Populations of striatal medium spiny neurons encode vibrotactile frequency in rats: modulation by slow wave oscillations

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Keywords: dorsolateral striatum, medium spiny neurons, somatosensory, vibrotactile

Word count: 2,821
Abstract

Dorsolateral striatum (DLS) is implicated in tactile perception and receives strong projections from somatosensory cortex. However the sensory representations encoded by striatal projection neurons are not well understood. Here we characterized the contribution of DLS to the encoding of vibrotactile information in rats by assessing striatal responses to precise frequency stimuli delivered to a single vibrissa. We applied stimuli in a frequency range (45-90 Hz) which evokes discriminable percepts and carries most of the power of vibrissa vibration elicited by a range of complex fine textures. Both medium spiny neurons and evoked potentials showed tactile responses that were modulated by slow wave oscillations. Further, medium spiny neuron population responses represented stimulus frequency on par with previously reported behavioural benchmarks. Our results suggest that striatum encodes frequency information of vibrotactile stimuli which is dynamically modulated by ongoing brain state.
**Introduction**

Striatum provides the major input nucleus to the basal ganglia, and as such is implicated in a number of both discrete and overlapping circuits critical to sensory-motor transformation and appetitive behaviour (Alexander et al., 1986; Yin et al., 2004; Bolam et al., 2000; DeLong and Wichmann, 2007). Dorsolateral striatum (DLS) lesions have implicated this region in automatic sensory-driven behaviours (Balleine et al., 2007; Tang et al., 2007; Tang et al., 2009). Some form of perceptual processing has been attributed to striatum. Thus striatal dysfunction plays a role in Parkinson’s disease and this condition produces impairments in somatosensory discrimination. The deficits are associated with impaired caudate activity and the effect is dissociable from motor impairments (Zia et al., 2003; Weder et al., 1999). In rats, somatosensory cortical columns corresponding to individual whiskers (the so called barrel cortex) send a strong projection to DLS, forming part of a sensory-motor corticostriatal loop (Alloway et al., 2006; Parent and Hazrati, 1995; Wright et al., 2001). DLS responds to pulsatile air puff stimuli applied to the whiskers (Pidoux et al., 2011; Syed et al., 2011). Little is know however about the role of striatum in encoding specific parameters of vibrotactile stimuli in frequency ranges which evoke discriminable percepts in behavioural tasks and carry most of the power of vibrissa vibration elicited by complex fine textures (Hipp et al., 2006; Gerdjikov et al., 2010). In cortex, sensory evoked potentials are strongly dependent on instantaneous UP/DOWN states (Petersen et al., 2003; Haslinger et al., 2006). Striatal UP/DOWN states are likely driven by cortex and/or oscillatory activity in thalamocortical loops, yet it is not known whether slow oscillations modulate tactile representations in this structure (O'Donnell and Grace, 1995; Kasanetz et al., 2008; Tseng et al., 2001; Mahon et al., 2001; Ushimaru
et al., 2012). To begin to address these questions, here we recorded striatal responses to precisely defined high frequency stimuli in urethane-anaesthetized rats. We found that populations of medium spiny neurons encode vibrotactile frequency. This encoding was subject to modulation by slow oscillations known to depend on cortical activity suggesting interplay between these structures in sculpting DLS tactile representations.

