Synaptic Alterations in Direct and Indirect Spiny Projection Neurons in the Q175 Mouse Model of Huntington's Disease

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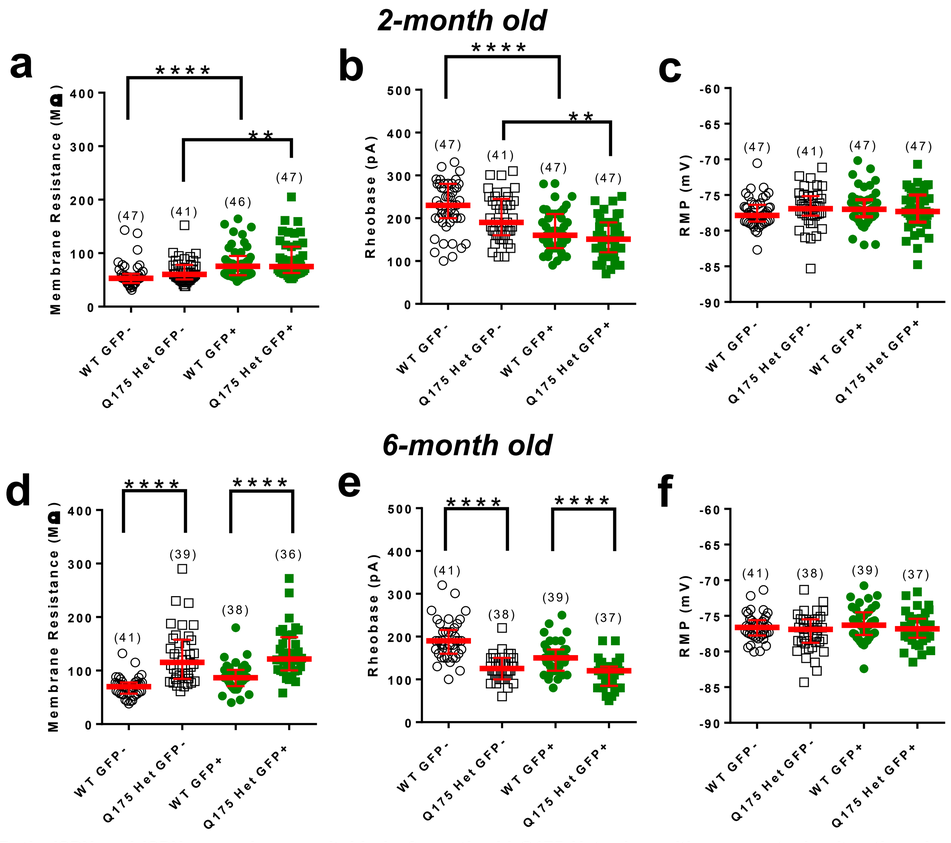
Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from an extended number of CAG repeats in the *Huntingtin (Htt)* gene. HD comprises cognitive and affective symptoms, as well as uncontrolled movement (chorea), which have been hypothesized to arise from a preferential vulnerability in the indirect pathway spiny projection neurons (iSPNs) preceding dysfunction of direct pathway (dSPNs) in the striatum (Zuccato, C., et al, 2010). In order to characterize this possible imbalance, we performed in vitro brain slice whole patch-clamp recordings from the Q175 heterozygous knock-in HD mouse model expressing GFP under the control of the D2 receptor promoter to identify dSPNs (GFP-negative) and iSPNs (GFP-positive). We asked to what extent alterations in intrinsic and synaptic properties in Q175 mice were selectively affected in each SPN subtype. Our experiments analyzed 1) intrinsic membrane properties in dSPNs and iSPNs from 2 and 6-month old Q175 mice; 2) spontaneous miniature excitatory synaptic currents (mEPSCs) in dSPNs and iSPNs from 2 and 6-month old Q175 mice; and 3) corticostriatal long-term potentiation (LTP) in dSPN and iSPNs using a Hebbian induction paradigm. Our findings demonstrate that although both dSPN and iSPNs exhibit a hyperexcitability phenotype, only iSPNs display decreased spontaneous glutamatergic transmission in 6-month old Q175 Het mice. Interestingly, we also demonstrate a selective impairment in corticostriatal LTP in dSPNs in 6-month old Q175 Het mice, whereas LTP in iSPNs was unaltered. These findings suggest that HD progression is not equally expressed in dSPNs and iSPNs in the striatum, and further analysis is necessary to understand how the emergence of these selective SPN alterations is related to HD progression.

