Pharmacology of currents underlying the different firing patterns of spinal sensory neurons and interneurons identified in vivo using multivariate analysis

Crawford I. P. Winlove and Alan Roberts
School of Biological Sciences, University of Bristol, Bristol, United Kingdom

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Winlove CIP, Roberts A. Pharmacology of currents underlying the different firing patterns of spinal sensory neurons and interneurons identified in vivo using multivariate analysis. J Neurophysiol 105: 2487–2500, 2011. First published February 23, 2011; doi:10.1152/jn.00779.2010.—The operation of neuronal networks depends on the firing patterns of the network’s neurons. When sustained current is injected, some neurons in the central nervous system fire a single action potential and others fire repetitively. For example, in Xenopus laevis tadpoles, primary-sensory Rohon-Beard (RB) neurons fired a single action potential in response to 300-ms rheobase current injections, whereas dorsolateral (DL) interneurons fired repetitively at 10–20 Hz. To investigate the basis for these differences in vivo, we examined drug-induced changes in the firing patterns of Xenopus spinal neurons using whole cell current-clamp recordings. Neuron types were initially separated through cluster analysis, and we compared results produced using different clustering algorithms. We used these results to develop a predictive function to classify subsequently recorded neurons. The potassium channel blocker tetraethylammonium (TEA) converted single-firing RB neurons to low-frequency repetitive firing but reduced the firing frequency of repetitive-firing DL interneurons. Firing frequency in DL interneurons was also reduced by the potassium channel blockers 4-aminopyridine (4-AP), catechol, and margatoxin; 4-AP had the greatest effect. The calcium channel blockers amiloride and nimodipine had few effects on firing in either neuron type but reduced action potential duration in DL interneurons. Muscarine, which blocks M-currents, did not affect RB neurons but reduced firing frequency in DL interneurons. These results suggest that potassium currents may control neuron firing patterns: a TEA-sensitive current prevents repetitive firing in RB neurons, whereas a 4-AP-sensitive current underlies repetitive firing in DL interneurons. The cluster and discriminant analysis described could help to classify neurons in other systems. Xenopus; spinal cord; electrophysiology; current clamp; whole cell recording

NEURONAL FIRING PATTERNS in response to injected current influence the way that neurons interact in neuronal circuits and have been well characterized, for example, in the spinal cord (Dale and Kuenzi 1997; Ruscheweyh et al. 2004), brain stem (Pape and McCormick 1995; Sivaramakrishnan and Oliver 2001), and cortex (Markram et al. 2004; Yuste et al. 2005). Firing patterns partly depend on the amplitude of the injected current (Beurrier et al. 1999; Chandler et al. 1994) and partly on the experimental methods; for example, the temperature (Heitler et al. 1977; Hodgkin and Katz 1949), the intactness of morphological processes (Abdulla and Smith 2001; Johnston et al. 1996), and the use of patch or sharp electrodes (Aiken et al. 2003; Li et al. 2004a). Nonetheless, it is broadly clear that a few similar firing patterns are seen in a wide range of species and anatomic locations.

Most commonly, in response to suprathreshold depolarizing current, neurons fire repetitively without adaptation at frequencies between 10 and 20 Hz. Typical examples include invertebrate neurons (Grolleau and Lapied 2000; Hodgkin 1948); spinal neurons in rats (Baccei and Fitzgerald 2005; Deuchars et al. 2001), turtles (Smith and Perrier 2006), lampreys (Buchanan 1993), and Xenopus tadpoles (Li et al. 2002); and many classes of cortical neuron (Cauli et al. 2000; Gonzalez-Burgos et al. 2005; Luhmann et al. 2000; Zhou and Hablitz 1996). A second pattern, adapting firing, especially within 500 ms, is less common (Buss et al. 2003; Iwasaki et al. 2008; Melnick et al. 2004a), although some neurons cease firing within 200 ms (Li et al. 2003, 2004b). A third pattern, delayed-onset repetitive firing, is of similar prevalence to adapting firing; delays vary in duration between preparations and between successive stimuli (Hochman et al. 1997; Houngsaard and Midtgaard 1988; Hsiao et al. 2007; McQuiston and Katz 2001; Ruscheweyh et al. 2004). Finally, single firing is the least common response to current injection (Iwasaki et al. 2008; Reyes et al. 1994; Waddell and Lawson 1990); the majority of single-firing neurons fire repetitive action potentials when larger currents are injected (Erisir et al. 1999; Garrido-Sanabria et al. 2007; Mo et al. 2002), but some remain single firing (Gamkrelidze et al. 1998; Lin 1997). Other firing patterns have been reported, such as bursting (McCormick et al. 1985) and chattering (Brumberg et al. 2000), but these are characteristic of specific cell types and anatomic regions.

What then are the functions of these firing patterns? In sensory neurons, firing frequencies code stimulus intensity (Getchell and Shepherd 1978; Hille 2001), adapting firing emphasizes new information and coincidence detection (Krahe and Gabbiani 2004; Sabourin and Pollack 2009), and delayed-onset firing integrates sustained stimuli (Prescott and De Koninck 2002; Russo and Houngsaard 1999). Firing patterns also influence the properties of synapses (Bi and Poo 1998; Zucker and Regehr 2002); interactions between firing patterns and synapses are likely to be important in network development (Feldman et al. 1999; Sun and Dale 1998) and the dynamic assembly of stimulus-specific networks (Fuhrmann et al. 2002; Gerstner et al. 1997).

Such intriguing functions have encouraged attempts to identify the intrinsic membrane properties that underlie specific neuronal firing patterns (Chandler et al. 1994; Houngsaard and Midtgaard 1988; Risner and Holt 2006; Smith and Perrier 2006; Szabo et al. 2002). The majority of such studies use in vitro preparations, yet evidence suggests that electrophysiological properties are altered by the loss of morphological pro-
cesses and intercellular interactions (Connors et al. 1982; Gurantz et al. 1996; Mathiesen and Maler 1988; Turrigiano et al. 1994). Consequently, results are difficult to relate to normal in vivo phenomena. However, although there is a clear need for in vivo studies, it is often hard to resolve cell types unambiguously; the anatomic techniques are very time consuming, so a simple and quick method for neuron classification is required.

We have therefore addressed two main questions using hatching *Xenopus* tadpole spinal neurons. First, can neurons recorded in vivo be classified accurately and reliably using multivariate analysis but only limited information? Second, can drug-induced changes in firing patterns provide insights into the basis of single and repetitive firing in *Xenopus* spinal neurons? Current-clamp recordings were made in vivo, and we used cluster analysis and discriminant analysis to determine a minimum set of parameters required to reliably distinguish single- and repetitive-firing neurons. We compared the electrophysiological properties of the resulting groups and used a wide range of channel blockers to examine drug-induced changes in their firing patterns. We found potassium channel blockers converted neurons between single and repetitive firing patterns, which suggests that potassium channels play a central role in determining firing patterns.

