Comparison of intrinsic membrane and synaptic properties of striatal medium spiny projection neurons from both D1-GFP and D2-GFP x Q175 mouse models of Huntington's disease

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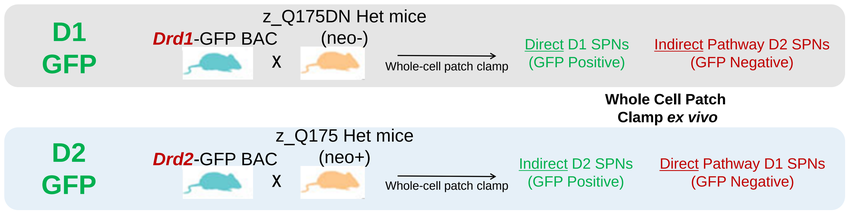
Abstract

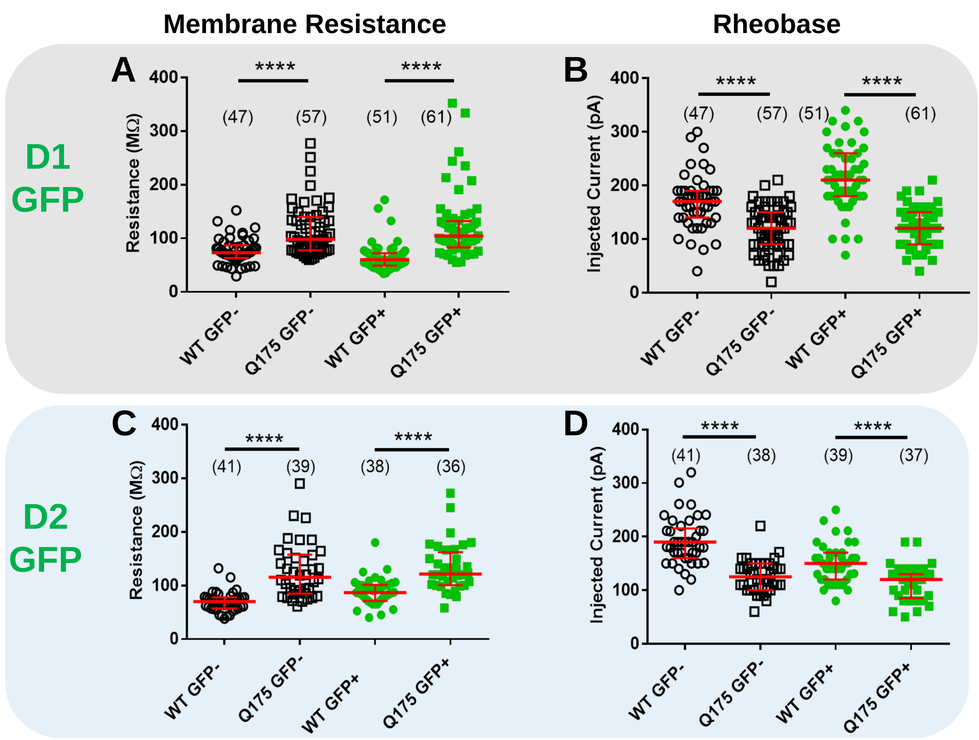
Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from an expanded number of CAG repeats in the huntingtin (Htt) gene. HD patients exhibit both cognitive and affective symptoms, as well as uncontrolled movements (chorea) which are thought to reflect pathological changes in striatal medium spiny projection neurons (SPN) from both the direct (dSPN) and indirect (iSPN) pathways. The Q175 knock-in mouse model of HD has been useful in revealing functional alterations in intrinsic membrane and synaptic properties of striatal SPNs. Q175 mice have been successfully crossed with mice expressing GFP under control of the promoters of either the dopamine D1 or D2 receptor genes. To the extent that 1) D1- and D2- driven GFP expression putatively labels dSPNs and iSPNs, respectively, and 2) that unlabeled SPNs represent the complementary pathway, comparisons of GFP+ and GFP- cells in either model should be equally valid. However, testing this assumption by directly comparing independent datasets obtained from these two mouse lines has not yet been reported. In this study, we performed whole cell patch-clamp recordings from GFP+ and GFP- striatal SPNs in brain slices from 6-month old WT and heterozygous Q175 (Q175) mice in D1-GFP and D2-GFP lines of Q175 mice. In cells from WT mice, membrane resistance values of putative dSPNs and iSPNs in the D1 line (i.e. GFP+ and GFP- cells, respectively) were nearly identical to those of the cognate cell types in the D2 line. Moreover, both dSPNs and iSPNs in Q175 mice from each line showed markedly and comparably elevated membrane resistance relative to WT controls. Similarly, both cell types in Q175 mice from each line exhibited significantly reduced rheobase compared to WT controls. Resting membrane potential and action potential properties remained uniform between genotypes and cell types in both lines. The frequency of miniature excitatory synaptic currents (mEPSCs) in iSPNs from Q175 mice was selectively decreased relative to that seen in WT mice in both D1-GFP and D2-GFP lines. The average mEPSC amplitude was unchanged across cell types in both lines and no genotypic differences were observed. These symmetrical findings help confirm that both D1-GFP and D2-GFP lines of Q175 mice can be used to assess the electrophysiological properties of putative direct and indirect pathway SPNs.