Methods

Electrophysiology: Brain slices were prepared from 2 or 6-month WT D2 GFP and Q175 Het D2 GFP mice (gender-balanced). The brain was rapidly removed and cooled in ice-cold oxygenated sucrose-ACSF (in mM): Sucrose 220; KCl 2.5; CaCl2 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 36ºC 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, MgSO4 1.2, NaH_2PO4 1.2, NaHCO3 26, glucose 11. For intrinsic properties and LTP experiments, whole-cell patch clamp recordings were made under IR/DIC optics from visually identified SPNs with pipettes (4-6MΩ) filled with potassium-based internal solution containing (in mM): K-gluconate 105, KCl 30, EGTA 0.3, HEPES10, $MgCl_2$ 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-methansulfonate 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 RMP or in voltage clamp at a holding potential of -80mV using pClamp 10 software. Rheobase (R_h) was determined by applying 300ms depolarizing current pulses of increasing amplitude. Membrane resistance (R_m) was determined with +5mV pulses using the automated "Membrane Test" function built into the Clampex software. Miniature excistory postsynaptic currents (mEPSCs) were isolated by including 0.5uM TTX and 40μM picrotoxin in the ACSF solution, recordings were filtered at 1KHz and collected continuously for 5 minutes at room temperature from each cell at a holding potential of -80mV. For LTP experiments, slices were superfused with oxygenated ACSF (in mM): NaCl 125; KCl 2.5, $CaCl_2$ 2, MgSO4 1, NaH_2PO4 1.25, NaHCO3 26, glucose 11, Na pyruvate 10 μM. Excitatory postsynaptic currents (EPSCs) between 50-200 pA in amplitude were evoked at a recording temperature of 30-32ºC by stimulating cortical axons (400 μsec stimulus duration) using a matrix stimulating electrode placed between layers 5-6 of the cortex. Hebbian LTP protocol consisted of 4 trains of 100 Hz stimulation coincident with postsynaptic depolarization from -80 to 0 mV for 1 second, with an inter-train interval of 10 seconds. Baseline and post-LTP values were collected at 0.1 Hz, and post-LTP changes in EPSC amplitude were normalized to the averaged EPSC collected in the last 3 minutes of baseline recording prior to LTP induction. Data was excluded if either access resistance or input resistance changed by more than 30% from baseline (Fino, E. et al, 2005).