**METHODS**

**Preparation.** Experiments and husbandry were in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act 1986 and received local ethical approval. Experiments were performed on hatching *Xenopus laevis* tadpoles (stage 37/38) (Nieuwkoop and Faber 1956) sired by the hormone-induced mating of adults from our breeding colony. An important feature of the *X. laevis* model system is that in contrast to many studies of mammalian neurons, *Xenopus* neurons are characterized at normal in vivo temperatures of 18–22°C. Tadpoles were anesthetized in MS-222 (1 mg/ml; Sigma-Aldrich, Poole, UK) and, after the dorsal fin was cut open with finely etched tungsten wire, paralyzed by immersion in saline containing α-bungarotoxin (10 μM, 30 min). Anesthetics were not used during experiments because hatching tadpoles are considered to be insentient.

Immobilized animals were secured to a layer of Sylgard on a rotatable stage with tungsten micropins and viewed with a dissecting microscope; rotation of the stage facilitated dissections and allowed us to optimize the illumination of neurons during whole cell recordings. RB neurons and dorsolateral (DL) interneurons are found more dorsally in the spinal cord (Roberts et al. 2000) and were exposed by dissection. First, the skin and muscle overlying the trunk region of the spinal cord were removed. After loose tissue and pigment cells were cleaned away, the neural canal was opened by cutting along the dorsal midline of the spinal cord. This revealed the somata of RB neurons, which are large and spherical (Clarke et al. 1984). The somata of DL interneurons are smaller than RB somata and lie immediately ventral to RB neurons (Li et al. 2001). The somata of DL interneurons could often be seen simply by rotating the stage on which the tadpole was mounted, although it was sometimes necessary to remove additional overlying ependymal cells (Roberts et al. 2000). Recordings were made throughout the spinal cord to randomize any rostral-caudal differences in neuron properties. Dissections and recordings were made in a chamber with two compartments, a larger one for dissection and a smaller one for recording, designed to minimize damage to prepared specimens. The recording chamber had a volume of 2 ml and could be perfused at rates ≥20 ml/min. Neurons were visualized under bright-field illumination using a ×40 water-immersion lens and an upright E600FN microscope (Nikon, Tokyo, Japan).

**Solutions and drugs.** Physiological extracellular solution, used during dissection and in the majority of current-clamp recordings, comprised (in mM) 115 NaCl, 3 KCl, 10 CaCl2, 2.4 NaHCO3, 1 MgCl2, and 10 HEPES (Li et al. 2002). Where 10 mM tetrodynammonium chloride was used, the extracellular sodium concentration was reduced to 105 mM (Li et al. 2002; Ribera and Spitzer 1990). The reliability with which seals formed between the electrode and the neuron membrane was improved by the high external calcium concentration. This probably reflects the action calcium has in facilitating ionic interactions between phospholipid headgroups, thereby stabilizing the neuron membrane (Ohki and Ohshima 1985). Extracellular solutions were adjusted to pH 7.4 and 250 mosM, filtered before use (<2 μm, grade 1575 Whatman filter paper; Sigma-Aldrich), and used within 1 mo of preparation. Pipette solutions comprised (in mM) 100 K-glucuronate, 2 MgCl2, 10 EGTA, 10 HEPES, 3 Na2ATP, and 0.5 Na2GTP (Li et al. 2002). We used MgCl2, Na2ATP, and Na2GTP to minimize current rundown (Orlowski and Grinstein 1997). Pipette solutions were adjusted to pH 7.3 and 230 mosM, frozen for storage, filtered before use (0.1 μm, Millex syringe filters; Sigma-Aldrich), and used within 1 h of thawing. Stock solutions of tetraethylammonium (TEA), 4-aminopyridine (4-AP), catechol, nimodipine, amiloride, and muscarine were frozen for storage, used within 1 wk of thawing, and supplied by Sigma-Aldrich. Margatoxin (MGX; Alomone Laboratories, Jerusalem, Israel) was frozen as stock and used within 1 day of thawing.

**Electrophysiology.** Patch pipettes were pulled from thin-walled borosilicate glass (TW-150F; World Precision Instruments, Sarasota, FL) on a Sutter Instruments P97 puller (Novato, CA), and had resistances of 2–5 MΩ when filled with pipette solution. The patch pipette was motor driven by an MX-7630R manipulator (Siskiyou Instruments, Grants Pass, OR), and visualized with a ×40 water-immersion lens (Nikon). Whole cell current-clamp measurements were made using an Axoclamp 2B amplifier in bridge mode (Molecular Devices, Sunnyvale, CA). Junction potentials were calculated according to Barry (1994), and the offset was adjusted on electrode immersion and again off-line if altered by subsequent solution changes. After formation of a gigahm seal, the membrane was ruptured by brief positive current pulses (5 × 1 nA, 3-ms duration, 5-ms separation). The capacitance compensation was adjusted after breakthrough and checked alongside the bridge balance during experiments. Only neurons that established a stable resting potential within 30 s of breakthrough were studied further. Whole cell recordings are susceptible to rundown over the course of experiments (Sakmann and Neher 1995). Neurons were therefore characterized within 5 min of breakthrough to minimize changes in firing patterns due to rundown. Drugs were bath-applied after dissection, 15 min before patch-clamp recordings were started. This ensured that drug concentrations were clearly defined when firing patterns were assessed, and we are not aware of any evidence of desensitization associated with the drugs used. Consequently, figures show different neurons in control and test conditions. Data were digitized using a CED Micro 1401-1 digitizer sampling at 40 kHz and were visualized using Signal 3.14 software (both from Cambridge Electronic Design, Cambridge, UK).