Methods

Electrophysiology: Brain slices were prepared from 6-month WT and heterozygous Q175 animals (gender-balanced) from Drd1-eGFP (D1-GFP) x Q175 or Drd2-eGFP (D2-GFP) x Q175 mouse breedings. The brain was rapidly removed and cooled in ice-cold oxygenated sucrose-ACSF (in mM): Sucrose 220; KCl 2.5; $CaCl_2$ 0.5, MgSO4 3, NaH2PO4 1.2, NaHCO3 26, glucose 5. Para-horizontal slices (300 micron) were prepared in ice-cold oxygenated ACSF then warmed to 36°C for 30min, allowed to cool to room temperature, and transferred as needed to a submerged slice chamber mounted on the stage of an upright microscope, perfused at 2 ml/min with oxygenated normal ACSF (in mM): NaCl 124; KCl 3.5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO4 1.2, $NaHCO_3$ 26, glucose 11. All recordings were made from GFP-positive neurons. For intrinsic properties, whole-cell patch clamp recordings were made under IR/DIC optics from visually identified SPNs with pipettes (4-6 MΩ) filled with potassium-based internal solution containing (in mM): K-gluconate 105, KCl 30, EGTA 0.3, HEPES 10, $MgCl_2$ 4, Na_2ATP 4, Na_3GTP 0.3, Tris-phosphocreatine 10, pH adjusted to 7.2. For mEPSC recording, the pipettes were filled with a cesium-based internal solution containing (in mM): Cs-methanesulfonate 110, EGTA 10, HEPES 10, TEA-Cl 10, NaCl 10, $CaCl_2$ 1, Mg-ATP 5, Na_2GTP 0.5, QX314-Cl 5, pH 7.2. Active and passive membrane properties were measured at room temperature either in current clamp at resting membrane potential or in voltage clamp at a holding potential of -80 mV using pClamp 10 software. Rheobase (R_h) was determined by applying 300 ms depolarizing current pulses of increasing amplitude. Membrane resistance (R_m) was determined with +5 mV pulses using the automated "Membrane Test" function built into the Clampex software. Miniature excitatory postsynaptic currents (mEPSCs) were isolated by including 0.5 μM TTX and 40 μM picrotoxin in the ACSF solution, recordings were filtered at 1 kHz and collected continuously for 5 minutes at room temperature from each cell at a holding potential of -80 mV. Data was excluded if either access resistance or input resistance changed by more than 30% from baseline.

