The Journal of Undergraduate Neuroscience Education (JUNE), Spring 2018, 16(2):R39-R43

AMAZING PAPERS IN NEUROSCIENCE
Characteristics, Emergent Properties and Functions of Somato-dendritic T- and L-Type Calcium Channels

Corey L. Cleland
Department of Biology, James Madison University, Harrisonburg, VA 22807.

The primary literature, as an adjunct to textbooks, lectures, problem sets, and laboratories, has become integral to most undergraduate neuroscience courses by extending learning to topics outside the scope of introductory textbooks, providing insight into experimental methods and design, and offering a platform for critical thinking, independent learning, and student presentations. While introductory and intermediate textbooks cover "Hodgkin-Huxley" (H-H) Na and K channels thoroughly, the characteristics of diverse calcium, chloride, and other sodium and potassium channels, and especially the resulting emergent cellular properties and their functional consequences, receive far less coverage. The specific aim of this report is to identify, summarize, and pedagogically evaluate six articles that describe the biophysical channel properties, resulting cellular emergent properties, and potential functions of two types of somato-dendritic calcium channels: type T- and L-type channels. The three-tier vertical organization (channel, emergence, function) across multiple channels (T-, L-type) will help students connect information across parallel and hierarchical levels of analysis.

Key words: education; primary literature; plateau potential; bursting; action potential

The primary literature, as an adjunct to textbooks, lectures, problem sets, and laboratories, has become integral to most undergraduate neuroscience courses (Cook-Snyder, 2016). Journal articles extend learning by covering topics outside the scope of introductory textbooks, providing insight into experimental methods and design (Hoskins, 2008), and offering a platform for critical thinking, independent learning, and student presentations (Harrington et al., 2015).

Although diverse in approach, depth, and style, introductory (e.g., Striedter, 2015; Luo, 2016) and intermediate level textbooks (e.g., Kandel et al., 2012) inevitably cover in detail the classic "Hodgkin-Huxley" (H-H) sodium and potassium channels from which the action potential emerges. In contrast, the characteristics of diverse calcium, chloride, and other sodium and potassium channels, and especially the resulting cellular emergent properties and their functional consequences, receive far less coverage. More advanced textbooks address these concepts (e.g., Johnston and Wu, 1994; Fain, 2014; Hammond, 2015), but are often too advanced for undergraduates. While it can be argued this more extensive coverage is outside the scope of an undergraduate neurobiology course, I have found that covering only the H-H channels in detail limits my ability to teach how the quantitative properties of channels (ion selectivity, activation and inactivation curves, time dependence, and role of intracellular ligands) can lead to a wide range of functionally important emergent properties such as delay, oscillation, spike frequency adaptation burst-tonic switching, and plateau potentials (Figure 1).

The specific aim of this report is to identify, summarize and pedagogically evaluate a group of related articles that describe the biophysical channel properties, resulting cellular emergent properties, and potential functions of two types of somato-dendritic calcium channels: T- and L-type channels. The six papers span three levels – biophysical channel properties, resulting cellular emergent properties, and potential functions. These articles, all of which I use in teaching an upper-level Neurobiology class, were pedagogically evaluated and chosen based on three criteria that I employ for all 10-12 empirical articles I assign my students to present in class.

- *Length.* Articles must be relatively short because I require students to read carefully all papers each semester and they are examined in detail (e.g., by changing the data in a figure and asking about implications, which requires a solid understanding). I often use articles from *Science*, *Nature* or brief report sections of journals rather than more extensive "parent" articles.

- *Lack of jargon/techniques that are not covered in class.* While there are many fabulous recent articles, the neuroscience techniques and jargon are often outside the scope of what is covered in my class, thus creating an unnecessary barrier to understanding.

- *Reinforce material covered in class.* While many teachers use articles to expand the course content, the articles I assign reinforce specific, often difficult (e.g., reversal potentials, Owens et al., 1996), topics covered in lecture.