**Measurement and analysis.** Action potentials (APs) were evoked by injecting depolarizing current, for either 10 ms, to examine individual APs, or 300 ms, to examine repetitive firing. APs were compared at rheobase, the minimum size current required to evoke firing; rheobase amplitudes varied between neurons and treatment groups. Several measurements of the voltage waveform were made; the first spike was measured in all cases (Fig. 1). The AP threshold was the voltage at which all-or-nothing firing occurred and was defined as the point at which dV/dt reached a critical level. The AP peak was the most depolarized potential reached during an AP. AP duration was the spike width measured at the potential midway between AP peak and AP threshold. The maximum rates of depolarization and repolarization and their times relative to AP peak were measured between the AP threshold and AP peak. The afterhyperpo-
larization potential (AHP) was the minimum potential recorded shortly after the AP peak. The resting membrane potential (RMP) was measured \( \sim 30 \text{ s} \) before test currents were injected. Input resistance (IR) was calculated from the average change in membrane potential caused by small negative current pulses administered at the RMP (5 pulses, \(-50\text{-pA amplitude}, 300\text{-ms duration})). The resulting changes in membrane potential occurred rapidly and, once established, were stable for the duration of the test pulse. This suggests hyperpolarizing pulses did not activate membrane currents (Sakmann and Neher 1995). Many neurons fired a single AP, so we report the number of APs (“AP number”) during 300-ms test pulses, rather than firing frequencies. Soma diameter was measured at the start of each recording using either an eyepiece graticule or digital photographs (Pixera Penguin 150CLM; Pixera, San Jose, CA); somata were categorized as \( 5–10, 10–15, \) or \( 15–20 \mu\text{m} \) in diameter.

We used several algorithms to separate the recorded neurons into groups with similar properties. Initially, neurons recorded in control conditions were examined using hierarchical cluster analysis, a method that avoids a priori assumptions about the number of groups present (Hill and Lewicki 2006; Manley 2008). Briefly, vectors described the position of individual objects (neurons) in a multidimensional space that was defined by separate axes for each parameter. Squared Euclidean distances were then used to group spatially close neurons, first individually and then as groups. We used Ward’s method for making these mergers, whereby groups were formed that yielded the least possible increase in the within-group sum of squares (Ward 1963). Parameters were normalized between 0 and 1 before vectors were calculated. Data were also analyzed using the K-means clustering algorithm (Hartigan and Wong 1979; MacQueen 1967), whereby objects are grouped into the number of clusters requested (K). Unlike Ward’s method, the K-means algorithm is iterative, so suboptimal categorizations of neurons can be corrected during analysis. Clusters were generated for increasing values of K, with analysis repeated at each value (\( > 100 \) replicates) to account for the effect of differences in the randomly generated starting point.

Silhouette plots (Rousseeuw 1987) were used to evaluate the optimum value of K (Karagiannis et al. 2009), whereby the average distance of a given neuron from members of the same cluster is compared with its distance from members of the next closest cluster:

\[
S(t) = \frac{b(t) - a(t)}{\text{Max}[a(t), b(t)]}
\]

where \( S(t) \) is the silhouette value, \( a(t) \) is the average distance of point \( i \) from members of group \( A \), and \( b(t) \) is the average distance from members of group \( B \). The inequality \( -1 \leq S(t) \leq 1 \) holds, and values approaching \(-1\) indicate misclassification. The average of \( S(t) \) across the whole data set, the silhouette width \( S(A) \), indicates cluster quality. Silhouette values were also used to compare the importance of correlations between cluster parameters in determining groups. If correlations between parameters were incidental, the average cluster quality would be unaffected by randomizing the data set (Helmstaedter et al. 2009). Data were randomized within parameters, thereby changing intermeasurement relationships without altering mean values or standard deviations.

Discriminant analysis was used to establish the relative importance of parameters in determining group membership (Huberty 1994; Lachenbruch and Goldstein 1979). Cluster analysis was repeated using only strongly discriminating parameters to establish the minimum set of parameters required to accurately categorize neurons. This minimum set of parameters was then used to calculate a classification function that could be used to categorize future recordings (Halabisky et al. 2006). This classification function had the following general form:

\[
P_g = C + (W_{11}X_1) + (W_{12}X_2) + \ldots + (W_{mn}X_m)
\]

where \( P \) is the classification function of group \( t \), \( C \) is a constant, \( W \) is the calculated Fisher coefficient for the parameters \( 1, 2, \ldots, m \), and \( X \) is the parameter’s value. Classification functions were calculated for the groups formed by cluster analysis, with parameters having different Fisher coefficients in each function. Following the recommendations of Hill and Lewicki 2006, individual neurons were assigned to the group whose classification function yielded the highest numerical value. Neurons were assigned to the same groups when classified on the basis of Mahalanobis distance (Mahalanobis 1936; McLachlan 2004), and classification was robust to leave-one-out analysis (Hill and Lewicki 2006).

ANOVA tests were used to examine cross-group differences if the underlying assumptions were met (Hill and Lewicki 2006); subsequent pairwise comparisons were made using Dunnett’s tests if variances were equal (Dunnett 1955) or using Games-Howell tests if variances were unequal (Games and Howell 1976). Parameters unsuitable for parametric analysis were compared using Kruskal-Wallis and Kolmogorov-Smirnov Z-tests (Hill and Lewicki 2006). Data measurements were made using Sigmal 3.14 (Cambridge Electronic Design) and Minitab 13 (State College, PA). Statistical tests, hierarchical cluster analysis, and discriminant analysis were done using SPSS 17 (SPSS, Chicago, IL); K-means cluster analysis and silhouette plots were done using MATLAB (The MathWorks, Natick, MA). Graphs were prepared using SigmaPlot 10.0 (Systat Software, Hounslow, UK). Data are means and SE; \( n \) indicates the sample size.

Classification. The aim of this study was to investigate the currents underlying the different firing patterns of two classes of spinal neuron by using drugs to block specific currents. Current-clamp recordings were made from 376 neurons in the dorsal spinal cord, where most neurons are skin-sensory RB neurons or sensory-pathway DL neurons (Li et al. 2001, 2004b; Sautois et al. 2007). A total of 61 recordings in control conditions were used in analyses designed to establish the minimal criteria required to reliably differentiate RB and DL neurons. Forty of these neurons were used in the initial analysis, with the remaining 21 used to verify the accuracy of the predictive algorithm.

First, we tested the null hypothesis that single and repetitive firing patterns are seen in an otherwise homogenous population. To do this, we used unsupervised hierarchical cluster analyses to group similar neurons without making a priori assumptions about the sample structure. Unsupervised cluster analyses have been used extensively to
define neuron classes (Garrido-Sanabria et al. 2007; Halabisky et al. 2006; Helmstaedter et al. 2009; Karube et al. 2004; Li et al. 2001; Prescott and De Koninck 2002). We used 12 parameters in our unsupervised cluster analysis, and the resulting dendrogram revealed the sample formed two large clusters, separated by a substantial distance (Fig. 2). The two-group classification was supported by the agglomeration schedule and suggested the control sample contained two physiologically distinct groups of neurons. Notably, single and repetitive firing patterns were segregated between the two classification groups. To validate this classification, we compared alternative clusterings of the same data produced by the K-means algorithm (Hartigan and Wong 1979; MacQueen 1967). This iterative method allows suboptimal categorizations of neurons to be corrected during analysis, unlike in hierarchical cluster analysis. However, because the final number of clusters is specified before analysis, it is important to carefully evaluate the effects of changing this quantity. We examined the effect of changing the number of clusters using silhouette plots, where width is proportional to cluster quality (see METHODS and Rousseeuw 1987). These plots confirmed that our sample data were best described by two groups (Fig. 2B: K = 2, 0.72; K = 3, 0.53; K = 4, 0.52; K = 5, 0.52; K = 6, 0.45; K = 7, 0.44).

