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

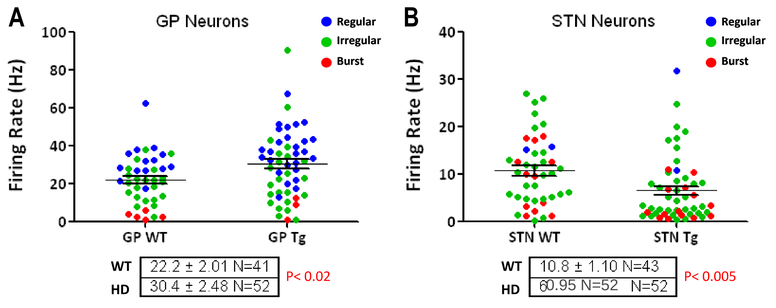
Chorea in Huntington's disease (HD) patients may be substantially due in part to a dysfunction of the indirect pathway (IP) of the basal ganglia [1-6]. D2-expressing striatal medium spiny neurons (MSN), giving rise to IP projections, appear more vulnerable to expression of mutant huntingtin (mHTT). A consequence of the preferential loss of striato- external pallidal (GPe) 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 month old), reported an age-dependent increase in mean firing rate of GP neurons and decrease in the mean firing rate of STN neurons *in vitro* (D.J. Surmeier, Northwestern Univ) 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 within dopaminoreceptive MSNs of the striatum 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 rats (8-13 months old) were anesthetized with Urethane (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 stereotaxic apparatus (David Kopf instrument) in a flat skull position. 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 (5.5-6.5 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 5-10 min 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 bioanalytical 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 rat brains were frozen then cut into 40 µm thick coronal sections using a cryostat to verify the recording locations. The data were assessed using one-way ANOVA/two 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



Dual simultaneous single unit recordings from GP and STN from 8 to13 months old transgenic BACHD rats revealed a significant increase of mean spontaneous firing rates in GP (P<0.02, panel A) and a significant decrease in STN (P<0.005, panel B), relative to the age- and strain-matched WT rats.

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

Firing Pattern	GP Firing (%)		STN Firing (%)	
	WT	TG	WT	TG
Regular	34	50	5	4
Irregular	54	44	60	62
Burst	12	6	35	35

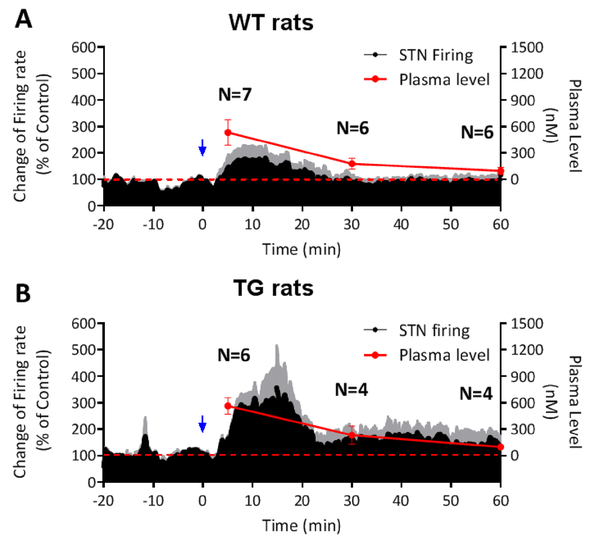
As compared to WT rats, in transgenic HD rats, the overall firing pattern of GP neurons appeared to be more regular with fewer irregular and burst-type firing neurons. In STN however, no clear differences in firing patterns between WT and Tg rats was detected.

MP-10 (bolus i.v.) effects on single unit discharges in GP and STN neurons of BACHD rats

Summary of Results

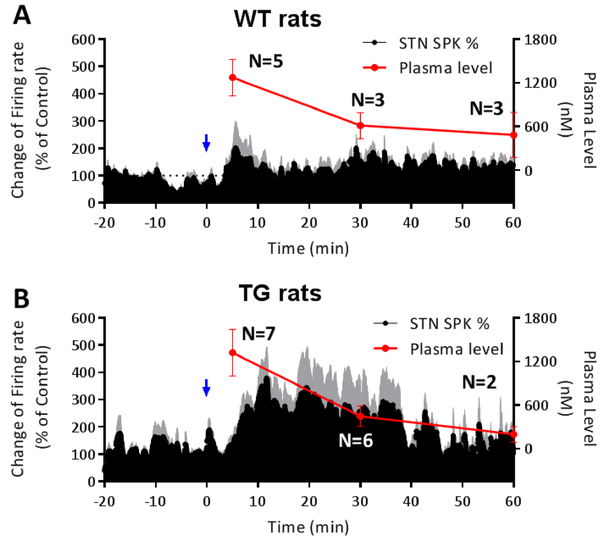
1. In 80% of rats enrolled, a single bolus IV injection of MP-10 at all doses studied elicited a robust increase in firing rate of STN neurons in a dose dependent manner (see below).
2. Comparable plasma concentration level of MP-10 was found in all the treated groups. However, in 20% of rats, administration of MP-10 did not produce significant change in the STN neurons firing rate. Since multiple reasons could contribute to the lack of response, non-responders were not included in this current analysis.
3. In contrast to STN, MP-10 treatments did not induce a significant change in GP neurons firing rate (data not shown).

