Early impairment of thalamocortical circuit activity and coherence in a mouse model of Huntington’s disease

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ABSTRACT
Huntington’s disease (HD) is a progressive, fatal neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances. There is no known cure for HD, but its progressive nature allows for early therapeutic intervention. Currently, much of the research has focused on the striatum, however, there is evidence suggesting that disruption of thalamocortical circuits could underlie some of the early symptoms of HD. Loss of both cortical pyramidal neurons (CPNs) and thalamic neurons occurs in HD patients, and cognitive, somatosensory, and attention deficits precede motor abnormalities. However, the role of thalamocortical pathways in HD progression has been understudied. Here, we measured single unit activity and local field potentials (LFPs) from electrode arrays implanted in the thalamus and primary motor cortex of 4-5 month-old male and female Q175 mice. We assessed neuronal activity under baseline conditions as well as during presentation of rewards delivered via actuation of an audible solenoid valve. HD mice showed a significantly delayed licking response to the reward stimulus. At the same time, neuronal activation to the reward was delayed in thalamic neurons, CPNs and fast-spiking cortical interneurons (FSIs) of HD mice. In addition, thalamocortical coherence increased at lower frequencies in HD relative to wildtype mice. Together, these data provide evidence that impaired cortical and thalamic responses to reward stimuli, and impaired thalamocortical coherence, may play an important early role in motor, cognitive, and learning deficits in HD patients.

1. Introduction
Huntington’s disease (HD) is a genetic, neurodegenerative disorder characterized by motor, psychiatric, and cognitive disturbances (Bates et al., 2002; Harper and Jones, 2002). The role of the dorsal striatum in the development of motor disturbances in HD has been well-studied, but evidence suggests that changes in the cerebral cortex precede those in the striatum, and disruptions in cognitive function precede motor disturbances (Beglinger et al., 2010; Lawrence et al., 1998; Paulsen et al., 2008). In addition to the well characterized loss of striatal medium-sized spiny neurons (MSNs) in HD, cortical pyramidal neurons (CPNs) are lost while some types of interneurons are spared (Vonsattel and DiFiglia, 1998; Waldvogel et al., 2015). Interestingly, in the striatum, parvalbumin (PV)-expressing interneurons also are lost (Reiner et al., 2013), while in the cortex, interneuron loss is region- and symptom-dependent (Kim et al., 2014). In addition, the number of perisomatic inhibitory contacts onto CPNs is significantly reduced in HD mouse models and in human cortex (Burgold et al., 2019).

A major role of the cerebral cortex in HD pathophysiology is supported by studies in genetic animal models. CPNs have been shown to be hyperexcitable in symptomatic mice and receive decreased inhibitory signals (Cummings et al., 2009; Spampanato et al., 2008). Reduction of the mutant (m)Htt gene in CPNs partially rescues behavioral and electrophysiological alterations in the striatum (Estrada-Sanchez et al., 2015; Wang et al., 2014). Cortical neuronal function is primarily regulated by cortico-cortical connectivity, local circuit GABAergic...
interneurons, and thalamic inputs. Thalamic motor nuclei receive inputs from the internal globus pallidus (GPI) and project to the motor cortex. In HD, it is believed that the early loss of indirect pathway MSNs results in disinhibition of the motor thalamocortical pathway (Albin et al., 1992; Deng et al., 2004; Reiner et al., 1988), although evidence suggests the disinhibition may also be due to alterations in the direct pathway (Andre et al., 2011). In addition to potential dysfunction in thalamocortical projections, CPNs that project to the thalamus are preferentially lost (Hedreen et al., 1991), suggesting potential reciprocal disruption in cortico-thalamocortical circuitry.

Corticothalaleral circuits have been extensively studied in HD (Blumenstock and Doudanova, 2020; Miller et al., 2011; Naze et al., 2018; Ponzi et al., 2020; Rothe et al., 2015), however, the role of the thalamus and the thalamocortical projection in HD has been understudied, even though some of the earliest sensory, attention, and cognitive deficits are clearly associated with thalamocortical circuits. In human HD, there is evidence for significant cell loss within the thalamus (Heinsen et al., 1999; Heisen et al., 1996), although it is not known if thalamic atrophy occurs in parallel or is secondary to cortical damage. Cell loss in thalamic nuclei is important because the thalamus is the principal gate for integration of sensory and motor information before it reaches target regions in the cortex and striatum. EEG studies have shown that the most common abnormality in HD patients is suppression of α-activity, which points to thalamic abnormalities (de Tommaso et al., 2003). It is therefore possible that thalamic and cortical alterations could underlie some of the early symptoms in HD.

