Differential impairments of cortico-striatal transmission onto direct and indirect pathway SPNs from Q175 mice

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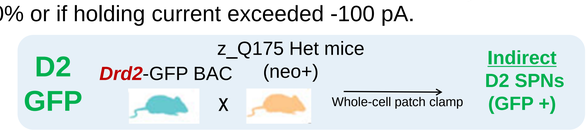
Abstract

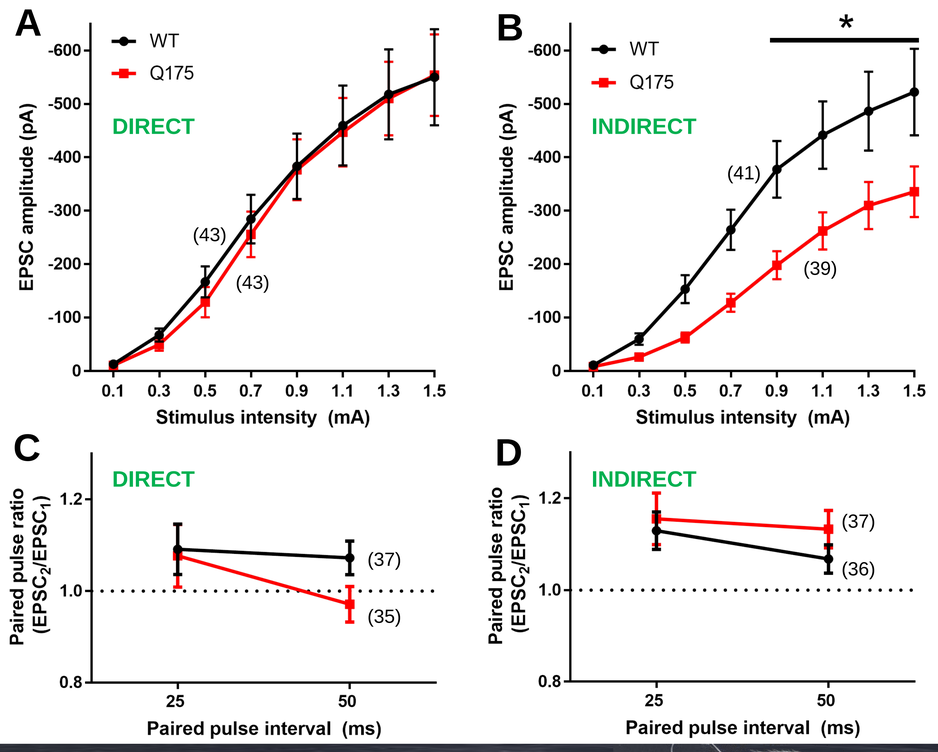
Changes in corticostriatal neurotransmission have been identified as an early pathophysiological event observed in Huntington's disease (HD), and model organisms have been a critical tool in furthering our understanding of these disease-driven processes. Examination of the Q175 mouse model of HD has uncovered changes in intrinsic membrane properties and excitability of both direct (D1 receptor-expressing) and indirect (D2 receptor-expressing) pathway medium spiny striatal projection neurons (SPNs). We have extended this work by probing the function of the direct and indirect corticostriatal pathways, using electrical stimulation of brain slices obtained from lines of Q175 mice expressing GFP in either D1-expressing (D1-GFP) or D2-expressing (D2-GFP) SPNs. We used three independent measures to assess excitatory presynaptic function: namely i) input-output relationship; ii) the relative size of the readily releasable pool (RRP) of synaptic vesicles; and iii) evoked Sr^{2+}-mediated asynchronous release from cortical afferents. We found that glutamatergic corticostriatal transmission was decreased in indirect pathway (D2-GFP) SPNs, but was similar to WT in the direct (D1) pathway at the same age. This impairment in indirect pathway transmission was observed at several stimulus intensities. Paired pulse ratios at 25 ms and 50 ms intervals were unaffected in Q175het animals, suggesting that release probability was largely unaffected. Estimates of RRP size from a single stimulus train suggested a significant reduction in the number of vesicles available for release in indirect pathway corticostriatal terminals, while direct pathway terminals in Q175het slices were not significantly different from WT. We also observed a decrease in the frequency of asynchronous EPSC events following stimulation of indirect pathway Q175het corticostriatal afferents compared to WT, while no such deficit was observed in the direct pathway. These data are consistent with a corticostriatal deficit in neurotransmission in the indirect pathway of 6-month old Q175het mice, suggesting a potential pathway-specific mechanism of corticostriatal dysfunction.

