Cell-type Specific Resonances
Shape the Responses of Striatal Neurons to Synaptic Input

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Running Title: Frequency Tuning of Striatal Neurons
ABSTRACT

Neurons respond to synaptic inputs in cell-type specific ways. Each neuron type may thus respond uniquely to shared patterns of synaptic input. We applied statistically identical barrages of artificial synaptic inputs to four striatal cell types to assess differences in their responses to a realistic input pattern. Each interneuron type fired in phase with a specific input frequency component. The fast-spiking interneuron fired in relation to the gamma-band (and higher) frequencies, the low-threshold spike interneuron to beta-band, and the cholinergic neurons to delta-band frequencies. Low-threshold spiking and cholinergic interneurons showed input impedance resonances at frequencies matching their spiking resonances. Fast-spiking interneurons showed resonance of input impedance, but at lower than gamma frequencies. The spiny projection neuron's frequency preference did not have a fixed frequency, but instead tracked its own firing rate. Spiny cells showed no input impedance resonance. Striatal interneurons are each tuned to a specific frequency band corresponding to the major frequency components of local field potentials. Their influence in the circuit may fluctuate along with the contribution of that frequency band to the input. In contrast, spiny neurons may tune to any of the frequency bands, by a change in firing rate.

INTRODUCTION

Like many forebrain structures, the striatum consists of a large number of principal neurons and a smaller and more diverse population of interneurons (Kawaguchi et al., 1995; Tepper et al., 2010a). The number of distinct interneuron types, and even the best system for categorizing them, have been debated for decades (Chang et al., 1982; DiFiglia et al., 1980; Fox and Rafols, 1971; Yelnik et al., 1991) and continue to be subjects of intense research (e.g. Gittis et al., 2010; Tepper et al., 2010a; 2010b). It is puzzling that there are so many different kinds of interneurons, and identifying their unique contributions to the striatal circuit may reveal key principles of its operation.

One difference between cell types is their selective firing in relation to specific frequency components of the local field potential (e.g. Berke et al., 2004; 2009; Courtemanche et al., 2003; Sharott et al., 2009; Sharott et al., 2012; Thorn and Graybiel, 2014; van der Meer et al., 2010). In these experiments, the local field potential recorded simultaneously with a neuron’s firing pattern is filtered by set of band-pass filters. Frequency selectivity is indicated by preferential firing of the neuron at fixed phase relative to the filtered field potential waveforms. For example, fast-spiking interneurons in the striatum preferentially fire in phase with gamma or higher frequency components of the field potential, whereas spiny neuron firing is usually in phase with lower frequency components (Berke, 2009; Sharott et al., 2009; van der Meer and Redish, 2009). Changes in the frequency spectrum of local field potentials parallel behavioral state, for example during performance of a learned task (e.g. Courtemanche et al., 2003; DeCoteau et al., 2007; Howe et al., 2011; Kalenscher et al., 2010; Leventhal et al., 2012; van der Meer and Redish, 2009). They also change in pathological conditions. For example, Parkinson’s disease is associated with an exaggerated beta (13-30 Hz) component of the local field potential in various brain regions including the
striatum (e.g. Brittain et al., 2014; Goldberg et al., 2004). The specific frequency tuning of neurons suggests that changes in input pattern differentially engage striatal neuron types and their associated subcircuit in the nucleus.

What is responsible for cell-type frequency tuning? Striatal neurons receive thousands of synapses from nearly as many different neurons, each of which has some ongoing activity. The resulting synaptic barrage is probably best viewed as a continuous stream of synaptic current, rather than a series of discrete inputs. The local field potential corresponds roughly to the sum of all synaptic currents in the vicinity (e.g. Schomburg et al., 2012), and so may reflect the collective input stream to neurons in a small region of the striatum. Of course, the input connections of each cell type are thought to be somewhat different, and it may be that each cell type samples a unique set of afferents carrying signals of distinct frequency composition. On the other hand, some afferent fibers carry signals that span the entire frequency range seen in the local field potential. Corticostriatal neurons contribute to both the high frequency gamma signals and the low (< 5 Hz) delta components simultaneously (Stern et al., 1997). The two oscillations are coupled, with gamma-locked firing in corticostriatal neurons occurring only during the depolarizing phase of the slower delta oscillation. Amplitude-coupled cross-frequency oscillations of this kind are also observed in striatal field potentials (López-Azacárate et al., 2013; Tort et al., 2008; von Nicolai et al., 2014). It thus may be valid to view the local field potential as an indirect measure of the input current stream being delivered to many or even all neuron types. If so, the frequency tuning differences among striatal cell types may be caused by intrinsic differences in the dynamics of their responses to periodic synaptic inputs.

Resonant and oscillatory neurons are known to be preferentially sensitive to inputs modulated at the natural frequency of their oscillation (Hutcheon et al., 1996; Ströhmann et al., 1994). We have previously shown that the striatal fast-spiking interneuron exhibits a minimum repetitive firing frequency in the gamma-frequency range, which should make it more sensitive to fluctuations in synaptic current at this frequency (Sciamanna and Wilson, 2011). Another striatal interneuron, the giant cholinergic interneuron, exhibits intrinsic low frequency oscillations (Wilson, 2005), and a third, the low-threshold spiking interneurons have calcium dependent oscillations at depolarized potentials (Beatty et al., 2012).

We tested the frequency selectivity of 4 of the best characterized striatal cell types: (1) the spiny projection neuron, which is the principal cell of the striatum, (2) the fast-spiking (FS) interneuron, which mainly targets the soma and proximal dendrites of spiny neurons, (3) the low-threshold spiking (LTS) interneuron, which targets distal dendrites, and (4) the giant striatal cholinergic (ACh) interneuron. We measured the frequency selectivity of these neurons using a technique analogous to that used with the field potential in vivo. We applied a broadband artificial synaptic barrage, and measured spike phase-locking to frequency components of that input stream. In contrast to the field potential studies, we could ensure that our artificial barrage of input current was statistically identical for all neuron types and consisted of equal contributions over a wide range of frequencies. Spiking resonance measured this way must be intrinsic in origin, not caused by differential connections. We also employed a second measure of
resonance, one that has been successful in quantifying subthreshold membrane resonance in many brain
areas (e.g. Fishman et al., 1979; Moore et al., 1993; Hutcheon et al., 1994; Pike et al., 2000; Hua et al.,
2009). This method uses a sinusoidal input with gradually changing frequency to measure the frequency-
dependent changes in input impedance that may contribute to the spiking resonance measured using the
first approach.

