Proportion of N-Type Calcium Current Activated by Action Potential Stimuli

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King, J. Darwin Jr. and Stephen D. Meriney. Proportion of N-type calcium current activated by action potential stimuli. J Neurophysiol 94: 3762–3770, 2005. First published August 17, 2005; doi:10.1152/jn.01289.2004. N-type calcium currents are important in many neuronal functions, including cellular signaling, regulation of gene expression, and triggering of neurotransmitter release. Often the control of these diverse cellular functions is governed by the spatial and temporal patterns of calcium entry in subcellular compartments. Underlying this issue is the effectiveness of action potentials at triggering calcium channel opening. Chick ciliary ganglion neurons were used as model cells to study the activation of N-type calcium current during action potential depolarization. Several different action potential shapes were recorded, used as voltage command templates, and altered such that control action potential–evoked currents could be compared with those elicited by broadened action potential command forms. Depending on the action potential shape used to activate calcium currents in chick ciliary ganglion neurons, and the temperature at which recordings were performed, varying proportions (II/Imax) of N-type calcium current could be activated. The largest proportion measured occurred using a broad action potential command to activate calcium current at 37°C (100%). The smallest proportion measured occurred using a fast, high-temperature–adjusted frog motoneuron nerve terminal action potential to activate calcium current at room temperature (10%). These data are discussed with respect to the impact on cellular signaling and the regulation of transmitter release.

INTRODUCTION

Different types of voltage-gated calcium channels have been identified with varying properties that are well suited for specific roles in different compartments of the cell (De Waard et al. 1996; Tully and Treistman 2004). In particular, N-type calcium channels have diverse functions that include cellular signaling (see Balkowiec and Katz 2002; Saffell et al. 1992; Sandler and Barbara 1999; Shah and Haylett 2000; Usachev and Thayer 1997), regulation of gene expression (see Brosenitsch and Katz 2001), and the calcium-triggered vesicle fusion that underlies neurotransmitter release (see Stanley 1997). The specific role that N-type calcium channels play in a neuron depends on the subcellular distribution of these ion channels, the macromolecular complex of other proteins with which they are tightly associated, and the patterns of calcium entry through these channels. The specific spatial and temporal patterns of calcium entry through N-type channels are governed by neuronal activity patterns and the response characteristics of N-type calcium channels. Some cellular events may be triggered by the combined flux through many local open channels, whereas others may be triggered by the flux through a single open channel.

N-type calcium currents are activated by action potential stimuli (Artim and Meriney 2000). We have studied the activation of N-type calcium channels by various action potential shapes (at room temperature and at 37°C) using parasympathetic chick ciliary ganglion neurons as model cells. Acute cultures of chick ciliary ganglion neurons provide a homogeneous population of neurons that, when grown on the appropriate substrate, do not elaborate neurites. As such, they provide the opportunity to study calcium current under excellent voltage control. At embryonic stage 40, chick ciliary ganglion neurons express about 74% N-type, about 24% L-type, with only about 2% resistant calcium current (White et al. 1997). These cells have little or no tonic G-protein modulation (DE Artim and SD Meriney, unpublished observations) and allow us to evaluate the proportion of current that can be activated by action potentials. Therefore we used this model system to study N-type calcium current activation during action potential stimuli of various shapes. We find that, depending on the action potential shape used and the temperature, action potentials can activate varying proportions (II/Imax) of N-type calcium current (ranging from 10 to 100%). Implications of these findings for various neuronal functions are considered.

METHODS

Cell culture

Ciliary ganglia were dissected from stage 40 White Leghorn chick embryos after rapid decapitation (Hamburger and Hamilton 1951) in Tyrode solution containing (in mM): 134 NaCl, 3 KCl, 3 CaCl2, 1 MgCl2, 12 glucose, and 20 NaHCO3 (pH 7.3). Dissected ganglia were incubated for 20 min in 0.08% trypsin in Ca2+-and Mg2+-free Tyrode solution at 37°C. Removal and inhibition of the trypsin was accomplished by washing three times in minimal essential medium (MEM) plus 10% heat-inactivated horse serum. Neurons were mechanically

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dissociated from ganglia by trituration through a polished Pasteur pipette. The suspension of cells was centrifuged at 100 × g for 6–8 min and resuspended in MEM plus 10% heat-inactivated horse serum. The cells were plated onto poly-d-lysine–coated 35-mm plastic dishes, incubated at 37°C in 5% CO₂, and used for experimentation after 1–4 h of incubation.

