Computational modeling of spike generation in serotonergic neurons of the dorsal raphe nucleus

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Abstract

Serotonergic neurons of the dorsal raphe nucleus, with their extensive innervation of limbic and higher brain regions and interactions with the endocrine system have important modulatory or regulatory effects on many cognitive, emotional and physiological processes. They have been strongly implicated in responses to stress and in the occurrence of major depressive disorder and other psychiatric disorders. In order to quantify some of these effects, detailed mathematical models of the activity of such cells are required which describe their complex neurochemistry and neurophysiology. We consider here a single-compartment model of these neurons which is capable of describing many of the known features of spike generation, particularly the slow rhythmic pacemaking activity often observed in these cells in a variety of species. Included in the model are ten kinds of voltage dependent ion channels as well as calcium-dependent potassium current. Calcium dynamics includes buffering and pumping. In sections 3-9, each component is considered in detail and parameters estimated from voltage clamp data where possible. In the next two sections simplified versions of some components are employed to explore the effects of various parameters on spiking, using a systematic approach, ending up with the following eleven components: a fast sodium current \( I_{Na} \), a delayed rectifier potassium current \( I_{KDR} \), a transient potassium current \( I_A \), a low-threshold calcium current \( I_T \), two high threshold calcium currents \( I_L \) and \( I_N \), small and large conductance potassium currents \( I_{SK} \) and \( I_{BK} \), a hyperpolarization-activated cation current \( I_H \), a leak current \( I_{Leak} \) and intracellular calcium ion concentration \( Ca_i \). Attention is focused on the properties usually associated with these neurons, particularly long duration of action potential, pacemaker-like spiking and the ramp-like return to threshold after a spike. In some cases the membrane potential trajectories display doublets or have kinks or notches as have been reported in some experimental studies. The computed time courses of \( I_A \) and \( I_T \) during the interspike interval support the generally held view of a competition between them in influencing the frequency of spiking. Spontaneous spiking could be obtained with small changes in a few parameters from their values with driven spiking. Spontaneous activity was facilitated by the presence of \( I_H \) which has been found in these neurons by some investigators. For reasonable sets of parameters spike frequencies between about 0.6 Hz and 1.2 Hz are obtained. Anodal break phenomena predicted by the model are in agreement with experiment. There is a considerable discussion of in vitro versus in vivo firing behavior, with focus on the roles of noradrenergic input, corticotropin-releasing factor and orexinergic inputs. Location of cells within the nucleus is probably a major factor, along with the state of the animal.
**Keywords:** Dorsal raphe nucleus, serotonergic neurons, computational model, pacemaking

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**Abbreviations**

5-HT, 5-hydroxytryptamine (serotonin); 5-HP, 5-hydroxytryptophan; AHP, afterhyperpolarization; acid; BK, big potassium channel; Ca, internal calcium ion concentration; CB, calbindin-D28k; CBP, calcium binding protein; CDI, calcium-dependent inactivation; CNS, central nervous system; CR, calretinin; CRF, corticotropin releasing factor; CSF, calcium source factor; D, duration (of spike); DA, dopamine; DRN, dorsal raphe nucleus; EPSP, excitatory post-synaptic potential; FURA-2AM, Fura-2-acetoxymethyl ester; GABA, gamma-aminobutyric acid; HPA, hypothalamus-pituitary-adrenal cortex; HVA, high-voltage activated; ISI, interspike interval; LVA, low-voltage activated; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; PV, parvalbumin; REM, rapid eye movement; SE, serotonin or serotonergic; SK, small potassium channel; SSRI, selective serotonin re-uptake inhibitor; TEA, tetra-ethyl ammonium chloride; TPH, tryptophan hydroxylase; TTX, tetrodotoxin; VGCC, voltage-gated calcium channel.

**Contents**

1. Introduction: a summary of the properties of DRN SE neurons ........................................4
2. Membrane currents ........................................................................................................10
3. Voltage-dependent potassium currents ........................................................................12
4. Calcium currents .........................................................................................................17
5. Calcium-dependent potassium currents .....................................................................30
6. Hyperpolarization activated cation current, $I_H$ ......................................................36
7. Fast transient sodium current, $I_{Na}$ .........................................................................37
1 Introduction: a summary of the properties of DRN SE neurons

The last several decades have seen intensive experimental programs in neuroscience, endocrinology, psychiatry and psychology aimed at elucidating the involvement and responses of the many components of the nervous and endocrine systems in stress and to understanding their role in the complicated phenomenon of clinical depression. Serotonergic neurons in the dorsal and other raphe nuclei, which extensively innervate most brain regions, have a large influence on many aspects of behavior, including sleep-wake cycles, mood and impulsivity (Liu et al., 2002; Miyazaki et al., 2011). With their influence on limbic and higher brain regions, they have important modulatory or regulatory effects on many cognitive, emotional and physiological processes (Lanfumey et al., 2005) including reward (Nakamura et al., 2008; Kranz et al., 2010; Hayes and Greenshaw, 2011). They have been strongly implicated in responses to stress and in the pathophysiology of major depressive disorder and other stress-related psychiatric disorders (Neumeister et al., 2004; De Kloet et al., 2005; Firk and Markus, 2007; McEwen, 2007; Joëls et al., 2007; Joëls, 2008;
The therapeutical efficacy of selective serotonin re-uptake inhibitors indicates the key role of serotonin in depression. The involvement of serotonergic neurons arises through a variety of physiological, neurophysiological and endocrine processes. For example, the synthesis of serotonin, which may occur in the soma, synaptic terminals and to a lesser extent in some dendrites (Cooper et al., 2003; Adell et al., 2002) is from tryptophan which is supplied through the diet and enters the brain from blood. Within serotonergic cells the amino acid undergoes hydroxylation to 5-hydroxytryptophan (5-HTP) via tryptophan hydroxylase (TPH) which is synthesized only in the cell bodies of serotonergic cells. The activity of TPH is modifiable by some stressors and pharmacological agents. For example, through glucocorticoid receptors, sound stress increases the activity of TPH (Singh et al., 1990).

Included in the targets of serotonergic neurons of the DRN are the hippocampus, amygdala, locus coeruleus, prefrontal cortex (PFC) and the paraventricular nucleus of the hypothalamus. Serotonergic input from the raphe into the hippocampus is important for regulating hippocampal neurogenesis (Balu and Lucki, 2009).

Endocrine interactions and stress

DRN SE neurons are known to be important mediators of endocrine (Chaouloff, 2000; Amat et al., 2004) and behavioral (Maier and Watkins, 1998) responses to stressors. Bambico et al. (2009) found changes not only in spontaneous 5-HT neuron single-spike firing activity after chronic uncontrollable stress, but also changes in the number of spontaneously-active 5-HT neurons. Thus stress affects serotonergic neurotransmission by altering the firing rate of raphe 5-HT neurons, as well as the synthesis, release and metabolism of the transmitter and the levels of pre- and postsynaptic 5-HT receptors (Linthorst and Reul, 2005).

The firing activity of DRN SE neurons is influential as serotonin (5-HT) is involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis mainly by an effect exerted at a hypothalamic level (Jørgensen et al., 2002). The serotonergic activation of the HPA axis occurs by direct activation of CRF neurons in the paraventricular nucleus of the hypothalamus (Liposits et al., 1987).

Stress may affect the serotonin system through corticotropin-releasing factor (CRF) fibers, which densely innervate the DRN where there are abundant CRF receptors (Pernar et al., 2004). DRN SE cells also have glucocorticoid receptors which are known to respond to high levels of cortisol (Laaris et al., 1995). Kirby et al. (2000) demonstrated in vivo that a dense innervation of the DRN by CRF appeared to be topographically organized and showed that endogenous CRF in the DRN could alter the activity of 5-HT neurons. In vitro studies also suggest that
CRF modulates serotonergic neurotransmission (Lowry et al., 2000). The effects of CRF were principally excitatory and limited to a subpopulation of serotonergic neurons. Evidence that stress activates DRN 5-HT neuronal activity is indicated by an increase in 5-HT release within both the DRN and its projection areas (Lanfumey et al., 2005).

**Firing activity**

In most species, serotonergic neurons of the raphe nuclei have been found in vivo to have a slow, tonic pattern of firing. Usually spontaneous firing is found in rat DRN SE cells at rates around 0.5 to 2 Hz, as for example in Aghajanian et al. (1968), Aghajanian and Haigler (1974), Aghajanian and Vandermaelen (1982), Allers and Sharp (2003) with rates depending on the physiological state of the animal (Trulson and Jacobs, 1979; Jacobs and Azmitia, 1992; Urbain et al., 2006). There have been studies in freely moving rats in response to various stimuli (Waterhouse et al., 2004). Some cells display bursting (Hájos et al., 1995, 1996; Schweimer et al., 2010). Aghajanian and Vandermaelen found that bursts of several spikes could be elicited with brief depolarizing currents of 0.5 to 1.0 nA.

The robustness of the steady firing under a variety of conditions was taken to imply that spiking in serotonergic neurons was generated by intrinsic tonic pacemaker mechanisms. In fact, numerous intracellular recordings from dorsal raphe neurons show that spikes arise from gradual depolarizing ramps (Aghajanian and Vandermaelen, 1982; Vandermaelen and Aghajanian, 1983; Park, 1987) rather than the arrival of excitatory post synaptic potentials, although the latter could be present (Segal, 1985).

DRN SE cells in vivo have a complex electrophysiological and neurochemical environment (Azmitia and Whitaker-Azmitia, 1995; Harsing, 2006; Lowry et al., 2008) and it is not surprising therefore that recordings of spiking activity in vivo, usually made with chloral hydrate as anesthetic, have produced variable results. In slice, the activity of DRN SE neurons is usually reduced, which is usually attributed to the lack of excitatory synaptic input, which seems to contradict the claims that excitatory input is not required for their usual pacemaker-type firing. In Segal (1985), some cells required depolarizing currents of magnitude over 0.25 nA to fire. Interestingly, Vandermaelen and Aghajanian (1983) found that whereas in vivo most cells were spontaneously active (spiking), most cells were silent in slice - see also Kirby et al. (2003). Pan et al. (1990) recorded spontaneous spiking in 8 of 104 neurons in slice. These findings contrast with earlier results by Mosko and Jacobs (1976) who claimed that the firing properties of raphe neurons in slice were mostly similar to in vivo, but the identification of the cells as serotonergic was not
attempted. With the application of either norepinephrine or the \( \alpha_1 \)-adrenoceptor agonist phenylephrine, silent cells in slice may usually be made to fire regularly (VanderMaelen and Aghajanian, 1983; Judge and Gartside, 2006). In the former study, the injection of depolarizing currents of from 0.05 to 0.4 nA was also used to induce firing.

**Biophysical properties**

A survey of biophysical properties of rat DRN SE neurons reveals the following encapsulated view (for details see Tuckwell, 2012b). They have resting membrane potentials ranging from -75 to -58 mV with an average value (13 studies) of -64.4 mV. Membrane time constants range from 7.4 to 53.5 ms, with a mean of 32.5 ms (8 studies) and input resistances from 30 to 600 M\( \Omega \) with a mean of 236 M\( \Omega \) (18 studies). Such properties may vary across subfields (Calizo et al., 2011). Firing rates are variable, an average in vivo rate being 1.37 Hz (16 studies), usually with chloral hydrate, and ranging from 0.1 to 3.5 Hz. For 3 studies in slice, the average rate was 1.07 Hz with range from 0.8 to 1.3 Hz. Spike durations are called long relative to many other types of neuron. It is difficult to give summary statistics for duration because of the many ways it is measured, but generally figures between 2 and 3.6 ms are cited, but sometimes up to 5 ms (Marinelli et al., 2004). Thresholds for firing are usually about 10 mV above resting potential and the afterhyperpolarization following spikes is on average 13.7 mV (9 studies). Spike amplitudes range from 61 to 92 mV with a mean of 77.3 mV (9 studies), but again various definitions are employed. Estimated soma area, in \( \mu^2 \), is from 1073 to 2400 and estimated total somadendritic area is from 3914 to 11158 \( \mu^2 \). Capacitance can be estimated from area using the 1 \( \mu F \) per square cm rule, and this gives a mean of 55 pF with a most likely value of 39 pF. Using instead the time constants and input resistances gives a higher mean of 89.5 pF. After considering all the data available on areas and capacitances, it was concluded that a typical DRN SE neuron has a soma dendritic area of 4000 \( \mu^2 \) and a capacitance of 40 pF, these being the assumed values throughout most of the computations reported in this article - see Table 17. A very large cell might have an area of 9000 \( \mu^2 \) and a capacitance of 90 pF.

**Inputs**

DRN SE neurons receive many inputs involving a variety of neurotransmitters (Jacobs and Azmitia, 1992) and the afferents are topographically organized (Lee et al., 2003; Hale and Lowry, 2011; Maximino, 2012), such as the important reciprocal connections between the DRN and locus coeruleus (Kim et al., 2004). The
transmitters include norepinephrine and 5-HT itself. Norepinephrine, acting via \( \alpha_1 \)-adrenoceptors, can accelerate the pacemaker activity of serotonergic neurons by closing potassium channels (Aghajanian and Sanders-Bush, 2002).

5-HT\(_{1A} \) receptors (autoreceptors) in the 5-HT cell soma and proximal dendrites exert negative feedback control over DRN cell firing. Such serotonin-activated inhibition at autoreceptors may arise from volume transmission from self or neighboring DRN SE neurons as well as by recurrent and other axonal endings. A second population of 5-HT\(_{1A} \) receptors is found throughout the dendritic field and modulates release of serotonin. Furthermore, 5-HT\(_{1B/D} \) receptors are found on the dendrites and preterminal axons where they modulate release of 5-HT (Stamford et al., 2000). Extrusion of 5-HT can occur through the reverse action of the serotonin transporter (Azmitia and Whitaker-Azmitia, 1995).

Intracellular recordings from dorsal raphe neurons in brain slices show that 5-HT\(_{1A} \) agonists and 5-HT hyperpolarize the cell membrane, decreasing input resistance by opening K\(^+ \) channels, and decreasing high-threshold calcium currents (Penington and Kelly, 1990). A small component of this calcium current shows sensitivity to L-type calcium channel blockers and about 50\% of what remains is sensitive to an N-type Ca\(^{2+} \) channel blocker (Penington et al., 1991). There is evidence that the opening of K\(^+ \) channels via 5-HT\(_{1A} \) receptors in dorsal raphe neurons (Penington et al., 1993) is mediated by a pertussis-toxin-sensitive G protein (Williams et al., 1988). There are also glutamatergic inputs directly on DRN SE neurons, from the PFC, lateral habenula, the hypothalamus and other regions, as revealed by glutamate transporter tracing (Soiza-Reilly and Commons, 2011).

The mPFC not only receives strong inputs from serotonergic neurons in the DRN but also sends projections to this nucleus. Electrophysiological investigations in the rat DRN reveal that most serotonergic neurons are inhibited by electrical stimulation of the mPFC, suggesting that this pathway is more likely to synapse onto neighboring GABA-ergic neurons rather than onto 5-HT cells (Jankowski and Sesack, 04). The mPFC thereby exerts control over the DRN and that may be involved in the actions of pharmaceutical drugs and drugs of abuse (Goncalves, 2009). Thus, in addition to feedback systems involving separate nuclei and structures, local networks are found within the dorsal raphe nucleus involving interactions between 5-HT and local inhibitory GABA-ergic and excitatory glutamatergic neurons (Aghajanian and Sanders-Bush, 2002).

**Perspective**

Mathematical modeling of neurophysiological dynamics has been pursued for many different nerve and muscle cell types. Some well known neuronal examples...
are thalamic relay cells (Huguenard and McCormick, 1992; Destexhe et al., 1998; Rhodes and Llinas, 2005), dopaminergic cells (Komendantov et al., 2004; Putzier et al., 2009; Kuznetsova et al., 2010), hippocampal pyramidal cells (Traub et al., 1991; Migliore et al., 1995; Poirazi et al., 2003; Xu and Clancy, 2008), neocortical pyramidal cells (Destexhe et al., 2001; Traub et al., 2003; Yu et al., 2008) and molluscan interneurons (Vavoulis et al., 2010). Cardiac myocytes have also been the subject of numerous computational modeling studies with similar structure and equivalent complexity to that of neurons (Faber et al., 2007; Williams et al., 2010).

It is a complicated task to model all the details of an SE neuron. Some authors have focused on models of synaptic release (Best et al., 2010) including the effects of SSRIs (Geldof et al., 2008). However, the dynamics of the various currents are important in determining the sequence of action potentials which will determine, despite the many complexities of the axonal branchings, the amounts of transmitter release. Hence the focus here is on the modeling of the process of spike generation which is fundamental as a prelude to understanding the responses of these cells to activation of both neurophysiological (including synaptic) and endocrinological receptors, as well as the effects of this spiking and subsequent 5-HT release at numerous sites.

The firing patterns of these cells have been much studied and many properties of the ionic currents underlying action potentials have been investigated (Aghajanian, 1985; Segal, 1985; Burlhis and Aghajanian, 1987; Penington et al., 1991, 1992; Penington and Fox, 1995, Chen et al., 2002). In the present work, modeling of spike generation in DRN SE neurons has emphasis on intrinsic properties, being a first step in the construction of a quantitative theory of how these neurons are influenced by their many inputs and how they influence their numerous target cells.

According to Aghajanian and Sanders-Bush (2002) and several others, the pacemaker activity of serotonergic neurons results from the interplay of several intrinsic ionic currents including a voltage-dependent transient outward potassium current $I_A$, a low-threshold inward calcium current $I_T$, and a calcium-activated outward potassium current. There have been conflicting ideas about the role of $I_A$ (Burlhis and Aghajanian, 1987; Segal, 1985). Since the basis of pacemaker-like activity in these cells is not yet fully understood, one of the aims of the present article is to investigate these purported mechanisms in a quantitative way.
2 Membrane currents

For a single compartmental neuron model, the differential equation for the membrane potential is,

\[ C \frac{dV}{dt} = -I, \quad V(0) = V_0, \]  

(1)

where \( I \) is the sum of all current types and \( V_0 \) is the initial value of \( V \), taken in most of what follows to be the resting membrane potential, \( V_R \). Here depolarizing currents are negative, \( V \) is in mV, and \( t \) is in ms so that if \( I \) is in nA, then the membrane capacitance is in nF. If the i-th component of the current is denoted by \( I_i \) so that \( I = \sum I_i \), then following the Hodgkin-Huxley (1952) model, generically and generally, each component current, \( I_i \), is taken as a product of activation and inactivation variables, a maximal conductance, \( g_{i,max} \), and a driving force which is \( V - V_i \) where \( V_i \) is usually at or near the Nernst equilibrium potential.

For noninactivating currents there is an activation variable \( m \) raised to a certain power \( p \geq 1 \), not necessarily an integer, so that

\[ I_i = g_{i,max} m^p (V - V_i). \]  

(2)

If the current inactivates, then the current contains an inactivation variable \( h \) which is usually raised to the power 1 so

\[ I_i = g_{i,max} m^p h(V - V_i). \]  

(3)

Sometimes the activation or inactivation variables depend on, or also on, calcium ion concentration. A constant field expression may be used instead of the linear term \( V - V_i \) - see Section 4 on calcium currents.

Activation and inactivation variables are determined by differential equations which are conveniently written in the forms

\[ \frac{dm}{dt} = \frac{m_\infty - m}{\tau_m}, \]  

(4)

\[ \frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \]  

(5)

where \( m_\infty \) and \( h_\infty \) are steady state values which depend on voltage. The quantities \( \tau_m \) and \( \tau_h \) are time constants which may also depend on voltage and/or calcium concentration. We adhere to the convention throughout that all parameters as
written are positive. For accounts of the dynamics of many types of current, see Levitan and Kaczmarek (1987) and Destexhe and Sejnowski (2001).

Table 1 gives the notation for the 11 membrane currents included in the present model, so that

$$C \frac{dV}{dt} = -[I_A + I_{KDR} + I_M + I_T + I_L + I_N + I_H + I_{Na} + I_{BK} + I_{SK} + I_{Leak}], \quad (6)$$

though in the computations various components are sometimes omitted. In addition there is a differential equation describing the evolution of the internal calcium ion concentration in terms of sources and a pump,

$$\frac{dCa_i}{dt} = f_{sources} - f_{pump}.$$  

Table 1: Notation for membrane currents

| Symbol  | Description                                      |
|---------|--------------------------------------------------|
| Voltage-gated potassium |                                                 |
| $I_A$   | Fast transient A-type                           |
| $I_{KDR}$ | Delayed rectifier potassium                    |
| $I_M$   | M-type potassium                                |
| Calcium |                                                 |
| $I_T$   | Low threshold calcium, T-type                   |
| $I_L$   | High threshold calcium, L-type                  |
| $I_N$   | High threshold calcium, N-type                  |
| Calcium-activated potassium |                                             |
| $I_{BK}$ | Large conductance channels                     |
| $I_{SK}$ | Small conductance channels                     |
| Hyperpolarization activated cation current |                                      |
| $I_H$   |                                                 |
| Fast transient sodium current |                                          |
| $I_{Na}$ |                                                 |
| Leak current |                                              |
| $I_{Leak}$ |                                              |
3 Voltage-dependent potassium currents

There is evidence for three types of voltage-gated potassium currents in DRN SE neurons. These are the transient $I_A$, and two which are usually considered to be non-inactivating or very slowly inactivating, being the delayed rectifier, $I_{KDR}$, and the M-current, $I_M$.

M-type potassium current, $I_M$

The M-current is associated with the potassium channel types $K_v$7.1 to $K_v$7.5 or the KCNQ family (Gutman et al., 2005). Such a current activates slowly and inactivates extremely slowly, more so than the usual delayed rectifier. M-type potassium currents are considered to play a significant role in adaptation (Storm, 1990; Benda and Herz, 2003). M-type potassium currents have been included in several computational models of neurons, especially hippocampal and cortical pyramidal cells (Lytton and Sejnowski, 1991; Poirazi et al., 2003; Vervaeke et al., 2006; Xu and Clancy, 2008). However, such currents are associated with extremely small conductance densities, being of the order 1000 to 4000 times less than that of the usual delayed rectifier.

The potassium M-current is written as

$$I_M = g_{M,max} m_M (V - V_M)$$

where $V_M$ is the reversal potential, taken as around the Nernst potential, $V_K$, for potassium. The activation variable $m_M$ satisfies an equation like (4) and the steady state is written

$$m_{M,\infty} = \frac{1}{1 + e^{-(V - V_{M_1})/k_{M_1}}}$$

with a time constant

$$\tau_{m_M} = \tau_{m,M_c}$$

a constant. In voltage clamp experiments on potassium currents similar to those in our recent report on $I_A$ (Penington and Tuckwell, 2012), in some cells we have observed a persistent current which seems to have properties similar to an M-current. An example is shown in Figure 1 where the current is decomposed into a component likely to be $I_A$, which declines exponentially, and a persistent component which is putatively an M-current with time constant of activation of about 50 ms and no sign of inactivation.
Figure 1: Decomposition of current under voltage clamp (-60 mV to 50 mV) in a putative DRN SE neuron. The exponentially decaying curve is likely to be $I_A$ whereas the persistent current is possibly $I_M$.

