Evaluation of MP-10, a PDE10 inhibitor, on BACHD transgenic rats using dual recording of single units in Globus Pallidus and Subthalamic nucleus

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Introduction
Chorea in Huntington's disease (HD) patients may be substantially due in part to a dysfunction of the indirect pathway (IP) of the basal ganglia [1-6]. D2-expressing striatal medium spiny neurons (MSN), giving rise to IP projections, appear more vulnerable to expression of mutant huntingtin (mHTT). A consequence of the preferential loss of striato-external pallidal GPes projections in HD patients would be expected to result in increased GPe firing rate, subsequently reduced STN firing rate, reduced activity of GPi and, ultimately, overactivity of the thalamus, resulting in chorea [1, 8]. Previous studies in the mouse BACHD model (6 months old), reported an age- dependent increase in mean firing rate of GP neurons and decrease in the mean firing rate of STN neurons in vivo (D.J. Surmey, Northwestern University) and in vivo (James Tepper, Rutgers University). The current study, using dual recording from GP and STN, demonstrated that comparable alterations in firing rates are also detected in another HD preclinical model, BACHD full length mHTT transgenic rats. The phosphodiesterase 10 (PDE10) is highly expressed in dopaminergic neurons of the substantia nigra and PDE10 inhibitors have been viewed as a potential treatment for schizophrenia. To provide a rationale for developing PDE10 inhibitors as a therapy for HD disease, we evaluated whether MP-10, a specific and potent PDE10 inhibitor, would be able to reverse the altered firing rate observed in BACHD rat. A PK/PD relationship was studied by collecting blood samples at 5, 30, and 60 minutes after compound administration.

Material and Methods
All experimental procedures involving animals have been conducted according to the established guidelines and were approved by Institutional Animal Care and Use Committee. Male BACHD rats and their WT littermate controls (8-13 months old) were anesthetized with Urheane (initial dose at 1.5 g/kg, i.p. Additional 0.3 g/kg was given as necessary during the surgical procedure) and surgically implanted with two catheters, one in the femoral vein and one in the femoral artery, for drug administration and blood sampling respectively. The animal was mounted on a stereotactic apparatus (David Kopf instrument) in a flat skull position. Two burr holes were drilled on the skull. One with stereotactic coordinates 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 2-1.7 mm (STN recording). The recording electrodes were advanced to reach the target coordinates of the GP (5.5-6.5 mm below the brain surface) and STN (6.8 to 7.5 mm below the dorsal surface). After a stable baseline recording was established, vehicle was given intravenously 5 minutes before an IV bolus injection of MP-10. Blood samples were taken about 5 min after vehicle injection (control), and 5, 30, and 60 minutes after compound IV injection for bianalytical measurement to establish PK/PD relationship. At the end of each experiment, the recording sites were marked by the microiontophoresis of Pontamine Skyblue (20 μA, 15 min). The rats brains were then cut into 40 μm thick coronal sections using a cryostal to verify the recording locations. The data were assessed using one-way ANOVA/2 way ANOVA or paired Student T-test, when appropriate. All data were expressed as mean ± SEM or as percentage of the baseline firing rate. A P value of less than 0.05 was deemed statistically significant.

Results
In BACHD rats, mean firing rates were significantly increased in GP and significantly decreased in STN.

0.18 mg/kg of intravenously injected MP-10 induced a robust increase in STN neuron mean firing rate in both WT (panel A) and TG rats (Panel B). To 30 min post MP-10 administration, both MP-10 induced firing rate increase and MP-10 plasma exposure diminished. The magnitude of STN firing rate increase appeared higher in TG rats than that in WT rats. N represents the numbers of units recorded at 5, 30, or 60 minutes after MP-10 injection. Blue arrows represent the time point of MP-10 IV administration.

Conclusions
1. Our data provide evidence that is complementary to the prevailing hypothesis in HD patients; expression of mHTT in rats alters the firing properties of neurons in the "indirect" pallidolusothalamic pathway.
2. MP-10 restored the STN firing rates in BACHD rats, which is consistent with a potential therapeutic action of PDE10 inhibitors for the treatment of HD. A
3. Although not statistically significant, we demonstrated a trend that MP-10 induced firing rate increase in STN was more sensitive in BACHD rats than in WT rats whereas comparable MP-10 PK was demonstrated between BACHD and WT rats. Delayed PD response (transit time ~10 min) and extended PK exposure contributed to prolonged increase of STN firing rate.

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