Characterization of Single Unit Firing Properties *In-Vivo* in Globus Pallidus and Subthalamic Nucleus in 7 Month-old z_Q175KI Transgenic and Wildtype Mice

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Introduction

Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disease that leads to deficits in motor control and cognitive/psychiatric functions. Chorea, a loss of motor control that is characteristic of HD physical symptoms, is thought to reflect a dysfunction of the indirect pathway (IP) drive arising from a deficit of striatal output to globus pallidus (GP), which in turn innervates subthalamic nucleus (STN). Due to the inhibitory GABAergic projections from GP to STN, it is expected that an increase in GP activity would lead to a decrease in STN neuronal discharge. Electrophysiological studies in basal ganglia slices from symptomatic transgenic BACHD rats carrying full length mutant huntingtin has shown increased GP and decreased STN firing consistent with this hypothesis[1].

We have now evaluated spontaneous single unit discharges in GP and STN *in-vivo*, in heterozygous (HET) and homozygous (HOM) z_Q175 mice, a knock-in mouse model of HD which carries either one wildtype and one mutant htt allele containing an expanded CAG repeats (HET), or two mutant Htt alleles (HOM)[2,3]. Six month-old HET, HOM and WT littermate mice were anesthetized using urethane, and extracellular single unit spikes were recorded in their GP or STN region. Expression of mutant Htt appeared to alter the firing rate of STN neurons, as firing rates in HET and HOM mice were lower than their WT littermate. Our data also suggest a gene dosage effect on decreasing firing rate in STN neurons, since the probability of detecting spontaneously active units in Homo mice is significantly lower than Het. in contrast to STN, expression of mutant Htt has no effect on GP neurons firing rate.

1. Zhong et al. (2013). Evaluation of MP-10, a PDE10 inhibitor, on BACHD transgenic rats using dual recording of single units in Globus Pallidus and Subthalamic nucleus. SFN poster 2013.
2. Heikkinen T et al. (2012) PLoS ONE
3. Menalled LB et al. (2012) PLoS ONE

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MATERIALS AND METHODS

Subjects.

Adult (6-8 months) WT and z_Q175 mice of both genders were used. They were treated in accordance with NIH guidelines for the humane treatment of animals. Mice had free access to food and water. The weight range was 19-35 g. In general, males weighed more than females, WT and HET mice weighed more than HOM by 15-30% (Table 1). Animals were housed in groups of 5 maximum in plastic cages under diurnal lighting conditions (12 hours on / 12 hours off).

Genotype	*WT*	*z_Q175KI HET*	*WT2*	*z_Q175KI HOM*
Weight (gram) [M, F]	*30.64±0.80* *23.78±0.44*	*29.21±0.55* *23.31±0.48*	*31.38±0.38* *24.88±0.64*	*24.81±0.38* *20.25±0.62*
Mean Age (month)	*7.0±0.20*	*7.3±0.23*	*7.6±0.21*	*7.4±0.17*
Body Temp (°C) [M, F]	*33.73±0.21* *33.35±0.10*	*33.22±0.18* *33.08±0.17*	*34.08±0.26* *33.68±0.31*	*33.50±0.19* *33.07±0.11*

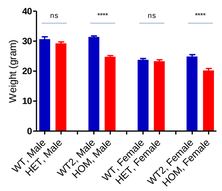


Table 1: Animal information. WT are littermates of HET mice, WT2 are littermates of HOM.

Analysis of single units

Analyses of recorded single units were done offline. Measures such as firing rate, autocorrelogram, ISI (inter-spike interval) and inter-spike variability (CV, coefficient of variation) were computed. For GP recordings, single units were further categorized as regular, irregular or burst firing pattern cells; their percentage breakdown is listed in Table 3. By visual inspection, units with ≥ 3 peaks in autocorrelogram were considered as regular pattern cells; units with ≤ 2 peaks were judged to be irregular cells (Ni et al., 2000). In general, regular pattern units fired at a faster rate and with a narrower ISI shape than irregular pattern units. Burst pattern cells fired at a very low rate relative to the regular and irregular pattern subsets, and they consisted 5-10% of overall GP neurons recorded. Furthermore, there were abnormal units with prolonged silent gaps between spikes (Table 3); they were only seen in units recorded from HOM mice. To categorize such units properly, the longest silent gap must be >5% of the total recording time, or >20 sec for a 5 min recording session. For STN, only irregular and burst pattern units were detected and recorded. The single unit recording information is listed in Table 2.

