In vivo characterization of the basal ganglia Direct, Indirect, and Hyperdirect pathways in the zQ175 heterozygous knock-in mouse model of Huntington's disease

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Introduction

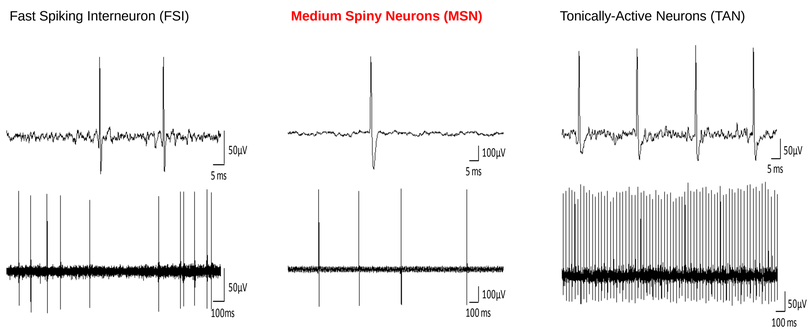
Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats in the *Huntingtin (HTT)* gene. HD patient brains reveal a devastation of the caudate-putamen and cortico-striatal-thalamo-cortical circuits are thought to be particularly affected. Choreic symptoms correlate well with D2-containing medium spiny neuron (MSN) loss, which originate the indirect pathway. MSN dysfunction has been extensively studied *in-vitro* in models of HD, but the functional consequences on the immediate downstream nuclei of the indirect pathway, the external Globus Pallidus and subthalamic nucleus (STN), remains unknown. Here we characterized a basal ganglia circuitry dysfunction relevant to the human HD condition. These functional *in-vivo* assays will allow for the assessment of novel compounds with potential disease modifying properties.

Material and Methods

Using *in vivo* electrophysiology we characterized the properties of cortical to basal ganglia transmission in early symptomatic, zQ175 heterozygous knock-in mice (Q175, 6-8 months old, both genders). zQ175 is a model mouse for early symptomatic HD. Under urethane anesthesia, responses of striatal MSNs and STN neurons were recorded as single units following stimulation of primary motor cortex (M1). I/O (stimulus-response) curves were generated by increasing the stimulus from sub threshold to one yielding a maximum response (0.2 – 1.6mA). We used the following stereotaxic coordinates for MSN recording : AP -0.1 to 0.75, LM 1.8-2.2 mm DV 1.0mm, and M1 stimulation AP 1.8 to 2.0 mm, LM 1.8-2.0 mm. DV 1.5-3.5 mm. For SN stimulation AP -3.2, LM 1.4mm.DV 4.32 mm. For STN recording. Cells originating the direct pathway were positively identified by responding to both orthodromic stimulation of the cortex and antidromic stimulation of SN.

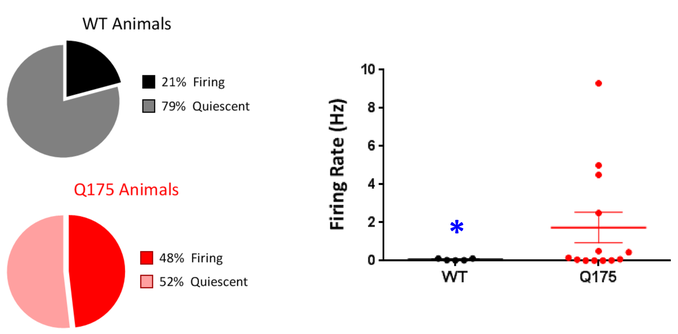
Results

Isolating MSN from other spontaneously firing neurons in Striatum



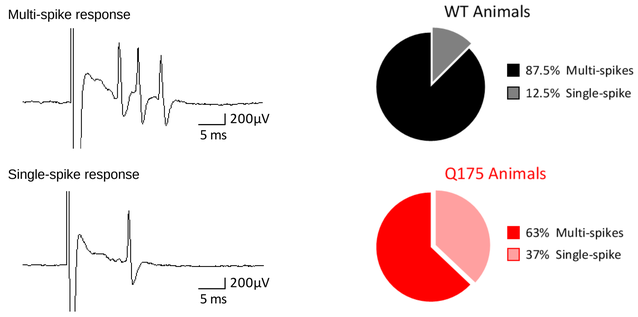
Representative traces of spontaneous single-unit activity that can be recorded in striatum. Fast spiking interneurons (FSI) are identified by the short duration spike (<1ms) and short latency. Medium spiny neurons (MSN) are identified by a long duration action potential (>1.0 ms) and long latency following M1 stimulation. Tonically active neurons (TAN) are identified by fast and regular firing and lack of response to M1 stimulation.