All three interneurons showed resonance, but in different frequency bands. Spiking responses of ACh
interneurons were selective for the delta frequency range (1-5 Hz), and were matched by a delta-band
subthreshold membrane resonance. LTS interneurons showed spiking resonance in the beta frequency
range, and a corresponding membrane potential resonance. FS cells showed spiking resonance specific
to sub-bands in the gamma frequency range and above (40-250 Hz), but their somatic membrane
resonance was restricted to lower frequencies. Spiny projection neurons showed no subthreshold
resonance, but each preferentially fired to frequency components of the input matching its own firing rate.
By altering firing rate, the spiny neuron may be tuned to be selective for inputs in any of the frequency
ranges.

MATERIALS AND METHODS

All experimental procedures were carried out according to the NIH Guidelines for the Care and Use of
Laboratory Animals and were approved by the University of Texas at San Antonio Institutional Animal
Care and Use Committee. For most experiments we used B6.FVB-Tg(Npy-hrGFP)1Lowl/J transgenic
mice (Jackson lab stock #006417), which express green fluorescent protein (GFP) under control of the
neuropeptide-Y (NPY) promoter (NPY-GFP). We used gene-positive offspring to record specifically from
LTS cells and gene-negative offspring to record from other striatal cell types. For some experiments, we
used slices from a cross of B6.Cg-Gt(ROSA)26Sor<tm14(CAG-tdTomato)Hze>/J (Jackson lab stock
#007914) with B6;129P2-Pvalb<tm1(cre)Arbr>/J (Jackson lab stock #008069). These mice expressed
tdTomato specifically in parvalbumin-expressing cells, and were used to target FS neurons. We used 3-8
week old mice of either sex. Mice were deeply anesthetized with 5% isoflurane and intracardially perfused
with ice-cold, oxygenated (95% O₂/5% CO₂) slicing medium containing (in mM): 2.5 KCl, 1.25 NaH₂PO₄,
10.0 MgSO₄, 0.5 CaCl₂, 26.0 NaHCO₃, 10.0 glucose, 230.0 sucrose, 1.0 Na-pyruvate, 1.0 Na-ascorbate,
and 0.05 μM glutathione. Striatal slices (300 μm thickness) were cut in the sagittal plane and transferred
to a holding chamber containing warmed (~35 °C), oxygenated physiological solution containing (in mM):
126.0 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 MgCl₂, 2.0 CaCl₂, 26.0 NaHCO₃, 10.0 glucose, 1.0 Na-pyruvate,
1.0 Na-ascorbate, and 0.05 μM glutathione for 30 m, then maintained at room temperature until used.
Slices were transferred to a submersion recording chamber and bathed (2-3 ml/min) with the same
oxygenated physiological solution maintained at 35 °C.

Micropipettes had tip resistances of 3-8 MΩ, and were filled with a solution containing 140.5 mM
KMeSO₄, 7.5 mM NaCl, 10.0 mM HEPES, and 0.5 μg/ml gramicidin. Gramicidin was freshly mixed in
DMSO (0.5 mg/ml), and diluted 1:1000 in filtered electrode solution. 20 μM Alexa Fluor 594 biocytin
(Molecular Probes, Eugene, OR) was added to the electrode solution to verify that perforation was maintained without rupturing the patch and to visualize the morphology of recorded neurons following rupture at the conclusion of the experiment.

Striatal neurons were visualized using an Olympus BX51WI microscope with a Coherent Chameleon-XR Ti:sapphire laser, Ultima scanhead and detectors (Prairie Technologies, Middleton, WI). Fluorescent emissions from GFP and Alexa Fluor 594, both excited at 810 nm, were separated using a dichroic beamsplitter (575 nm) and barrier filters (525/70 and 607/45) from Chroma (Rockingham, VT). Image stacks were deconvolved using AutoQuant X (Media Cybernetics, Bethesda, MD), and maximum projection images were generated using Imaris (Bitplane, Zurich, Switzerland).

Data were acquired using a Multiclamp 700B amplifier (Molecular Devices, Union City, CA), filtered at 10 kHz, and digitized at 20 kHz using a HEKA ITC-18 digitizer, and collected using Igor Pro (WaveMetrics, Lake Oswego, OR). All recordings were performed in the perforated patch configuration, and no correction was made for liquid junction potential. Analysis was done using Mathematica (Wolfram Research, Champaign, IL). Membrane potential was simultaneously digitized with a National Instruments PCIe 6251 digitizer in another computer running RTXI (www.rtxi.org), which was used for applying artificial synaptic conductances.

**Spiking Resonance**

Spiking resonance tests employed either a Poisson-triggered barrage of artificial synaptic currents or synaptic conductances. Both were generated in real time by the recording apparatus, so that each barrage was unique, and the injected current was digitized at 20 kHz and recorded along with the cell membrane response. Synaptic conductance barrages consisted of Poisson-triggered bi-exponential conductances with 0 mV reversal potential, 0.1-0.5 nS peak, 0.25 ms rising time constant, and 2.5 ms decay time constant. Firing rates were varied by constant current injection. The frequency spectrum of the resulting current signal was limited by the decay time constant of the individual artificial synaptic conductance (Fig. 1A). The artificial synaptic conductance barrage produced currents that were very similar to those used for current injection. Because the reversal potential was far from the membrane potential during most of the interspike interval, there was very little effect of driving force variation on the shapes of the resulting artificial synaptic currents. The main difference between these stimuli was the introduction of a fluctuating shunt conductance and sublinear synaptic summation. In both cases, it is the synaptic current that should phase-lock firing, so we digitized the current generated by the dynamic clamp and used it as the input signal for the analysis of phase locking.