**Parameter values for $I_M$**

Half-activation potentials for $I_M$ have been obtained in subtypes expressed in Chinese hamster ovary cells and ranged from -28.7 mV to -11.6 mV with corresponding slope factors from 10.1 to 12.9 (Tatulian et al., 2001). In various modeling and other studies the half-activation potentials have ranged from as low as -50 mV to -20 mV with slope factors around 9 or 10. Time constants for activation have been from about 50 ms to 200 ms, depending in some cases on $V$. The following set of parameters (Table 2) may be chosen for $I_M$, where $V_K$ is the Nernst potential for potassium.

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{M1}$  | -30   | $\tau_{m,M_t}$ | 100   |
| $k_{M1}$  | 9     | $V_M$     | $V_K$ |

**Transient potassium current, $I_A$**

The transient potassium current $I_A$ was documented in early studies of DRN SE neurons by, for example, Aghajanian (1985), Segal (1985) and Burlhis and Agha-
janian (1987). $I_A$ has been posited to play an important role in determining the cell’s firing rate. However, there have been conflicting ideas about the role of $I_A$ (Burlhis and Aghajanian, 1987; Segal, 1985). Six kinds of $K_v$ channels have been linked to $I_A$ (Gutman et al., 2005) but according to Serôdio and Rudy (1998) and other studies, in the mammalian brain, somatodendritic $I_A$ currents are likely to be carried by channels of the Shal family $K_v4.1, K_v4.2$ and $K_v4.3$. Using in situ hybridization histochemistry, Serôdio and Rudy (1998) found that in the DRN, $K_v4.3$ signals were strong, $K_v4.2$ weak and $K_v4.1$ below background. However, Pearson et al. (2006) in their study of DRN and locus coeruleus, found evidence for $K_v4.2$ in the DRN of adult rats. In a recent report (Penington and Tuckwell, 2012), we have examined various data which suggested that the channels carrying $I_A$ in the putative serotonergic neuron analyzed are of the $K_v4.2$ type, but there was insufficient evidence to completely rule out the other two Shal members. In support of this idea are the findings of Bekkers (2000) who postulated that in rat layer 5 cortical pyramidal cells, $I_A$ was carried by $K_v4.2$ channels, and of Kim et al. (2005), who found that $K_v4.2$ is the main contributor to $I_A$ in hippocampal CA1 pyramidal cells, determining several basic characteristics of the action potential, including its width.

In their model of thalamic relay cells, Huguenard and McCormick (1992), employed two types of $I_A$ with somewhat different kinetic properties. However, we do not have evidence for any but one type which we denote simply by $I_A$ with the form

$$I_A = g_{A,\text{max}} m_A^4 h_A(V - V_A)$$

where $m_A$ and $h_A$ satisfy equations like (6) and (7). The power 4 is used for $m_A$ based on our previous observations (Penington and Tuckwell, 2012), whereas other authors have used 3. The steady state activation is written

$$m_{A,\infty} = \frac{1}{1 + e^{-(V - V_{A_1})/k_{A_1}}}$$  \hspace{1cm} (11)$$

with a time constant

$$\tau_{m_A} = a_A + \frac{b_A}{\cosh((V - V_{A_2})/k_{A_2})}$$  \hspace{1cm} (12)$$

where there are 4 parameters $a_A, b_A, V_{A_2}, k_{A_2}$. The steady state inactivation variable is set to

$$h_{A,\infty} = \frac{1}{1 + e(V - V_{A_3})/k_{A_3}}$$  \hspace{1cm} (13)$$
with corresponding voltage-dependent time constant,

$$
\tau_{m_A} = c_A + \frac{d_A}{\cosh((V - V_{A_4})/k_{A_4})}.
$$

(14)

Parameter values for $I_A$

The standard set of kinetic parameters for $I_A$ is based on one given in Huguenard and McCormick (1992), with suitable approximations for the time constants. In the case of the time constant for inactivation, the approximation is valid over values of $V$ which are likely to occur during the activity of a DRN SE neuron. The use of these parameters is validated by the results obtained below in simulation of some voltage clamp experiments of Burlhis and Aghajanian (1987) on DRN SE neurons in slice.

Table 3: Standard set of parameters for $I_A$

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{A_1}$ | -60   | $V_{A_3}$ | -78   |
| $k_{A_1}$ | 8.5   | $k_{A_3}$ | -6    |
| $a_A$     | 0.37  | $c_A$     | 19    |
| $b_A$     | 2     | $d_A$     | 45    |
| $V_{A_2}$ | -55   | $V_{A_4}$ | -80   |
| $k_{A_2}$ | 15    | $k_{A_4}$ | 7     |

Delayed rectifier potassium current, $I_{KDR}$

Evidence for a delayed rectifier potassium current in DRN SE neurons has been given by Penington et al. (1992) and Liu et al. (2002) who found it was substantial in the repolarization phase of action potentials. There are apparently no explicit data on the properties of $I_{KDR}$ in these cells, but since the original modeling of squid axon action potential by Hodgkin and Huxley (1952) there have been numerous approaches to a quantitative framework.

We take the form for this current to be as

$$
I_{KDR} = g_{KDR,\text{max}} n^m (V - V_{KDR})
$$

(15)
where \( n \) (the traditional symbol) is the activation variable which satisfies a differential equation like (4), \( V_{KDR} \) is the reversal potential, and \( n_k \) is usually an integer between 1 and 4, inclusively. The steady state activation is written

\[
n_\infty = \frac{1}{1 + e^{-(V - V_{KDR1})/k_{KDR1}}} \tag{16}
\]

and the time constant

\[
\tau_n = a_{KDR} + \frac{b_{KDR}}{\cosh((V - V_{KDR2})/k_{KDR2})} \tag{17}
\]

**Parameter values for \( I_{KDR} \)**

Table 4 gives parameters on the activation used in various studies (some of which are in conjunction with experiment) and it can be seen that all integer powers from 1 to 4 have been used for \( n \), with 4 the value in the squid axon model (Hodgkin and Huxley, 1952). We will choose the power 1 in the absence of specific data as the properties are then simpler to relate to the steady state activation function, but also examine other values with appropriate changes in other parameters. It can be seen that there is considerable variability assumed or measured in the kinetic properties of \( I_{KDR} \) in various neurons. Note that Traub et al. (1991) values were also used by Migliore (1995) and Xu (2008) and that in Bekkers (2000) the index may vary with \( V \).

**Table 4: Examples of parameters for activation of \( I_{KDR} \)**

| Source             | Form | \( V_{KDR1} \) | \( k_{KDR1} \) | \( V_{Rest} \) | \( \tau_{min} - \tau_{max} \) | \( V \) at \( \tau_{max} \) |
|--------------------|------|----------------|----------------|---------------|-----------------------------|-----------------------------|
| Traub (1991)*      | \( n \) | -18.2          | 10             | -60           | 0.8-4.2                    | -31                         |
| Belluzzi (1991)    | \( n \) | -6.1           | 8              | -75           | 2.5-25                     | -27                         |
| Schild (1993)      | \( n^2 \) | -7.2           | 11.8           | -51           | 4-64                       | -32                         |
| Bekkers (2000)     | \( n^1 \) | -9.6           | 13.2           | -             | 1.5-20                     | -20                         |
| Traub (2003)       | \( n^4 \) | -29.5          | 10             | -62 to -68    | 0.25-4.6                   | -10                         |
| Poirazi (2003)     | \( n^2 \) | -42            | 2              | -70           | 2.3-3.5                    | -                           |
| Molineux (2005)    | \( n \) | -35            | 4              | -             | -                          | -                           |
| Komendantov (2007) | \( n^3 \) | -18.3          | 10             | -58           | 1-2-6.3                    | -34                         |
| Anderson (2010)    | \( n \) | -25            | 4              | -65 to -60    | -                          | -                           |
Based on the properties of the repolarization and afterhyperpolarizations in spikes in DRN SE neurons, it is postulated that the activation time constant for $I_{KDR}$ would not be very large (less than 5 ms) and that it would be mainly activated between about -40 mV and +20 mV. Thus, considering the various values given in Table 4, the following parameters (Table 5) are taken as the standard set. The reversal potential is taken to be $V_K$.

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{KDR_1}$ | -15 | $b_{KDR}$ | 4 |
| $k_{KDR_1}$ | 7 | $V_{KDR_2}$ | -20 |
| $a_{KDR}$ | 1 | $k_{KDR_2}$ | 7 |

To estimate the conductance associated with the delayed rectifier potassium current we appeal to some voltage clamp activation experiments done with and without TEA on dissociated cells. The methods were described in Penington and Tuckwell (2012). Results for one DRN serotonergic cell are shown in Figure 2. From the uppermost recordings (clamps from -110 mV to 0 mV), the final current amplitude without TEA minus the final amplitude with TEA gives 711 pA. Mathematical modeling of the potassium delayed rectifier current then yields an associated conductance of 0.0085 µS and since a dissociated cell has a capacitance of about 20 pF (Penington and Fox, 1995), this translates to 0.425 nS/pF, at room temperature. Allowing for an increase due to an increase in temperature, a figure of 0.641 nS per pF seems reasonable for the maximal conductance of $I_{KDR}$ at body temperature.

4 Calcium currents

Calcium currents, which are found in all excitable cells, have been generally divided into the two main groups of low-threshold or low-voltage activated (LVA) and high-threshold or high-voltage activated (HVA). The former group contains only the T-type (T for transient) and the latter group consists of the types L, N, P/Q and R (L for so called long-lasting, N, either for neither T nor L, or neuronal, P for Purkinje, and R for resistant). Calcium channels have up to four subunits, $\alpha_1$, $\alpha_2$, $\delta$, $\beta$ and $\gamma$, which may exist in different forms, and which modulate the conductance and dynamical properties of the channel (Dolphin, 2006, 2009; Davies et
Figure 2: Current versus time with voltage clamp in activation experiments with TTX alone (left column) and TTX + 20 mM TEA (right column). Steps from -120 mV to a maximum of 0 mV. Markers 100 ms and 100 pA.

al., 2010). The ten forms of the conducting pore subunit, α₁, lead to an expansion of the above groups (Catterall et al., 2005; Dolphin, 2009) to 10 main subtypes. According to the accepted nomenclature, L-type channels consist of the subtypes Caᵥ1.1 – Caᵥ1.4. The remaining “high-threshold” currents, P/Q, N and R are respectively Caᵥ2.1 -Caᵥ2.3 and the T-current subtypes are Caᵥ3.1 – Caᵥ3.3. Within the subtypes, various configurations of other subunits lead to channels, with quite different properties (Dolphin, 2009). One cannot therefore ascribe definite parameters in the dynamical description of calcium currents based on the subtypes. L-type channels, for example, display a wide variety of activation properties (Tuckwell, 2012a). The properties of channel types may not be the same in all locations of the same cell. For example, somatic N-type channels do not inactivate in rat supraoptic neurons but do in synaptic terminals of the same cell and in other cell types (Joux et al., 2001).

**Calcium T-type current, Iₜ**

The T-type (low threshold) calcium current has been implicated in pacemaker activity in some cells and also in bursting activity (Perez-Reyes, 2003; Catterall et al., 2005) and amplification of dendritic inputs (Crandall et al., 2010). The three main subtypes are designated Caᵥ3.1, Caᵥ3.2 and Caᵥ3.3 or α₁G, α₁H and α₁I, respectively. Since the pioneering experimental studies of Segal (1985) and Burlhis and Aghajanian (1987), spike generation and the pacemaker cycle in DRN SE neurons have been posited to be triggered by T-type currents. See also Jacobs and Azmitia (1992) and Aghajanian and Sanders-Bush (2002).
Mathematical expressions for $I_T$

The mathematical form for the T-type current has usually been chosen to be the linear expression (Destexhe et al., 1994, 1996; Rybak et al., 1997; Amini et al., 1999; Sanchez et al., 2003),

$$I_T = g_{T,max} m_T^2 h_T (V - V_{Ca,rev}),$$  \(18\)

although some authors have $m_T^3$ rather than $m_T^2$ (Rhodes and Llinas, 2005). $m_T$ and $h_T$ satisfy differential equations like (4) and (5). The advantage of this form for $I_T$ is that the associated conductance can be compared with other ion channels for which the linear form is usually employed - see the simplified model in section 10. However, because the linear form may sometimes lead to inaccuracies especially for calcium ion currents and in particular for low internal calcium ion concentrations (Belluzzi and Sacchi, 1991; Huguenard and McCormick, 1992; Poirazi et al., 2003), it is often desirable to use the constant field form

$$I_T = k_T m_T^2 h_T V \left[ \frac{C_{a_i} - C_{a_o} e^{-\frac{2V}{V_0}}}{1 - e^{-\frac{2V}{V_0}}} \right],$$  \(19\)

where

$$k_T = 1000 A \rho T P_T^s \frac{4F}{V_0}.$$  \(20\)

Here $A$ is membrane area in sq cm, $\rho_T$ is the channel density, $P_T^s$ is the single channel permeability in $\text{cm}^3 \text{per second}$, $C_{a_i}$, $C_{a_o}$ are the (time dependent) internal and external concentrations of Ca$^{2+}$ in mM, $F$ is Faraday’s constant (96000 Coulombs/Mole),

$$V_0 = \frac{RT}{F},$$  \(21\)

and the factor 1000 converts the current to nA. The time constants for activation and inactivation are denoted by $\tau_{m_T}$ and $\tau_{h_T}$.

For current through some Ca$^{2+}$ channels, there is not only voltage-dependent inactivation but also inactivation by internal calcium, called calcium dependent inactivation (CDI). When present, CDI can exert a considerable effect (Tuckwell, 2012a). However, for the low threshold current $I_T$, there is no CDI (Budde et al., 2002; Dunlap, 2007), but it was included in a model of a CA1 pyramidal cell (Poirazi et al., 2003). We will assume there is no CDI for $I_T$ in DRN SE neurons.
Voltage clamp data: $m_{T,\infty}, h_{T,\infty}$

Voltage clamp data are available for activation and inactivation of $I_T$ in various cell types and some data are reviewed in Perez-Reyes (2003) and Catterall et al. (2005). For the steady state activation we put

$$m_{T,\infty} = \frac{1}{1 + e^{-\left(V - V_{T1}\right)/k_{T1}}},$$

where $V_{T1}$ is the half-activation voltage and and $k_{T1}$ is the slope factor. Similarly for the steady state inactivation

$$h_{T,\infty} = \frac{1}{1 + e^{\left(V - V_{T3}\right)/k_{T3}}}.$$  \hspace{1cm} (23)

Table 6 gives values of the constants $V_{T1}$, $k_{T1}$, $V_{T3}$ and $k_{T3}$ obtained for some CNS cells. All experimental data are for dissociated cells. There are also data (not shown) for the three subtypes $\alpha_1 G$, $\alpha_1 H$ and $\alpha_1 I$, expressed in HEK-293 cells (Klöckner et al., 1999). The half-activation potentials ranged from -45.8 mV to -43.8 mV, the half-inactivation potentials ranged from -72.8 mV to -72.0 mV and the slope factors for inactivation ranged from -4.6 mV to -3.7 mV. In their modeling of thalamic relay cells, Rhodes and Llinás (2005) used the data of Huguenard and McCormick (1992), shifted in the direction of depolarization by 5 mV to prevent a large $I_T$ at rest. Destexhe et al. (1998) also shifted the same data by a few mV in the hyperpolarizing direction to allow for different external $Ca^{2+}$ concentrations and in the depolarizing direction as well to obtain agreement with their experimental observations.

| Neuron type | $V_{T1}$ | $k_{T1}$ | $V_{T3}$ | $k_{T3}$ |
|-------------|----------|----------|----------|----------|
| Thalamic relay, Huguenard (1992) | -57 | 6.2 | -81 | -4 |
| Thalamic relay, Huguenard & Prince (1992) | -59 | 5.2 | -81 | -4.4 |
| Thalamic reticular, Huguenard & Prince (1992) | -50 | 7.4 | -78 | -5.0 |
| Thalamic relay, Destexhe (1998) | -56 | 6.2 | -80 | -4 |
| Thalamic relay model, Rhodes (2005) | -63 | 6.2 | -80 | -4 |
Table 7: Maximal currents for $I_T$ in a DRN SEneuron: $V$ from -80 to $V_1$

| $V_1$ (mV) | $I_{max}$ Expt (pA) | $I_{max}$ | $I_{max}$ |
|------------|---------------------|-----------|-----------|
| -35        | 230                 | 230       | 230       |
| -40        | 161                 | 228       | 160       |
| -45        | 93                  | 208       | 83        |
| -50        | 44                  | 165       | 32        |

For presumed DRN SE neurons (dissociated), some voltage clamp data for $I_T$ were given by Penington et al. (1991). For a holding potential of -80 mV, records of $I_T$ were given for test potentials of $V_1 = -35, -40, -45, -50$ mV and estimates of the peak currents are given in column 2 of Table 7. Using the kinetic data of Huguenard and McCormick (1992), with $V_{T_1} = -57$ mV, gave the peak currents in column 3 which are not close to the experimental values. Only if $V_{T_1}$ was shifted to -44 mV, with or without a similar shift in the inactivation curve, was it possible to obtain an approximate agreement with the experimental data. Such discrepancies could be due to a number of factors such as temperature, differing compositions of intracellular and extracellular fluids or that in the experiments 5 mM $Ba^{2+}$ was the charge carrier. However, many reports (Huguenard and Prince, 1992; Bourinet et al., 1994; Rodriguez-Contreras and Yamoah, 2003; Durante et al., 2004; Goo et al., 2006) indicate that barium shifts the kinetics in the hyperpolarizing direction relative to calcium, although in one study a depolarizing shift was noted (Lipscombe et al., 2004).

**Time constants**

The corresponding time constants have been written in several ways including a constant plus the reciprocal of the sum of two exponentials (McCormick and Huguenard, 1992; Destexhe et al, 1994; Rybak et al. 1997; Rhodes and Llinás, 2005) and a Gaussian (Amini et al., 1999). For activation the following form was able to fit well several of the functions employed

$$
\tau_{mT} = a_T + \frac{b_T}{\cosh((V - V_{T_2})/k_{T_2})},
$$

where there are 4 parameters $a_T, b_T, V_{T_2}, k_{T_2}$.

For the inactivation time constant $\tau_{hT}$ the same forms were used as for $\tau_{mT}$ by Destexhe et al. (1994), Rhodes and Llinás (2005) and Amini et al. (1999), and
a function with discontinuous derivative was used by McCormick and Huguenard, (1992) and Rybak et al. (1997). A good fit was found possible with the form used by Amini et al. (1999), namely

\[ \tau_{h,T} = c_T + d_T e^{-\left(\frac{(V - V_T^4)}{k_T^4}\right)^2}, \]  

(25)

where there are 4 parameters \( c_T, d_T, V_T^4, k_T^4 \). In some calculations, such as those mentioned in the previous subsection, the forms given by Huguenard and McCormick (1992) were employed.

Voltage clamp results of Burlhis and Aghajanian (1987)

Where possible, in order to obtain estimates of the properties of various component currents, experimental data can be compared with theoretical predictions. Burlhis and Aghajanian (1987) performed several experiments in brain slice, designed to examine quantitatively the currents underlying pacemaking in intact rat DRN SE neurons. In one such experiment, called an “anodal break” (see also Section 12), a single-electrode voltage clamp was applied with a depolarizing step from -80 mV to -56 mV as seen in their Figure 2B.

![Figure 3: Steady state activation and inactivation functions versus membrane potential for \( I_A \) and \( I_T \) with parameters from Huguenard and McCormick (1992). Here are plotted \( m_{T,\infty}^2 \) and \( m_{A,\infty}^4 \).](image)
With the start of the depolarizing step, a transient outward current was observed with a peak magnitude of about 615 pA at around 7 ms. This was followed by a slower but transient inward current of peak magnitude about 92 pA around 67 ms. The early component was posited to be the fast transient potassium current $I_A$ and the inward current was the transient $I_T$ calcium current, being the component responsible for what Burlhis and Aghajanian (1987) called the prepotential which occurred before an action potential. These two component currents were modeled under the voltage clamp, -80 mV → -56 mV, using the parameters of activation and inactivation, including steady state activation and inactivation functions and time constants given for thalamic relay cells given by Huguenard and McCormick (1992). Note that the latter cells have resting potentials very similar (about -63 mV) to those DRN SE neurons.

In this modeling, the constant field form for $I_T$ was used. The steady state values of $m_{T,\infty}$ and $m_{A,\infty}$ and also $h_{T,\infty}$ and $h_{A,\infty}$ are shown in Figure 3 where it can be seen that the half-activation values are about -56 mV for $I_T$ and about -48 mV for $I_A$. A computed result is shown in Figure 4. The top part shows the voltage step and the lower part shows the time course of the sum of $I_A$ and $I_T$. The maximal conductances had to be estimated by trial and error which gave approximately for $I_A$, the value $g_{A,max} = 0.479 \mu S$. The estimated value of $k_T$ defined by Equ. (20) which resulted in the $I_A + I_T$ curve in Figure 4 was $k_T = 0.0825$.

Figure 4: Model results for $I_A + I_T$ for comparison with the voltage clamp results, -80 mV → -56 mV, of Figure 2B in Burlhis and Aghajanian (1987).
Table 8: Conductance densities for $I_A$ for various cells

| Source           | Cell type                           | $g_{A,\text{max}}$ (µS cm$^{-2}$) |
|------------------|-------------------------------------|-----------------------------------|
| Lytton (1991)    | Cortical pyramid                    | 100000                            |
| Amini (1999)     | Midbrain dopaminergic               | 445                               |
| Athanasiades (2000) | Medullary respiratory              | 6730                              |
| Traub (2003)     | Cortical pyramid                    | 30000                             |
| Komendantov (2007) | Magnocellular neuroendocrine       | 15000 to 20000                    |
| Saarinen (2008)  | Cerebellar granule                  | 445                               |
| Penington (2012) | DRN                                 | 1600                              |
| This study       | SE DRN                              | 12000                             |

The estimate of $g_{A,\text{max}}$ translates to a density of about 12000 µS per cm$^2$, on the assumption of a soma-dendritic area of 4000 µm$^2$. This figure is compared with those from some other neurons in Table 8 where an extremely broad range of values is seen, from a smallest value of 445 µS per cm$^2$ to 100000 µS per cm$^2$. With the estimated value $g_{A,\text{max}}$ from the voltage-clamp measurements of Burlhis and Aghajanian (1987), the predicted time course for $I_A + I_T$ shown in Figure 4 is in broad agreement with the experimental results. However, this is only a heuristic result which may be useful as a quantitative guide, because of the unknown effects of various other membrane currents and fluxes which might affect the experimental results.