Many parameters used in these cluster analyses correlated with other parameters. Although correlations were relatively small ($r = \sim 0.1$–0.4), they were also widespread, which prevented the exclusion of individual parameters. It was important to establish whether these correlations reflected genuine biological relationships or simply coincidental associations. We therefore randomized the data set and repeated the K-means analyses; over multiple iterations, coincidental correlations should be as numerous in the randomized data set as in the original data (Helmstaedter et al. 2009; Karagiannis et al. 2009). After randomization, the average silhouette plot was significantly reduced in width [K = 2: original = 0.72 ± 0.19 (SE) vs. randomized = 0.43 ± 0.11; $P < 0.0001$].

Next, we developed a predictive discriminant function to categorize future recordings as either group I or group II neurons using a minimal number of measurements (see METHODS). First, the parameters used in clustering were entered into a discriminant analysis to determine their relative importance in determining group membership. The resulting discriminant function coefficients indicated the most strongly discriminating variables were IR (−0.751), RMP (−0.409), AP threshold (0.395), soma size (0.465), and AP duration (0.600) (Supplemental Table S1). (Supplemental Material for this article is available online at the Journal of Neurophysiology website.) Of these, we used RMP, IR, and soma size to calculate a predictive discriminant function (associated Fisher coefficients are shown in Supplemental Table S2). These three measurements were all very quick and easy to make. We
did not use AP threshold or AP duration in this analysis because preliminary work had shown these values were drug sensitive and therefore would be less useful during subsequent studies of drug effects.

The predictive discriminant function significantly predicted group membership (Wilk’s lambda, \( P < 0.001 \), classified all neurons to the same group as the cluster analysis, and was unaffected by leave-one-out analysis (group I = 100%; group II = 100%). We examined the validity of the predictive function further by using it to categorize an additional sample of 21 neurons recorded in control conditions. When these neurons were included in a combined cluster analysis with the original group of 40 control neurons, their categorization was the same as predicted. This is illustrated by the dendrogram in Fig. 3A. Also illustrated (Fig. 3B) is the numerical difference between the classification scores calculated for each neuron using the two classification functions. The size of this number indicates the security of the neuron’s classification. Notably, even

Fig. 3. A: hierarchical cluster analysis based on 3 parameters. Sixty-one neurons recorded in control conditions were clustered on the basis of soma size, RMP, and IR. Group I (blue circles) and group II neurons (red squares) used in the 12-parameter cluster analysis (Fig. 2) are shown, alongside additional neurons used to examine the reliability of the classification procedure (yellow circles and squares). These additional neurons are labeled with the group predicted by the predictive discriminant function; in all cases, their position in the dendrogram matches the predicted position. B: for predictive categorization, 2 classification scores were calculated for each neuron; neurons were assigned the group whose classification function yielded the highest score. The greater the difference between the 2 scores, the more reliable the categorization. The smallest difference between scores is seen in group I neurons close to the group II cluster.
when classification scores were similar, cluster analysis showed that neurons were correctly classified. The size of the difference between classification scores was monitored when drugs were used, because this could indicate that classification became less reliable.

RESULTS

Neurons in the hatching X. laevis spinal cord. Twelve neuron classes have been identified in vivo in the X. laevis spinal cord at the developmental stage examined in this study, using electrophysiological and anatomic methods (Li et al. 2007; Sautois et al. 2007). Of these, RB neurons (Clarke et al. 1984; Li et al. 2003) and DL interneurons (dIN) fire a single AP when depolarizing current is injected. Five other classes of neuron in the Xenopus spinal cord fire repetitively in response to current injection: motoneurons (Li et al. 2002), ascending (aIN) inhibitory interneurons (Li et al. 2002), and three classes of sensory pathway interneurons, dorsolateral commissural (dlc) (Li et al. 2003), dorsolateral ascending (dla) (Li et al. 2004b), and excitatory commissural interneurons (ecIN) (Li et al. 2007). The connections between these neurons were recently reviewed (Roberts et al. 2010), and the detail of this understanding may strike readers from other fields. Differences have been reported in the onset of firing and rates of adaptation, but these were not considered in the current study, in which we compared the properties of single- and repetitive-firing neurons.

Properties of group I and II neurons. Neurons in the Xenopus spinal cord differ in their morphological and electrical properties (Li et al. 2001; Sautois et al. 2007). In dorsal regions, large neuron somata (∼20 μm; Fig. 4A) projected from the spinal cord and typically fired a single AP within a few milliseconds of current injection (Fig. 4, B–E). When larger currents were injected, most of these neurons continued to fire a single AP, although a subset fired one or two additional APs (Fig. 4E). Neuron somata in more ventral regions were smaller (∼8 μm) and contained fewer yolk platelets (Fig. 4B). These neurons fired repetitively following current injection, with the number of APs positively correlated with current amplitude (Fig. 4, F and G). Some features of repetitive-firing neurons were heterogeneous: in some neurons the membrane potential rose rapidly during interspike intervals (Fig. 4F), whereas in others the potential had a plateau phase (Fig. 4G); in many neurons APs were smaller after the first spike (Fig. 4, E and G). Compared with repetitive-firing neurons, individual APs in single-firing neurons were longer in duration and had more depolarized thresholds (Fig. 4H; see Table 2).

Cluster analysis indicated the control sample contained two groups, neurons that fired a single AP on depolarization and neurons that fired repetitively. As a first step toward understanding the basis of single and repetitive firing patterns, we compared values of the parameters that were not required to classify neurons. Mean values for the parameters used in classification are presented in Table 1, and properties based on spike measurements for each group are presented in Table 2. In single-firing group I neurons, the rheobase current was significantly higher, the AP threshold was significantly more depolarized, and AP duration was significantly longer. The maximum rates of depolarization and repolarization were significantly higher in group II. The AP peak and the AHP were not significantly different between groups.