Relationship between stimulatory effects of MP-10 (0.18 mg/kg bolus i.v.) on single unit discharges in STN neurons and its plasma concentration level in BACHD and WT rats



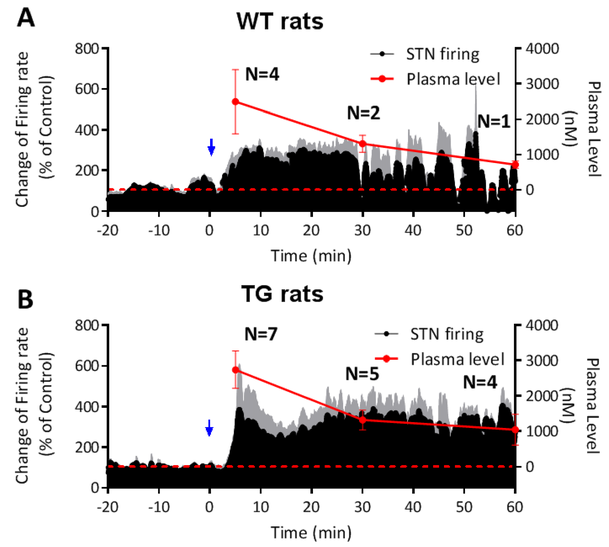
0.18 mg/kg of intravenously injected MP-10 induced a robust increase in STN neuron mean firing rate in both WT (panel A) and TG rats (Panel B). 30 to 60 min post MP-10 administration, both MP-10 induced firing rate increase and MP-10 plasma exposure diminished. The magnitude of STN firing rate increase 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.

Relationship between stimulatory effects of MP-10 (0.52 mg/kg bolus i.v.) on single unit discharges in STN neurons and its plasma concentration level in BACHD and WT rats



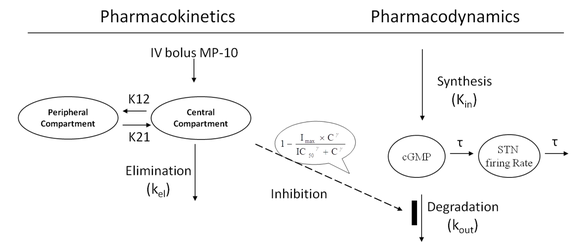
Intravenous injection of 0.52 mg/kg of MP-10 induced an increase in firing rates of STN neurons in both WT (panel A) and TG rats (Panel B). The firing rate increase, as well as plasma concentration level of MP-10, decreased with time and reached close to the baseline levels 60 minutes after compound administration. Note that the magnitude of STN firing rate increase appears higher in TG rats than that in WT rats.

Relationship between stimulatory effects of MP-10 (1.5 mg/kg bolus i.v.) on single unit discharges in STN neurons and its plasma concentration level in BACHD and WT rats



1.5 mg/kg of MP-10 (IV) induced a robust increase of STN neurons firing rate in both WT (panel A) and TG rats (Panel B). There is a good relationship between firing rate increase and plasma level of MP-10. Both the firing rats and plasma level of MP-10 were still elevated at 60 min and unlike lower doses the elevation in firing rate did not return to baseline level over this time period.

MP-10, pharmacokinetic/pharmacodynamic relationships



	Imax	IC50_TG (ng/ml)	IC50 Ratio WT/TG	Kout (min^{-1})	Tau (min)
Estimate	0.48	24	2	301	10.5
90% CI	0.30 – 0.61	0.2 – 149	0.2 – 699	4.8 - 39485	5.5 – 23.3

PK/PD model structure and parameter estimation. A two-compartment PK model and transduction PD model adequately described the observed PK/PD profiles. Imax, unit maximum inhibition of cGMP turnover rate; IC50, drug concentration associated with half maximum inhibition of cGMP turnover rate; Kout, cGMP turnover rate; Tau, signal transit time between cGMP and STN firing rate change.

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" pallidosubthalamic pathway.
2. MP-10 restored the low STN firing rates in BACHD rats, which is consistent with a potential therapeutic action of PDE10 inhibitors for the treatment of HD.
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 while comparable MP-10 PK was demonstrated between BACHD and WT rats.
4. Delayed PD response (transit time ~ 10 min) and extended PK exposure contributed to prolonged increase of STN firing rate

References and Acknowledgments

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