Method

Animals and surgery

Experiments were performed under urethane anesthesia in eight male Wistar rats (Charles River Laboratories Wilmington, MA, USA) weighing between 350-500g. Rats were injected with an initial dose of 1.25 mg/kg urethane which was supplemented with a second 0.25 mg/kg injection 20 min later. Pinch and corneal reflexes were monitored throughout the experiment and the rat received 0.15 mg/kg top-ups if necessary to maintain anaesthesia. This dosing regimen is similar to what was used previously to assess tactile encoding in rat barrel cortex (Arabzadeh et al., 2006; Hirata and Castro-Alamancos, 2011). A similar regimen has also been used to study cortical contributions to UP and DOWN states in medium spiny neurons (Kasanetz et al., 2006). Thus the current approach provides results which are directly comparable with these studies. Body temperature was monitored rectally and maintained at 37 Cº using a homeothermic pad (Harvard Apparatus, Boston, MA, USA). For fluid replacement, 5% glucose was continuously administered via an infusion pump (3 mL/hour, s.c.; Instech, K. D. Scientific, Holliston, MA, USA). Glycopyrronium bromide (40µL/kg, i.m.; Anpharm, Warsaw, Poland) was given to reduce respiratory tract secretions. Animals were fixed to a stereotaxic frame and the head was adjusted so that lambda and bregma were on the same horizontal plane. A left-side craniotomy was
performed to provide access to DLS, leaving dura mater intact. To prevent corneal
desiccation Lacri-Lube Eye Ointment (Allergan, Wesport, Ireland) was applied to the
eyes. Surgeries were carried out under institutional ethics approval and a project license
granted by the UK Home Office under the Animals (Scientific Procedures) Act 1986.

Tactile stimulation

The whisker stimulator was constructed from a glass capillary (1 mm o.d.) glued to a
piezo bender (Physik Instrumente, Karlsruhe, Germany). The tip of the capillary was
further thinned through heating until a whisker hair could rest snugly inside the tip
opening. Voltage commands were programmed in Matlab (Mathworks, Natick, MA,
USA) and delivered using custom-written LabVIEW software (National Instruments,
Austin, TX, USA). The stimuli consisted of brief pulsatile deflections (single-period
cosine wave, 100 Hz, duration 10 ms) presented to the right C1 whisker for 1 sec at inter-
pulse intervals of 22, 17, 13 and 11 ms corresponding to frequencies of 45, 60, 75 and 90
Hz (amplitude 12°, i.e. 5 mm distance from the whisker base) (Figure 1a,b). The length
of the glass capillary and point of attachment of the piezo element were optimized to
remove ringing of the stimulator. Calibration with a phototransistor (HLC1395,
Honeywell, Morristown, NJ, US) showed that differences in amplitude and peak velocity
between frequencies were smaller than three percent. The capillary tip was positioned
5 mm away from the skin and tilted at an angle of 155° to 175° against the whisker such
that the vibrissa rested against the inside wall of the capillary, ensuring that the stimulator
engaged the whisker immediately.
Electrophysiological recordings and analysis

Wideband signals were acquired continuously via an op-amp based headstage amplifier (HST/8050-G1-Gr, 1x gain, Plexon Inc., Dallas, TX, USA), passed through a preamplifier (PBX2/16wb, 1000x gain; Plexon Inc., Dallas, TX, USA) and digitized at 40 kHz. Recording electrodes consisted of quartz glass-coated platinum/tungsten wires pulled and ground to custom shapes in our laboratory (shank diameter 80 µm; diameter of the metal core 23 µm; free tip length ~8 µm; impedance, 1-3MΩ; Thomas Recording, Giessen, Germany). DLS recording electrodes pierced dura and were advanced into DLS using established coordinates and covered DLS areas receiving extensive barrel cortex input and known to respond to airpuffs delivered to the whisker (Hoffer and Alloway, 2001; Syed et al., 2011). DLS penetrations were located AP -0.8 to -1.5mm, ML 2 to 5mm, DV 4 to 7mm (Figure 1c). All data processing was done offline. For spike sorting the raw signal was band-pass filtered 300-3,000Hz and spikes were sorted using the Matlab-based Wave_Clus software to yield single-unit spike trains (Quiroga, Nadasdy, & Ben-Shaul, 2004). Wave_clus performs unsupervised spike detection and sorting using wavelets and super-paramagnetic clustering. All automatic detection thresholds and sorting solutions were examined individually and adjusted if needed. Field potentials were recorded from the same electrode and were downsampled to 5,000 Hz and evoked responses extracted from the raw data using a 200 Hz low-pass Butterworth filter. Responses were averaged over 20 trials. Single unit tactile responses were calculated by adding spikes in a 500 msec window after stimulus onset and subtracting the baseline firing rate calculated over 100 msec. To investigate the effect of DLS slow oscillations on tactile responses, LFPs were low-pass filtered < 5Hz using a second order Butterworth
DLS tactile evoked potentials are modulated by slow oscillations

