Dysregulated Information Processing by Medium Spiny Neurons in Striatum of Freely Behaving Mouse Models of Huntington’s Disease

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INTRODUCTION

The striatum, which serves as the information processing hub of the basal ganglia, integrates sensorimotor, cognitive, and motivational information arising from the entire cortical mantle to guide behavioral output (Graybiel 1995; Groves 1983). Medium spiny neurons (MSNs), which consist of >90% of neurons in the striatum, constitute its sole output system (Bolam et al. 2000; Gerfen and Wilson 1996; Groves 1983), thus representing a critical node for information coding in the basal ganglia (Murer et al. 2002). In fact, increasing evidence implicates MSNs in the behavioral phenotype of Huntington’s disease (HD), an autosomal dominant condition caused by an expansion of a polyglutamine (CAG) repeat in exon 1 of the Huntington gene (Macdonald et al. 1993). Although the loss of these cells may play a role in the cognitive, emotional, and motor symptoms of HD, recent evidence suggests that the onset and progression of the behavioral phenotype is likely caused by deficits in corticostriatal information processing that precede cell death (for review see Cepeda et al. 2007). Mice that model HD, for example, show behavioral symptoms long before significant cell loss (Carter et al. 1999; Menalled et al. 2003). These behavioral changes, moreover, are accompanied by changes in the intrinsic properties of MSNs. When studied in vitro, MSNs show exaggerated glutamate-dependent responses, more depolarized resting membrane potentials, reductions in both inwardly and outwardly rectifying K+ currents, increased input resistance, and increased intracellular Ca2+ levels (Cepeda et al. 2001; Klapstein et al. 2001; Lafort et al. 2001; Li et al. 2004; Zeron et al. 2002). It is important to understand, however, how MSN malfunction contributes to spike output during behavior, which depends on the spatiotemporal convergence of glutamatergic signals arising from cortex (Kita 1996; Wilson and Kawaguchi 1996). To investigate how MSN output is compromised in HD, we used chronically implanted microwire electrodes to record from MSNs in symptomatic HD mice and wild-type (WT) controls as they behaved freely in an open field. To assess the generality of our results we used both transgenic and knock-in (KI) models. R6/2 mice, which are transgenic for the human HD gene, are the most characterized model and express a rapidly progressive HD phenotype considered to reflect juvenile HD (Carter et al. 1999; Lione et al. 1999; Murphy et al. 2000). The KI model, which contains a chimeric mouse/human HD gene, resembles adult onset HD, and the behavioral symptoms are less severe compared with R6/2 mice (Dorner et al. 2007; Menalled et al. 2003).

Apart from spike rate, which provides an index of the overall level of activation, we measured burst activity, which includes spike clusters of varying rates and duration, and correlated firing, which identifies temporal interactions between the activity of neuron pairs (Perkel et al. 1967), and may represent information coding by cell assemblies (Sakurai et al. 1999; Salinas and Sejnowski 2001). Computationally, bursts ensure reliable information transmission between neurons (Izhikevich et al. 2003; Lisman 1997), a process likely to be impaired in HD (Cepeda et al. 2007). Because correlated firing is a dynamic process related to behavioral output (Salinas and Sejnowski 2001), it is also likely disrupted in HD. Cross-correlations, which measure the degree of correlated firing, can also provide a measure of oscillatory spike activity, a process that has been implicated in other basal ganglia disorders (for reviews see Geve et al. 2006; Hammond et al. 2007; Murer et al. 2002). To provide a better understanding of correlated firing and burst activity, we assessed coincident bursting between pairs of MSNs (Legendy and Salcman 1987; Lisman 1997). Our results suggest an important role for both...
bursting and correlated firing in information coding by MSNs. Dysregulation of this coding scheme, moreover, is a key component of HD pathophysiology, regardless of the severity of HD symptoms or the genetic construct of the mouse models.

METHODS

Animals and care

Male transgenic R6/2 mice (B6CBA-Tg[NHDexon1]62Gpb), which express an expanded CAG repeat in exon 1 of the human HD gene, and WT littermate controls are based on the C57BL/6J*CBA/J background strains. R6/2 mice and their corresponding WT s were obtained from The Jackson Laboratories (Bar Harbor, ME) at 5–6 wk of age. On arrival, the mice were housed individually and allowed 1 wk of habituation. Nine WT and seven R6/2 mice ranging in age between 6 and 9 wk with mean age of 7.5 ± 0.3 and 7.7 ± 0.3 wk, respectively, were used. This age range in R6/2 mice corresponds to a time of early symptom development extending to approximately the midpoint of symptom severity (Carter et al. 1999). Homozygous male and female KI mice and WT littermates were bred in our colony from heterozygous pairs obtained from an established colony at the University of California, Los Angeles (Menalled et al. 2003). The KI mice express a chimeric mouse/human exon 1 of the HD gene inserted into the mouse gene via homologous targeting of W9.5 ES cells from a 129sv background strain. Seventeen WT mice (male, n = 9; female, n = 8) ranging in age between 10 and 46 wk with mean age of 21.8 ± 1.5 wk, respectively, were used. Fourteen KI mice (male, n = 9; female, n = 5) ranging in age between 10 and 31 wk with mean age of 22.3 ± 1.3 wk were also used at these ages, KI mice show a subtle HD phenotype and are considered symptomatic (Dorner et al. 2007; Menalled et al. 2003).