The stimulus waveform was decomposed into complex components using the discrete Fourier transform, and these were filtered into overlapping bands of 2 or 10 Hz width, centered every 1 or 5 Hz respectively. The filters consisted of a Tukey window ($\alpha = 0.25$) that was zero everywhere except for the frequency band of interest. This yielded a set of filtered components of the inputs (Fig. 1B). The phase angle of each action potential was expressed as a vector with the same angle and a length of $1/n$, where $n$ is the total number of spikes, and the vectors were added (Fig. 1C). The length of the vector sum, called
Vector Strength, is a measure of coherence between firing and the filtered waveform (Fisher, 1993). The vector strengths for the filtered stimulus waveforms were combined to make a frequency spectrum (Fig. 1D). The statistical significance of frequency dependent phase-locking was tested using the Kolmogorov-Smirnov (K-S) test for deviation from a uniform phase distribution, corrected for multiple comparisons using the Bonferroni correction.

**Membrane Resonance:**

It was impossible to use current clamp recording to measure membrane impedance resonance in the LTS interneurons or ACh interneurons, because they fire spontaneously, and current clamp measurements were always contaminated by action potentials unless the cells were hyperpolarized outside of their normal membrane potential range. We used an equivalent voltage clamp method for measuring membrane resonance while controlling spiking.

Each cell was tested with 12 repeats of a frequency sweep stimulus consisting of a sine wave of linearly increasing frequency (Fig. 2A). Each series consisted of 6 traces of sine waves of initially positive slope, and 6 initially negative. In some cases we reversed the order of frequencies, sweeping from high to low frequency. This never changed the result. The current responses to each set of 6 traces with the same sign were averaged (Fig. 2B). For each average we calculated the discrete Fourier transform of both the input voltage waveform and the output current, and the complex impedances were obtained for each frequency step as the ratio of the voltage and current. Complex impedances were plotted as an impedance locus (Fig. 2C). Cell capacitance is an essential component of a cell’s resonance, so whole cell capacitance correction was disabled. Access resistance is seen as a positive shift on the real axis. We measured the access resistance using pulses and corrected the data by shifting each point in the impedance locus accordingly (Fig. 2C). The real and imaginary parts of the corrected impedance at any point may be positive or negative, but the impedance magnitude, which is the distance from the origin to each point (marked Z) is always positive. We calculated the impedance as a function of frequency, and averaged the results for the positive-going and negative-going traces for each cell (Fig. 2D).

All data are presented as mean ± standard deviation unless otherwise noted. Error bars in figures are standard errors of the mean.

**RESULTS**

Most experiments were performed using slices from a mouse line in which green fluorescent protein (GFP) is expressed under the control of the neuropeptide Y (NPY) promoter (NPY-GFP). This was to facilitate identification of LTS neurons, which are by far the most difficult to identify without a direct reporter. We also recorded from spiny cells and ACh interneurons in these mice and their GFP-negative littermates. Candidate spiny neurons and ACh interneurons were targeted by size and shape in interference optics. In initial experiments we also identified candidate FS interneurons by appearance in interference optics in the NPY-GFP mice. In later experiments we obtained striatal slices from another mouse line, a cross between a parvalbumin-Cre mouse and a floxed td-Tomato reporter mouse in which
FS cells were fluorescent. Cell identities were always confirmed using morphological and physiological characteristics. We employed perforated patch recordings to maintain stable consistent recordings over the 30-90 minutes required to complete our experimental protocol. After completion of the experiment, the patch was ruptured and the cells filled with Alexa 594 for morphological identification.

Fast Spiking Neurons

We recorded from 16 striatal FS neurons, 7 from the NPY-GFP mouse and 9 from the parvalbumin-td-Tomato mouse. FS cells recorded from the two lines were indistinguishable, and the results were pooled. Nine FS neurons were used to study spiking resonance (i.e. phase-locking to frequency components of the artificial synaptic barrage), and all were used to characterize membrane resonance (frequency-dependence of input impedance using frequency-swept sine waves). In 7 cells, we used sine wave sweeps from 0 to 60 Hz at two or more different holding potentials (-80 mV, -60 mV, -40 mV). In 9 cells we used a broader range of frequencies (0-260 Hz).

The striatal FS cells in our sample exhibited the morphological and physiological features associated with this cell-type (Bracci et al., 2003; Kawaguchi, 1993; Koós and Tepper, 1999; Sciamanna and Wilson, 2011). They had compact, symmetrical highly branched dendritic fields and dense axonal arborizations mostly localized in the same volume as the dendrites (Fig. 3A). They were silent in slices, with membrane potentials averaging -76 mV (± 10 mV). Their input resistances (measured at the resting potential) varied from 41 to 157 MΩ (74 ± 35 MΩ). When depolarized to fire they showed a characteristic stuttering firing pattern, consisting of variable-length episodes of firing at a nearly constant rate separated by periods of silence with subthreshold oscillations (Fig. 3B). We measured the minimum repetitive firing rate (f_{min}) as the reciprocal of the modal interspike interval at rheobase (Sciamanna and Wilson, 2011). The f_{min} was consistent across measurements for individual neurons but varied over a wide range among neurons (19-265 spikes/s). Most (9/16) had f_{min} values between 19 and 80 spikes/s, but there were a smaller number of neurons that could not fire repetitively except at much higher frequencies (Fig. 3I).

Gamma-frequency Spiking Resonance in FS Neurons

We studied spiking resonance in 5 cells recorded in slices from the NPY-GFP mice, and 4 cells in slices from the parvalbumin-tdTomato mouse. FS cells do not fire spontaneously, so we applied constant current to drive the cell to fire in the stuttering pattern. We adjusted the current to be slightly above rheobase, so that the mean firing rate substantially less than f_{min}. At these rates, the cells fired in doublets or episodes of spikes at f_{min}, separated by periods of silence (Fig. 3C&F). To imitate the high density of individually subthreshold synaptic inputs expected in vivo, we used a dense barrage (1000/s) of small (20 pA) brief (τ_{decay} = 2.5 ms) artificial synaptic currents. The holding current was then adjusted to achieve approximately the same firing rate that was achieved using constant current alone (Fig. 3D&G). In cells with f_{min} near 40 Hz, the artificial synaptic barrage was sufficient to disturb the firing pattern and make the stuttering pattern barely discernible. In cells with higher f_{min}, episodes of high frequency firing continued but were much briefer, more frequent and less regular. We calculated the phase-locking of FS cells to
frequency components in the barrage of artificial synaptic currents or conductances. The current waveform was decomposed into overlapping frequency bands of 10 Hz width, spaced every 5 Hz from 5 to 300 Hz. We calculated the phase of each action potential relative to each of the filtered current waveforms. These measurements were converted to unit vectors, scaled by the number of spikes, and added. The length of the vector sum (Vector Strength) was used to quantify phase-locking at each frequency. Vector Strength could take values between 0 (meaning no phase-locking) and 1 (meaning all spikes were at a fixed phase on the filtered current waveform). The statistical significance of frequency specific phase-locking for each cell was tested using the K-S test (see Methods).

