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NeuReal: an interactive simulation system for implementing artificial dendrites and large hybrid networks

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Abstract

The dynamic clamp is a technique which allows the introduction of artificial conductances into living cells. Up to now, this technique has been mainly used to add small numbers of ‘virtual’ ion channels to real cells or to construct small hybrid neuronal circuits. In this paper we describe a prototype computer system, NeuReal, that extends the dynamic clamp technique to include i) the attachment of artificial dendritic structures consisting of multiple compartments and ii) the construction of large hybrid networks comprising several hundred biophysically realistic modelled neurons. NeuReal is a fully interactive system that runs on Windows XP, is written in a combination of C++ and assembler, and uses the Microsoft DirectX application programming interface (API) to achieve high-performance graphics. By using the sampling hardware-based representation of membrane potential at all stages of computation and by employing simple look-up tables, NeuReal can simulate over 1000 independent Hodgkin and Huxley type conductances in real-time on a modern personal computer (PC). In addition, whilst not being a hard real-time system, NeuReal still offers reliable performance and tolerable jitter levels up to an update rate of 50 kHz. A key feature of NeuReal is that rather than being a simple dedicated dynamic clamp, it operates as a fast simulation system within which neurons can be specified as either real or simulated. We demonstrate the power of NeuReal with several example experiments and argue that it provides an effective tool for examining various aspects of neuronal function.

Keywords

Dynamic Clamp; Thalamus; Oscillations; Computer Simulation; Gap Junctions

INTRODUCTION

The dynamic clamp is a technique which allows computer-simulated conductances to be introduced into living cells in real-time (Robinson and Kawai, 1993; Sharp et al., 1993a,b). The system operates by continuously sampling the membrane potential of an excitable cell at relatively high rates (typically 10-50 kHz) and then based on these samples and a set of differential equations stored in a computer which describe one or more voltage- and time-dependent conductances, calculating and injecting the membrane current that would result in the cell if these conductances were actually present (Fig. 1A). As such, the dynamic clamp creates ‘virtual’ conductances in real cells with the properties of these conductances being under the complete control of the experimenter. Whilst not being without its limitations, the most obvious being the introduction of current at a point source, this technique has proved to be a valuable tool for probing the function of excitable cells (Prinz et al., 2004). This is particularly true for neurons where the dynamic clamp has been extensively used to

introduce both virtual intrinsic conductances, in order to examine the basic properties of single cells, as well as artificial synaptic conductances in order to construct and investigate small hybrid neuronal networks (Prinz et al., 2004).

To provide accessible dynamic clamping several research groups have constructed their own dynamic clamp systems (reviewed in Prinz, et al., 2004; but see also Nowotny, et al., 2006). Each of these systems has its own particular advantages and features with some emphasising ease-of-use and installation (Pinto et al., 2001; Nowotny et al., 2006) whilst others have focused on achieving hard real-time performance (Butera et al., 2001; Dorval et al., 2001; Kullmann et al., 2004; Raikov et al., 2004; Müniz et al., 2005). However, in nearly all cases, these systems have been designed to allow the introduction of a relatively small number of conductances (typically <20) or to construct hybrid circuits comprising only a few cells. In this paper we describe a Windows XP-based prototype computer system, NeuReal, which extends the normal remit of the dynamic clamp system in two key ways. Firstly, this system allows the easy attachment of artificial, i.e. simulated, dendritic tree structures, comprising multiple compartments, to real neurons (Fig. 1B) (Hughes et al., 1998b,1999a; Dorval et al., 2001). Secondly, it facilitates the construction of hybrid neuronal networks that can contain several hundred biophysically realistic modelled cells (Fig. 1B). In delivering these two features, NeuReal provides a novel approach for investigating two technically demanding questions: i) How does dendritic structure affect the integrative properties of individual neurons? and ii) How are the properties of single neurons shaped by extensive network activity?

NeuReal currently employs the Axon Digidata 1200 to perform analogue to digital (ADC) and digital to analogue (DAC) conversions and can operate reliably at sampling/update rates of up to 50 kHz. The simulation speed of NeuReal is achieved by maintaining the sampling hardware-based representation of membrane potential at all stages of computation, using simple look-up tables to compute expensive functions and by utilising direct hardware access for performing all graphics operations during experiments. In tests run on a modern PC, at a step-size of 0.1 ms NeuReal was able to simulate 1380 Hodgkin and Huxley type conductances in real-time. However, even on a modestly-powered PC possessing a 700 MHz Pentium processor we were able to use NeuReal to attach an artificial dendritic tree structure comprising 155 compartments, each possessing up to 4 distinct active conductances, to a real thalamocortical (TC) neuron maintained *in vitro*. Importantly, rather than being simply a dedicated dynamic clamp system, NeuReal is essentially a simulation system within which individual neurons can be specified as either real or simulated. We believe that this represents a clear conceptual departure from more specialized dynamic clamp systems and one which provides a highly flexible experimental tool for studying neuronal function.