All mice were housed individually in the departmental animal colony under standard conditions (12-h light/dark cycle with lights on at 07:30) with unrestricted access to food and water. Both the housing and experimental use of animals followed the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee.

Genotype and CAG repeat length

Genomic DNA was extracted from tail tissue samples in 25 μL cell lysis buffer (50 mM Tris, pH 8.0; 25 mM EDTA; 100 mM NaCl; 0.5% IGEPA LA-630; 0.5% Tween 20) and proteinase K (10 mg/mL; 60 μg/reaction) at 55°C for 2 h with gentle mixing after the first hour. DNA was diluted with 400-μL filter-sterilized high performance liquid chromatography (HPLC) water, heated to 100°C for 10 min, centrifuged for 2 min at 17,000 × g, and stored at 4°C. Polymerase chain reaction (PCR) and analytical agarose gel electrophoresis were used to determine CAG repeat length. Primers were 31,329 (5'-ATG AAG GCC TTC GAG TCC ACG ACG TTC-3') and 33,934 (5'-GCC GGC TGA GGA AGC TGA GAA-3') (Mangiarini et al. 1996). Each reaction consisted of 2.0 μL DNA template, 0.4 μL each primer (20 μM stock solution), 7.2-μL filter-sterilized HPLC water, and 10.0 μL 2× Biomix Red (Bioline USA, Taunton, MA) for 20 μL total volume. Cycling conditions were 94°C for 30 s followed by 30 cycles of 94°C for 30 s, 62°C for 45 s, and 72°C for 90 s, with a final elongation at 72°C for 10 min. Electrophoresis of samples was performed in 3.0% NuSieve 3:1 analytical agarose (Lonza Rockland, Rockland, ME) with 0.2 μg/mL ethidium bromide at 5 V/cm for 180 min using a 100-base pair (bp) ladder as DNA standard. Gels were evaluated with Kodak Image Station 4000R and Kodak Molecular Imaging software (Carestream Molecular Imaging, New Haven, CT) to confirm genotype and determine CAG repeat length. Using Clone Manager software (Sci-Ed Software, Cary, NC), we aligned primers to the huntingtin gene sequence acquired from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). Alignment of primers to template indicated that the DNA fragment amplified by PCR is 86 bp longer than the CAG repeat region. Computer analysis of fragment migration against the 100-bp standard showed that our experimental R6/2 mice had 111.3 ± 1.6 (mean ± SE) repeated CAG codons and the KI mice had 125.3 ± 2.3 CAG repeats.

Surgical procedures

Mice were anesthetized with a mixture of chloral hydrate and sodium pentobarbital (chloropent, 0.4 ml/100 g, administered intraperitoneally) and were mounted in a stereotaxic frame in preparation for subsequent single-unit electrophysiology. The scalp was shaved and swabbed with betadine; after lidocaine (20 mg/ml) was injected subcutaneously (sc), an incision was made at the midline to expose the skull. Holes were drilled through the skull over the striatum, the exposed dura was removed, and multiwire bundle electrodes were lowered slowly into striatum. Stratium coordinates were +0.5 mm anterior and ±1.6 mm lateral to bregma and 3.0 mm ventral to skull surface (Paxinos and Franklin 2001). Two additional holes were drilled for stainless steel anchor screws. The electrode assembly was permanently attached to the skull by means of dental acrylic. Antibiotic cream was applied to the surgical site to prevent infection. Lactated Ringer solution (1 ml) was administered sc to counteract dehydration. All mice were allowed 1 wk of postsurgical recovery, during which they were monitored for signs of pain and other health complications.

Electrophysiology

Each electrode bundle was made in-house and consisted of three 25-μm Formvar-insulated stainless steel recording wires (California Fine Wire, Grover Beach, CA) and one 50-μm uninsulated stainless steel ground wire twisted together. Two bundles were friction-fitted to gold pin connectors in a custom nylon hub (6-mm diameter). Electrode impedances were typically 1 MΩ. The electrode assembly was small, lightweight, and well tolerated by the mice so that they could behave freely.

All experiments were conducted during the light phase of the diurnal cycle and were 20 min in duration. During recording, the electrode assembly was connected to a lightweight flexible wire harness equipped with field-effect transistors that provide unity-gain current amplification for each of the six microwires. The home cage (16 × 27.5 cm) of each mouse served as an open field and consisted of clear Plexiglas walls (12 cm) and standard bedding. The home cage was placed in a sound-attenuating and shielded recording chamber. The harness was connected to a swiveling commutator, allowing the mice to behave freely.