**Electrophysiological recording techniques**

Recordings of calcium current from chick ciliary ganglion neurons were performed using the whole cell patch-clamp technique (Hamill et al. 1981). External bath solution was as follows (in mM): 100 NaCl, 50 TEA-Cl, 10 HEPES, 5 glucose, 5 KCl, 5 CaCl₂, 2 MgCl₂, and 1 μM tetrodotoxin (pH 7.3). The use of 5 mM external calcium (to increase current amplitude), instead of the more physiologic 2 mM calcium, results in a slight shift in calcium current activation (attributed to increased surface charge) to more positive voltages (predicted to be <5 mV; see Smith et al. 1993). Pharmacological agents α-conotoxin (α-CgTX) from the venom of the cone shell Conus geographus (GVIA, 500 nM) and nitrendipine (1 μM in 0.01% DMSO) were added to isolate L- or N-type calcium currents, respectively. A combination of α-CgTX GVIA and nitrendipine blocks about 98% of calcium current in these neurons (White et al. 1997). Borosilicate glass pipettes were pulled on a Flaming/Brown micropipette puller (Sutter Instruments, Model P-97), coated with Sylgard (Dow Corning) and fire polished to a diameter with a measured electrical resistance of 0.5–2 MΩ. Internal solution used in the patch pipettes included (in mM): 120 CsCl, 10 HEPES, 11 EGTA, 5 TEA-Cl, 1 CaCl₂, and 4 MgCl₂. To retard calcium current rundown, the following were added to the internal solution fresh daily (in mM): 4 Mg-ATP, 0.3 Na-GTP, and 0.1 leupeptin. Correction was made for a ~6 mV liquid junction potential before all recordings. Series resistance averaged 6.0 ± 0.4 MΩ (means ± SE; n = 26) and was compensated by 85%. Cell capacitance averaged 12.0 ± 0.9 pF. Data were leak subtracted using a P/4 protocol and collected through the use of the Axopatch 200A amplifier (Axon Instruments) and the pClamp 6.0 software package (Axon Instruments) running on a Pentium processor–based computer.

**Calcium current activation and analysis**

Calcium current was activated by square voltage steps and action potential waveforms. Representative action potential waveforms, to be used as voltage commands, were recorded using the fast current-clamp mode of an Axopatch 200B amplifier from two model preparations: cultured parasympathetic chick ciliary ganglion neuron somata and cultured Xenopus frog motoneuron nerve terminal variability (taken from Pattillo et al. 1999). These action potentials were digitized and the resulting waveforms were used as voltage commands. At room temperature (20–22°C), the ciliary ganglion action potential had a resting potential of −60 mV, a peak amplitude of +25 mV, and a duration at half-amplitude of 2.1 ms. At 37°C, duration at half-amplitude shortened to 0.65 ms. The motor nerve terminal action potential waveform had a resting potential of −60 mV, a peak voltage of +30 mV, and a duration at half-amplitude of 0.85 ms. At 37°C, duration at half-amplitude was shortened to 0.24 ms. To assess the proportion of calcium current activated, the action potential waveforms were altered such that the duration of the peak (most depolarized) voltage was 0.02, 0.06, 0.26, 0.46, 0.66, 0.86, 1.06, 1.26, 1.46, 2.46, 3.46, or 4.46 ms. In the control nerve action potentials, the duration at the peak was 0.06 ms.

For analysis, each current amplitude was normalized to the peak tail current amplitude recorded with the 3.46-ms peak duration action potential. Rundown was adjusted for by using a double-pulse protocol (64-ms interpulse interval), which allowed comparison of the calcium current elicited by the test action potentials of varying duration (first pulse) with the 3.46-ms peak-broadened action potential (second pulse). By comparing the calcium current evoked by these test action potentials to the calcium current that could be evoked by a standard broad-duration action potential, we estimated the proportion of current (I/Iₜₚₘₚₚ) that could be evoked by an action potential waveform. Data were plotted relative to the duration of the action potential at half-amplitude. Even with prolonged strong-voltage depolarizations not all available calcium channels will be open at any one point in time. We broadened action potential shapes until I/Iₜₚₘₚ approached a maximum at or near 1.0 (defined as 100%). For these studies, this is defined as maximal current activation. Maximal current activation does not represent a condition in which all available calcium channels are open.

