Differential Contributions of Shaker and Shab K⁺ Currents to Neuronal Firing Patterns in *Drosophila*

I-Feng Peng and Chun-Fang Wu

Department of Biological Sciences, University of Iowa, Iowa City, Iowa

Submitted 22 September 2006; accepted in final form 24 October 2006

Peng I-F, Wu C-F. Differential contributions of Shaker and Shab K⁺ currents to neuronal firing patterns in *Drosophila*. J Neurophysiol 97: 780–794, 2007. First published November 1, 2006; doi:10.1152/jn.01012.2006. Different K⁺ currents participate in generating neuronal firing patterns. The *Drosophila* embryonic “giant” neuron culture system has facilitated current- and voltage-clamp recordings to correlate distinct excitability patterns with the underlying K⁺ currents and to delineate the mutational effects of identified K⁺ channels. Mutations of Sh and Shab K⁺ channels removed part of inactivating *Iₖ* and sustained *Iₖ*, respectively, and the remaining *Iₖ* and *Iₖ* revealed the properties of their counterparts, e.g., Shal and Sh channels. Neuronal subsets displaying the delayed, tonic, adaptive, and damping spike patterns were characterized by different profiles of K⁺ current voltage dependence and kinetics and by differential mutational effects. Shab channels regulated membrane repolarization and repetitive firing over hundreds of milliseconds, and Shab neurons showed a gradual decline in repolarization during current injection and their spike activities became limited to high-frequency, damping firing. In contrast, Sh channels acted on events within tens of milliseconds, and Sh mutations broadened spikes and reduced firing rates without eliminating any categories of firing patterns. However, removing both Sh and Shal *Iₖ* by 4-aminopyridine converted the delayed to damping firing pattern, demonstrating their actions in regulating spike initiation. Specific blockade of Shab *Iₖ* by quinidine mimicked the Shab phenotypes and converted tonic firing to a damping pattern. These conversions suggest a hierarchy of complexity in K⁺ current interactions underlying different firing patterns. Different lineage-defined neuronal subsets, identifiable by employing the GAL4-UAS system, displayed different profiles of spike properties and K⁺ current compositions, providing opportunities for mutational analysis in functionally specialized neurons.

Correspondence: C-F. Wu, Dept. of Biological Sciences, University of Iowa, Iowa City, IA 52242 (E-mail: chun-fang-wu@uiowa.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Shal channels. We also took advantage of the GAL4-UAS system (Brand and Perrimon 1993) to demonstrate different profiles of $K^+$ current kinetics and firing properties in cell-lineage defined neuronal subsets and their specific alterations by Sh and Shab mutations. Preliminary accounts of this work have appeared in abstract form (Peng and Wu 2004).

**METHODS**

**Drosophila stocks**

The wild-type (WT) strain was Canton S. Two Shab mutant alleles, $Shab^1$ (caterpillar, a hypomorph) (Hegele et al. 1999) and $Shab^3$ (a null) (Singh and Singh 1999), were used in this study. Two Sh null alleles, $Sh^{55}$ and $Sh^{133}$ (Haugland and Wu 1990; Tanouye and Ferrus 1985; Wu and Haugland 1985; Zhao et al. 1995), were from the original collection of Dr. S. Benzer of Cal Tech. For upstream activating sequence-green fluorescent protein (UAS-GFP) expression experiments, the GH146-Gal4 UAS-CD8-GFP/CyO line (Wong et al. 2002) was a gift from Dr. J. Wang (University of California, San Diego), and two separate 201Y-Gal4 and UAS-CD8-GFP lines from Dr. Y. Zhong (Cold Spring Harbor Lab) were used to produce recombined homozygous lines. All fly stocks were maintained at room temperature with standard fly medium.

**Single embryo “giant” neuron culture**

The “giant” neuron culture system derived from dissociated gastrula neuroblasts has been previously described (Saito and Wu 1991; Zhao and Wu 2001; Yao and Wu 2000; Zhao and Wu 1997). Briefly, the interior content of stage 7–8 embryos was sucked out with a glass micropipette and then dispersed in culture medium on an uncoated coverslip. The culture medium contains 80% *Drosophila* Schneider medium and 20% fetal bovine serum (GIBCO, Invitrogen, Carlsbad, CA) with the addition of 200 ng/ml insulin, 50 μg/ml streptomycin, and 50 μg/ml penicillin. Drugs without specific labeling were from Sigma (St. Louis, MO). To generate multi-nucleated “giant” neurons from neuroblasts, cytochalasin B (CCB, 2 μg/ml) was added on the first day to arrest cytokinesis (Wu et al. 1990). CCB was removed by replacing the culture medium with CCB-free medium 24 h after plating. Actin filaments were restored within one day, as indicated by pattern stained by phalloidin in filopodia and lamellipodia (Berke et al. 2006). In Gal4-UAS GFP expression experiments, fluorescence was first detected in subsets of neurons between 12–24 h after plating. The intensity peaked after ~2–3 days.

**Electrophysiology**

Whole cell patch-clamp recording of cultured “giant” neurons has been previously described (Saito and Wu 1991; Yao and Wu 1999, 2001; Zhao and Wu 1997). Recording electrodes were prepared from 75-μl glass micropipettes (VWR Scientific, Chicago, IL). The tip opening of the electrodes had a diameter of ~1 μm and an input resistance of 3–5 MΩ in bath solution. The bath solution contained (in mM) 128 NaCl, 2 KCl, 4 MgCl₂, 1.8 CaCl₂, and 35.5 sucrose, buffered at pH 7.1 with 5 mM HEPES. The solution for filling patch pipettes contained (in mM) 144 KCl, 0.5 CaCl₂, and 5 EGTA, buffered at pH 7.1 with 10 mM HEPES. Recordings were performed on the soma (diameters ranging from 13 to 18 μm) from 2- to 4-day-old cultures by using a patch-clamp amplifier (Axopatch 1B, Axon Instruments, Foster City, CA). The seal resistance was usually >5 GΩ and junction potentials were nulled before establishing the whole cell configuration. A PC computer, an A/D-D/A converter and pClamp software (version 5.5.1, Axon Instruments) were used to generate the current- and voltage-clamp commands and for data acquisition. During current-clamp experiments, membrane potential was maintained at or near the resting level (around −60 mV) by applying polarizing current. Experiments were carried out at room temperature.

Several biophysical parameters in Table 2 were determined as previously described (Yao and Wu 1999). Briefly, currents were activated by step depolarization from a holding potential of −80 mV at 20-mV increments up to +60 mV. Boltzman fit was performed (reversal potential = −80 mV) to determine the half-activation voltage ($V_{1/2}$). The recovery from inactivation protocol involved 500-ms twin pulses to +20 mV with varying interpulse intervals. Steady-state (st-st) inactivation was determined with a 500-ms pre-conditioning pulse (to −20 or 0 mV) followed by a +60-mV test pulse 5 ms afterward.