Because we and others have found no advantage of using triangulation (tetrode mode) to isolate single units in striatum, single-channel mode was used for all our recordings (for discussion on this topic see Costa et al. 2004). Extracellular spike activity was routed through multiple-channel preamplifiers with 500× gain and 154-Hz to 8.8-kHz band-pass filters (Plexon, Dallas, TX). The signals were digitized at 40 kHz and acquired by the Multichannel Acquisition Processor system (Plexon). This system and its companion software Sort Client (Plexon) provide direct computer control over amplification, filtering, signal detection, spike-waveform discrimination, and storage. We were able to discriminate and record simultaneously at least four signals per channel for a maximum of 24 possible units per recording session. Units were sorted on-line prior to the recording session and care was taken to ensure that all units were isolated with enough rigor to eliminate the need for post hoc off-line sorting. Thus a voltage threshold ≥2.5-fold background noise was established and about 1,000 waveform samples were collected to define a waveform template via principal component analysis. The raw signal for each unit was routed to an oscilloscope and audio monitor to facilitate unit discrimination by matching the analog signal with the digitized template. To maximize the probability that units consisted of only one
signal, autocorrelation and interspike interval (ISI) analyses for each unit were used to detect the presence of the absolute refractory period. Using these procedures, we found that template drift during the recording sessions was minimal. We took care to avoid recording the same unit on multiple channels by excluding any unit from one of the channels if they had suspiciously similar spike activity. The typical yield was three to six units per recording session.

All mice participated in multiple recording sessions and units were often recorded on the same wire as that of a previous session. We did not assume that a common source generated each signal because electrode drift and subtle changes in behavioral state cannot guarantee positive detection of single units over multiple sessions (Lewicki 1998). Therefore we treated all units in each recording session as independent entities.

**Histology**

To verify electrode placement in striatum on completion of all recording sessions, mice were killed with an overdose of chloropent (>twofold the surgical dose) and a current pulse (30 µA for 10 s) was passed through each active microwire to mark recording sites. Mice were then transcardially perfused with saline followed by 10% potassium ferrocyanide [K$_4$Fe(CN)$_6$] in 10% paraformaldehyde to produce small blue deposits at the site of the recording electrode (“Prussian blue” reaction). Brains were removed, postfixed in 10% paraformaldehyde for 1 h, and cryoprotected in 30% phosphate-buffered sucrose. The brains were then frozen; coronal sections (60 µM) were then cut on a sliding microtome and mounted on gelatin-subbed slides. The sections were stained with cresyl violet and examined under a light microscope to confirm microwire location. Only recordings with clear electrode placements in striatum were used in the analysis.

**Behavioral analysis**

To determine whether group differences in overall behavioral activation could explain our electrophysiology results, a subset of mice (n = 6 for each group) were videotaped for the 20-min recording session. Behaviors were coded by independent observers who were blind to genotype. Behavioral epochs included exploration, grooming, and quiet rest. Exploration was defined as any behavioral activation including locomotion, rearing, sniffing, and digging, while excluding bouts of grooming. Grooming was coded as stereotyped grooming lasting ≥10 s. Quiet rest was defined as the absence of all movement lasting ≥5 s. Hindlimb flicks, which are a common symptom in R6/2 mice (Carter et al. 1999) and are absent in KI mice, are also coded.

**Spike train analysis**

Electrophysiological data for each recording session were analyzed by means of Neuroexplorer (Nex Technologies, Littleton, MA). All analyses were performed on single-unit data collected over the entire 20-min recording session. Firing rate was calculated by dividing the spike trains into 1-s bins (spikes/s). To assess spike-train variability, the coefficient of variation of interspike intervals (CV ISIs) was calculated by dividing the SD of all ISIs in a train by the mean ISI of the train. We used the Poisson surprise method to detect bursts in spike trains (Legenday and Salcman 1985). The burst surprise method assumes that each spike train is a random Poisson process and defines a burst as a cluster of spikes in which the inclusive ISIs of that cluster significantly deviate from a Poisson distribution. Bursts are constructed by adding successive spikes at the beginning and end of the burst until the Poisson-surprise value maximizes the probability of the burst; this is equivalent to burst duration. The surprise value is defined as the negative natural logarithm of the probability that a series of spikes in a given time interval is significantly different from that expected from a Poisson process with the same mean firing rate. Thus the surprise value provides an estimate of the statistical significance of each burst in the spike train. Therefore the surprise value is an index of how intense or “surprising” the ISIs of a particular burst are compared with other ISIs in the same train. Here we used a minimum burst surprise value of 5, which estimates that bursts occur about 150 times (P < 0.007) more frequently than would be expected in a Poisson spike train with the same mean firing rate (Homayoun et al. 2005). Following convention, the burst surprise method excludes spike doublets as bursts (Aldridge and Gilman 1991; Legendy and Salcman 1985). The burst surprise method is a rigorous detector of bursts because it is not sensitive to fluctuations in average firing rate and treats each spike train as an independent entity. The method, moreover, is well established for detecting bursts in striatum and other basal-ganglia structures (Aldridge and Gilman 1991; Stanford et al. 2007; Wichmann and Soares 2006). We used it to measure burst rate (number of bursts/min), burst duration, burst surprise value, as well as ISIs, frequency of spikes, and percentage of spikes within a burst.