N-type calcium channels have been shown to have multiple gating modes that have a variable probability of opening (see Delcour et al. 1993), with a predicted mean probability of opening around 0.5 estimated from prolonged exposure to voltages reached by action potentials under physiological conditions (see Colecraft et al. 2000; Lee and Elmslie 1999). Consequently the Iₜₚₘₚ max values cited in this report are an overestimate of the actual probability of channel opening during an action potential. Estimating the actual probability of channel opening under the various conditions reported here is complicated by several factors, including potential differences in the time during an action potential waveform at which maximal probability of opening occurred relative to the time at which peak current was measured, and the amount of deactivation that occurred between these two times. Within each condition studied (action potential shape and temperature), there were no changes in the voltage at which peak current was measured as action potentials were broadened. As such, within each experimental condition, there were no changes in driving force for calcium entry.

To evaluate the kinetics of calcium current activation, currents were activated by 5-ms steps from −80 to 0 mV. Kinetics of calcium current activation were measured by fitting a single exponential to the current trace beginning at the time that current began to flow inward and ending at the time of maximal current (see Jones and Marks 1989).

**RESULTS**

**Calcium current activation during action potential depolarization**

Before isolating N-type calcium current, we evaluated the activation of all calcium currents expressed in ciliary ganglion neurons elicited by a motor nerve terminal action potential waveform. Figure 1A shows the proportion (I/Iₜₚₘₚ max) of ciliary ganglion calcium current activated by a nerve terminal action potential at room temperature (20–22°C). These nerve terminal action potentials activated only 36.5 ± 3.7% (means ± SE) of maximal calcium current in ciliary ganglion neurons (arrow in Fig. 1A). This relatively small proportion of total calcium current activation led us to investigate isolated calcium channel types. We evaluated the activation of L- or N-type currents selectively by exposure to either 1 μM nitrendipine (to isolate N-type) or 500 nM α-CgTX GVIA (to isolate L-type). After isolation of either N-type or L-type current, we estimated the proportion of current activated by the nerve terminal action potential. A significantly greater proportion of L-type calcium current (73.2 ± 12.6%) than N-type (32.8 ± 6.3%) was evoked by the nerve terminal action potential (Fig. 1B; P < 0.001, two-tailed Student’s t-test, independent groups, n = 12 for L-type and n = 18 for N-type). Representative current examples are shown in Fig. 1C.

We also evaluated the activation of calcium currents expressed in ciliary ganglion neurons during an action potential recorded from a ciliary ganglion neuronal soma. Figure 2A
FIG. 1. Effects of action potential broadening on calcium current activation in chick ciliary ganglion neurons. A: plot of calcium current elicited by motor nerve terminal action potentials of varying duration at half-amplitude, normalized to the current elicited by the broadest action potential ($I_{Ca}$/max). Nerve terminal action potential (arrow) activated $36.5 \pm 3.7\%$ of maximal calcium current. B: plot of calcium current as described in A after isolation of L- (open circles) or N-type (filled circles) calcium currents through the use of $\omega$-conotoxin ($\omega$-CgTX) from the venom of the cone shell Conus geographus (GVIA) (500 nM) or nitrendipine (1 $\mu$M), respectively. Nerve terminal action potential activates $73.2 \pm 12.6\%$ of L-type current (arrow, open circle) but only $32.8 \pm 6.3\%$ of N-type current (arrow, filled circle). Inset: nerve terminal action potential voltage command and associated calcium current for both L-type (left) and N-type (right) calcium currents. C: representative current traces and voltage commands for both L- and N-type calcium currents. Starting with the nerve terminal action potential, every other trace has been omitted for clarity. In A and B, data are fit to a single exponential function.