In a second voltage clamp experiment, Burlhis and Aghajanian (1987) used CsCl-filled electrodes to suppress $I_A$. The holding potential was again -80 mV and the peak inward current was plotted against test potential in their Figure 5D. According to Puil and Werman (1981), Cs$^+$ blocks not only $I_A$ but also $I_{KDR}$ and calcium-activated potassium currents (but see also Sanchez et al., 1998). A model of an DRN SE neuron with all potassium currents blocked was employed to examine the current-voltage relation and the results are shown in Figure 5. There is broad agreement with the experimental result as there is a fairly sharp upswing in the peak inward current from about -70 mV to -50 mV, indicating that $I_T$ has been switched on at about the right voltages to give the prepotentials which trigger spikes in these cells.
Figure 5: An attempt to emulate voltage-clamp results of Burlhis and Aghajanian (1987, Figure 5D). From a holding potential of -80 mV, test potentials gave rise to computed peak inward currents of magnitudes shown, indicating broad agreement with the experimental results.

Parameter values for $I_T$

The standard set of kinetic parameters for $I_T$ given in Table 9 is mainly taken from Huguenard and McCormick (1992), with suitable approximations for the time constants. In the case of the time constant for inactivation, the approximation is valid over values of $V$ which are likely to occur during the activity of a DRN SE neuron.

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{T_1}$ | -57   | $V_{T_3}$ | -81 m |
| $k_{T_1}$ | 6.2   | $k_{T_3}$ | -4    |
| $a_T$     | 0.7   | $c_T$     | 28    |
| $b_T$     | 13.5  | $d_T$     | 300   |
| $V_{T_2}$ | -76   | $V_{T_4}$ | -81   |
| $k_{T_2}$ | 18    | $k_{T_4}$ | 12    |
Calcium L-type current, $I_L$

There are four main subtypes of L-type calcium currents, designated, according to the accepted nomenclature, $\text{Ca}_v.1.1 - \text{Ca}_v.1.4$. In most central nervous system cells the subtypes are mainly $\text{Ca}_v.1.2$ or $\text{Ca}_v.1.3$. According to a recent review (Tuckwell, 2012a), L-type calcium currents often inactivate by both voltage-dependent and calcium dependent mechanisms (CDI). The general form for this current in the linear version is similar to that of $I_T$ in Equation (18) with $L$ replacing $T$ throughout. Similarly with the constant field formalism the current is

$$I_L = k_L m_L^2 h_L f_L V, \frac{\left[ \text{Ca}_i - \text{Ca}_o e^{\frac{-2V}{V_0}} \right]}{1 - e^{\frac{-2V}{V_0}}}$$  \hspace{1cm} (26)$$

where most of the symbols are as defined for $I_T$ except that now $f_L$ represents the CDI. $m_L$ and $h_L$ satisfy differential equations like (4) and (5). For neurons the powers 1 and 2 are about equally frequently employed for $m_L$. The equation for $f_L$ is

$$\frac{df_L}{dt} = \frac{f_L,\infty - f_L}{\tau_f},$$  \hspace{1cm} (27)$$

where $f_L,\infty(\text{Ca}_i)$ is the steady state value $f_L(\text{Ca}_i, \infty)$ and $\tau_f$ is a time constant which may depend on $\text{Ca}_i$. Here $f_L,\infty$ is defined as

$$f_L,\infty = \frac{1}{1 + \left( \frac{\text{Ca}_i}{K_f} \right)^n}$$  \hspace{1cm} (28)$$

where $K_f$, in mM, is the value of $\text{Ca}_i$ at which the steady state inactivation has half-maximal value. Knowledge of $\tau_f$ is scant, and one can adopt the usual assumption that it is very small (especially relative to $\tau_{h,L}$) so that $f_L$ is set equal to its steady state value. We will also adopt the usual value $n = 1$ (Standen and Stanfield, 1982). Thus we may put

$$I_L = k_L m_L^2 h_L V, \frac{1}{1 + \left( \frac{\text{Ca}_i}{K_f} \right)^n} \left[ \text{Ca}_i - \text{Ca}_o e^{\frac{-2V}{V_0}} \right] \frac{1}{1 - e^{\frac{-2V}{V_0}}}$$  \hspace{1cm} (29)$$

Assumptions about the parameters for $I_L$ are not expected to have grave consequences for the spiking dynamics because the contributions from the L-type current are presumed to be less than about 10% of the whole cell calcium currents, based on the observations of Penington et al. (1991) that the L-type contribution was small
(about 4%) in dissociated cells and that the threshold for activation is not likely to occur around potentials near the threshold for action potentials.

For the steady state L-type activation we put

$$m_{L,\infty} = \frac{1}{1 + e^{-(V-V_{L_1})/k_{L_1}}}$$  \hspace{1cm} (30)

and

$$\tau_{m_L} = a_L + \frac{b_L}{\cosh((V+V_{L_2})/k_{L_2})},$$  \hspace{1cm} (31)

where there are 4 parameters $a_L, b_L, V_{L_2}, k_{L_2}$. These two functions, $m_{L,\infty}$ and $\tau_{m_L}$ were well-fitted, in accordance with experimental data, to the more complicated forms in Athanasiades et al. (2000) and Komendantov et al. (2007). The same remarks apply to the steady state voltage-dependent inactivation variable which is set to

$$h_{L,\infty} = \frac{1}{1 + e^{(V-V_{L_3})/k_{L_3}}}$$  \hspace{1cm} (32)

with a voltage-independent time constant,

$$\tau_{h,L} = const.$$  \hspace{1cm} (33)

**Parameter values for $I_L$**

For DRN SE neurons there are no explicit voltage clamp data from which kinetic parameters for activation and inactivation can be estimated for $I_L$. In Penington et al. (1991), single channel currents (slope 23 pS) were given for L-type with voltages ranging from about -20 mV to +30 mV. It is likely that the L-type current in these cells belongs to the high neuron group summarized in Table 3 of Tuckwell (2012a). Aghajanian (personal communication) found that L-channel blockers had little effect on firing rates, which implies that these channels are not of the pacemaking type as they are in dopaminergic neurons (see Discussion), in which the activation occurs at much lower voltages, as in the low neuron group of Tuckwell (2012a).

The average data is employed as the standard set in Table 10 in the present article.

**Calcium N-type current, $I_N$**

In voltage clamp experiments on DRN SE neurons, Penington et al. (1991) found evidence that among high-threshold calcium currents, N-type channels contributed
Table 10: Standard set of parameters for $I_L$

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{L1}$  | -18.3 | $k_{L1}$  | 8.4   |
| $k_{L1}$  | 15    | $V_{L3}$  | -42   |
| $a_L$     | 0.5   | $k_{L3}$  | 13.8  |
| $b_L$     | 1.5   | $\tau_{h_L}$ | 200  |
| $V_{L2}$  | -20   |           |       |

about 40% of total calcium current in dissociated cells. Most of the remaining fraction was not blocked by $\omega$-conotoxin and was called N-like. These two components are here lumped together as N-type. Whether CDI is appreciable for N-type calcium currents is uncertain (Budde et al., 2002), although some evidence for it has been obtained in chick dorsal root ganglion neurons (Cox and Dunlap, 1994) and rat sympathetic neurons (Goo et al., 2006). Amini et al (1999), in their model of midbrain dopamine neurons, included CDI (steady state only) for N-type calcium but without any voltage-dependent inactivation.

There have been a few voltage clamp studies of high threshold calcium currents in DRN SE neurons (Penington et al., 1991; Penington and Fox, 1995). Analysis of the I-V relations has been performed under the constant field assumption and the form of the current and its kinetic properties estimated. These analyses lead to the conclusion that the N-type current can be written

$$I_N = k_N m_N^2 h_N V \frac{[Ca_i - Ca_o e^{-\frac{2V}{V_o}}]}{1 - e^{-\frac{2V}{V_o}}}$$

where $m_N$ and $h_N$ satisfy differential equations like (4) and (5). For activation variables we put

$$m_{N,\infty} = \frac{1}{1 + e^{-(V-V_{N1})/k_N1}}$$

and

$$\tau_{m_N} = a_N + \frac{b_N}{\cosh((V-V_{N2})/k_N2)}.$$  

If the calcium N-type channels located on the soma (Penington et al., 1991) have similar properties to those examined in Joux et al. (2001), then they do not inactivate, as incorporated in the model of Komendantov et al. (2007). Some inactivation does occur however in DRN SE neurons and others (Cox and Dunlap, 1994; Lin et
al., 1997; Goo et al., 2006) so we put for inactivation variables

\[ h_{N,\infty} = \frac{1}{1 + e(V - V_{N3})/k_{N3}} \]  

(37)

and a (large) voltage-independent time constant

\[ \tau_{hN} = \text{const.} \]  

(38)

**Parameter values for \( I_N \)**

Deducing kinetic parameters from voltage clamp data for \( I_N \) is also made difficult because of the many confounding factors such as temperature, mixtures of currents, buffers and pipette solutions and nature of the charge carrier, which is rarely calcium. From the data of Figure 6 in Penington and Fox (1995), the following parameter values were estimated (voltages in mV, times in ms): \( V_{N1} = -13.5 \), \( k_{N1} = 9 \), \( a_N = 0.305 \), \( b_N = 2.29 \), \( V_{N2} = -20 \), \( k_{N2} = 20 \), \( V_{N3} = -50 \), \( k_{N3} = 20 \), and \( \tau_{hN} = 1000 \). In their studies of variants of N-type channels expressed in sympathetic ganglia, Lin et al. (1997) found \( V_{N1} \) values from -13.8 to -6.2, \( V_{N3} \) values from -53 to -43, \( \tau_{mN} \) values from 3.3 to 5.8 and \( \tau_{hN} \) values from 317 to 1567. In another study (unpublished data) we found \( V_{N1} = -15.2 \) and \( k_{N1} = 9 \). Furthermore, Tris-PO\(_4\) in the pipette shifted the activation by several mV to the left. These values may be compared with those employed by Komendantov et al. (2007), \( V_{N1} = -11 \) and \( k_{N1} = 4.2 \). From these estimates, the following set of parameters (Table 11) was chosen as standard for N-type. The value of \( k_N = 0.412 \) gives a peak current of about 3.8 nA with a clamp from a holding potential of -60 mV.

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| \( V_{N1} \) | -8    | \( k_{N2} \) | 15    |
| \( k_{N1} \) | 7     | \( V_{N3} \) | -52   |
| \( a_N \)   | 1     | \( k_{N3} \) | 12    |
| \( b_N \)   | 1.5   | \( \tau_{hN} \) | 1000 |
| \( V_{N2} \) | -15   | \( k_N \)   | 0.206 |

Table 11: Standard set of parameters for \( I_N \)
5 Calcium-dependent potassium currents

Calcium entry into neurons (and other cell types) may activate certain calcium-dependent potassium ion channels which usually leads to hyperpolarizing effects. There are 4 main types of such channels in neurons, viz, the large conductance BK channel (or $K_{Ca1.1}$) and the small conductance SK channels, SK1, SK2 and SK3 ($K_{Ca2.1}$, $K_{Ca2.2}$ and $K_{Ca2.3}$, respectively). According to Camerino et al. (2007) BK channels are involved in a number of diseases including hypertension, coronary artery spasm, urinary incontinence, stroke, epilepsy and schizophrenia.

Apart from the magnitudes of their conductances, the BK and SK types have differing activation properties and pharmacology. The activation of SK channels is purely calcium-dependent whereas that of BK channels depends both on calcium ion concentration and membrane potential. SK channels are selectively blocked by apamin from bee venom whereas BK channels are blocked by micromolar concentrations of TEA and certain scorpion-derived toxins such as iberiotoxin. For reviews and discussions of the properties of these $Ca^{2+}$-dependent potassium channels see Faber and Sah (2003), Muller et al. (2007), Fakler and Adelman (2008), Faber (2009) and Adelman et al. (2011). The role of BK channels is usually considered to be the hastening of repolarization after a spike with a consequent shortening of spike duration as demonstrated, for example, in rat CA1 pyramidal cells (Shao et al., 1999; Gu et al., 2007; Loane et al., 2007), cerebellar Purkinje cells (Womack and Khodakhah, 2002), hippocampal granule cells (Müller et al., 2007, Jaffe et al., 2011) and dorsal root ganglion cells (Scholz et al., 1998). Blocking of BK channels may lead to bursting in some neurons (Traub et al., 2003). SK channels are involved in the medium duration (of order 100 ms) afterhyperpolarization following a spike which leads to a lengthening of the interspike interval.

As noted above, calcium entry into neurons can be via several kinds of voltage-dependent calcium channel (VGCC) called L, N, P/Q, R and T-types, but not all are equally efficacious in the activation of $Ca^{2+}$-dependent potassium currents. In CA1 pyramidal cells Marrion and Tavalin (1998) showed that SK channels were only activated by L-type $Ca^{2+}$ currents, whereas BK channels were only activated by N-type currents, the latter also being the case for CA1 granule cells (Muller et al., 2007).

However, the situation differs from one neuron type to another. SK currents can also be coupled to N-type, P/Q-type, R-type or T-type (Sah and Davies, 2000; Faber and Sah, 2003; Loane et al., 2007). BK channels have also been found to be driven by R-type, L-type and P/Q-type (Fakler and Adelman, 2008). Sah and Davies (2000) contains a summary of couplings of various VGCCs with SK and
BK channels in various neurons. Close coupling of VGCCs to BK occurs when or because they are very close and there is evidence that the two kinds of channel are coassembled (Grunnet and Kaufmann, 2004; Fakler and Adelman, 2008). On the other hand, SK channels are not usually so tightly connected with VGCCs, although SK2 and T-type VGCCs are possibly co-assembled in some cells. In midbrain dopamine neurons, depolarization by L-type calcium currents occurs as part of the pacemaker cycle and the AHP in these cells is the result of the activation of SK channels by T-type calcium currents (Adelman et al., 2012).

Calcium-dependent potassium currents have long been implicated as the basis of the long (several hundred ms) afterhyperpolarization (AHP) following spikes in DRN SE neurons and hence a major component of pacemaker-like activity in these cells (Vandermaelen and Aghajanian, 1983; Segal, 1985; Burlhis and Aghajanian, 1987; Freedman and Aghajanian, 1987; Penington et al., 1992; Aghajanian and Sanders-Bush, 2002; Kirby et al., 2003; Beck et al., 2004). Direct experimental evidence has been provided by Freedman and Aghajanian (1987), Pan et al. (1994), Scuvecé-Moreau et al. (2004), Rouchet et al. (2008), Crespi (2009) and Crawford et al. (2010). The recordings of Pan et al. (1994) and Scuvecé-Moreau et al. (2004) show clearly the inhibiting effect of apamin on the afterhyperpolarization in DRN SE neurons. Since only SK channels are blocked by apamin, it is clear that the AHP is mainly due to SK channel activation.

It is claimed that in rat brain BK channels are driven mainly by N-type VGCCs (Loane et al., 2007) so we will assume that this is the case in DRN SE neurons and that N-type calcium current contributes to the repolarization phase by activating BK channels. However, there is evidence for the colocalization and coassembly of L-type VGCC and BK in some parts of rat brain (Grunnet and Kaufmann, 2004), so despite the relatively small magnitude of the L-type Ca\(^{2+}\) current in 5-HT cells of the dorsal raphe, there remains the possibility of an L-type-BK coupling.

Anatomical investigations have been made of the distribution of BK and SK channels in mouse and rat brain, with similar findings in both species. In the first such study, Knaus et al. (1996) found that in rat, BK channel density was low in the brainstem relative to areas such as frontal cortex. A more detailed enumeration of BK densities in mouse brain (Sausbier et al., 2006) gives the numbers in the dorsal raphe nucleus as “few”, compared with a maximum of “very high”, thus corroborating the findings in rat brain. Stocker and Pedarzani (2000) found that in the rat DRN and MRN, SK1 were absent, SK2 were at a “moderate” level and SK3 were “high”. In B9 serotonergic neurons, both SK2 and SK3 were found to be “high”. High levels of SK3 were found in the brainstem of mouse (Sailer et al., 2004) and rat (Sailer et al., 2002).
Modeling the BK current

The dual dependency of the BK current on Ca\(^{2+}\) concentration and voltage makes its description a little more complex than that for purely voltage-dependent channels. Some of the first attempts at the inclusion of BK currents in spiking neuron models seem to be those of Yamada et al. (1989) for a cell of the bullfrog sympathetic ganglion, and Lytton and Sejnowski (1991) for cortical pyramidal cells. Schild et al. (1993) included a calcium-dependent potassium current to account for spike frequency adaptation in neurons of the rat nucleus tractus solitarii. More recently, with additional data available, mathematical descriptions of calcium-dependent potassium currents, including those through BK channels, have been handled in various ways (Traub et al., 2003; Xiao et al. 2004; Komendantov et al., 2007; Saarinen et al., 2008; Jaffe et al., 2011). A simple model in which the calcium dependence is ignored was proposed by Tabak et al. (2011) and is explored in the simplified model in this article.

The current through BK channels is designated by \(I_{BK}\). The following equation describes this current on the assumption that the reversal potential is the Nernst potential for \(K^+\),

\[
I_{BK} = g_{BK,max} m_{BK}(V - V_K),
\]

(39)

where \(g_{BK,max}\) is the maximal conductance and \(m_{BK}\) is the activation variable. The constant field form may also be employed (Sun et al., 2004). It is assumed here that there is no explicit inactivation variable, although inactivation has been found when the BK channel has certain subunits (Xia et al., 1999) and has been documented in some other cases (Vergara et al. 1998; Sun et al., 2004; Gu et al., 2007). The observation that the BK current often closely follows the calcium current indicates that the decline may be reasonably accurately described as deactivation. Furthermore, Marcantoni et al. (2010) found in mouse chromaffin cells that the L-type current through Ca\(_v\)1.3 channels is coupled to fast inactivating BK and as we have discussed above, it is most likely that in DRN SE neurons the L-type channels are .

With the simplification of no explicit inactivation

\[
\frac{d m_{BK}}{dt} = \frac{m_{BK,\infty} - m_{BK}}{\tau_{m_{BK}}},
\]

(40)

where the steady state activation and time constant depend on both \(V\) and \(Ca_i\).

Empirically based estimates of the steady state activation function \(m_{BK,\infty}(Ca_i, V)\) have been available since Barrett et al. (1982). The data are usually characterized by fitting a family of Boltzmann functions of voltage at various values of \(Ca_i\), where
the half-activation potential and the slope factor depend on $Ca_i$. Many such data sets are available (for example, Cui et al., 1997; Scholz et al. 1998; Womack and Khodakhah, 2002; Sun et al., 2004; Thurm et al., 2005; Sweet and Cox, 2008) in a variety of preparations, which leads to very different parameter sets. Much of the data involves levels of $Ca_i$ (and voltages) which are considerably higher than the ambient values expected in the normal functioning of DRN SE (or any other) neurons. This raises the question of how to interpret $Ca_i$ values in formulas for the activation functions and time constants. Since calcium ion concentrations just on the inside of BK channels, for example, just after the passage of $Ca^{2+}$ are much higher than the ambient averages (Fakler and Adelman, 2008), the latter need to be scaled up by a numerical factor $\alpha_{BK}$ when using formulas such as (41) and (44) below, which have been from data obtained using inside-out patch recording.

On examination such data, those of Womack and Khodakhah (2002) were chosen, whereby a family of well-fitting Boltzmann functions was found to be, with half-activation potentials $V_{BK,h}$ and slope factors $k_{BK}$:

$$m_{BK,\infty}(Ca_i, V) = \left(1 + e^{-\frac{V - V_{BK,h}(Ca_i)}{k_{BK}(Ca_i)}}\right)^{-1},$$  

where

$$V_{BK,h}(Ca_i) = -40 + 140e^{-(Ca_i - 0.7)/7}$$  

and

$$k_{BK}(Ca_i) = 11 + 0.03Ca_i,$$

with calcium ion concentrations in $\mu$M and voltages in mV. The value of the half-activation potential $V_{BK,h}$ asymptotes to -40 mV at large calcium ion concentrations, displaying behavior similar to that shown in Latorre and Brauchi (2006). The steady state activation function described by these last three equations is plotted in Figure 6.

Empirical data on activation time constants for BK channels also exhibit much variability (Cui et al., 1997; Womack and Khodakhah, 2002; Sun et al., 2004; Thurm et al., 2005) which reflects the diversity of the preparations. As a rare example where data at the same $(Ca_i, V)$ point are given in two studies, the time constant of activation reported at $Ca_i = 10\mu$M and $V = 20mV$ is 0.25 ms in rat inner hair cells (Thurm et al., 2005) and 1.3 ms in Xenopus motor nerve terminals (Sun et al., 2004). Data are sometimes not given explicitly over ranges of values of $Ca_i$ or $V$ which would enable one to construct a function of these two variables suitable for use in a mathematical model. Since an action potential is only a few ms in duration
it seems that for BK currents to affect this quantity the activation time constant should be not much greater than an ms at the corresponding values of \(Ca_i\) and \(V\). Consideration of the various data sets led to the use of that of Thurm et al. (2005) to approximate the activation time constant for BK, as the values were given over ranges of \(Ca_i\) and \(V\) expected to occur during the action potential. Reasonable fits over the ranges of \(0 \leq Ca_i \leq 10\) (in \(\mu\)M) and \(-100 \leq V \leq 100\) (in mV) were obtained on putting

\[
\tau_{m_{BK}}(Ca_i, V) = 1.345 - 0.12Ca_i + V(0.0004Ca_i - 0.00455).
\]  (44)

This yields a plane which is illustrated in Figure 7.