**Functional categories of regenerative potentials, spike patterns and voltage-activated $K^+$ currents**

Cultured “giant” neurons could generate nonregenerative and regenerative membrane potentials on current injection. The regenerative responses included graded oscillations with single or multiple peaks as well as all-or-none action potentials with clear thresholds (Fig. 1) (Saito and Wu 1991; Zhao and Wu 1997). With a fixed duration (400 ms) and current injection intensity (2–3 times the threshold level), all-or-none action potential firing patterns could be further divided into five categories (modified from Zhao and Wu 1997): single spike: only a single action potential was generated at different levels above threshold; delayed: the onset of spike activity showed a significant delay (>100 ms); tonic: action potential in the spike train occurred at
regular intervals with onset latency <100 ms and $f_{\text{peak}}/f_{\text{rest}} > 0.7$, where $f$ represents instantaneous spike frequency in Hz; adaptive: spike frequency decreased over time with latency <100 ms and $f_{\text{peak}}/f_{\text{rest}} < 0.7$; and damping: the spike amplitude diminished over time.

Voltage-activated $K^+$ currents were isolated in saline containing TTX (10 nM) and Cd$^{2+}$ (0.2 mM) to eliminate inward Na$^+$ and Ca$^{2+}$ currents as well as outward Ca$^{2+}$-activated $K^+$ currents (Saito and Wu 1991; Yao and Wu 1999; Zhao and Wu; 1997). Voltage-clamp measurements were performed on the soma with step depolarization from a holding potential of −80 mV, at 20-mV increments up to +60 mV. Under these conditions, four types of total outward current responses (Types 1–4, T1–4, Fig. 4A) were distinguished based on their kinetics and ratios of $I_d/I_p$, where $I_p$ represented the peak current and $I_d$ the sustained component measured at the end of pulses. T1 and T2 currents were characterized by a larger inactivating component ($I_d/I_p < 0.5$), with a single ($\tau_1$)- and double ($\tau_1$ and $\tau_2$)-exponential decay kinetics, respectively. In contrast, T3 and T4 neurons exhibited a larger sustained component ($I_d/I_p > 0.5$) with a double ($\tau_1$ and $\tau_2$)- or single ($\tau_2$)-exponential decay kinetics, respectively. Currents were normalized to membrane capacitance for current density estimates in pA/pF.

**RESULTS**

**Full range of excitability patterns in Drosophila neurons**

To gain a general overview of the excitability patterns in Drosophila neurons, we performed current-clamp experiments on a large sample of individual, isolated neurons in single-embryo cultures. We observed both nonspiking membrane potential changes and spike activities, resembling a variety of excitability patterns previously described in different species, including Drosophila (Choi et al. 2004; Kuppers-Munther et al. 2004; Mee et al. 2004; O’Dowd 1995; Renden and Brodie 2003; Schmidt et al. 2000) and other invertebrates and vertebrates (Harris-Warrick and Marder 1991; Shepherd 2004).

During 400-ms step current injection, nonspiking neurons were unable to generate all-or-none action potentials (57% nonspiking vs. 43% spiking neurons) but still displayed different waveforms, including nonregenerative potentials and graded regenerative potentials with single- or multiple-peak responses (Fig. 1A). Similar nonspiking neurons have been described in insect nervous systems for a specific mode of signal processing (Burrows 1996). Among spiking neurons, the all-or-none repetitive firing patterns can be further divided into delayed, tonic, adapting, damping, or single-spike categories, based on spike onset time, and adaptation in spike frequency and amplitude (see Fig. 1 and METHODS for criteria). We found that each neuron retained the characteristics of a particular firing pattern in response to varying stimulus intensities, allowing a consistent, functional classification of neuronal subpopulations (Fig. 1, C and D). The proportions of neurons in each subcategory of nonspiking and spiking neurons were in general agreement with previously published results in the same preparation (cf. Saito and Wu 1991; Zhao and Wu 1997; with the additional categories of single-spike and damping firing patterns). Among the mono-, bi-, and multipolar cells examined in this culture system (cf. Saito and Wu 1991), we found no strict correlations between membrane excitability patterns with these morphological types (data not shown).

**Distinct alterations of membrane excitability in Sh and Shab mutant neurons**

The elimination of specific K$^+$ currents by Sh and Shab mutations led to contrasting consequences in the regulation of neuronal excitability. Effects of Sh mutations have been documented in a number of in vivo preparations (Salkoff and Wyman 1981; Tanouye et al. 1981; Ueda and Wu 2006; Wu and Haugland 1985). In the “giant” neuron culture system, removal of Sh channels by a null mutation ($Sh^M$) resulted in quantifiable modifications in neuronal excitability patterns. Sh cultures contained a lower proportion of spiking neurons on current injection compared with WT cultures (Fig. 2C, WT vs. Sh, 43 vs. 30%, $P < 0.05$, $\chi^2$ test), but the proportions of the four multiple spike firing patterns remained unaltered (Fig. 2, A and C). It was also evident that $Sh^M$ caused distinct modifications of action potential properties, including broadened action potentials during delayed and tonic firing and a slower firing rate in neurons of the damping category (Fig. 3 and Table 1).
Although Sh $I_{\text{A}}$ plays a role in shaping the delayed firing pattern, neurons in Sh cultures showed only a slight, statistically insignificant shortening of spike onset time [means ± SE (n): WT, 193 ± 17 ms (11); Sh, 157 ± 22 ms (6)]. However, Sh neurons with delayed firing patterns had a higher voltage threshold for action potential initiation (Table 1).

In contrast, elimination of Shab current distorted the neuronal excitability patterns in a qualitative manner. There was a significant increase in the proportion of nonspiking cells, and the categories defining spike activities in WT and Sh could no longer describe the major spike patterns in Shab cultures (Fig. 2C). Most spiking neurons in Shab cultures displayed damping firing patterns (80%, Fig. 2C). In addition, we found a progressive damping of membrane regenerative activity during prolonged current injection in Shab cultures, which appeared as an abnormal decline in the membrane repolarization level, coupled with dwindling spike amplitude during repetitive firing (Fig. 2B). A declining membrane repolarization level between the initial and final regenerative events was frequently >20 mV among both nonspiking and spiking Shab neurons (top two traces in Fig. 2B; C, ■). These observations suggest that Shab currents play a major role in maintaining neuronal polarization during repetitive firing or during nonregenerative activities over a time course of hundreds of milliseconds. It is worth noting that cells displaying such a repolarization decline were also found in a smaller population of WT and Sh neurons (Fig. 2C, ■), suggesting that low levels of Shab expression also occur in a small subset of normal neurons. However, repetitive spikes in Shab neurons were characterized by an abnormally high firing rate and dwindling amplitude, which were not observed in either WT or Sh cultures (Fig. 3C and Table 1).