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, $MgSO_4$ 3, NaH_2PO_4 1.2, $NaHCO_3$ 26, glucose 5. Para-horizontal slices (300 μm) were prepared in ice-cold oxygenated ACSF then warmed to 36ºC for 30 min, allowed to cool to room temperature, and after 1 h of recovery transferred as needed to a submerged slice chamber mounted on the stage of an upright microscope and 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, osmolarity ~300 mOsm, pH 7.3-7.4. All recordings were made from GFP-positive neurons. Whole-cell patch-clamp recordings from SPNs from both lines were performed in the dorsal striatum at room temperature using fire-polished pipettes (5-7 MΩ) 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, QX-314 5, pH adjusted to 7.2 with KOH. ACSF was supplemented with 40 μM picrotoxin. Glutamatergic EPSCs (V_h= -80 mV) were evoked with a matrix bipolar stimulating electrode positioned in or near corpus callosum. Input-output curves were collected over a fixed range of increasing stimulus intensities, n=3 sweeps/ stimulus intensity (0.1 to 1.5 mA, 0.2 mA increments), with total stimulus duration of 0.4 ms (bipolar pulse), delivered at a frequency of 0.067 Hz. Paired-pulse experiments were performed by delivering two pulses separated by inter-stimulus intervals of 25 and 50 ms. For estimates of the RRP size, whole cell recordings were performed as described above for I-O curves. A single train of 40 biphasic square wave stimuli were delivered at 20 Hz at an intensity that evoked 50% of the I-O maximum response. For RRP analysis, cumulative sum plots of raw EPSC amplitudes were plotted for each cell, and a linear regression was fit to the last 20 points. From these the y-intercepts were calculated and served as the estimate of the size of RRP (# vesicles x quantal content). For asynchronous release experiments, whole-cell patch clamp recordings were made from SPNs with pipettes (5-7 MΩ) 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. ACSF was supplemented with 40 μM picrotoxin and 50 μM D-AP5. Synchronous EPSCs were evoked with electrical stimulation at 0.05 Hz, with stimulus intensity adjusted to evoke responses with amplitudes ranging between 300-400 pA. After obtaining stable baseline, ACSF was switched to a Ca2+-free ACSF containing 6 mM strontium chloride ($SrCl_2$) to desynchronize evoked vesicular release. Collection of asynchronous events began once the synchronous evoked EPSC response reached a steady state. Events were analyzed within a 400ms window, beginning 50 ms after the stimulus artifact. The number of sweeps collected per cell was targeted to ≥ 100 aEPSCs for frequency analysis. Contamination by mEPSCs were estimated (in the ACSF containing strontium) and corrected by measuring spontaneous events in a 400 ms window immediately prior to the stimulus. Data was discarded if R_a changed by more than 30% or if holding current exceeded -100 pA.