**Modeling the SK current**

This current is comparatively straightforward to describe, as its activation is purportedly only dependent on \(Ca_i\). We will assume that there is only one kind of SK channel, SK3, which was noted above to be prevalent in the DRN. If SK2 are present then they can be included in the same terms as their properties are very similar (Coetzee et al., 1999; Barfod et al., 2001). Thus

\[
I_{SK} = g_{SK,max}m_{SK}(V - V_K),
\]  (45)
Figure 7: Activation time constant for BK channels as a function of membrane potential $V$ and internal $\text{Ca}^{2+}$ concentration in $\mu M$. Adapted from Thurm et al. (2005).

where

$$\frac{dm_{SK}}{dt} = \frac{m_{SK,\infty} - m_{SK}}{\tau_{m_{SK}}},$$

though some authors have put $m_{SK}^2$ instead of $m_{SK}$ in Equ (45) (Yamada et al., 1989; Destexhe et al., 1994). There is evidently no explicit inactivation process, as the decay of $I_{SK}$ is governed by $\text{Ca}^{2+}$ dynamics (Teagarden et al., 2008). The steady state activation is steeply dependent on $\text{Ca}_i$ and is written

$$m_{SK,\infty} = \frac{Ca_i^n}{Ca_i^n + K_c^n}$$

where $n$ is the Hill coefficient and $K_c$ is the EC$_{50}$ which is the value of $\text{Ca}_i$ at which the activation $m_{SK,\infty} = 0.5$. SK channels have $K_c$ values between 300 and 800 nM and Hill coefficients between 2 and 5, where the ranges given by Vergara et al. (1998) and Stocker (2004) have been combined. The time constant $\tau_{m_{SK}}$ is about 5 ms (Fakler and Adelman, 2008), the range 5-15 ms being given by Stocker (2004) and calcium dependency for this quantity was employed by Yamada et al. (1989)
and Destexhe et al. (1994). Sometimes the time constant has been ignored so that $m_{SK}$ is always at its steady state value (Komendantov et al., 2007). Considering that time scales of several hundred milliseconds are relevant in the spontaneous activity of many DRN SE neurons, such an approximation would be reasonable except perhaps when the cells are in bursting mode.

6 Hyperpolarization activated cation current, $I_H$

This current, which is is elicited by hyperpolarizations relative to rest, is slow to activate and does not inactivate (McCormick and Pape, 1990; McCormick and Huguenard, 1992; Pape, 1996; Robinson and Siegelbaum, 2003). In DRN SE neurons a similar current activating below -70 mV, which we denote by $I_H$, was described by Williams et al. (1988). $I_H$ may be enhanced by activation of certain 5-HT receptors thus preventing excessive hyperpolarization and tending to increase SE neuron firing rates (Aghajanian and Sanders-Bush, 2002). The current has been written in various forms and is often omitted in mathematical models, though it often plays a role in pacemaking - see the Discussion for details.

In the absence of specific data we put, as in McCormick and Huguenard (1992),

$$I_H = g_{H,\text{max}} m_H (V - V_H)$$

(48)

where $V_H$ is the reversal potential, usually taken as about -40 mV, $m_H$ satisfying an equation like (4). The steady state activation is given by

$$m_{H,\infty} = \frac{1}{1 + e^{(V - V_{H_1})/k_{H_1}}}$$

(49)

with time constant well fitted by

$$\tau_{m_H} = \frac{a_H}{\cosh((V - V_{H_2})/k_{H_2})}.$$  

(50)

**Parameter values for $I_H$**

The parameters for $I_H$ are slightly modified from those of Huguenard and McCormick (1992) to make the current activate at around -70 mV and have an activation time constant around 300 to 1000 ms (Williams et al., 1988). Thus the following set of parameters was chosen as standard for $I_H$. 

Table 12: Standard set of parameters for $I_H$

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{H1}$  | -80   | $V_{H2}$  | -80   |
| $k_{H1}$  | 5     | $k_{H2}$  | 13    |
| $a_H$     | 900   | $V_H$     | -45   |

7 Fast transient sodium current, $I_{Na}$

The only sodium current included is the transient $I_{Na}$ which, when blocked by TTX in DRN SE neurons, reduces spike amplitude by about 60 mV or more (Segal, 1985; Burlhis and Aghajanian, 1987). The current is given by the classical form

$$I_{Na} = g_{Na,max}n^3h_{Na}(V - V_{Na})$$  \hspace{1cm} (51)

with activation variable $m_{Na}$ and inactivation $h_{Na}$. For the steady state activation we put

$$m_{Na,\infty} = \frac{1}{1 + e^{-(V - V_{Na_1})/k_{Na_1}}}$$  \hspace{1cm} (52)

with corresponding time constant

$$\tau_{m,Na} = a_{Na} + b_{Na}e^{-(V - V_{Na_2})/k_{Na_2}}$$  \hspace{1cm} (53)

which fits well the forms used by some authors (McCormick and Huguenard, 1992; Traub et al., 2003) but not all. The steady state inactivation may be written

$$h_{Na,\infty} = \frac{1}{1 + e^{(V - V_{Na_3})/k_{Na_3}}}$$  \hspace{1cm} (54)

with corresponding time constant fitted with

$$\tau_{h,Na} = c_{Na} + d_{Na}e^{-(V - V_{Na_4})/k_{Na_4}}$$  \hspace{1cm} (55)

Parameter values for $I_{Na}$

The properties chosen for fast sodium transient currents in neuronal models are diverse, especially with respect to time constants of activation and inactivation, which might be explained by the various types of $Na_{x}e$ channels, where $x$ is 1.1 to 1.9 (Catterall et al., 2005). Some examples which illustrate this are given Table
13. Note that the entry for the Hodgkin-Huxley squid axon model gives potentials relative to zero as resting level, which was near -60 mV (Hodgkin and Katz, 1949; Tuckwell, 1988, Chapter 2).

Table 13: Examples of parameters for $I_{Na}$

| Source                  | $V_{Na1}$ | $k_{Na1}$ | $V_{Na3}$ | $k_{Na3}$ | $\tau_{m,max}$ | $\tau_{h,max}$ |
|-------------------------|-----------|-----------|-----------|-----------|-----------------|-----------------|
| Hodgkin (1952)          | +25       | 10        | +2.7      | 7.5       | 0.5             | 8               |
| Belluzzi (1991)         | -36       | 7.2       | -53.2     | 6.5       | 0.3             | 22              |
| Traub (2003)            | -34.5     | 10        | -59.4     | 10.7      | 0.16            | 0.16            |
| Komendantov (2007)      | -34.6     | 6.2       | -61.6     | 6.8       | 0               | 29.1            |
| This study              | -33.1     | 8         | -50.3     | 6.5       | 2               | 8               |

Voltage clamp experiments previously performed on a putative DRN SE neuron enable estimates of parameters for sodium current kinetics to be made. Current versus voltage values were obtained with a holding potential of -80 mV and steps to various test potentials from -60 mV to +60 mV. Using $G/G_{max}$ gave a half-activation potential (for $m^3$) of -30.73 mV and a slope factor of 4.62. However, it is useful to employ the methodology in Penington and Tuckwell (2012) to obtain estimates of the parameters of $m_{Na,\infty}$ and $\tau_{m,Na}$ as well as $g_{Na,max}$. Current versus time curves for test potentials of -35 mV and -40 mV were obtained and that for -35 mV is shown in Figure 8.

The maximum amplitude of the current and its time of occurrence are used to determine best fitting curves proportional to $m^3h$ as in Penington and Tuckwell (2012). This procedure yielded the following two equations (in nanoamps),

\[ 1.12 = 62.73g_{Na,max}m_{Na,\infty}^3(-35) \] \tag{56}
\[ 0.657 = 75.33g_{Na,max}m_{Na,\infty}^3(-40). \] \tag{57}

It is convenient to assume that very nearly, $m_{Na,\infty}(0) = 1$, which yields, in conjunction with the experimental maximum current at the test potential $V=0$ and a supposed ratio of $\tau_{h,Na}$ to $\tau_{m,Na}$ at large depolarizations from two other experimental studies of sodium currents (Belluzzi et al., 1991; Yoshino et al., 1997),

\[ 2.15 = 11.9g_{Na,max}m_{Na,\infty}^3(0). \] \tag{58}

From this last equation the estimate is $g_{Na,max}=0.18 \mu S$ for a dissociated cell. It is then possible to estimate $m_{Na,\infty}(-35) = 0.462$ and $m_{Na,\infty}(-40) = 0.364$. Using
the estimated values of $m_{Na,\infty}$ at $V=0$, -35 and -40 mV, the Boltzmann parameters are obtained as in Table 14.

To find $\tau_{m,Na}$ as a function of $V$ we use the values estimated from voltage clamp data $\tau_{m,Na}(-35) = 0.15$ ms and $\tau_{m,Na}(-40) = 0.2$ ms. Considering the positions of the maximum of $\tau_{m,Na}$ relative to the half-activation potential in other studies of sodium channel dynamics, it is reasonable to assume that the maximum of $\tau_{m,Na}$ is in fact 2 ms at $V = -40$. Assuming too an asymptotic minimum of 0.05 ms at very large depolarizations and very large hyperpolarizations leads to the estimates of the four parameters in $\tau_{m,Na}$ given in Table 14.

For the steady state inactivation, in accordance with findings of Belluzzi et al. (1991), it is supposed that the half-inactivation potential is -50.3 mV at 17.2 mV in the hyperpolarizing direction from the half activation potential. The time constant for inactivation $\tau_{h,Na}$ was estimated from voltage clamp data at two test potentials to give 2.41 ms at -35 mV and 6.24 ms at -40 mV. Assuming as found in other studies that the maximum value of $\tau_{h,Na}$ occurs at about 7 mV positive to the half-inactivation potential and that this maximum is 8 ms with a minimum of 0.5 ms yields the parameters for $\tau_{h,Na}$ and $h_{Na,\infty}$ given in Table 14. Note that the value
of $k_{Na_1}$ has been reduced from its estimated value of 12.3 to 8, which is more in accordance with the average of the values found in other preparations. The standard set of parameters for $I_{Na}$ kinetics is summarized in Table 14.

| Parameter | Value | Parameter | Value |
|-----------|-------|-----------|-------|
| $V_{Na_1}$ | -33.1 | $V_{Na_3}$ | -50.3 |
| $k_{Na_1}$ | 8 | $k_{Na_3}$ | 6.5 |
| $a_{Na}$ | 0.05 | $c_{Na}$ | 0.5 |
| $b_{Na}$ | 0.15 | $d_{Na}$ | 7.5 |
| $V_{Na_2}$ | -40 | $V_{Na_4}$ | -43 |
| $k_{Na_2}$ | 7.85 | $k_{Na_4}$ | 6.84 |

8 Leak current, $I_{Leak}$

In the Hodgkin-Huxley (1952) model, a leak current was inserted in the differential equation for $V$ with its own equilibrium potential and conductance. This small current was stated to be composed of chloride and other ions, and the conductance did not depend on $V$. One motivation for including a leakage current is to take account of ion flows by active transport (pumps), although some authors take such transport into account explicitly. More recently, specific channels for potassium (Goldstein et al., 2001) and sodium (Tremblay et al., 2011) leak currents have been found. In the present model, as can be discerned from the properties of DRN SE neurons discussed above, there is not expected to usually be a zero ion flux at rest because many of these neurons fire spontaneously without afferent input. We therefore insert a token leak current which is carried by sodium and potassium ions. Based on a dynamical balance equation the following expressions are obtained for the leak current which is composed of a potassium ion and sodium ion contribution.

$$I_{leak} = I_{K,leak} + I_{Na,leak}$$ (59)

$$I_{K,leak} = g_{K,leak}(V - V_K)$$ (60)

$$I_{Na,leak} = g_{Na,leak}(V - V_{Na})$$ (61)

$$g_{K,leak} = \frac{(V_R - V_{Na})}{(V_K - V_{Na})} \frac{1}{R_m}$$ (62)
\[ g_{Na,\text{leak}} = \frac{1}{R_{in}} - g_{K,\text{leak}} \]  

(63)

where \( V_K \) and \( V_{Na} \) are Nernst potentials, \( V_R \) is the resting potential and \( R_{in} \) is the input resistance of the cell.

9 Calcium ion dynamics

The intracellular calcium ion concentration \( C_{a_i} \) varies in space and time throughout the cytoplasm. It may undergo increases due to the inward flow of \( Ca^{2+} \) through VGCCs and because of release from intracellular stores, including endoplasmic reticulum and mitochondria, and release from buffers. Decreases occur by virtue of the pumping of \( Ca^{2+} \) to the extracellular compartment, absorption by various buffers and the return of ions to the intracellular stores. Many of these processes are subjects of ongoing research so that accurate quantitative modeling is not able to be carried out with certainty. Calcium buffers have important effects on neuronal firing and synaptic transmission and altered expressions of them are associated with many diseases including Alzheimer’s and Parkinson’s, epilepsy, schizophrenia and depression (Schwaller, 2007). Buffers can increase firing rate by reducing the amount of \( Ca^{2+} \) available to activate BK and SK channels. We first discuss some of the relevant properties of buffers before formulating a quantitative description of \( Ca^{2+} \) dynamics.

The overall scheme for the rate of change in intracellular calcium ion concentration has three components due to inward current \( I_{Ca} \) through voltage gated calcium channels, buffering and pumping,

\[ \frac{dC_{a_i}}{dt} = \left. \frac{dC_{a_i}}{dt} \right|_{\text{Buff}} + \left. \frac{dC_{a_i}}{dt} \right|_{I_{Ca}} + \left. \frac{dC_{a_i}}{dt} \right|_{\text{Pump}} . \]  

(64)

We will deal with each of these terms separately.

**Calcium buffering**

Calcium binding proteins are classified as either principally sensors, such as the ubiquitous calmodulin, or buffers, which bind \( Ca^{2+} \) when sufficiently large local increases in \( C_{a_i} \) occur. The main buffers in neurons are parvalbumin (PV), calbindin-D28k (CB) and calretinin (CR). Assuming that binding of buffer \( B \) to \( Ca^{2+} \) is according
to the simple scheme

\[ \text{Ca}^{++} + B \xrightleftharpoons[k_{off}]{k_{on}} \text{CaB}, \quad (65) \]

with \( k_{on} \) in units of \( \mu M^{-1}s^{-1} \) and \( k_{off} \) in units of \( s^{-1} \). Here and in the following, \( \text{Ca}^{++} \) bound to \( B \) is denoted as simply \( \text{Ca} \). The dissociation constant is

\[ K_d = \frac{k_{off}}{k_{on}} \quad (66) \]

in \( \mu M \) and as pointed out by Schwaller (2010), its value usually far exceeds the resting level of \( \text{Ca}_i \) so that most buffer is free in resting conditions. Fast buffers have a relatively large value of \( k_{on} \). Generally PV is considered to be relatively slow, CB is faster and CR is the fastest. Table 15 gives typical approximate (average) parameters for the three main buffers, based on several reports (Bortolozzi et al., 2008; Cornelisse et al., 2007; Faas et al., 2007; Faas and Mody, 2011; Nägerl et al., 2000; Schwaller, 2007, 2009, 2010). Here CBP is calcium binding protein. Note that various concentrations of other metallic ions such as \( \text{Mg}^{2+} \) can in some cases alter the parameters for calcium binding. In the case of CR, the kinetics of binding depend on the state of occupancy and can change as \( \text{Ca}_i \) changes (Schwaller, 2009) which makes computational descriptions difficult.

### Table 15: Kinetic parameters for buffers

| CBP | \( K_d \) (nM) | \( k_{on} \) (\( \mu M^{-1}s^{-1} \)) | \( k_{off} \) (s\(^{-1} \)) | Remarks |
|-----|---------------|---------------------------------|-----------------|---------|
| PV  | 50            | 19                              | 0.95            |         |
| CB  | 195           | 12                              | 2.34            | High affinity, slow |
| CB  | 490           | 80                              | 39.2            | Low-medium affinity, fast |
| CR  | 68            | 310                             | 21.1            | R sites |
| CR  | 28000         | 1.8                             | 50.4            | T sites |
| CR  | 36000         | 7.3                             | 262.8           | EF5     |

The concentration of a buffer is an important variable and is unfortunately the least available, except for a few cell types such as hair cells (Bortolozzi et al., 2008; Hackney et al., 2005) cerebellar neurons (Hackney et al., 2005 and Schwaller, 2010, and references therein) and hippocampal neurons (e.g., Müller et al., 2005).
Considering many reports and omitting extremely high values, the concentration ranges in neurons are given as approximately 50-150 µM for PV, 40-350 µM for CB and 20-70 µM for CR. Nonspecified buffer concentrations were given as 45 µM (fast) and 250 µM (slow) by Sah (1992). Computational models have often followed Yamada et al. (1989) who employed a buffer with a concentration of 30 µM in the shell just interior to the membrane and 3 µM elsewhere. The kinetic parameters were $K_d = 1 \, \mu M$ and $k_{on} = 100 \, \mu M^{-1}s^{-1}$. Several other authors have followed the approach of Schild et al. (1993).

**Buffer types in the DRN**

With immunocytological methods, studies have been made of the gross details of the distribution of PV, CB and CR across various structures. These include rat brain (Celio, 1990; Résibois and Rogers, 1992; Rogers and Résibois, 1992), rat hindbrain (Arai et al., 1992) and macaque brainstem (Parvizi and Damasio, 2003). There have also been studies specifically targeting the DRN of the squirrel monkey (Charara and Parent, 1998) and chinchilla (Jaworska-Adamu and Szalak, 2009, 2010). In the results of most of these works, it is not possible to know with certainty which of the many kinds of cell in the DRN (see for example, Lowry et al., 2008) are the ones that contain the CBP.

Certain generalizations have been made concerning the distributions of the three buffers under consideration. According to Résibois and Rogers (1992), PV is usually found in GABA-ergic neurons. Further, PV, CB and CR are mostly concentrated in different nuclei, but in some nuclei many neurons are positive for more than one of these three calcium buffer proteins. Also, CR is not generally found in major cell groups which are serotonergic such as occur in DRN. Schwaller (2007) claims that a greater variety of neurons express CB than PV and that, in accordance with Résibois and Rogers (1992) and Rogers and Résibois (1992), CB and CR are rarely found in the same cells.

Concerning PV, (Celio, 1990) and Schwaller (2007) suggest little if any PV is found in the rat DRN and Parvizi and Damasio (2003) found no PV positive cells in the macaque mesencephalic raphe nuclei. However, inter-species differences probably exist, because Charara and Parent (1998) found that in the squirrel monkey DRN, there were a few 5-HT-ergic neurons that contained PV. Jaworska-Adamu and Szalak (2009) found only weak PV immunostaining in the chinchilla DRN. It seems reasonable to conclude from the available evidence that PV is probably not frequently present in DRN SE neurons of the rat.

In both squirrel monkey and macaque, many DRN neurons were found to contain...
this CB. Jaworska-Adamu (2010) found intense staining for CB in nearly all DRN cells. It may be concluded that CB is a frequently occurring calcium buffer in the DRN, including the 5-HT containing cells. Concerning CR, it seems from the pictorial results of Charara and Parent (1998), that CR may be quite prevalent in the squirrel monkey DRN. In rat, Arai et al. (1991), found many small and medium-sized cells in the lateral parts of the DRN contained CR, with few in the central part. Jaworska-Adamu and Szalak (2009) also found CR containing cells in the chinchilla DRN, but it is not known if these were serotonergic.

In summary, from the above observations, it may be concluded that in rat the DRN SE neurons are most likely to have CB as their main buffer, but that some cells may use CR. With modeling, one can test if the parameters for CB or CR lead to very different results if all other parameters are the same.

Modeling of the buffering contributions

The term \( \frac{dC_{a_i}}{dt} \Big|_{B_{aff}} \) appearing in Equ (64) will be described on the assumption that the endogenous buffer is in fact calbindin-D28k. Many approaches to modeling a contribution such as this have appeared in recent years, some of which (Schmidt et al., 2003; Canepari and Vogt, 2006; Schmidt and Eilers, 2009) follow the relatively simple independent sites scheme set forth in Nägerl et al. (2000) and some, such as Nadkarni et al. (2010) and Modchang et al. (2010) use more complete and hence complicated kinetic schemes for CB to Ca\(^{2+}\) binding. Pursuing the simpler description, we let the concentration of the \( k \)-th buffer site, with \( k = 1, 2, 3, 4 \), be \( B_k(t) \) at time \( t \) and let the reaction with Ca\(^{2+}\) be

\[
Ca^{++} + B_k \xrightleftharpoons[b_k]{f_k} CaB_k,
\]

where now the more succinct symbols \( f_k \) and \( b_k \) are used for forward (on) and backward (off) reaction rates. Applying standard chemical kinetics formulas gives the contribution to the rate of change of intracellular calcium ion concentration \( C_{a_i}(t) \)

\[
\frac{dC_{a_i}}{dt} \Big|_{B_{aff}} = \sum_{k=1}^{4} \left[ b_k [CaB_k](t) - f_k B_k(t) C_{a_i}(t) \right]
\]

where \([CaB_k](t)\) is the concentration of bound \( k \)-th buffer. Following Yamada et al. (1989) we let the total (bound and free) concentration of buffer site \( k \) be the
B_{k,tot} and note that

\[ B_{k,tot} = B_k(t) + [CaB_k](t), \]  \hspace{1cm} (69)\

which enables one to eliminate the bound buffer-calcium terms. The buffering contribution becomes

\[ \frac{dCa_i}{dt} \bigg|_{Buff} = \sum_{k=1}^{4} \left[ b_k(B_{k,tot} - B_k(t)) - f_kB_k(t)Ca_i(t) \right]. \]  \hspace{1cm} (70)\

The 4 subsidiary equations are

\[ \frac{dB_k}{dt} = b_k \left( B_{k,tot} - B_k(t) \right) - f_kB_k(t)Ca_i(t). \]  \hspace{1cm} (71)\

These previous two equations enable one to march forward in time, for example with an Euler scheme, in which, the buffering contribution only gives

\[ Ca_i((j + 1)\Delta t) = Ca_i(j\Delta t) + \Delta t \sum_{k=1}^{4} \left[ b_k \left( B_{k,tot} - B_k(j\Delta t) \right) - f_kB_k(j\Delta t)Ca_i(j\Delta t) \right]. \]  \hspace{1cm} (72)\

and

\[ B_k((j + 1)\Delta t) = b_k \left( B_{k,tot} - B_k(j\Delta t) \right) - f_kB_k(j\Delta t)Ca_i((j\Delta t). \]  \hspace{1cm} (73)\

**Calcium currents**

The term \( \frac{dCa_i}{dt} \) in (64) has several components. The presence of L-type, N-type and T-type Ca\(^{2+}\) currents has been verified in DRN SE neurons. A further calcium current, called \( I_{Ca,Raphe} \) was also found with similar electrophysiology to the N-type current, but it was not blocked by \( \omega \)-Conotoxin-GVIA (Penington and Fox, 1995). In the quantitative treatment of calcium currents we treat N-type and \( I_{Ca,Raphe} \) all as N-type.

Because the calcium-activated potassium currents may distinguish the type of Ca\(^{2+}\) channel which activates them, we keep track of the changes in the various calcium currents which have a total designated by

\[ I_{Ca} = I_L + I_N + I_T, \]  \hspace{1cm} (74)\

and must also decompose the intracellular calcium concentration into its sources. It is assumed that the resting level \( Ca_{i,R} \) is the value at \( t = 0 \) and that

\[ Ca_i(t) = Ca_{i,R} + Ca_{i,L}(t) + Ca_{i,N}(t) + Ca_{i,T}(t). \]  \hspace{1cm} (75)
However, as a simplifying assumption the total intracellular calcium concentration is used if the constant field expressions are employed and in determining the rates of buffering and pumping.

