The first known disparity between area X and mammalian striatum concerns its efferent connections—area X projects directly to the thalamus (Bottjer et al. 1989; Okuhata and Saito 1987), whereas the mammalian striatum communicates with the thalamus only through the globus pallidus and substantia nigra (Parent and Hazrati 1995). Furthermore, the projection neurons of area X are not spiny neurons, in sharp contrast to mammalian striatum, even though the vast majority of neurons in area X are spiny neurons and are electrophysiologically virtually identical to mammalian striatal spiny projection neurons (Farries and Perkel 2002b). Instead, the projection neurons of area X are a sparsely distributed population of relatively large cells that have few dendritic spines (Bottjer et al. 1989; Luo and Perkel 1999) and contain the palidal marker LANT6 (Reiner et al. 2004a). Farries and Perkel (2002b) identified a rare class of neurons in area X that morphologically resemble these cells and that electrophysiologically resemble a cell type found in the mammalian globus pallidus. They hypothesized that the thalamus-projecting cells of area X correspond to this “pallidum-like” cell type, effectively making area X a mixture of striatum and globus pallidus. This hypothesis received some confirmation when Farries et al. (2005) demonstrated that at least some pallidum-like cells do in fact project to the thalamus.

Given the highly specialized nature of area X, it is tempting to believe that this unusual organization is limited to the song system and does not reflect a more general divergence in the basal ganglia of birds and mammals. However, large regions of the songbird striatum outside of area X also contain pallidum-like neurons (Farries and Perkel 2000) and have morphologically similar cells that project directly to the thalamus (Farries and Perkel 2002a). Moreover, a direct striatothalamic projection is not unique to songbirds—parrots also have a striatal region that projects to the thalamus (Striedter 1994). This part of the parrot striatum is also implicated in vocal control and/or learning, suggesting that a striatothalamic projection could be specifically associated with the ability to learn complex vocalizations. However, the available evidence indicates that the vocal control systems of parrots and songbirds evolved independently (Farries 2001; Striedter 1994), arguing against this notion and implying that songbirds and parrots inherited their striatothalamic projections from a common avian plan shared by most birds. This possibility is further supported by evidence...
for a direct striatothalamic projection in the domestic chick, *Gallus domesticus* (Székely et al. 1994).

To test the hypothesis that a striatum/pallidum hybrid organization is common to many avian taxa and is not specifically linked to vocal learning, we sought to characterize the morphology and intrinsic electrophysiological properties of neurons in the basal ganglia of an avian species that does not learn its vocalizations. We selected the domestic chick for this task, in part because the evidence for an “anomalous” striatothalamic projection in that species (Székely et al. 1994) provides some a priori expectation that chick striatum might contain the pallidum-like neurons found in songbirds. This choice of species is also recommended by the fact that *Gallus* belongs to an avian taxon (order Galliformes) that is an outgroup to most other avian taxa (Sibley and Ahlquist 1990) and hence is well-placed to provide information about the ancestral features of avian striatum. Finally, the domestic chick is used as a model system for learned behaviors (passive avoidance, reinforcement learning) that involve the striatum (Csillag 1999; Izawa et al. 2001). Our data should be useful in understanding the physiological mechanisms underlying these forms of learning and may help identify mechanistic parallels between these learned behaviors and vocal learning in songbirds, parrots, and hummingbirds.

Some of these data were previously published in abstract form (Farries and Perkel 2002a).

**METHODS**

**Preparation of brain slices**

White leghorn chickens were hatched from eggs and incubated in E. W Rubel’s animal facility until used. Brain slices were prepared as described for zebra finches by Stark and Perkel (1999), and procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. Briefly, chicks were anesthetized with isoflurane and killed by decapitation. The brain was anesthetized with isoflurane and killed by decapitation. The brain was

**Histology and immunocytochemistry**

After recording, slices were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for ≥2 h (usually ≥12 h). Slices were then transferred to a 30% sucrose solution in 0.1 M PB for cryoprotection and resectioned to 50-μm thick with a freezing microtome. Biocytin-filled neurons were most often visualized by processing with the avidin/biotin/horseradish peroxidase kit (ABC Elite Kit, Vector Laboratories, Burlingame, CA) using diaminobenzidine (Sigma), the VIP kit (Vector Laboratories), or the NovaRED kit (Vector Laboratories) as the peroxidase substrate. Biocytin-filled neurons were sometimes visualized by incubation with Cy2- or Cy3-conjugated streptavidin (1:200, Jackson ImmunoResearch, West Grove, PA); this method was always used when visualization was paired with immunostaining. For ChAT immunostaining, sections were incubated in the primary antibody (1:100, Chemicon International, Temecula, CA) for 5 day at 4°C followed by a Cy2- or Cy5-conjugated secondary antibody (1:100, Chemicon) overnight at 4°C.

**Measurement of electrophysiological parameters**

Most basic electrophysiological parameters were measured as described in Farries and Perkel (2000). Because the membrane resistance of our recorded cells could depend strongly on membrane potential, we defined the input resistance in a manner independent of the potential at which the cell happened to rest. The input resistance was defined as the maximum membrane resistance (point of maximum slope) found on the steady state current-voltage relation at potentials more negative than −50 mV. We also calculated the ratio of maximum resistance to minimum resistance (“inward rectification ratio”), so the full range of membrane resistance exhibited by a neuron can always be reconstructed from the data we present. We measured two new electrophysiological parameters, burst index and sag index, that are not described in Farries and Perkel (2000). Those parameters are defined in RESULTS.

In our analysis, we sometimes computed a large number of linear regressions between measured parameters that we had no a priori reason to expect to be related. Because of the large number of calculations performed, a significance criterion of \( P = 0.05 \) for each linear regression can yield a high probability of finding at least one “significant” relationship (a slope significantly different from zero) where no real relationships exist. To account for this, we adjusted our criterion for significance for each linear regression to a level that would produce a probability of 0.05 for finding one or more significant relationships among a total of \( N \) regression calculations where no real relationships exist (Shaffer 1995). If \( p \) is the significance criterion for individual regressions, the probability \( P \) of finding one or more “false positives” is \( P = 1 - (1 - p)^N \). Using \( P = 0.05 \) and solving for \( p \), we obtain the desired significance criterion: \( p = 1 - 0.95^{1/N} \). For our data, this method agreed with the more commonly used Bonferroni correction. All linear regressions and statistical tests were performed using the PRISM program (GraphPad Software, San Diego, CA).

Values reported in the form \( X ± Y \) are mean ± SD.