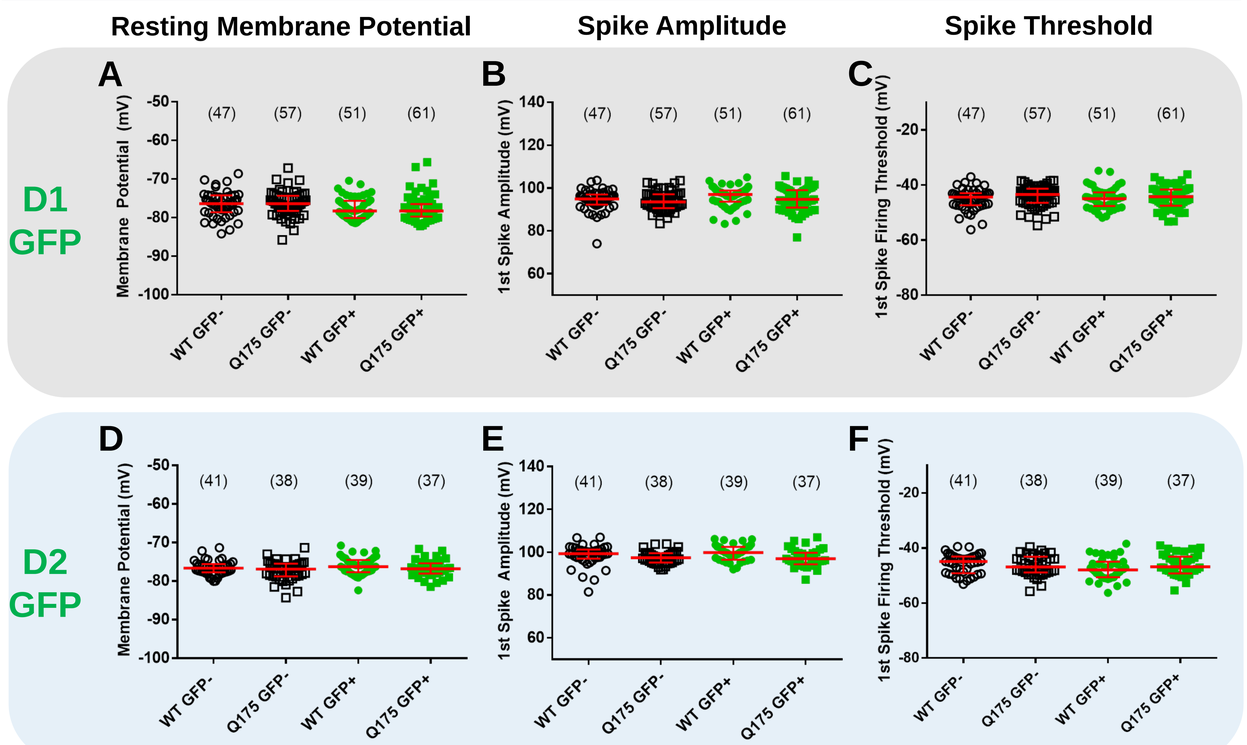


Membrane resistance and rheobase changes correspond to a hyperexcitable phenotype in both dSPNs and iSPNs of 6-month Q175 het mice