Hyperexcitability in Q175 Het Mice Emerge in Parallel in dSPNs and iSPNs



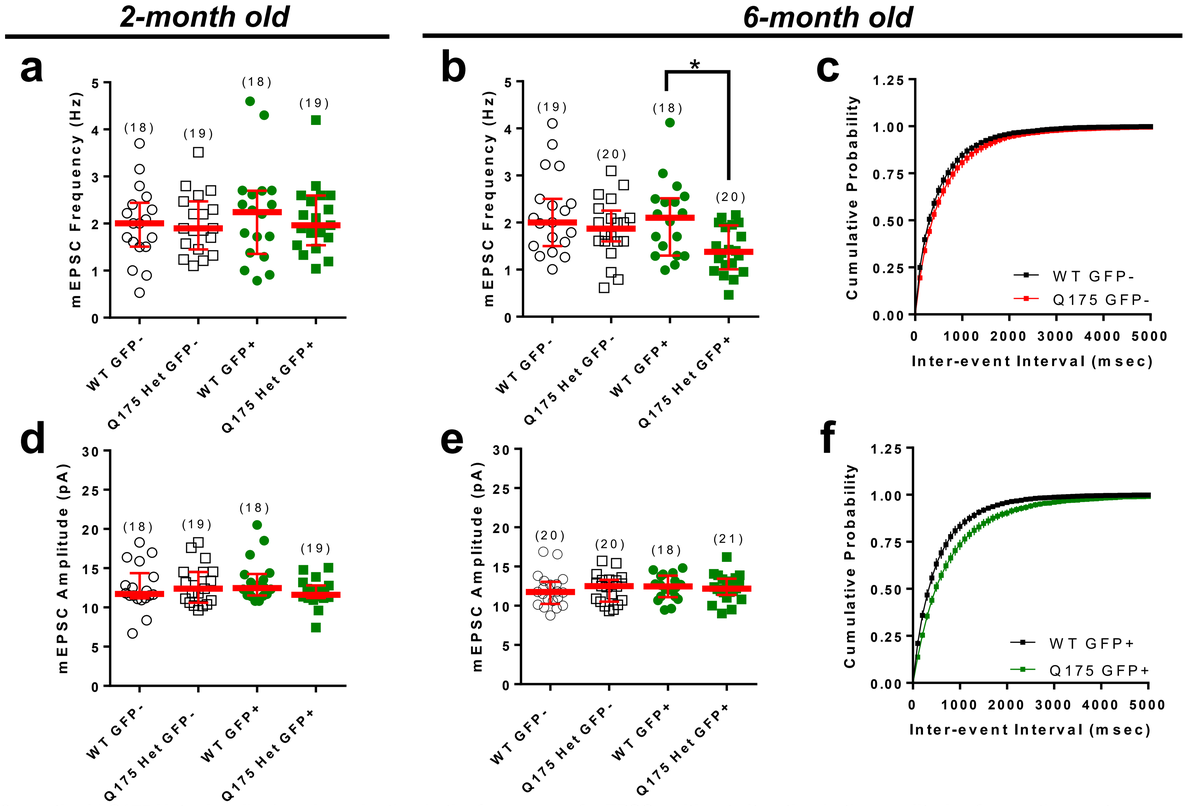
Both dSPN and iSPNs were hyperexcitable in 6-month old Q175 Het mice, with no genotypic alterations in 2-month old in either cell type.

[a-c] iSPNs (GFP+) were more excitable than dSPNs (GFP-) in 2-month old mice for both genotypes, with iSPNs displaying higher membrane resistances (panel a) and lower rheobase currents (panel b). Resting membrane potential (RMP) (panel c) was not different between iSPNs and dSPNs in either genotype. No genotypic alterations were detected in 2-month old mice for membrane resistance and rheobase.

[d-f] Both iSPNs and dSPNs displayed elevated membrane resistance (panel d) and reduced rheobase current (panel e) in 6-month old Q175 Het mice. Resting membrane potential was unaltered across genotype and cell types in 6-month old mice. (panel f)

Data presented as scatter-dot plots displaying medians and interquartile ranges. The number of SPNs per cell type and genotype is built-in to the graphs in parenthesis. Statistical analysis was performed using Kruskal-Wallis One-way ANOVA with Dunn's post-hoc test for multiple comparisons (p<0.01**; p<0.0001****). ,

Decreased Glutamatergic Transmission in iSPNs from 6-month old Q175 Mice



iSPNs from 6-month old Q175 Het mice displayed a reduction in the frequency of mEPSCs without alteration in the amplitude of synaptic events.

