

GDNF, CDNF and MANF have divergent effects on nigrostriatal dopamine neurochemistry in rats

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1. INTRODUCTION

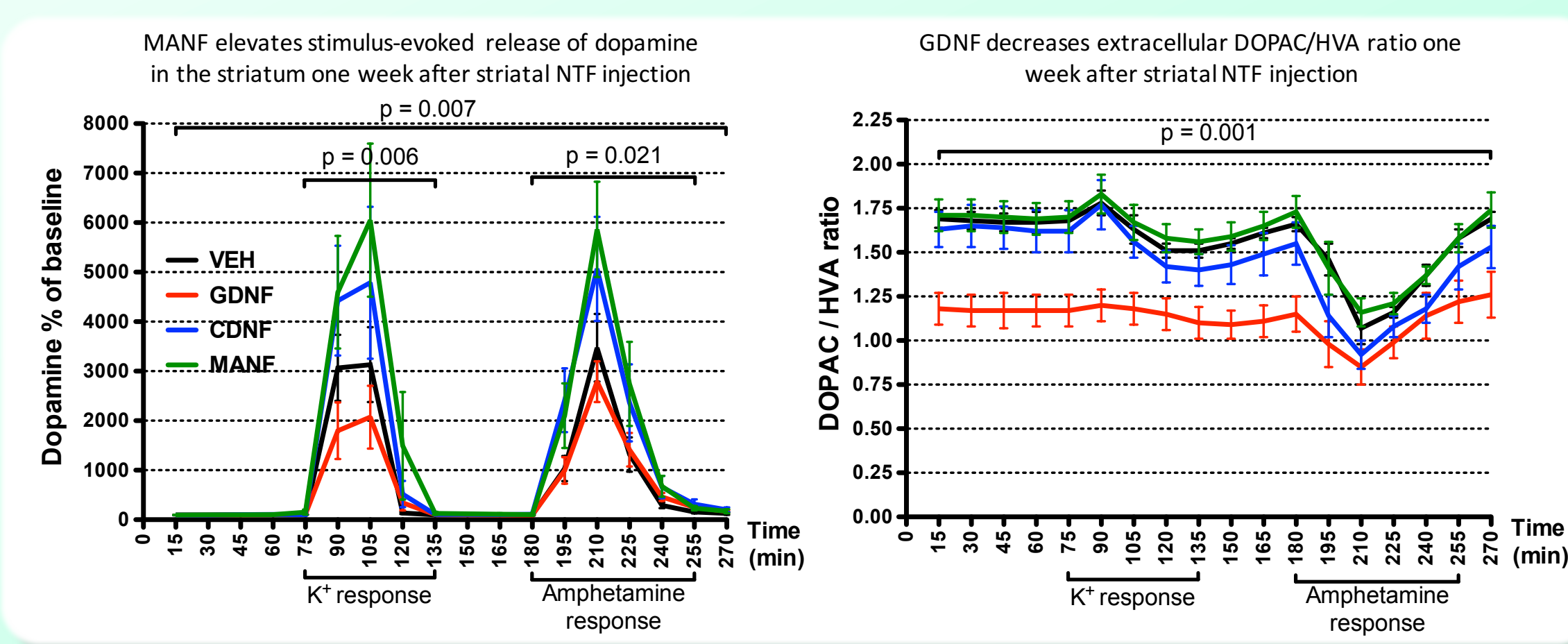
Neurotrophic factors (NTFs) hold potential as disease-modifying therapies for Parkinson's disease (PD). Glial cell line-derived neurotrophic factor (GDNF), cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) have shown neurorestorative effects on nigral dopaminergic neurons in various animal models of PD (1-3). We have earlier compared effects of the NTFs on nigrostriatal dopamine neurochemistry using *in vivo* microdialysis in intact, freely-moving rats (4). In the present study we wanted to further elucidate differences between the NTFs seen in the microdialysis study.