**RESULTS**

In our primary set of experiments, we obtained stable recordings from a total of 165 neurons in the basal ganglia of chicks aged 4–20 days posthatch (34 birds), a sample comprising 149 cells in the striatum and 16 cells in the globus pallidus (formerly known as the “paleostriatum primitivum”) (see Reiner et al. 2004b). We focused our attention on the medial striatum (formerly the “lobe parolfactorius”) because this is the region of songbird striatum that is known with...
certainty to contain pallidum-like cells (Farries and Perkel 2000, 2002b). Of our recorded striatal cells, 124 were located in the medial striatum with the remainder (25 cells) in the lateral striatum (formerly the “paleostriatum augmentatum”). Of the 149 striatal recordings, we recovered 52 cells sufficiently well labeled with biocytin to obtain a clear view of their dendritic morphology. Two major morphological classes were evident—cells whose dendrites bore five or more dendritic spines per 10 μm of length (and generally had at least twice that density), called “spiny neurons,” and whose dendrites exhibited no more than one spine or spine-like process per 10 μm (“aspiny neurons”). Electrophysiological and morphological measurements of striatal neurons are listed in Table 1, divided into groups based on dendritic morphology and spontaneous activity. Table 2 presents the significant differences between these groups. In a separate set of recordings, we focused on presumptive striatal aspiny neurons, in an effort to determine which of these cells might express choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine. During these experiments, we immediately discarded any cells that appeared to be spiny neurons, based on intrinsic electrophysiological properties. Such cells were not analyzed further. Thus these data constitute a heavily biased sample and for that reason are reported separately.

**Intrinsic electrophysiological properties of striatal spiny neurons**

The majority of biocytin-labeled cells recovered in chick striatum (43 of 52, 83%) were spiny neurons. In mammals and songbirds, striatal spiny neurons exhibit characteristic and highly conserved physiological properties, namely, a rapid decrease in membrane resistance during hyperpolarization (fast inward rectification) and a ramping response to depolarizing current injection that triggers action potentials that are substantially delayed relative to the onset of the current pulse (Farries and Perkel 2000; Jiang and North 1991; Kita et al. 1984; Nisenbaum et al. 1994). Many of our chick striatal spiny neurons exhibited these properties, which define what one might call the “canonical” striatal spiny neuron; an example is shown in Fig. 1. However, chick striatal spiny neurons commonly deviated from this canonical pattern in a number of different ways.

**TABLE 1. Properties of chick striatal neurons**

<table>
<thead>
<tr>
<th></th>
<th>Spiny Neurons</th>
<th>Quiescent Unlabeled Neurons</th>
<th>Active Unlabeled Neurons</th>
<th>Active Aspiny Neurons</th>
<th>Quiescent Aspiny Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>90</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>−74 ± 8</td>
<td>−71 ± 11</td>
<td>NA</td>
<td>NA</td>
<td>−53 ± 11</td>
</tr>
<tr>
<td>Spontaneous firing rate, Hz</td>
<td>NA</td>
<td>NA</td>
<td>4.5 ± 5.6</td>
<td>5.2 ± 1.8</td>
<td>NA</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>513 ± 301</td>
<td>441 ± 232</td>
<td>454 ± 270</td>
<td>252 ± 142</td>
<td>629 ± 689</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>40 ± 13</td>
<td>40 ± 13</td>
<td>43 ± 6</td>
<td>32 ± 8</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>1.4 ± 0.7</td>
<td>1.6 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>−12 ± 3</td>
<td>−11 ± 4</td>
<td>−11 ± 4</td>
<td>−16 ± 5</td>
<td>−13 ± 9</td>
</tr>
<tr>
<td>AHP time to peak, ms</td>
<td>9.6 ± 13.8</td>
<td>7.4 ± 8.0</td>
<td>8.3 ± 3.0</td>
<td>8.7 ± 5.8</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>Burst index</td>
<td>1.6 ± 0.7</td>
<td>1.6 ± 0.6</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Sag index</td>
<td>0.07 ± 0.09</td>
<td>0.05 ± 0.09</td>
<td>0.07 ± 0.04</td>
<td>0.28 ± 0.11</td>
<td>0.07 ± 0.11</td>
</tr>
<tr>
<td>Delay to first spike, ms</td>
<td>339 ± 132</td>
<td>279 ± 147</td>
<td>93 ± 51</td>
<td>181 ± 110</td>
<td>108 ± 80</td>
</tr>
<tr>
<td>Inward rectification ratio</td>
<td>0.23 ± 0.15</td>
<td>0.28 ± 0.18</td>
<td>0.50 ± 0.28</td>
<td>0.47 ± 0.14</td>
<td>0.41 ± 0.24</td>
</tr>
<tr>
<td>Soma diameter, μm</td>
<td>11.5 ± 1.8</td>
<td>NA</td>
<td>17.2 ± 7.7</td>
<td>13.0 ± 2.9</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. The sample of “active aspiny neurons” does not include the four pallidum-like neurons tested for choline acetyltransferase (ChAT) immunoreactivity. AP, action potential; AHP, afterhyperpolarization.

**TABLE 2. Significant differences among striatal neurons**

<table>
<thead>
<tr>
<th>Differences</th>
<th>43/90</th>
<th>7/5/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mV</td>
<td>−74 ± 8</td>
<td>NA</td>
</tr>
<tr>
<td>Quiescent aspiny*</td>
<td>−71 ± 11</td>
<td>4.5 ± 5.6</td>
</tr>
<tr>
<td>Active aspiny‡</td>
<td>NA</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>Quiescent unlabeled*</td>
<td>−53 ± 11</td>
<td>NA</td>
</tr>
</tbody>
</table>

Significant differences are expressed as follows: x < y, z means that the average value of that parameter for group x is less than that for groups y and z. Differences were identified by 1-way ANOVA followed by Tukey’s pairwise post hoc comparisons if the means were found to be different at the P = 0.05 level. Significance levels: *P < 0.05; †P < 0.01; ‡P < 0.001. Note: ANOVA on action potential duration showed that the means were significantly different, but none of the pairwise comparisons found a significant difference at the P = 0.05 level.

One way in which chick spiny neurons could differ from canonical spiny neurons is in the pattern of action potentials evoked by depolarizing current pulses. Some spiny neurons responded to depolarizing current with an initial burst of two to four action potentials followed by regular firing at a lower rate. Such cells could (but did not always) resemble the canonical spiny neuron in other respects; an example of a bursting spiny neuron is shown in Fig. 2. To quantify the propensity of neurons to engage in burst firing, we defined a “burst index”: the mean interspike interval (ISI) during a current pulse divided by the minimum ISI in that pulse, averaged over all current pulses that evoked more than two spikes. Neurons that fire with perfect regularity would have a burst index of one, while any deviation from uniform ISI duration yields a burst index greater than one. This measure of “burstiness” does not crisply distinguish between spike rate accommodation and burst firing, but qualitatively one can say that moderate spike rate accommodation corresponds to a burst index of <2.0, whereas cells with a burst index >2.5 would be described as “bursting.” Most of our tracer-filled striatal spiny neurons were nonbursting (40 of 43, 93%; burst index range: 1.01–2.02), but three spiny neurons (7% of the total sample) had a burst index >2.5 (range: 2.91–4.92); a histogram showing the full distribution of burst indices appears in Fig. 5A.
Another way in which chick striatal spiny neurons could diverge from the canonical pattern is in their response to hyperpolarizing current pulses. Normally, spiny neurons in mammalian and songbird striatum exhibit very fast (“time-independent”) inward rectification that causes the membrane potential to reach a steady state value soon after the onset of the current pulse (Fig. 1A). In contrast, a number of chick striatal spiny neurons demonstrated time-dependent inward rectification in which the membrane resistance only gradually decreases during hyperpolarization, so that a “sag” appears in the voltage trace recorded during the current pulse (Fig. 3). We quantified this time-dependent inward rectification with a “sag index,” defined as the difference between the minimum voltage achieved during the largest hyperpolarizing current pulse (i.e., the membrane potential at the bottom of the sag) and the steady-state voltage deflection of that pulse, divided by the