Vibrotactile stimuli elicited evoked field potential responses in DLS (Figure 2a). Studies done in cortex show a strong relationship between the phase of low frequency LFP of deep cortical layers and intracellularly recorded neuronal UP/DOWN states (Saleem et al., 2010). We low-pass filtered field potential traces < 5Hz and used a Hilbert transformation (Figure 2b,c) to derive the instantaneous phase of spontaneous striatal LFP. Instantaneous LFP phase at stimulus onset calculated in this manner was evenly distributed across the 0-360 deg waveform cycle as would be expected with random stimulus presentations (mean resultant vector length of the phases at onset = 0.06, p = 0.3). We next calculated the slope of the evoked potential curve between 0-200 ms after stimulus onset and plotted it against the phase of the slow oscillations assessed at stimulus onset on a trial by trial basis (compare (Wyble et al., 2000)). The magnitude of the tactile response showed a clear modulation by oscillation phase (Figure 1d). The response was strongest during the 0-180º portion of the slow wave oscillation corresponding to a DOWN state and it was virtually absent at 270º corresponding to the
UP state oscillation. Evoked potentials however did not appear to be modulated by stimulus frequency, even when controlling for slow oscillation phase.

*Populations of MSNs encode vibrotactile stimulus frequency during DOWN/UP transitions*

Medium spiny projection neurons (MSNs) represent more than 90% of rat striatal neurons and unlike DLS interneurons are characterized by a relatively low firing rate (Rymar et al., 2004; Berke et al., 2004). To ensure only this cell type entered our data set, we recorded units with low baseline activity (< 5Hz) and the firing rates we observed (1.37 spikes/s ± 1.25, mean ± s.d.) are consistent with previous studies (Mowery et al., 2011; Barnes et al., 2005; Schmitzer-Torbert and Redish, 2008; Sharott et al., 2009; Berke et al., 2004). Further we noted that the spike waveforms of the recorded units showed peak to valley intervals (mean ± SEM) of 735 ±57 μsec and peak widths of 372 ± 15 μsec, which are in agreement with previously reported MSN extracellular waveform characteristics (Wiltschko et al., 2010). We recorded from 35 neurons. Consistent with previous work showing subthreshold responses to tactile stimuli in intracellular recordings but a weak spike response when single pulses were used (Pidoux et al., 2011), here with the delivery of high frequency stimuli only a small percentage of cells responded to a 45Hz stimulus. Seven out of the 35 recorded units (20%) showed a significant change in firing rate during the 500 ms after stimulus onset ($p < .05$; Wilcoxon signed-rank test). In all cases the response was a small increase in firing (.53 spikes/s ± .18). When stimulating at 60Hz, we obtained 23% responsive units (.52 spikes/s ± .14), at 75Hz 23% (.54 spikes/s ± .49) and at 90Hz 11% (.70 spikes/s ±.34).
Thus tactile responses of individual MSNs to stimuli at different frequencies were sparse, similar to responses to pulses (Pidoux et al., 2011). To investigate a possible contribution of UP/DOWN state transitions, instantaneous LFP phase was used to construct phase histograms of MSN spike times (Figure 3a,b) (Saleem et al., 2010). Spontaneous MSN firing was highest during the negative component of the LFP wave consistent with recent combined intracellular and LFP recordings in cortex showing a mean phase distribution of the low pass-filtered LFP trace of 200-225° during UP states (Figure 3c) (Saleem et al., 2010). Thus our DLS results are consistent with the previously observed phase relationship between cortical membrane states and slow oscillation phase.

