Dual single unit recording in Globus Pallidus (GP) and Subthalamic nucleus (STN) in anesthetized rats: a comparison of GP and STN firing patterns in wild type and BACHD transgenic rats

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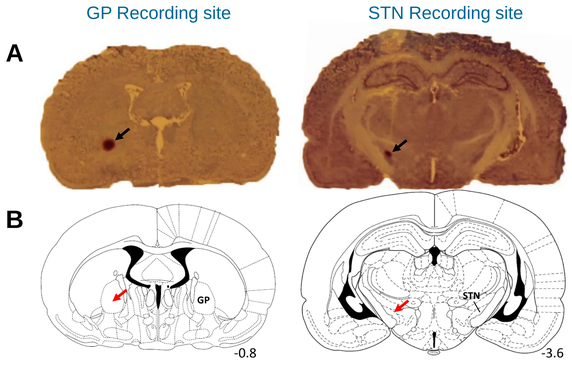
Introduction

Chorea in Huntington's disease (HD) patients has been proposed to arise predominantly from indirect pathway (IP) dysfunction [1-4] and D2-expressing striatal medium spiny neurons (MSN) giving rise to IP projections appear more vulnerable to mutant huntingtin (mHtt) insult [4, 5]. Consistent with this, BACHD transgenic and Q175 knock in mouse models demonstrate age-dependent alterations in firing activity of downstream nuclei within the IP; namely an increase in mean firing rate of D2 MSN-innervated globus pallidus (GP) neurons and a corresponding decrease in the mean firing rate of pallidal-innervated subthalamic nucleus (STN) neurons *in vitro* and *in vivo* (D.J Surmeier and J.Tepper, CHDI personal communication). We have tested this finding in a full length BAC transgenic Huntington's disease rat model, by recording intrinsic firing rates from GP and STN nuclei *in vivo* at a behaviorally symptomatic age.

Material and Methods

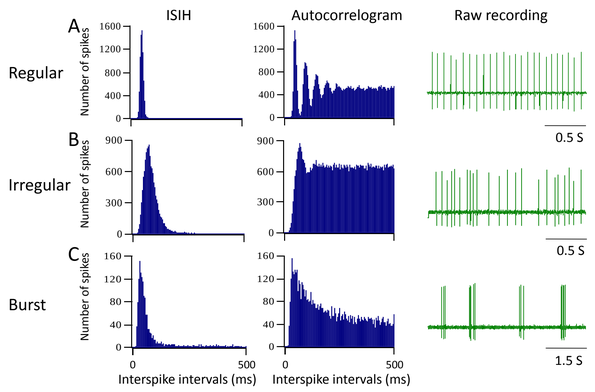
Male BACHD rats and their WT littermate rats (8-10 months old) were anesthetized with Urethane (initial dose at 1.5 g/kg, i.p.) and surgically implanted with two catheters, one in the femoral vein and one in the femoral artery, for drug administration and blood sampling. The animal was mounted on a stereotaxic apparatus (David Kopf instrument) in a flat skull position. Core temperature was maintained at 37^{0}C by a heating pad. To gain access to the STN and GP recording sites, the micro-electrodes were advanced by a dual single axis in vivo micromanipulator system (Scientifica, United Kingdom) mounted on two kopf stereotaxic holders. Two burr holes were drilled on the skull. One with stereotaxic coordinate of AP -0.8 to 1.3 mm, Lateral 3-4 mm (GP recording, with a 10 degree angle) and the other at AP -3.2 to -3.9, Lateral 2.1-2.7 mm (STN recording). The recording electrodes were advanced to reach the target coordinates of the GP (5.5-5.7 mm below the brain surface) and STN (6.8 to 7.5 mm below the dorsal surface).
At the end of each experiment, the recording sites were marked by the microiontophoresis of Pontamine Skyblue (-20 µA, 15 min). Each rat was given an overdose of Urethane. The brain was immediately removed and fixed in 4% Para formaldehyde for 4 hrs and placed in phosphate buffered saline (PBS) with 20% sucrose over night. The brains then were frozen and cut into 40 µM thick coronal section using a cryostat. The sections were mounted on gelatin-coated slide and stained with Cresyl Violet in order to determine the location of the recording sites.
The data were assessed using one-way ANOVA/two way ANOVA or paired Student T-test, when appropriate. All data are expressed as mean SEM or as percentage of the baseline firing rate. A P value of less than 0.05 was deemed statistical significant.