![Fig. 4. Firing patterns of group I and II neurons. A: photograph of the neuron in which the activity illustrated in C was recorded. The view is from the tadpole’s right side and shows the neuron somata projecting from the spinal cord and in contact with the recording pipette; the top of the image is dorsal and the right side is rostral. B: photograph of the neuron in which the activity illustrated in F was recorded. The somata is smaller than that in A and contains fewer yolk platelets. C–E: firing in group I neurons is illustrated in response to 300-ms current injections at rheobase (top trace) and at 3 times this amplitude (3×; bottom trace); the rheobase varied between neurons and was not analyzed. Many dorsal neurons fired a single AP at both current amplitudes. In E, a subset of group I neurons fired a single AP at rheobase and 1 or 2 additional APs at higher current levels. F and G: repetitive firing in group II neurons fired repetitively at rheobase (top traces), with more APs fired at higher current levels (3×; bottom traces). H: superimposed traces show single-firing neurons (shaded line) had long-duration APs and were typically preceded by a relatively slow depolarizing phase, whereas repetitive-firing neurons (black line) had shorter duration APs and lower thresholds.](http://jn.physiology.org/doi/10.1152/jn.00579.2010/supplement)
Table 1.  Mean and median values used for categorization

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>$-70.2 \pm 0.8$</td>
<td>$-58.1 \pm 0.7$</td>
</tr>
<tr>
<td>IR, MΩ</td>
<td>$348.3 \pm 33.6$</td>
<td>$1582.9 \pm 53.5$</td>
</tr>
<tr>
<td>Soma diameter, μm</td>
<td>15–20</td>
<td>5–10</td>
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</table>

Values are means ± SE (n = no. of neurons) for resting membrane potential (RMP) and input resistance (IR), whereas soma diameter is expressed as the median size category, for group I and II neurons.

2000). Nonetheless, comparisons with other previous studies do allow inferences to be drawn about the identity of the neurons we examined.

Only two classes of Xenopus spinal neuron fire a single AP when depolarizing current is injected, RB neurons (Clarke et al. 1984; Li et al. 2003) and dIN neurons (Li et al. 2007; Sautois et al. 2007). Both anatomic and physiological evidence suggests that group I primarily contained RB neurons. Regarding anatomy, group I neurons were recorded from the cut dorsal edge of the spinal cord and had large spherical somata. Such features are characteristic of RB neurons, which form a nearly continuous column near the dorsal surface of the spinal cord (Clarke et al. 1984; Li et al. 2003; Roberts et al. 2000). The somata of dIN neurons are generally smaller than RB somata, lie more ventrally in the spinal cord and are not found in a dorsolateral position (Clarke et al. 1984; Li et al. 2007). A previous study (Sautois et al. 2007) showed that some physiological properties of RB and dIN neurons are similar, including IR, AP duration, AP threshold and AHP. However, other physiological differences between the group I neurons that we recorded and dIN neurons recorded previously (Sautois et al. 2007) further suggest that our sample mainly contained RB neurons. Specifically, compared with the dIN neurons reported by Sautois et al. (2007), group I neurons had a more negative RMP (group I = $-70.2$ mV vs. dIN = $-51$ mV), a more depolarized AP peak (group I = $44.8$ mV vs. dIN = $29$ mV), and a more rapid rate of depolarization (group I = $140.8$ mV/ms vs. dIN = $85$ mV/ms). In combination, these comparisons suggest that group I neurons were primarily RB neurons and can reasonably be referred to as such.

Neurons lying in the dorsolateral spinal cord comprise several previously characterized classes of Xenopus neurons: excitatory dla (Li et al. 2004b), dlc (Li et al. 2003), and eclN (Li et al. 2007) neurons and inhibitory aIN neurons (Li et al. 2002). All four of these neuron classes fire repetitively and have similar somata size and many similar physiological properties (Sautois et al. 2007). Because we cannot securely distinguish group II neurons as members of a single neuron class, we termed group II neurons “DL” to reflect their dorsolateral location.

Drug-induced changes in firing patterns. Next, we compared drug-induced changes in the firing patterns of RB and DL neurons as a first step toward understanding how differences in channel expression underlie contrasting firing patterns. We describe results seen with potassium channel blockers, calcium channel blockers, and muscarine.

Potassium channel blockers. Potassium currents define firing patterns in many neurons (Connor and Stevens 1971b; Erisir et al. 1999; Reyes et al. 1994; Yarom et al. 1985). We examined the role of the delayed rectifier current with 10 mM TEA (Barish 1986; Ribera and Spitzer 1990) and the role of transient potassium currents with the blockers MGX (1 nM) (Garcia-Calvo et al. 1993; Knaus et al. 1995), 4-AP (2 mM) (Connor and Stevens 1971b; Ribera and Spitzer 1990), and catechol (10 μM) (Ito and Maeno 1986; Kuenzi and Dale 1998). Changes in the properties of RB and DL neurons caused by these drugs are summarized in Table 3; RMP and IR, properties used to classify neurons, were unaltered by drugs. We also compared the average difference between classification scores for RB and DL neurons to see whether drugs reduced the difference between scores; there were no significant effects in either population, although the SE was increased in DL neurons (see Supplemental Fig. S1).

TEA converted RB neurons from firing a single AP to firing repetitively (Fig. 5, A and B). In contrast, TEA reduced the firing frequency of DL neurons (Fig. 5, A and B). TEA increased AP duration in both RB and DL neurons, although at the first spike, this effect was only statistically significant in RB neurons; APs increased in duration over the course of activity in both cell types. TEA significantly reduced the maximum rate of repolarization in RB neurons and reduced the AHP in both neuron classes. 4-AP reduced firing frequencies and the rheobase current in DL neurons (Fig. 5, A and C) and shifted the AP threshold to more negative potentials in both RB and DL neurons. AP duration was increased in RB and DL neurons by 4-AP, but the change was not statistically significant. 4-AP significantly reduced the maximum rate of repolarization in DL neurons but did not alter repolarization in RB neurons. Catechol had no effect on RB neurons, but in DL neurons it reduced the number of APs, the rheobase current, the AP threshold, and the maximum rate of repolarization (Fig. 6, A and B). In catechol, there was no change in DL neuron AP duration. MGX reduced the firing frequency in DL neurons (Fig. 6, A and C) but did not significantly alter any of the parameters measured in RB neurons. MGX reduced the AP threshold and increased AP duration in RB neurons. In DL neurons, MGX reduced AP duration, the AP peak, and the maximum rate of repolarization. None of the potassium blockers used altered IRs or RMPs.