More spontaneous activity and higher firing rate in MSN from Q175 compared to WT



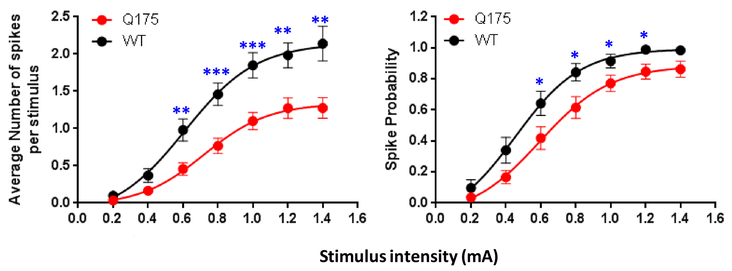
The percentage of spontaneously active MSN recorded in Q175 mice was higher than in WT mice (48%, vs. 21%). The mean firing rate of MSN recorded in Q175 mice was significantly higher than in WT mice (P<0.05, 1.74Hz vs. 0.07Hz.) WT N=24, Q175 N=27

Lower percentage of multi-spike responses in MSN from Q175 compared to WT



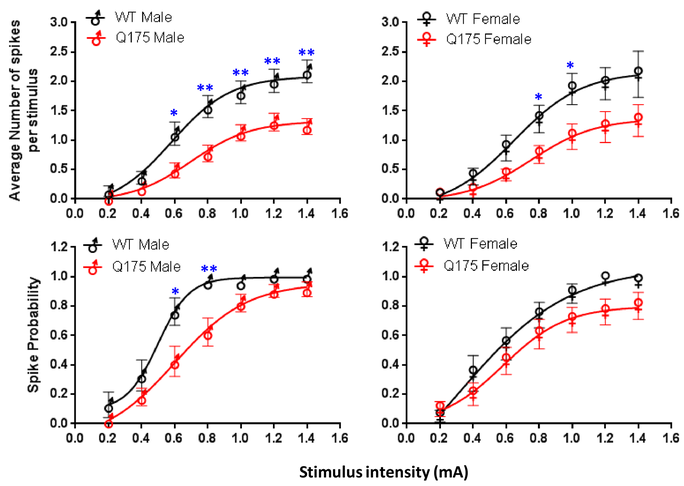
Using sub maximal stimulation intensities, 87% of MSN in WT mice showed multi-spike responses compared to 63% of MSN in Q175. Left: A representative trace of a MSN response showing multiple spikes (upper trace) and a single spike (lower trace), WT N=24, Q175 N=27

Q175 MSN are less sensitive to M1 stimulation than WT for both the number of spikes/stimulus and spike probability



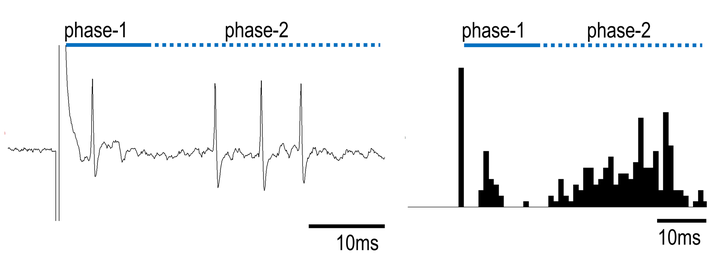
Stimulus-response plots of MSN in Q175 and WT mice. Unitary responses were quantified by averaging the number of spikes during a 2s window following repeat stimulation (30x). Data are gender mixed. WT N=24(Male=11, Female=13. Q175 N=27 (Male=16 Female=11). *P<0.05, **P<0.01, ***P<0.001

The Q175 phenotypic difference is more pronounced in male than in female mice



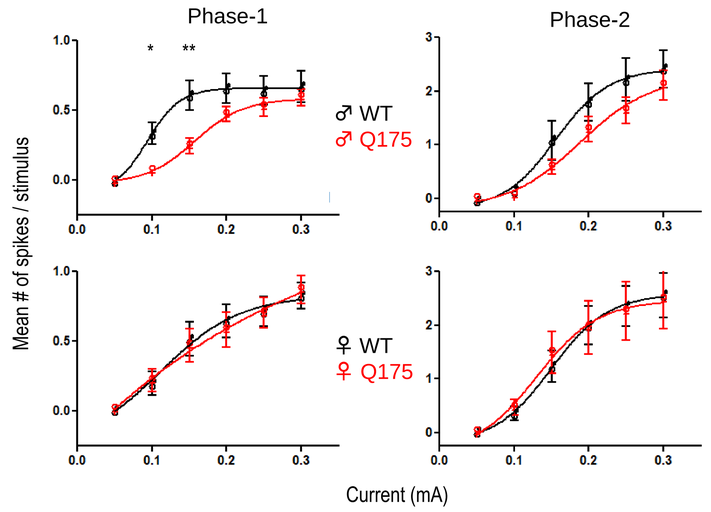
Stimulus-response plots of MSN in Q175 and WT mice separated by gender. Top: average number of spikes/stimulus. Bottom: spike probability. The phenotypic difference is present in both genders but stronger in male animals. WT N=24(Male=11, Female=13. Q175 N=27 (Male=16 Female=11). *P<0.05, **P<0.01.