FIG. 1. Intrinsic and firing properties of the “canonical” spiny neuron, recorded in medial striatum. A: responses of this spiny neuron to a series of 500-ms current pulses delivered from rest (−82 mV). The size of the current pulses is plotted immediately below the voltage traces. B: response of this cell to larger current pulses (amplitude noted to the left of each trace), illustrating its firing properties. Scale is the same as in A. C: photomicrograph of this neuron labeled with biocytin. This panel is a montage of 2 images taken at different focal planes. Scale bar is 10 μm. D: graph of the steady-state voltage deflections from rest produced in this neuron as a function of injected current. E: action potential frequency evoked in this neuron as a function of injected current. This firing rate is defined as the inverse interspike interval averaged over the current pulse, and thus excludes the “delay period” prior to the 1st spike and is not defined for traces containing only 1 spike. F: the delay from the onset of the current pulse to the occurrence of the 1st action potential as a function of injected current. G: instantaneous firing rate (inverse interspike interval) over the course of the current pulse for 0.20-, 0.28-, and 0.35-nA current pulses, illustrating spike rate accommodation. The data in E–G were calculated from the average response to 2 repetitions of each current pulse. This cell had a burst index of 1.37, a sag index of 0.03, and an inward rectification ratio of 0.10 (these measurements were used to quantify the differences between canonical spiny neurons and other spiny neurons observed in chick striatum; see text for definitions).

Another way in which chick striatal spiny neurons could diverge from the canonical pattern is in their response to hyperpolarizing current pulses. Normally, spiny neurons in mammalian and songbird striatum exhibit very fast (“time-independent”) inward rectification that causes the membrane potential to reach a steady state value soon after the onset of the current pulse (Fig. 1A). In contrast, a number of chick striatal spiny neurons demonstrated time-dependent inward rectification in which the membrane resistance only gradually decreases during hyperpolarization, so that a “sag” appears in the voltage trace recorded during the current pulse (Fig. 3). We quantified this time-dependent inward rectification with a “sag index,” defined as the difference between the minimum voltage achieved during the largest hyperpolarizing current pulse (i.e., the membrane potential at the bottom of the sag) and the steady-state voltage deflection of that pulse, divided by the

FIG. 2. Intrinsic and firing properties of a bursting spiny neuron, recorded in medial striatum. A: responses of this spiny neuron to a series of 1,000-ms current pulses delivered from rest (−81 mV). The size of the current pulses is plotted immediately below the voltage traces. B: response of this cell to larger current pulses (amplitude noted to the left of each trace), illustrating its firing properties. Scale is the same as in A. C: photomicrograph of this neuron labeled with biocytin. This panel is a montage of 2 images taken at different focal planes. Scale bar is 10 μm. D: graph of the steady-state voltage deflections from rest produced in this neuron as a function of injected current. E: action potential frequency evoked in this neuron as a function of injected current. This firing rate is defined as the inverse interspike interval averaged over the current pulse, and thus excludes the “delay period” prior to the 1st spike and is not defined for traces containing only 1 spike. F: the delay from the onset of the current pulse to the occurrence of the 1st action potential as a function of injected current. G: instantaneous firing rate (inverse interspike interval) over the course of the current pulse for 0.20-, 0.28-, and 0.35-nA current pulses, illustrating early bursting behavior followed by regular firing at a much lower rate. This cell had a burst index of 2.91, a sag index of 0.01, and an inward rectification ratio of 0.28.

J Neurophysiol • VOL 94 • JULY 2005 • www.jn.org
steady-state voltage deflection. Hence, a cell with only time-dependent inward rectification would have a sag index of zero, whereas a cell whose maximum voltage deflection is twice as large as the steady-state deflection would have a sag index of one. The majority of spiny neurons exhibited relatively little time dependence in their inward rectification (35 of 43 cells had a sag index of \(\leq 0.1\)), but several others exhibited a considerable amount of time-dependent inward rectification (8 cells, 19\%) with sag indices ranging from 0.11 to 0.46. Figure 5B shows the full distribution of sag indices.

A third major difference between a subset of chick spiny neurons and the canonical spiny neuron lies in the ramping response to depolarizing current injection that precedes the onset of firing. In mammals and songbirds, this response is mediated by depolarization-activated, slowly inactivating potassium currents (Farries and Perkel 2000, 2002b; Nisenbaum and Wilson 1995; Nisenbaum et al. 1994), which can delay the appearance of the first action potential by hundreds of milliseconds. Many of our recorded chick spiny neurons did indeed exhibit this behavior but a substantial number did not; Fig. 4 shows an example of such a cell. We quantified this behavior by measuring the average amount of time that passed from the onset of depolarizing current pulses to the appearance of the first action potential, for current pulses that evoked the fewest number of spikes (ideally, just one). Eleven chick spiny neurons (26\%) had a spiking delay of \(<250\) ms, whereas striatal spiny neurons in songbirds usually exhibited a delay of \(>300\) ms when driven by 500-mS current pulses just above rheobase (Farries and Perkel 2000, 2002b). These unusual chick spiny neurons could begin firing as little as 25 ms after the onset of the smallest current pulse capable of evoking action potentials (full distribution shown in Fig. 5C).

Two final electrophysiological features marked off certain chick spiny neurons from the more orthodox variety: a relative lack of inward rectification (fast or slow) and an inability to sustain firing throughout suprathreshold depolarizing current pulses. The degree of inward rectification is quantified by the “inward rectification ratio,” the minimum membrane resistance achieved during hyperpolarization divided by the input resistance (hence greater inward rectification corresponds to a smaller ratio). The cells shown in Figs. 2–4 display considerably less inward rectification than the “canonical” spiny neuron shown in Fig. 1 (inward rectification ratios of 0.27–0.29 vs. 0.10), but they still fall within the upper part of the range observed for striatal spiny neurons in songbirds (Farries and Perkel 2000, 2002b). However, some chick spiny neurons exhibited inward rectification (IR) ratios outside of that range: nine cells had IR ratios \(>0.3\) (21\% of the total) and three cells had IR ratios exceeding 0.5 (Fig. 5D). As for the ability to sustain firing, three cells (7\%) could not continue to fire action potentials for \(>250\) ms given any amount of injected current; one of these cells could fire no more than a single spike during a current pulse.