METHODS

Fast current computation

Fundamental to the construction of NeuReal is the ability to rapidly simulate realistic neurons and networks. The following section briefly outlines how such computations are carried out. Some of these details have already been published in descriptions of an earlier version of NeuReal in preliminary form (Hughes et al., 1998b,1999a). All neurons were modelled according to the discretized cable equation:

$$C_m dV_k/dt = I_{\text{parent}} + \Sigma I_{\text{child}} - (\Sigma I_{\text{intrinsic}} + \Sigma I_{\text{synaptic}} + I_{\text{inj}}) \quad [1]$$

where C_m is the membrane capacitance, V_k is the membrane potential of compartment k , $I_{intrinsic}$ and $I_{synaptic}$ are the sum of intrinsic ionic currents and synaptic currents respectively, I_{inj} is the injected current and I_{parent} and I_{child} represents the current contributions of the parent compartment and a child compartment respectively and are given by:

$$I_{parent} = \gamma_k (V_{parent} - V_k) \quad [2]$$

$$I_{child} = \gamma_{child} (V_{child} - V_k) \quad [3]$$

where γ_k is the coupling conductance between the parent compartment and compartment k , γ_{child} is the coupling conductance between compartment k and a child compartment, and V_{parent} and V_{child} are the respective membrane potentials of the parent and child compartments. Both γ_k and γ_{child} are of the form $\gamma_k = (\pi \cdot d_k^2) / (4R_a \cdot l_k)$ where d_k and l_k are the diameter and length of compartment k and R_a is the axial resistivity.

Each voltage-dependent intrinsic ionic current, $I_{intrinsic}$, was modelled using standard Hodgkin and Huxley formalism (Hodgkin and Huxley, 1952) as follows:

$$I_{intrinsic} = g_{max} m^p h^q \cdot (V_m - E_r) \quad [4]$$

Where g_{max} is the maximal conductance, m and h are the activation and inactivation variables respectively (with p and q their respective indices), and V_r is the reversal potential. Both m and h are governed by the first order equation:

$$dx/dt = (x_{\infty} - x) / \tau_x \quad (x=m \text{ or } h) \quad [5]$$

where $x_{\infty} = f(V_m)$ gives the steady-state value of x and $\tau_x = f(V_m)$ gives the time constant of activation or inactivation.

Synaptic currents are modelled in NeuReal according to the methods described by Destexhe et al. (1994). Briefly, the relationship between the concentration of neurotransmitter in the synaptic cleft, $[T]$, and presynaptic voltage, V_{pre} , is approximated by:

$$[T] = T_{max} / [1 + \exp(-(V_{pre} - 2)/5)] \quad [6]$$

where T_{max} is the maximal concentration of transmitter and is usually taken as 1mM. The kinetics of the synaptic current are then modelled according to the scheme:

where C and O represent channels in closed and open states respectively, and α and β are voltage-independent forward and backward rate constants. If r is defined as the fraction of receptors in the open state, then it can be described by:

$$dr/dt = \alpha [T] (1 - r) - \beta r \quad [8]$$

and the postsynaptic current is given by:

$$I_{synaptic} = g_{max} r (V_m - V_r) \quad [9]$$

As indicated above, the calculation of an intrinsic ionic current $I_{\text{intrinsic}}$ at some point in time, t , and for some membrane potential, V_m , is performed according to equations [4] and [5]. If it is further assumed that V_m remains constant for some integration interval Δt then we can solve [5] analytically which gives:

$$x_{t+\Delta t} = x_{\infty} - (x_{\infty} - x_t) \exp(-\Delta t / \tau_x) \quad [10]$$

In NeuReal, both x_{∞} and τ_x need to be calculated in real-time on the basis of the most recently acquired (for dynamic clamp experiments) or calculated (for pure simulations) V_m value. However, because this usually involves the costly computation of exponential functions, and since V_m is represented by the Digidata board as a 12 bit signed integer value which can only take on 4096 different values, it is more efficient if x_{∞} and τ_x are represented in look-up tables which are indexed directly by the value obtained from the Digidata board or calculated by the system. Although the range of x is usually from 0-1, it is profitable (see below) to map these values onto the interval (0,255) before tabulation. Also, because equation [10] still contains an exponential term, it is advantageous to assume a constant sampling interval, Δt , and tabulate $\exp(-\Delta t / \tau_x)$ instead of merely τ_x (Hughes et al., 1998b, 1999a; see also Butera et al., 2001). Two disadvantages of this approach are: i) if Δt changes, then the table has to be rebuilt, although this is not a problem unless there is a need for a constantly changing sampling rate, and ii) it is difficult to incorporate interactive acceleration and deceleration into the control of kinetics, i.e. including an on-the-fly alterable scaling factor λ , which is incorporated as follows:

$$\exp(-\Delta t / (\lambda \tau_x)) \quad [11]$$

If, however, it is assumed that λ can only take on a finite set of 16 values, say for example 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8 & 2.0, then using a two-dimensional array of floating point numbers for $\exp(-\Delta t / (\lambda \tau_x))$, which is indexed by V_m and i_{idx} (i.e. a cross reference index ranging from 0 to 15 and which corresponds to 0.1 to 2.0 as listed above). With this approach, calculation of an activation or inactivation variable requires one look-up, one floating point multiplication, two floating point subtractions, one integer addition and a 4 bit shift operation. To compute the proportion of open channels, i.e. $m^p h^q$, another table which gives $m^p h^q$ based on two indices, m & h which have been mapped onto the interval (0,255) (see above) can be used. To obtain a value from this table, we simply need one look-up and one bitwise shift operation. If we wish to keep the maximal conductance and reversal potential as interactively alterable variables then in order to calculate the final intrinsic ionic current value, two additional floating multiplications and a subtraction are required (see equation [4]). Synaptic currents can be considered as a special case of an intrinsic current where $r \equiv m$, $p=1$, $q=0$, $r_{\infty} = \alpha / (\alpha + \beta)$ and $\tau_r = 1 / (\alpha + \beta)$. Although the kinetics are now simplified due to a lack of voltage-dependence, it is also now necessary to build a 4096 entry table for the function described in equation [6]. Electrical synapses were considered as a special case whereby $r=1$ and V_r is replaced by V_{pre} in equation [9].

If it is intended to incorporate currents calculated in the above manner to a compartment of a neuronal model (i.e. equation [1]) then as long as the membrane capacitance is specified in the correct units (pF), no further scaling needs to be performed (since the same number representing 1pA for I , represents 1mV for V_m). The resulting V_m value can then be used to index the activation, inactivation and time constant tables and facilitate further current computation. If, on the other hand, the calculated currents are to be injected into a real neuron via the Digidata 1200 and Axoclamp then hardware scaling issues must be taken into account.

Although the above procedures are described for the 12 bit integer format of membrane potential provided by the Digidata 1200 board they can easily be adapted for higher resolution formats (e.g. 16 and 18 bit). Also, whilst the choice of 8 bit resolution for look-up tables for inactivation and activation variables is so that the system can function on modest computer hardware with limited memory, the accuracy can obviously be improved by increasing the resolution of the activation and inactivation variables when more memory is available, e.g. to 16 or 32 bits, with no loss in performance. Lastly, further performance improvements can be easily achieved at the expense of on-the-fly interactive control, e.g. by including the maximal conductance, driving force and, in the case of current injection, the hardware scaling as part of a look-up table (Butera et al., 2001)

Hardware interfacing and performance

The Digidata 1200 is an ADC/DAC device that is able to simultaneously sample and output 12 bit signals at up to 333 kHz. In NeuReal the Digidata is programmed in assembler such that voltage output signals are updated asynchronously by simply writing to the appropriate DAC data register whereas acquisition of input signals is timed by the on board clock. To ensure a fast access to acquired data the main simulation loop of NeuReal continuously polls the ADC FIFO (first in, first out) empty flag (-EF in the ADC/DAC control register) to check for the arrival of a new sample. As soon as this sample is available, the next action to take place is the writing of the most recently computed output current value. This ensures that the true time between outputted values is as close to the integration step-size as possible. To assess performance of the system we used the low level 64-bit RDTSC (read time stamp counter) Pentium assembler command which provides a precise readout of the number of CPU clock cycles that have elapsed since the last processor reset. Thus, by calling this instruction during every simulation cycle for a large number of consecutive cycles, storing it in a temporary array and then writing this array to a text file at the end of the experiment, we can obtain accurate statistics regarding the jitter of the system (see also Butera et al., 2001).

Programming, graphics and key features of NeuReal

All other programming and user interface design and construction were done with Microsoft Visual C++. The organisation of the primary classes in NeuReal, which mainly comprise neurons, dendritic trees, conductances and synapses is shown in Fig. 2A. Templates of basic objects are constructed using easy-to-use editing windows and can then be stored in a NeuReal database file. Based on these templates a network is then constructed which is the primary object upon which an experiment or simulation is performed (see results). To provide high-performance graphics for visualizing experimental data we used the DirectX library. This library contains a collection of routines which allow direct access to the graphics memory space and which can take advantage of the onboard acceleration features that are present on different graphics cards. Fig. 2B shows the main interface and the basic oscilloscope display and interactive control panel components. NeuReal also provides a facility for recording up to 6 channels of data during an experiment which can then be replayed or exported to a text file. The source code for Neureal can be downloaded from www.thalamus.org.uk/html/software.html.

In vitro electrophysiology

Procedures involving experimental animals were carried out in accordance with local ethical committee guidelines and the U.K. Animals (Scientific Procedure) Act, 1986. All efforts were made to minimize the suffering and number of animals used in each experiment. Young adult cats (1-1.5 kg) were deeply anaesthetized with a mixture of O₂ and NO₂ (2:1) and 2.5% isoflurane, a wide craniotomy performed and the brain removed. Sagittal slices (450-500 µm) of the thalamus containing the dorsal lateral geniculate nucleus (LGN) were

prepared and maintained as described previously (Hughes et al., 2002a,b; Hughes et al., 2004). For recording, slices were perfused with a warmed (35 ± 1 °C), continuously oxygenated (95% O₂, 5% CO₂), artificial cerebrospinal fluid (ACSF) containing (mM): NaCl (134); KCl (2); KH₂PO₄ (1.25); MgSO₄ (1); CaCl₂ (2); NaHCO₃ (16); glucose (10). Intracellular recordings, using the current clamp technique, were performed with standard-wall glass microelectrodes filled with 1M potassium acetate (resistance: 80-120 MΩ) and connected to an Axoclamp-2A amplifier (Axon Instruments, Foster City, USA) operating in bridge mode. Impaled cells were identified as TC neurons using established electrophysiological criteria (Hughes et al., 2002a,b; Hughes et al., 2004).