All FS cells showed statistically significant phase-locking, which was frequency-dependent, as indicated by a maximum in the vector strength versus frequency curve (e.g. Fig. 3E&H). We compared the frequency at this peak ($f_0$) to $f_{\text{min}}$. The $f_0$ for all cells corresponded to the $f_{\text{min}}$ (Fig. 3J), as indicated by the strong linear correlation between these measures ($r = 0.99, p < 0.01$). In some cells, a clear second peak of phase-locking could be seen at twice $f_{\text{min}}$ (e.g. Fig. 3H). The group curve for 9 neurons, with frequency normalized to $f_0$ is shown in Fig. 3K. It is remarkable that in the cells with the highest $f_{\text{min}}$, strong phase-locking occurred at frequencies that were only weakly present in the original stimulus.

**Somatic Membrane Resonance in FS Cells but Not in the Gamma Range**

We measured membrane resonance in voltage clamp using sine wave sweeps with linearly increasing frequency. We expected resonances near 40 Hz, so we initially used a frequency sweep from 0 to 60 Hz over 20 seconds. After recognizing that some FS cells in our sample had much higher minimum firing rates, we repeated the experiment in an additional set of cells using a frequency sweep up to 260 Hz. The second series of experiments yielded no new resonances beyond those seen in the first series. Even in cells with very high $f_{\text{min}}$, the membrane potential resonance, measured from the soma, was restricted to lower frequencies. Thus the results reported here are for our original (0-60 Hz) sample, which gave the highest resolution in the frequency range of membrane resonance.

We measured membrane resonance at three different holding potentials. We expected that resonance would be evident at membrane potentials between -50 and -40 mV, at which the cells linger and oscillate between episodes of firing (Bracci et al., 2007; Fig.1). Note that the experiment could not have been performed at this voltage using current clamp recording, because the measurements would be disturbed by episodes of firing. Our voltage clamp recordings usually (5 of 7 cells) had sufficient control to prevent firing at this potential. Holding voltages were corrected off-line for error due to access resistance, so varied somewhat among cells. We took additional measurements near the FS cells’ resting membrane potential (-80 mV) and at an intermediate potential (-60 mV). Example impedance spectra at all three voltages for a representative striatal FS cell are shown in Fig. 4A-C. There was little or no resonance seen at -80 mV or at -60 mV. There was a resonance in the -40 to -50 mV range (Fig 4C). However, the peak frequency for this membrane resonance was always lower than the frequency of the spiking resonance or $f_{\text{min}}$ measured in the same cell. The $f_{\text{min}}$ for the cell whose spectra are shown in Fig. 4A-C was 55 spikes/s, but the membrane potential resonance peaked near 20 Hz. There was no correlation
between the peak frequency of membrane resonance and the $f_{\text{min}}$, and the peak membrane resonance was always less than 30 Hz. Membrane potential resonance depended on voltage-activated sodium current, being blocked by treatment with 1 µM tetrodotoxin ($n = 4$, not shown).

To facilitate comparisons at different voltages, the curves were normalized by DC input resistance (Fig. 4D). To determine the statistical significance of the resonance, we used a repeated measures analysis of variance (ANOVA) over the initial part of the group resonance curve. This tested for an initial increase in impedance preceding the inevitable decline at high frequency. The impedance at -44 mV showed a significant increase from 0.2 to 10 Hz ($F = 2.50; df = 39,156; p < 0.01$), whereas there was no corresponding increase over the initial part of the curve (0.2-3.0 Hz) at -60 mV ($F = 1.93; df = 10,60; p > 0.05$) or at -80 mV ($F = 1.56; df = 19,114; p > 0.05$ measured from 0.2-5.0 Hz).

These data indicate that the resonance of the somatic and proximal dendritic membrane accessible by somatic voltage clamp is not adequate to generate the spiking resonance of FS cells. Perhaps the high frequency resonance is associated with the action potential trigger zone, which is probably located on the axon, as it is on cortical FS cells (Goldberg et al., 2008).

**LTS Cells**

We studied the responses of 58 striatal LTS interneurons in slices from 38 NPY-GFP mice. Care was taken to distinguish recordings from LTS cells from the neurogliaform interneuron type labeled in the striatum of NPY-GFP mice (Ibáñez-Sandoval et al., 2011). LTS cells had ovoid somata and extended, sparsely branched dendritic trees (Fig. 5A). All cells also showed the characteristic membrane potential and spiking responses to depolarizing and hyperpolarizing current pulses associated with this neuron type (Fig. 5B), consisting of a moderate depolarizing sag when hyperpolarized by negative current pulses, and a characteristic rebound burst when hyperpolarizing pulses were terminated (e.g. Kawaguchi, 1993; Ibáñez-Sandoval et al., 2011, Beatty et al. 2012). All cells identified as LTS cells showed spontaneous firing during perforated patch recordings, with firing rates from 1.8 to 18.6 spikes/s (mean 9.0 ± 4.0).