AIM 1

Determine the effects of GDNF, CDNF and MANF on *in vivo* activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis

AIM 2

Study whether GDNF changes the activity of dopamine metabolizing enzymes catechol-O-methyltransferase (COMT) and monoamine oxidases A and B (MAO-A and MAO-B)



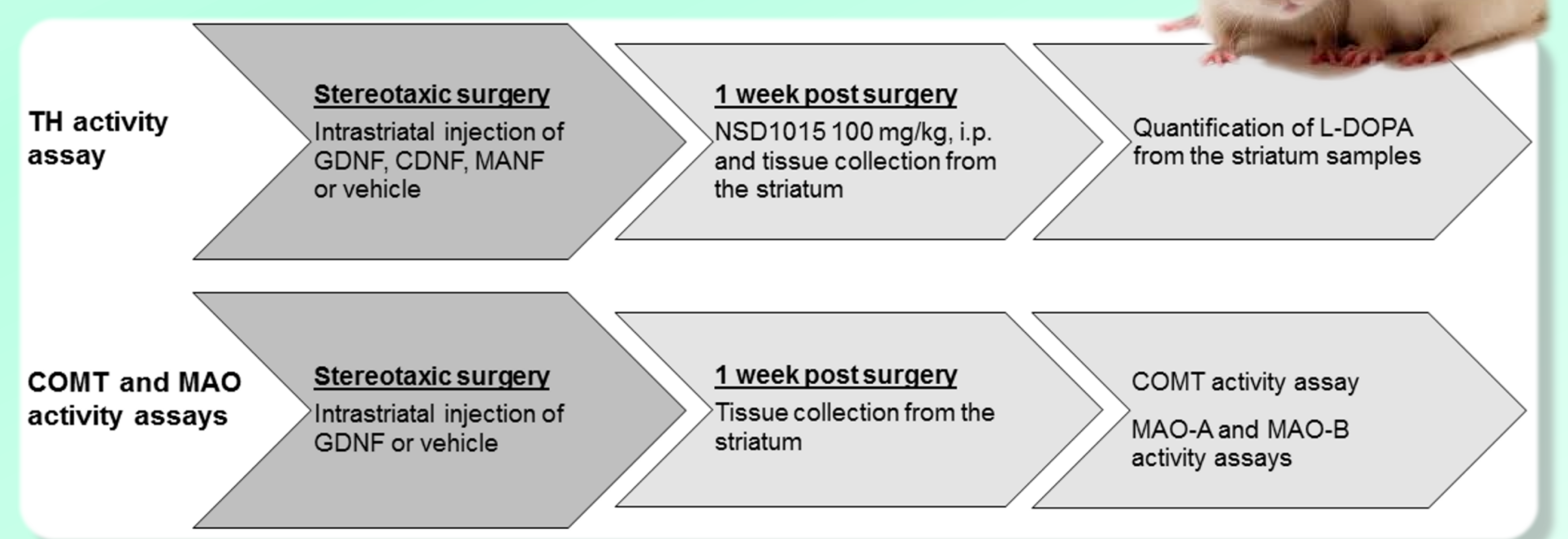
2. MATERIALS AND METHODS

Cohort 1: *in vivo* TH activity measurement

- An unilateral injection of hGDNF, hCDNF or hMANF (10µg/5µl) or vehicle (PBS) was made into the left dorsal striatum of young adult male RccHan:WIST rats
- Seven days later, rats were administered with an aromatic amino acid decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD1015) 100 mg/kg, i.p. Rats were sacrificed and dorsal striatum samples were collected 30 min after the NSD1015 injection
- The amount of accumulated L-3,4-dihydroxyphenylalanine (L-DOPA) in the samples was analyzed with an HPLC system equipped with an electrochemical (EC) detector