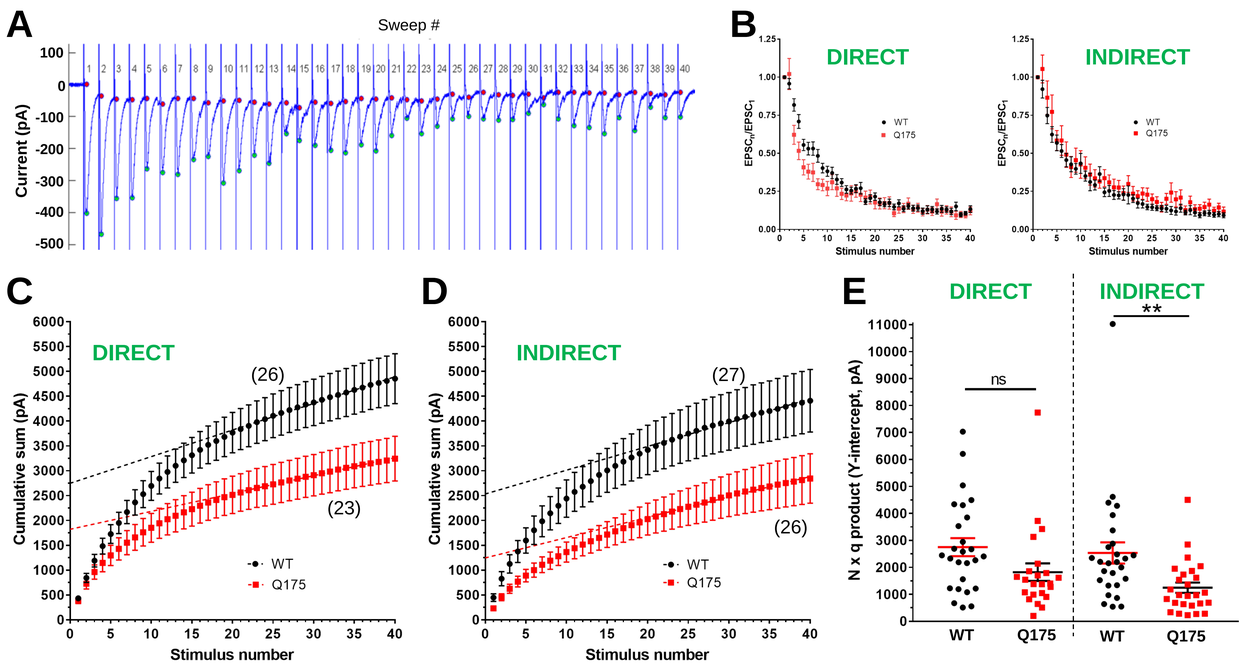


Glutamatergic corticostriatal transmission was decreased in indirect pathway SPNS of Q175het mice



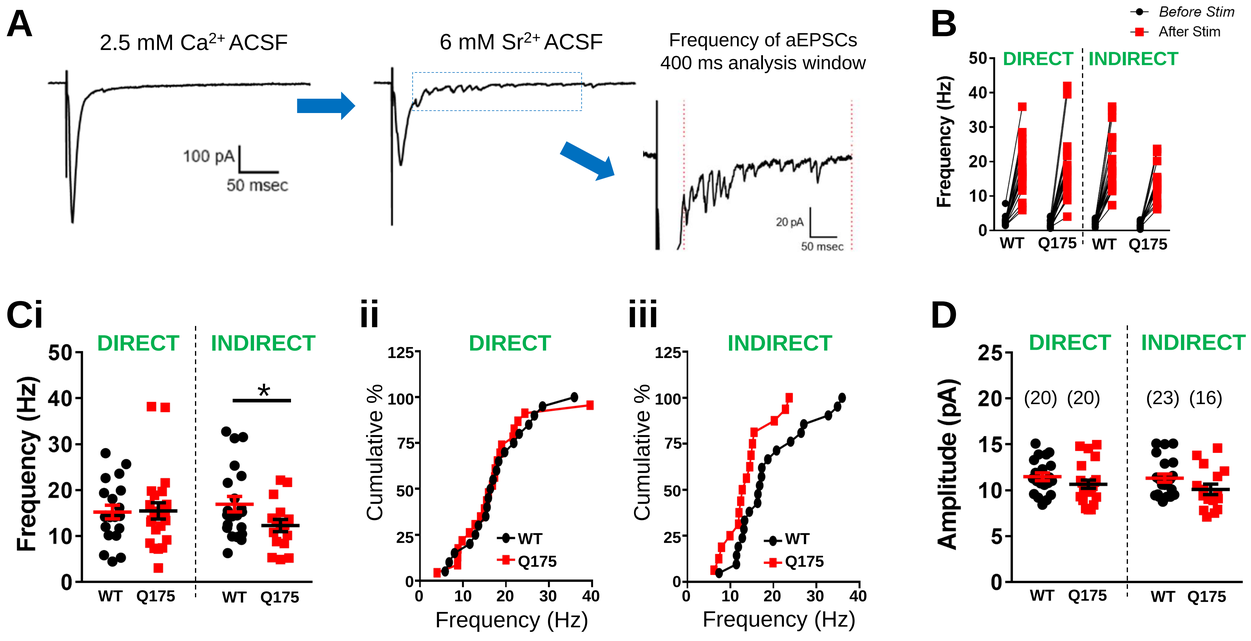
Evoked corticostriatal EPSCs in D1 receptor-containing (direct pathway) and D2 receptor-containing (indirect pathway) SPNs. A and **B)** input-output curves obtained from stimulation of cortical afferents to SPNs of D1-GFP (A) or D2-GFP (B) WT or Q175het mice. Values shown are mean +/- SEM, number of cells (n) indicated in parentheses. *p<0.05, significant effect of genotype for stimulus intensities as indicated by 2-way repeated measures (RM) ANOVA with Bonferroni's multiple comparisons test. No significant differences observed in input-output relationship of direct pathway SPNs. **C** and **D)** Paired pulse ratios obtained from stimulation of cortical afferents to SPNs of D1-GFP (C) or D2-GFP (D) WT or Q175het mice. Values shown are mean +/- SEM, number of cells (n) indicated in parentheses. No significant differences in PPR were observed.