Overall, chick striatal spiny neurons exhibited an extraordinary variety of intrinsic properties and could differ from canonical spiny neurons in at least five different ways (burstiness, sag, ramping response/delayed spiking, overall inward rectification, and ability to sustain firing). If we define the canonical spiny neuron as one with a burst index of \(<2.5\), sag index of \(<0.15\), delay to first spike exceeding 300 ms, inward rectification ratio under 0.3, and possessing the ability to fire throughout at least some current pulses (not counting the delay period that precedes the first spike), 20 of our 43 tracer-filled striatal spiny neurons conformed to the canonical phenotype—a substantial fraction, but still less than half of all spiny neurons. Of the 23 noncanonical spiny neurons, 12 diverged from the canonical pattern in only parameter, while the remaining 11 cells differed in two or more ways. The border between canonical cells and other spiny neurons was generally not sharp; the histograms of Fig. 5 show peaks centered on the canonical values that are largely contiguous with “anomalous”
rations between the four distinguishing characteristics displayed in Fig. 5 (burst index, sag index, delay, and inward rectification ratio) and all other measured electrophysiological parameters (Table 1). Because this entailed computing 34 separate linear regressions among variables that we had little a priori reason to believe are related, we used a stringent criterion for statistical significance for each regression: \( P < 0.0015 \) (see METHODS). Only two relationships proved to be significant: cells with more depolarized resting potentials tended to have less overall inward rectification (i.e., higher inward rectification ratio; slope: \( 0.009, r^2 = 0.35, P < 0.0001; \) Fig. 6A, ●) and shorter spike delay (slope: \(-7.80, r^2 = 0.34, P < 0.0001; \) Fig. 6B, ●). We also noted that cells exhibiting time-dependent inward rectification showed some trend toward resting at more depolarized membrane potentials (Fig. 6C, ●), although the slope of this relationship was not significantly different from zero (slope: \( 0.003, r^2 = 0.08, P = 0.06 \)). Finally, we observed a trend toward larger action potential amplitudes in spiny neurons that engaged in burst firing (not significant; slope: \( 0.005, r^2 = 0.15, P = 0.01; \) Fig. 6D, ●).

The extraordinary variability among chick striatal spiny neurons greatly complicates the task of classifying unlabeled or poorly labeled neurons. We had hoped to find a correlation between cell morphology and physiology that would allow us to classify these unlabeled neurons as “spiny” or “aspiny” based on their intrinsic electrophysiological properties—especially because nearly two-thirds of our recorded striatal neurons (97 of 149) could not be so classified based on dendritic morphology. Unfortunately, the variability among spiny neurons precludes the possibility of using most electrophysiological parameters to sort the unlabeled cells with a high degree of certainty. Only one feature sharply distinguishes spiny striatal neurons from aspiny cells: spontaneous activity. Five of nine aspiny striatal neurons (fully described in the following text) spontaneously fired action potentials, whereas no spiny neuron did. Thus we can be reasonably certain that the spontaneously

![Fig. 4](image-url) Intrinsic and firing properties of a spiny neuron that lacks a ramping response and delayed spiking when injected with depolarizing current, recorded in medial striatum. A: responses of this spiny neuron to a series of 500-ms current pulses delivered from rest (−66 mV). The size of the current pulses is plotted immediately below the voltage traces. B: response of this cell to larger current pulses (amplitude noted to the right of each trace), illustrating its firing properties. Scale is the same as in A. C: photomicrograph of this neuron labeled with biocytin. Scale bar is 10 μm. D: graph of the steady-state voltage deflections from rest produced in this neuron as a function of injected current. E: action potential frequency (average inverse interspike interval) evoked in this neuron as a function of injected current. F: the delay from the onset of the current pulse to the occurrence of the 1st action potential as a function of injected current. G: instantaneous firing rate (inverse interspike interval) over the course of the current pulse for 0.08-, 0.14-, and 0.30-nA current pulses. The data in E–G were calculated from the average response to three repetitions of each current pulse. This cell had a burst index of 1.69, a sag index of 0.27, and an inward rectification ratio of 0.27.

Among cells departing from the canonical phenotype, no obvious groupings or subtypes were evident; cells could exhibit “anomalous” properties in virtually any combination. We sought some patterns in the data by calculating linear regressions between the four distinguishing characteristics displayed in Fig. 5 (burst index, sag index, delay, and inward rectification ratio) and all other measured electrophysiological parameters (Table 1). Because this entailed computing 34 separate linear regressions among variables that we had little a priori reason to believe are related, we used a stringent criterion for statistical significance for each regression: \( P < 0.0015 \) (see METHODS). Only two relationships proved to be significant: cells with more depolarized resting potentials tended to have less overall inward rectification (i.e., higher inward rectification ratio; slope: \( 0.009, r^2 = 0.35, P < 0.0001; \) Fig. 6A, ●) and shorter spike delay (slope: \(-7.80, r^2 = 0.34, P < 0.0001; \) Fig. 6B, ●). We also noted that cells exhibiting time-dependent inward rectification showed some trend toward resting at more depolarized membrane potentials (Fig. 6C, ●), although the slope of this relationship was not significantly different from zero (slope: \( 0.003, r^2 = 0.08, P = 0.06 \)). Finally, we observed a trend toward larger action potential amplitudes in spiny neurons that engaged in burst firing (not significant; slope: \( 0.005, r^2 = 0.15, P = 0.01; \) Fig. 6D, ●).

The extraordinary variability among chick striatal spiny neurons greatly complicates the task of classifying unlabeled or poorly labeled neurons. We had hoped to find a correlation between cell morphology and physiology that would allow us to classify these unlabeled neurons as “spiny” or “aspiny” based on their intrinsic electrophysiological properties—especially because nearly two-thirds of our recorded striatal neurons (97 of 149) could not be so classified based on dendritic morphology. Unfortunately, the variability among spiny neurons precludes the possibility of using most electrophysiological parameters to sort the unlabeled cells with a high degree of certainty. Only one feature sharply distinguishes spiny striatal neurons from aspiny cells: spontaneous activity. Five of nine aspiny striatal neurons (fully described in the following text) spontaneously fired action potentials, whereas no spiny neuron did. Thus we can be reasonably certain that the spontaneously
active unlabeled cells are aspiny and treat the remaining (quiescent) unlabeled cells as putative spiny neurons. Obviously, these “putative spiny neurons” very likely include some misclassified aspiny neurons, since not all aspiny neurons were spontaneously active. We can estimate how many neurons might be misclassified: of the 52 biocytin-labeled striatal neurons (spiny or aspiny), 48 (92%) were quiescent (reassuringly close to the proportion of quiescent unfilled cells: 90 of 97, or 93%). Of the 48 quiescent labeled striatal neurons, 4 were in fact aspiny (8%), suggesting that we can expect that something on the order of 7 “putative spiny neurons” will be misclassified. Any conclusions drawn from these cells must take that into account.