To gain insights into the dynamics and interactions of cortex and thalamus in HD, we used high-density silicon probes to simultaneously record from the motor cortex and thalamus of young Q175 mice, a knock-in model of adult-onset HD, during a behavioral task. The results indicate delayed cortical and thalamic responses to reward stimuli, as well as impaired thalamocortical coherence.

2. Methods

Animals: Mouse use and experimental procedures were performed in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committee at the University of California, Los Angeles (UCLA). Nine wild-type (WT) and 9 Q175/+– aged 4–5 mo (when motor deficits start) male and female mice (WT: 4 females and 5 males; Q175: 3 females and 6 males) were used for behavior and in vivo electrophysiological recordings. Data from males and females were pooled for analysis since we did not observe consistent differences between sexes.

Surgery: The first surgery was performed to cement head bars on top of the skull for head restraint. During the head bar surgery, mice were deeply anesthetized with isoflurane (1–1.5%) and placed in a stereotaxic frame (Model 1900, Kopf Instruments). The skin above the skull was removed and the skull was leveled in the x, y, and z directions. Stainless-steel head bars (laser cut at Mainstay Manufacturing) were then positioned, with the medial portion over the parietal plates and the lateral portion extending over the ears, and were cemented in place (Metabond, Parkell). A layer of silicone elastomer (Kwik-Cast, World Precision Instruments) was placed over any exposed skull followed by another layer of cement to prevent infections. Mice were given one week to recover before handling.

Once mice acquired the behavioral task (see behavior section), a second surgery consisting of craniotomies above the motor cortex and thalamus was performed the day before the recording session. During the craniotomy surgery, mice were again deeply anesthetized with isoflurane and placed in a stereotaxic frame. The silicone elastomer and cement were removed from above the skull while keeping the head bars in place. A rectangular cranioectomy (1.8 mm × 1 mm) was drilled above the motor cortex and thalamus with ±0.2 mm buffer from the final electrode placement (see electrophysiology section) and the skull was carefully removed so as not to disturb the dura mater. A small hole was drilled above the cerebellum for reference electrode placement. A piece of Surgifome (Ethicon) soaked in artificial cerebral spinal fluid (aCSF) was placed above the exposed brain to absorb any blood and keep the brain moist. The Surgifome and exposed skull were again covered with a layer of silicone elastomer.

Behavior: One week following the head bar surgery, mice were handled and habituated to head restraint and reward exposure to 10% sweetened condensed milk for a week. Following recovery from surgery, mice were food deprived to maintain ∼90% of their baseline weight and only fed once daily after each training session. They received water ad libitum. Following habituation, mice were positioned on top of a Styrofoam treadmill allowing free movement in the forward and reverse direction with a cannula placed in front of their mouth for milk delivery. On the first day of training, the cannula was positioned so that a drop of milk protruding from the cannula would make contact with their mouth. On all following days, the cannula was placed further away from the mouth where the mouse's tongue could reach the milk droplet but the droplet was not immediately felt by the mouse. An audible solenoid valve actuation (Part 161 T010, Neptune Research) was used to deliver the reward stimulus. Mice were trained for 2 weeks to lick for milk in response to the solenoid valve actuation for up to 40 trials. Licking was continuously monitored through an infrared lickometer (UCLA machine shop) placed in front of the reward delivery tube and milk droplet (Fig. 1A). Once the mouse was consistently responding by licking the milk droplet after the solenoid valve actuation for 40 consecutive trials with a random inter-trial interval of 15–30 s, the mouse was considered trained and ready for recordings.

Electrophysiology: On the recording day, mice were head restrained on the Styrofoam treadmill and the milk delivery system was positioned in front of the mouse as described above. The silicone elastomer and Surgifome were removed and aCSF was added to the brain to keep it moist. The electrodes were carefully positioned over the motor cortex and thalamus and slowly lowered with a micromanipulator (MP-285, Sutter Instruments). Electrodes consisted of silicon microprobes (Yang et al., 2020) with 256 electrodes divided across 8 prongs; 4 prongs containing a total of 128 electrodes were placed in the primary motor cortex (prong tip positions: 1.2 mm anterior, 1.25 to 2.25 mm lateral, and 2.0 mm ventral relative to bregma) and another 4 were placed in the thalamus (−1 mm anterior, 0.6 to 1.6 mm lateral, and 3.75 mm ventral relative to bregma). The silicon microprobes were coated with a fluorescent dye (Di-D, Thermo Fisher) to verify placement of the probes (Fig. 1B). An Ag/AgCl reference electrode was placed in the cerebellum. Electrode insertion was monitored with a surgical microscope (Zeiss OPMI pico) to ensure the probes were not deflection during the insertion. After reaching the target depth, a drop of mineral oil was placed on the exposed brain surface to prevent the brain from drying. Electrodes were allowed 30 min to stabilize before recordings began. A 10 min baseline session was recorded in which the mouse was allowed to move on the Styrofoam treadmill but no milk was delivered. After the baseline session, the mouse was presented with 40 reward stimulus trials in the same manner as the training session.