RESULTS

Basic operation and testing of NeuReal

Any experiment in NeuReal is essentially a simulation of a network object which must consist of at least one neuron. Each neuron is based on a neuron type or template which in turn is built up from a variety of other objects such as intrinsic and synaptic conductances and dendritic tree structures (Fig. 2A). Up to 2 neurons in any one network can be specified as real meaning that their somatic membrane potential is obtained from a real neuron via a distinct recording electrode, amplifier and the ADC hardware, rather than being computed, and that all somatic currents calculated for that neuron are actually injected via the amplifier and recording electrode. Thus, to perform a basic dynamic clamp experiment involving the injection of one or more artificial conductances to a single cell, a network is created consisting of one neuron that possesses the required number and type of intrinsic conductances and which is specified as real. Additional neurons can then be added and modified within the network to produce increasingly complex types of hybrid or purely simulated cells and networks. Thus, NeuReal acts primarily as a real-time simulation system that is fully interactive, i.e. parameters can be modified on demand, and with which different types of dynamic clamp experiments can be performed yet represent merely a subset of possible experiments.

To test the capacity of NeuReal to simulate large numbers of conductances and neurons in real-time we first constructed a simple one compartment TC neuron template possessing a leak conductance, a fast inactivating Na⁺ current (I_{Na}), a delayed rectifier K⁺ (I_{Kdr}), an inactivating T-type Ca²⁺ current (I_T) and a hyperpolarization-activated mixed cation current (I_h) (see Appendix) (Toth and Crunelli, 1992; Hughes et al., 1998a,1999b; Emri et al., 2000). We then built a network object consisting of an ever increasing quantity of purely simulated instances of these neurons and assessed at which point the ability of NeuReal to simulate them at faster than real-time broke down (Fig. 3A). For an integration step-size of 0.1 ms, equivalent to a update/sampling rate of 10 kHz in a dynamic clamp experiment, on a PC possessing a 2.26GHz Intel Pentium processor and 2 GB of RAM, NeuReal could simulate 345 neurons at sub-real-time speeds (Fig. 3A). This equates to the parallel simulation of 690 inactivating and 690 non-inactivating conductances or, in other words, 2070 independent activation/inactivation variables.

One of the main technical challenges when performing dynamic clamp experiments is limiting and controlling the amount of jitter, i.e. the variability in the update cycle interval (Butera et al., 2001; Dorval et al., 2001; Raikov et al., 2004). This is a particular problem when using operating systems that are not designed for real-time tasks such as Windows. Therefore, to test the amount of jitter present in NeuReal we collected data for 200000 samples over 20 trials from the Digidata board at sampling frequencies from 10 to 100 kHz. Because different scheduling priorities can be assigned to programs running on Windows we repeated this procedure for the priority classes NORMAL, ABOVE_NORMAL, HIGH and REALTIME. In doing so we found that jitter predictably increased with update frequency

and became generally worse as program priority was lowered (Fig. 3B). However, despite obviously not matching the performance achieved with hard real-time systems, we deemed that up to 50 kHz the REALTIME priority class displayed a tolerable level of variability (Fig. 3B, filled black squares).

We next tested the ability of NeuReal to perform a basic dynamic clamp function. In particular, we compared the results of injecting an artificial T-type Ca^{2+} current (I_T) ($g_T=60$ nS) and hyperpolarization-activated mixed cation current (I_h) ($g_h=40$ nS) into a passive 'model' cell (Axon Instruments CLAMP-1U model cell) (Fig. 3C₂) with those from a pure simulation using the same currents in a neuron with identical passive properties (input resistance=50 M Ω and membrane capacitance=470 pF) (Fig. 3C₁). We also compared the results of these experiments with a pure simulation performed using conventional floating point operations (Fig. 3C₃). As shown in Fig. 3C, the output in each scenario was virtually indistinguishable (Fig. 3C₄). We also tested the ability of NeuReal to perform a basic dynamic clamp experiments on a real TC neuron. Specifically, we demonstrated the ability of NeuReal to recreate the I_T -mediated low-threshold Ca^{2+} potential (LTCP) following block of endogenous I_T with Ni^{2+} (Fig. 4) (Hughes et al., 1999b). This experiment also allowed us to highlight the value of providing the capacity to interactively alter key parameters such as the acceleration and deceleration of kinetics and maximal conductances because we often found that to achieve an accurate replication of the original LTCP required modifications in the kinetics (Fig. 4B).