Effects of calcium channel and M-current blockers. Amiloride (40 μM), a blocker of low-voltage-activated T-type calcium

Table 2.  Properties of group I and II neurons in control conditions

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Number of APs</td>
<td>1</td>
<td>6.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rheobase current, mV</td>
<td>299.3 ± 16.2</td>
<td>112.9 ± 9.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>$-20.8 \pm 1.4$</td>
<td>$-25.5 \pm 1.2$</td>
<td>0.018</td>
</tr>
<tr>
<td>AP peak, mV</td>
<td>44.8 ± 1.4</td>
<td>45.9 ± 1.4</td>
<td>0.579</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maximum rate of depolarization, mV/ms</td>
<td>140.8 ± 6.4</td>
<td>156.5 ± 6.1</td>
<td>0.041</td>
</tr>
<tr>
<td>Maximum rate of repolarization, mV/ms</td>
<td>53.1 ± 4.3</td>
<td>80.1 ± 3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>$-47.6 \pm 1.6$</td>
<td>$-50.3 \pm 1.6$</td>
<td>0.255</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. of neurons) with the exception of the number of action potentials (APs), which are median values. Parameters were compared using independent sample t-tests, except for the number of APs, where a Kolmogorov-Smirnov Z-test was used. P values are given for group I vs. group II. AHP, afterhyperpolarization potential.
channels (Tang et al. 1988), increased the rheobase current in RB neurons (Table 4), but firing patterns in response to long-duration pulses (300 ms) were unaltered in both RB and DL neurons. Amiloride reduced the AP peak slightly in RB and greatly in DL neurons (Table 4 and Fig. 7, A–D). In DL neurons, amiloride reduced the AP duration and the maximum rate of depolarization. In RB neurons, amiloride hyperpolarized the AHP.

Nimodipine (20 μM), a blocker of high-voltage-activated L-type calcium channels (Mogul and Fox 1991), reduced the AP peak in RB neurons and DL neurons (Table 4 and Fig. 7, A, B, E, and F). In DL neurons, nimodipine reduced the AP duration and the maximum rate of depolarization and had a variable effect on AP number: firing was reduced in 2/5 neurons, an effect that was not statistically significant.

Muscarine (10 μM), which antagonizes M-currents via an incompletely understood intracellular pathway (Jentsch 2000; Marrion 1997), slightly reduced AP duration in RB neurons (Table 5). In DL neurons, muscarine reduced the number of APs (Fig. 8) and the rheobase current.

DISCUSSION

This study had two main aims. First, we used analytical techniques to establish whether a few parameters could reliably distinguish single- and repetitive-firing neurons in vivo. Second, we examined drug-induced changes in neuron properties to gain insights into which membrane currents determine firing patterns.

Neuron classification. Many in vivo investigations have characterized neurons in the Xenopus tadpole spinal network in terms of their morphology, transmitter type, and synaptic interactions (Aiken et al. 2003; Li et al. 2002, 2003, 2004b). Such extensive measurements are time consuming and limit the time available during a short-term whole cell recording to characterize the intrinsic membrane properties that determine

### Table 3. Effects of potassium channel blockers on properties of RB and DL neurons

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TEA</th>
<th>4-AP</th>
<th>Catechol</th>
<th>MGX</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>13</td>
<td>16</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>IR, MΩ</td>
<td>248.3</td>
<td>330.7</td>
<td>309.9</td>
<td>343.6</td>
<td>324.7</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>−70.2</td>
<td>−73.8</td>
<td>−70.7</td>
<td>−68.3</td>
<td>−69.4</td>
</tr>
<tr>
<td>Number of APs</td>
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<td>4.0†</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Rheobase current, pA</td>
<td>229.3</td>
<td>270.6</td>
<td>228.7</td>
<td>283.8</td>
<td>165.0</td>
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<tr>
<td>AP threshold, mV</td>
<td>−20.8</td>
<td>−15.4</td>
<td>−31.7†</td>
<td>−27.8</td>
<td>−32.8†</td>
</tr>
<tr>
<td>AP peak, mV</td>
<td>44.8</td>
<td>51.7</td>
<td>45.1</td>
<td>40.3</td>
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<tr>
<td>AP duration, ms</td>
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<td>1.8</td>
<td>1.1</td>
<td>1.9*</td>
</tr>
<tr>
<td>Maximum rate of depolarization, mV/ms</td>
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<td>118.4</td>
<td>147.7</td>
<td>163.1</td>
<td>156.3</td>
</tr>
<tr>
<td>Maximum rate of repolarization, mV/ms</td>
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<td>23.8†</td>
<td>52.4</td>
<td>65.0</td>
<td>51.5</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>−47.6</td>
<td>−29.7†</td>
<td>−55.5</td>
<td>−52.4</td>
<td>−54.2</td>
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<td>13</td>
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<td>14</td>
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<td></td>
<td>1582.9</td>
<td>1303.3</td>
<td>1578.3</td>
<td>1454.3</td>
<td>1786.3</td>
</tr>
<tr>
<td></td>
<td>112.9</td>
<td>82.0</td>
<td>73.1*</td>
<td>67.1*</td>
<td>165.0</td>
</tr>
<tr>
<td></td>
<td>−25.5</td>
<td>−26.1</td>
<td>−37.4†</td>
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<td>45.4</td>
<td>41.3</td>
<td>46.9</td>
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<tr>
<td></td>
<td>0.9</td>
<td>2.0</td>
<td>1.7</td>
<td>1.0</td>
<td>1.9*</td>
</tr>
<tr>
<td></td>
<td>156.5</td>
<td>135.6</td>
<td>137.4</td>
<td>141.6</td>
<td>156.3</td>
</tr>
<tr>
<td></td>
<td>80.1</td>
<td>57.1</td>
<td>45.7†</td>
<td>40.3†</td>
<td>51.5</td>
</tr>
<tr>
<td></td>
<td>53.1</td>
<td>23.8†</td>
<td>52.4</td>
<td>65.0</td>
<td>51.5</td>
</tr>
</tbody>
</table>

Mean values in control conditions and in the presence of drugs are compared for each parameter, with the exception of AP number, where the median value is reported. IR and RMP were used to classify neurons and were unaltered by the drugs used. *P value between 0.0001 and 0.05; †P value <0.0001.

![Fig. 5. Effects of tetraethylammonium (TEA) and 4-aminopyridine (4-AP) on firing patterns of Rohon-Beard (RB) and dorsolateral (DL) neurons during 300-ms rheobase current injection. A: control. RB neurons (left) fired a single AP, whereas DL neurons (right) fired repetitively. B: TEA, 10 mM. RB neurons fired repetitively, with AP duration increasing over the course of activity. DL neurons fired fewer APs than in control conditions. These examples, and those in C, are from different neurons than those used to illustrate firing in control conditions. C: 4-AP, 2 mM. RB neurons fired a single AP; in DL neurons, firing frequency was reduced. In both RB and DL neurons, 4-AP increased AP duration.](http://jn.physiology.org/)
properties that would be unaltered by drugs and could therefore be used in a wide range of studies.