To determine the percentage of coincident bursts, which are defined as bursts from two units that overlap in time (Lisman 1997), we calculated the number of burst overlaps and divided it by the sum of bursts between the two units. The mean time that bursts were coincident, which we refer to as coincidence duration, was also calculated. Coincident bursting and coincidence duration were determined for each pairwise comparison in each session.

To assess correlated spike activity between two spike trains, cross-correlation histograms (CCHs) were constructed for each pairwise comparison (Kirkwood 1979; Perkel et al. 1967) in each 20-min recording session. All CCHs were constructed based on 1-ms bins and a ±1-s time lag from the zero bin. The CCHs were smoothed using a Gaussian filter with a bin width of 3. Significant peaks, which indicate correlated firing, in both the raw and smoothed CCHs, were identified using a 99% confidence interval by assuming the null hypothesis that each spike train is a Poisson process and that firing between neuronal pairs is independent (i.e., flat cross-correlogram) (Abeles 1982). We found no difference in the proportion of significant peaks between the raw and smoothed CCHs. For clarity, only smoothed CCHs are presented.

**Statistical analysis**

All statistical analyses used GraphPad Prism (GraphPad Software, San Diego, CA). A two-way ANOVA was used to test differences in behavior. Mann–Whitney U tests were used to compare firing rate, CV ISI, and bursting parameters between HD and WT mice of both models. Nonparametric statistics are appropriate because of the significant deviation from normality and a lack of homogeneous variances in spike-train samples (Zar 1999). Therefore our data are presented as either the median of the mean values or the median of the percentage mean values. Pearson’s chi-square ($\chi^2$) test was used to determine differences in the proportions of cross-correlations with significant peaks between HD and WT animals.

**RESULTS**

All mice were behaviorally active for the duration of all recording sessions. Exploratory activity (locomotion, rearing, sniffing, and occasional digging) predominated between bouts of grooming and quiet rest. There was no main effect of group across all behaviors (Fig. 1; $P = 1$). Although this behavioral pattern appeared in both HD lines and their respective WTs, HD mice also displayed symptomatic activity, which included tremor and overall motor slowness (see Carter et al. 1999; Dorner et al. 2007; Rebec et al. 2002, 2006). These symptoms were more pronounced in R6/2 mice, consistent with the more aggressive form of HD that appears in this line (Carter et al. 1999). For example, unlike KIs, R6/2 mice also displayed hindlimb flicks (25 ± 5.8, mean ± SE for the 20-min session).
A transgenic (62 WT and 68 R6/2) and KI groups (86 WT and 66 mice were pooled for each genotype in the KI line. Single-unit isolations represent putative striatal MSNs neurons for each genotype were pooled across age. Although logical parameter in both HD mouse lines. Therefore recorded though not significantly, more active compared with WT mice for the KI line (129sv strain) (see Fig. 1).

In accord with previous data (Holmes et al. 2002), WT mice for the R6/2 line (C57BL/6J*CBA/J strain) were generally, al-

There was no main effect of group across behaviors (two-way ANOVA, \( P = 1, n = 6 \) for each group, mean ± SE).

There was no correlation between age and any electrophysiological parameter in both HD mouse lines. Therefore recorded neurons for each genotype were pooled across age. Although subtle sex differences in behavior have been observed in KI mice (Dorner et al. 2007), we found no gender differences in any electrophysiological parameters. Thus data from male and female mice were pooled for each genotype in the KI line.

Single-unit isolations represent putative striatal MSNs

We recorded 130 and 152 individually isolated units from the transgenic (62 WT and 68 R6/2) and KI groups (86 WT and 66 KI), respectively. Histological analysis confirmed that all microwire electrode placements were in dorsal striatum (Fig. 2A). All recorded neurons from WT mice had low firing rates, were bursty in nature, and exhibited periods of near-quiescent activity. Regardless of genotype, waveform amplitudes and durations were typically 100–300 \( \mu V \) and 0.8–1.5 ms, respectively. Moreover, the late positive phase of all extracellularly recorded waveforms had durations \( >300 \mu s \). Analysis of peak and valley widths indicated a common waveform pattern in all groups across all sessions (Fig. 2B). These spike patterns and waveform characteristics are typical of MSNs (Aldridge and Gilman 1991; Berke et al. 2004; Courtemanche et al. 2003; Rebec et al. 2006; Teagarden and Rebec 2007; Wilson and Groves 1981). Furthermore, indicative of their burst activity, MSNs show unimodal and positively skewed ISI profiles, as well as nonoscillatory autocorrelation histograms that exhibit a double-sided peak near the zero bin (see Fig. 2C) (Berke et al. 2004; Courtemanche et al. 2003; Mahon et al. 2006; Wilson 1993). It is thus likely that our unit recordings represent MSNs in striatum.

Altered firing activity of MSNs in HD mice

To determine differences in spike activity recorded from WT and HD mice, we calculated spike rate and CV ISI across all recording sessions. Consistent with previous data (Rebec et al. 2006), R6/2 units discharged significantly faster than WT (Fig. 3A; \( P = 0.012 \); see Fig. 8 for raw data). The ISI pattern, moreover, was more random in R6/2 compared with WT as indicated by CV ISI values in R6/2 clustering closer to 1 than those of WT (Fig. 3B; \( P = 0.032 \)). In KI mice, however, firing rate (Fig. 3C) and CV ISI (Fig. 3D) were not different from those of WT (\( P = 0.56 \) and \( P = 0.39 \), respectively). Therefore compared with their respective WTs, only R6/2s showed a faster rate and a more random pattern.