BACKGROUND

T- and L-type calcium channels, beyond sharing selectivity for calcium, differ in activation, inactivation, time course, and pharmacology. In particular, the membrane potential at which T-type channels inactivate is relatively more positive compared to H-H sodium and L-type calcium channels. L-type channels importantly lack rapid inactivation, thus creating persistent currents (Hammond, 2015). Although not discussed in this paper, there are also N- and P-type

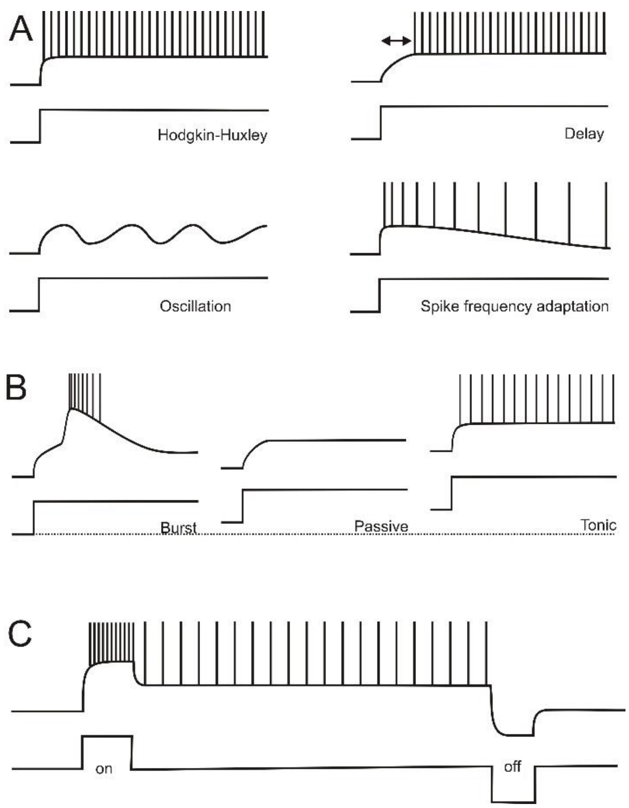

Figure 1. Emergent cellular properties result from diverse ion channels. Cartoon of membrane potential and spiking behavior of representative neurons in response to step depolarization. *A*. Classic "Hodgkin-Huxley" neuron, Delay, Oscillation and Spike Frequency Adaption arising potentially from diverse sodium, calcium and potassium channels. *B*. Burst-tonic switching arising from T-type calcium channels. The resting membrane potentials are progressively more positive in each panel due to progressively more injected baseline current, resulting in two possible states – bursting and tonic responses to depolarization. *C*. Plateau potential arising from L-type calcium channels.

calcium channels that have their own distinct characteristics (Johnston and Wu, 1994).

Expressed in neurons, these calcium channels underlie the emergent properties of burst-tonic switching (T-type) and plateau potentials (L-type) in neurons (Huguenard and McCormick, 1994; Kiehn and Eken, 1998). For burst-tonic switching (Figure 1B), the central concept is that the resting potential must be especially negative (-80 mV) to remove T-type channel inactivation, thus allowing rapidly adapting activation to create a slow calcium potential and a burst of sodium action potentials. In the absence of removing inactivation, depolarization results in only passive responses or tonic firing of action potentials. Thus, somewhat paradoxically, hyperpolarization *increases* the sensitivity of the neuron to stimulation.

For plateau potentials (Figure 1C), the central concept is that once activated by a brief depolarization, the non-(slowly) inactivating L-type calcium channels support an inward calcium current great enough to maintain a depolarization that keeps the activation gates open. Thus, plateau potentials could persist indefinitely. The plateau potential can be turned off, however, by a brief hyperpolarization that closes the activation gate, thus eliminating the persistent depolarization. Interestingly, plateau potentials in spinal motoneurons were not recognized for over 20 years of intracellular recording, possibly because anesthesia or spinalization may have allowed potassium channels, which could sufficiently counter the calcium-mediated depolarization, to remain open. Once identified, early hypotheses focused on network rather than cellular explanations (Hultborn et al., 1975); only later was the endogenous origin of motoneuronal plateau potentials appreciated (Hounsgaard et al., 1984).

Functionally, burst-tonic switching, shown in Figure 1B, has been implicated in thalamic neurons switching between sleep-wake cycles (Hirsch et al., 1983; Sherman, 2001; McCormick, 2014), learning and memory (Leresche and Lambert, 2017), nociception (Bourinet et al., 2016), and disease (Nilius and Carbone, 2014). Plateau potentials have been shown to potentially underlie postural stability (Eken and Kiehn, 1989), memory (Malik and Johnston, 2017), and locomotion (Tong and McDearmid, 2012)

PAPER DESCRIPTIONS

The six papers below describe two of calcium channels at three levels – biophysical channel properties, resulting cellular emergent properties, and potential functions.

Nowycky et al. (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity [Biophysical: both T- and L-type].

Llinás and Jahnsen (1982) Electrophysiology of mammalian thalamic neurones in vitro [Emergent: T-type].

Hounsgaard and Kiehn (1985) Ca dependent bistability induced by serotonin in spinal motoneurons [Emergent: L-type].