Like confirmed spiny neurons, the putative spiny neurons were highly variable in their intrinsic properties. Thirty-one of these cells (34%) qualified as “canonical spiny neurons” given the definition used in the preceding text, compared with 47% of the biocytin-labeled spiny neurons. Interestingly, if one assumes that the seven expected misclassified cells could not possibly qualify as canonical spiny neurons, so that the effective pool of “potential” canonical cells is only 83, the proportion of canonical spiny neurons is still substantially lower (37 vs. 47%) than among the confirmed spiny neurons, perhaps suggesting that the true number of misclassified cells is higher than our estimate. Five putative spiny neurons (6%) had a burst index >2.5, 8 cells (9%) had a sag index over 0.1, 38 cells (42%) had a spike delay <250 ms, 31 cells (34%) had an inward rectification ratio >0.3, and 7 cells (8%) could not sustain firing throughout suprathreshold current pulses. The contribution of putative spiny neurons to the full distribution of each of these parameters (capacity for sustained firing excepted) is shown by open bars in Fig. 5. Like confirmed spiny neurons, putative spiny neurons resting at more depolarized membrane potentials tended to have less overall inward rectification (slope: 0.006, \( r^2 = 0.13, P = 0.0004 \); Fig. 6A, △), shorter spiking delays (slope: -5.87, \( r^2 = 0.18, P < 0.0001 \); Fig. 6C, △), and a larger time-dependent component in their inward rectification (not significant; slope: 0.002, \( r^2 = 0.07, P = 0.01 \); Fig. 6C, △). This last trend approached significance when data from confirmed and putative spiny neurons were considered together (slope: 0.002, \( r^2 = 0.06, P = 0.003 \)). Putative spiny neurons did not exhibit any trend toward larger spike amplitudes with increasing burst index (slope: 0.003, \( r^2 = 0.006, P = 0.46 \); Fig. 6D, △).

Differences in age and temperature cannot explain most of the variation among spiny neurons

We looked for some parameter that could explain the enormous variability we observed in chick striatal spiny neurons. One obvious candidate is the age of the bird: we recorded from chicks at a wide range of ages (4–20 days posthatch). We calculated linear regressions between age and all of our measured electrophysiological parameters. Because 12 separate linear regressions were computed, our threshold for statistical significance was \( P = 0.004 \) (see Methods). No relationship had a slope significantly different from zero, whether confirmed spiny neurons were considered alone or in combination with putative spiny neurons. The two relationships that came closest to significance were decreases in action potential duration (slope: \(-0.07, r^2 = 0.15, P = 0.009\); Fig. 7A, △) and inward rectification ratio (slope: \(-0.009, r^2 = 0.05, P = 0.13\); Fig. 7B, △) with increasing age. Both of these relationships were pushed further from significance when putative spiny neurons were included (\( P = 0.04 \) and \( P = 0.96 \) for action potential duration and inward rectification ratio, respectively; Fig. 7A, △).

We also considered whether differences in recording temperature could explain some of the variation in the properties of spiny neurons, again calculating 12 linear regressions using a significance criterion of \( P = 0.004 \). Only two relationships proved to be significant: action potential duration (slope: \(-0.10, r^2 = 0.22, P = 0.001\); Fig. 7C, △) and amplitude (slope: \( 0.0004 \), Fig. 6C, △).
Intrinsic electrophysiological properties of aspiny neurons in chick striatum

Five of nine (56%) biocytin-labeled aspiny neurons spontaneously fired action potentials at regular intervals (range: 3–8 Hz), all recorded in medial striatum (Fig. 8). This characteristic sets them apart from most cell types yet identified in mammalian striatum but is reminiscent of a spontaneously active pallidum-like cell type found in the striatum of songbirds (Farries and Perkel 2000, 2002b). Like the “pallidal” cells of songbird striatum, these spontaneously active neurons exhibited time-dependent inward rectification (sag index range: 0.12–0.40) and were capable of sustained firing at high rates (>100 Hz for ≥500 ms in 3 of 3 cells tested). They showed some spike rate accommodation, especially in the early phase of large depolarizing current pulses, but were not prone to burst firing (burst index range: 1.10–1.30). Although these cells were quite similar to the pallidum-like cells of songbird striatum with regard to intrinsic electrophysiological properties, they differed considerably in their morphology. These cells had relatively thick, infrequently branching, nonvaricose dendrites, whereas the pallidum-like cells of songbird striatum had extremely thin, profusely branched neurites that bore many varicosities (Farries and Perkel 2000, 2002b). In fact, these pallidum-like cells of chick striatum are morphologically much more similar to mammalian pallidal neurons than their counterparts in songbird striatum.

The four quiescent aspiny striatal neurons we recorded (3 in medial striatum, 1 in lateral striatum) were quite heterogeneous and did not appear to constitute a single coherent cell type. One of these neurons resembled the spontaneously active aspiny cells in all respects save its lack of spontaneous firing (sag index: 0.24, burst index: 1.20, could sustain firing >100 Hz for ≥500 ms); it rested at a relatively depolarized potential (~59 mV). Another quiescent aspiny neuron somewhat resembled the “fast-spiking” interneuron type identified in mammalian and songbird striatum (Farries and Perkel 2002b; Kawaguchi 1993), in that it exhibited fast inward rectification (inward rectification ratio: 0.06, sag index: 0.03) and displayed a “chattering” pattern of spiking (short spike trains irregularly interrupted by pauses in firing). However, it differed from such “fast-spiking” neurons by having relatively long duration action potentials (1.49 ms). The two remaining quiescent aspiny neurons were unable to sustain firing throughout depolarizing current pulses; one of them could not fire more than one action potential during a given pulse.

We compared the spontaneously active biocytin-labeled cells (all aspiny) to the seven spontaneously active unlabeled cells (firing at 1–16 Hz) to see if they could all be representatives of a single physiologically defined cell type. In fact, these two groups exhibited some differences. Unlabeled spontaneously active cells had less time-dependent inward rectification than most of the labeled cells (sag index range: 0.03–0.14) and
only one of them (of 4 cells tested) could sustain high-frequency firing (>100 Hz) for ≥500 ms. Interestingly, the one cell that demonstrated a capacity for sustained high-frequency firing was also the one with the most time-dependent inward rectification (sag index of 0.14, within the range of the tracer-filled spontaneously active neurons) and highest spontaneous firing rate (16 Hz). Among the 90 quiescent unlabeled striatal neurons (i.e., “putative spiny neurons”), two possessed the pallidum-like intrinsic properties of time-dependent inward rectification (sag indices of 0.26 and 0.33) and the ability to sustain firing at >100 Hz. They could represent further examples of the one pallidum-like, yet quiescent, biocytin-labeled aspiny neuron. However, it is perfectly possible that they are spiny neurons—indeed, the spiny neuron shown in Fig. 3 also met these “pallidum-like” criteria.