**Beta-frequency Spiking Resonance in LTS Interneurons**

Spiking resonance was studied in 31 identified LTS cells in slices from 17 animals. Because LTS interneurons fired spontaneously, we applied a constant hyperpolarizing current to compensate for the average current in the artificial synaptic barrage and return the cell to approximately its spontaneous rate. Firing rate was further adjusted over a range from about 4 to 40 spikes/s by passage of additional constant current, allowing us to measure spiking resonance at various firing rates. Barrages of conductance inputs were also tested using conductance clamp through the somatic electrode. Individual simulated synaptic conductances had rise time constants of 0.25 ms, decay time constants of 2.5 ms, a reversal potential of 0 mV, and peak conductances of 0.1-0.5 nS. Similar results were obtained for artificial synaptic current and synaptic conductance barrages. Because of summation of conductances, there was both an average conductance and a resulting constant current. The current (but not the conductance) was calculated and removed using a hyperpolarizing offset.
All LTS neurons showed statistically significant spiking resonance in the 10-30 Hz range (Fig. 5C-E). Changes in firing rate did not alter phase-locking at higher frequencies, but did at low frequencies. When cells were firing much slower than their normal spontaneous rate, they would phase-lock to a wide range of low frequencies. Phase-locking to frequency components above 30 Hz dropped off similarly at all firing rates. Similar results were obtained using conductance, rather than current, stimuli (Fig. 5D). LTS cells did not show large individual variations in the frequency of their spiking resonance, and so it was not necessary to normalize the group curve for peak frequency (Fig. 5E). We attempted to drive firing above 30/s, to determine whether the peak frequency of spiking resonance would follow firing rate when the rate exceeded the natural resonant frequency. Most LTS cells would not maintain consistent firing at rates higher than 30 spikes/s for the time required for our test (3 min). Thus the high frequency curve shown in Figure 5E represents a subset of neurons for which this test could be performed. Peak spiking resonance did not shift much with rate, but as the cells’ firing rates increased, they became increasingly selective for the 15-30 Hz components of the input.

**Beta-frequency Membrane Resonance in Striatal LTS Interneurons**

We measured membrane resonance at -80 mV (outside the range of the normal membrane potential trajectory) or -50 mV (within the range of interspike membrane potentials). LTS neurons show membrane potential oscillations at potentials positive to -40 mV (Beatty et al., 2012) so it was important to test for resonance at depolarized potentials. In most neurons, action currents interrupted our measurements at membrane potentials too near the action potential threshold, but in a few cells we were able to hold the cells as depolarized as -30 mV long enough to complete the test. The value of the clamped voltage was corrected for voltage error caused by the series resistance. These experiments were performed in a total of 32 neurons in slices from 25 animals.

When measured at -80 mV or -50 mV, there was no significant resonance that could be responsible for the beta-range phase-locking seen with artificial synaptic current barrages (Fig. 6A-D). At more depolarized voltages, we consistently observed resonance in the beta-frequency range (Fig. 6C). We tested the statistical significance of that resonance for our sample using the repeated measures ANOVA. The resonance at the depolarized membrane potentials, measured as a trend in the impedance curve between 0.1 and 10 Hz, was statistically significant ($F = 5.8; \text{df} = 79,474; p < 0.01$).

Blockade of sodium channels with TTX did not change the impedance profile at -80 mV, or at -50 mV, although at that voltage there is substantial persistent sodium current. TTX also did not eliminate the resonance at -30 mV (Fig. 6G&H). Although smaller on average in the sample, the resonance in TTX had the same frequency profile and was still statistically significant ($F = 4.64; \text{df} = 20, 320; p < 0.01$).

**Cholinergic Interneurons**

We recorded from 24 ACh interneurons in slices from 19 mice. Their identities were confirmed on the basis of their broad action potentials, large spike afterhyperpolarizations, distinctive responses to intracellular current pulses, and characteristic morphology (Fig. 7A&B) (Bennett and Wilson, 1999;
In response to hyperpolarizing current steps they showed strong sags and long duration rebounds during which they often fired, but which never evoked a high frequency burst. In response to depolarizing steps, ACh neurons showed a rapidly adapting firing pattern followed by a long lasting afterhyperpolarization. All ACh interneurons were spontaneously active.

**Delta-frequency Spiking Resonance in Cholinergic Interneurons**

We studied spiking resonance of 9 cells using artificial synaptic current or conductance barrages. It was impossible to change the average firing rate of ACh interneurons using constant current injection as we did for LTS and spiny neurons. They showed large brief changes in rate in response to 1 s constant current pulses, but a powerful spike frequency adaptation rapidly restored firing to a narrow range near the spontaneous firing rate. Spike frequency adaptation in these cells is caused by a calcium-dependent slow afterhyperpolarization current (Goldberg and Wilson, 2005). Attempts to slow firing by constant current evoked a strong HCN current that returned firing to near-baseline levels. We measured spiking resonance at the natural firing rate for these cells in our sample, on average $1.37 \pm 0.53$ spikes/s. Firing rate during injection of simulated synaptic currents for measurement of spiking resonance averaged $1.61 \pm 0.55$ spikes/s.

For analysis of spiking resonance at such low firing rates, we filtered the input waveform into components using 1 Hz bandwidths. An example response to conductance noise is shown in Fig. 7C.

This neuron was firing at 2.1 spikes/s, and showed practically no phase-locking at all for frequencies above 5 Hz. All ACh neurons showed statistically significant spiking resonance, but only to very low frequency components, in the 1-5 Hz frequency range. The results for the sample of 9 neurons are shown in Fig. 7D.

**Membrane Resonance in Cholinergic Interneurons**

We studied membrane resonance at two different holding potentials in 17 ACh neurons. One set of measurements was taken at -80 mV, which is below the normal trajectory of the membrane potential during its spontaneous repetitive firing. At this voltage, there is no contribution from the persistent sodium current that drives spontaneous activity (Bennett et al., 2000), but it is within the activation range of hyperpolarization-activated Kir current and HCN currents, which can produce oscillations in these cells in the hyperpolarized voltage range (Wilson, 2005). We also measured membrane resonance at the most positive voltage that we could maintain in voltage clamp without interference of action currents. This voltage varied among neurons between -50 and -60 mV and was well within the range of the persistent sodium current activation. At both hyperpolarized and depolarized voltages we observed a strong resonance that peaked in the 1-3 Hz range (Fig. 7E-G). The peak frequencies at the two membrane potentials were similar, but on average much stronger resonance was seen at the more depolarized membrane potential (Fig. 7G). The resonance was statistically significant at both potentials, as indicated by an increase in impedance over frequencies less than or equal to 2 Hz ($F$ at -50 mV = 8.8; $df$ = 8,64, $p < 0.01$; $F$ at -80 mV = 19.7, $df$ = 8,72, $p < 0.01$).
To determine the potential participation of persistent sodium channels in the resonance of ACh interneurons, we repeated the measurements after blockade of sodium channels with TTX. This treatment had no effect on resonance at -80 mV (Fig. 7H), but abolished the resonance seen at -50 mV (Fig. 7I).