Hounsgaard et al. (1984) Intrinsic membrane properties causing a bistable behaviour of α-motoneurones [Emergent: L-type].

Hirsch et al. (1983) Sleep-related variations of membrane potential in the lateral geniculate body relay neurons of the cat [Functional: T-type].

Eken and Kiehn (1989) Bistable firing properties of soleus motor units in unrestrained rats [Functional: L-type].

These papers are amazing in two ways. First, several (Llinas and Jahnsen, 1982; Hounsgaard et al., 1984; Nowycky et al., 1985) revolutionized our understanding of ion channel diversity and functional implications. Prior to their publication, the prevailing view in mammals was that neurons were electrically simple, "platonic," neurons that created complex behaviors through complex synaptic inter-connections (Llinas, 1988). Nowycky et al. (1985) underscored the point that neurons were not electrically simple in that they had multiple channels for a single ion, especially regarding calcium (Tsien and Barrett 2005).

Llinas and Jahnsen (1982) demonstrated that these new and diverse channels could create unexpected emergent spiking behavior. Hounsgaard et al. (1984) exemplified the concept, demonstrating that a specific behavior, the long-lasting component of the stretch reflex, arose from cellular rather than network properties as was previously believed (Hultborn et al. 1975). Although the other three papers did not transform our understanding, they completed the functional and mechanistic story of T-type calcium channels.

Second, most of the six papers are amazing for teaching in that they perfectly match the criteria I employ for papers in my neurobiology course – lack of jargon, brevity and reinforcement of concepts covered in neurobiology course.

Channels

Nowycky et al. (1985) is the classic, often cited (n=2316, Google Scholar, March, 2018) and brief *Nature* article that provides the biophysical descriptions of T- and L-type (and N-type) calcium channels – voltage and time dependencies of activation and inactivation – using both whole cell voltage and patch clamp current recording of channel activity. The central findings of their paper were three-fold. First, single neurons contained all three types of channels; previously it was believed that different types of calcium channels would be largely found in different neurons. Second, a new type of calcium channel was identified, the N-type, giving rise to three distinct categories of channels. Finally, they first coined the terms L-, T- and N-type (Tsien and Barrett, (2005) that have persisted to today. Voltage clamp records and current-voltage (I-V) curves, although they take some effort to understand, closely match typical textbook examples. The patch clamp channel records, although typical of records at that time, are slightly “messier” than textbook examples because a large number of channels are present in the patch; nevertheless, they are readily understood. From a pedagogical perspective, probably the only limitation is that their experiments are descriptive rather than hypothesis driven.

Emergent Cellular Properties

The article by Llinas and Jahnsen (1982) on T-type channels, cited 623 times (the longer parent articles, Jahnsen and Llinas, 1984a and 1984b, are cited 1130 and 1041 times), combines both a descriptive and hypothesis driven approach using current clamp intracellular recording of membrane potential. The article is short but addresses several topics. Specifically, in only three pages, the authors demonstrate clearly for the first time membrane potential and spiking bistability, determine the dependence of bistability on resting potential, evaluate pharmacologically the ionic basis and argue that switching between states is all-or-none. The experiments also demonstrate effective use of intracellular current injection to vary resting membrane potential. The paper relies on, but does not explain, orthodromic versus antidromic axonal excitation, which can be skipped without affecting the remaining concepts.

Two short articles by Hounsgaard and colleagues (Hounsgaard et al., 1984; Hounsgaard and Kiehn, 1985) describe plateau potentials. First clearly described in cat motoneurons, plateau potentials have now been described in diverse neurons and preparations (Kiehn and Eken, 1998). However, most of the more complete articles (e.g., Hounsgaard et al., 1988) are pedagogically less suitable to my teaching (too long, difficult or containing educationally less useful figures). In these two papers, the authors use intracellular current clamp recording of membrane potential to describe plateau potentials, their dependence on resting potential, mediation by calcium, and modulation by serotonin actions on potassium channels. Together, the findings provide a complete description of emergent behaviors at both the cellular and channel level. While the figures are clean and easily understood, because of the shortness of the papers students may need some assistance in understanding the experimental design.

Function

The literature on the functional consequences of these two emergent cellular properties of calcium channels is much less extensive and the papers more difficult to understand. Nevertheless, it is important for students to connect cellular behaviors, often observed in in-vitro preparations, with functional consequences for in-vivo preparations and normal behavior.