FIG. 2. Effects of action potential broadening on calcium current activation in chick ciliary ganglion neurons using a recorded ciliary ganglion neuron action potential. A: plot of calcium current elicited by action potentials of varying durations, normalized to the current elicited by the broadest action potential ($I_{Ca}$/max). Ciliary ganglion neuron action potential (arrow) activated $82.4 \pm 2.3\%$ of maximal calcium current. B: plot of calcium current as described in A after isolation of N-type calcium currents through the use of nitrendipine (1 $\mu$M). Ciliary ganglion neuron action potential activated $76.9 \pm 3.3\%$ of N-type current (arrow). Inset: ciliary ganglion neuron action potential voltage command and associated calcium current for N-type calcium currents. In A and B, data are fit to a single exponential function. C: representative current traces and voltage commands for N-type calcium currents. Starting with the ciliary ganglion neuron action potential, every other trace has been omitted for clarity.
shows the proportion of total current activated by the ciliary ganglion action potential at room temperature. These action potentials are much broader than those recorded from the motoneuron nerve terminals and, as a result, are more effective at activating calcium current, with 82.4 ± 2.3% activated using the native ciliary ganglion action potential waveform. In this case, eliminating the L-type channel contribution (using 1 μM nitrendipine) and examining solely N-type current had little effect on the proportion of current activated by a native ciliary ganglion action potential (76.9 ± 3.3%; see Fig. 2B). Figure 2C shows representative currents activated by the ciliary ganglion action potential as it is broadened. Because these native ciliary ganglion action potential waveforms are so effective at activating N-type current, and N-type channels are the focus of our study, we did not examine the activation of isolated L-type channels using this action potential waveform.

**Gating characteristics of N- versus L-type current**

Next we focused on potential differences between N- and L-type channel gating that could underlie the observed differences in current activation during a nerve terminal action potential (Fig. 1B). Representative traces (see Fig. 3B) demonstrate the faster time course of activation for L-type compared with N-type current during a step depolarization that activates maximal calcium current (from −80 to 0 mV). The kinetics of activation (τ) for L-type and N-type currents averaged 0.65 ± 0.07 and 1.85 ± 0.11 ms, respectively, and these were significantly different (means ± SE; P < 0.001, one-way ANOVA, n = 10 for L-type and n = 7 for N-type; see Fig. 3A), consistent with previously reported work (see Kasai and Neher 1992). Differences in the kinetics of activation for N- and L-type channels may explain the proportion of current activated by a motoneuron nerve terminal action potential (Fig. 1B) because faster kinetics of activation of the L-type channels would predict a greater activation of available calcium current with such a brief action potential stimulus.

**Effects of temperature on activation of N-type calcium current during action potential depolarization**

Although we found that a nerve terminal action potential is not very effective at gating N-type calcium current at room temperature, we questioned whether the same would be true at mammalian physiological temperatures of 36–38°C. Sabatini and Regehr (1996) used whole cell recordings and calcium imaging in the rat cerebellum to document the effects of temperature on the timing of calcium influx during an action potential. At 37°C they showed that the delay between the peak of the action potential and calcium influx was significantly reduced as a result of rapid kinetics of channel gating. First, we measured the kinetics of activation of N-type channels at 37°C. Consistent with previous observations (see Van Lunteren et al. 1993), we found that N-type channel gating was significantly faster at 37°C such that current activated with a τ of 0.31 ± 0.03 ms (means ± SE; P < 0.001, one-way ANOVA, n = 9; Fig. 3). This faster activation at higher temperature would be predicted to yield a greater proportion of current activation during an action potential.