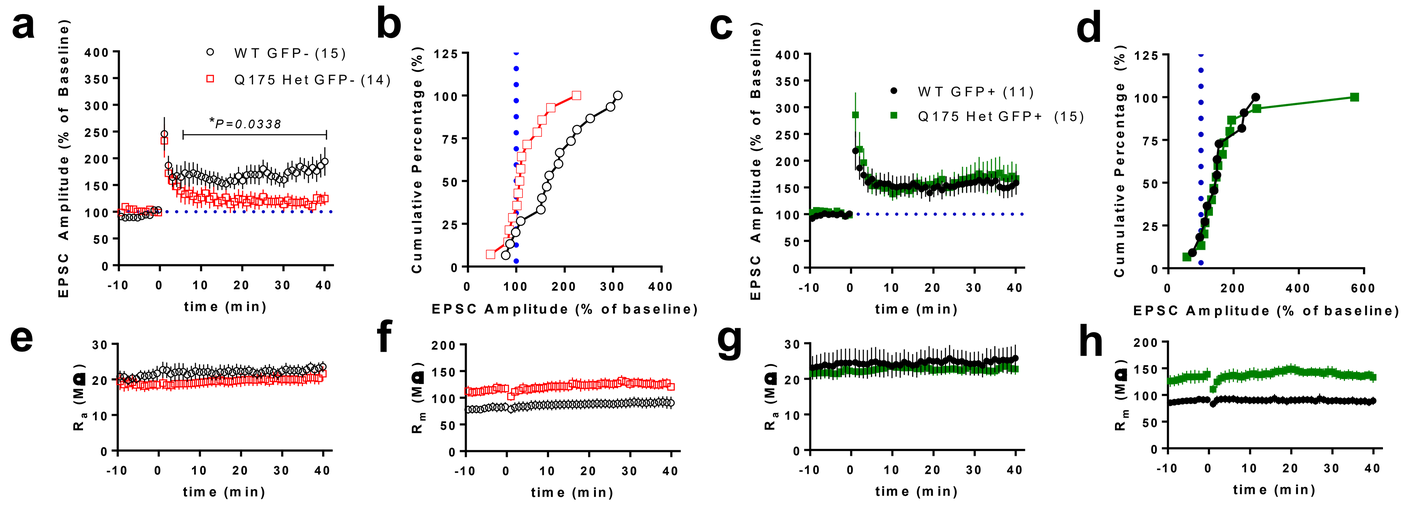
[a and b] Frequency of mEPSCs was unaltered across cell type and genotypes in 2-month old Q175 Het mice (panel a). In 6-month old mice, a reduction in the frequency of mEPSCs was detected in iSPNs from Q175 Het mice, while frequency of mEPSCs was not altered in dSPNs (panel b).

[d and e] Amplitude of mEPSCs was not affected in either cell type and genotype in 2-month old (panel d) and 6-month old mice (panel e).

[c and f] Cumulative probability for inter-event interval did not shift in dSPNs (panel c), whereas iSPNs displayed a rightward shift in the cumulative probability for inter-event interval (panel f)

For panels a, b, d, and e, data presented as scatter-dot plots displaying medians and interquartile ranges. The number of SPNs per cell type and genotype is built-in to the graphs in parenthesis. Statistical analysis was performed using Kruskal-Wallis One-way ANOVA with Dunn's post-hoc test for multiple comparisons (p<0.05*)

Corticostriatal Hebbian LTP was Impaired in dSPNs from 6-month old Q175 Mice



Hebbian LTP in corticostriatal synapses was impaired in dSPNs from 6-month old Q175 Het mice, whereas LTP expression in the iSPN pathway was unaffected.

[a-d] LTP was decreased in dSPNs from Q175 Het mice (panel a and b), while LTP in iSPNs was unaltered (panel c and d). Each data point in the cumulative percentage plot (b and d) was calculated by averaging post-LTP baseline EPSCs between 30-40 min post LTP induction for each cell recorded.

[e-h] Stable access resistance (Ra) (panel e and g) and membrane resistance (Rm) (panel f and h) were utilized as inclusion criteria as part of the LTP analysis.

Data presented as mean and standard error of the mean (SEM). The number of SPNs per cell type and genotype is built-in to the graphs in parenthesis. Genotypic differences in dSPNs was detected using Two-way ANOVA to analyze mean differences at intervals 5-40 min, 10-40 min, 20-40 min, and 30-40 min post LTP induction.

Conclusions

1. SPN hyperexcitability in the Q175 HD model emerge in parallel in dSPNs and iSPNs
2. Decreased glutamatergic transmission was detected in iSPNs from 6-month old Q175 HD mice
3. A corticostriatal LTP deficit was identified in dSPNs from 6-month old Q175 HD mice, whereas iSPNs showed no differences when LTP was induced using a Hebbian induction paradigm
4. The emergence of changes in intrinsic and synaptic properties in the Q175 HD mouse model is more complex than just a preferential vulnerability to the iSPN pathway preceding alterations in dSPN pathway. Further studies are necessary to understand the cellular adaptations occurring in both SPN cell types from Q175 HD mice, and how they are related to the progression of HD.

Acknowledgments and disclosures

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