Voltage-activated outward $K^+$ currents

The variety of firing patterns found in individual WT neurons and the extent of their disruptions in Sh and Shab cultures suggest the possibility of differential expression of $K^+$ channels. Cultured “giant” neurons facilitated space-clamp control for analyzing the components of the total voltage-activated $K^+$ currents that can be isolated in the presence of TTX and Cd$^{2+}$ (Saito and Wu 1991; Yao and Wu 1999, 2001). A comprehen-

Table 1. Membrane potentials of $K^+$ channel mutants

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>All Spiking</th>
<th>Single Spike</th>
<th>Delayed</th>
<th>Tonic</th>
<th>Adaptive</th>
<th>Damping</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>$-53.7 ± 1.1$</td>
<td>$-55.3 ± 1.2$</td>
<td>$-47.6 ± 2.3$</td>
<td>$-58.6 ± 2.3$</td>
<td>$-57.1 ± 2.6$</td>
<td>$-53.0 ± 8.0$</td>
<td>$-52.4 ± 8.1$</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>NA</td>
<td>$-25.2 ± 1.3$</td>
<td>$-21.2 ± 4.4$</td>
<td>$-23.0 ± 1.4$</td>
<td>$-24.9 ± 1.3$</td>
<td>$-28.4 ± 1.5$</td>
<td>$-26.0 ± 1.0$</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>NA</td>
<td>$7.0 ± 0.4$</td>
<td>$8.8 ± 1.2$</td>
<td>$7.2 ± 1.2$</td>
<td>$6.8 ± 0.6$</td>
<td>$6.0 ± 0.5$</td>
<td>$6.9 ± 0.5$</td>
</tr>
<tr>
<td>AP frequency, Hz</td>
<td>106</td>
<td>$24.5 ± 0.8$</td>
<td>NA</td>
<td>$25.8 ± 2.3$</td>
<td>$22.8 ± 1.3$</td>
<td>$26.8 ± 1.6$</td>
<td>$25.8 ± 1.3$</td>
</tr>
<tr>
<td>n</td>
<td>150</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>15</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Sh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>$-52.3 ± 1.9$</td>
<td>$-56.5 ± 3.0$</td>
<td>$-61.0 ± 2.5$</td>
<td>$-53.8 ± 6.4$</td>
<td>$-53.5 ± 3.2$</td>
<td>$-56.5 ± 3.2$</td>
<td>$-67.3 ± 7.8$</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>NA</td>
<td>$-22.0 ± 1.6$</td>
<td>$-24.5 ± 1.0$</td>
<td>$-17.4 ± 1.9^*$</td>
<td>$-26.5 ± 1.7$</td>
<td>$-27.5 ± 1.4$</td>
<td>$-22.2 ± 1.0$</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>NA</td>
<td>$9.0 ± 0.8^*$</td>
<td>$7.5 ± 0.5$</td>
<td>$11.3 ± 1.1^*$</td>
<td>$9.4 ± 0.6^*$</td>
<td>$7.5 ± 0.5$</td>
<td>$6.6 ± 0.9$</td>
</tr>
<tr>
<td>AP frequency, Hz</td>
<td>44</td>
<td>$21.6 ± 0.6^*$</td>
<td>NA</td>
<td>$22.9 ± 2.7$</td>
<td>$22.0 ± 2.3$</td>
<td>$28.2 ± 4.5$</td>
<td>$19.3 ± 1.2^*$</td>
</tr>
<tr>
<td>n</td>
<td>150</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>15</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Shab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>$-52.1 ± 2.1$</td>
<td>$-46.2 ± 2.9^*$</td>
<td>—</td>
<td>$-52.0 ± 4.0$</td>
<td>—</td>
<td>$-63.0$</td>
<td>$-17.3 ± 5.0$</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>NA</td>
<td>$-22.4 ± 1.1$</td>
<td>—</td>
<td>$-23.4 ± 1.6$</td>
<td>—</td>
<td>$-19.6$</td>
<td>$-23.4 ± 1.9$</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>NA</td>
<td>$5.4 ± 0.5$</td>
<td>—</td>
<td>$3.5 ± 0.6$</td>
<td>—</td>
<td>8</td>
<td>$5.5 ± 0.4$</td>
</tr>
<tr>
<td>AP frequency, Hz</td>
<td>42</td>
<td>$42.2 ± 1.7^{**}$</td>
<td>—</td>
<td>$53.5 ± 0.9$</td>
<td>—</td>
<td>43</td>
<td>$41.9 ± 4.6^{**}$</td>
</tr>
<tr>
<td>n</td>
<td>150</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>15</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Electrophysiological parameters are presented as means ± SE (mean ± range if n < 3). Action potential (AP) duration was measured for the initial spike at the inflection point. AP frequency is determined from the second half of the repetitive firing during current injection (see METHODS). NA, not applicable; —, not encountered; *P < 0.05, **P < 0.01, one-way ANOVA against wild type (WT) for the cases of all spiking and damping categories and t-test for the rest, where Shab samples are limited.
sive voltage-clamp study could reveal the K⁺ current composition and kinetic profile in each neuronal subpopulation.

WT neurons displayed voltage-activated K⁺ currents with different degrees of inactivation in response to step depolarization (950 ms). The total K⁺ currents expressed by individual neurons could be classified into four types (T1-4, Fig. 4A) according to inactivation kinetics and the ratio of sustained/peak currents (Iₛ/Iₚ, see METHODS or Fig. 4 for criteria). In WT cultures, T3 neurons represented the largest population (~40.3%), whereas T1 neurons represented the smallest (11.5%, Fig. 4B). In terms of current density (Fig. 4C), a larger Iₚ and a smaller Iₛ were found in both T1 and T2 neurons, yielding a lower Iₛ/Iₚ ratio. For current decay kinetics (Fig. 4D), the sole decay component in T1 neurons (τ₁, 100–300 ms) was much slower than the fast component in T2 and T3 (τ₁, <100 ms) but faster than their slow component τ₂. By contrast, T4 neurons were characterized with a single slow decay component τ₂ (>400 ms).

Distinct kinetics of voltage-activated K⁺ currents in Sh and Shab cultures

The properties of Sh- and Shab-mediated K⁺ currents could be revealed by comparing recordings on WT and null mutant cultures. In general, ShM cultures exhibited a great reduction in the peak transient current, Iₚ, along with a substantial increase in the T3 subpopulation (Fig. 5, A and C, P < 0.05). In contrast, Shab neurons displayed a severely reduced sustained component, Iₛ, and consequently T1 and T2 neurons became dominant in Shab cultures (Fig. 5, B and C, P < 0.01).