Implementation of artificial dendrites

To test the implementation of artificial dendrites we made additional intracellular recordings from TC neurons of the cat LGN and attached a 155 compartment dendritic tree structure possessing various types of ion channel distributions. This dendritic tree was based on the morphology of an X cell in the LGN (Fig. 5) (Bloomfield et al., 1987; Emri et al., 2000) (see Appendix) and possesses a simplified symmetrical branching pattern. To illustrate the ability of NeuReal to operate efficiently on a modestly powered computer, these experiments were run with a PC possessing a 700 MHz Pentium processor and 256 MB of RAM. In a first set of experiments we injected a simple positive current pulse at the soma whilst designating the dendritic tree as fully passive. In doing so we noted a minimal backpropagation of somatic action potential activity into the dendritic tree that was substantially attenuated beyond the first branch point (Fig. 5A). We then repeated this experiment with an active dendritic tree possessing a uniform distribution of I_{Na} and I_{Kdr} channels (25 and 12 mS/cm², respectively) (Fig. 5B). In this scenario, we noted that action potentials actively propagated into distal portions of the dendritic tree with minimal attenuation (Fig. 5B).

Previous modelling work has suggested that the ability of TC neurons to generate intrinsic δ oscillations at ~1-2 Hz (McCormick and Pape, 1990; Leresche et al., 1991) is not solely dependent on somatic T-type channels but is also influenced by dendritic T-type channels and the balance between the conductance of dendritic T-type channels and K^+ channels (Emri et al., 2000). To test this we performed a second series of experiments where 8 identical artificial dendritic trees with a high uniform concentration of T-type channels (2 mS/cm²) as in (Emri et al., 2000) were attached to a real LGN TC neuron that was otherwise not exhibiting spontaneous oscillatory activity (Fig. 6). In doing so, we found that characteristic oscillatory activity could be induced at the soma following artificial dendritic attachment as long as i) sufficient dendritic I_h was present (0.5 mS/cm²) and ii) the dendritic leak conductance was reduced from 0.062 to 0.04 mS/cm² (Fig. 6A and B). In these experiments, we also included a small amount of I_{Kdr} (2 mS/cm²). Interestingly, because of the small amount of K^+ conductance present on the dendrites in this scenario, introduction of a even moderate amount of dendritic Na^+ conductance (6 mS/cm²) led to oscillations taking

on a more paroxysmal appearance in the dendrites that was clearly reflected at the soma (Fig. 6B).

Hybrid networks

In a final set of experiments, we tested the ability of NeuReal to implement a large hybrid network simulation. Again, these experiments were performed with a modestly powered PC possessing a 700 MHz Pentium processor. Our previous work has indicated that the cat LGN contains a specialized subset of TC neurons which show intrinsic rhythmic bursting at ~10 Hz (high-threshold, HT, burst cells) and which are connected by electrical synapses (Hughes et al., 2004; Hughes and Crunelli, 2005). These properties lead to the generation of waxing and waning field oscillations at 10 Hz resembling *in vivo* alpha rhythms (Hughes et al., 2004; Hughes and Crunelli, 2007). To test the effect of these network oscillations on conventional tonic firing, i.e. relay mode, TC neurons which can also be linked to HT burst cells via electrical synapses (Hughes et al., 2002a), we constructed an artificial HT burst cell network and connected it via artificial electrical synapses to a real tonic firing TC neuron (Fig. 7).

The artificial network consisted of 49 HT bursting cells that were randomly interconnected by electrical synapses such that on average each cell was connected to 2.2 others (Fig. 7A). All HT bursting cells had equivalent properties (see Appendix) except that a random amount of steady current between 40 and 60 pA was applied to each cell. Under these conditions, the isolated network generated a waxing and waning population oscillation at ~8 Hz which closely resembled that observed in recent experiments (Fig. 7B) (Hughes et al., 2004; Hughes and Crunelli, 2007). Examination of individual cells showed that this waxing and waning was related to spontaneous and apparently random shifts in synchrony between individual cells (Fig. 7B). We then connected the network to a real tonic firing TC neuron by 3 additional electrical synapses originating from distinct HT burst cells (Fig. 7A and D). This led to the tonic firing TC neuron receiving a clear rhythmic modulation of membrane potential from the network (Fig. 7D, bottom left) with this modulation able to powerfully influence spike timing (Fig. 7D, bottom right).

DISCUSSION

We have described a Windows XP-based prototype computer system, NeuReal, which provides an easy-to-use fast simulation platform for facilitating the attachment of multi-compartmental artificial dendrites to real neurons and for implementing large hybrid neuronal networks. We demonstrate this system with a series of simple experiments in LGN TC neurons which highlight both the feasibility of the artificial dendrite concept as well as the benefits of examining large hybrid networks.