STN neurons respond to M1 stimulation in 2 phases



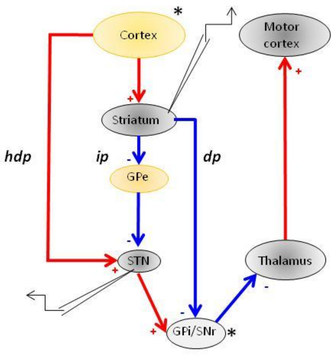
LSTN neuron responds to M1 stimulation in two phases (bars) First phase is monosynaptic and has a latency of 2-15 ms, the second phase is polysynaptic and has a latency of 16-50 ms. Right: histogram plots of responses to 50 repeat stimuli clearly separate the two response phases.

Neurons of the Subthalamic Nucleus (STN) in Q175 mice are less responsive to cortical stimulation than in WT

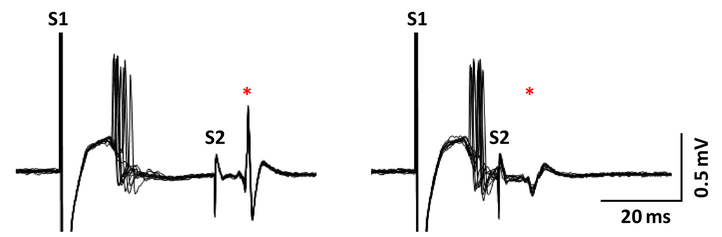


Stimulus-response plots of evoked single-unit activity in Q175 STN neurons separated by gender. Left: Data represent the first monosynaptic phase following M1 stimulation, STN neurons in Q175 male mice are less sensitive to M1 stimulation but the difference is gender specific and absent in female mice (B left bottom). Right: Same data but plotted for the second phase in the response to M1 stimulation, differences are not significant in either male or female mice. *, p<0.05, **, p<0.01.

Detecting MSNs of the direct pathway



Schematic of the recording (stars) and stimulation (electrodes) sites. Indicated are the direct (dp), indirect (ip) and hyperdirect (hdp) pathways that are involved in motor control. Stimulation of cortex leads to excitatory responses in Striatum and subthalamic nucleus (STN) via the hyperdirect pathway. Those cells in striatum that respond to antidromic stimulation of the substantia nigra reticulata (SNr) are part of the direct pathway. Gpe: external globus pallidus, Gpi internal globus pallidus.



Collision model used to positively identify MSNs involved in the direct pathway. Stimulus electrode 1 (S1) is located in M1 cortex, a second electrode (S2) is located in the SNr. **Left:** S1 stimulation activates the MSN with a 18 ms latency, subsequent stimulation with S2 antidromically activates the same neuron with very short latency. (star). **Right:** shortening the inter-stimulus interval between S1 and S2 to 20ms suppresses the antidromic activation of the MSN.

Summary and Conclusions

1) MSN in striatum and neurons of the STN showed a shifted stimulus-response curve in a mouse model of early symptomatic HD, the zQ175 Het mice. The shift is more pronounced in male *vs* female mice and indicates dysfunction of striatal and STN neurons to cortical stimulation.
2) MSN neurons in the zQ175 Het mice are spontaneously more active but respond less often with multi-spikes. This could point to a model of cortical hyperexcitability either due to altered intrinsic properties of Q175 motor neurons or disinhibition of the cortical-striatal network paired with concurrent deficits in synaptic transmission. Q175 MSN also have an increased membrane resistance as previously shown by our lab, which could contribute to the increase in spontaneous activity.
3) The phenotypic differences described for Q175 mice are more pronounced in male mice (for MSN) and absent in female mice (STN). This has implications on future experimental design when testing compounds for disease modifying properties.
4) We have implemented a method to detect MSN neurons originating the direct pathway using antidromic stimulation of the Substantia Nigra. Although the numbers of "SN positive MSN" obtained so far are low, this method holds promise to separate out and identify pathway-specific etiology of HD.

References and Acknowledgments

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