**FIG. 1.** Behavioral activity during the 20-min recording sessions. Behaviors were coded as time epochs; thus each behavior corresponds to the length of time (s) mice engaged in a particular behavior during the recording session. There was no main effect of group across behaviors (two-way ANOVA, \( P = 1, n = 6 \) for each group, mean ± SE).

**FIG. 2.** Identification of putative medium spiny neurons (MSNs) in dorsal striatum. A: coronal sections of mouse brain that show the location of histologically verified microwire bundle placements in striatum (modified from Paxinos and Franklin 2001). Numbers indicate distance (in mm) anterior to bregma and shading indicates the regions where electrode tips were identified. B: multiple (40) consecutive traces of waveforms recorded from MSNs isolated in striatum of wild-type (WT) and R6/2 mice (top pair) and WT and knock-in (KI) mice (bottom pair). Waveforms from both genotypes and from both Huntington’s disease (HD) lines had comparable magnitudes and duration. The black waveform is the mean of the consecutive waveforms (gray). Scale bar: 500 \( \mu s \). C: interspike interval (ISI, left) and autocorrelation (right) histograms (1-ms bins) corresponding to the WT unit at the top of B. Note the burst profiles in both histograms and the lack of oscillatory activity.
Dysregulated burst pattern of MSNs in HD mice

We next explored differences in burst activity by defining two properties of bursts. The burst pattern, which includes burst rate, percentage of spikes within a burst, and the surprise value, measures burst activity with respect to the background firing pattern. The burst structure, which includes burst duration, spike rate within bursts, and ISI in bursts, measures properties of individual bursts without regard to the background firing pattern. Units recorded from WT mice had higher burst rates (bursts/min) than those from R6/2s (Fig. 4A; \(P = 0.009\)). WT units, moreover, had a greater percentage of spikes that participated in burst activity (Fig. 4B; \(P < 0.001\)) and greater burst surprise values than those of R6/2 units (Fig. 4C; \(P = 0.0054\)).

Unlike the R6/2 line, there was no difference in burst rate between WT and KI mice (Fig. 4D; \(P = 0.3\)), but WT units had a greater percentage of spikes that participated in bursts (Fig. 4E; \(P = 0.011\)) and the bursts were more surprising (Fig. 4F; \(P < 0.001\)). Analysis of burst structure revealed no differences between WT and HD mice from both the R6/2 and KI lines (Table 1; \(P > 0.05\)). Thus burst pattern but not burst structure is dysregulated in both HD lines compared with WT.

Correlated firing is decreased in HD mice

To investigate the temporal relationship of spikes recorded between pairs of units, we constructed CCH matrices for all pairwise comparisons within each recording session. This resulted in a large number of correlation pairs: 88 in WT, 111 in
TABLE 1. Burst structure for WT and HD mice in the R6/2 and KI lines

<table>
<thead>
<tr>
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<th>WT</th>
<th>R6/2</th>
<th>KI</th>
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<tbody>
<tr>
<td></td>
<td>Burst duration, s</td>
<td>0.66 (0.51–1.1)</td>
<td>0.94 (0.52–1.57)</td>
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<tr>
<td></td>
<td>Spike rate in bursts, s</td>
<td>31.20 (17.3–46.3)</td>
<td>21.30 (14.2–43.6)</td>
</tr>
<tr>
<td></td>
<td>ISI in bursts, s</td>
<td>0.06 (0.03–0.10)</td>
<td>0.07 (0.03–0.11)</td>
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Values are medians (25th and 75th percentiles).

R6/2, and 135 in WT, 108 in KI. Peaks that exceed the 99% confidence limit in the CCH indicate correlated firing between pairs of units, whereas peaks below this limit are considered noncorrelated (Abeles 1982). Correlated firing is a prominent feature in both WT lines. In fact, a large proportion of the unit pairs showed correlated firing in the CCHs, suggesting that multiple units within a population are correlated. The correlated activity, moreover, is not oscillatory; rather, the peaks are bimodal with asymptotes near the zero bin. Analysis of correlated firing in the HD models, however, revealed a marked reduction in correlated units. Figure 5, A and B shows representative CCH matrices for simultaneously recorded units in striatum of WT and R6/2 mice, respectively. Note the large proportion of correlated firing in the WT CCH matrix (Fig. 5A) compared with the R6/2 CCH matrix (Fig. 5B). Figure 5, C and D shows representative CCH matrices for simultaneously recorded units in striatum of WT and KI mice, respectively. Overall, correlated firing in HD animals is decreased compared with their respective WTs (Fig. 6A: $\chi^2 = 35.2, P < 0.001$; Fig. 6B: $\chi^2 = 12.7, P < 0.001$).