The usual and often-used assumption is made that the change of the calcium concentration due to flux through the voltage-gated channels occurs within a thin shell just inside the cell membrane with ascribed volume $v$ (Standen and Stanfield, 1982; Yamada et al., 1989), which might depend on the type of current but this complication will not be included. Then, with $F$ denoting Faraday’s constant, for the rate of change in internal calcium due to the L-type current

$$\frac{dCa_{i,L}}{dt} = -\frac{I_L}{2Fv}$$

and similarly for the N-type and T-type currents. A calcium source factor CSF with a maximum value of 1 and a minimum value of 0 is multiplied by the current terms to limit the magnitude of the internal calcium ion concentration, if necessary.

**Calcium pumping**

Intracellular $Ca^{2+}$ levels are kept low after surges of current through VGCCs and the maintenance of such levels is brought about by buffering and active transport to intracellular stores or to the extracellular space. The two main pumping mechanisms are the PMCA (plasma membrane $Ca^{2+}$ ATP-ase) pump and the NaX $Na^{+}$-$Ca^{2+}$ exchange pump (Carafoli, 2002). Only a few models of single neurons (for example, Lytton and Sejnowski, 1991; Amini et al., 1999) have included the NaX pump but all include versions of the PMCA pump. Some authors have employed a linear form (Komendantov et al, 2007; Cornelisse et al., 2007) or voltage-dependent forms (Rybak et al., 1997) for the latter but most often the form utilized is the simple Michaelis-Menten one (for example Good and Murphy, 1996; Lumpkin and Hudspeth, 1998)

$$\left.\frac{dCa_i}{dt}\right|_{Pump} = -K_s\frac{Ca_i}{Ca_i + K_m},$$

where $K_s$ determines the maximal pumping rate and $K_m$ is the dissociation constant which is the value of $Ca_i$ for half-maximal rate. It seems that the reasoning of Lumpkin and Hudspeth (1998) for using this simple form and not including NaX, is sound, namely that there is a lack of reliable data for the required parameters in more complex models.
10 Simplified model

It is convenient to first see if some of the firing properties of DRN SE neurons can be predicted with a model which has the main elements of the model described in Sections 2 to 9 but has a few simplifications. In the first instance, the latter consist of (a) ignoring the BK current (b) for calcium currents, using the form of (18) with an assumed fixed calcium reversal potential rather than the constant field form (c) omitting the M-type potassium current (d) simplifying the equations used to describe the buffering of calcium to those employed by Rybak et al. (1997), so that the differential equation for internal calcium concentration becomes

$$\frac{dC_{ai}}{dt} = -CSF(I_L + I_N) \cdot \frac{1 - PB(t)}{2F_v} - K_s \cdot \frac{C_{ai}}{C_{ai} + K_m}$$

where the fraction of calcium which is bound is

$$PB(t) = \frac{B_{tot}}{C_{ai} + B_{tot} + K_d},$$

$K_d$ being the dissociation constant defined in (66). In addition, an applied current $\mu$, which may be positive (hyperpolarizing) or negative (depolarizing) is added. This applied current can be experimentally applied or have its origin in synaptic inputs such as the noradrenergic input from locus coeruleus or inhibitory inputs from neighbouring DRN SE cells or within nucleus GABA-ergic neurons.

Equation (6) for the voltage now becomes

$$C \frac{dV}{dt} = -[I_A + I_{KDR} + I_T + I_L + I_N + I_H + I_{Na} + I_{SK} + I_{Leak} + \mu]$$

(78)

where the equations describing the individual components are given in the individual sections describing them. For time constants of activation and inactivation variables, a further simplification is sometimes made that they are not voltage dependent as given in their text descriptions. For example, the time constant $\tau_{m,Na}$, which depends on 4 parameters in Equ. (53), may be replaced with the single parameter

$$\tau_{m,Na} = \tau_{m,Na,c}$$

(79)

e tc.
Table 16: Activation and inactivation parameters

| Parameter         | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 |
|-------------------|---------|---------|---------|---------|---------|
| $V_{Na_1}$        | -33.1   | $c_T$   |         | $\tau_{h,N}$ | 1000    |
| $k_{Na_1}$        | 8       | $d_T$   | 300     | $V_{A_1}$ | -60     |
| $V_{Na_3}$        | -50.3   | $V_{T_4}$ | -81   | $k_{A_3}$ | 8.5     |
| $k_{Na_3}$        | 6.5     | $k_{T_3}$ | 12   | $V_{A_3}$ | -78     |
| $\tau_{m,Na_c}$  | 0.2     | $V_{L_1}$ | -18.3 | $k_{A_3}$ | 6       |
| $\tau_{h,Na_c}$  | 1       | $k_{L_1}$ | 8.4  | $a_A$ | 0.37    |
| $V_{KDR_1}$       | -15     | $a_L$   | 0.5    | $b_A$ | 2       |
| $n_k$             | 1       | $b_L$   | 1.5    | $V_{A_2}$ | -55     |
| $k_{KDR_1}$       | 7       | $V_{L_2}$ | -20  | $k_{A_2}$ | 15      |
| $a_{KDR}$         | 1       | $k_{L_2}$ | 15  | $c_A$ | 19      |
| $b_{KDR}$         | 4       | $V_{L_3}$ | -42  | $d_A$ | 45      |
| $V_{KDR_2}$       | -20     | $k_{L_3}$ | 13.8 | $V_{A_4}$ | -80     |
| $k_{KDR_2}$       | 7       | $\tau_{h,L}$ | 200 | $k_{A_4}$ | 7       |
| $V_{T_1}$         | -57     | $V_{N_1}$ | -8   | $V_{H_1}$ | -80     |
| $k_{T_1}$         | 6.2     | $k_{N_1}$ | 7   | $k_{H_1}$ | 5       |
| $V_{T_3}$         | -81     | $a_N$   | 1     | $a_H$ | 900     |
| $k_{T_3}$         | 4       | $b_N$   | 1.5   | $k_{H_2}$ | 13      |
| $a_T$             | 0.7     | $V_{N_2}$ | -15 | $V_{H_2}$ | -80     |
| $b_T$             | 13.5    | $k_{N_2}$ | 15  | $K_c$ | 0.000025 |
| $V_{T_2}$         | -76     | $V_{N_3}$ | -52 | $\tau_{m,SK}$ | 5      |
| $k_{T_2}$         | 18      | $k_{N_3}$ | 12  | $TF$ | 13.27   |

Table 17: Cell properties, Ca dynamics, Equilibrium potentials

| Parameter         | Value 1 | Value 2 | Value 3 |
|-------------------|---------|---------|---------|
| $V_K$             | -93     | $F$     | 96500   |
| $V_{Ca,rev}$      | 60      | $Ca_o$  | 2       |
| $V_R$             | -60     | $Ca_{i,R}$ | 0.00005 |
| $V_H$             | -45     | $B_{tot}$ | 0.03    |
| $V_{Na}$          | 45      | $K_d$   | 0.001   |
| $R_{in}$          | 2.415e08 | $K_s$ | 1.25e-06 |
| $C$               | 0.04    | $K_m$   | 0.0001  |
| $A$               | 4000    | CSF     | 0.7     |
| $d$               | 0.1     | $\mu$   | 0       |

In the simplified model there are 89 parameters, many of which have been given in the text. Tables 16-18 give the set of parameter values, referred to as Set 1, which
Table 18: Maximal conductances

| Channel          | Maximal Conductance |
|------------------|---------------------|
| $g_{Na,max}$     | 2                   |
| $g_{N,max}$      | 0.5                 |
| $g_{KDR,max}$    | 0.5                 |
| $g_{A,max}$      | 0.479               |
| $g_{T,max}$      | 0.0825              |
| $g_{IH,max}$     | 0.005               |
| $g_{L,max}$      | 0.0825              |
| $g_{SK,max}$     | 0.01                |

are first employed in the simplified model. This is a trial set which will be modified as necessary in the sequel. Table 16 contains the parameters required to describe the steady state activation and inactivation functions as well as the time constants for the various channels. Table 17 gives ionic equilibrium potentials and several cell properties including parameters required to describe calcium buffering and pumping. Finally Table 18 gives 8 maximal conductances, being for the 7 voltage-gated channels and the calcium-activated potassium current (SK). All potentials are in mV, all times are in ms, all ionic concentrations are in mM, all conductances are in $\mu$S, A is in square microns, d is in microns, C is in nF, and F is in .coulombs/mole.

Figure 9: Isolated spike in the simplified model with set 1 parameters as in Tables 16, 17 and 18, and a depolarizing applied current $\mu = -0.17$ nA. The resting potential is -60 mV.
11 Computed solutions

The differential equations of the model were integrated with an Euler method of step size 0.004 ms, the results being indistinguishable from those obtained by Runge-Kutta techniques. Initial conditions were chosen as values at resting potential and resting ion concentrations. In the following the equilibrium potential for Ca$^{2+}$ was set at +60mV.

Preliminary results for the 9-component model (78)

With no applied current the results for Set 1 parameters showed no evidence of spontaneous spiking. The membrane potential drifted down from resting value of -60 mV to almost be at about -65 mV in 25 ms. It is clear that the depolarizing forces of low threshold calcium $I_T$ and fast sodium current were overwhelmed by the negative drive from the transient A-type potassium current $I_A$ and the delayed rectifier potassium current. With an applied depolarizing current $\mu < 0$, the same situation prevailed until it reached a level of $\mu = -0.17$ nA. With this value of $\mu$, as depicted in Figure 9, a spike was emitted at about $t=55$ ms, with maximum value $+27.5$ mV, minimum $-80$ mV and a duration D (always taken to be the spike width at $-40$ mV) of 1.4 ms. The magnitudes of both the fast sodium current and the delayed rectifier potassium current were both very large (about 18 nA). Even after a very long time interval, there was no subsequent spike as $V$ tended to remain at a new equilibrium value around -71 mV. There was a pronounced afterhyperpolarization as found in DRN SE cells, but the behavior of $V$ after the spike was not typical for these neurons.

Consideration of simplest model with only Na and K

With a multi-component neuronal model there are of course a large number of varying properties for different choices of parameters. Just using three values of each of 89 parameters would result in over 700,000 combinations. With so many parameters it is difficult to see which are responsible for important spiking properties. Many chosen combinations led to satisfactory spike trains with properties similar to experiment, but often with durations which were unacceptably long. Other combinations led to spontaneous activity but spikes had uncharacteristic large notches on the repolarization phase. Doublets and triplets were often observed, and although these are found in experimental spike trains, it was desirable to find solutions depicting the regular spiking and relatively smooth voltage trajectories usually observed.
Hence a gradual increase in complexity was employed starting with the usual basic two currents in action potential generation.

In this subsection attention is focused on the spike properties of duration and to a lesser extent inter-spike interval (ISI). In subsequent sections the various other component currents are added. We illustrate with two choices of parameters for the sodium and potassium variables $m_{Na,\infty}$, $h_{Na,\infty}$ and $n_{\infty}$, one set being chosen from the above parameter set and another based in part on those of Belluzzi and Sacchi (1991) which we denote by Set 2. For the latter we also use the resting potential from Kirby et al. (2003) and the cell capacitance, both being for rat DRN SE cells. The two sets of parameters are summarized in Table 19.

| Parameter      | Set 1 | Set 2 |
|----------------|-------|-------|
| $V_{Na_1}$     | -33.1 | -36   |
| $k_{Na_1}$     | 8     | 7.2   |
| $V_{Na_3}$     | -50.3 | -53.2 |
| $k_{Na_3}$     | 6.5   | 6.5   |
| $V_R$          | -60   | -67.8 |
| $\tau_{m,Na_1}$| 0.2   | 0.1   |
| $\tau_{h,Na_1}$| 1.0   | 2.0   |
| $C$            | 0.04  | 0.08861|
| A              | 4000  | 8861  |
| $V_{KDR_1}$    | -15   | -6.1  |
| $k_{KDR_1}$    | 7.0   | 8.0   |
| $n_k$          | 1     | 1     |
| $\tau_{n,KDR_1}$| -    | 3.5   |
| $a_{KDR}$      | 1     | -     |
| $b_{KDR}$      | 4     | -     |
| $V_{KDR_2}$    | -20   | -     |
| $k_{KDR_2}$    | 7     | -     |
| $g_{Na,max}$   | 2.00  | 1.5   |
| $g_{KDR,max}$  | 0.5   | 0.5   |

Both of these reduced model parameter sets led to repetitive spiking with the addition of a small depolarizing current. Typical spike trains are shown in Figure 10 and Table 20 lists some of the details of the spike and spike train properties.
Spiking for the second parameter set has a lower threshold for (repetitive) spiking, a longer ISI at threshold, a longer spike duration and a larger spike amplitude. For both parameter sets, most of these spike properties are in the ranges observed for DRN SE neurons so that such sets could form the basis of a more complete model in accordance with Equ. (6) as explored below.

Figure 10 shows well defined spikes with abruptly falling repolarization phases to a pronounced level of hyperpolarization followed by a steady increase in depolarization until an apparent spike threshold is reached. DRN SE neurons are usually characterized as having long plateau-like phases in the latter part of the ISI and this is a feature shown in Figure 11 for spikes elicited near the threshold for spiking for both sets of parameters. The plateau for the second set is nearly three times as long as that for the first set.

Graphs of the frequency of repetitive spiking versus depolarizing input current are shown in Figure 12. In both cases it seems that at a particular value of \( \mu \) the frequency jumps from zero to values of 3.0 Hz for set 1 and 1.1 Hz for set 2, rather than increasing continuously from zero. Thus these models with the chosen parameters would be classified as type 2 neurons (Hodgkin, 1948; Tateno et al., 2004) whereby the instability of the rest point is due to an Andronov-Hopf bifurcation rather than a saddle-node bifurcation as in Type 1 neurons. These results can be
Figure 11: Showing long plateaux in repetitive spiking in the reduced model (Na-K) for the parameter sets of Table 19, Set 1 (top part) and Set 2 (lower part). In both cases $\mu$ is very close to the threshold for firing.

compared with those for the complete simplified model in Section 12.

| Property              | Set 1 | Basic set 2 |
|-----------------------|-------|-------------|
| Spike threshold       | $\mu = -0.0342$ | $\mu = -0.018$ |
| ISI at threshold      | 331 ms | 948 ms      |
| Spike duration (-40 mV) | 1.6 ms | 2.9 ms      |
| Max V                 | +8    | +19.4       |
| Min V                 | -90.0 | -91.2       |

Four-component model with a high threshold $\text{Ca}^{2+}$ current and $I_{SK}$

We wish here to include in the model the spike-induced influx of calcium ions and calcium-activated potassium currents. For the former, we reduce the components described in Section 4 to just N-type $\text{Ca}^{2+}$ and for the latter only an SK current
Figure 12: Frequency of action potentials versus magnitude of depolarizing current for the two above parameter sets with only two components. Note the different ordinate scales.

is included along with calcium buffering and pumping. Thus these results would apply when the currents $I_A$, $I_T$, $I_H$ are blocked. For these computations three of the parameters of the N-type Ca$^{2+}$ activation and inactivation were changed to $V_{N_1} = -20$, $V_{N_3} = -50$ and $k_{N_3} = 10$, representing a general high threshold Ca$^{2+}$ current.

Two sets of results for $V$, the internal Ca$^{2+}$ concentration, and $I_{SK}$ are shown in Figure 13, where also the near threshold value of $\mu = -0.035$ was employed. In the results shown in the left column of Figure 13, the following maximal conductances were employed: $g_{N,max}=0.1$, $g_{Na,max}=2$, $g_{KDR,max}=0.5$, $g_{SK,max}=0.003$, so that the sodium and potassium conductances are the same as in Set 1 of Table 19. The ISI is about 550 ms, the maximal values of $I_{Na}$, $I_{KDR}$, $I_{KCA}$ and $I_{N}$ are about 10, 13, 0.03 and 0.7 nA, respectively and the maximal intracellular Ca$^{2+}$ concentration is only about 400 nM, the resting level being 50 nM. There is a plateau in the latter phase of the ISI as the threshold for the subsequent spike is approached gradually.

In order to reduce the magnitudes of $I_{Na}$ and $I_{KDR}$, the following set of maximal conductances was employed: $g_{N,max}=0.1$ (as before) $g_{Na,max}=1$, $g_{KDR,max}=0.25$, $g_{SK,max}=0.015$, and the results are shown in the right-hand column of Figure 13. Whereas the ISI is little changed with a value of about 544 ms, the membrane
potential during the ISI has a decidedly different form in that it remains at the most hyperpolarized levels for more than half of the ISI and then turns fairly sharply upward to reach threshold for firing at similar values (-50 mV) as for the first set of conductances. Now however, the maximal values of $I_{Na}$, $I_{KDR}$, $I_{SK}$ and $I_{N}$ are about 3.5, 5.5, 0.1 and 0.9 nA, respectively and the maximal intracellular Ca$^{2+}$ concentration is about 600 nM. The form of $I_{SK}$ is also quite different in the two cases considered, there being a rapid increase (in absolute value) and equally rapid decrease around the time of occurrence of the spike for the second set of conductances.

Figure 13: Showing the following three quantities versus time during spiking: voltage ($V$) (top), intracellular calcium ion concentration ($Ca_i$) (middle) and calcium-activated potassium current ($I_{KCA}$) (bottom). In the left-hand column the key parameters are $g_{N,max}=0.1$, $g_{Na,max}=2$, $g_{KDR,max}=0.5$, $g_{SK,max}=0.003$ (set 1 above) and in the right-hand column, the same conductance for N-type Ca$^{2+}$ but $g_{Na,max}=1$, $g_{KDR,max}=0.25$, $g_{SK,max}=0.015$ (set 2).

**Changes of calcium pump strength**

If the calcium pump is reduced in strength so that the intracellular calcium concentration does not decline so rapidly after Ca$^{2+}$ influx from the high threshold current $I_{N}$, then the value of $m_{SK,\infty}$ in (46) remains high and hence $I_{KCA}$ is slow.
to decline. This leads to a prolongation of the ISI as is seen in the records shown in Figures 14 and 15. In Figure 14, which shows membrane potential versus time during the ISI, the top two records were obtained with the first set of conductances and the bottom two records with the second set, as described in the previous two paragraphs. It can be seen that despite the lengthening or shortening of the ISI by decreasing or increasing the calcium pump strength, respectively, the general form of the response is not altered. That is to say, for the first conductance set there is still a prolonged and gradual plateau-like approach to threshold, whereas for the second set, V remains near its most hyperpolarized levels for half or more of the ISI and swings upward to threshold rather sharply towards the end. The corresponding time courses of the intracellular Ca\(^{2+}\) concentration are shown in Figure 15.

![Figure 14](image_url)

Figure 14: Effects of increased and decreased calcium pump strength, described by the parameter \(K_s\) in Equ. (77) on membrane potential during spiking. In the top two records \(g_{Na,max}, g_{KDR,max}, g_{SK,max}\), \(I_N\) conductances from set 1 are used and in the bottom two records the set 2 values as described in the caption of the previous figure. The numbers on the plots indicate increases (1.5) or decreases (0.5) in \(K_s\) values relative to the standard value.

**Smaller \(I_{Na}\) and \(I_{KDR}\) and the AHP**

In the above simulations for set 1 and set 2 parameters, the maximal sodium conductances were 2.0 \(\mu S\) and 1.0 \(\mu S\) respectively and the maximal values of \(g_{KDR,max}\)
Figure 15: Intracellular Ca$^{2+}$ concentrations corresponding to the voltage trajectories in the previous figure for two sets of conductances and two pump strengths.

were 0.5 and 0.25 $\mu$S, respectively. Although the properties of the spikes as shown in Figures 10, 11 and 14, 15 could serve as a basis for observed spikes, the sodium and potassium currents were fairly large, being of the order of 10 nA.

A sodium current of about 9 nA was given for hypothalamic magnocellular neuroendocrine cells (Komendantov et al. 2007), but judging by measured or computed currents in cells expected to be similar to DRN serotonergic cells (Milescu et al., 2008; Milescu et al., 2010), the sodium currents are likely to be of order 2-3 nA. Further support for this estimate comes from analysis of a clear image of a spike in Kirby et al. (2003). The derivative of the voltage trajectory at the upswing of the spike is estimated at about 63 volts per second. From the same report the given resistance and time constant lead to an estimate of 0.0886 nF for the capacitance, which combined with the value of $dV/dt$ give a current of about 5.6 nA. Assuming this is about 90% sodium current results in an estimate of 5 nA. On the other hand, since the capacitance estimate may be high (Tuckwell, 2012b), if the value 0.04 nF is used then the estimate of the sodium current is about 2.4 nA, in accordance with the estimate from the articles of Milescu et al. (2008, 2010). In conclusion, the maximal sodium current in DRN serotonergic neurons is likely to be about 2-3 nA with the possibility of values as high as 5 nA. One expects the delayed rectifier potassium current to be of approximately the same or smaller magnitude.
In fact, recall the value $g_{Na,max}=0.18 \, \mu S$ estimated in section 7 which was for a dissociated cell at room temperature. The dissociated cell has a small capacitance of perhaps only 20 pF (Penington and Fox, 1995) which means a capacitance of 9 nS per pF at room temperature or perhaps about 13.5 nS per pF at body temperature. Thus an average sized cell of capacitance 40 pF (set 1 cell) would have $g_{Na,max}=0.54 \, \mu S$ and a larger cell with a capacitance of 88.61 pF (set 2 cell, Table 19) a value $g_{Na,max}=1.2 \, \mu S$. The first of these values is considerably less than the ad hoc value of 2 $\mu S$ in Tables 18 and 19, but the second value is close to the value in set 2 of Table 19. In a similar vein, the conductance for delayed rectifier potassium current was given as 0.641 nS/pF in Section 3.3 which translates to 0.0256 $\mu S$ for an average cell with $C=40$ pF and 0.0567 $\mu S$ for a larger cell with $C=88.61$ pF.

In investigating the effects of changing various parameters on the spike train properties, a systematic approach would require a large amount of space, as each of the about 90 parameters does have an important influence. Small changes in just one parameter can radically alter the nature of spiking. With the above new estimates for $g_{Na,max}$ and $g_{KDR,max}$, a number of runs, reported in Table 21, were performed.