Cohort 2: *ex vivo* COMT, MAO-A and MAO-B activity measurements

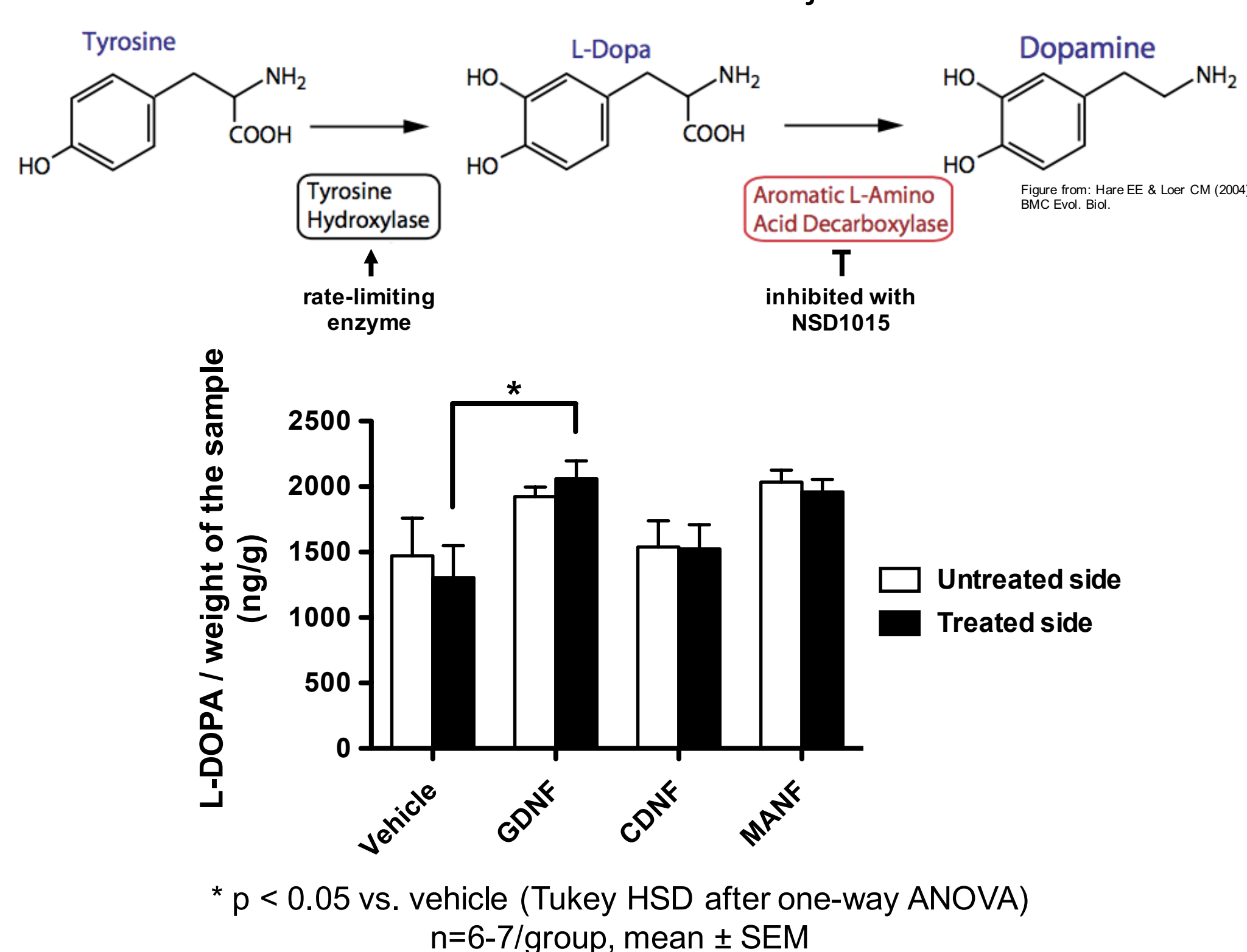
- An unilateral injection of hGDNF (10µg/5µl) or vehicle (PBS) was made into the left dorsal striatum of young adult male RccHan:WIST rats
- Seven days later, dorsal striatum samples were collected and total protein concentration in the samples was determined using a bicinchoninic acid method
- Total COMT activity assay:** samples were incubated in phosphate buffer containing S-adenosyl-L-methionine and 3,4-dihydroxybenzoic acid. Reaction products, vanillic and isovanillic acid, were analyzed with an HPLC system coupled with an EC detector
- MAO-A and -B activity assay:** Monoamine oxidase assay kit (Sigma-Aldrich) was utilized. In the assay MAO reacts with p-tyramine forming H₂O₂ which is measured by a fluorimetric method