Coincident bursting contributes to correlated firing

To determine the relationship between the occurrence of bursts and correlated firing, we calculated the percentage of coincident bursts, which are bursts that overlap in time from one unit to another (see Lisman 1997), and the mean time during which the bursts overlapped (coincidence duration)
between each pairwise comparison in each recording session.

First, we asked whether there was a difference in coincident bursts and coincidence duration between genotypes. We found a reduction in the percentage of coincident bursts in both HD lines compared with their respective WTs (Fig. 7A; P = 0.038; Fig. 7D: P < 0.001). There was, however, no difference in coincidence duration between WT and HD mice of both lines (data not shown; P = 0.19 for the R6/2 line and P = 0.078 for the KI line). To characterize the occurrence of bursts between correlated units or noncorrelated units regardless of genotype, we grouped the genotypes for each HD line. We found that a higher percentage of coincident bursts occur in correlated units than in noncorrelated units in both HD lines (Fig. 7B; P < 0.001) and in the KI line (Fig. 7E; P < 0.001). Similarly, the coincidence duration was longer in correlated units than in noncorrelated units in both HD lines (Fig. 7C: P < 0.001; Fig. 7F: P < 0.001). Thus the degree of coincident bursting appears to influence correlated activity between neuronal pairs, suggesting a novel role of population coding by MSNs.

**Burst activity reflects the proportion of correlated firing in WT mice**

Although often not considered, strain-dependent genetic variations in mice can contribute to a host of physiological and behavioral differences between mouse strains (Crawley et al. 1997; Holmes et al. 2002; Nguyen 2006). Interestingly, we found that striatal units from WT mice for the R6/2 line (C57BL/6J* CBA/J) have fewer correlated spikes (44%) compared with those from WT mice for the KI line (129sv) (78%) (Fig. 8A; χ² = 26.1, P < 0.001). The difference in correlated firing may be explained by the differences in burst activity between the strains. Specifically, spike trains from C57BL/6J* CBA/J mice had fewer spikes that participated in bursts (Fig. 8B; P = 0.03), shorter burst durations (Fig. 8C; P < 0.009), and lower burst surprise values (Fig. 8D; P < 0.001) compared with 129sv mice. The C57BL/6J* CBA/J mice also had fewer coincident bursts (Fig. 8E; P < 0.001) and shorter coincident durations (Fig. 8F; P = 0.016) compared with 129sv mice. There were, however, no differences in firing rate, CV ISI, or any other parameter that we measured. Differences in the proportion of correlated firing and the level of burst activity between the two strains can likely be attributed to variations in background genetics and/or behavioral phenotype.

**Figure 9 shows examples of long-timescale spike rasters,** corresponding to units in the CCH matrices in Fig. 5, and their inclusive bursts from all groups. Note that spike trains from WT mice from both lines exhibit a more bursty spike pattern than that of HD mice. There also is a higher percentage of coincident bursts between units in WT compared with R6/2 and KI mice.

**DISCUSSION**

Our results show that, during behavior, information processing at both the single-neuron and population level is compromised in striatum of symptomatic HD mouse models compared with WT littermates. At the single-unit level, R6/2 but not KI mice have elevated and more regular firing than that of WTs. This is likely explained by the fact that R6/2 mice exhibit a robust and aggressive HD behavioral phenotype, whereas KI mice express a more subtle profile throughout adulthood (Carter et al. 1999). Burst activity, however, was reduced in both HD lines, suggesting that dysregulated burst coding is a common feature of HD regardless of symptom severity. Activity at the population level also was impaired in both HD models, as revealed by reduced correlated spikes compared with those in WT mice.
Identification of putative MSNs

Although our preparation does not allow for the direct identification of recorded neurons, it is likely that our neuronal sample represents MSNs. For one, as the most common neuron in the striatum (Bolam et al. 2000), MSNs are likely to dominate extracellular recordings in freely behaving animals because behavior routinely elevates MSN activity (Sandstrom and Rebec 2003). In addition, MSNs have an asymmetric waveform, in which the duration of the late positive phase of the waveform exceeds that of the early negative deflection (see Fig. 1B) (Berke et al. 2004). Furthermore, the activity of MSNs is characterized by periods of high-frequency discharges in close temporal relation (burst activity) interspersed with periods of low frequency or near quiescence (see Fig. 8 and Wilson 1993; Wilson and Groves 1981). These waveform- and spike-pattern profiles characterize the neurons in our sample. In contrast, tonically active neurons, which are the presumed cholinergic interneurons consisting of only 5% of the neuronal population in striatum (Graveland and DiFiglia 1985), have high firing frequencies, lack burst activity, and exhibit long-duration and high-amplitude waveforms (Courtemanche et al. 2003; Wilson et al. 1990). We can also rule out the possibility that the parvalbumin-staining GABAergic interneurons contributed to our sample population because they have high firing frequencies and very short duration waveforms, including a short late positive deflection (Berke et al. 2004; Kawaguchi 1993). Based on these criteria, we classified our neuronal sample as MSNs.