We found that single- and repetitive-firing neurons could be distinguished independently of their firing patterns using measurements of just three properties: neuron size, IR, and RMP (Figs. 2 and 3). A predictive classification function based on all three parameters reliably classified neurons in the original sample and neurons from an additional separate sample. The implications of several technical aspects that affect these analytical techniques were discussed above, but one key issue remains: the significant correlation between IR and neuron size. The accuracy with which groups are established using cluster analysis may be reduced if subsets of parameters are highly correlated (Helmstaedter et al. 2009; Hill and Lewicki 2006; Manley, 2008). However, the reduced classification accuracy we observed when only one or two of these parameters were used suggests this was not the case.

Behavioral significance of RB and DL neuron firing patterns. There were significant differences between RB and DL neurons in properties that were not used for classification. RB neurons repolarized more slowly than DL neurons and had longer duration APs, suggesting each class of neuron expressed different channel types, or similar channel types at different densities. During development, the duration of APs in RB neurons decreases (Baccaglini and Spitzer 1977), and there is likely to be a corresponding reduction in their synaptic output (Hochner et al. 1986; Wall and Dale 1994). However, the relatively long AP duration we observed may contribute to the ability of a single action potential in a single RB neuron to initiate swimming (Clarke et al. 1984; Li et al. 2003; Sautois et al. 2007). The highly negative RMP and depolarized AP thresholds of RB neurons could be adaptations for regulating the excitability of the neurons, which act as the gatekeepers that determine whether tactile stimuli initiate behavioral responses.

The DL group of neurons may comprise several classes of neurons (see above), and each has different roles in behavior (Li et al. 2007; Sautois et al. 2007). As such, repetitive firing must have different functions in each neuron class. Notably, neurons fire repetitively during stronger, slower, struggling movements, which are probably initiated by the recruitment of multiple RB neurons during large and sustained stimuli (Soffe 1997) and which in turn drives repetitive firing in eClN neurons (Li et al. 2007). A specific role in struggling is also likely to underlie the repetitive firing of inhibitory aIN neurons (Li et al.

Table 4. Effects of calcium channel blockers on properties of RB and DL neurons

<table>
<thead>
<tr>
<th></th>
<th>RB</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Amiloride</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Amiloride</td>
</tr>
<tr>
<td>Number of APs</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Number of APs</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Rheobase current, pA</td>
<td>229.3</td>
<td>427.1†</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>−20.8</td>
<td>−26.8</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>−20.8</td>
<td>−26.8</td>
</tr>
<tr>
<td>AP peak, mV</td>
<td>44.8</td>
<td>32.9*</td>
</tr>
<tr>
<td>AP peak, mV</td>
<td>44.8</td>
<td>32.9*</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Maximum rate of depolarization, mV/μs</td>
<td>140.8</td>
<td>154.6</td>
</tr>
<tr>
<td>Maximum rate of repolarization, mV/μs</td>
<td>53.1</td>
<td>59.9</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>−47.6</td>
<td>−62.9*</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>−47.6</td>
<td>−62.9*</td>
</tr>
</tbody>
</table>

Mean values in control conditions and in the presence of drugs are compared for each parameter, with the exception of AP number, where the median value is reported. *P value between 0.0001 and 0.05; †P value <0.0001.
An individual action potential in an RB neuron will evoke a single action potential in sensory pathway dlc and dla interneurons and leads to swimming (Li et al. 2003, 2004b). As soon as the swimming pattern is established, as well as during struggling, dlc and dla interneurons are inhibited. The need for repetitive firing in dla and dlc neurons is therefore unclear. It is possible that very brief repetitive firing in dla and dlc neurons helps to ensure the initiation of swimming and helps to amplify the skin stimulation signal (Li et al. 2007). More speculatively, changes in the repetitive firing frequency of dla and dlc neurons could provide a mechanism for coding stimulus strength, as seen in other systems (Connor and Stevens 1971a). A final possibility is that the repetitive firing pattern is misleading and is never actually seen in response to natural stimuli. The ability of dla and dlc neurons to fire repetitively in response to unnatural stimuli could arise as a secondary consequence of other more important features, for example, the short duration of the action potential.

Potassium channel blockers. We compared the effects drugs had on the properties of RB and DL neurons and found potassium channels defined different aspects of firing in each class of neuron. TEA converted RB neurons from firing single APs to firing repetitively; no other drug had such dramatic effects on RB neurons, but TEA has had similar effects in other preparations (Brodin and Grillner 1986; Schwarz and Puil 1997). This result indicates that in RB neurons, TEA-insensitive membrane currents are sufficient for repetitive firing and that such repetitive firing is normally prevented by TEA-sensitive currents. Voltage-clamp recordings from *Xenopus* neurons in vitro suggest these TEA-sensitive channels pass a delayed rectifier potassium current (O’Dowd et al. 1988; Ribera and Spitzer 1990; Wall and Dale 1994). The AHP peak was reduced by TEA in both RB and DL neurons (Table 3 and Fig. 5). Similar effects have been reported previously in neurons

| Table 5. Effects of muscarine on properties of RB and DL neurons |
|---------------|--------|--------|--------|
|               | RB     | Muscarine | DL     | Muscarine |
| n             | 36     | 11      | 28     | 7        |
| Number of APs | 1.0    | 1.0     | 6.5    | 3.0*     |
| Rheobase current, pA | 229.3 | 246.5  | 112.9  | 61.5†    |
| AP threshold, mV | −20.8 | −28.9  | −25.5  | −27.2    |
| AP peak, mV    | 44.8   | 37.5    | 45.9   | 44.2     |
| AP duration, ms| 1.2    | 1.0*    | 0.87   | 0.77     |
| Maximum rate of depolarization, mV/ms | 140.8 | 162.1  | 156.5  | 143.0 |
| Maximum rate of repolarization, mV/ms | 53.1  | 57.4    | 80.1   | 72.8     |
| AHP peak, mV | 47.6  | 49.6    | 50.3   | 30.8     |

Mean values in control conditions and in the presence of drugs are compared for each parameter, with the exception of AP number, where the median value is reported. *P value between 0.0001 and 0.05; †P value <0.0001.
MGX reduced the AP threshold (Table 3). Like the majority of which also blocks transient potassium currents (Garcia-Calvo 1992). El Manira 2001; Ito and Maeno 1986; Sah and McLachlan neurons (Kuenzi and Dale 1998) and in other species (Hess and
membrane currents. There is some evidence that 4-AP blocks negative shift in AP threshold.

First, they reduced the rate of repolarization (Table 3; Figs. 5 and 6). The reduced firing of DL neurons in the presence of potassium blockers suggests that the currents mediated by these channels are essential for high-frequency repetitive firing, and such a role in repetitive firing has been clearly shown in lamprey spinal neurons (Hess and El Manira 2001).