Analysis: Latency to lick was measured as the time from reward delivery (i.e., solenoid actuation) to the first lick. Lick rates were determined as the number of licks/s up to 8 s after reward (50 ms bins). In some trials, the mice were licking immediately prior to solenoid delivery and continued to lick during and after solenoid delivery. Since it is unknown if the licking immediately post solenoid delivery was specific to the solenoid presentation, trials in which a mouse licked within 1 s prior to solenoid delivery were removed from analysis. There was no significant difference in the number of trials that were removed between WT and Q175 mice (mean ± SEM, 15.6 ± 4.8% for WT and 10.7 ± 2.4% for Q175 mice). Latency to lick and lick rates were averaged by mouse so that each data point represents the mean of all licking trials for a given mouse. A Student's t-test was used to measure difference in lick latency. A two-way ANOVA was used to measure difference in lick rate. Lick rate for the two-
way ANOVA was calculated from solenoid actuation to 1 s post solenoid actuation with 0.1 s bins.

For analysis of neural activity, spike sorting was performed using custom, semi-automated software written in MATLAB (MathWorks). To minimize globally correlated signal artifacts, the mean background signal was removed from each electrode by subtracting the mean voltage of all electrodes on the corresponding silicon prong. Signals were then filtered between 600 and 6500 Hz to detect spikes. Spikes were detected on the spike trough with a signal-to-noise ratio (SNR) threshold of ~3. Spike waveform trough-to-peak duration was used to distinguish putative FSIs from CPNs in M1. Units with a trough-to-peak duration of less than 0.475 ms and baseline firing greater than 0.1 Hz were classified as FSIs, while units with a trough-to-peak duration between 0.55 and 1.25 ms and baseline firing rate between 0.05 and 10 Hz were classified as CPNs (Bakhurin et al., 2016). Units were spike sorted using custom MATLAB scripts (Shobe et al., 2015), which automatically generated waveform-based templates from the first 5 min of the recording session and matching the rest of the spikes to those templates (Fig. 1C). All units were manually inspected and removed if waveforms appeared to represent a signal artifact. Single-unit firing was calculated in time steps of 50 ms and smoothed by convolving with a Gaussian kernel (SD = 250 ms). Signals were down-sampled offline to 1 kHz for local field potential (LFP) analysis. Relative power and thalamocortical coherence were calculated at frequencies of 0–55 Hz (0.25 Hz bins). Firing rate was determined 3 s before and up to 8 s post reward (5 ms bins) for thalamic neurons, CPNs, and cortical FSIs. Firing rates were then normalized using z-scores based on the mean baseline firing rate. Data were binned into smaller time bins in order to perform two-way ANOVAs from 0 to 6 s post reward and 0 to 1 s post reward. Data analyzed from 0 to 6 s post reward were binned into 6 bins of 1 s each. Data analyzed from 0 to 1 s were binned into 10 bins of 0.1 s each. The maximum firing rate z-score for each neuron was determined from 0 to 6 s post reward and used to determine the latency to maximum firing for each neuron. Data for latency to maximum firing rate for each neuron were plotted as a histogram with data binned into intervals of 0.6 s using z-scores and plotted for 6 s.

For LFP data, we averaged the LFP signals across all 128 channels such that we only used 1 mean LFP signal from each mouse. Welch's power spectral density estimate was used to determine the relative power of the cortical and thalamic LFP data with a window length of double the LFP sampling rate and non-overlapping samples. Baseline LFP data were taken as the average LFP signal over a 5 min window in the baseline session. LFP post reward stimulus was taken as the mean LFP signal for 2 s post solenoid presentation and averaged across all solenoid presentations. The total power was determined from 0 to 100 Hz and used to determine relative power from 0 to 100 Hz. Two-way ANOVAs were used to determine significant differences in relative power of LFP signals with post hoc Holm-Sidak tests. Since no significant differences were found above 10 Hz, relative power below 10 Hz was only included in the figures. To determine significant differences in
relative delta power, the relative power was summed from 1 to 4 Hz for each mouse and a t-test was used to determine significant differences in relative delta power between WT and Q175 mice. Given the large variability in thalamic relative LFP delta power, we separated out the delta LFP power by prong to determine if there were any medial-lateral effects. We averaged the LFP signal from each prong and used a two-way ANOVA to determine any potential statistical differences.