It is important to note that evidence suggests that MSNs belonging to the striato-pallidal (indirect) pathway are more vulnerable to HD than those belonging to the striatonigral (direct) pathway (Deng et al. 2004). One could hypothesize that HD-induced changes in MSN firing patterns should reflect the two striatal populations. Extracellular recording techniques, however, cannot distinguish between MSNs belonging to either the direct or indirect pathways. No such distinction, moreover, was evident in our data. It is also relevant that a sizable number of striatal MSNs contribute axon collaterals to both the direct and indirect pathways (Levesque and Parent 2005), making it difficult to generalize about possible differences between striato-pallidal and striato-nigral neurons in HD.

Altered temporal spike pattern of MSNs in HD mice

In line with our previous report (Rebec et al. 2006), we found that R6/2 mice have elevated firing frequencies and a more random firing pattern than those of WT. In contrast, units from KI mice were not different from their respective WT's in either rate or CV ISI. Increased firing in the R6/2 line is likely caused by the hyperexcitability of these neurons, which have more depolarized resting membrane potentials, concomitant with selective enhancement of N-methyl-D-aspartate (NMDA) currents and increased flux of intracellular Ca$^{2+}$ in vitro (Ariano et al. 2005; Cepeda et al. 2001; Klapstein et al. 2001; Levine et al. 1999). The lack of an elevated rate in the KI mice might reflect the more subtle HD phenotype in the KI line compared with R6/2s. For example, the hyperexcitability that characterizes MSNs in HD models is more dramatic in R6/2 mice compared with the TgCAG100 or YAC72 HD models, which, like the KI model, exhibit longer disease progressions and more subtle phenotypes than R6/2s (Ariano et al. 2005; Carter et al. 1999; Cepeda et al. 2001; Menalled et al. 2003).

Burst firing, which is a prominent feature of MSNs (Aldridge and Gilman 1991; Wilson 1993), is thought to increase the fidelity of synaptic communication (Izhikevich et al. 2003;
We found a reduction in the burst rate and percentage of spikes in bursts in R6/2 mice compared with WTs. Bursts in R6/2 mice were also less salient with respect to background firing, as indicated by decreased burst surprise values (Legendy and Salcman 1985). Like R6/2 mice, KIs showed a reduction in both the percentage of spikes in bursts and burst surprise values compared with those of WT. KIs, however, did not show a lower burst rate, perhaps because spike trains from KI and WT mice had comparable CV ISIs. Overall, there were no differences in burst structure between WT and HD mice. It thus appears that, although MSNs from HD animals have the capacity to generate bursts, the magnitude of bursting with regard to the background firing is reduced. Bursts not only increase the reliability of information transfer, but they are also important inducers of synaptic plasticity (Huerta and Lisman 1995; Lisman 1997). In fact, long-term potentiation, a major form of synaptic plasticity in striatum (Charpier and Deniau 1997; Wickens et al. 2003), is reduced in the R6/2 mouse (Kung et al. 2007). Thus a reduced level of burst activity in striatum of HD mice may result in altered information flow to downstream structures such that the fidelity of the signal is lost and synapses become resistant to modification.

Correlated firing is a prominent feature of MSNs that is reduced in HD

Correlated firing, which provides insight into the functional connectivity of networks, is thought to play a key role in regulating the flow of neuronal information (Abeles et al. 1994; Salinas and Sejnowski 2001). To provide a measure of the temporal activity in populations of MSNs, we computed CCHs.
for each pairwise comparison (Abeles 1982). We found that a large proportion of MSNs exhibit correlated, but nonoscillatory, activity. There was also a marked reduction of correlated firing in both HD lines. The reduction in correlated firing is not likely due to differences in spontaneous firing rate relative to the WT groups. The probability of correlated firing actually increases in spike trains with high firing rate. In fact, despite higher firing frequencies there was a dramatic reduction in correlated firing in R6/2 compared with that in WT. Our methodology, moreover, limits the possibility that differences in correlated firing occur by chance. Recordings were obtained, for example, while mice were behaving freely in an open field, and no experimental stimuli were available to co-modulate the firing rate of MSN pairs to produce artificial correlations. Additionally, to ensure an accurate reflection of population activity, data were recorded continuously for 20 min to provide a large sample for constructing our CCHs. Finally, although our spike-sorting parameters minimized sorting errors, models suggest that sorting errors in fact reduce the significance of correlated firing (Pazienti and Grün 2006). Thus correlated firing and its reduction in HD likely constitute a real property of MSNs.