Until relatively recently, dendrites were considered to be passive structures that act to transmit distally-generated synaptic potentials to the site of action potential initiation. It is now well known though that such structures are endowed with a large variety of active ionic conductances which contribute greatly to determining the integrative properties of individual neurons (Hausser et al., 2000). However, the practical difficulties associated with directly investigating active dendritic structures are widely acknowledged and present considerable technical issues (Davie et al., 2006). This is especially true for distal or thin dendrites (Nevian et al., 2007) and for non-planar structures which can be easily compromised in brain slice preparations (Williams and Stuart, 2000). One solution to these problems is to use multicompartmental models which are constrained by a combination of available data and the requirement to realistically reproduce specific somatic activities (De Schutter and Bower, 1994; Traub et al., 1994; Mainen and Sejnowski, 1996; Emri et al., 2000). Despite the value of this approach an obvious drawback is that the results are to some extent always

dependent on estimation and affected by incomplete data. The attachment of artificial dendrites to real neurons provides a compromise between direct electrical recording of dendrites and pure modelling. For example, whilst simulated dendrites are subject to the same issues that affect a pure simulation, the signals under investigation in these dendrites are at least partly dictated by the activity of a real neuron (e.g. Fig. 5). Similarly, it allows examination of how different types of dendritic morphology and ion channel distributions can influence the electrical behaviour of a real soma (e.g. Fig. 6).

Since we first introduced the concept of artificial dendrites (Hughes et al., 1998b, 1999a) we are only aware of one other publication which has explored this idea (Dorval et al., 2001). However, to our knowledge, the current study is the first to demonstrate its application to real neurons. As such, we provide results which endorse the view that low frequency (~1-2 Hz) oscillatory activity in LGN TC neurons involves an active interaction of both somatic and dendritic T-type Ca^{2+} channels (Emri et al., 2000). Our results also indicate that a reduction of dendritic leak K^{+} conductance may be an important element in bringing about such oscillations. This is consistent with the known pro-oscillatory effects of activating metabotropic glutamate receptors (mGluRs) on TC neurons which are known to be located on distal dendrites and which are negatively coupled to leak K^{+} channels (Hughes et al. 2002b; Hughes et al., 2004).

We have also demonstrated the ability of NeuReal to implement a sizeable hybrid network. This enabled us to show how a specialized group of electrically coupled pacemaker cells might influence relay mode firing in conventional tonic firing TC neurons. More generally, and by employing more powerful computers, NeuReal offers the viable opportunity to examine how the activity of real neurons is shaped by being embedded in networks of several thousand biophysically realistic modelled cells which are entirely under the interactive control of the experimenter.

Compared to other systems which provide dynamic clamping the main advantage of NeuReal is the ability to rapidly construct and simulate a large number of intrinsic and synaptic conductances. The system is easy to use, possessing an intuitive Windows interface for object editing, rolling oscilloscope-like displays for visualizing data, a fully interactive experimental interface that allows all key parameters to be altered on demand and the ability to record, replay and export data. NeuReal offers a reasonable level of performance up to a sampling rate of 50 kHz. However, unlike other systems, NeuReal does not deliver so-called hard real-time performance, whereby the timing of the input and output of analogue voltage signals is precisely controlled and deterministic within a set of strict criteria, and because of the nature of the Windows operating system jitter is not normally distributed with occasionally long delays of a several milliseconds occurring. The accuracy of the system is also limited by the tabulation techniques and by the 12-bit integer representation of membrane potential provided by the ADC/DAC board. Whilst we fully acknowledge the importance of these issues our initial primary objective was solely to build a prototype system that could demonstrate the feasibility of implementing realistic artificial dendrites and large hybrid networks, and which could do so in an easy to use manner and on modest and readily available hardware. Having achieved this, our next aim is to address the more precise demands of hard real-time computing and enhance the accuracy of the numerical methods whilst maintaining a high level of performance and usability. With respect to this, whilst other systems which deliver hard real-time performance have used extensions to the Linux operating system (Butera et al., 2001; Dorval et al., 2001; Raikov et al., 2004; Mūniz et al., 2005), the emergence of extensions to the Windows XP and Windows Vista operating systems that also offer hard real-time performance might provide a powerful combination of ease-of-use and high performance. Indeed, our preliminary tests using real-time extensions to Windows XP indicate that such a system is a viable prospect. We also plan to implement

the use of more advanced ADC/DAC hardware interfaces and provide support for importing neuronal models built with other systems (e.g. Bower and Beeman, 1997; Hines and Carnevale, 1997; Gleeson et al., 2007) along similar, but ultimately more generic, lines to the RT-NEURON system (Debay et al., 2004). RT-NEURON is a real-time version of the NEURON simulation environment which operates under the linux operating system and which provides similar functionality to the system described here. It has been used extensively in a number of studies in recent years (Derjean et al., 2003; Rudolph et al., 2004; Wolfart et al., 2005; Pospischil et al., 2007).

Finally, an important aspect of NeuReal is that rather than being simply a dedicated dynamic clamp system, it is essentially a modelling system which allows the construction of hybrid real-artificial neuron simulations within which simple dynamic clamp experiments are just a small subset. As such, we believe that NeuReal represents a clear conceptual departure from more specialized dynamic clamp systems and has significant potential as a research tool which, if used appropriately taking into consideration the well known general limitations of dynamic clamp experiments (Prinz et al., 2004), greatly extends the range of experimental options that are immediately available to the investigative electrophysiologist.