Thalamocortical coherence between 0 and 55 Hz was calculated using MATLAB’s mscohere function with a hanning window that produced 0.5 Hz divisions from the coherence of LFP data. Coherence was measured between each pair of thalamus and cortical recording prongs. Since 4 prongs were in the thalamus and cortex each, the coherence was determined for a maximum of 16 prong pairs for each mouse. Baseline coherence was measured as the mean thalamocortical coherence during the 5 min of the baseline session. Resting coherence data were analyzed at delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–55 Hz) bands using two-way ANOVAs with post hoc Holm-Sidak tests. The coherence during the behavioral session was measured in 0.1 s bins using MATLAB’s mscohere function with a hanning window again to produce 0.5 Hz divisions. Mean coherence for WT and Q175 mice was calculated for each frequency bin at each time bin in relation to cue delivery. Mean coherence was then plotted as a colorogram for both WT and Q175 where the x-axis represented time and the y-axis represented frequency. The color on the colorogram, from blue to red, represents the magnitude of coherence. Lower coherence is represented in blue and higher coherence is represented in red. To quantify differences in coherence, we separated the colorogram into 3 time windows: pre stimulus (mean of coherence from 2 s prior to the cue), early post stimulus (mean of coherence from the onset of the cue to 2 s post cue), and late post stimulus (mean of coherence from 2 to 6 s post cue). For statistical analyses, the mean time windows were separated into frequency bins with each of the following frequency ranges: delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–55 Hz). Two-way ANOVAs were then used to analyze significant differences in coherence within each frequency band between genotypes during the 3 different time bins with post hoc Holm-Sidak tests.

Histology and electrode position determination: Following each recording session, the mouse was sacrificed and perfused with 4% paraformaldehyde to fix the brain tissue. Coronal sections were cut at 30 µm through the motor cortex and thalamus. Sections were visualized under a fluorescent microscope to verify electrode location based on the location of Di-D staining left from the tip of the prongs (Fig. 1B).

Statistical Tests: Appropriate ANOVA, t-tests, and Chi-Square tests were performed using SigmaPlot (SigmaStat) to determine significant differences between group means. Post hoc Holm-Sidak tests on ANOVAs were performed when appropriate. Only significance values above 0.01 were included for two-way ANOVAs of neuronal firing whereas a cut-off of 0.05 was used to determine significance for LFP and coherence data. Chi-square tests were used to measure differences in latency to maximum firing for the histograms.

3. Results

3.1. Delayed lick latency

To determine deficits in reaction time in Q175 mice, we measured the latency to the first lick and the instantaneous lick rate immediately following the auditory conditioned stimulus. Q175 mice showed a significant delay in latency to first lick following the reward stimulus compared to WT mice (Fig. 2A; n = 9 WT and 9 Q175 mice, t = −3.06, p = 0.007). The lick latency in Q175 mice was more than twice that of WT mice. We also observed this delay when measuring instantaneous lick rate from the initiation of the reward stimulus to 1 s post reward stimulus (Fig. 2B, main effect of genotype: F(1,160) = 6.6, p = 0.01).

3.2. Single unit activity

To determine if the behavioral differences were related to changes in neural activity in the thalamocortical pathway, the firing rate of thalamic and motor cortical neurons of WT and Q175 mice were measured while mice were performing the behavioral task. Cortical neurons were separated into CPNs and FSIs. There was no significant difference in the mean number of neurons recorded per mouse between genotypes in any of the cell types (Table 1). Given the variance in baseline firing rates for neurons, we generated a z-score of the firing rate over time to normalize the firing rate at any given point to the average baseline firing rate for that cell. The mean baseline firing rates prior to the delivery of the reward stimulus did not differ between WT and Q175 mice for each neuron type (Table 2).