Reduced RRP size in indirect pathway corticostriatal terminals of Q175het mice



Estimations of the size of the RRP at direct and indirect pathway corticostriatal synapses of WT and Q175het mice. A) Example trace showing EPSCs in response to a train of 40 pulses delivered at 20 Hz. Initiation (red dots) and EPSC peak (green dots) as indicated, to calculate EPSC amplitude. **B)** Synaptic depression plots obtained from train stimulation of cortical inputs to direct (left) and indirect (right) pathway SPNs. **C and D)** Cumulative EPSC amplitude plots generated from responses of D1-GFP (Direct, C) or D2-GFP (indirect, D) WT or Q175het SPNS to train stimulation. Dashed lines indicate linear regression slope fit to last 20 values. Data expressed in B, C and D are expressed as group means +/- SEM, number of cells (n) in parentheses in C and D. **E)** Y-intercept values generated from cumulative EPSC plots as in C and D, indicating the product of the total number of vesicles available for release (N) and the quantal content (q). Each data point represents one cell, group means +/- SEM as indicated. **p<0.01 by one-way ANOVA, Bonferroni post-hoc test.

Corticostriatal aEPSC frequency is reduced in indirect pathway SPNs of Q175het mice



Asynchronous release is impaired in the indirect pathway. A) Asynchronous release allows biasing of synaptic activity towards corticostriatal transmission. Experimental scheme showing how synchronous EPSC breaks down with Sr^{2+} substitution and aEPSCs appear. **B**) Frequency plots demonstrating the enhanced frequency of 'miniature' synaptic events following stimulation under conditions of asynchronous release. **Ci)** Group data for frequency of aEPSCs observed, following stimulation, in direct or indirect pathway SPNs. *p<0.05, unpaired T-test (2-tailed). Means +/- SEM, number of cells (n) as indicated in panel D. **Cii and Ciii)** Data as represented in Ci, expressed as cumulative probability plots. **D)** aEPSC amplitudes observed in direct or indirect pathway SPNS following stimulation. Data expressed as means +/- SEM.

Conclusions

1) **We observed a deficit in 6-month old Q175het mice targeting indirect pathway SPNS, using three independent measures of corticostriatal function:**
   - **A significant impairment in the input-output relationship**
   - **A significant decrease in the observed size of the RRP of synaptic vesicles in corticostriatal terminals**
   - **A significant reduction in corticostriatal aEPSC frequency, consistent with a reduction in the number of corticostriatal synapses**
2) **These deficits were not observed in recordings targeting direct pathway SPNs**
3) **The corticostriatal dysfunction associated with HD pathophysiology may initially target indirect pathway, D2 receptor-expressing SPNs**

Acknowledgments and disclosures

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