**Striatal “pallidum-like” neurons are not cholinergic interneurons**

Our discovery of spontaneously active aspiny neurons in chich medial striatum suggests that at least a portion of chich striatum, like songbird striatum, is functionally a mixture of striatum and globus pallidus. On the other hand, the cholinergic interneurons of mammalian striatum can be spontaneously active in vitro, especially in brain slices heated above room temperature (Bennett and Wilson 1999). Mammalian cholinergic interneurons also exhibit time-dependent inward rectification (Kawaguchi 1992, 1993), like our chick striatal pallidum-like neurons. This raises the possibility that our pallidum-like neurons are simply striatal cholinergic interneurons. Chick pallidum-like neurons do differ significantly from mammalian and songbird striatal cholinergic interneurons—cholinergic interneurons exhibit a much longer duration afterhyperpolarization (AHP) and have not been reported capable of sustained firing at rates ≥100 Hz (Bennett and Wilson 1999; Farries and Perkel 2002b; Kawaguchi 1992, 1993). However, our finding that chick striatal spiny neurons can differ considerably from their counterparts in mammals and songbirds demands that we entertain the possibility that the physiological properties chick striatal cholinergic interneurons have diverged as well. To test the possibility that the chick striatal pallidum-like neurons are modified cholinergic interneurons, we made a concerted effort to record more of these neurons and test them for ChAT immunoreactivity. We successfully recovered four biocytin-labeled pallidum-like neurons, identified by spontaneous activity (9.0 ± 0.8 Hz, range: 8–10 Hz), time-dependent inward rectification (0.32 ± 0.08, range: 0.23–0.38), and the ability to sustain firing at >100 Hz (4 of 4 cells). None of these cells exhibited ChAT immunoreactivity; an example is shown in Fig. 9.

**Intrinsic electrophysiological properties of neurons in the globus pallidus**

We recorded 16 cells in the chick globus pallidus (properties summarized in Table 3), and recovered 12 biocytin-labeled neurons. Ten of the biocytin-labeled neurons (83%) were aspiny, whereas the remaining 2 cells had spiny dendrites and were morphologically indistinguishable from striatal spiny neurons. Eight of the aspiny neurons (80%) were spontaneously active, almost all at relatively low rates (1–5 Hz; 7 of 8 cells), but with one outlier that fired spontaneously at 45 Hz. The spontaneously active aspiny neurons exhibited time-dependent inward rectification (sag index 0.35 ± 0.16, range: 0.10–0.58), but the quiescent ones did not (sag indices of 0.01 and 0.02). Five of the spontaneously active aspiny neurons (63%) were able to sustain firing at high rates (>100 Hz for ≥500 ms); an example of such a cell is shown in Fig. 10. Because the avian globus pallidus contains neurons immunoreactive for choline acetyltransferase (Li and Sakaguchi 1997; Medina and Reiner 1994; Roberts et al. 2002), the enzyme that synthesizes acetylcholine, we tested six of our biocytin-labeled pallidum-like neurons, identified by spontaneous activity (≥500 ms); an example of such a cell is shown in Fig. 10.

**TABLE 3. Properties of chick pallidal neurons**

<table>
<thead>
<tr>
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<th>Quiescent Neurons</th>
<th>Active Neurons</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>−69 ± 13</td>
<td>NA</td>
</tr>
<tr>
<td>Spontaneous firing rate, Hz</td>
<td>NA</td>
<td>8.1 ± 13.9*</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>561 ± 735</td>
<td>437 ± 144</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>43 ± 22</td>
<td>42 ± 18</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>1.3 ± 1.0</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>AHP peak, mV</td>
<td>−14 ± 11</td>
<td>−16 ± 6</td>
</tr>
<tr>
<td>AHP time to peak, ms</td>
<td>4.3 ± 3.1</td>
<td>11.4 ± 8.5</td>
</tr>
<tr>
<td>Burst index</td>
<td>2.0 ± 1.8</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td>Sag index†</td>
<td>0.04 ± 0.05</td>
<td>0.34 ± 0.16</td>
</tr>
<tr>
<td>Delay to first spike, ms</td>
<td>232 ± 154</td>
<td>206 ± 145</td>
</tr>
<tr>
<td>Inward rectification ratio‡</td>
<td>0.54 ± 0.23</td>
<td>0.30 ± 0.13</td>
</tr>
<tr>
<td>Soma diameter, μm</td>
<td>15.0 ± 4.1</td>
<td>17.2 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. *if an extreme outlier is excluded (see text), this value is 3.5 ± 1.5 Hz. †Significant difference, P = 0.0002; ‡significant difference, P = 0.02.
pallidal cells for ChAT immunoreactivity. Only one cell proved to be immunoreactive, shown in Fig. 11.

Of the two spiny neurons we recovered in the globus pallidus, one exhibited most of the features of canonical striatal spiny neurons (burst index: 1.66, sag index: 0.02, inward rectification ratio: 0.35, delay: 469 ms), whereas the other was quite different (burst index: 1.88, sag index: 0.15, inward rectification ratio: 0.53, delay: 170 ms). Of the four cells for which no tracer fill was recovered, one was spontaneously active (3 Hz) but differed from the tracer-filled spontaneously active neurons in its propensity for burst firing (burst index: 4.51) during small depolarizing current pulses or on rebound following the offset of hyperpolarizing current pulses. A second (quiescent) unfilled pallidal neuron also exhibited burst firing.

FIG. 10. Intrinsic and firing properties of a spontaneously active neuron in the globus pallidus. A, top: spontaneous firing in this cell; no current was injected. Bottom: responses of this neuron to a series of 500-ms current pulses delivered from a hyperpolarized potential (−60 mV, maintained by continuous injection of −0.03 nA). The size of the current pulses is plotted immediately below the voltage traces. B: response of this cell to larger current pulses (amplitude noted to the right of each trace). Scale is the same as in A. C: confocal image of this neuron labeled with biocytin, constructed from the brightest point projection of 29 optical sections and displayed with an inverted lookup table. Scale bar is 20 μm. D: graph of the steady-state voltage deflections from rest produced in this neuron as a function of injected current. E: action potential frequency (average inverse interspike interval) evoked in this neuron as a function of injected current. F: the delay from the onset of the current pulse to the occurrence of the 1st action potential as a function of injected current. G: instantaneous firing rate (inverse interspike interval) over the course of the current pulse for 0.04-, 0.15-, and 0.30-nA current pulses. The data in E–G were calculated from the average response to 2 repetitions of each current pulse. This cell had a burst index of 1.09, a sag index of 0.10, and an inward rectification ratio of 0.44.

FIG. 11. Intrinsic and firing properties of a ChAT-immunoreactive neuron in the globus pallidus. A, top: spontaneous firing in this cell; no current was injected. Bottom: responses of this neuron to a series of 500-ms current pulses delivered from a hyperpolarized potential (−58 mV, maintained by continuous injection of −0.07 nA). The size of the current pulses is plotted immediately below the voltage traces. B: response of this cell to larger current pulse (amplitude noted to the right of the trace). Scale is the same as in A. C: confocal image of this neuron labeled with biocytin, constructed from the brightest point projection of 36 optical sections; bottom: ChAT immunoreactivity in this neuron. The biocytin was visualized with Cy3-conjugated streptavidin, whereas the ChAT immunoreactivity was visualized with a Cy2-conjugated secondary antibody. Scale bar is 10 μm. D: graph of the steady-state voltage deflections from rest produced in this neuron as a function of injected current. E: action potential frequency (average inverse interspike interval) evoked in this neuron as a function of injected current. F: the delay from the onset of the current pulse to the occurrence of the 1st action potential as a function of injected current. G: instantaneous firing rate (inverse interspike interval) over the course of the current pulse for 0.04-, 0.15-, and 0.30-nA current pulses. The data in E–G were calculated from the average response to 2 repetitions of each current pulse. This cell had a burst index of 1.48, a sag index of 0.35, and an inward rectification ratio of 0.17.
firing (burst index: 6.10). The two remaining unfilled neurons did not conform to any noteworthy pattern.