3. RESULTS

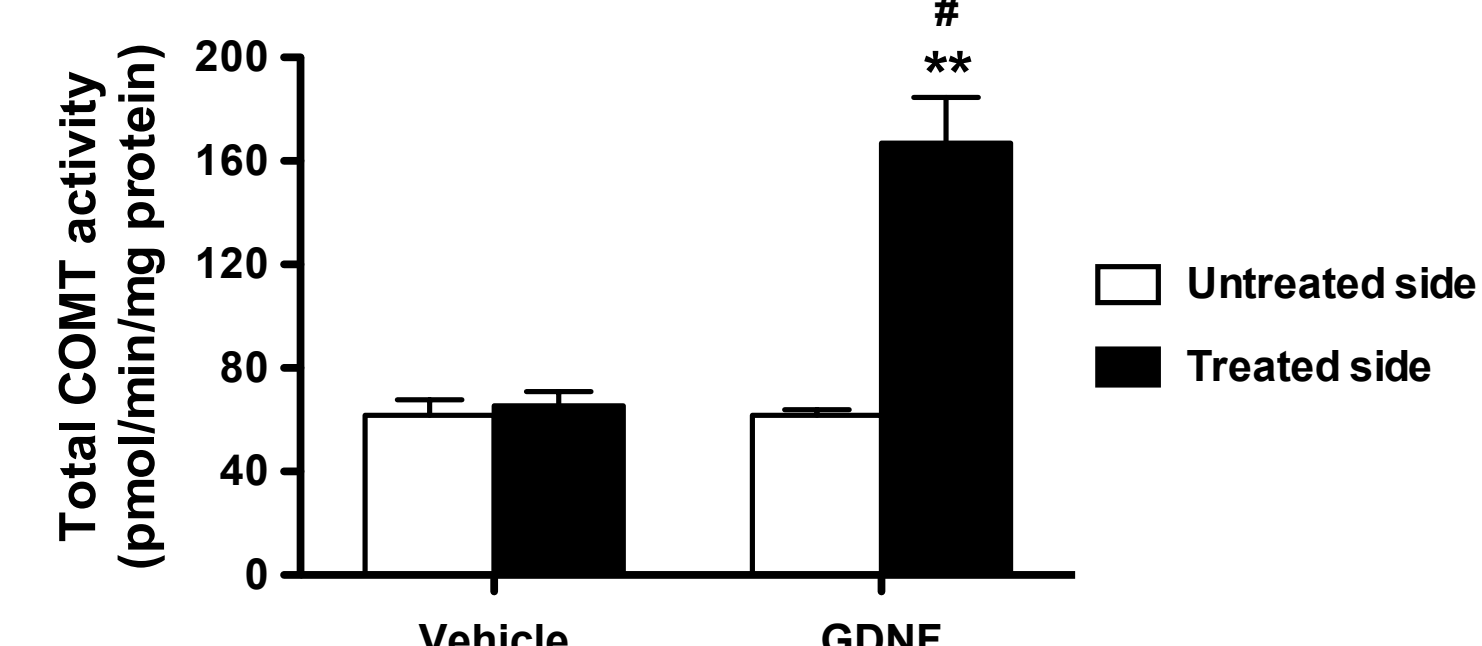
AIM 1

In vivo TH activity in the striatum is increased one week after striatal GDNF injection