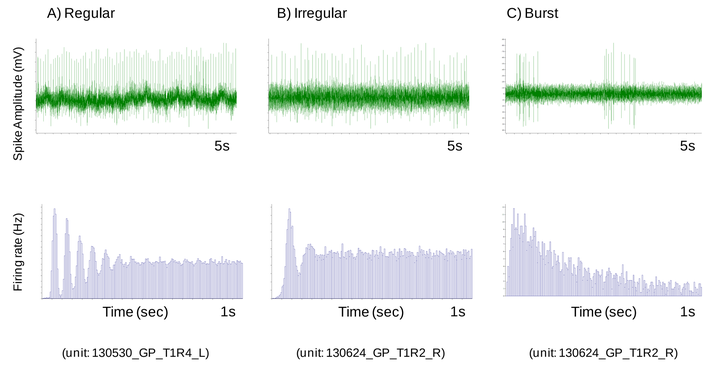
Genotype	WT1		z_Q175KI HET		WT2		z_Q175KI HOM	
	GP	STN	GP	STN	GP	STN	GP	STN
Animal Count [Total, M, F]	16 [8,8]	16 [5,11]	18 [13,5]	23 [14,9]	17 [7,10]	10 [5,5]	19 [11,8]	10 [7,3]
Cell Count	49	27	52	30	120	19	123	17
Cell Yield/Recording	3.1	1.3	2.7	1.2	6.0	1.1	4.7	0.7

Table 2: Single unit information. WT are littermates of HET mice, WT2 are littermates of HOM.

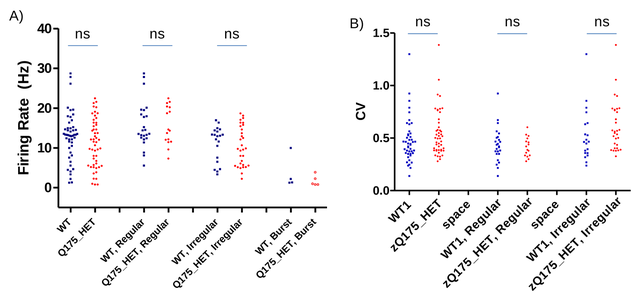
Genotype	WT1	z_Q175KI HET	WT2	z_Q175KI HOM
Regular (# of cells, %)	25, [51%]	16, [31%]	66, [55%]	70, [57%]
Irregular (# of cells, %)	20, [41%]	31, [60%]	47, [39%]	43, [35%]
Burst (# of cells, %)	4, [8%]	5, [9%]	7, [6%]	10, [8%]
Abnormal (# of cells, %)	0, [0%]	0, [0%]	0, [0%]	6, [5%]

Table 3. Cell count and percentage distribution of different firing patterns in GP.

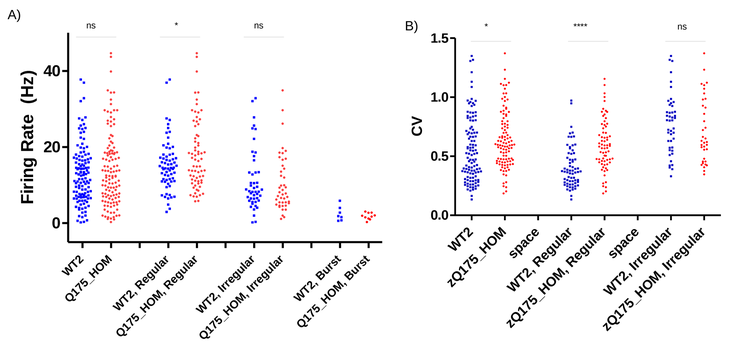
Globus Pallidus (GP)



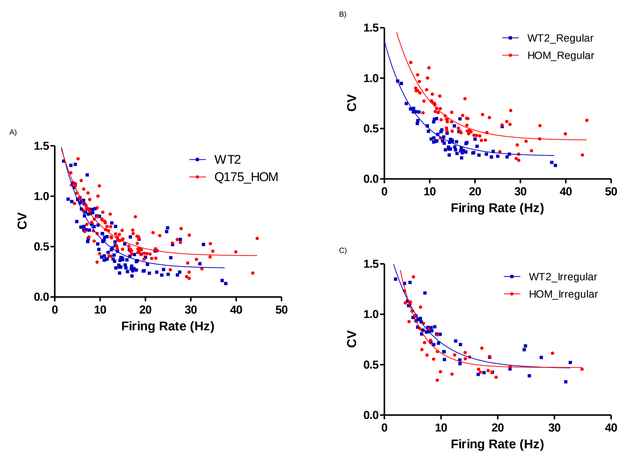
Representative examples of three different firing patterns in GP (Ni et al., 2000). The upper panels show a 5-sec window of single unit spiking activity; the lower panels are autocorrelograms.



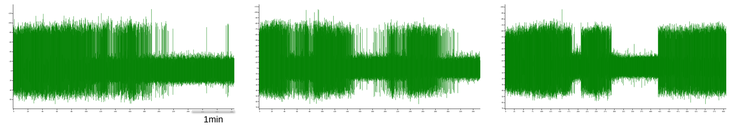
No difference in GP firing rates or inter-spike variability between WT (blue) and z_Q175 HET (red) mice at 7 months of age. A) Firing rates. B) CV - coefficient of variation. ns, not significant.