Histology confirmation of the recording sites in Globus Pallidus (GP) and Subthalamic nucleus (STN)



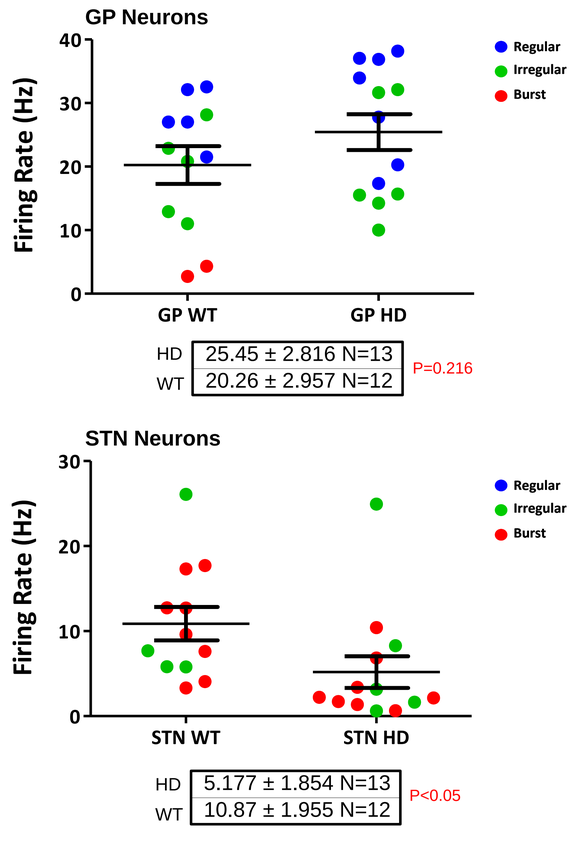
A frontal section showing the recording sites (Black arrows) in GP and STN marked by microiontophoresis injection of Pontamine Skyblue (-20 µA, 15 min) (A), and schematic pictures showing recording sites on corresponding brain sections (B). The numbers represent the posterior distance (mm) from Bregma. Data was excluded if the recording site was found to be outside of the target area.

Three distinct basal firing patterns exist in both GP and STN



Firing patterns of neurons recorded from GP and STN in BACHD and WT rats are each depicted by an interspike interval histogram ISIH (left), an autocorrelogram (middle) and a sample recording of spontaneous activity (right). A. Regular firing neurons exhibit a symmetrical ISIH distribution and an autocorrelogram with at least three peaks. B. Neurons with irregular firing exhibit an asymmetrical ISIH distribution and an autocorrelogram with 1 or 2 peaks. C. Activity of neurons with a burst firing pattern exhibit a positively skewed distribution, and an autocorrelogram without a clearly resolved peak.

In BACHD rats, mean firing rates showed a non-significant increase in GP and a significant decrease in STN



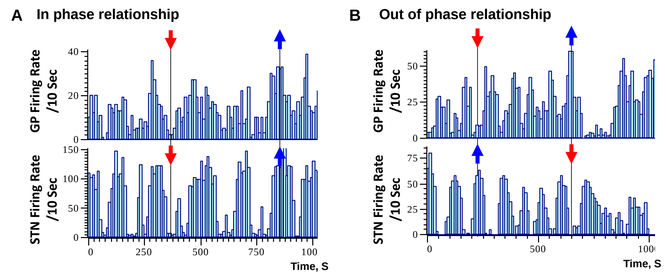
Dual simultaneous single unit recordings from GP and STN of 8-10 month transgenic BACHD rats reveal a non-significant increase of mean firing rates in GP (P=0.22, upper panel) and a significant decrease in STN (P<0.05, lower panel) relative to age and strain matched WT rats (WT: n=8; HD: n= 7).

Distribution of the firing patterns for GP and STN neurons in BACHD and WT rats

	WT Rats(n=13)		BACHD Rats(n=12)	
Firing Pattern	GP (%)	STN (%)	GP (%)	STN (%)
Regular	42	0	54	0
Irregular	42	42	46	38
Burst	16	58	0	62

In HD rats, the overall firing pattern of GP neurons appears to become more regular with fewer burst-type cells. The firing pattern of STN neurons exhibits the opposite trend, with fewer regular firing cells and proportionally more burst-type cells.

Dynamic changes in unit firing can be observed between GP and STN neurons



Dual single unit recordings from GP and STN reveal either an *in-phase* or *out-of-phase* relationship between the two nuclei. A. An *in-phase* relationship is represented by firing rates that change in the same direction (increase or decrease) in both GP and STN. B An *out-of-phase* relationship is represented by firing rates that change in the opposite direction in GP and STN.

Summary and Conclusion

- Our current findings provide evidence that mHtt affects firing properties of the pallidosubthalamic pathway in rodent HD models similar to those reported in clinically afflicted HD patients [1].
- The contribution of extrinsic versus intrinsic factors underlying these changes are being explored.
- Our dual recording approach in HD rodents may be a useful model for assessing compounds that revise abnormal activity of the indirect pathway.

References

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