Table 1

|                | WT total | WT cells/mouse | Q175 total | Q175 cells/mouse |
|----------------|----------|----------------|------------|------------------|
| CPN            | 155      | 17.22 ± 3.14   | 250        | 27.78 ± 5.07     |
| Cortical FSI   | 51       | 5.67 ± 1.35    | 66         | 7.33 ± 2.91      |
| Thalamus       | 104      | 11.56 ± 3.04   | 169        | 18.78 ± 3.05     |

Fig. 2. Latency to lick following presentation of a reward stimulus for WT (black) and Q175 (red) mice. A. Mean latency to first lick following presentation of the reward stimulus. Error bars represent SEM. Colored dots above and below the bars represent mean lick latencies for individual mice. ** represents p < 0.01. B. Mean lick rate average from 0.5 s prior to auditory solenoid trigger to 1 s following the trigger at 0.05 samples/s. * represents p < 0.05 for the main effect of genotype. Solenoid trigger occurred at time 0. Shaded areas represent SEMs of 9 WT and 9 Q175 mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.2.1. Thalamic neurons

The most striking response was seen in thalamic neurons where neuronal activity was clearly triggered by the presentation of the reward stimulus in both WT and Q175 mice. This can be seen in both the heat map of firing rates for individual neurons (Fig. 3A) and averaged firing rate over time (Fig. 3B and C). The heat maps show a clear maximal firing rate band immediately post presentation of the reward stimulus, and both the raw firing and z-score firing rates show a peak firing rate at about 0.1 s post reward stimulus presentation (Fig. 3B and C). This peak in thalamic firing occurs prior to the peak in lick rate (Fig. 3B bottom graph). Although both WT and Q175 mice have thalamic neurons that are precisely timed to the reward stimulus, the mean firing rate in Q175 mice was significantly reduced during the first sec post reward stimulus (Fig. 3B and C; Genotype x Time: F(9,2700) = 4.9, p < 0.001; post hoc: 0–1 s t = 3.6, p < 0.001). When smaller time bins were examined, the reduced response occurred during the first 200 ms post reward stimulus (Fig. 3B and C; Genotype x Time: F(9,2700) = 4.9, p < 0.001; post hoc: 0–100 ms t = 7.5, p < 0.001, 100–200 ms t = 4.1, p < 0.001). However, thalamic neurons in Q175 mice showed a delay in maximum firing in response to the cue with a significant right shift in the histogram plot (Fig. 3D; \( \chi^2(20) = 64.0, p < 0.001 \)) as well as a delayed, significant increase in firing rate in Q175 mice compared to WT mice from 3 to 5 s post reward stimulus presentation (Fig. 3B; Genotype x Time: F(5,1620) = 5.5, p < 0.001; post hoc: 3–4 s t = 2.7, p < 0.01, 4–5 s t = 2.6, p = 0.01).

3.2.2. Cortical pyramidal neurons

Firing of CPNs was not as precisely timed to the reward stimulus presentation as that of thalamic neurons (compare Figs. 3 & 4). Only about 30 to 40% of neurons in Q175 and WT mice, respectively, were tuned to the reward stimulus (Fig. 4A). The reduction in the proportion of tuned Q175 CPNs was reflected as a significant reduction in the mean z-score firing from 0 to 0.3 s (Fig. 4B; Interaction of Genotype and Time: F(5,2418) = 2.6, p = 0.03, post hoc 0–1 s: t = 3.1, p = 0.002 and 4C; Main Effect of Genotype: F(1,4103) = 36.8, p < 0.001; post hoc 0–0.1 s: t = 2.8, p = 0.005; post hoc 0.1–0.2 s: t = 2.9, p = 0.004; post hoc 0.2–0.3 s: t = 3.1, p = 0.002). Similar to the thalamic, the reduction in firing rate immediately post reward stimulus in Q175 compared to WT mice may be due to a delay in the response of Q175 CPNs (Fig. 4A). Although not statistically significant, there is a small increase in firing rate of Q175 CPNs around 3–4 s post reward stimulus (Fig. 4B). However, given the wide variability in latency to maximum firing, there was no statistically significant difference in the latency to maximum firing between WT and Q175 CPNs (Fig. 4D).