**DISCUSSION**

Our data show that at least part of the chick striatum contains neurons with pallidum-like morphology and electrophysiological properties, supporting our hypothesis that a mixing of striatal and pallidal cell types is common to most or all avian taxa. Chick striatum also contains a population of spiny neurons that share all of the defining characteristics of striatal spiny neurons in mammals and songbirds. However, many chick striatal spiny neurons are quite different from their mammalian and oscine counterparts and display considerable diversity in their electrophysiological properties.

**Comparison of chick and songbird striatum**

The vast majority of neurons recorded in songbird striatum, inside and outside of the specialized vocal control region known as area X, are spiny neurons (Farries and Perkel 2000, 2002b). In songbirds, spiny neurons constitute a fairly homogeneous physiological cell type defined primarily by the presence of fast inward rectification on hyperpolarization and a depolarization-activated, slowly inactivating K⁺ current (Farries and Perkel 2000, 2002b), features shared with mammalian striatal spiny neurons (Nisenbaum and Wilson 1995; Nisenbaum et al. 1994). The firing properties of songbird spiny neurons are characterized by delay from the onset of the current pulse to the appearance of the first action potential, followed by regular spiking with moderate spike rate accommodation (Farries and Perkel 2000, 2002b). Like songbird striatum, the vast majority of biocytin-labeled cells recovered in chick striatum were spiny neurons (defined morphologically). Nearly half of these spiny neurons closely resembled the striatal spiny neurons of mammals and songbirds. However, the majority of chick spiny neurons differed from their mammalian and oscine counterparts in their subthreshold responses, firing properties, or both. The possible significance of this finding is discussed below. Although we place these neurons in a single morphological class, it is possible that more subtle morphological features covary with their physiological characteristics, a possibility that we did not explore.

Another major cell type found in songbird striatum is a spontaneously active cell type known as the “aspy, fast-firing” (AF) class (Farries and Perkel 2000, 2002b), a cell type never reported in mammalian striatum yet very similar to some cell types identified in the mammalian pallidum (Bengtson and Osborne 2000; Nakanishi et al. 1990). We established that the medial striatum of chicks contains a similar pallidum-like cell type. As in songbirds, these cells were spontaneously active, exhibited time-dependent inward rectification and were capable of sustained firing at very high rates. The pallidum-like neurons in chicks did, however, differ from those of songbirds in two ways. First, they fired spontaneously at a lower rate on average: 5.2 ± 1.8 Hz in chick striatum versus 18.4 ± 11.6 Hz in area X (Farries and Perkel 2002b) and 14 ± 5 Hz in songbird striatum outside of area X (Farries and Perkel 2000). Second, they had a rather different dendritic morphology—chick pallidum-like neurons had much thicker, less frequently branched dendrites that lacked the varicosities that are so prominent on songbird AF cells. The morphological pattern exhibited by chick neurons may be closer to the ancestral condition, given that they better resemble true pallidal neurons, but direct support for this supposition would require that this trait be found in other avian taxa.

Although chick pallidum-like neurons more closely resemble songbird AF cells than any other songbird striatal cell type, the morphological differences between these neurons in chicks and songbirds raise the possibility that chick pallidum-like neurons represent a modified interneuronal cell type rather than projection neurons innervating pallidum-typical targets. This possibility is reinforced by the fact that chick striatal spiny neurons can be quite different from their songbird counterparts; perhaps some chick striatal interneurons have also acquired new characteristics, and their resemblance to songbird AF cells is merely coincidental. If chick pallidum-like neurons are in fact a modified version of one of the other known striatal interneuron types found in mammals and songbirds, they are most likely cholinergic interneurons, given their electrophysiological properties.

However, the pallidum-like neurons we tested for ChAT immunoreactivity did not contain this cholinergic marker. We cannot rule out the possibility that chick pallidum-like neurons are modified versions of one of the other known striatal interneuron classes, “fast-spiking” and “low-threshold spike” cells, but if they are, the fact that their newly acquired characteristics happen to make them more closely resemble another cell type in songbird striatum (the AF cell) is quite a coincidence indeed. Given the currently available evidence, we think it is more likely that chick pallidum-like neurons are homologues of songbird AF cells and are indicative of the same kind of mixing of striatal and pallidal cell types exhibited by songbird striatum.

If chick pallidum-like neurons are homologues of songbird AF cells, then the presence in chick striatum of homologues of the three mammalian striatal interneuron types (Kawaguchi 1993) remains an open question. We did not unambiguously identify these cell types in chick striatum, although some of our recorded cells could represent examples of these types. Given the rarity of these cell types in songbird and mammalian striatum, our data cannot provide strong evidence for their absence from chick striatum. Indeed, Farries and Perkel (2000) found only one of the three interneuron classes in songbird striatum, whereas Farries and Perkel (2002b) found all three classes using a larger sample size. On the other hand, one of these cell types, the cholinergic interneuron, may be intrinsically harder to find in chick striatum than in mammals or songbirds. In mammals, cholinergic interneurons are distributed throughout the striatum (Kimura et al. 1984; Phelps et al. 1985), and in songbirds they are scattered through most of the striatum but are excluded from a caudal region of lateral striatum (Li and Sakaguchi 1997; personal observations). Our ChAT immunostained material suggests that chicks also have this excluded region, but it appears to be much larger than in songbirds, possibly encompassing the majority of chick striatum. If this informal observation proves to be accurate (we are not aware of any published studies of ChAT immunoreactivity in chick striatum that could confirm or refute it), it could explain our failure to observe the striatal cholinergic cell type in this study.
Comparison of chick and mammalian globus pallidus

Like the striatum, the avian globus pallidus resembles the mammalian pallidal complex histochemically and anatomically (Medina and Reiner 1995). They do differ in that the mammalian dorsal pallidum is divided into two segments with distinct connections, while the avian pallidum is not, but even that difference may be superficial—Reiner and colleagues have argued that the cellular components of both mammalian pallidal segments, as defined by connections and histochemistry, are present in the avian globus pallidus (Jiao et al. 2000). Our results in chick pallidum are consistent with the hypothesis that pallidal characteristics are highly conserved across amniotes. All components of the mammalian pallidum contain neurons that are spontaneously active in vitro and capable of sustained high-frequency firing (Bengtson and Osborne 2000; Nakanishi et al. 1990; Nambu and Lináš 1994), like many of our chick pallidal neurons. Some studies, but not all, report time-dependent inward rectification in a subset of mammalian pallidum neurons (Bengtson and Osborne 2000; Cooper and Stanford 2000; Nakanishi et al. 1990) as we do for chick pallidum. Both segments of the mammalian dorsal pallidum contain bursting neurons (Kita and Kitai 1991; Nakanishi et al. 1990; Nambu and Lináš 1994), again mirroring our results in chicks. Even our finding of pallidal neurons resembling striatal spiny neurons does not set chick pallidum apart—similar cells have been reported in the globus pallidus of guinea pigs (Nambu and Lináš 1997). Despite these similarities, there may still be important physiological differences between avian and mammalian pallidum. Our sample size here is relatively small and is backed up only by an even tinier sample of pallidal neurons recorded in zebra finches (Farries and Perkel 2000).