In addition to reducing the number of action potentials, 4-AP and catechol affected several other properties of DL neurons. First, they reduced the rate of repolarization (Table 3; Figs. 5 and 6); only TEA altered this parameter in RB neurons, suggesting different channels mediate repolarization in each class of neuron. Second, 4-AP and catechol shifted the AP threshold to more negative values (Table 3; Figs. 5 and 6). This suggests that 4-AP, which had the same effect in RB neurons, blocks a current that plays a role in preventing the onset of depolarization; similar effects have been reported in other studies (Segal et al. 1984; Tell and Bradley 1994; Vydyanathan et al. 2005). Finally, 4-AP and catechol reduced the rheobase current (Table 3; Figs. 5 and 6), an effect compatible with the negative shift in AP threshold.

Given the similarity of the effects of 4-AP and catechol, it is likely they block the same channel type, and therefore similar membrane currents. There is some evidence that 4-AP blocks transient potassium currents in Xenopus neurons (Ribera and Spitzer 1990) and considerable evidence of such an effect in other preparations (Catacuzzeno et al. 2008; Risner and Holt 2006; Vydyanathan et al. 2005). Catechol has also been reported to block a transient potassium current in Xenopus neurons (Kuenzi and Dale 1998) and in other species (Hess and El Manira 2001; Ito and Maeno 1986; Sah and McLachlan 1992).

The final potassium channel blocker to consider is MGX, which also blocks transient potassium currents (Garcia-Calvo et al. 1993; Knaus et al. 1995). In both RB and DL neurons, MGX reduced the AP threshold (Table 3). Like the majority of potassium channel blockers, MGX increased the AP duration in RB neurons (Table 3), suggesting the MGX-sensitive current aids repolarization. However, in DL neurons, MGX was unique among potassium channel blockers in reducing AP duration. Given this effect in DL neurons, it is surprising that MGX also reduced the overall number of APs and the rate of repolarization (Table 3; Fig. 6). Such effects are difficult to reconcile but may relate to the associated reduction in the AP peak. We are not aware of other studies that might allow us to propose a mechanistic basis for these results.

In conclusion, these data suggest that potassium currents prevent repetitive firing in RB neurons, whereas currents with different pharmacological sensitivity enable fast repolarization in DL neurons. It must be a priority for future work to examine these currents directly and to identify their molecular basis. One clear direction for these investigations is provided by the observation that TEA antagonism is characteristic of Kv1.1 channels (Alexander et al. 2008; Hopkins 1998) and that RB neurons at stage 37/38 express xKv1.1 mRNA, the Xenopus homolog of mammalian Kv1.1 (Ribera and Nguyen 1993). Candidate channels in DL neurons include members of the Kv3 family, which underlie high-frequency repetitive firing in other systems (Baranauskas et al. 2003; McKay and Turner 2004).

These and many other studies have also highlighted the important roles played by accessory subunits in regulating channel activity. In Xenopus, xKv1.1 mRNA expression spans the locations of RB and DL soma (Ribera and Nguyen 1993), whereas the expression of xKvβ4 β-subunits is restricted to regions that contain DL soma (Lazaroff et al. 1999). xKvβ4 dramatically alters the properties of heterologously expressed xKv1.1 currents (Lazaroff et al. 2002); could it be that localized expression of xKvβ4 also underpins the differentiation of firing patterns?

Calculated and M-current blockers. In RB neurons, amiloride increased the rheobase current injection and reduced the AP peak (Table 4; Fig. 7). Because amiloride blocks low-voltage-activated T-type calcium channels (Fox et al. 1987; Tang et al. 1988), this suggests currents carried by T-type channels contribute to depolarization in stage 37/38 RB neurons; such a role has been reported in Xenopus RB neurons at stage 42 (Sun and Dale 1997). In DL neurons, amiloride also affected depolarization, greatly reducing the AP peak and the rate of repolarization (Table 4 and Fig. 7). AP duration was also reduced in DL neurons, presumably reflecting the fact APs did not overshoot in the presence of amiloride.

Nimodipine blocks high-voltage-activated L-type calcium channels (Mogul and Fox 1991), and these may play a role in the calcium-dependent excitability that emerges early in the development of Xenopus neurons (Baccaglini and Spitzer 1977). However, despite RB neurons originating during gastulation (Lamborghini 1980), nimodipine had little effect on their properties: a small reduction in the AP peak (Table 4 and Fig. 7). In DL neurons, nimodipine had remarkably similar effects to amiloride, shortening the AP duration, reducing the maximum rate of depolarization, and lowering the AP peak. These data suggest current passed by L-type calcium channels plays a key role in initiating and sustaining depolarization in DL neurons; in the absence of these currents, neurons repolarize more rapidly, shortening APs. The fact that nimodipine did not reduce the rate of repolarization in RB neurons may indicate that sodium, rather than calcium, is the principal
inward current that mediates depolarization in these neurons. An important limitation to such an interpretation is that the similar effects seen with amiloride and nimodipine in DL neurons could indicate a lack of selectivity.

M-currents can regulate neuronal excitability (Adams et al. 1982; Brown and Adams 1980), and we therefore examined the effects of muscarine, which indirectly blocks M-currents (Jentsch 2000; Marrion 1997). Firing in RB neurons was unaffected by muscarine, but AP duration slightly decreased (Table 5 and Fig. 8). This could result from muscarine acting nonspecifically to block L-type calcium channels (Allen and Burnstock 1990; Scroggs et al. 2001; Svirsksis and Hounsgaard 1998), the presence of which in these neurons is indicated by the nimodipine sensitivity described above. In DL neurons, muscarine reduced the rheobase current, suggesting an M-current prevents depolarization (Halliwell and Adams 1982; Madison and Nicoll 1984). More surprisingly, muscarine reduced firing in DL neurons (Table 5 and Fig. 8); more commonly, muscarine increases the number of stimulus-evoked APs (Aiken et al. 1995; Perez et al. 2009). This effect could result from blocking of L-type calcium channels but should be clarified using specific M-current blockers such as XE991 (Wang et al. 1998).

Conclusion. We have established that multivariate analysis enables neuronal cell types to be resolved and predictively classified using only a few simple measurements. Such methods are likely to be of particular interest to others who perform experiments in vivo or in other complex preparations. Using these techniques, we have identified several features of single- and repetitive-firing neurons that may underlie their distinctive firing patterns. We also found that potassium channels play a central role in differentiating neuronal firing patterns. In particular, a TEA-sensitive current prevents repetitive firing in RB neurons, whereas a 4-AP-sensitive current underlies repetitive firing in DL interneurons.

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We thank Drs. R. W. Meech and S.R. Soffe for help and advice at all stages of this work.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

References


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