The most notable effect of the Shab mutations was a conspicuous absence of neurons displaying T4 kinetics (Fig. 5C), indicating that Shab codes for the IK channels that mediate the major sustained K⁺ currents in T4 neurons. Furthermore, T3 neurons, the major subpopulation in WT, were also severely reduced in Shab cultures, again reflecting a major contribution of Shab currents to the sustained component in these cells. Conversely, T1 cells, a minor subpopulation in WT cultures, became excessively abundant at the expense of drastically reduced T3 and T4 neurons in Shab cultures (Fig. 5, B and C).

Figure 6 summarizes a detailed analysis of current density and kinetics in T1–T4 neurons in WT and mutant cultures. The overabundance of T1 cells in Shab cultures was presumably due to a conversion from Shab-affected neurons, primarily T4 and T3, on removal of a sustained component. This new T1 subpopulation displayed distinct properties as reflected in an emergence of cells with an abnormally short decay constant τ₁ and a lowered Iₛ density, compared with WT T1 neurons (Fig. 5C). WT vs. Shab, Iₛ in pA/pF, 12 ± 2 vs. 8 ± 1, P < 0.01; τ₁ in ms, 246 ± 11 vs. 167 ± 4 ms, P < 0.01). Similar results have been obtained from another independently isolated allele, Shab' (data not shown).

The histograms for Sh neurons also provide clues about the properties of non-Sh Iₐ, presumably mediated by Shal channels. As illustrated in Fig. 6, the current density and kinetics distributions of the remaining transient current in Sh cultures were quantitatively distinct from WT cultures. Neurons in Sh cultures showed a reduction in the average density of transient Iₚ (Fig. 6A, WT vs. Sh, 27.4 ± 0.2 vs. 21.7 ± 0.2, P < 0.01) and lacked a group of cells that expressed extremely large Iₚ currents seen in WT and Shab cultures (extreme outliers, >48 pA/pF, see Fig. 6A, - - -). Thus some neurons of the dominant T3 category in Sh cultures (Fig. 5C) were likely converted from T1 and T2 categories after the removal of Sh transient Iₛ. This putative conversion is supported by the fact that a subpopulation of Sh T3 neurons exhibited a lower Iₛ density (Fig. 6A, WT vs. Sh, 27 ± 2 vs. 21 ± 2 pA/pF, P < 0.01) and prolonged τ₁ (Fig. 6C, note the log scale, 45 ± 1 vs. 56 ± 2 ms, P < 0.01), resembling the characteristics of τ₁ in WT T1 neurons.

In addition, differences in biophysical properties between Sh- and non-Sh Iₐ (Table 2) provided explanations for distinct action potential and firing pattern phenotypes of Sh neurons (Table 1). In voltage-clamp recordings, T1–T3 Sh neurons had a more positive half-activation voltage (~10 mV shift), and T1 Sh neurons inactivated at a more positive potential (~36 vs. ~50 mV for half-inactivation) in a steady-state inactivation protocol (Table 2). Furthermore, the inactivating components in T2 and T3 Sh neurons showed a slower recovery from inactivation than WT neurons in a paired-pulse protocol (~107–122 vs. 74–84 ms for half recovery, Table 2). Importantly, no significant changes were observed in T4 neurons between Sh and WT cultures, supporting the idea that the current in T4 neurons consists of mostly Shab currents.
K⁺ CURRENT SUBTYPES IN SPIKE PATTERN GENERATION

Properties of membrane excitability and corresponding K⁺ current kinetics

Our correlation studies further established that the extent of delay in spike initiation found in T1–T3 neurons is determined by the strength of transient current. The delay in spike onset was inversely proportional to the \( I_s/I_p \) ratio (Fig. 8). T1 and T2 neurons exhibited a smaller \( I_s/I_p \) ratio and tended to have longer delays in firing than did T3 neurons (Fig. 8A). Notably, the longest delay in spike initiation was found in T1 neurons (Fig. 8B), which had the slowest decay and the smallest \( I_s/I_p \) ratio (Figs. 4D and 6C).

Current- and voltage-clamp correlation further demonstrated that Shab \( I_K \) is crucial in action potential repolarization during repetitive firing. Removal of Shab current significantly increased the proportion of nonspiking neurons, and the remaining spiking neurons were predominantly of the damping type (Fig. 2). Nonspiking Shab cells generally displayed T1 (Fig. 9B, top traces) or T2 kinetics, whereas spiking cells displayed only T3 kinetics (5 of 5, \( I_s/I_p >0.5 \), Fig. 9B, bottom). These altered excitability patterns indicate the essential role of Shab currents in maintaining repetitive firing. Even though Shab T3 neurons retained a substantial sustained component, presumably reflecting \( I_K \) currents mediated by Shaw and other non-inactivating currents, that component was not sufficient for normal spike repolarization to maintain full-blown repetitive firing, leading to dwindling oscillations.

The “repolarization decline” phenotype was associated with both spiking and nonspiking Shab neurons that exhibited T1, T2, or T3 current kinetics (Fig. 9B, cf. Fig. 2B). Figure 9C summarizes the extent of “repolarization decline” as a function of \( I_s/I_p \) ratio in neurons of different genotypes. Pooled data from WT and Sh cultures illustrate an inverse correlation between the strength of sustained K⁺ current (\( I_s/I_p \) ratio) and the level of repolarization decline. In general, a more severe repolarization decline during current clamp was seen in T1 or T2 neurons (with an \( I_s/I_p \) ratio <0.5), rather than in T3 and T4 cells, for both WT and Sh cultures. Significantly, the most serious repolarization decline was observed in Shab neurons, regardless of their \( I_s/I_p \) ratios (Fig. 9C).

4-aminopyridine and quinidine effects on outward voltage-activated K⁺ currents and firing patterns

We used specific K⁺ channel blockers to contrast the acute pharmacological removal of \( I_A \) and \( I_K \) from the effects of Sh and Shab null mutations. It is well established that 4-aminopyridine (4-AP) at low concentration blocks transient \( I_A \) in different preparations including Drosophila larval motor axons and cultured embryonic neurons (Ueda and Wu 2006; Wu et al. 1989; Zhao and Wu 1997). Quinidine selectively blocks a component of delayed \( I_K \) in muscles at low concentrations (Singh and Singh 1999; Singh and Wu 1989, 1990) and enhances motor axon excitability and neuromuscular transmission in Drosophila larvae (Ueda and Wu 2006; Wu et al. 1989).