An increase in inter-spike variability and a small but significant increase in firing rates for GP regular pattern cells in z_Q175 HOM (red) mice at 7 months of age. **A)** Firing rates. **B)** CV. The increase in spike jitter in HOM mice is most salient in the regular pattern cells (middle two columns). ns, not significant; *, p < 0.05; ****, p < 0.0001.

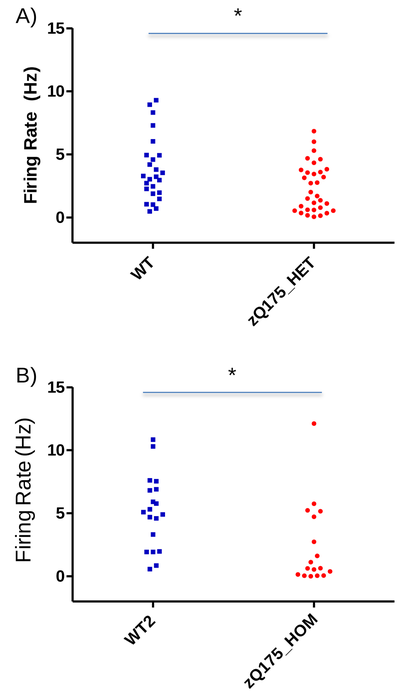


Regression analyses (nonlinear exponential fit) of the CV measure show significant increase in inter-spike variability for Q175 HOM mice. **A)** All units combined. **B)** Regular pattern cells. Most if not all the difference comes from the regular pattern cells **C)** irregular pattern cells.



Abnormal GP units with prolonged silent gaps; they were only seen in HOM mice. Six such units were identified in 3 HOM animals. To categorize these units properly, the longest silent gap must be >5% of the total recording time (>20 sec for a 5-min recording session).

Subthalamic Nucleus (STN)



Reduced single unit activity in STN for both z_Q175 HET and HOM mice (red). **A&B)** HET and HOM mice show a significant decrease in its spontaneously active single unit discharges. **C)** For HOM mice, in addition to reduced single unit activity that was recorded, there were signs of a quiet STN as indicated by lower probability of detecting spontaneously active neurons (recording success rate: WT 15/18; HOM 13/25. Fisher's exact test, p = 0.05). This suggests that the reduction in STN neuronal activity in HOM mice is likely to be underestimated. *, p < 0.05.

Summary and Discussion

SUMMARY

GLOBUS PALLIDUS (GP) :

- No difference in overall firing rate (p=0.17) or inter-spike variability (p=0.12) is detected in z_Q175KI HET mice as compared to WT mice.
- HOM mice, comparable to HET no difference in overall firing rate (p=0.29) was detected, however HOM mice showed an increased in inter-spike variability (CV: coefficient of variation, p=0.026) compared to WT.
  - The increase in CV measure for GP neurons in HOM mice is most salient for the regular pattern cells (p < 0.0001). Concurrently, regular pattern cells in HOM mice also showed a small but significant increase in firing rates compared to WT (p=0.037).
  - The regression plots of firing rate vs. CV show clearly that the biggest group separation is between regular pattern cells for HOM and WT mice.
  - Abnormal units with prolonged silent gaps were found only in HOM mice.

SUBTHALAMIC NUCLEUS (STN) :

- Reduced overall firing rate in z_Q175KI HET vs. WT (p=0.03).
- Reduced overall firing rate in z_Q175KI HOM vs. WT (p=0.01).
- For HOM mice, the extent of firing rate decrease in STN is likely underestimated. This postulate is based on the reduced probability of detecting spontaneously active single units in HOM mice (recording success rate: WT, 15/18 = 0.83; HOM, 13/25 = 0.52; Fisher's test, p=0.05).

DISCUSSION

- The lack of rate difference in GP in this animal model at 6 months of age could either stem from less sensitivity of GP neurons to IP dysfunction, or could be due to GP heterogeneity in firing rate and pattern that makes detection of abnormalities in single unit activity harder in GP than STN. Alternatively, an impairment of the hyperdirect pathway (excitatory glutamatergic cortico-STN projection) could result in a hypoactive STN with minimum impact in GP activity, at least during the early stage of HD neurodegeneration in the z_Q175 mouse model.

- The increase in inter-spike variability for GP regular pattern cells in HOM mice could result from extrinsic factors such as possible impairment of the nigrostriatal pathway (Ni et al., 2000), or intrinsically due to changes in neuronal ion channel composition such as the calcium-activated SK channel (Deister et al., 2009), or both. An increase in intrinsic spike variability could reduce the amount of information that can be encoded by spike times.