**Runs 1-5 of Table 21**

With the estimated values for $g_{Na,max}$ (0.54) and $g_{KDR,max}$ (0.0256), model responses (with just Na and K) to a depolarizing current of 0.05 nA were computed using the remaining Set 1 values of Table 19 for sodium and potassium DR currents. This gave rise to a rapidly oscillating solution, similar to action potentials at the beginning, but dying out after 20 ms at an equilibrium value of about -20 mV. Changing the value of $n_k$ to 2, which moves the $I_{KDR}$ activation in the depolarizing direction, led to an even briefer oscillatory response, stabilizing again at about -20 mV. With $n_k$ again at 1, the time constant for potassium activation (17) was increased by putting $b_{KDR}=14$ so it had a maximum of 15 ms. Then with $\mu$ still at -.05 nA, repetitive spiking occurred with an ISI of 15 ms, duration (-40) of 5 ms, $V_{max}=11.5$ mV, $V_{min}=-55$ mV, $I_{Na,max}=2.0$ nA and $I_{KDR,max}=1.4$ nA.

Several sets of results are summarized in Table 21. In all cases, $n_k=1$, $\mu=-0.03$ nA and the value of $b_{KDR}=14$ in the potassium activation time constant. In all runs except the 8th, a regular train of action potentials emerged. In Run 1, as in the case with $\mu=-.05$ nA, the time constants for sodium activation and inactivation (called just $\tau_m$ and $\tau_h$ here) are constant (Table 19, 1st column). The ISI is slightly longer at 19 ms, and generally there are only small changes in spike train properties, including the maximum sodium and potassium currents (called just
INA and IK in Table 21). The duration D is the same at 5.0 ms. In Run 2, the time constants for sodium are given their full voltage-dependent forms (denoted by vd in Table 19) as in Equations (53) and (55) with parameters as in Table 14. This increases $I_{Na}$ significantly, leads to more extreme values of V, shortens the duration to D=3.9 ms and increases the ISI to 22 ms. With the maximal sodium conductance $g_{Na,max}$ reduced by 25% to 0.41 µS, (Run 3), the ISI is hardly changed, but the maximal voltage and sodium current are significantly less. The duration is reduced by 0.1 ms to 3.8 ms. A further reduction in $g_{Na,max}$ to 0.35 µS (Run 4) leads to a sodium current about as estimated from experiment at 5.4 nA, without much change in the remaining properties in Table 19. Run 5 also includes the leak current (see section 8), all other parameters being as in Run 4. The effect on the ISI is large as it increases from 23 to 103 ms, with a concomitant drop in maximal sodium current. The remaining spike properties are not greatly different, except D is greater at 4.6 ms.

Table 21: Effects of changing some parameters on spiking

| Run | GNA | GK | $\tau_m$ | $\tau_h$ | Leak | GN | GSK | $V_{\text{max}}$ | $V_{\text{min}}$ | ISI | D | INA | IK | $Ca_{i,\text{max}}$ |
|-----|-----|-----|--------|---------|------|----|-----|--------------|-------------|-----|----|-----|----|----------------|
| 1   | 0.54| 0.0256| c     | c       | -    | -  | -   | 10           | -55         | 19  | 5.0| 1.8 | 1.4| -              |
| 2   | "   | "   | "    | vd     | vd   | -  | -   | 30           | -60         | 22  | 3.9| 7.4 | 1.9| -              |
| 3   | 0.41| "   | "    | "     | -    | -  | -   | 24           | -60         | 22  | 3.8| 5.4 | 1.7| -              |
| 4   | 0.35| "   | "    | "     | -    | -  | -   | 20           | -58         | 23  | 4.0| 4.6 | 1.5| -              |
| 5   | "   | "   | "    | "     | yes  | -  | -   | 7.4          | -56         | 103 | 4.6| 2.9 | 1.0| -              |
| 6   | "   | "   | "    | "     | 0.003| 0.012| 7.4 | -56         | 255         | 4.8 | 2.9| 1.0 | 100| -              |
| 7   | "   | "   | "    | "     | 0.006| "  | 7.5 | -65         | 491         | 4.7 | 2.9| 1.05| 170| -              |
| 8   | "   | 0.032| "   | "     | "    | 0.006| "  | No         | AP          |      |    |     |    | -              |
| 9   | 0.41| "   | "    | "     | "    | "  | 11  | -64         | 383         | 4.1 | 3.6| 1.4 | 156| -              |
| 10  | "   | "   | "    | c     | c    | "  | "  | -13         | -79         | 750 | 10 | 0.65| 0.33| 300            |
| 11  | 0.35| 0.0256| vd | vd     | "    | 0.012| "  | -7.5        | -81.5       | 1100| 5.3| 2.8 | 1.05| 393           |
| 12  | 1.62| 0.0768| vd | vd     | "    | "  | "  | 39          | -79         | 475 | 2.4| 17  | 4.8 | 295            |
| 13  | 1.08| 0.0768| vd | vd     | "    | "  | "  | 33          | -76         | 422 | 2.4| 12  | 4.3 | 248            |

**Runs 6-13 of Table 21**

In Run 6, the high threshold calcium current $I_N$ is present with a maximum (denoted by GN in the Table) of $g_{N,max}=0.003$ µS, and at the same time the calcium-dependent potassium (SK) current is switched on with a maximal conductance...
(denoted in the table by GSK) of \( g_{SK,max} = 0.012 \, \mu \text{S} \). Without any change in the resulting sodium and potassium delayed rectifier current, nor in the maximum and minimum voltages, the ISI is more than doubled to 255 ms, the spike duration \( D \) is slightly increased to 4.8 ms and the internal calcium concentration rises to 100 nM at its maxima.

In Run 7, the only change in parameters from Run 6 is the doubling of the N-type calcium conductance to \( g_{N,max} = 0.006 \, \mu \text{S} \), with a consequent deeper AHP, a slightly less duration \( D \), and a much longer ISI of 491 ms. The maximum internal calcium concentration is now 170 nM. The sodium and delayed rectifier potassium currents are little altered. The spikes have an appearance similar to those of some DRN SE cells, and some of their properties are shown in Figure 16. The properties shown are \( V \), the internal calcium concentration, the fast sodium and N-type calcium currents and the potassium currents \( I_{KDR} \) and \( I_{SK} \).

Because the AHP was not as deep as usually found in DRN SE cell spikes, the value of \( g_{KDR,max} \) was increased by 25 % in Run 8, with all other parameters the same. The result was that no spiking at all emerged. To counter this, in Run 9, the value of \( g_{Na,max} \) was increased back up to 0.41 as in Run 3. Spiking was re-established but compared to the Run 7 spike, the maximum of \( V \) was increased to 7 mV and the minimum increased to -64 mV, whereas the spike duration was reduced to 4.1 ms and the ISI was also decreased to 383 ms, the maximum \( Ca_i \) was only 156 nM, and the maxima of \( I_{Na} \) and \( I_{KDR} \) were greater at 3.6 and 1.4 nA, respectively.

Another run (Run 10) with the same parameters was performed except with constant rather than \( V \)-dependent time constants for \( I_{Na} \), which resulted in a much greater ISI at 750 ms and a much greater spike duration of 10 ms. At the same time \( I_{Na} \) and \( I_{KDR} \) were very small. Whereas the minimum voltage was -79 mV, the maximum was also decreased to -11 mV. Run 11 is the same as Run 7 but with increased \( g_{N,max} = 0.012 \). This causes a lengthening of the ISI to 1100 ms, the duration is somewhat larger at 5.3 ms and the internal \( Ca^{2+} \) increases to 393 nM, being more than doubled.

**Effects of changes to parameters for \( I_N \)**

In order to determine the influence of the parameters for activation and inactivation of the N-type calcium current, the following (denoted by N-type set 2) were used (with all else as in Run 11), as estimated from a voltage-clamp study (Penington and Fox, 1995): \( V_{N_1} = -13.5, \; k_{N_1} = 9, \; a_N = 0.305, \; b_N = 2.29, \; V_{N_2} = -20, \; k_{N_2} = 20, \; V_{N_3} = -50, \; k_{N_3} = 20 \; \tau_{h,N} = 1000 \). The data are for barium as charge carrier, room temperature and with EGTA and may be different for native cells at
body temperature.

For the results, defining the summarizing 7-vector

\[
X = [V_{\text{max}}, V_{\text{min}}, D, \text{ISI}, I_{\text{Na,max}}, I_{\text{KDR,max}}, Ca_{i,max}].
\]

this gave, \(X = [87.3, -65.6, 5.1, 500, 2.85, -1.05, 175]\), differing significantly only in \(V_{\text{min}}\), ISI and \(Ca_{i,max}\). In this and all of the above runs 1-11, the spike duration was longer than that expected for these cells, in contrast with the value 1.6 ms in Table 20, Set 1.

With the time constants and (larger) conductances for \(I_{\text{Na}}\) and \(I_{\text{KDR}}\) as in set 1 of Table 19, and with N-type set 2, a more satisfactory result was obtained, \(X = [26.8, -80, 3.1, 587, 6.3, -5.1, 349]\). The results of these last two runs are not given in Table 21. Similarly with the original set 1 for N-type, Run 12 in Table 21, with increased \(g_{\text{Na,max}}\) and \(g_{\text{KDR,max}}\), a very reasonable duration \(D=2.4\) ms was obtained but with very large \(I_{\text{Na}}\) and a smaller ISI. With \(g_{\text{Na,max}}\) reduced by 33%, the duration was unchanged - see Run 13 in Table 21. With \(\mu = 0\) in the last run, no spikes emerged, nor when the value of \(g_{\text{KDR,max}}\) was reduced to 0.0512 or further to 0.0384.

![Graphs](image)

Figure 16: Spike properties for Run 7 (see Table 21) described in the text and with parameters as given in Table 19. The ISI is 491 ms, or a frequency of 2.04 Hz.
Effects of $I_{BK}$ and changing capacitance and area

In this subsection the effects of altering the cell capacitance $C$ and area $A$ are briefly investigated as well as the effects of including a simplified model for BK current (Tabak et al., 2011). The results are given in Table 22, being labeled S1 to S6. In all these runs the following maximal conductances are employed: $g_{Na,max}=0.675$, $g_{KDR,max}=0.0768$, $g_{N,max}=0.024$ (double that in the last line of Table 21) and $g_{SK,max}=0.012$.

In S1, the dramatic effect of increased $g_{N,max}$ is seen, especially in that the ISI is much longer at 1253 ms, well within the range of firing rates of many DRN SE neurons. In S2, the cell capacitance is increased by 25% and this change induces an irregular pattern of ISIs, at least for the first several seconds, the pattern being long, short, long, short, with values given in column 7 of Table 22. The spike duration is also increased as is also the peak internal Ca$^{2+}$ concentration. There appeared some small undulations in the potential near the end of the ISIs as spikes were approached. When the total area $A$ is increased by 25%, as in S3, the train was regular again, with a decreased ISI, a little-changed spike duration and a slightly smaller internal Ca$^{2+}$ concentration. In a case of increases in both area and capacitance, (S4), a regular train arose with an ISI of 932 ms and spike duration 3 ms.

As the spike durations in most of the runs so far have been at the long end of the range of those commonly reported for DRN SE neurons, it is of interest to consider the effects of potassium BK currents, which are often posited to play a role in speeding up the process of spike repolarization. In the mathematical model for BK currents presented in Section 5.1, the values of internal Ca$^{2+}$ required to make the conductance significant are above those obtained in the computations reported above. Hence a simpler more direct model was employed as in Tabak et al. (2011) whereby the steady state activation depends only on voltage. The half-activation voltage was set at -20 mV, the slope constant at 2 mV, both as in Tabak et al. (2011) and the time constant at 2 ms, regardless of voltage, which is at the low end of the range in Tabak et al. (2011). There was, as usual, no inactivation for BK channels (see Section 5.1).

Runs S5 and S6 include BK current with conductances equal to 1/3 and 2/3, respectively, of the potassium delayed rectifier current. The effects of the inclusion of BK current here were surprising as even though the spike duration was reduced from 2.65 ms to 2.4 and 2.2 ms for S5 and S6 respectively, the spike train became irregular. For the smaller BK conductance (S5), long and short ISIs alternated, being 1147 and 876 ms respectively and the peak internal Ca$^{2+}$ concentration also alternated. For the larger BK conductance (S6), the pattern of ISIs was long, short,
short, long, short, short etc. at least for the first several seconds.

Table 22: Induced firing with $\mu = -0.03$: changes in C, A and effects of BK

| Run | GBK | C    | A    | $V_{max}$ | $V_{min}$ | ISI   | D  | INA | IK   | $Ca_{i,\text{max}}$ |
|-----|-----|------|------|-----------|-----------|-------|----|-----|------|---------------------|
| S1  | -   | 0.04 | 4000 | 20.7      | -81       | 1253  | 2.65 | 6.9 | -3.6 | 380                 |
| S2  | -   | 0.05 | 4000 | 16.7      | -81       | 1360, 1678 | 3.0  | 6.6 | -3.4 | 400                 |
| S3  | -   | 0.04 | 5000 | 21.2      | -80.5     | 826   | 2.7  | 7.0 | -3.7 | 344                 |
| S4  | -   | 0.05 | 5000 | 17.2      | -81       | 932   | 3.0  | 6.8 | -3.5 | 383                 |
| S5  | 0.0256 | 0.04 | 4000 | 18.6      | -79.7     | 876, 1147 | 2.4  | 6.9 | -3.2 | 335                 |
| S6  | 0.0512 | 0.04 | 4000 | 19.5      | -80.0     | 815, 1064 | 2.2  | 6.9 | -3.2 | 295                 |

Spontaneous firing with 6 components

DRN SE and other raphe SE cells often fire spontaneously in a pacemaker fashion, so it is of interest to explore this aspect in a computational model, at first with just $I_{Na}$, $I_{KDR}$, $I_N$, $I_{SK}$ and $I_{BK}$ and the leak current as in the last section. Because only small depolarizing currents were required to induce firing in the most of the runs of the previous section, it seemed that a small increase in excitability could induce spontaneous firing. This was achieved with just one change, namely an increase in the value of $k_{Na_1}$ from its prior value 8 to 10 mV. A set of results for $\mu = 0$ is given in Table 23. In all the runs of this section, which are given a P label, the V-dependent forms for the sodium and potassium time constants are used, the sodium conductance is $g_{Na,max}=0.675$, $g_{KDR,max}=0.0768$ and $g_{SK,max}=0.012$. Further, here the activation parameters of $I_N$ are left the same, with $V_{N_1} = -25 mV$ and $k_{N_1} = 7$.

In the first of this set of runs, P1, the N-type Ca$^{2+}$ conductance is 0.012. The cell fires regularly with an ISI of 467 ms and the duration of the spikes is 2.3 ms. An increase in $g_{N,max}$ to 0.24, as in P2, increases the ISI to 982 ms and the maximum internal Ca$^{2+}$ to 512 nM. The effects of increases in cell capacitance C and area A are explored in P3 to P5. As a general rule, increasing the capacitance leads to increases in the ISI, spike duration and peak internal calcium concentration whereas increasing the area decreases the ISI and the peak internal calcium ion concentration.

The simplified BK current model, as described above, is included in runs P6 and P7. Compared to P2 which has the same C and A values, the ISI is shortened somewhat but most significantly the spike duration is reduced to 2.15 ms in P6 and 2.0 in P7, these values being in the experimental range for DRN SE neurons. In
contrast to the runs S5 and S6 (induced firing), the spike trains in the pacemaker
runs P6 and P7 with BK current were regular with a constant ISI.

Table 23: Pacemaker firing with $\mu = 0$ and $k_{Na_1} = 10$, changes in C,A and effect of
BK

| Run | GN  | GBK | C  | A  | $V_{max}$ | $V_{min}$ | ISI | D  | INA | IK | $Ca_i, max$ |
|-----|-----|-----|----|----|-----------|----------|-----|----|-----|----|-----------|
| P1  | 0.012 | -   | 0.04 | 4000 | 26       | -79      | 467 | 2.3 | 8.4 | -4.0 | 250       |
| P2  | 0.024 | -   | 0.04 | 4000 | 27       | -84      | 982 | 2.45| 8.4 | -4.0 | 512       |
| P3  | 0.024 | -   | 0.05 | 4000 | 23.5     | -84.2    | 1099| 2.8 | 8.3 | -4.0 | 554       |
| P4  | 0.024 | -   | 0.04 | 5000 | 27       | -83.5    | 790 | 2.4 | 8.5 | -4.1 | 430       |
| P5  | 0.024 | -   | 0.05 | 5000 | 23.3     | -83.8    | 887 | 2.75| 8.3 | -4.0 | 445       |
| P6  | 0.024 | 0.0256 | 0.04 | 4000 | 25.5     | -83.6    | 830 | 2.15| 8.4 | -3.3 | 423       |
| P7  | 0.024 | 0.0512 | 0.04 | 4000 | 23.9     | -83.0    | 710 | 2.0 | 8.5 | -3.4 | 362       |

Low threshold calcium current, $I_T$

The low threshold Ca$^{2+}$ current $I_T$ has often been ascribed a role in pacemaking
in DRN SE neurons (for example, Aghajanian and Sanders-Bush, 2002). In this
subsection we investigate the effects of the inclusion of this component in the model
for which some results without $I_T$ were given in Table 23, making the number of
components 7, including leak current. Experimentally this would correspond to the
block of $I_A$ and $I_H$, the L-type Ca$^{2+}$ current, which is relatively small in these cells,
being approximately combined with the N-type.

Here the value of $g_{T, max}$ was estimated from voltage-clamp data in Penington et
al. (1991), yielding a value of approximately 0.05 $\mu$S for a cell at room temperature,
this estimate therefore being considered low for a cell at body temperature. A value
of $g_{T, max}=0.125$ $\mu$S is a reasonable upper limit, which compares favorably with an
erlier estimate of 0.180 $\mu$S from the voltage-clamp data in Burlhis and Aghajanian
(1987) - but see also the next subsection where $I_A$ is included.

When, in a run labeled T1, the higher value (0.125) was employed for $g_{T, max}$,
using the same parameters as in P7 of Table 23 (with other conductances as in the
runs (S) of Table 22), there was an early spike with a small notch at 15 ms, a doublet
at 300 ms, and spike which did not repolarize for over 100 ms, with a resultant huge
increase in internal Ca$^{2+}$ concentration. This contrasts to the regular train in P7
with $g_{T, max}=0$. In run T2, with $g_{T, max}$ at the lower estimate of 0.05 $\mu$S there were
Figure 17: Doublet spikes appear at regular intervals (about 1500 ms) when the $I_T$ conductance is around 0.05 $\mu$S in Run T2 of the 6-component model.

Figure 18: Consecutive spikes with an ISI of 734 ms when the $I_T$ conductance is at 0.025 $\mu$S (Run T4).

Initially 4 short ISIs involving singlet spikes with concomitant $Ca_i$ levels of about 500 nM, followed by a regular train of doublets with an ISI of 1557 ms, $V_{\text{max}} = 28$ mV, then after the doublet $V_{\text{min}} = -84$ mV, and a $Ca_i$ rising to about 945 nM. The doublet spike is pictured in Figure 17 and has the appearance of doublets reported for DRN SE neurons by Hájós et al. (1996).
Because the internal Ca\textsuperscript{2+} concentration became somewhat elevated, a stronger calcium pump (constant \(K_s\) in Table 17) was tested, as in run T3, which made the spike train more irregular. Initially there were 6 singlets with notches of increasing magnitudes on their repolarizing edges, with \(V_{max}\) values 22 mV, AHPs to -82 mV and durations about 2.1 ms. This was followed, at intervals of over 2000 ms, in sequence, by a doublet, a triplet, and then several quartets which eventually degenerated into a damped oscillation. The internal Ca\textsuperscript{2+} concentration for the first spikes grew to about 400 nM, with later levels around 3400 nM. Surprisingly, in Run T4, with \(g_{T,max}\) reduced to 0.025, somewhat below the lower estimate, a very regular spike train occurred with the following properties: \(X = [25.5, -84.2, 734, 9.1, -3.5, 462]\) and \(I_{T,max} = 0.2\) nA. The last three ISIs all had values of 734 ms. A pair of spikes is shown in Figure 18, indicating a small notch on the repolarization phase.

With the same value of \(g_{T,max}\), the half-activation and half-inactivation potentials of \(I_T\) were increased by 5 mV in Run T5A and decreased by 5 mV in run T5B. In the former case, the spike train properties were similar to those described in the previous example, but the notch on repolarization was smaller. In the latter case, however, the membrane potential took on a new character between spikes as now there was a smooth broad undulation (notch) preceding the spike as \(V\) retreated and paused before swinging up to threshold, similar to the appearance of some spiking in DRN SE cells in Bayliss et al. (1997). There was no discernible notch on the repolarization phase, and the \(I_T\) current was noticeably smaller with a maximum value of only 0.03 nA. The ISI was about 702 ms.

The next four runs, designated T7-T10, were designed to ascertain the relative roles of \(I_T\) and \(I_{Na}\) in spike triggering, especially in pacemaker mode. The higher (+5 mV) half-activation and half-inactivation potentials were employed for \(I_T\). In T7, the smallest value of \(g_{T,max} = 0.025\) \(\mu\)S was used with \(g_{Na,max}\) smaller by 25\% at 0.54 \(\mu\)S. Previously this value of the T-type conductance gave rise to bursting, but now the spike train was perfectly regular with an ISI of 642 ms and spike duration 2.05 ms. Further, there was no sign of a notch either in the late part of the ISI or the repolarization phase, but the maximum of \(I_T\) was quite small at 0.028 nA. In T8, with the higher \(g_{T,max} = 0.05\) and the same \(g_{Na,max}\), a regular spike train also ensued with an ISI of 705 ms, maximum \(I_T\) of 0.59 nA and with a small notch on the repolarization phase of the spikes. In run T9, as depicted in Figure 19, the sodium conductance was returned to its higher (by 25 \%) value and with \(g_{T,max} = 0.05\), there was now no bursting with an ISI of 802 ms, a small post-spike notch and a maximum \(I_T\) of 0.725 nA. Noteworthy is that the notch is higher at about -60 mV, compared with about -73 mV in the previous figure. It seems that the higher the notch, the
closer the cell is to bursting mode.

Figure 19: Spikes with an ISI of 802 ms in run T9 with higher half-activation and half-inactivation potentials.

Finally, in run T10, the value of $g_{Na,max}$ was decreased to 0.405 $\mu$S (0.75 x 0.54) and $g_{T,max}$ was increased to 0.075 $\mu$S. In this case spiking halted after 5 spikes, the 4th being a doublet and the 5th being a multiple oscillation accompanied by rises in $Ca_i$ to about 4000 nM.

**Inclusion of $I_A$: 8 component model**

A strong outward current, labeled as $I_A$, was found in DRN SE neurons by Segal (1985) and studied in Aghajanian (1985), Burlhis and Aghajanian (1987) and Penington and Tuckwell (2012). Such a current has been postulated as exerting considerable control over spiking activity. Here we first describe two interesting results obtained with fairly small $I_A$ and $I_T$, and then obtain a very satisfying result by using conductances estimated from voltage-clamp data, in which $I_A$ increases in amplitude throughout the ISI and concomitantly $I_T$ decreases, reinforcing the idea that competition between these two components is an important factor in determining the ISI. Finally, in 11.8.3, a methodical analysis results in the culmination of computations of notch-free spikes which are often reported for these cells.