We extracted the 4-AP-sensitive component from the difference between voltage-clamp responses before and after 4-AP treatment (Fig. 10, A and B, top). The peak current (\( I_p \)) density in 4-AP-treated neurons was analyzed for individual current kinetic categories (T1–T4, Fig. 10, A and B, bottom). As expected, the effect of 1 mM 4-AP treatment on WT cultures
TABLE 2: Properties of individual current types

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WT</th>
<th>Sh</th>
<th>Shab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current type</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>$I_v$ half-activation (pA/pF)</td>
<td>23 ± 2</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>$I_v$ half-recovery time (ms)</td>
<td>67 ± 5</td>
<td>74 ± 8</td>
<td>84 ± 11</td>
</tr>
<tr>
<td>$I_{\text{A,inact}}$ st-st inactivation (pA/pF)</td>
<td>-50 ± 4</td>
<td>-53 ± 3</td>
<td>-43 ± 3</td>
</tr>
<tr>
<td>$V_{1/2}$ mV</td>
<td>68 ± 3</td>
<td>70 ± 15</td>
<td>87 ± 15</td>
</tr>
</tbody>
</table>

Data indicate means ± SE (n). Currents were activated by step depolarization from a holding potential of −80 mV at 20-mV increments up to +60 mV. Boltzmann fit was performed to determine the half-activation voltage (reversal potential = −80 mV). Recovery from inactivation protocol involved 500-ms twin pulses to +20 mV with varying interpulse intervals. Steady-state (st-st) inactivation was determined with a 500-ms preconditioning pulse (to −20 or 0 mV) and 5 ms afterward followed by a +60-mV test pulse. NA, not applicable; ND, not determined; *, P < 0.05; **, P < 0.01. One-way ANOVA was performed across WT, Sh and Shab parameters. Paired t-tests against WT were performed for some Sh parameters when Shab data are not available.

J Neurophysiol • VOL 97 • JANUARY 2007 • www.jn.org

was most drastic in T1 and T2 neurons, which predominantly expressed transient currents (Fig. 10A). We found that the 4-AP effects also held true for Sh T1 and T2 neurons (Fig. 10B), indicating that 4-AP can suppress both Sh $I_A$ and the remaining non-Sh transient currents. In either WT or Sh cultures, most of the T4 neurons, which expressed little inactivation currents, showed negligible sensitivity to 4-AP (Fig. 10A), with only a small 4-AP-sensitive transient component occasionally found in some T4 neurons (see example traces in Fig. 10B). As shown in Fig. 10, 4-AP treatment decreased the peak currents in T1 and T2 neurons more severely than in T3 and T4 neurons. The remaining 4-AP-insensitive currents in the treated neurons displayed T3- and T4-like kinetics, consistent with the apparent population conversion observed in Sh cultures, in which removal of Sh currents led to an excess of T3 and T4 neurons with diminished current amplitude (Figs. 4 and 5). In current-clamp experiments, 4-AP treatment of WT neurons converted delayed firing patterns into damping spike patterns (Fig. 11, A and B). 4-AP treatment of Sh cultures also converted delayed, and some tonic, to damping neurons (Fig.
ment. In contrast, neurons in 10 C but lacked T4, neurons (Fig. 5), showed little reduction components from voltage-clamp responses before and after quinidine treatment (Fig. 10).

FIG. 7. Correlation of K+ current types to firing patterns. Current- and voltage-clamp recordings performed consecutively on the same cells provided direct correlations between firing patterns and voltage-activated K+ current kinetics. Neurons with delayed firing could exhibit T1, T2, or T3, but not T4, current kinetics. In contrast, tonic, adaptive and damping neurons displayed T3 and T4 kinetics.

11B), indicating that non-Sh (presumably Shal), as well as Sh, I_A channels participate in the generation of delayed and tonic firing patterns.

To manipulate sustained K+ currents, a low dosage of 50 μM quinidine was used to avoid nonspecific effects on other K+ channels that became detectable at concentrations >100 μM (data not shown). Extraction of quinidine-sensitive components from voltage-clamp responses before and after quinidine treatment (Fig. 10, C and D, top) distinguished different components of the sustained current (I_S) in neurons displaying T1–T4 kinetics (Fig. 10, C and D, bottom). In WT cultures, a major component of the sustained current was removed in T3 and T4 neurons by quinidine (Fig. 10C), whereas T1 and T2 neurons showed much less sensitivity (<10% reduction, Fig. 10C). Note that, in this case, T3 and T4 current kinetics could be converted to T1- or T2-like kinetics after quinidine treatment. In contrast, neurons in Shab cultures, which contained T1–T3, but lacked T4, neurons (Fig. 5), showed little reduction in I_S amplitude, and the kinetics of the total K+ current remained unaltered after quinidine treatment (Fig. 10D). These results indicate that quinidine specifically blocks Shab currents, which contribute substantially to the sustained components in T3 and T4 but little to T1 and T2 neurons.

Current-clamp experiments in WT cultures showed that quinidine affected the tonic and adaptive spike activities but did not alter the damping firing pattern (Fig. 11C). In fact, most tonic and adaptive neurons examined in WT cultures were converted into the damping category, whereas the damping firing encountered in Shab cultures (cf. Fig. 2) remained unaltered after drug treatment. Thus the combined voltage- and current-clamp data support the idea that the quinidine-sensitive Shab current (Fig. 10, C and D) is required for maintaining membrane repolarization during repetitive firing (Figs. 2 and 11, C and D).

A sequence of sensitivity to drug treatment among the different firing patterns emerges from the preceding results, suggesting a hierarchy of complexity of K+ current interactions among neurons displaying different firing patterns. As a whole, the most frequently observed conversions are from the delayed to damping firing patterns after 4-AP treatment and from tonic to damping after quinidine treatment (Fig. 11). However, conversions between delayed and tonic firing patterns on drug treatment have not been observed. These observations suggest that the damping firing pattern requires weaker or fewer K+ current components, whereas participation of 4-AP- and quinidine-sensitive components is critical for generating the delayed and tonic patterns, respectively. We also occasionally encountered conversions from tonic to adaptive and adaptive to damping but not vice versa (Fig. 11). Additional mutations and pharmacological agents may be employed in future investigations to elucidate this apparent hierarchy of complexity of the K+ current interactions in different firing patterns.

K+ channel distribution in “cell lineage-defined” subpopulations of neurons

In view of the wide range of excitability patterns and the underlying currents described in the preceding text, we explored the possibility of distinct expression patterns of K+ channels in more restricted, identifiable neuronal subsets in the “giant” neuron culture. We utilized enhancer detection lines to drive expression of the GFP marker in different neuronal lineages. We chose to focus on two specific subsets of neurons, marked by the GH146 and 201Y Gal4 drivers. In larval and adult preparations, these two drivers label olfactory glomerular projection neurons and their postsynaptic target, the Kenyon cells in the mushroom bodies (Stocker et al. 1997; Yang et al. 1995).

We found that only relatively small subsets of neurons were GFP-positive (Fig. 12A) in GH146 (7.1 ± 3.5%, n = 10) and
unlabeled neurons (Fig. 12).