We next tested if slow oscillation phase affects DLS tactile responses. To do this, we extracted the instantaneous phase of striatal LFP at the onset of each stimulus. Within each unit (i.e., individual trials serving as replications), phase did not appear to modulate the relationship between stimulus frequency and firing rate ($p_s > .10$ for the phase x stimulus interaction term in individual unit multiple regressions). However in the population (i.e., individual cell averages serving replications), the tactile response was related to LFP phase at stimulus onset (Figure 4). This was supported by a phase (4 quadrants) x stimulus (4 frequencies) repeated measures ANOVA [main effect of phase, $F(3, 102) = 10.31, p < .01$; interaction, $F(9, 306) = 1.86, p = .056$]. We tested different amplitude (2 to 12°) x frequency (45-90Hz) combinations on a subset of cells ($n = 17$). This did not reveal any amplitude effects, but confirmed the phase x frequency interaction [$F(9, 27) = 2.34, p = .017$]. The interaction was followed up with simple one-way ANOVAs at each phase interval, which showed a clear frequency response for
stimuli arriving between instantaneous phases of 90 and 180° \[ F(3, 102) = 3.24, p = .025 \].

In previous work, cortical DOWN states showed a mean phase distribution of 290-320° (compared to 110-135° during UP states; here 0° corresponds to the positive peak in the slow oscillation, cf. Figure 1c) thus the 90-180° quadrant roughly corresponds to the transition from Down and Up states. Specifically firing rate was significantly higher for 45Hz compared to 75 and 90 Hz and for 60 Hz compared to 90 Hz (pair-wise comparisons, \( p < .05 \)). The effect of frequency was not significant in other phase intervals. We conclude that tactile responding is sparse and shows no stimulus frequency modulation at the level of individual MSNs. However firing rate was related to stimulus frequency in the population and this effect was dynamically modulated by slow oscillation phase: vibrotactile frequency was encoded during the transition from DOWN to UP states.

Spike-phase coding refers to the encoding of information in the relative timing of neuronal activity to slow background rhythms (Kayser et al., 2009). To assess whether the timing of spikes relative to the ongoing slow oscillation is related to stimulation frequency, we calculated the average LFP slow oscillation phase of all recorded neurons in a 2 sec window prior to tactile stimulation as well as during the 1 sec of tactile stimulation separately for each of the 4 stimulation frequencies. However a 4 x 2 (frequency x window) within-subjects ANOVA across neurons showed no effect of frequency or analysis window (\( p_s > .5 \)). Thus we found no evidence for spike-phase coding of frequency information in this system.

**Discussion**
Striatum is increasingly being recognized as contributing to sensory processing in addition to its traditional role as a motor structure (Schneider et al., 1987; Sathian et al., 1997; Zia et al., 2003). Here we characterized the encoding of vibrotactile information in rat DLS assessing striatal responses to frequency stimuli applied to a single vibrissa. Our results suggest that populations of striatal neurons are in principle able to represent vibrotactile frequency in this system. This property of MSNs is modulated by slow wave oscillations likely originating in cortex or thalamocortical loops.

We found that field potentials obtained within the DLS projection field of the barrel cortex show robust responses to tactile vibrations but carry no information about stimulus parameters. LFP reflects neural activity summed over some distance from the recording location which may to some extent explain the loss of stimulus detail observed here (Kajikawa and Schroeder, 2011). To address this, we characterized the tactile response of individual MSNs recorded within the DLS to higher frequency stimuli (45-90 Hz) which were shown to elicit a discriminable percepts in head-fixed rats. We found that the MSN tactile response to this stimulus set was sparse. This observation is consistent with recent intracellular work showing superior tactile responses in subthreshold cellular activity and only weak action potential generation (Pidoux et al., 2011). However we also demonstrate that stimulus parameters of tactile vibrations in the higher frequency range are well represented in the population response. It is also noteworthy that these population results fit quite closely behavioural discrimination performance observed in head-fixed rats in earlier work (Gerdjikov et al., 2010). This is compatible with some form of sensory encoding carried out in DLS to support its role in automatic stimulus-
response behaviours observed in lesion studies (Ding et al., 2010; Yin et al., 2008).

However we acknowledge that this is correlational evidence and further work is necessary to elucidate the possible role of DLS medium spiny neurons in tactile perception.