3.2.3. Cortical FSIs

Cortical FSIs in WT mice were tightly tuned to the reward stimulus with a rapid initial increase in firing, whereas Q175 FSIs seemed to persist in firing once it was initiated (Fig. 5). Similar to the other neurons

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**Table 2**

Mean baseline firing rates ± SEM of CPNs, Cortical FSIs, and Thalamic neurons from 9 WT and 9 Q175 mice.

|        | WT     | Q175   |
|--------|--------|--------|
| CPN    | 1.88 ± 0.20 | 2.13 ± 0.68 |
| Cortical FSI | 4.46 ± 1.24 | 4.06 ± 0.96 |
| Thalamus | 6.72 ± 0.85 | 6.91 ± 2.09 |

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**Fig. 3.** Firing rate of thalamic neurons following the reward stimulus. A. Heat maps of normalized mean firing rate of 104 WT (top) and 169 Q175 (bottom) thalamic neurons recorded from all mice relative to reward stimulus presentation. White/yellow indicates time periods of maximal firing rate for a given neuron and dark red represents minimal firing rates. Each row on the y-axis represents a different neuron where the neurons are sorted by their firing latency in relation to the cue. Time is represented along the x-axis from 3 s before the cue to 8 s post-cue. B. Top graph shows the raw firing rate over 11 s (3 s before and 8 after reward stimulus) where the neuronal activity was clearly triggered by the presentation of the reward mouse within the same time frame as the neuron firing rate graphs. Lighter colored area represents SEM of neurons. Middle graph shows mean firing rate z-score averaged for neurons recorded from all mice relative to reward stimulus presentation. White/yellow indicates time periods of maximal firing rate for a given neuron and dark red represents minimal firing rates. Each row on the y-axis represents a different neuron where the neurons are sorted by their firing latency in relation to the cue. Time is represented along the x-axis from 3 s before the cue to 8 s post-cue. C. Expanded representation of the mean firing rate z-score from 0 to 0.3 s (Fig. 4B; Interaction of Genotype and Time: F(5,2418) = 2.6, p = 0.03, post hoc 0–1 s: t = 3.1, p = 0.002 and 4C; Main Effect of Genotype: F(1,4103) = 36.8, p < 0.001; post hoc 0–0.1 s: t = 2.8, p = 0.005; post hoc 0.1–0.2 s: t = 2.9, p = 0.004; post hoc 0.2–0.3 s: t = 3.1, p = 0.002). Similar to the thalamic, the reduction in firing rate immediately post reward stimulus in Q175 compared to WT mice may be due to a delay in the response of Q175 CPNs (Fig. 4A). Although not statistically significant, there is a small increase in firing rate of Q175 CPNs around 3–4 s post reward stimulus (Fig. 4B). However, given the wide variability in latency to maximum firing, there was no statistically significant difference in the latency to maximum firing between WT and Q175 CPNs (Fig. 4D).
recorded in the thalamocortical circuit, the initial firing response (0.1–0.2 s) in Q175 FSIs was significantly decreased compared to that of WT mice (Fig. 5D; Genotype x Time: F(9,1150) = 179.3, p < 0.001; post hoc: t = 3.6, p < 0.001). For the firing rate z-score, there was a clear peak followed by a rapid decrease in the firing rate over time in WT mice (Fig. 5B). However, in Q175 mice, there was a more gradual drop in firing rate with a time scale that more closely matched the licking rate profile (Fig. 5B). This persistent firing in Q175 FSIs was reflected as a significant increase in firing rate z-score for Q175 mice compared to WT mice from 1 to 4 s post reward stimulus presentation (Fig. 5B; Main effect of Genotype: F(1,690) = 25.8, p < 0.001; post hoc: t = 3.4, p < 0.001; post hoc 1–2 s: t = 4.0, p < 0.001; post hoc 3–4 s: t = 2.9, p = 0.004). In addition to sustained firing, FSIs in Q175 mice showed a delay in latency to maximum firing compared to WT mice (Fig. 5D; χ²(9) = 17.5, p = 0.04).

Overall, neurons in the thalamocortical circuit of Q175 mice have a reduced initial response following reward stimulus presentation but have an increased delayed response compared to WT mice.

### 3.3. Baseline delayed and thalamic LFP and coherence

Since there were clear differences in individual thalamic and cortical neuronal responses to the reward stimulus, the global activity of the thalamus and motor cortex and their cross-communication using LFPs and coherence were measured. To determine if there were baseline differences in thalamic and cortical activity without any discrete external cues, baseline LFP signals were collected while the mouse was suspended on a ball for 5 min before any reward stimuli were delivered. During the baseline period, there were no statistically significant differences in the broad relative power spectrum (Figs. 6A and B, left graphs) but when delta power was isolated from the relative power, Q175 mice showed significantly higher delta power in the cortex (Fig. 6A right; t = −2.5, p = 0.02) compared to WT mice but no difference in the thalamus (Fig. 6B, right graph). Given the large variability in delta LFP power in the thalamus, we separated out the LFP data by prong to determine if there were any medial-lateral difference in the thalamic LFP. However, there were no genotype or positional differences in relative delta power by prong (data not shown). Relative power was not significantly different at any other frequency range suggesting that, at baseline, there are only modest differences in the LFP between Q175 and WT mice.