In addition, these two neuronal subsets displayed distinct excitability properties in current-clamp experiments. We observed both spiking and nonspiking activities in GH146(+) and 201Y(+) cells (Fig. 12E). However, action potential threshold was significantly more positive and duration was longer in GH146(+) cells, as compared with 201Y(+) cells or unlabeled neurons (Fig. 12E and Table 3). Consistent with the observations in the general population, the Shab mutation produced a characteristic repolarization decline in both spiking and nonspiking cells (7/8) and converted repetitive firing into a damping pattern (2/2) in the 201Y (+) subset (compare Figs. 12E and 2B).

Discussion

This study demonstrates that the Drosophila “giant” neuron culture system can provide a bridge between heterologous expression systems and in vivo preparations to facilitate the study of how firing patterns are generated by interactions of molecularly identified channel subtypes and controlled by genes of interest. Our current-clamp study has shown a diversity of firing patterns in WT cultures (Fig. 1). Voltage-clamp recordings on the same cells further revealed the relationship of firing patterns and the compositions of underlying K+ currents (Fig. 7). In addition, manipulating K+ channel compositions by employing mutations and pharmacological agents provided independent lines of evidence for the distinct contributions of each K+ current component to the control of membrane excitability.

Elimination of a sustained K+ current component and deterioration of firing patterns in Shab neurons

In Drosophila muscles, it has been demonstrated that IA is eliminated by Sh mutations (Haugland and Wu 1990; Salkoff and Wyman 1981) and IK is affected by Shab mutations (Singh and Singh 1999). Mutations of either a go-go (egg), another gene encoding a K+ channel subunit, can also reduce IA and IK in muscles (Zhong and Wu 1991). Several studies propose a different K+ channel expression pattern in neurons: IA channels are encoded by both Sh and Shaw (Baker and Salkoff 1990; Choi et al. 2004; Gasque et al. 2005; Yao and Wu 2001), but major component of IK is produced by Shab channels (Tsunoda and Salkoff 1995). Consistently, in “giant” neuron cultures, Sh and Shab null mutations only reduced, but did not eliminate IA or IK (Fig. 5), indicating the presence of a substantial non-Sh component for IA and a minor non-Shab component for IK in neurons.

Our results provide a first description of the striking phenotype of Shab neurons. During sustained current injection, Shab neurons rely on other noninactivating K+ currents for action potential repolarization as the transient IA becomes progressively inactivated. The resultant abnormal damping spike pattern and unusual regenerative activities contrast the roles of Shab and non-Shab channels (including Shaw) (cf. Hodge et al. 2005). In most Shab neurons, spiking or nonspiking, we observed a novel “repolarization decline” phenotype that reflects a failure in maintaining a steady level of membrane polarization (Figs. 2 and 9). Furthermore, the spiking activity in Shab cultures was restricted to the damping firing pattern and was coupled with T3 current kinetics (Figs. 2 and 9). Consistently, quinidine-treatments, which specifically remove Shab currents in WT neurons (Fig. 10), closely mimicked the “repolarization decline” phenotype and converted spiking patterns into damping firing (Fig. 11).
m al action potentials in response to brief stimuli (Fig. 2), but prolonged current injection causes abnormal high-frequency, run-away spikes that degenerate into dwindling oscillations. WT neurons expressing both Shab and non-Shab sustained currents fire full-blown action potentials /H11011 25 Hz on average (Table 1). In contrast, T3 Shab neurons (I{sub S}/I{sub P} > 0.5) that retain substantial non-Shab currents, including Shaw, generate high-frequency firing (up to 60 Hz) either immediately on depolarization or with a gradual development (Fig. 3 and Table 1). Interestingly, it has been shown that pharmacological blockade or mutations of Shaw-like Kv3 channels disable the high-frequency firing (up to 1 kHz) often found in certain neuronal types in the hippocampus, basal ganglia, and auditory nuclei (Lau et al. 2000; Rudy and McBain 2001). Because the exact dynamics of membrane repolarization can determine the timing of recovery of inward Na{sup +} and Ca{sup 2+} currents from inactivation for resetting another cycle of spike activity, the balancing act between Shab and non-Shab I{sub K} currents can enrich spike frequency control during repetitive firing.

**Interaction between I{sub K} and I{sub A} in repetitive firing**

An interesting parallel of the striking Shab phenotype during repetitive firing is observed at the larval neuromuscular junction (Ueda and Wu 2006). With single nerve stimuli, Shab synaptic transmission appears normal. During high-frequency nerve stimulation, an explosive neuromuscular transmission (up to 10-fold gain, termed the “big bang” phenomenon) could suddenly occur when cumulative inactivation of I{sub A} reaches a critical level. The phenomenon can also be induced in WT by repetitive stimulation following quinidine treatment and by single nerve stimuli in quinidine-treated Sh preparations (Ueda and Wu 2006). The generation of a plateau membrane potential in the motor axon terminals, which are enriched with both Na{sup +} and Ca{sup 2+} channels, has been proposed to account for this phenomenon when both I{sub A} and I{sub K} are weakened by mutations, drugs, or activity-dependent inactivation. These observations are reminiscent of the “repolarization decline” in Shab neurons and quinidine-treated WT neurons during prolonged current injection (Figs. 2 and 9). Taken together, an intricate interaction between slowly inactivating I{sub K} and fast inactivating I{sub A} is
importance during the dynamic process of repetitive firing for maintaining the cycles of membrane excitation and repolarization.

Notably, simple removal of Shab current by acute quinidine treatment on WT cultures converted firing patterns to damping rather than nonspiking activities (Fig. 11). However, in Shab cultures, a drastic increase of nonspiking neurons was observed (Fig. 2C), contrary to the expectation of increased excitability caused by reduced \( I_A \). A clue to this unexpected finding is provided by the observation that in some nonspiking Shab cells, regenerative oscillations could still be initiated when the transient \( I_A \) was suppressed by 4-AP or a depolarizing prepulse (data not shown). Voltage-clamp measurements (Fig. 6A) yielded direct evidence for an increase in the inactivating \( I_A \) in Shab cultures. The peak total current \( (I_P) \), which represents the sum of peak \( I_A \) and \( I_K \), remained undiminished despite the fact that the sustained current component \( (I_S) \) was significantly decreased in Shab neurons (Figs. 5B and 6A). In contrast, pharmacological removal of Shab currents reduced both \( I_S \) (~35%, Fig. 10B) and \( I_P \) (~30%, data not shown). Our observations resemble the homeostatic regulation of ion channel previously reported in other preparations. When neuronal spike activity is manipulated, a homeostatic regulation can be initiated to adjust the relative abundance of ion channels and other proteins (Guan et al. 2005; Spitzer 1999; Turrigiano and Nelson 2004). Over-expression of Shal \( I_A \) in lobster neurons triggers a compensatory increase of hyperpolarization-activated inward \( I_K \) (MacLean et al. 2003). Therefore a compensatory up-regulation of the transient \( K^+ \) current component in Shab cultures could account for the lower percentage of spiking cells (Fig. 2). Overexpression of transient \( I_A \) could prevent spike initiation in the standard current-clamp protocols em-

**FIG. 11.** Differential sensitivities of different firing patterns to \( K^+ \) channel blockers among WT, Sh, and Shab neurons. A: representative traces showing conversion of the delayed to damping firing pattern in a WT neuron after 1 mM 4-AP treatment. The 4-AP effects on the firing patterns are summarized in the plot. Data from the same cells are connected (--). The conversion in the representative traces is indicated (gray symbols) in the plot. In contrast to its drastic effects on the delay firing pattern, 4-AP had little effect on tonic, adaptive and damping patterns. B: in Sh cultures, delayed and some tonic neurons were sensitive to 4-AP and their firing patterns became adaptive and damping. C: in WT cultures, quinidine application converted tonic and adaptive firing to the damping and mimicked the "repolarization decline" phenotype commonly seen in Shab cultures (cf. Fig. 8). D: spiking Shab neurons (predominantly damping, cf. Fig. 2) showed low sensitivity to quinidine.