**Results with small $g_{A,max}$, $g_{T,max}$**

In the first of these preliminary runs, called A1 here, the following maximal conductances (in $\mu$S) are employed: $g_{Na,max}=0.594$, $g_{KDR,max}=0.0768$, $g_{N,max}=0.024$, 67
$g_{SK, max} = 0.012$, $g_{BK, max} = 2g_{KDR, max}/3$, $g_{A, max} = g_{T, max} = 0.1$. The activation and inactivation parameters for $I_A$ and $I_T$ are as given in Table 16, the value of the pump strength $K_s$ is fairly small at $0.3125 \times 10^{-6}$ and $V_{N_1}$ is set at the relatively low value of -25 mV. The resulting voltage trajectory is shown in Figure 20 and the regular train of spikes have the following characteristics. $X = [16mV, -77, 2.1, 1185, 6.55, -2.75, 433]$, a small notch with magnitude about 1.5 mV at the bottom of the fast post-spike repolarization and no prolonged plateau at the end of the ISI. The other maximum current amplitudes are $I_{T, max} = 0.026$, $I_{A, min} = -0.12$ and $I_{N, max} = 0.45$.

Figure 20: The membrane potential showing consecutive spikes with an ISI of 1185 ms, with $g_{A, max} = g_{T, max} = 0.1$, as described in 11.8.1.

In the following run, called A2, only 1 change was made, namely to increase $g_{N, max}$ by 50% to 0.036, but this resulted in a radical change in the nature of the spikes, which are depicted in Figure 21. A very long plateau developed as the membrane potential lingered for over 2000 ms about 5 mV above resting level before an oscillation of growing amplitude called pre-spike oscillation, similar to that observed in some cells (Atherton and Bevan, 2005) finally triggered a spike. Apart from the very long ISI of about 3480 ms, the remaining spike properties such as maximum $V$, minimum $V$, spike duration and magnitudes of $I_{Na}$, $I_{KDR}$, $I_A$ and $I_T$ were practically the same as in the run with smaller $g_{N, max}$. Figure 22 shows that during the long plateau, the internal Ca$^{2+}$ concentration remained practically constant at a low level, down from a somewhat higher peak value of 480 nM.
Figure 21: Spikes obtained when in the previous parameter set only one change was made, consisting in an increase in the N-type Ca\(^{2+}\) conductance.

Figure 22: Time course of internal Ca\(^{2+}\) concentration in the ISI during the spiking shown in the previous graphic.

**Further voltage clamp estimates of conductances for \(I_A\) and \(I_T\)**

Using the updated model obtained with 7 components (plus leak), a careful re-analysis was performed for the voltage-clamp data in Burlhis and Aghajanian (1987), but using the linear form for the calcium current and not the constant field form.
Recall that two sets of clamps were performed, from -80 mV to -56 mV and from -65 mV to -56 mV. In this analysis, variations on the activation and inactivation parameters for $I_T$ and $I_A$, namely $V_{T1}$, $V_{T3}$, $V_{A1}$, $V_{A3}$, were tested against the values given in Table 16, which were essentially those from Huguenard and McCormick (1992). There were 8 variations for each of the two holding potentials as each half-activation or half-inactivation potential was increased or decreased by 5 mV. The conclusion was that the values in the Table could not be improved upon. The final values of the maximal conductances resulting from this analysis were (in $\mu$S) $g_{A,max}=0.75$ and $g_{T,max}=0.265$. These values were used in conjunction with the conductances $g_{Na,max}=0.594$, $g_{KDR,max}=0.0768$, $g_{N,max}=0.024$, $g_{SK,max}=0.012$, $g_{BK,max}=2g_{KDR,max}/3$ and a small depolarizing current of $\mu = -0.1$ nA. This set of parameters will be referred to as run A3.

Results are shown in Figures 23 and 24. In the first of these Figures, the two top panels show the time course of the membrane potential $V$ in mV and the internal Ca$^{2+}$ concentration $Ca_i$ in mM versus time in ms. In the lower 4 panels are plotted the currents $I_{Na}$, $I_{KDR}$, and $I_{N}$ on expanded time scales and $I_{SK}$ all in nA. In the membrane potential trajectory a small notch (a few mV) can be seen at the base of the falling edge of the spike. The membrane potential climbs steadily to the threshold of the succeeding spike. The spike properties are $X = [11.9, -74, 2.2, 1363, 5.3, -2.3, 300]$ and $I_{N,max} = 0.34nA$ $I_{SK,min} = -0.18nA$ and $I_{BK,min} = -1.55$ nA. In Figure 24 are shown the details of the currents $I_A$ and $I_T$ during the ISI. According to these calculations, both currents peak abruptly during the spike but in the period until the subsequent spike $I_A$ gets progressively but slowly more negative, while $I_T$ reaches a maximum at about 400 ms (in this example) post spike and then declines steadily until it jumps up to trigger the next spike. The competition between $I_A$ and $I_T$ is therefore a factor in determining the length of the ISI, as postulated by many. Thus, these calculated activities can be compared with the proposed mechanisms of pacemaking in these cells as summarized by Jacobs and Azmitia (1992).

During an action potential, calcium enters serotonergic neurons through a high-threshold calcium channel. This is followed by a large (15-20 mV) AHP generated by a calcium-activated potassium conductance. This AHP results in a long relative refractory period, thus preventing discharges in bursts and insuring slow rates of firing. As the AHP decreases (due to sequestration and/or extrusion of calcium), it deinactivates a low-threshold calcium current and an early transient outward potassium current (IA). The currents generated by the activation of these two voltage-dependent channels are opposed, with IA tending to slow the rate of depolarization.
and the low-threshold calcium conductance increasing it. Under normal conditions, the calcium conductance is stronger, thus leading to a shallow ramp depolarization, which ultimately reaches threshold, fires, and, as the calcium enters the cell, reinitiates the sequence of events. The slope of this ramp is what determines the rate of discharge of serotonergic neurons.

![Graph of variables](image)

**Figure 23**: Time courses of several variables versus time during spiking and during the ISI for Run A3. Top left, membrane potential $V$, top right, internal $\text{Ca}^{2+}$ concentration with a maximum of 300 nM. In the bottom four panels, from left to right, are shown the fast sodium current, the high threshold calcium current, the delayed rectifier potassium current and the calcium-dependent (SK) potassium current. Note the different time scales for the faster and slower currents.

**Notch-free spikes**

As seen above, many spikes generated by the model, including those in the last subsection, which have taken account of the major known currents operating in
Figure 24: The computed time courses of $I_T$ (top panel) and $I_A$ (bottom panel) during and between spikes in the model with 7 component currents plus leak for Run A3.

DRN SE cells, have notches of various magnitudes, appearing usually on the falling edge of the spike. Such notches are likely to have their origin in equilibrium points such as spiral points in the 8 or 9-dimensional space in which the trajectories move. Notches in DRN SE spikes are not uncommon - see for example Hájós et al. (1996). The majority of reported spikes, however, are notch free, or possibly with such small notches they are not noticeable.

As a first step towards finding the factors which might lead to a notch or its absence, the parameters $V_{T1}$ and $V_{T3}$, being the half-activation and half-inactivation potentials for $I_T$, were varied over several mV from their standard values. The notch size with the standard values was 4.5 mV at the base of the spike, as seen in Figure 23. All the tested values resulted in similar sized notches, so it was concluded that notch size was practically independent of the magnitudes of these two parameters.

Secondly the parameter $g_{SK,max}$ was varied from its standard value of 0.012. Smaller values of $g_{SK,max}$ gave rise to larger notches, up to 7 or more mV, whereas larger values gave smaller notches and also in some cases an undulating plateau.
Thirdly, the strength of the calcium pump, through the parameter $K_s$ in Table 17, was varied. Increases in this parameter led to larger notch sizes and faster spiking. With smaller values, notch size also grew to over 8 mV and spiking became extremely slow.

Finally, the maximal conductance $g_{KDR,max}$ was varied from its standard value of $3 \times 0.0256$. The notch sizes for $g_{KDR,max}=2.5 \times 0.0256$, $2.0 \times 0.0256$ and $1.75 \times 0.0256$ were respectively 4.5, 2.7 and 0.15 mV indicating that the notch size varied in an inverse fashion to the delayed rectifier conductance. Somewhat amazingly when the value of $g_{KDR,max}$ was set at $1.5 \times 0.0256$, the spikes subsequent to the first, which had a very small notch of about 0.15 mV, became absolutely notch free. The results of such a run, called A4, are depicted in Figure 25, the quantities appearing therein being the same as in Figure 23 for run A3.

![Figure 25: Voltage, current and internal Ca$^{2+}$ concentration (as in Figure 23) for the regular spike train in Run A4 where notch-free spikes were obtained after reduction of the conductance of the potassium delayed rectifier current.](image-url)
12 Spontaneous activity with and without $I_H$

The question as to whether DRN SE neurons require excitatory synaptic input to fire regularly in pacemaker fashion has been much discussed. It is generally expected that in slice the amount of synaptic input would be small or zero, so the fact that there are spontaneously active neurons in slice indicates that synaptic input may not be required. Note, however, that Segal (1985) shows a neuron in slice with definite EPSPs.

In this section we focus on modeling the possibility of regular spiking with no external drive so that $\mu = 0$. It was not so easy to achieve this without $I_H$, the general finding being that small changes in many parameters would frustrate the occurrence of regular firing, leading to such results as bursting either in doublets or higher multiplets and long duration spikes with concomitant enormous increases in internal $Ca^{2+}$ concentration.

There are doubtless many combinations of parameters which give rise to regular spontaneous activity without the introduction of $I_H$ and here we report on one such set. The significant features of this set are (a) there is no $I_H$ nor any $I_L$ (b) the fast sodium conductance is relatively much larger at $g_{Na,max}=1.35$ and $V_{Na1} = -33.1$ mV is at its standard value (c) $V_{Ni} = -25$ mV (d) the magnitudes of $g_{A,max}=0.3$ and $g_{T,max}=0.15$ are considerably reduced (e) the area and capacitance have their standard values of $A=4000$ and $C=0.04$ (f) the magnitudes of $g_{KDR,max}=0.3$ (g) the calcium pump strength is $K_s = 0.625 \times 10^{-6}$ (h) the parameter CSF which determines the effective increase in $Ca_i$ due to $Ca^{2+}$ influx from $I_N$ is decreased to 0.25 compared with the standard value of 0.7.

With these changes spontaneous activity ensued but did not settle to regular firing until after over 20 spikes. When regular (fast) spiking was established, with an ISI of only 282 ms, the spikes had the following properties

$$X = [34, -77, 1.9, 282, 16, -4.05, 248].$$

Further, a large post-spike notch with an increment of 8.5 mV occurred at -71 mV.

Spontaneous activity in the complete simplified model, including $I_L$

The presence of $I_H$ makes it easier to achieve spontaneous spiking activity. First we note that diverse spike patterns can be obtained with various calcium pump rates for certain parameter sets, as illustrated in Figure 26. Here the values of the area, capacitance and maximal sodium conductance are set at higher than standard values, being 0.08 nF, 5200 sq microns and 1.188 $\mu$S. Remaining conductances, in $\mu$S are
$g_{KDR,max}=1.5 \times 0.0256$, $g_{T,max}=0.95 \times 0.265$, $g_{A,max}=0.9 \times 0.75$ and $g_{IH,max}=0.012$. Also, in mV, $V_{N_{a,1}} = -36.1$, $k_{N_{a,1}} = 10$, $V_{N_{a,3}} = -53.3$, $V_{T,1} = -57$, $V_{N1} = -25$ with other parameters taking standard values.

When the pump strength is $K_s = 0.25 \times 1.25 \times 10^{-6}$, the spike train is a regular sequence of singlets with an ISI = 2102 ms and duration 3.2 ms, as depicted in the top record of Figure 26. The level of $Ca_i$ reaches 575 nM. A somewhat larger pump strength of $K_s = 0.35 \times 1.25 \times 10^{-6}$ leads eventually, as seen in the middle panel of Figure 26, to a regular pattern of spikes with an ISI of 1501 ms, but initially there is a short interval terminating with a doublet and then a much longer interval before the train settles to its periodic form. During the latter part of the ISI a small kink is seen in the voltage trajectory. Similar kinks have been observed in spike trains of some DRN SE neurons (Park, 1987; Li and Bayliss, 1998).

At a still higher pump strength $K_s = 0.40 \times 1.25 \times 10^{-6}$, the spike train becomes more irregular, settling to alternating long and short ISIs of durations about 1500 and 1000 ms, respectively. The short ISIs display no kink but the longer ones have a pronounced kink about 75% into the ISI, similar to those found in some midbrain dopamine neurons (Neuhoff et al., 2002). Initially there is a very short interval which in this case terminates with a triplet.

Figure 26: Spike trains in the model with $I_H$, but not $I_L$, with three different calcium pump strengths, $K_s$, in multiples of $1.25 \times 10^{-6}$. Note the presence of a doublet (D) and a triplet (T) with the stronger pumps.
Spontaneous firing with only small changes in parameters from those for driven activity

The properties of the spikes in runs A3 and A4 were close to those of DRN SE neurons, but in those runs there was an applied depolarizing current of magnitude \( \mu = -0.1 \text{ nA} \) required for spiking. It was found that with very minor changes to, for example, only 5 parameters together with the introduction of \( I_H \), spontaneous pacemaker-like activity ensued. The very small changes made were, as summarized in Table 24, 5\% in the half-activation potentials for \( I_{Na} \), \( I_T \) and \( I_A \), as well as a 5\% change in the slope factor for the steady state sodium activation. Further, the maximal sodium conductance, \( g_{Na,max} \), was reduced to 0.955 of its value. There are three sets of runs here based on this slightly altered set, labeled SS, SSL and F.

| Parameter | Driven (A3) | Spontaneous (SS1) |
|-----------|-------------|-------------------|
| \( V_{Na_1} \) | -33.1       | -34.755           |
| \( k_{Na_1} \) | 10          | 10.5              |
| \( V_{T_1} \) | -57         | -54.15            |
| \( V_{A_1} \) | -60         | -57               |
| \( g_{Na,max} \) | 0.594       | 0.567             |
| \( g_{IH,max} \) | 0           | 0.012             |
| \( \mu \) | -0.1        | 0                 |

Table 25: ISIs of pacemaker spikes with \( I_H \) but not \( I_L \)

| Run | \( g_{IH,max} \) | \( V_{N_1} \) | \( V_{N_3} \) | \( K_s \) | \( g_{N,max} \) | ISI |
|-----|-----------------|--------------|--------------|----------|--------------|-----|
| SS1 | 0.012           | -25          | -50          | 0.25 x 1.25 x 10^{-6} | 0.024 | 1950          |
| SS2 | 0.012           | -25          | -50          | 0.25 x 1.25 x 10^{-6} | 0.024 | No spikes    |
| SS3 | 0.012           | -10          | -35          | 0.25 x 1.25 x 10^{-6} | 0.024 | 1200          |
| SS4 | 0.012           | -10          | -45          | 0.25 x 1.25 x 10^{-6} | 0.0462 | 1640         |
| SS5 | 0.012           | -10          | -45          | 1.25 x 1.25 x 10^{-6} | 0.0462 | 1500         |

Before describing results with all components, we consider the set (SS) in which pacemaker activity is observed with \( I_H \) but \( I_L \) is not included. There are 5 such
runs described in Table 25. Table 24 gave the values of the parameters which differ between SS1 (spontaneous) and A3 (driven). In Table 25 there are only 5 parameters, $g_{IH,max}$, $V_{N_1}$, $V_{N_3}$, $K_s$ and $g_{N,max}$, which are varied. The half-activation potential of $I_N$ could be higher than -25 mV, so the value -10 mV was tested. However, in either case $I_N$ is only activated substantially above action potential threshold so this change probably only has a minor effect. The value of $g_{N,max}$ was also estimated from voltage-clamp data in Penington et al. (1991) to give the value 0.0462 µS, which is the value used in runs SS4 and SS5, but again, this change had only small effects. The actual value in native cells at body temperature is not available.

For these parameter sets, the computed model ISIs ranged from 1200 to 1950 ms. All the spike trains were regular, and only in the last run of this group, SS5, was there a small kink about half way along the ISI (cf Park, 1987; Li and Bayliss, 1998; see also spiking in mouse locus coeruleus, de Oliveira et al., 2010). All other spike trains had properties resembling those often observed in rat DRN SE neurons, which we note may have frequencies of discharge as low as 0.1 to 0.25 Hz (Aghajanian et al., 1978; Hájós et al., 1995; Bambico et al., 2009) corresponding to ISIs as large as 2500 to 10000 ms.

**Spontaneous firing in the complete simplified model**

The complete simplified model has the 10 currents

\[ I_A, I_{KDR}, I_T, I_L, I_N, I_H, I_{Na}, I_{SK}, I_{BK}, I_{Leak}, \]

the M-type potassium current being omitted here, but to be included in future work. It is rather worth pondering that such a complex system, of 16 nonlinear differential equations, admits smooth and regular periodic solutions, especially in light of the fact the biological existence of such periodic solutions is essential for the proper functioning of many neurons in the mammalian and other nervous systems. In the calculations described so far, it was apparent that the ranges are narrow of all of the 90 parameters in the computational model in which regular periodic spiking occurs. For the proper functioning of the neurons and hence the brain, this implies there is much scope for the occurrence of serious problems in maintaining required or normal activity.

Altogether about 30 different parameter sets in the 10-component current model were used to explore the roles of some parameters, of which 24 will be described briefly. These are the two sets SSL (i.e., SS with added L-type) and F (for final) there being 14 sets in the SSL-sequence and 10 in the F-sequence. In the SSL set,
for which results for some spike properties are given in Table 26, the surface area of the cell was always taken to be the standard value 4000 sq microns, except in the last two runs SSL13 and SSL14, and \( g_{IH,\text{max}} \) was always 0.012 \( \mu \text{S} \). There are only seven parameters which vary in this set. Thus an idea, albeit limited, of how the spike train is affected by changes in the parameters of the two higher threshold \( \text{Ca}^{2+} \) currents, \( I_N \) and \( I_L \) as well as the \( \text{Ca}^{2+} \) pump rate and area can be obtained.

Generally larger pump rates make the ISI shorter (compare SSL2 with SSL3 and SSL6 with SSL7 and SSL8) and larger values of \( g_{N,\text{max}} \) make the ISI longer (compare SSL4 with SSL5). This is because higher pump rates clear internal \( \text{Ca}^{2+} \) faster which has less chance to activate \( I_{SK} \) channels and higher \( g_{N,\text{max}} \) (or \( g_{L,\text{max}} \)) leads to more calcium influx with subsequent activation of \( I_{SK} \). Increasing the half-activation potentials of \( I_L \) and \( I_N \) made the ISI smaller (compare SSL6 with SSL9), because there was less calcium influx to activate \( I_{SK} \). Increasing the calcium pump strength can induce repetitive firing which is absent for smaller pump strengths (compare SSL10 and SSL11). Comparing SSL12 and SSL13, an increase in area \( A \) causes a speeding up of the spiking, but in comparing SSL13 and SSL14, there is a trade-off between the inhibitory effect of a weaker calcium pump and the excitatory effect of a larger area, the former dominating so that the ISI increases.

The final set of results, labeled F, is given in Table 27. Here there are only 4 parameters which vary, the following parameters being fixed: \( g_{L,\text{max}} = 0.00462 \), \( g_{N,\text{max}} = 9g_{L,\text{max}} \), \( g_{IH,\text{max}} = 0.012 \), and the half-activation and inactivation potentials for \( I_L \) and \( I_N \), as in the last row of Table 26. The parameters that varied were the area, \( A \), the pump strength, \( K_s \), and the sodium and \( I_T \) conductances \( g_{Na,\text{max}} \) and \( g_{T,\text{max}} \). ISIs ranged from a smallest value of 506 ms to a largest value of 1694 ms. A few spike trains (F2, F3) were irregular but mostly they were periodic. In the ISI there were instances of kinks, changes of slope (mainly not great) and plateaux, but no notches. The spikes in runs F4, F6, F7, F9 and F10 were smooth as usually observed for DRN SE neurons, and the spike trains were regular periodic. Investigations in which \( g_{T,\text{max}} \) varied indicated that this component was probably the likely origin of kinks when they occurred.

From the results in Table 27, comparing F1 and F2 again demonstrates the slowing down of the spike train by a decreased calcium pump strength. Decreasing the area \( A \), as from F2 to F3, can change the character of the spike train from steady state regular to an alternating long-short sequence. Comparing F3 with
Table 26: ISIs of spontaneous pacemaker spiking in the complete model: SSL sequence

| Run   | $V_{L1}$ | $V_{L3}$ | $g_{L,max}$ | $V_{N1}$ | $V_{N3}$ | $g_{N,max}$ | $K_s$ | ISI   | Remarks                                                                                           |
|-------|----------|----------|-------------|----------|----------|-------------|------|-------|--------------------------------------------------------------------------------------------------|
| SSL1  | -15      | -25      | 0.01        | -25      | -50      | 0.024       | 3.125 x 10^{-7} | 3000  | Plateau 1000 ms                                    |
| SSL2  | -20      | -50      | 0.01        | -10      | -45      | 0.0462      | 3.125 x 10^{-7} | 2757  | Similar to SSL1                                   |
| SSL3  | -20      | -50      | 0.01        | -10      | -45      | 0.0462      | 3.90625 x 10^{-7} | 1819  | Slope change mid ISI                             |
| SSL4  | -20      | -50      | 0.01        | -10      | -45      | 0.0693      | 4.6875 x 10^{-7} | 2700  | Late rise in $V_{g_{N,max}}$, 50% larger $Ca_{i,max}$ 1100 nM                                      |
| SSL5  | -20      | -50      | 0.01        | -10      | -45      | 0.0231      | 4.6875 x 10^{-7} | 1114  | Kink in $V_{g_{N,max}}$, 50% smaller $Ca_{i,max}$ 380 nM                                        |
| SSL6  | -20      | -50      | 0.01        | -10      | -35      | 0.024       | 3.125 x 10^{-7} | 1980  | Smooth trajectory, $dV/dt$ steadily decreases during the ISI                                   |
| SSL7  | -20      | -50      | 0.01        | -10      | -35      | 0.024       | 4.6875 x 10^{-7} | 1200  | Kink mid ISI                                     |
| SSL8  | -20      | -50      | 0.01        | -10      | -35      | 0.024       | 3.125 x 10^{-7} | 1932  | Slope change mid ISI                             |
| SSL9  | -15      | -50      | 0.01        | -5       | -35      | 0.024       | 3.125 x 10^{-7} | 1476  | Slope change mid ISI                             |
| SSL10 | -25      | -50      | 0.01        | -10      | -45      | 0.0362      | 3.125 x 10^{-7} | -     | 1 spike only                                     |
| SSL11 | -25      | -50      | 0.01        | -10      | -45      | 0.0362      | 3.90625 x 10^{-7} | 2400  | Larger $g_{N,max}$                                |
| SSL12 | -20      | -45      | 0.00462     | -10      | -45      | 0.04158     | 3.90625 x 10^{-7} | 1659  | Stronger pump                                    |
| SSL13 | -20      | -45      | 0.00462     | -10      | -45      | 0.04158     | 3.90625 x 10^{-7} | 1346  | Slope change mid ISI                             |
| SSL14 | -20      | -45      | 0.00462     | -10      | -45      | 0.04158     | 3.125 x 10^{-7} | 1454  | Slope change mid ISI                             |

F4, a decrease in calcium pump strength can change an irregular spike train into a regular one and a small change in this parameter can introduce a kink in the voltage trajectory during the ISI. A comparison of F1 and F6 shows that a small increase
in $g_{Na,max}$ and a concomitant decrease in $g_{T,max}$ can produce smooth trajectories between spikes rather than ones with a marked change of slope, but that the ISI is hardly changed. From F6 to F7 a decrease in area is inhibitory as well as the reduction in $g_{T,max}$, but the spike train remains smooth and regular. F8 and F9 demonstrate the speeding up of spiking with increased area, and in F9 an increase in $g_{Na,max}$ and a concomitant decrease in $g_{T,max}$ leads to a plateau towards the end of the ISI. Comparing F9 and F10 further demonstrates how a larger area leads to a smaller ISI. Finally, in FURA-2AM measurements under various conditions, it was found that the resting internal $Ca^{2+}$ concentration was about 32 nM, but when this value was employed rather than 50 nM, the spike properties differed very little, including ISI and the maximal $Ca_i$.