Thalamocortical coherence was then measured during the baseline session to determine thalamocortical communication. In thalamocortical coherence, a peak in the power spectrum typically occurs in the alpha frequency range (Bollimunta et al., 2011; Nunez et al., 2001). During the baseline session, WT mice show the signature increased alpha thalamocortical coherence, whereas in Q175 mice, the alpha and beta ranges of 8–33 Hz were decreased compared to WT mice (Fig. 6C & D; Main effect of Genotype: F(1,2574) = 160.5, p < 0.001; post hoc: 8 Hz t = 4.1, p < 0.001, 11.6 Hz t = 4.8, p < 0.001, 15.2 Hz t = 4.8, p < 0.001, 18.8 Hz t = 5.6, p < 0.001, 22.4 Hz t = 5.5, p < 0.001, 26 Hz t = 4.2, p < 0.001, 29.6 Hz t = 3.9, p < 0.001, 33.2 Hz t = 3.2, p = 0.002). Thalamocortical coherence was significantly increased at lower frequency bands including delta frequencies (Fig. 6C & D; Main effect of Genotype: F(1,2002) = 12.8, p < 0.001), specifically at 2.5 Hz (post hoc: t = 2.3, p = 0.02), and theta frequencies (Fig. 6C & D; Main effect of Genotype: F(1,2574) = 9.6, p < 0.001), specifically between 5 and 7 Hz (post hoc: 5 Hz t = 2.6, p = 0.009, 5.5 Hz t = 2.4, p = 0.02, 6 Hz t = 2.4, p = 0.02, 6.5 Hz t = 2.1, p = 0.03) in Q175 mice compared to WT (Fig. 6C & D). This change in thalamocortical coherence during the baseline session, suggests that there are disruptions in thalamocortical communication in the absence of a discrete external cue, and the increased thalamocortical
coherence in Q175 mice at lower frequency bands is consistent with pathologies of thalamocortical dysrhythmia (TCD).

3.4. Cortical and thalamic LFPs and coherence during behavior

To determine whether these disruptions also occurred during behavior, we measured oscillatory changes in the thalamus and cortex around the presentation of the reward stimulus. LFPs were measured from the time of the reward stimulus and up to 2 s post reward stimulus presentation. There were no significant differences in LFP signals at any time points following the reward stimulus and up to 2 s post reward stimulus presentation. There were no significant differences in LFP signals at any time points following the reward stimulus and up to 2 s post reward stimulus presentation. Lighter colored area represents SEM. Bottom graph represents mean firing rate z-score averaged by neuron over time. Data were binned into 1 s intervals for statistical analysis. Lighter colored areas represent SEM. C. Expanded representation of the mean firing rate z-score from −0.2 to 1 s. Data were binned into 0.1 s intervals for statistical analysis. D. Histogram plot of latency to maximum firing for M1 FSIs. * represents p < 0.01 and ** represents p < 0.001 for two-way ANOVAs and post hoc Holm-Sidak tests for panels B and D. * represents p < 0.05 for Chi square test for panel C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

HD is characterized by motor, psychiatric, and cognitive disturbances and also is associated with an increase in reaction time (Bates et al., 2015; Jahanshahi et al., 1993). In the present study, a simple cued-response task was used and reaction times of Q175 mice at 4–5 months of age were measured. At this age, behavioral deficits begin to emerge in heterozygous Q175 mice (Heikkinen et al., 2012; Menalled et al., 2012). Consistent with early motor dysfunction, a delayed licking behavior in response to a reward stimulus in Q175 mice compared to WT was observed. Given this clear behavioral difference occurring in the early symptomatic stage, the neural substrates of this delayed behavioral
response were examined as they could indicate circuit disruptions occurring early during disease progression. In light of previous evidence of cortical and thalamic dysfunction in HD (de Tommaso et al., 2003; Heinsen et al., 1999; Orth et al., 2010; Schippling et al., 2009), we hypothesized that this early behavioral deficit could be due to a disruption in cortico-thalamocortical circuitry. Indeed, the data obtained are consistent with early impairment of thalamocortical circuit activity. Unlike the more rapid responses observed in WT mice, individual neuron activity in Q175 mice in both the thalamus and cortex showed a slowed, more gradual response profile following the reward stimulus. This slowed response occurred as a decrease in the initial firing rate in thalamic neurons as well as CPNs and