Because the *Xenopus* preparation does not tolerate patch-clamp recording at 37°C (Meriney, unpublished observations), we could not record a native action potential from motoneuron nerve terminals at this temperature. Instead, we altered the room-temperature–recorded nerve terminal action potential shape as it would be predicted to be changed at this higher temperature. We scaled this nerve terminal action potential with respect to the duration at the base, duration at half-amplitude, rise time, and repolarization time in a manner that was proportional to that shown by Sabatini and Regehr (1996). This scaled action potential shape is consistent with the time course of action potentials recorded from other preparations at this temperature (Borst and Sakmann 1998; Sabatini and Regehr 1996). This fast (rescaled) motor nerve terminal action potential was then used to investigate calcium current activa-
tion at 37°C. Using this native action potential, N-type activation was not significantly changed (76.9 ± 3.3% at room temperature vs. 71.8 ± 8.8% at 37°C; \( P = 0.55 \), Student’s \( t \)-test; see Fig. 5A). Apparently, temperature-dependent changes in action potential shape almost exactly offset those in calcium current kinetics for this relatively broad native action potential waveform. Representative currents are shown in Fig. 5B. The most obvious difference in the currents evoked by the ciliary ganglion action potentials when compared at room temperature and at 37°C was the steepness of the relationship between the proportion of current activated and the broadening of the action potential. At 37°C maximal activation was achieved with only slight broadening (filled circles in Fig. 5A) compared with that at room temperature (open circles in Fig. 5A). This temperature-dependent increase in steepness of the relationship was observed using both action potential waveforms (motor nerve terminal and ciliary ganglion; compare Fig. 4B and Fig. 5A).

To more thoroughly investigate the effects of temperature and action potential waveform shape, we mixed conditions to

FIG. 4. Effects of motor nerve terminal action potential broadening at 37°C on calcium current activation in chick ciliary ganglion neurons. A: nerve terminal action potential waveforms used at room temperature (dotted line) and 37°C (solid line) are shown for comparison. B: plot of normalized N-type calcium current activation (as shown in Fig. 1) at 37°C (filled circles) in comparison with the room-temperature data from Fig. 1B (open circles). Nerve terminal action potentials (arrows) activated 60.3 ± 5.7% of maximal current at 37°C compared with 32.8 ± 6.3% at room temperature. Data are fit to a single exponential function. Inset: nerve terminal action potential voltage command and associated calcium current recorded at 37°C. C: representative current traces and voltage commands for the data collected at 37°C. Starting with the nerve terminal action potential, every other trace has been omitted for clarity.

FIG. 5. Effects of action potential broadening on calcium current activation recorded at 37°C in chick ciliary ganglion neurons using a recorded ciliary ganglion neuron action potential as a voltage command. A: plot of normalized N-type calcium current activation recorded at 37°C (filled circles) in comparison with the room-temperature data from Fig. 2B (open circles). Ciliary ganglion neuron action potentials (arrows) activated 71.8 ± 8.8% of maximal current at 37°C compared with 76.9 ± 3.3% at room temperature. Data are fit to a single exponential function. Inset: ciliary ganglion neuron action potential voltage command and associated calcium current recorded at 37°C. C: representative current traces and voltage commands for the data collected at 37°C. Starting with the ciliary ganglion action potential, every other trace has been omitted for clarity.
examine effects on N-type calcium current activation. Figure 6A demonstrates that the native ciliary ganglion action potential shape recorded at 37°C (0.65-ms duration at half-amplitude) activated about 25% ($I_{\text{max}}/I_{\text{dip}} = 0.25$) of available N-type current recorded at room temperature (where $t_{\text{act}} = 1.85$ ms). However, when the ciliary ganglion action potential recorded at room temperature was used at 37°C (Fig. 6B), this broadest action potential shape (2.1-ms duration at half-amplitude) activates maximal N-type calcium current (100%; $I_{\text{max}}/I_{\text{dip}} = 1.0$). At this temperature, N-type calcium current activates very quickly ($t_{\text{act}} = 0.31$ ms). When the motor nerve terminal action potential is adjusted to reflect the fast kinetics expected at high temperature (0.24-ms duration at half-amplitude), a very small proportion of N-type current is recorded at room temperature (10%; see Fig. 6C). As expected, using the motor nerve terminal action potential recorded at room temperature (0.85-ms duration at half-amplitude) to record calcium current at 37°C, this native action potential shape activated a very large proportion (about 90–95%) of N-type calcium current (see Fig. 6D).

**DISCUSSION**

We have shown that action potentials are not always effective at activating calcium current compared with maximal activation evoked by a broadened waveform. As would be expected, broad action potentials are more effective at activating calcium current than brief action potentials. Furthermore, because calcium current activation kinetics can vary, this aspect of channel gating is also critical in the determination of the proportion of current that will be activated by an action potential. The proportion of calcium current activated by an action potential can be important in several respects.