**FIG. 12.** \( K^+ \) current kinetics and firing properties in cell lineage-defined neurons. A: phase and fluorescent images of neurons dissociated from GH146-Gal4, UAS-GFP embryos demonstrating selective GFP labeling. Scale bar: 25 μm. B and C: representative current traces and distributions of \( K^+ \) current kinetics types showing predominant T3 kinetics among GH146(+) cells and more abundant T2 kinetics among 201Y(+) cells (P < 0.05 for both subpopulations, \( \chi^2 \) test against the distribution of the total population in culture; cf. Fig. 4B). Both Sh(1-12) and Sh(2-8) mutations altered the distribution of current types among 201Y(+) neurons (P < 0.05). Note that in the 201Y(+) neuronal subpopulation, the Sh mutation caused a conversion of dominant T2 to excessive T3 and T4 neurons and that no T4 neurons were found in Shab cultures (*). Total, n = 134; GH146(+), n = 32; 201Y(+), n = 34; Sh 201Y(+), n = 22; 201Y(+), n = 23. D: box plots for \( I_P \) and \( I_S \) density. Note that some 201Y(+) neurons expressed excessively large \( I_S \). In contrast to a significantly reduced \( I_S \) in GH146(+) cells, in the 201Y(+) subpopulation, Sh mutation reduced \( I_P \), whereas Shab mutation reduced \( I_S \). * P < 0.05, 1-way ANOVA. E: spiking and nonspiking cells recorded from GH146(+) and 201Y(+) neurons. Broadened action potentials were common in GH146(+) cells. Characteristic damping firing patterns along with repolarization decline were frequently observed in 201Y(+) Shab neurons.
indicating that the remaining Shal channels inactivate more cultures (Baker and Salkoff 1990; Gasque et al. 2005), is in agreement with previous reports on pupal and larval and Steven 1971). Nevertheless, such damping firing patterns repolarization deteriorates during repetitive firing (cf. Connor delayed and other firing patterns.

Alteration of action potential duration and firing frequency in Sh neurons

In contrast to Shab channels, Sh channels play a role in regulating rapid events within a millisecond time scale. We observed broadened action potentials in Sh neurons with delayed and tonic firing patterns (Fig. 3 and Table 1). This demonstrates a role of action potential repolarization for Sh channels, consistent with greatly prolonged action potentials previously documented in the cervical giant fiber of Sh mutants (Tanouye and Ferrus 1985).

A well-established function of transient K\(^+\) currents is pertinent to the control of spike initiation time during excitatory inputs (Hille 2001). Our mutational and pharmacological analyses confirm the important but overlapping roles of Sh and Shal channels in controlling spike initiation (Figs. 7 and 10) (cf. Choi et al. 2004; Zhao and Wu 1997). Neurons exhibiting delayed firing persisted in within only a small subset of neurons, \(I_{\text{A}}\) channels are exclusively or predominantly encoded by Sh (Baker and Salkoff 1990). Furthermore, no disproportional reduction in any of the categories of firing patterns was observed in Sh cultures (Fig. 2), indicating that Sh and Shal \(I_{\text{A}}\) may serve redundant roles in suppressing transient depolarization, and thus the action of Shal \(I_{\text{A}}\) alone could retain the dynamic manifestation of the delayed and other firing patterns.

Delayed or tonic firing in WT and Sh cultures could be converted into damping patterns after 4-AP treatment (Fig. 11), suggesting that with total elimination of transient \(I_{\text{A}}\), spike repolarization deteriorates during repetitive firing (cf. Connor and Steven 1971). Nevertheless, such damping firing patterns were distinct from those observed in Shab or quinidine-treated WT neurons, in that the high-frequency oscillations typical of Shab neurons were never observed (Figs. 1, 2, 9, and 11). In summary, a clear rank of firing rate was observed in the damping patterns of the three genotypes: Shab > WT > Sh (Table 1).

The slower decay of transient \(I_{\text{A}}\) in Sh cultures (Table 2) is in agreement with previous reports on pupal and larval cultures (Baker and Salkoff 1990; Gasque et al. 2005), indicating that the remaining Shal channels inactivate more slowly than Sh channels. This is also consistent with the conclusion based on Sh and Shal channel expression experiments in the frog oocyte (Wei et al. 1990). Our results also suggest that Shal channels follow a slower kinetics of recovery from inactivation (Table 2), something that awaits confirmation in other preparations. However, our steady-state inactivation measurements of \(I_{\text{A}}\) in Sh cultures suggest that Shal channels inactivate at voltages more positive than that for Sh channels (Table 2), contrary to the preceding reports (Baker and Salkoff 1990; Gasque et al. 2005). The reason for the discrepancy is unknown, although expression patterns of the Sh and Shal splice variants as well as their potential association with auxiliary channel subunits could differ among the embryonic, larval and pupal cultures used in these studies. Splice variants of the Sh products are known to mediate K\(^+\) currents of varying degrees of inactivation when expressed heterologously in Xenopus oocytes (Iverson et al. 1988; Timpe et al. 1988). Similarly, subtypes of mammalian Kv1 channels also display different inactivation properties (Coetzee et al. 1999; Stumhmer et al. 1989).

In some cases, 4-AP did not completely eliminate the transient component in T3 cells in WT and Sh cultures (2 of 5 cells for both genotypes, Fig. 10, A and B). Such 4-AP insensitive transient component may reflect low sensitivity to 4-AP in certain splicing isoforms of Sh or Shal subunits. Alternatively, inactivating isoforms of Shaw may exist, since a mammalian homologue of Shaw, Kv3.4, mediates inactivating K\(^+\) currents (Coetzee et al. 1999).