**Spiny Neurons**

We recorded from 27 spiny neurons in slices from 18 mice. Spiny neurons’ identities were confirmed by their distinctive physiological properties and their densely spine-laden dendrites observed after filling with Alexa 594 at the end of the experiment (Fig. 8A). They had deeply hyperpolarized resting membrane potentials, low input impedances, rapid inward rectification, and a ramp-like depolarization to the firing point near rheobase (Fig. 8B).

**Firing Rate Dependent Spiking Resonance in Spiny Neurons**

Spiking resonance was studied in 17 striatal spiny neurons in slices prepared from 11 animals. We applied 150-450 pA constant current to generate repetitive firing at rates ranging from 2 to 30 spikes/s, and superimposed barrages of artificial synaptic current or excitatory conductances on this background constant current. We varied the firing rate of the neuron by changes in either the level of constant current or the average frequencies of the artificial synaptic barrage. Changing firing rates by alterations in constant current was simpler, in that it preserved the variance of the synaptic current barrage, and the results presented here will be from the 9 cells tested in that way. Similar results were obtained by changing the average frequency of the synaptic barrage. In 8 cells we also used simulated synaptic conductance waveforms (0.35-0.6 nS in amplitude).

All spiny neurons showed statistically significant phase-locking to a range of frequency components centered on the firing rate of the neuron (Fig. 8C). In contrast to the results for interneurons, the firing rate of the spiny neuron was the sole determinant of selectivity of phase-locking to frequency components of the artificial synaptic barrage. Spiny cells would not maintain firing at rates above 30 Hz for long periods of time, which limited the rates at which we were able to test phase-locking. Over the range studied, this result was highly reproducible across cells (Fig. 8D). Similar results were also obtained for barrages of excitatory conductances (not shown).

**Absence of Membrane Resonance in Spiny Neurons**

We focused on two membrane potentials: one near the resting potential and one near the Up state seen in spiny neurons *in vivo* (e.g. Wilson and Kawaguchi, 1996). Spiny neurons are known to possess voltage-dependent currents that are activated in the subthreshold range. Near the resting membrane potential, or Down state, (about -80 mV), they are controlled by a hyperpolarization-activated potassium current, Kir (e.g. Nisenbaum and Wilson, 1995). *In vivo*, the neurons may be depolarized by a tonic excitatory synaptic barrage to a membrane potential near -60 mV (the Up state), at which the Kir current is effectively deactivated. At that potential the input resistance is increased, both because the Kir channel is deactivated and because of positive feedback from persistent sodium current (Nisenbaum and Wilson, 1995). We tested 10 spiny cells for membrane resonance at -80 mV (Down state) and 11 cells at -60 mV.
(Up state). A subset of cells (17 and 8 respectively) were tested again at these potentials in the presence of 1 µM TTX. At -80 mV, there was no sign of resonance and no effect of TTX (Fig. 8E). The average input resistance was near 80 MΩ. The low-frequency input impedance of the spiny neurons was much higher at -60 mV. This was partly attributable to the persistent sodium current, because TTX application reduced the input impedance by about half (Fig. 8F). Despite the presence of this regenerative voltage-dependent current, there was no apparent resonance in the spiny neuron at -60 mV, either in the control or in TTX solution. During application of TTX, it was possible to test the spiny neuron for resonance at voltages that would be near threshold in the untreated cell, and again no resonance was evident (not shown).

The phase-locking of spiny neurons to components of the synaptic barrage cannot be attributed to intrinsic resonance of the membrane potential. Instead, it is the tendency of repetitively firing neurons to phase-lock with periodic components of their input that match their firing rate, even if those components are buried in a complex, noisy barrage of synaptic currents.

**DISCUSSION**

Knowing the amplitude and time course of synaptic current tells something, but not everything about the strength of a synapse. A synapse’s effect also depends on the temporal sequence of activity in which it is embedded. Synapses activated repeatedly at a rate commensurate with the resonant frequency of a postsynaptic neuron gain enormously in importance. Inputs that do not may be rendered ineffective (e.g. Izhikevich et al., 2003). In a network with ongoing activity, the frequency relationships between neurons strongly influence their functional connectivity.

All neurons exhibit some kind of resonance when they fire. Some neurons also have resonances because of interacting subthreshold voltage-sensitive ion channels (e.g. Hutcheon and Yarom, 2000). Even in neurons lacking membrane resonance, the ionic currents generated by action potentials and afterhyperpolarizations create a resonance of their own. The responsiveness of a repetitively firing neuron to an input depends on the timing of the input relative to the neuron’s interspike period. Periodic inputs arriving at the right time to influence the firing of one action potential will be most effective if they arrive at about the same time relative to the next. Thus neurons are most responsive to synaptic inputs from other neurons firing at the same mean rate (e.g. Richardson et al., 2003; Higgs and Spain, 2013).

A neuron may have more than one resonant frequency, for example if it has an intrinsic membrane resonance at one frequency and is firing at a different rate. Computer simulations by Richardson et al. (2003) show that these may interact in a conditional way, at least for cells with relatively simple dynamics. For the cell model they used, if the firing rate was lower than the membrane resonance frequency, membrane resonance dominated the overall response, whereas spiking resonance dominated at higher firing rates.

We studied spiking resonance using a random barrage of either depolarizing current or conductance transients. The additional membrane conductance introduced by our conductance stimulus had no discernable effect on the spiking resonance of the neurons, suggesting that resonance might be relatively
robust to changes in synaptic load. However, the presence of ongoing inhibition in vivo is likely to increase the effective membrane conductance beyond the level used here, and might modulate the frequency or strength of cellular resonance.