**FIG. 6.** Evaluation of calcium current activation using various combinations of action potential waveforms and recording conditions. A: plot of normalized N-type calcium current activation when the ciliary ganglion action potential that was recorded at 37°C (see Fig. 5) was used to activate calcium current recorded at room temperature. This control action potential activated 27.8 ± 4.1% of maximal calcium current. B: plot of normalized N-type calcium current using the ciliary ganglion action potential recorded at room temperature (see Fig. 2) to activate calcium currents at 37°C. This control action potential activated 107.4 ± 2.4% of maximal current (data are fit with a linear regression). C: plot of normalized N-type calcium current activation when the motor nerve terminal action potential that was scaled as it is predicted to change at 37°C (see Fig. 4) was used to activate calcium current recorded at room temperature. This control action potential shape activated 11.7 ± 2.0% of maximal current. D: plot of normalized N-type calcium current using the motor nerve terminal action potential recorded at room temperature (see Fig. 1) to activate calcium currents at 37°C. This control action potential shape activated 94.0 ± 1.9% of maximal current. For plots A, C, and D, data are fit to a single exponential function. In all plots the control action potential duration (arrow) is altered at the peak as described above.
Cellular signaling

Calcium is the intracellular signal for many cellular events. As these events have become more fully understood, it has become clear that the detailed characteristics of intracellular calcium elevations are very important. The specific transmembrane source and subcellular localization, as well as the pattern, time course, and magnitude of calcium entry, can be critical in determining the cellular events that are triggered (Bałkowiec and Katz 2000, 2002; Fields et al. 1997). Furthermore, at subcellular sites of calcium entry there may be mechanisms to amplify the calcium signal through calcium-induced calcium release from intracellular stores (Tully and Treistman 2004). These issues have often been studied in neurons with respect to the regulation of gene expression and the plasticity of synaptic transmission. In terms of calcium entry through voltage-gated calcium channels, different calcium channel types appear to be coupled to the regulation of different genes (Brosenitsch and Katz 2001; Finkbeiner and Greenberg 1998). Previous work using a high potassium stimulation paradigm may have favored study of the role of L-type, and overlooked the contribution of N-type calcium channels, because the N-type may inactivate during prolonged high potassium stimulation (Nowycky et al. 1985). Using physiological patterns of stimulation in primary sensory neurons, Brosenitsch and Katz (2001) have shown that the calcium flux through N-type channels appears to trigger the expression of several immediate early genes and tyrosine hydroxylase. This is likely attributable to a subcellular co-localization of particular channel types with specific signaling cascades. The reliability of this local signaling will depend on the probability that channels open during action potential stimuli.

In terms of synaptic plasticity, long-term changes in synaptic strength are known to be triggered by calcium entry (Artola and Singer 1993; Lisman 1989; Lisman and Goldring 1988; Miller and Kennedy 1986). Recently, it has been shown that the specific temporal pattern of calcium entry can determine the type of synaptic plasticity that results (Ismailov et al. 2004; Yang et al. 1999; Zucker 1999). The patterns of calcium entry during activity depend critically on the probability that voltage-gated calcium channels open during these physiological stimuli. With a low probability of specific channels opening during an action potential, calcium entry is predicted to be sporadic at particular entry sites, be restricted to small subcellular compartments, and generally not accumulate during low-frequency action potential activity or during very short bursts of higher-frequency activity. On the other hand, if channels have a high probability of opening during action potential activity, the local "flood" of calcium could create a larger intracellular cloud that temporarily overcomes local buffering, and this signal would be predicted to be consistent after each action potential stimulus. In this scenario, calcium signals would be more reliably generated to accurately report action potential activity in the cell.

Voltage-gated calcium channels are common targets for G-protein modulation. The proportion of calcium channels that are normally activated by an action potential can have important implications related to the potential for neuromodulation. In cases where there is a very high probability for calcium channel opening during an action potential, there is not much room for modulation that would increase channel open probability, but a large dynamic range over which inhibitory modulation could act. If calcium channels have a very low probability of responding during action potential stimuli, the opposite would be true and there would be much more room for modulation that would serve to increase calcium entry. Furthermore, neuromodulation often targets potassium channels that indirectly alter calcium entry by changing action potential shape. Even subtle changes in action potential shape can have significant effects on calcium entry (Patillo et al. 1999). Furthermore, based on the steepness of the relationship between action potential duration and $I_{\text{Na}}(\text{max})$ observed at $37^\circ$C (filled symbols of Fig. 4B and Fig. 5A), we predict that slight differences in action potential shape will have more dramatic effects on action potential–evoked activation of N-type calcium current at $37^\circ$C than that at room temperature.