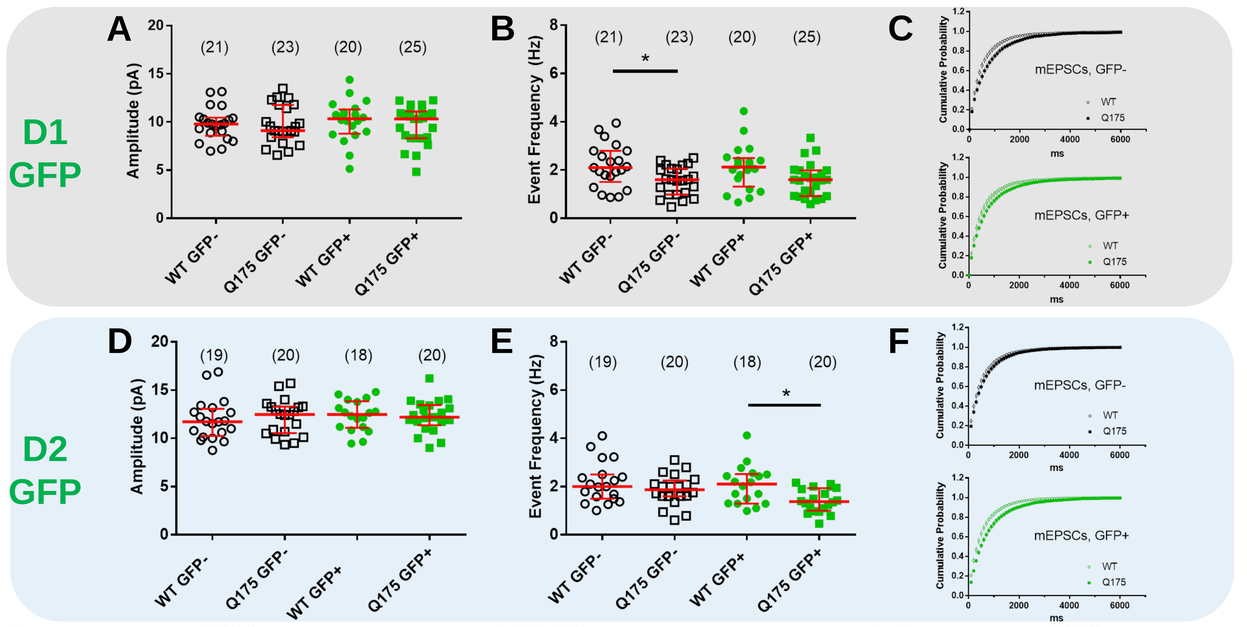
Membrane resistance (R_m; **A** and **C**) is significantly elevated in Q175 dSPNs and iSPNs from both D1-GFP and D2-GFP mouse lines. Rheobase (R_h; values obtained from the cells in A and C) is concurrently significantly reduced in Q175 dSPNs and iSPNs from both D1-GFP and D2-GFP mice (**B** and **D**). Data is presented as group medians +/- interquartile ranges, number of cells per group shown in parentheses. ****p<0.0001, Kruskal-Wallis 1-way ANOVA with Dunn's post-hoc test for multiple comparisons.

Resting membrane potential and AP properties are not affected in 6-month Q175 mice



Other measures of intrinsic excitability remain unaffected in the HD phenotype. Resting membrane potential was not significantly different between WT and Q175 dSPNs and iSPNs from D1-GFP (**A**) or D2-GFP (**D**) mouse lines. Measured action potential properties (spike amplitude, **B** and **E**) and spike threshold (**C** and **F**) are also unchanged in both dSPNs and iSPNs of Q175 mice of both lines. Data is presented as group medians +/- interquartile ranges, number of cells per group in parentheses. No significant differences observed, Kruskal-Wallis 1-way ANOVA with Dunn's post-hoc test for multiple comparisons.

Glutamatergic transmission is impaired in iSPNs of 6-month Q175 het mice



The amplitude of mEPSC events is unaffected in Q175 SPNs regardless of mouse line (**A, D**). However mEPSC frequency is reduced in Q175 iSPNs, but not dSPNs, a difference observed in both D1-GFP (**B**) and D2-GFP (**E**) mouse lines. Inter-event intervals were calculated for recordings made from D1-GFP (**C**) and D2-GFP (**F**) mouse lines using mEPSC frequency data of cells in B and E to show the distribution of all events. Data is presented as group medians +/- interquartile ranges, number of cells per group in parentheses. *p<0.05, Kruskal-Wallis 1-way ANOVA with Dunn's post-hoc test for multiple comparisons.

Conclusions

1) SPN hyperexcitability in the Q175 HD model is evident at 6 months of age, and is observed in dSPNs and iSPNs of both D1-GFP and D2-GFP Q175 mice. The physiological basis for this difference remains under investigation, and occurs in the context of other measures of intrinsic excitability remaining unchanged in the HD phenotype.

2) Decreased mEPSC frequency, potentially indicative of presynaptic impairments in glutamatergic transmission, was observed in iSPNs from both D1-GFP and D2-GFP Q175 HD mice

3) Parallel observations in both D1-GFP and D2-GFP Q175 mouse lines have identified robust measures that correlate with HD pathogenesis, making the Q175 Het mouse model a powerful tool for *i)* investigating HD pathogenesis and progression in both direct and indirect pathways, and *ii)* preclinical testing to develop HD-modifying and -management therapies

Acknowledgments and disclosures

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