Mechanisms of Subthreshold Resonance

Subthreshold resonance has been studied in a variety of neuron types, and the mechanisms responsible for resonance are well known (e.g. Fishman et al., 1979; Koch, 1984; Moore et al., 1993; Pike et al., 2000; Hutcheon and Yarom, 2000; Narayanan and Johnston, 2008). Resonance arises from a timing mismatch between opposing ionic currents as they respond to a change in an input current. In its simplest form, resonance arises because restorative ionic currents, (which oppose a change in membrane potential in response to a perturbation), evolve slower than the membrane potential change. For example, resonance in the original Hodgkin and Huxley equation for the squid axon arises because there is a delay in the onset of the restorative processes (sodium channel inactivation and potassium channel activation) after the onset of the membrane potential changes that trigger them (e.g. Koch, 1984). Resonance may be amplified by the presence of fast regenerative currents that accelerate changes in membrane potential (e.g. Ströhmann et al., 1994). The same processes that make membranes resonant are also often responsible for the generation of subthreshold membrane potential oscillations, and cells showing subthreshold oscillations are expected to show subthreshold resonance at the same frequency.

In ACh interneurons the frequency of spiking resonance matched that of subthreshold resonance, and both corresponded to the rate of autonomous firing of these cells. ACh interneurons have two different subthreshold oscillations, both at frequencies close to the resonance found here (Wilson, 2005; Wilson and Goldberg, 2006). One depends entirely on hyperpolarization-activated currents, and creates oscillations at membrane potentials below -50 mV (Wilson, 2005). This interaction is probably the origin of the resonance we have seen in the very hyperpolarized membrane potential range in ACh interneurons. Another oscillatory subthreshold membrane potential mechanism in those cells relies on calcium entry through voltage-gated subthreshold activated Ca\textsubscript{v}1 (L-type) channels, and a restorative current flowing through calcium-dependent potassium channels activated by the resulting calcium influx (Goldberg and Wilson, 2005). This oscillation is seen near spike threshold, and may be the origin of resonance at these potentials. Why then was this resonance sensitive to blockade of sodium channels by TTX? A large proportion of the calcium currents, and presumably the potassium currents also, are located in the dendrites (Goldberg et al., 2009). Perhaps transfer of depolarization into dendrites from our somatic voltage clamp is enhanced by dendritic sodium channels.

The mechanism of membrane resonance in LTS interneurons is unknown. They are autonomous oscillators, firing at rates between 1 and 30 spikes/s in the absence of synaptic input. They display spiking and membrane resonance in the beta frequency range (10-30 Hz). The beta frequency membrane resonance persisted after blockade of sodium channels with TTX. LTS interneurons show underlying TTX-insensitive membrane potential oscillations when depolarized into the voltage range of their
resonance. Perhaps the oscillations and resonance share a similar mechanism. The oscillations persist after blockade of sodium channels, but are sensitive to blockade of calcium channels (Beatty et al. 2012).

In contrast to the other cells, the ionic mechanism of subthreshold oscillation and resonance in FS interneurons resides in the spiking channels themselves, as indicated by its sensitivity to TTX. However, FS cells showed unexpectedly low frequency membrane resonances that did not match the frequency of their spiking resonances. Perhaps it is the axonal, not the somatic membrane resonance that is responsible for the powerful and obvious spiking resonance of this neuron. We have previously suggested that the axonal membrane lying between the soma and the spike trigger zone has an especially low membrane resistance during stuttering activity because of the activation of the subthreshold, slowly inactivating potassium channel Kv1 (Sciamanna and Wilson, 2011). These channels are predicted to produce a large voltage attenuation between the soma and the axonal trigger zone, which would isolate our measurement of resonance from the highly resonant membrane of the axon.

Periodic Synaptic Inputs to the Striatum

The evidence for periodicity in the synaptic inputs to the striatum is persuasive, although indirect. Local field potentials recorded in the striatum show a temporal inhomogeneity of frequency components, with peaks in power that come and go with changes in behavior (e.g. Courtemanche et al., 2003; DeCoteau et al., 2007; Howe et al., 2011; Leventhal et al., 2012; van der Meer and Redish, 2009; Kalenscher et al., 2010). There are peaks near 1 Hz (delta), at 5-10 Hz (theta), 12-30 Hz (beta), and 30-80 Hz (gamma). Sometimes there are also components at higher frequencies. Some of this periodicity is driven by cortico-striatal afferents. Individual corticostriatal neurons exhibit membrane potential oscillations consisting of a mixture of different periodicities, showing both gamma- and delta-periodic firing at the same time, with gamma oscillations riding on the depolarizing phase of the delta membrane potential oscillation (Cowan and Wilson, 1994; Stern et al., 1997). Individual corticostriatal neurons usually do not fire periodically, but their irregular firing is phase-locked to the underlying membrane potential oscillation (Stern et al., 1997), so the composite input stream from these neurons will contain both frequencies.

Firing Rate: The Medium, not the Message

Each individual interneuron type studied here is tuned to align with one of the functionally important frequency bands of the striatal field potential. As the degree of input coherence within these bands shifts during behavior, this predicts a corresponding shift in the coherence of firing in each interneuron and its corresponding intra-striatal subcircuit. These responses are not necessarily changes in firing rate, but in spike timing. They would result in changes in coherence between the afferent input stream and the neuron’s spike output, and between neurons of a cell-type sharing the periodic input. Increased coherence among neurons would enhance their influence on the spike timing of the spiny projection neurons independently of firing rate.
The spiking resonance of the spiny neuron shows that it too has a preference for inputs in some frequency range. But its resonance is variable, and depends on firing rate. When spiny neurons change rate, they tune to a new input frequency band, and to the interneurons whose firing is locked to that frequency. Unlike the interneurons studied here, spiny neurons have no intrinsic natural frequency and their firing rate may be readily varied over a wide range. They are capable of sustained firing in the low portion of the gamma range, but they can fire at much higher rates for a few seconds. During those transient rate changes, their spike timing may become entrained to commensurate frequency components in the activity of their afferents and interneurons (Wilson, 2009).