During slow wave sleep, quiet wakefulness and some forms of anesthesia, brain states are characterized by low frequency, large amplitude membrane potential changes (Petersen et al., 2003; Steriade et al., 1993). These so called UP and DOWN states are an attractive model for understanding how internally generated states may impact the neural representation of sensory stimuli (Haslinger et al., 2006; Watson et al., 2008). We found that both tactile evoked potential and MSN responses are dynamically modulated by brain state, and that the optimal representation is achieved during LFP phases consistent with DOWN to UP transitions (0-180º). This result is consistent with tactile responses obtained in cortex, where multiunit responses are a smooth function of prestimulus LFP that peaks between 90 -180º and is mostly flat between 270-360º (Haslinger et al., 2006). Previously reported findings that cortical responses to sensory input are modulated by UP/DOWN state transitions suggesting that these oscillations play a role in information processing or gating (Hasenstaub et al., 2007; Petersen et al., 2003; Civlillico and Contreras, 2012). In the current study we show that a similar gating mechanism operates at the level of striatum. The gain adjustment of DLS sensory input observed here may relate to specific sensory tasks or arousal states, which also affect brain state (West, 1998; Castro-Alamancos, 2004).
This is the first report of striatal responses to precise vibrotactile stimuli in behaviourally relevant frequency ranges in the rat whisker system. These stimuli evoke discriminable percepts in behavioural tasks and carry most of the power of vibrissa vibration elicited by a range of complex fine textures. We found that the striatal representation of specific stimulus parameters was sparse and that population responses in striatum appear to represent well stimulus parameters. The tactile stimulus representation obtained in DLS parallels previously reported behavioural benchmarks. DLS slow oscillations appear to play a permissive role in these tactile representations.
Acknowledgement

TGH was funded by a Wellcome Trust Scholarship. The authors thank Julia Stalleicken for feedback during the preparation of the manuscript and for valuable discussions throughout the project.
References


Figure Captions

Figure 1. Tactile stimulation and recording. a: Schematic illustration of the experimental setup. b: Cutouts from the pulsatile stimulus waveforms (see Methods) delivered at frequencies of 45 - 90Hz. One pulse represented a single period of a sinusoid starting from the curve minimum. c: Example Nissl-stained coronal section indicating vertical tract left by the recording electrode. CPu = Caudate putamen (striatum); ec = external capsule; LV= lateral ventricle; S1 = primary somatosensory cortex.

Figure 2. Dorsolateral striatum local field potential (LFP) responses to precise single-whisker frequency stimuli. a: Evoked tactile potentials averaged across all trials and sessions showed a robust response which was not modulated by stimulus frequency. b: Example of instantaneous phase calculations using a Hilbert transform. The gray line shows instantaneous phase (right axis) superimposed on the corresponding LFP trace (left axis). The Hilbert transform is based on a 5 Hz low-pass filter whereas the field potential trace shows unfiltered LFP. c: average LFP data from the trace illustrated in b. as a function of instantaneous phase. d: magnitude of the LFP response calculated as slope of the evoked potential varied with the phase of the ongoing LFP slow oscillation, however frequency was not encoded at either phase. Response magnitudes are averaged across all trials and sessions.

Figure 3. Spontaneous medium spiny neuron activity related systematically to the phase of the ongoing LFP oscillations which were used to infer UP and DOWN states (see text). a and b: Representative single unit activity. From left to right: spike waveforms and
averages; time stamps of spiking activity relative to the ongoing LFP oscillations recorded simultaneously from the same electrode; angle histograms of instantaneous LFP phase at the time of spike occurrence. $r$ is the length of the mean phase angle vector and represents the strength of directionality. ** < .001 (Rayleigh’s test). c: average LFP phase angles at the time of spike occurrence for all 35 medium spiny neurons recorded in this study. Average phase angles are represented by the angle of the resultant vectors whereas resultant vector length represents the magnitude of the directionality effect.

Figure 4. Population responses of medium spiny neurons encoded stimulus frequency in an oscillation-dependent manner. Spike responses were separated according to the instantaneous oscillation phase of the LFP at stimulus onset. Responses were significant for stimuli presented during the 90-180° phase of the ongoing oscillations (* < .05). The tactile response observed here maps well onto psychophysical discrimination thresholds derived in head-fixed rats in previous work (see text).
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Figure 1
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Figure 4