model appears to be able to reproduce the slow oscillation in NRT neurons in vivo. However, there are differences in the properties of the slow oscillation in NRT neurons in vitro and in vivo. In vivo, the slow oscillation is not affected by anesthesia (Steriade et al., 1993c; Contreras and Steriade, 1995; Steriade et al., 1996; Timofeev and Steriade, 1996), whereas in vitro, the slow oscillation is abolished by anesthesia (Steriade et al., 1993c; Contreras and Steriade, 1995; Steriade et al., 1996; Timofeev and Steriade, 1996). This suggests that the slow oscillation in NRT neurons in vitro is not a true representation of the slow oscillation in NRT neurons in vivo.
network oscillations in the whole brain. Rather, it is likely that, in NRT neurons, the slow oscillation frequency (see below) might be differentially regulated in vivo. Either way, intrinsic up and down states in NRT neurons endow these cells with the ability to “resonate” within the frequency of slow waves, which in turn would actively reinforce ongoing network oscillations.

A role for various neurotransmitters in supporting the slow oscillation

A recent in vitro study has shown that thyrotropin-releasing hormone (TRH) is also able to bring about a slow oscillation in NRT neurons (Broberger and McCormick, 2005) with similar properties to those shown here and by us previously (Crunelli et al., 2005). Because TRH and mGluR agonists both decrease \( I_{\text{leak}} \) in NRT neurons, it appears that the slow oscillation is a latent property of these cells in vitro that can be supported by the activation of several different neuromodulatory pathways that are active in vivo and which target this current. Consistent with this, decortication, a procedure that eliminates the cortical mGluR pathway to the thalamus, drastically reduces the proportion of NRT neurons that exhibit a slow oscillation in vivo (to 8%) (Timofeev and Steriade, 1996), a finding that fits well with our experiments showing that electrical stimulation of corticothalamic fibers in vitro can bring about a slow oscillation via mGluR1a activation. That a small amount of NRT neurons in vivo still generate a slow oscillation after decortication is paralleled by our finding that a minority of NRT neurons recorded extracellularly in vitro demonstrate a spontaneous slow oscillation in control conditions (9%), and is also coherent with activation of other receptors apart...
from mGluR1a being able to induce this activity. Nevertheless, for the slow oscillation to be present to any great extent in NRT neurons in vivo, an intact cortical input appears to be necessary. This ultimately points to the cortex as having primary responsibility for initiating the slow rhythm, a proposal backed up by its presence in vivo after thalamectomy (Steriade et al., 1993b).

The ionic basis of the slow (<1 Hz) oscillation in NRT neurons

Although not demonstrating a spontaneous slow oscillation, a previous in vitro investigation has highlighted the ability of NRT neurons to generate intrinsic plateau potentials, which are similar to the equivalent up states shown here (Kim and McCormick, 1998). However, at odds with our results is the finding that these plateau potentials are dependent on persistent Na⁺ channels. Interestingly, a recent in vivo study has also ascribed a role for a persistent Na⁺ current in the generation of plateau potentials in NRT neurons (Fuentebalba et al., 2005). In our study, TTX did not block either the slow oscillation or equivalent up states. However, TTX did initially cause a specific block of tonic action potential firing during the up state while sparing the action potentials comprising the bursts marking the transition between the down and up state. Our initial suspicion was that this was because of a preferential block of persistent Na⁺ channels by TTX (Tennigkeit et al., 1998). However, a similar result was obtained in the model, where a reduction of transient Na⁺ current mimicked the early effect of TTX. Thus, a persistent Na⁺ current plays no role in the slow oscillation in NRT neurons. Rather, both experiments and modeling show that equivalent up states, and therefore the slow oscillation, are primarily dependent on $I_{\text{window}}$.

A common, although not ubiquitous, feature of the slow oscillation was a slow or sag-like depolarization during the down phase. Because this was also present after TTX treatment, it could not be attributed to an Na⁺-activated K⁺ current (Kim and McCormick, 1998; Bhattacharjee and Kaczmarek, 2005). Rather, it is caused by an $I_h$ current because it was consistently blocked by the selective h-channel blocker, ZD7288 (BoSmith et al., 1993). This is consistent with the observation of similar sag-like potentials in NRT neurons by others (Brunton and Charpak, 1997; Zhang and Jones, 2004), and with the intense localized expression of the HCN2 subunit, and moderate expression of the HCN3 and HCN4 subunits, in the rat NRT (Monteggia et al., 2000; Notomi and Shigemoto, 2004). In all cases, blocking the slow depolarization with ZD7288 also eliminated the slow oscillation demonstrating that $I_h$ is crucial to this activity. An identical result was obtained when $I_h$ was removed from our computational model.

Although not central to slow oscillation generation, $I_{\text{K(Ca)}}$ plays an important role in controlling its frequency because apamin caused a substantial increase in the duration of both the up and down states. After apamin treatment, it also became much easier to observe a slow depolarization during the down state. This is important because in some instances of the slow oscillation, a clear slow depolarization was not evident. However, one such oscillation was still abolished by the h-channel blocker ZD7288, suggesting that $I_h$ is active in cells lacking an obvious slow depolarization but that its effect is partially occluded. Such occlusion may be attributable to the presence of a large $I_{\text{K(Ca)}}$ in these cells, which has a shunting effect during the down state.

Consistency with previous in vitro studies and a proposed role for electrical synapses in synchronizing the NRT slow oscillation

A recent study using slices from young rats has also examined the effects of mGluR activation on NRT neurons (Long et al., 2004). In this investigation, the authors describe the appearance of subthreshold membrane potential oscillations at ~10 Hz. Although

![Diagram](image-url)
under normal conditions we did not see similar activity in our study, oscillations at close to 10 Hz were sometimes observed during equivalent up states recorded in the presence of TTX. This suggests that while not being overt under normal conditions, a 10 Hz oscillation is still present in cat NRT neurons and might influence firing rate during the slow oscillation up state. The 10 Hz oscillation described by Long et al. (2004) was synchronized between closely situated cells via electrical synapses (Landisman et al., 2002). Because evidence for electrical synapses is present in recordings from NRT neurons in the adult cat in vivo (Fuentesalba et al., 2004), the possibility exists that electrical synapses might play a role in synchronizing the slow oscillation. Indeed, the particular effectiveness of electrical synapses at transmitting low-frequency events (Landisman et al., 2002; Long et al., 2004) makes them ideally suited to this role. A similar scenario might also exist for TC neurons (Hughes et al., 2004), where electrical synapses are also present (Hughes et al., 2002b), raising the prospect that a certain degree of slow (<1 Hz) wave synchronization can occur locally in the thalamus.

References
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