Fig. 6. A. and B. show relative power of cortex and thalamus LFPs respectively, during baseline recordings from 9 WT and 9 Q175 mice. Left graph shows relative power from 0 to 10 Hz. Black (WT) and red (Q175) lines indicate relative power mean at a given frequency and the shaded bar surrounding the line indicates the SEMs. The box plots on the right show total relative Delta power (1–4 Hz) where boxes represent the 25–75% distribution, white (WT) or black (Q175) line in box represents the mean, gray lines represent the median, and tails represent the ranges of data. C. Thalamocortical coherence from 0 to 59 Hz. Shaded bars surrounding the lines indicate SEM. However, the error is so small that it is difficult to visualize. D. Zoomed in thalamocortical coherence from 0 to 10 Hz for easier visualization of lower frequencies. * in the box and whisker plots indicate $p < 0.05$ as determined by a t-test. *, **, and *** in the line plots represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, as determined by a two-way ANOVA with post hoc Holm-Sidak tests.
Fig. 7. A. and B. show relative power of cortex and thalamus LFPs respectively, within 2 s post reward stimulus from 9 WT and 9 Q175 mice. Left graph shows relative power from 0 to 10 Hz and right shows total relative Delta power (1–4 Hz) where boxes represent the 25–75% distribution, line in box represents the mean and tails represent the range of data. On left, black (WT) and red (Q175) lines indicate relative power mean at a given frequency and the shaded bar surrounding the line indicates the SEM. On right, box and whisker plots show total relative Delta power (1–4 Hz) where boxes represent the 25–75% distribution, white (WT) or black (Q175) line in box represents the mean, gray lines represent the median, and tails represent the ranges of data.

FSIs in Q175 compared to WT mice. Both thalamic neurons and cortical FSIs showed an increased latency to maximum firing and a delayed increase in firing that was present from 3 to 5 s in thalamic neurons and 1–4 s in cortical FSIs in Q175 compared to WT mice. In addition to individual neuron activity, LFP relative power in the cortex and thalamus was measured during a baseline state in which no reward stimuli were presented, as well as during a 2 s window immediately post cue presentation. Across the frequency spectrum, subtle differences in LFP signatures during the baseline period were found where an increase in inhibitory neurons activity in the cortex, it is possible that the decrease in gamma thalamocortical coherence is due to a compensatory increase in cortical interneuron activity. In fact, when individual neuron activity in cortex was measured, persistent activity of FSIs in Q175 mice was found following the reward stimulus that was not present in WT mice. It is possible that this persistent FSI activity is modulating the decrease gamma thalamocortical coherence seen in Q175 compared to WT mice.

While we do not know the exact source of the changes in the thalamocortical circuit, it is not likely due to earlier dysfunctions in the striatum. Anatomical studies demonstrated that the thalamostriatal projections are affected earlier than the corticostriatal pathway in a similar model, the CAG140 model (Deng et al., 2014). In a previous study from our lab examining electrophysiological changes in striatal output regions including the SNr and GPe in symptomatic HD mice, we found pre- and postynaptic changes in the striatal output regions (Barry et al., 2018). As the striatum does not project directly to the thalamus, but via SNr/GPi, we believe that cell dysfunction in the striatum is unlikely the culprit of the thalamocortical changes found in this study. Instead, we believe that early cognitive and behavioral changes are primarily due to altered thalamocortical activity, which subsequently affects the corticostriatal pathway. Importantly in the present context,
experimental data from three different HD models and computational simulations based on single-cell recordings in behaving animals demonstrated coherent bursting cell assembly dynamics in striatum of WTs but not in HD models (Ponzi et al., 2020). This loss of coherence was due to the cortical-striatal disconnect underlying cognitive and motor symptoms in HD (Cepeda and Levine, 2020). However, our present data do not preclude the possibility of early corticostriatal disruptions, and more experiments are needed to identify the possible source of thalamocortical alterations.

In conclusion, the present study is the first to show a disruption in thalamocortical circuit activity in early symptomatic Q175 mice as young as 4–5 months. Such miscommunication in thalamocortical circuits also occurs in other pathways and other HD models, e.g., the corticostratal pathway and pathways originating from striatal projection neurons (Barry et al., 2018; Cepeda and Levine, 2020; Veldman and Yang, 2018). However, considering that alterations in thalamocortical circuits occur prior to overt motor symptom onset, the thalamocortical circuit may be an important therapeutic target for early treatment of HD.

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