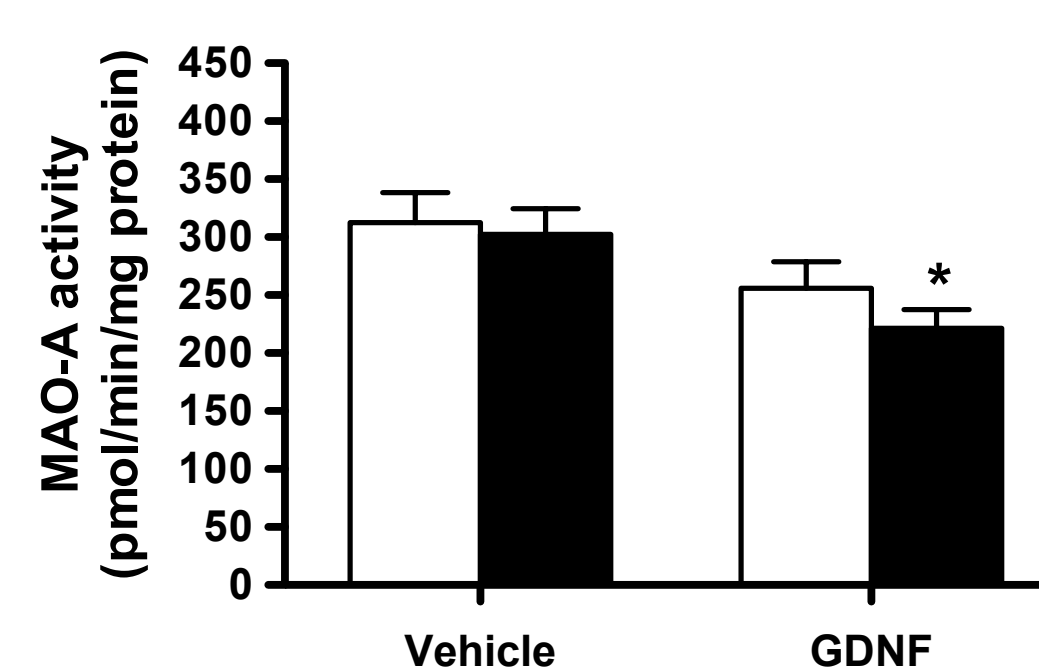


AIM 2

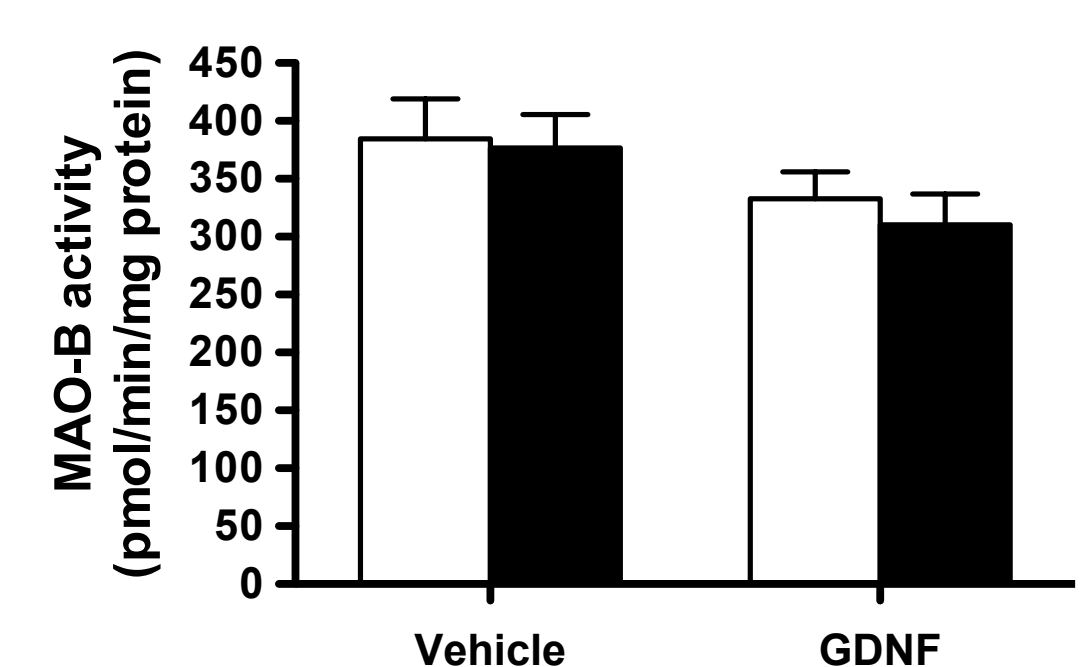
COMT activity in the striatum is increased one week after striatal GDNF injection



MAO-A activity in the striatum is decreased one week after striatal GDNF injection



MAO-B activity in the striatum remains unchanged one week after striatal GDNF injection



** p < 0.001; * p = 0.011 vs. vehicle (unpaired two-tailed t-test) # p < 0.001 vs. untreated side (paired two-tailed t-test) n=7-8/group, mean ± SEM

4. CONCLUSIONS

- ✓ Striatal TH activity is increased in GDNF-treated rats
- ✓ Changes in TH activity after NTF treatment do not correlate with the altered stimulus-evoked release of dopamine seen in the earlier study
- ✓ Striatal COMT activity is increased and MAO-A activity decreased in GDNF-treated rats
- ✓ Effects of GDNF on the activity of COMT and MAO-A can explain the reduced DOPAC/HVA ratio observed in the earlier study

REFERENCES

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