Correlated spike activity within networks can arise from an external common drive and/or functional connections within the population, both of which may occur in striatum. For example, different cortical areas project topographically to distinct regions of striatum and converge onto discrete populations of MSNs (Alexander et al. 1986; Flaherty and Graybiel 1994; Smith et al. 1998). In addition, the striatal microcircuits—consisting of MSNs, GABAergic interneurons, and cholinergic interneurons—provide local shaping of MSN spike patterns. MSN axons exhibit profuse local collaterals within striatum (Kawaguchi et al. 1990; Wilson and Groves 1980) and make functional, fast-acting γ-aminobutyric acid (GABA)—mediated synaptic connections between other MSNs (Czubayko and Plenz 2002; Plenz 2003; Rebec and Curtis 1988; Tunstall et al. 2002). Similarly, GABAergic interneurons tightly regulate the timing of MSN activity (Carrillo-Reid et al. 2008; Plenz and Kitai 1998). Cholinergic interneurons have also been shown to modulate the efficacy of the corticostriatal synapse (Calabresi et al. 2000; Wang et al. 2006). We suggest that correlated firing by MSN is largely driven by a loose coordination of external input sources arising primarily from excitatory cortical networks (Raz et al. 1996). In fact, both cortical stimulation and bath application of NMDA are sufficient to synchronize burst activity in assemblies of striatal cells in vitro (Carrillo-Reid et al. 2008). On the other hand, the striatal microcircuit might shape the fine temporal structure of correlated firing by tuning MSN activity. Regardless of the precise mechanism, correlated firing by MSNs seems to be a prominent feature of striatal information processing.

**Coincident bursting is compromised in HD**

The large proportion of correlated firing in WT animals, despite relatively low firing frequencies of MSNs, suggests that the temporally coordinated activity within MSN populations occurs during periods of high-frequency spikes, such as the activity exhibited in burst firing. To assess this phenomenon, we calculated the occurrences of coincident bursts, which overlap in time between pairs of units. Indeed, we found that WT mice have more coincident bursts than HD mice of both lines. It thus appears that dysregulated burst patterns in both HD lines contribute to fewer coincident bursts than WT. We also showed that pairs of neurons that display correlated firing, regardless of genotype, have more coincident bursts and longer coincidence durations than pairs of neurons that do not display correlated firing. Coincident bursts, by producing high-frequency discharges in close temporal relation between units, likely contribute to correlated firing among MSNs. Because multiple synaptic inputs are required to elicit action potentials, coincident bursts are thought to optimize neuronal signaling by increasing the signal-to-noise ratio of the input (Lisman 1997). Therefore coincident bursting in MSNs likely enhances the efficacy of signals that project from striatum to its target structures. Decreased coincident bursting in HD, however, would disrupt information processing between striatum and its targets, thereby compromising basal ganglia output.

**Differences in bursting reflect the proportion of correlated firing in WT mice**

Units from C57BL/6J*CA/J mice had fewer correlated units compared with those from 129sv mice. Although reduced correlated firing occurs concomitantly with reduced bursting, the firing rates of both WT groups were not different. We therefore suggest that the amount of correlated firing is tightly regulated by the degree of bursting. A difference in bursting between the two genetically inbred strains is consistent with the fact that C57BL/6J*CA/J and 129sv mice differ greatly in their behavior and physiology (Crawley et al. 1997; Holmes et al. 2002; Nguyen 2006). Regardless of the baseline activity of MSNs in the WT mice, both HD models showed marked reductions in both correlated firing and burst activity.

**Mechanisms for altered information processing of MSNs in HD**

MSNs, the key target of HD, seem to be selectively more vulnerable to HD than other striatal neurons (Cepeda et al. 2007; Fusco et al. 1999). Although MSNs are highly enriched in huntingtin (Fusco et al. 1999), the function of this protein is far from understood. It is clear, however, that alterations in voltage-gated conductances, receptor sensitivity, and synaptic communication in MSNs occur as a consequence of HD neuropathology (for review see Cepeda et al. 2007). In HD mice, for example, MSNs have reduced inwardly and outwardly rectifying K⁺ currents and express decreased levels of the K⁺ channel subunit proteins Kir2.1, Kir2.3, and Kv2.1 (Ariano et al. 2005). Because hyperpolarization of MSNs is in part driven by the Kir2 family of inwardly rectifying K⁺ channels (Nisenbaum and Wilson 1995), HD may prevent MSNs from reaching hyperpolarized potentials. This is consistent with evidence that MSNs from HD mice are locked in a depolarized state (Klapstein et al. 2001). Interestingly, MSN excitability may also result from decreased glutamate uptake, a major problem in HD (Miller et al. 2008). There is also a reduction and thinning of MSN dendritic spine densities in R6/2 mice (Klapstein et al. 2001), which likely compromises integration of afferent information.

Unlike MSNs, tonically active cholinergic interneurons in striatum appear resistant to degeneration in HD (Fusco et al. 1999). Although some of our unit activity recorded from HD
mice appears similar to activity from striatal cholinergic interneurons, which exhibit fast firing rates and few bursts (Courttemanche et al. 2003; Wilson et al. 1990), it is unlikely that they contributed to our neuronal sample. First, all units recorded in HD mice had waveform characteristics that resembled MSNs. Second, not all units in our HD sample fired at high rates. In fact, KI units showed no increase in firing rate compared with that of WT units. Striatal cholinergic interneurons are also known to produce correlated firing (Kimura et al. 2003; Raz et al. 1996). In contrast, our HD units showed a marked reduction in correlated firing. It also seems unlikely that the cholinergic interneurons dominated our HD sample given the small number of these cells relative to MSNs in striatum (Graveland and DiFiglia 1985). Overall, our results, which suggest an important role for both bursting and correlated firing in MSN information processing, implicate dysregulation of this mechanism in HD pathophysiology.

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