Although the tonic firing pattern is insensitive to 4-AP in WT neurons, 4-AP blockade of the remaining Shal currents in Sh neurons converts the tonic firing pattern to adaptive or damping in two out of three cases (Fig. 11, A and B). Apparently, the currents contributing to the tonic firing pattern have been reconfigured in some Sh neurons relative to WT neurons. In addition, the population of nonspiking neurons is also increased in Sh cultures (Fig. 2C), contrary to the expectation that removal of Sh currents leads to hyperexcitability. Again, the possibility of downregulation of inward Na\(^+\) and Ca\(^{2+}\) currents in Sh neurons must be considered, similar to the case for Shab mutant neurons. Furthermore, upregulation of Shal or other transient currents in Sh mutant neurons is possible. However, over-expression of non-Sh transient currents would be rather limited because a significant reduction of \(I_{\text{P}}\) was still evident in Sh cultures (Fig. 6). Taken together, these observations suggest diverse and cell-specific compensatory mechanisms still await further exploration in the heterogeneous population of the nervous system.

### TABLE 3. Firing and K\(^+\) current properties of lineage-defined neuronal subsets

<table>
<thead>
<tr>
<th>Property</th>
<th>Total</th>
<th>GH146 (+)</th>
<th>201Y (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mV</td>
<td>-54 ± 1 (106)</td>
<td>-52 ± 2 (7)</td>
<td>-49 ± 4 (7)</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>-25 ± 1 (71)</td>
<td>-14 ± 2* (7)</td>
<td>-22 ± 3 (7)</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>7.0 ± 0.4 (71)</td>
<td>12.3 ± 1.4* (7)</td>
<td>8.7 ± 1.1 (7)</td>
</tr>
<tr>
<td>(I_{\text{A}}) half-activation voltage, mV</td>
<td>-15 ± 4 (113)</td>
<td>-10 ± 2* (25)</td>
<td>-14 ± 2 (32)</td>
</tr>
<tr>
<td>(I_{\text{P}}) half-recovery time, ms</td>
<td>86 ± 6 (13)</td>
<td>158 ± 8* (7)</td>
<td>93 ± 7 (9)</td>
</tr>
</tbody>
</table>

See Tables 1 and 2 legends for explanation.
Conversion of firing pattern categories after removal of $K^+$ current components

Both Sh and Shab mutations alter the abundance of the individual kinetic categories of total voltage-activated $K^+$ currents among cultured neurons (Figs. 5 and 6). This redistribution conceivably leads to a population conversion of neuronal types in mutant cultures. For example, part of the more populated T1-like neurons in Shab cultures reflects a conversion from T2 and T3 neurons on removal of Shab I$_K$ as indicated by the unusually fast decay kinetics in some Shab T1 cells that resemble the decay time course of WT T2 and T3 neurons (Fig. 6). Similarly, the overpopulated T3 cells in Sh cultures might be converted from T1 and T2 neurons (Fig. 6), reflecting enhanced representation of Shal currents. The assumption of population conversion on removal of Sh and Shal channels is corroborated by the results of drug treatments in both voltage- and current-clamp experiments (Figs. 10 and 11) that reveal differential expression of $K^+$ channels required for generation of different firing patterns. Notably, neurons with delayed firing patterns were converted by 4-AP to damping patterns but were not affected by quinidine, suggesting an abundance of transient I$_A$ coupled with a deficiency of Shab I$_K$ in this cell category (Fig. 11). In contrast, quinidine converted tonic to damping firing patterns (Fig. 11) (cf. Zhao and Wu 1997), indicating a strong dependence on Shab I$_K$ during tonic firing. These observations suggest that the damping firing pattern requires actions of fewer $K^+$ channel subtypes. In other words, an apparent order of complexity exists for $K^+$ current interactions that underlie different firing patterns, suggesting an interesting scheme, in which differential expression or modulation of $K^+$ channels (Jonas and Kaczmarek 1996; Yao and Wu 2001) may generate a diversity of neuronal firing patterns to fulfill the specific tasks and functional plasticity of neuronal circuits.

$K^+$ current compositions and firing patterns in identifiable neuronal subsets

Several Drosophila Gal4 lines have been used to drive targeted expression of UAS-GFP to identify specific neuronal subsets in larval or pupal culture systems (Gasque et al. 2005; Jiang et al. 2005; Su and O’Dowd 2003; Wright and Zhong 1995). Using this approach, we demonstrated that in embryonic “giant” neuron cultures, neurons of different cell lineages displayed characteristic excitability properties and $K^+$ current kinetics (Fig. 12 and Table 3). We observed a substantial inactivating $K^+$ current component in 201Y(+) cells in embryonic “giant” neuron cultures, similar to that reported for cultured neurons dissociated from larval mushroom body (Gasque et al. 2005). It should be noted that channel distribution among different neuronal compartments in dissociated neuron cultures might not exactly match that of native neurons with their interacting partners in vivo and thus may modify the firing pattern of particular neuronal subpopulations. Moreover, in cell division-arrested embryonic neurons, the channel expression profile may reflect a combination of expression patterns of the neuronal descendants. It is known that the progeny of one insect neuroblast can develop different excitability properties (Goodman et al. 1980).

Despite these caveats, our previous electrophysiological and Ca$^{2+}$ imaging studies on the same preparation have indicated a disparity between soma and neurites in $K^+$ channel distribution (Berke et al. 2006; Saito and Wu 1991) similar to the pattern found in neurons of other in vivo preparations (Baro et al. 2000). In addition, the present physiological results of different $K^+$ currents are directly comparable to the measurements from acutely dissociated CNS neurons of Drosophila larvae, pupae and adults in terms of kinetic categories, current compositions and mutational effects (Wu et al. 2001; Xu et al. 2005; T. X. Xu, P. Jiang, C. F. Wu, and T. L. Xu, unpublished data). Therefore the “giant” neuron culture system can serve the purpose to link data of different levels that obtained from heterologous expression systems and in vivo preparations. In this system, results from electrophysiological analyses can be integrated with Ca$^{2+}$ imaging to reveal functional specialization in different neuronal lineages (Berke and Wu 2002; Berke et al. 2006; Jiang et al. 2005). Combining the baseline information obtained in neuronal cultures with in vivo studies will help elucidate the cellular mechanisms that enable the neuronal circuits of interest to perform different functional tasks in Drosophila (e.g., Baines 2003; Choi et al. 2004; Gu and O’Dowd 2006; Koenig and Ikeda 1983; Lee and Wu 2006; Tanouye and Wyman 1980; Thomas and Wyman 1984; Wang et al. 2001; Wang et al. 2003; Wilson et al. 2004).

Acknowledgments

We thank Dr. S. Singh for providing the mutant lines used in this study. We thank Dr. A. Ueda and J. Lee for comments on the manuscript. We also appreciate technical advice from Dr. W.-D. Yao and genetic constructs from Dr. B. Berke.

Grants

This work was supported by National Institutes of Health Grants HD-18577 and NS-26528.

References

K⁺ CURRENT SUBTYPES IN SPIKE PATTERN GENERATION