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APPENDIX

Description of voltage-gated currents

In the following descriptions, g_x and E_x are the respective maximal conductances and reversal potentials.

Fast inactivating Na^+ current, I_{Na} :

$$I_{\text{Na}} = g_{\text{Na}} m^3 h (V - E_{\text{Na}})$$

$$\tau_m = 1 / (\alpha_m + \beta_m) \text{ and } m_{\infty} = \alpha_m \tau_m$$

$$\tau_h = 1 / (\alpha_h + \beta_h) \text{ and } h_{\infty} = \alpha_h \tau_h$$

$$\alpha_m = 0.32 (V + 49.3) / [1 - \exp(-(V + 49.3) / 4)]$$

$$\beta_m = 0.28 (V + 22.3) / [\exp((V + 22.3) / 5) - 1]$$

$$\alpha_h = 0.128 \exp(-(V + 45.4) / 18)$$

$$\beta_h = 4 / [\exp(-(V + 22.4) / 5) + 1]$$

Delayed rectifier K^+ current, I_{Kdr} :

$$I_{\text{Kdr}} = g_{\text{Kdr}} m^4 (V - E_{\text{K}})$$

$$\tau_m = 1 / (\alpha_m + \beta_m) \text{ and } m_\infty = \alpha_m \cdot \tau_m$$

$$\alpha_m = 0.159 (V + 53.7) / [1 - \exp(-(V + 53.7) / 5)]$$

$$\beta_m = 2.48 \cdot \exp(-(V + 55.2) / 40)$$

Low-threshold Ca^{2+} current, I_T :

$$I_T = g_T m^3 h (V - E_{\text{Ca}})$$

$$m_\infty = 1 / \{1 + \exp[-(V + 63) / 7.8]\}$$

$$h_\infty = 1 / \{1 + \exp[(V + 83.5) / 6.3]\}$$

$$\tau_m = \lambda_m [2.44 + 0.0250 \exp(-0.0984 \cdot V)]$$

$$\tau_h = \lambda_h [19.15 + 0.07171 \exp(-0.1054 \cdot V)]$$

Where $\lambda_m = \lambda_h = 1.0$, unless otherwise stated, and $\lambda_m = 0.1$ and $\lambda_h = 0.3$ for the HT burst cell model (Hughes et al., 2004).

Hyperpolarization-activated mixed cation current, I_h :

$$I_h = g_h m^3 h (V - E_h)$$

$$m_\infty = 1 / \{1 + \exp[(V + 75) / 5.5]\}$$

$$\tau_m = 20820 \exp(0.0614V) \text{ if } V \leq -77.58$$

$$\tau_m = 29.54 \exp(-0.0458V) \text{ if } V > -77.58$$

For all simulations we also included a leak K^+ current with a reversal potential of -95 mV.

Geometric properties of the artificial dendritic tree

The dendritic tree structure used in this study was identical to that used in the recent modelling study by Emri et al. (2000). Briefly, it has a somato-dendritic path of symmetrical geometry with 5 levels of hierarchy and bifurcations at each level. The respective lengths of each level, starting from the most proximal, are 20, 17.5, 4, 55 and 75 μm and their respective diameters are 3.7, 2.3, 1.5, 0.9 and 0.6 μm . Each dendritic branch is subdivided into 5 independent compartments of less than 0.05 electrotonic length (L). The axial resistivity, $R_a = 0.33 \Omega\text{cm}$ and the membrane capacitance, $C_m = 1 \mu\text{F}/\text{cm}^2$. The membrane resistivity, $R_m = 15 \text{ k}\Omega\text{cm}^2$ m and was adjusted to yield a total electrotonic length of $L = 0.53$.

HT burst cell network

HT burst cells were modelled as a single compartment with a surface area of $5000 \mu\text{m}^2$ giving a total membrane capacitance of 50 pF. Each cell possessed I_{Na} , I_{Kdr} , I_{T} , I_{h} and I_{Leak} with maximal conductances of 1677 nS, 400 nS, 50 nS, 40 nS and 3 nS respectively. All electrical synapses had a conductance of 500 pS.

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Figure 1. Extending the dynamic clamp concept

A. Schematic representation of the basic dynamic clamp setup. The membrane potential (V_m) of a real neuron is sampled by a DAC and transmitted to a computer. Based on this V_m and a set of equations stored in the computer, which describe one or more voltage- and/or time-dependent conductances, a current is calculated. The computer then sends an appropriate voltage command via an ADC back to the recording amplifier which instructs the amplifier to inject the calculated current. **B.** In its simplest manifestation the dynamic clamp injects a current which mimics the activity of an intrinsic conductance. However, this current can also represent a synaptic conductance allowing the construction of hybrid circuits between real and artificial neurons (top left and right, red circles represent real cells,

blue circles represent simulated cells). These can be of varying complexity with the size being determined by computational power and algorithmic efficiency. The dynamic clamp can also calculate the axial currents that would be presented to the soma of a real neuron if various hypothetical dendritic structures were attached (i.e. artificial dendrites, bottom left). The artificial dendrite and hybrid network concepts can also be combined to produce more realistic hybrid scenarios (bottom right).