It has often been reported that striatal spiny neurons transiently change firing rates in temporal relation to the performance of a motor task, or in response to the motivational or informational content of a sensory signal. These rate changes of spiny neurons have long been assumed to be the signal sent by the striatum to its synaptic targets in the globus pallidus and substantia nigra. However, there is some evidence that oscillatory neurons in those targets are sensitive to timing of their inputs, so might be more sensitive to spike-timing coherence than to the mean rate of the striatal output (Wilson, 2013). The much-studied changes in firing rate of spiny cells may not in themselves comprise the signal sent from the striatum to its targets. Rate changes may serve primarily to produce a shift in the frequency band in which coherence of spike timing is embedded, and that coherence, rather than the rate change, may be the signal to which the targets of striatal output neurons respond.

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**FIGURE CAPTIONS**

Figure 1. Measurement of spiking resonance. A. Spectrum of the artificial synaptic barrage. B. Top panel: Filtered synaptic barrage (blue), and spike times (red lines). Inset at right shows how the phase of each spike time ($\phi$) was measured relative to zero-crossings in the filtered waveforms. Bottom panel: corresponding trace of the original membrane potential. C. Calculation of Vector Strength by addition of vectors for each action potential. Vector Strength was scaled so that it ranges from 0 (no consistent phase-locking) to 1 (all spikes occurred at the same phase on the filtered waveform). D. Example spectrum of phase-locking consisting of vector strengths over a range of input frequencies.

Figure 2. Measurement of membrane resonance. A. An input voltage command. The frequency of the sinusoidal increased from 0 Hz at the start of the sweep to 40 Hz at the end. B. Corresponding membrane current. Resonance is apparent as a decrease in the current amplitude. C. Complex impedance locus uncorrected for access resistance ($R_A$), (in red) and after correction (in black). The points increase in frequency going clockwise through the graph. D. Impedance amplitude spectrum obtained from the impedance (Z) measured at each frequency value in C.

Figure 3. Spiking resonance in FS neurons. A. FS cell after filling with Alexa 594 (electrode still present). B. Upper panel: High frequency stuttering response. Lower panel: A single episode of firing during the stuttering pattern, showing subthreshold oscillations. C-E. An example FS neuron with spiking resonance in the gamma range. C. Firing in short episodes of 40 spikes/s separated by brief silent periods. The minimum repetitive firing rate ($f_{\text{min}}$) is the modal instantaneous firing rate at rheobase. D. Upper panel: Artificial synaptic current barrage. Lower panel: Firing of the same neuron in C, at about the same rate, but made irregular by the presence of the artificial synaptic current barrage. E. Vector strength for phase-locking to the frequency components of the input barrage. Note the peak in phase-locking corresponds approximately to $f_{\text{min}}$. F-H, the same as C-E, but for another neuron with a much higher $f_{\text{min}}$. I. Histogram of $f_{\text{min}}$ values for the sample of 16 FS neurons. J. Relationship between $f_{\text{min}}$ and the peak of the vector strength curve ($f_0$). K. Group resonance curve, frequency normalized to each cell's $f_0$. Error bars are SEM.

Figure 4. Membrane resonance in striatal FS neurons. A-C. Measurements from one neuron at 3 different holding potentials. A. Holding near the resting membrane potential reveals little or no resonance. B. Holding at a potential intermediate between the resting potential and threshold shows no resonance. C. Holding near threshold, at a potential corresponding to the silent period between episodes during stuttering, yields a strong resonance near 20 Hz. D. Average membrane resonance for the sample of FS neurons, normalized by the DC input resistance at each holding potential. Error bars are SEM.

Figure 5. Spiking resonance in LTS Neurons. A. An LTS cell after filling with Alexa 594 (electrode still present). B. The response of an LTS neuron to hyperpolarizing (black) and depolarizing (gray) current pulses. Note the presence of spontaneous firing and the rebound LTS afterhyperpolarization. C. Spiking resonance to artificial synaptic currents at the spontaneous firing rate (black) and at slower and faster rates. Maximal phase-locking occurs in the beta frequency range. D. Spiking resonance in the same cell in response to an artificial synaptic conductance barrage. E. Group curve for spiking resonance in all LTS cells studied. Error bars are SEM.
Figure 6. Membrane resonance in striatal LTS Interneurons. A-C. Impedance measurements from a neuron in control solution. A. Absence of resonance at a -80 mV. B. Resonance is also absent at -50 mV. C. Resonance in the beta range is present at a depolarized potential. D. Membrane resonance for the sample of LTS neurons in control solution, normalized for their low frequency (0.3 Hz) impedance. E-H. Membrane resonance in LTS cells is not abolished by TTX. E-G. An LTS cell after blockade of sodium channels with TTX. H. Group curve for the sample of neurons in TTX. Error bars are SEM.

Figure 7. Spiking and membrane resonance of cholinergic interneurons. A. Morphology of a cholinergic interneuron after filling with Alexa 594 at the end of the experiment (electrode still present). B. Responses of an ACh neuron to depolarizing (red) and hyperpolarizing (black) current pulses. Note hyperpolarizing sag response and rebound afterhyperpolarization, and spike frequency adaptation. C. Spiking resonance in the delta frequency range. D. Spiking resonance for the sample of ACh interneurons. E-F. Delta-frequency membrane resonance in an ACh interneuron at -80 mV (E) and at a more depolarized level near the firing threshold (F). G. Average membrane resonance at the two holding potentials for the sample of ACh cells. H. TTX-insensitivity of membrane potential resonance at -80 mV. I. Membrane resonance at -50 mV was abolished by TTX. Error bars are SEM.

Figure 8. Firing-rate dependent phase-locking but absence of membrane resonance in spiny neurons. A. A spiny cell after filling with Alexa 594 (electrode still present). B. Responses of striatal spiny neurons to current pulses, including strong inward rectification and ramp-like depolarization preceding spiking. C. Spiking resonance in a spiny neuron to the artificial synaptic current barrage, at various firing rates. Peak of phase-locking occurs at the cell’s firing rate. D. Spiking resonance for the sample of spiny neurons at three different firing rates. E. Absence of membrane resonance at -80 mV (Down state). F. Absence of membrane resonance, but large TTX sensitivity of impedance at more depolarized but still subthreshold (Up state) membrane potentials. Error bars are SEM.

REFERENCES