Although only cited 99 times (longer similar article in humans cited 185 times), the medium length article by Eken and Kiehn (1989) on plateau potentials bridges the gap between observing plateau potentials in the spinal cord slice preparation and intact behaving animals. In a clever and understandable series of hypothesis-driven experiments using electromyography, the authors demonstrate that plateau potentials are present in rat motoneurons and at least in part determine firing rate in intact rats. The clever experimental designs do metaphorically sit on educational tipping point of being both challenging to understand but experimentally elegant.

Burst-tonic switching has been shown to underlie diverse behaviors. However, functional articles, especially those on sleep (e.g., Steriade et al., 1993), contain extensive jargon related to electroencephalogram frequencies and sleep-wake characteristics, making the articles unnecessarily difficult and obscuring the central point of cellular bistability. However, an older article (Hirsch et al., 1983), cited 278 times, provides a clear example of bistability correlated with sleep cycle obtained from intracellular recordings in intact cats, itself an accomplishment for the time. While the medium length article may not warrant being assigned as independent reading, presentation of the key finding in lecture would round out the multilevel treatment of L-type channels.

AUDIENCE

I have used these six papers in my upper-level course, *Neurobiology*, which focuses on the cellular level but delves secondarily into molecules and simple circuits. Although pre-requisites are minimal, most students have taken an introductory neuroscience or physiology course. The level is above Kandel et al. (2012) but below more advanced texts (Johnston & Wu, 1994; Fain, 2014); I use readings from all three in the class. The class is enrolled by 24 mostly seniors and some junior students and includes computer simulation laboratories that reinforce lecture material (relevant wet laboratories are taught in a separate course that I teach, *Experimental Neurobiology)*. In particular, the freely available *Electrophysiology of the Neuron* by Hounsgaard and McCormick (1994) and accompanying simulation software (http://eotn.stanford.edu/ and an updated version at http://www.eotnprogram.org/) illustrate bistability and other emergent properties, though it does not include plateau potentials. The program *Neurosim* (http://www.biosoft.com/w/neurosim.htm), though more complex and costly (Revest, 1995), does provide a model neuron exhibiting plateau potentials. Each student presents, in detail over 30 minutes, a single assigned paper that all students are required to have read. Approximately 25% of each exam covers material from the papers, often requiring a solid understanding on the part of the students. In order to ensure high quality presentations, I often review the article and later the presentation individually with each student (30-60 minutes for each session).

VALUE

Although these papers vary in difficulty, only one (Nowycky et al., 1985) requires an understanding of voltage clamp and patch clamp current recording; most rely on simple intracellular (current clamp) recordings, extracellular and electromyogram (Eken and Kiehn, 1989) recording. The explanations of the experimental designs, especially in the shorter papers, are often limited and require my guidance for full understanding. However, most of students would understand much of the content without assistance.

Few courses would benefit from requiring that students read all six articles, both because of the amount of narrowly targeted material for an undergraduate class and the varying educational benefit of the papers. Specifically, I have found Llinás and Jahnsen, (1982), Hounsgaard et al. (1984), and Hounsgaard and Kiehn (1985) especially valuable. The paper by Nowycky and colleagues (1985) is scientifically important but educationally of less value and requires understanding of voltage/patch clamping. Regarding the two papers on functional consequences, Eken and Kiehn (1989) is hypothesis-driven and requires critical thinking, but is somewhat divergent from typical course content. The content from Hirsch et al. (1983) is probably best covered in lecture. One educational approach is to present the material over both lecture and assigned reading as appropriate to the aims of the course. For example, the material on channels could be presented in lecture (~20 minutes if the students are prepared), emergent properties in required articles, and functional implications in lecture (~10 minutes).

In summary, this group of six articles provide a multilevel understanding of two different calcium channels that could further students' understanding of the causal relationships between ion channels, emergent cellular properties, and the behavior of neural systems.

REFERENCES

Bourinet E, Francois A, Laffray S (2016) T-type calcium channels in neuropathic pain. Pain 157 Suppl 1:S15-22.

Cook-Snyder DR (2017) Using case studies to promote student engagement in primary literature data analysis and evaluation. J Undergrad Neurosci Educ 16:C1-C6.

Eken T, Kiehn O (1989) Bistable firing properties of soleus motor units in unrestrained rats. Acta Physiol Scand 136:383-394.

Fain GL (2014) Molecular and cellular physiology of neurons, 2nd ed. Cambridge, MA: Harvard University Press.

Hammond C (2015) Cellular and molecular neurophysiology, 4th ed. Amsterdam, Netherlands: Academic Press.