Regulation of transmitter release

We have shown that a nerve terminal action potential recorded from a cultured frog motor synapse was not very effective at activating N-type calcium current at room temperature. These data lead us to hypothesize that very few of the N-type calcium channels at a frog motor nerve terminal will open with each action potential invasion. Recently, Wachman et al. (2004) used a fast calcium imaging approach at the adult frog neuromuscular junction to provide evidence that very few of the calcium channels at this synapse open during an action potential. Furthermore, they demonstrated that the spatial distribution of calcium entry at release sites was altered by calcium channel blockers in a manner consistent with very few—perhaps only one—calcium channel opening underlying each action potential—evoked calcium entry site. Interestingly, when a binomial analysis was applied to imaged calcium entry domains in this neuromuscular preparation, the predicted mean probability of calcium channel opening during a single action potential was about 0.12 (Luo et al. 2005). This is remarkably consistent with the proportion of N-type calcium current ($I_{\text{Na}}(\text{max}) = 0.25$) recorded from Xenopus presynaptic varicosities in vitro when activated by the motor nerve terminal action potential (Poage and Meriney 2002) after multiplication by the predicted mean opening probability of N-type calcium channels previously reported in the literature during long, strong depolarizations (about 0.5; Colcarafa et al. 2000; Lee and Elmslie 1999). Furthermore, our data from ciliary ganglion cell soma reported here are also consistent with these observations.

This low open probability further suggests that when vesicles are triggered to fuse by the calcium flux through N-type calcium channels, each vesicle fusion event might be triggered by the opening of a single N-type calcium channel. This hypothesis was first proposed by Yoshikami et al. (1989), who measured dose–response relationships between various calcium channel blockers and transmitter release at the adult frog neuromuscular junction, and performed computational analyses that led them to hypothesize that the activity of a single N-type calcium channel mediates vesicle fusion at an individual transmitter release site. Evidence in favor of this idea has previously been reported at other synapses controlled by calcium influx through N-type channels. Stanley (1993) used a combination of patch-clamp recordings of single N-type calcium channels and a chemiluminescent method to detect transmitter release in the calyciform presynaptic terminal of the
chick ciliary ganglion to show that single N-type calcium channel openings can trigger vesicle fusion. Further support for this idea was provided in a computational study (Bertram et al. 1996). These studies suggest that, in these preparations at room temperature, the flux of calcium through a single N-type calcium channel opening normally triggers each transmitter release event.

The results at the frog neuromuscular junction and chick ciliary ganglion are in contrast with what has been reported at the rat calyx of Held and hippocampal mossy fiber synapses where action potentials appear to be very effective (70–85%) at activating the predominately P/Q-type channels at these CNS synapses at room temperature (Bischofberger et al. 2002; Borst and Sakmann 1998). These data have led to the conclusion that a majority of presynaptic calcium channels open with each action potential, and the flux through many open channels sums to create the calcium trigger for vesicle fusion (Bischofberger et al. 2002; Borst and Sakmann 1998). At the rat calyx of Held, direct patch-clamp studies led to the conclusion that more than 60 calcium channel openings provide the calcium flux to trigger each vesicle fusion event (Borst and Sakmann 1996).

If different channel types show differences in their activation kinetics, this may explain some of the observed differences among preparations. Consistent with this possibility, previous work comparing P/Q-type channels with N- and L-type channels provides data to suggest that P/Q-type channels may activate with faster kinetics than N-type channels (Bischofberger et al. 2002; Mintz et al. 1992; Sather et al. 1993). However, measured differences between channel types in particular preparations may not be universally applicable because activation kinetics may vary significantly between splice variants of the same channel type and depend on specific interactions with auxiliary subunits (see Lin et al. 1997, 1999, 2004).

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