Diversity in the physiological properties of striatal spiny neurons in chicks

One of our main findings is that the majority of striatal spiny neurons in chicks differ from striatal spiny neurons in mammals and songbirds. This difference might be explained by the condition of chick brain slices—perhaps the slicing process damages or otherwise alters striatal spiny neurons in chicks. In support of this notion, one could note that songbird striatum also contained spiny neurons with “anomalous” firing properties in varying proportions (most common in area X of zebra finches, less common in other species or in zebra finch striatum outside of area X) that were interpreted as unhealthy and not representative of neurons in intact striatum (Farries and Perkel 2000, 2002b). However, the anomalous spiny neurons of songbirds were quite different from the noncanonical spiny neurons in chicks. First, the songbird neurons exhibited firing properties characteristic of damaged neurons: they either could not fire repetitively at all or fired action potentials of unusually long duration and small amplitude; the vast majority of chick neurons did not. Furthermore, the anomalous neurons of songbirds continued to show the canonical spiny neuron responses to subthreshold current pulses, fast inward rectification on hyperpolarization and a ramping response to depolarization; this is not true of many chick spiny neurons. With the possible exception of cells that were incapable of sustained firing (accounting for only 13% of noncanonical spiny neurons), we think that tissue damage is unlikely to explain the unusual properties of chick spiny neurons.

Another possible explanation for the unusual properties of chick spiny neurons is immaturity—perhaps these neurons were recorded in the middle of a developmental process that culminates with the canonical spiny neuron. This is certainly possible given the young age of our birds, but there are some factors that make this interpretation less likely. First, the domestic chick is highly precocial, able to feed itself and engage in basic adaptive behaviors almost immediately after hatching. Moreover, in chicks within the age range we studied the striatum is involved in some forms of learning (Csillag 1999; Izawa et al. 2001) and behavioral control (Izawa et al. 2003), indicating that it must be mature enough to perform at least some functions. Another factor weighing against an interpretation that classifies noncanonical spiny neurons as immature is a comparison to immature spiny neurons in mammals. Although immature mammalian striatal spiny neurons, like some (but not all) noncanonical chick spiny neurons, express relatively little inward rectification, they do not express time-dependent inward rectification or burst firing (Belleau and Warren 2000; Cepeda et al. 1991; Tepper et al. 1998). Unlike our noncanonical chick spiny neurons, immature mammalian spiny neurons tend to have low spine density (Cepeda et al. 1991; Tepper et al. 1998) and in at least some studies exhibited delayed spiking comparable to that of mature spiny neurons (Belleau and Warren 2000; Cepeda et al. 1991). We did not observe any of the age-related changes reported in maturing mammalian spiny neurons, including changes in resting potential, action potential shape (duration and amplitude), input resistance, and inward rectification (Belleau and Warren 2000; Cepeda et al. 1991; Tepper et al. 1998). For these reasons, we do not regard physiological immaturity as a leading explanation for the unusual properties found in many chick spiny neurons; nevertheless, it is still a possibility and could be tested by recording in adult chickens.

If the unusual properties of some chick spiny neurons are not due to poor health or incomplete development, then they are probably attributes acquired over the course of evolution. The question then becomes, at least with respect to the avian basal ganglia, which phenotype is closer to the ancestral state? Because the physiological properties of striatal spiny neurons are so highly conserved between mammals and songbirds, the most parsimonious hypothesis would take songbird striatal spiny neurons as the better representatives of the ancestral condition and treat the properties of chick spiny neurons as evolutionarily derived. This hypothesis, unlike the reverse (regarding songbird spiny neurons as derived), requires only one postulated evolutionary change, somewhere in the lineage leading to chickens. However, given that only two avian orders have been sampled to date (Passeriformes and Galliformes), one cannot regard as ruled out the possibility that the physiological similarities between striatal spiny neurons in mammals and songbirds result from convergent evolution rather than common inheritance. Here, it is worth noting that Farries and Perkel (2000) suggested that much of avian striatum could be homologous to the mammalian ventral striatum and extended amygdala (a category that includes the central nucleus of the amygdala, among other things) not the dorsal striatum (caudate and putamen). Studies of the mammalian central nucleus of the amygdala have reported spiny neurons that sometimes resemble striatal spiny neurons (Martina et al. 1999) but often differ from them in ways similar to chick striatal spiny neurons,

J Neurophysiol • VOL 94 • JULY 2005 • www.jn.org
including a propensity for burst firing, lack of delayed spiking, and the presence of some time-dependent inward rectification (Martina et al. 1999; Schiess et al. 1999). Moreover, the hypothesis that much of avian striatum is homologous to the mammalian ventral striatum and extended amygdala could explain the presence of pallidum-like neurons in avian striatum: mammalian ventral striatum contains some projection neurons with pallidum-like morphology (Bolam et al. 1981; Fisher et al. 1986) and the mammalian extended amygdala has some efferents more typical of the pallidum than striatum (de Olmos and Heimer 1999), including a projection to the thalamus (Canteras et al. 1995; Zahm et al. 1999). It is conceivable that chick striatum contains spiny neurons that differ from those in mammalian dorsal striatum because it is derived from a different sector of the subpallium (i.e., one that includes the extended amygdala), and that songbird striatum has come to more closely resemble mammalian dorsal striatum through convergent evolution.

Conclusion

Comparisons of the mammalian and avian basal ganglia have revealed a great deal of conservation in anatomical organization (Reiner 2002) and physiological properties (Farries and Perkel 2000, 2002b). Nevertheless, some noteworthy differences have been discovered in songbirds, including a direct projection from the striatum to the thalamus (Bottjer et al. 1989; Okuhata and Saito 1987) and the presence of neurons that physiologically resemble neurons found in the mammalian globus pallidus (Farries and Perkel 2000, 2002b). Here, we have shown that the striatum of the domestic chick also contains pallidum-like neurons, making it more likely that this characteristic is common to all birds rather than an adaptation limited to only a few avian groups. We would like to know if these pallidum-like neurons project to the thalamus as a subset do in songbirds (Farries et al. 2005). Unfortunately, the proposed projection from chick medial striatum to the thalamus (Székely et al. 1994) has not yet been confirmed through retrograde tracing, so we cannot even compare the morphology of chick pallidum-like neurons to chick striatal neurons projecting to the thalamus. The fact that medial striatum of both songbirds and chicks contain pallidum-like neurons and play a role in some forms of learning hint at functional and mechanistic parallels that could be useful in understanding how both systems work. On the other hand, our finding that the striatal spiny neurons of chicks can be quite different undermines that notion to some degree; the functional significance of these differences can only be guessed at present. It seems that comparative studies of basal ganglia in annmotes can still spring some surprises in spite of a high degree of evolutionary conservatism.

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