Figure 2. Structure and appearance of NeuReal

A. Diagram showing the main objects in NeuReal and their relationships to each other. All classes in NeuReal are, by convention, prefixed with the letter C. An open circle denotes zero or many multiplicity, with a filled circle representing one or many multiplicity. An asterisk denotes 'any number' whereas a specified number denotes the actual quantity that can be related for each particular case. The specific attributes and operations for each class are omitted for clarity. **B.** Main interface of NeuReal and an enlargement of one of the three basic oscilloscope-like display control panels. Each oscilloscope-like display shows two neurons and can be configured to operate in either a sweep or roll mode. Using dropdown

menu boxes and slider controls, for each neuron being displayed, the user can select which compartment to view and change several parameters relating to the components of that compartment, i.e. injected current, maximal conductances, activation and inactivation kinetics and reversal potentials etc.

Figure 3. Basic testing of NeuReal

A. Graph showing the mean actual time taken per 0.1 ms integration step for different numbers of simultaneously modelled TC neurons on a PC possessing a 2.26 GHz Intel Pentium processor and 2 GB of RAM (calculated from 100000 consecutive cycles). **B.** Graph showing the variability of the update cycle for NeuReal at different frequencies and for different Windows priority classes. **C.** Voltage response of a NeuReal-simulated, simple one compartment neuron possessing I_T and I_h ($g_T = 60\text{nS}$ and $g_h = 40\text{nS}$) to 700ms negative current steps of amplitude 200, 300 and 400pA (**1**). Shown below are the voltage responses of a 'model' cell, with identical passive parameters to the same current step protocol following the introduction of I_T and I_h ($g_T = 60\text{nS}$ and $g_h = 40\text{nS}$) using NeuReal as a simple dynamic clamp system (**2**). Shown further below is an equivalent simulation using standard floating point library functions (**3**). The bottom panel (**4**) shows a comparison of the three results shown in **1-3**.

Figure 4. Using NeuReal to do basic dynamic clamp experiments

A. Responses of a TC neuron in the cat LGN show a characteristic LTCP and associated burst of action potentials at the offset of negative current steps, i.e. rebound burst (top traces). This LTCP is blocked by 0.5 mM Ni^{2+} (middle trace) but can be reinstated using NeuReal to introduce an artificial I_T ($g_T=50\text{nS}$, $\lambda_m=0.15$ & $\lambda_h=1.2$, bottom traces). **B.** Similar experiment performed in the presence of tetrodotoxin showing how interactively altering the activation and inactivation kinetics can facilitate a more accurate reproduction of the LTCP.

Figure 5. Backpropagation of action potentials in passive and active artificial dendrites

A. Series of traces at different locations, as indicated by the grey arrows, showing the response of a TC neuron following the attachment of the illustrated artificial dendritic tree to a 100 pA positive current step injected at the soma. Note the rapid attenuation of action potentials in the dendritic tree. **B.** Same experiment but following the addition of I_{Na} (25 mS/cm²) and I_{Kdr} (12 mS/cm²) to the dendrites shows a minimal decline in action potential amplitude even at relatively distal sites.

Figure 6. Induction of δ (~1-2 Hz) oscillations through the attachment of artificial dendrites

A. Attachment of 8 identical artificial dendritic trees (same properties as in Fig. 5) possessing I_T (2 mS/cm²), I_h (0.5 mS/cm²) and I_{Kdr} (2 mS/cm²) to a TC neuron which did not show spontaneous oscillatory activity in control conditions brings about realistic δ oscillations at the soma. The pattern in the change in frequency of these oscillations with respect to varying steady current (bottom plot) applied at the soma is extremely similar to that shown previously for spontaneously oscillating cells (McCormick and Pape, 1990; Leresche et al., 1991). **B.** Oscillations require a small amount of dendritic I_h and take on a more paroxysmal appearance in the dendrites when even moderate amounts of dendritic I_{Na} are introduced.

Figure 7. Implementation of a large hybrid network

A. Schematic representation of the implemented hybrid network. The simulated part consists of 49 HT burst cells randomly connected by electrical synapses (black lines) as indicated. The real cell is connected to 3 independent HT burst cells, also by electrical synapses. The conductance of all electrical synapses was 500 pS. **B.** Examination of the HT burst cell network in isolation shows that the population output (average membrane potential of all cells, band-pass filtered at 2-15 Hz) is a waxing and waning oscillation at ~8 Hz (top trace). The traces 1, 2 and 3 show the activity of three different HT burst cells as indicated in **A**. The enlargements at the bottom show how periods of high-amplitude population activity are related to increased synchrony between HT burst cells (yellow shaded area). **C.** Activity of a real TC neuron in the absence of any hybrid network input. **D.** Activity of the same cell when the hybrid network is attached. The population output is shown at the top. Note how the membrane potential of the real cell becomes modulated by the network input which is highlighted by the population triggered subthreshold membrane potential average shown to the bottom left. Also note how this modulation can powerfully influence spike timing as indicated by the spike timing histogram shown to the bottom right.