A set of computed results for a run very similar to F7 is given in Figures 27 and

![Figure 27: Results showing spontaneous pacemaker activity ($\mu = 0$) for the complete model in a run very similar to F7 in Table 27. All parameters are given in Table 28. Top two panels show the membrane potential and internal $Ca^{2+}$ concentration during an ISI. The bottom left 3 panels show the details of the three currents $I_{Na}$, $I_N$, $I_{KDR}$ during a spike and the remaining panel shows the calcium-dependent current $I_{SK}$ during the ISI.](image-url)
Table 27: Results for ISIs for pacemaker spiking in the complete model, F sequence

| Run | A  | $K_s$         | $g_{Na,max}$ | $g_{T,max}$ | ISI | Remarks                  |
|-----|----|---------------|--------------|-------------|-----|--------------------------|
| F1  | 6000 | $3.90625 \times 10^{-7}$ | 0.567        | 0.265       | 1148 | Slope change mid ISI     |
| F2  | 6000 | $6.25 \times 10^{-7}$      | 0.567        | 0.265       | 506  | Irregular start          |
| F3  | 4000 | $6.25 \times 10^{-7}$      | 0.567        | 0.265       | 500,1200 | Alternate short,long     |
| F4  | 4000 | $5 \times 10^{-7}$         | 0.567        | 0.265       | 500,1200 | Regular train            |
| F5  | 4000 | $5.46875 \times 10^{-7}$   | 0.567        | 0.265       | 1500 | Kink                     |
| F6  | 6000 | $3.90625 \times 10^{-7}$   | 0.594        | 0.22525     | 1145 | Regular smooth spikes    |
| F7  | 4000 | $3.90625 \times 10^{-7}$   | 0.594        | 0.1855      | 1694 | Regular smooth spikes    |
|     |     |               |              |             |     | $Ca_{i,max}$ 550 nM      |
| F8  | 6000 | $6.25 \times 10^{-7}$      | 0.594        | 0.22525     | 719  | Slope change mid ISI     |
| F9  | 6000 | $6.25 \times 10^{-7}$      | 0.675        | 0.14575     | 770  | Plateau at end of ISI    |
| F10 | 4000 | $6.25 \times 10^{-7}$      | 0.675        | 0.14575     | 1157 | Small slope change end ISI |

Figure 28: Results for the same parameter set as in Figure 27. The currents $I_T$, $I_A$ and $I_H$ are shown during an ISI and the currents $I_L$ and $I_{BK}$ are shown during a spike.
28. The currents which remain extremely small for most of the ISI, namely $I_{Na}$, $I_{KDR}$, $I_L$, $I_N$ and $I_{BK}$ are shown only during the spike, whereas the remaining 4 currents are shown throughout the ISI. Again, the interplay between $I_T$ and $I_A$ is seen to be important in that both are activated in similar voltage ranges and tend to oppose one another. No measurements have been made of any of these currents, during either a spike nor during the ISI, with which to compare the numerical results.

**Summary of parameters for spontaneous activity**

We give here a complete list of the parameters for the run F7 of Table 27 for which spikes were regular with an ISI of 1694 ms corresponding to a frequency of 0.59 Hz, as depicted in Figures 27 and 28. This parameter set, based as much as possible on experimental data, may be used as a starting point for more complex models with a more complete knowledge of the characteristics of each component, though of course there is always large amount of variability from cell to cell and under different conditions so there are no definitive sets of parameters. In Table 28, corresponding to the initial Table 16, are given the parameters for activation (and inactivation if appropriate) of the 9 channel types $I_{Na}$, $I_{KDR}$, $I_T$, $I_L$, $I_N$, $I_A$, $I_H$, $I_{SK}$ and $I_{BK}$. Table 28 differs slightly in format from Table 16, as parameters for the simple $I_{BK}$ current have been added and the time constants for sodium are V-dependent. Table 17 of cell properties, calcium dynamics and equilibrium potentials is the same for F7, except for the one parameter for the calcium pump strength, which is now $K_s = 3.90625 \times 10^{-7}$. Table 29 gives the maximal conductances, all of which differ from those in Table 18, and there is the additional conductance for $I_{BK}$. In all, there are 90 parameters as Faraday’s constant is not a parameter.
### Table 28: Activation and inactivation parameters for run F7

| $V_{Na_1}$ | -34.76 | $c_T$ | 28 | $\tau_{h,N}$ | 1000 |
| $k_{Na_1}$ | 10.5 | $d_T$ | 300 | $V_{A_1}$ | -57 |
| $V_{Na_3}$ | -50.3 | $V_{T_4}$ | -81 | $k_{A_1}$ | 8.5 |
| $k_{Na_3}$ | 6.5 | $k_{T_4}$ | 12 | $V_{A_3}$ | -78 |
| $a_{Na}, b_{Na}$ | 0.05, 0.15 | $V_{L_1}$ | -20 | $k_{A_3}$ | 6 |
| $V_{Na_2}, k_{Na_2}$ | -43, 6.84 | $k_{L_1}$ | 8.4 | $a_A$ | 0.37 |
| $V_{KDR_1}$ | -15 | $a_L$ | 0.5 | $b_A$ | 2 |
| $n_k$ | 1 | $b_L$ | 1.5 | $V_{A_2}$ | -55 |
| $k_{KDR_1}$ | 7 | $V_{L_2}$ | -20 | $k_{A_2}$ | 15 |
| $a_{KDR}$ | 1 | $k_{L_2}$ | 15 | $c_A$ | 19 |
| $b_{KDR}$ | 14 | $V_{L_3}$ | -45 | $d_A$ | 45 |
| $V_{KDR_2}$ | -20 | $k_{L_3}$ | 13.8 | $V_{A_4}$ | -80 |
| $k_{KDR_2}$ | 7 | $\tau_{h,L}$ | 200 | $k_{A_4}$ | 7 |
| $V_{T_1}$ | -54.15 | $V_{N_1}$ | -10 | $V_{H_1}$ | -80 |
| $k_{T_1}$ | 6.2 | $k_{N_1}$ | 7 | $k_{H_1}$ | 5 |
| $V_{T_3}$ | -81 | $a_N$ | 1 | $a_H$ | 900 |
| $k_{T_3}$ | 4 | $b_N$ | 1.5 | $k_{H_2}$ | 13 |
| $a_T$ | 0.7 | $V_{N_2}$ | -15 | $V_{H_2}$ | -80 |
| $b_T$ | 13.5 | $k_{N_2}$ | 15 | $K_c$ | 0.000025 |
| $V_{T_2}$ | -76 | $V_{N_3}$ | -45 | $\tau_{m,SK}$ | 5 |
| $k_{T_2}$ | 18 | $k_{N_3}$ | 10 | $V_{BK}, k_{BK}$ | -20, 2 |

### Table 29: Maximal conductances

| $g_{Na,max}$ | 0.594 | $g_{N,max}$ | 0.04158 |
| $g_{KDR,max}$ | 0.0384 | $g_{A,max}$ | 0.75 |
| $g_{T,max}$ | 0.22525 | $g_{IH,max}$ | 0.018 |
| $g_{L,max}$ | 0.00462 | $g_{SK,max}$ | 0.012 |
| $g_{BK,max}$ | 0.0256 |

### 13 Anodal break

Some experimental results on DRN SE neurons are able to be compared with model predictions. In Burlhis and Aghajanian (1987), some anodal break experiments were reported in which a sustained hyperpolarizing input current was suddenly removed,
with and without TTX.

Figure 29: Computed model anodal break voltage response with (assumed zero fast sodium conductance) and without TTX to a hyperpolarizing current applied for 100 ms.

For the former case, with $g_{Na,max}=0$, in simulations a hyperpolarizing current $\mu = 0.1$ nA was applied for 100 ms using the same parameter set as in run F10 of Table 27. The result is shown in the left panel of Figure 29 where it is seen that during the application of the hyperpolarizing current, V decreases from resting level of -60 mV to about -79 mV with a relatively small time constant of about 7 ms, which is similar to the average value reported in Li et al. (2001). In another run a time constant of about 30 ms was obtained, which is more in the range of reported values for the majority of DRN SE neurons. When released from the hyperpolarizing current, the voltage did swing up past rest to about -56 mV and eventually returned to resting level, mimicking approximately the result in Figure 3A of Burlhis and Aghajanian (1987). The same experiment without TTX produced a spike on release as depicted in the right hand panel of Figure 29, with a large notch at about -63 mV.
14 f/I curves

There do not appear to be published explicit frequency versus magnitude of applied current in DRN SE neurons. There are data on firing rate versus concentration of norepinephrine and phenylephrine (Judge and Gartside, 2006). For example, firing rate increased from about 1.25 Hz to 1.6 Hz when 10 µM phenylephrine was applied. The computational model developed here was used to find frequency versus applied current (f/I) curves for two sets of parameters. In one set the cell fired spontaneously and even with a very small hyperpolarizing current, and in the other case the cell only fired if a depolarizing current of at least 77 pA was applied. The computed f/I curves are shown in Figure 30. In both cases, the firing rate increases as $-\mu$ increases as expected; but it was not expected that the firing rate would decrease slightly at values of the depolarizing current 0.45 nA in the spontaneously active cell and 0.55 nA in the non-spontaneously active cell. Furthermore, just above these values the behaviors were quite different as the spontaneously active cell entered a regime of very rapid firing (76.9 Hz) of single spikes with $\mu = -0.5$ nA whereas the non spontaneously active model neuron at $\mu = -0.6$ nA began to fire in rapid but regular triplets.

![Figure 30: Calculated f/I curves for the cases of a spontaneously active neuron and a neuron with a threshold for firing of 77 pA. Note the slight downturns at large depolarizing currents.](image-url)
15 Discussion

Serotonergic neurons of the dorsal (and other) raphe have complex electrophysiology and neurochemistry about which much is known (Jacobs and Fornal, 1995; Azmitia and Whitaker-Azmitia, 1995; Aghajanian and Sanders-Bush, 2002; Harsing, 2006; Lanfumey et al., 2008; Lowry et al., 2008; Hale and Lowry, 2011) including their role in the therapeutical effects of SSRIs and other antidepressants (Piñeyro and Blier, 1999; Guiard et al., 2011; Haenisch and Bönisch, 2011).

Nevertheless, it is a remaining challenge to determine the nature and functioning of the networks in which these neurons are involved, including their inputs from both nervous and endocrine systems and the effects they have on the many cells they influence. Considering the important roles played by these neurons in the physiological and psychological functioning of most mammalian species, it is not surprising that a more complete understanding is being pursued in various laboratories. Many of the details of the projections of DRN neurons and their interconnections have recently been determined (Vasudeva et al., 2011; Waselus et al., 2011), some with the use of genetic techniques (Bang et al., 2012). It is expected that quantitative models of the systems affected by and affecting DRN SE neurons will play an important role in confirming hypotheses concerning their involvement.

Opposing views have been stated concerning whether DRN SE cells’ apparent pacemaker activity is autonomous or does in fact require some external excitatory influence. There are many central neurons that have apparent spontaneous pacemaker activity, but in each cell type the mechanisms are seemingly different. In midbrain dopamine (DA) neurons, particularly of the substantia nigra in rats (Kang and Kitai, 1993; Wilson and Callaway, 2000; Durante et al., 2004; Atherton and Bevan, 2005; Foehring et al., 2009) and mice (Puopolo et al., 2007), L-type calcium currents, activated at relatively low membrane potentials have been found to be primarily involved though other voltage-gated calcium currents are present (Cardozo and Bean, 1995). The oscillations are set up primarily as an interaction between the L-type Ca$^{2+}$ and the calcium-dependent potassium current, with significant effects on frequency and regularity of spiking by SK3 channel activity (Wolfart et al., 2001), $I_A$ (Liss et al., 2001), buffering (Neuhoff et al., 2002; Foering et al., 2009) and $I_H$ (Neuhoff et al., 2002). The latter current is also important in pacemaking activity in many neuron types (Pape, 1996) including some hippocampal and thalamo-cortical neurons (Maccaferri and McBain, 1996; Luthi and McCormick, 1999).

Locus coeruleus neurons seem to have quite different pacemaking mechanisms from those in DA neurons. In rats, these cells are spontaneously active both in vivo and in slice with frequencies between 0.4 and 4 Hz (Aghajanian et al., 1983; Alreja...
and Aghajanian, 1991). Calcium-dependent potassium currents are the principal factor in the post-spike afterhyperpolarization and frequency of firing (Aghajanian et al., 1983) and availability of cAMP is required for sustained firing (Alreja and Aghajanian, 1991). In mice, de Oliveira et al. (2010, 2011) found that pacemaking in the majority of locus coeruleus cells was mainly due to sodium and potassium currents and that calcium currents were negligible during the ISI. There are differences between rat and mouse, and development is also a factor (de Oliveira et al., 2011).

Other examples of cells exhibiting pacemaker-like activity include thalamic neurons (Mulle et al., 1986), cells in the suprachiasmatic nucleus (Jackson et al., 2004; Brown and Piggins, 2007; Itri et al., 2010), brainstem neurons involved in the control of respiration (Richerson, 1995; Rybak et al., 2003; Ptak et al., 2009; Dunmyre et al., 2011; Ramirez et al., 2011) cardiac cells (Brette et al., 2006; Mangoni et al., 2003; Benitah et al., 2010; Vandael et al., 2010) and uterine cells (Rihana et al., 2009). Further examples are summarized in Ramirez et al. (2011), which contains a detailed discussion of pacemaking in preBötzinger complex neurons where $I_H$ and a persistent sodium current, $I_{NaP}$, play significant roles.

Turning attention to DRN SE neurons, it has long been recognized that they undergo slow and regular spiking in a variety of conditions in most mammalian species with a rate of about 0.5 to 2 per second in the quiet waking state, higher in waking arousal, less in slow-wave sleep and zero in REM sleep (Jacobs and Fornal, 1995; Aghajanian and Sanders-Bush, 2002). Such rates may also be broadly species dependent. The result of such regular firing is a fairly constant rate of release of serotonin in the many target areas (Jacobs and Fornal, 1995), both local and remote, but that the rate depends on physiological state. Since, according to Azmitia and Whitaker-Azmitia (1995), nearly every neuron in the brain is close to a serotonergic fiber and influenced either synaptically or by volume diffusion by serotonin, the consequence of the state-dependent release is a modulation of the activity of the whole brain activity as well as effects at spinal targets such as motoneurons.

During regular almost periodic firing, there is observed the sequence of current flows already alluded to in previous sections and satisfactorily predicted by the computational model. Such a sequence has been described by many authors (e.g., Burtis and Aghajanian, 1987; Jacobs and Azmitia, 1992; Pinedo and Blier, 1999). After a spike there is hyperpolarization due to calcium-dependent potassium current, followed by a fairly slow ramp-like approach to threshold, often with a plateau, as internal calcium is cleared and $I_A$ and $I_T$ compete, with $I_T$ assisting in or leading to the threshold crossing as $I_A$ retards the approach.

Concerning whether DRN SE neurons are endogenous pacemakers whose regular
firing or pacemaker-like activity does not require any external stimulation, some early reports stated that there were only minor differences between in vitro and in vivo firing rates (Mosko and Jacobs, 1976; Jacobs and Azmitia, 1992) which supported the claim of intrinsic mechanisms for pacemaking. Some authors were skeptical (Crunelli et al., 1983) whereas many expressed the view that the ramp-like approach to threshold was sufficient evidence of the non-necessity of external influences (Jacobs and Azmitia, 1992; Aghajanian and Sanders-Bush, 2002).

However, Vandermaelen and Aghajanian (1983) had found that most cells in vitro were less active than in vivo, which was supposed due to reduced excitatory drive. Pan et al. (1990) and Kirby et al. (2003) found only a small fraction of cells were active in slice, the latter authors claiming that such observations were probably evidence that noradrenergic input was cut off (see also Ohliger et al., 2003).

Convincing physiological evidence for a role of noradrenergic drive was presented by Baraban and Aghajanian (1980) who also provided anatomical evidence that noradrenergic input directly synapsed on DRN SE neurons (Baraban and Aghajanian, 1981). The physiological evidence was that α-adrenoceptor antagonists reduced the firing rates, although there was a possibility that the DRN SE neurons were subsequently able to recover and revert to their usual activity. Further, locus coeruleus cells are silent during REM sleep, during which DRN SE cells are also silent. Levine and Jacobs (1992) also noted that there was a dense noradrenergic input to the DRN and that the lack of effect of iontophoretic applications of noradrenergic agonists was possibly due to the already maximal noradrenergic input from locus coeruleus. Nevertheless, noradrenergic antagonists did suppress firing in anesthetized animals, but interpretation of this effect is complicated by the effects of some of these drugs on sodium channels.

The noradrenergic excitation of DRN SE cells was established as being via by α₁-adrenoceptors and the mechanism of action of α₁-adrenoceptor agonists was explored by Aghajanian (1985) who found that $I_A$ was reduced by them, resulting in shorter plateaux and faster firing. However, with noradrenergic inputs there are uncertainties, as opposing effects can be elicited by the stimulation of different receptor subtypes, such as the α₁- and α₂-adrenoceptors (Pudovkina et al., 2003; Bortolozzi et al. (2003); Pudovkina and Westerink, 2005; Harsing, 2006; O’Leary et al., 2007).

Since in brain slice preparations, most of the afferent inputs are removed, then the probable loss of most of the noradrenergic excitation is only one factor leading to a change in firing rate. As one example, inhibitory inputs, such as those originating in forebrain but mediated locally, will also be cut off, which would compensate to an unknown degree the loss of noradrenergic excitation.
In vitro preparations are also likely to have reduced or removed excitatory inputs from orexinergic neurons in the lateral hypothalamus (see Ohno et al., 2008 for review) and from histamine neurons as well as noradrenergic inputs which would contribute to their silence (Brown et al. 2002). Orexin afferents, which are dense in the DRN, affect both serotonergic neurons and GABA-ergic neurons such that at low levels the effect is primarily excitatory but at higher levels is inhibitory (Liu et al., 2002). The effects of orexinergic input on DRN SE cells are dependent on the state of arousal of the animal (Takahashi et al., 2005) having little effect during wakefulness but significant effect during REM and slow wave sleep. There are also differences in the strength of orexinergic inputs received by the dorsal raphe and medial raphe nuclei (Tao et al., 2006), with orexin A strongly targeting the DRN. An interesting feedback loop exists between the orexinergic and serotonergic systems, as the latter inhibit orexin neurons through 5-HT1A receptors and associated GIRK currents (Ohno et al., 2008). Del Cid-Pellitero and Garzón (2011) have reported that there are loops involving the mPFC, the DRN and hypothalamic orexin neurons, which further complicates the matter of in vitro versus in vivo firing of DRN SE cells.

Also relevant is the dense innervation of the DRN by CRF fibers as mentioned in the Introduction. Experiments using either urocortin or CRF have shown that at small doses SE cells are inhibited but at high doses excited (Kirby et al., 2000; Ordway et al., 2002; Pernar et al., 2004; Abrams et al., 2004). However Lowry et al. (2000) found that in vitro, application of CRF led to rapidly increased firing in DRN SE cells, but only in the ventral DRN. Additionally, CRF excites locus coerulueus neurons (Ordway et al., 2002; Snyder et al., 2012) which in turn excite many DRN SE neurons. Another relevant effect on DRN SE cells is from substance P neurons which excite them through a high density of receptors in the DRN (Ordway et al., 2002). In conclusion, there are many factors involved in the differences between in vivo activity and in vitro activity so that a generalization is not possible. The most important factor is probably the variation of input patterns from one location to the other (Abrams et al., 2004; Lowry et al., 2008). One source of variability in the findings in different experiments could be the concentrations of ions such as $K^+$, $Mg^{2+}$ and $Ca^{2+}$ in the extracellular fluid.

The computations reported in the present article constitute a first step in the development of a quantitative description of the firing activity of DRN SE neurons. It has been demonstrated that these neurons may fire in a regular pacemaker fashion with or without any external drive and that only small differences in the properties of some ion channels can lead to one or the other mode of pacemaker activity. The present results are preliminary because of uncertainties in the properties of many
of the component currents. Further, simplifications and omissions have been made in the model in order to obtain some insight into the behavior of these neurons with their very complex set of ion channels and inputs. Apart from more elaborate descriptions of some of the dynamics of some of the component currents, future work will involve additional exploration of the changes in spiking activity as functions of changes in many of the parameters as well as including the effects of the various neurotransmitters affecting the activity of DRN SE neurons, as alluded to in the present discussion. One important transmitter is serotonin itself, especially through its activation of autoreceptors that reduce the firing rate. However, the serotonin involved in this self-inhibition may be released non-synaptically from dendrites of the same or neighbouring cells making the calculation of this effect more complex than that for post-synaptic potentials.

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