Harrington IA, Grisham W, Brasier D, Gallagher SP, Gizerian SS, Gordon RG, Hagenauer MH, Linden ML, Lom B, Olivo R, Sandstrom NJ, Stough S, Vilinsky I, Wiest MC (2015) An instructor's guide to (some of) the most amazing papers in neuroscience. J Undergrad Neurosci Educ 14:R3-R14.

Hirsch JC, Fourment A, Marc ME (1983) Sleep-related variations of membrane potential in the lateral geniculate body relay neurons of the cat. Brain Res 259:308-312.

Hoskins SG (2008) Using a paradigm shift to teach neurobiology and the nature of science-a C.R.E.A.T.E.-based approach. J Undergrad Neurosci Educ 6:A40-A52.

Hounsgaard J, Kiehn O (1985) Ca++ dependent bistability induced by serotonin in spinal motoneurons. Exp Brain Res 57:422-425.

Hounsgaard J, Hultborn H, Jespersen B, Kiehn O (1984) Intrinsic membrane properties causing a bistable behaviour of alpha-motoneurones. Exp Brain Res 55:391-394.

Hounsgaard J, Kiehn O, Mintz I (1988) Response properties of motoneurones in a slice preparation of the turtle spinal cord. J Physiol (Lond) 398:575-589.

Huguenard J, McCormick DA (1994) Electrophysiology of the neuron: an interactive tutorial. Oxford, England: Oxford University Press.

Hultborn H, Wigström H, Wängberg B (1975) Prolonged activation of soleus motoneurones following a conditioning train in soleus Ia afferents — a case for a reverberating loop? Neurosci Lett 1:147-152.

Jahnsen H, Llinas R (1984a) Electrophysiological properties of guinea-pig thalamic neurones: an in vitro study. J Physiol (Lond) 349:205-226.

Jahnsen H, Llinas R (1984b) Ionic basis for the electro-responsiveness and oscillatory properties of guinea-pig thalamic neurones in vitro. J Physiol (Lond) 349:227-247.

Johnston D, Wu SM (1994) Foundations of cellular neurophysiology. Cambridge, MA: MIT press.

Kandel E, Schwartz J, Jessell T, Siegelbaum S, Hudspeth AJ (2012) Principles of neural science, 5th ed. New York, NY: McGraw-Hill.

Kiehn O, Eken T (1998) Functional role of plateau potentials in vertebrate motor neurons. Curr Opin Neurobiol 8:746-752.

Leresche N, Lambert RC (2017) T-type calcium channels in synaptic plasticity. Channels 11:121-139.

Llinás R (1988) The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. Science 242:1654-1664

Llinás R, Jahnsen H (1982) Electrophysiology of mammalian thalamic neurones in vitro. Nature 297:406-408.

Luo L (2016) Principles of neurobiology. New York, NY: Garland Science.

Malik R, Johnston D (2017) Dendritic GIRK channels gate the integration window, plateau potentials, and induction of synaptic plasticity in dorsal but not ventral CA1 neurons. J Neurosci 37:3940-3955.

McCormick DA (2014) Membrane Potential and Action Potential. In: From molecules to networks: an introduction to cellular and molecular neuroscience (Byrne JH, Heidelberger R, Waxman MN JL, eds) pp351-376. San Diego, CA: Academic Press.

Nilius B, Carbone E (2014) Amazing T-type calcium channels: Updating functional properties in health and disease. Pflügers Arch 466:623-626.

Nowycky MC, Fox AP, Tsien RW (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 316:440-443.

Owens DF, Boyce LH, Davis MB, Kriegstein AR (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414-6423.

Revest P (1995) Neurosim for Windows: By WJ Heitler, BIOSOFT, Trends Neruroscì 18:556.

Sherman SM (2001) Tonic and burst firing: dual modes of thalamocortical relay. Trends Neurosci 24:122-126.

Steriade M, McCormick DA, Sejnowski TJ (1993) Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679-685.

Striedter GF (2015) Neurobiology: a functional approach. Oxford, England: Oxford University Press.

Tong H, McDearmid JR (2012) Pacemaker and plateau potentials shape output of a developing locomotor network. Curr Biol 22:2285-2293.

Tsien RW, Barrett, CF (2005) A brief history of calcium channel discovery. In: Voltage-gated calcium channels (Zamponi GW, ed) pp27-47. New York: Kluwer Acdemic/Plenum Publishers.

Received January 14, 2018; revised March 20, 2018; accepted April 19, 2018.

The author thanks students in Neurobiology 445 for their continuing feedback on the use of primary literature in class.

Address correspondence to: Dr. Corey L. Cleland, Department of Biology, MSC 7801, 951 Carrier Dr., Harrisonburg, VA 22807. Email: clelancl@jmu.edu