TACTICS

Translational Adolescent and Childhood Therapeutic Interventions in Compulsive Syndromes

SEVENTH FRAMEWORK PROGRAMME

MS29 – Effect of pharmacological treatment on white matter integrity and functional connectivity

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1. Executive Summary

This document reports on the effects of a subchronic memantine treatment on structural and functional connectivity in an adolescent rat model of compulsive checking behavior. In this adolescent model, compulsive behavior was induced by twelve repeated injections with the dopamine receptor agonist quinpirole in combination with placement in an open field. The subchronic memantine treatment was given for seven consecutive days between the 10^{th} and 12^{th} quinpirole injection. We measured compulsive behavior before and after treatment, and demonstrated that the memantine treatment did not significantly reduce compulsive behavior in this adolescent rat model. Correspondingly, MRI-based measures of structural and functional connectivity within the frontostriatal circuitry were not altered after the subchronic memantine treatment. The subchronic memantine treatment however inhibited the developmental body weight increase in both control and quinpirole-injected rats. The absence of a therapeutic effect may be partly explained by model-treatment interactions between the dopaminergic (model) and glutamatergic (treatment) system.

2. Milestone report

Compulsivity is the repetitive, irresistible urge to perform certain behaviors without voluntary control, and can be considered to be a cross-disorder trait of psychiatric disorders like obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD) (Jacob et al., 2009). Current treatment strategies for these disorders, that typically focus on diminishing symptoms associated with a specific diagnosis, are often ineffective (Accordino et al., 2016; Franklin and Foa, 2011). This stresses the need for alternative treatment approaches, for example by focusing on cross-disorder traits, e.g. compulsive behavior, so treatment can be tailored to specific symptom domains.

Development of treatment approaches that focus on compulsive behavior requires knowledge of the underlying neural circuits. Compulsive behavior has been associated with structural and functional abnormalities within the frontal cortico-striatal-thalamo-cortical (CSTC) circuits in humans, as demonstrated with magnetic resonance imaging (MRI) (Figee et al., 2016; Montigny et al., 2013). Within the CSTC circuits, compulsive behavior may either be caused by hyperactivity within the striatal component or by a failure of top-down control of the frontal cortical regions over the striatal component (Fineberg et al., 2010). It has been theorized that this top-down cortical control is mediated by the neurotransmitter glutamate (Sesack et al., 2003). This points towards a relationship between compulsive behavior and altered glutamate concentrations, which is further supported by the high density of glutamate receptors in the frontostriatal circuits (Monaghan et al.,

1985) and dysregulation of glutamatergic signaling in individuals with ASD and OCD (Naaijen et al., 2015; Pittenger et al., 2011).

The role of glutamate in patients with OCD and ASD may imply that anti-glutamatergic drugs could be effective as medication against compulsivity (Mechler et al., 2017). One potential drug is the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine, an FDA approved drug used in the clinic for the symptomatic treatment of Alzheimer's disease (see for a systematic review and meta-analysis: Matsunaga *et al*, 2015). Memantine has shown some first promise as a successful treatment against OCD in an animal model and in human adults with OCD (Egashira et al., 2008; Ghaleiha et al., 2013; Haghighi et al., 2013) and is therefore also the drug tested in the TACTICS clinical trial (WP6 (Häge et al., 2016)). Since three-quarters of people with OCD experience their first symptoms in mid childhood, OCD is suggested to be a neurodevelopmental disorder (Boileau, 2011). Therefore, it is important to assess the treatment potential of memantine in this developmental period (Mechler et al., 2017). In addition, mechanistically it remains unclear how memantine exerts its therapeutic effects on neural circuits involved in OCD. Therefore, we investigated whether memantine would be able to reduce compulsive behavior in adolescent rats, by influencing structural and functional connectivity in the frontostriatal circuitry.

We used a model of compulsive checking behaviour in adolescent rats, which we have recently developed in Task 1 (MS22) based on a similar model in adult animals (Szechtman *et al.,* 1998). Five week-old juvenile Sprague Dawley rats were subcutaneously injected with quinpirole (0.5 mg/kg; n=16; Quinpirole group) or saline (n=16; Control group), twice a week during 6 weeks, resulting in a total of 12 injections. Each injection was followed by placement in the middle of a large open field table with four objects. The rat's locomotion was recorded for 30 minutes with a camera fixed to the ceiling, and the home-base was determined as the most frequently visited zone at the open field table. Checking behavior was characterized for the home-base and included the following parameters: frequency of checking (total number of visits of the home-base during the 30 min recording time), length of checks (average time of a visit at the home-base), recurrence time of checking (average time spent in other areas before returning to the home-base) and stops before returning to the home-base (average number of other areas the rat visited before returning to the home-base) (Szechtman *et al*, 1998; Tucci *et al*, 2014). Rats were randomly assigned to memantine or saline treatment, and the experimenters were blinded for this treatment assignment. Rats received daily intraperitoneal injections of memantine (20 mg/kg/day (Sekar et al., 2013)) (Quinpirole group: n=8, Control group: n=8) or saline (Quinpirole group: n=8, Control group: n=8) for seven consecutive days, starting the day after the 10th quinpirole or saline injection. MRI acquisition and compulsive behavior assessment were done after the 10th quinpirole/saline injection (pre-treatment) and after

the 12th quinpirole/saline injection (post-treatment). The MRI session consisted of an anatomical MRI, followed by resting-state fMRI acquisition and diffusion-weighted MRI. We determined structural and functional connectivity between the frontal cortex and striatum. Functional connectivity is measured as the Fisher's Z-transformed correlation coefficient and structural connectivity as fractional anisotropy (FA).

One control rat died during the post-memantine MRI acquisition because of respiratory problems caused by excessive mucus. In addition, the behavioral recording of one quinpirole-injected rat was incomplete and the MRI scans of one control and one quinpirole-injected rat were affected by artifacts. Therefore, final groups consisted of 14 quinpirole-injected rats (saline-treated: n=7; memantine-treated: n=7) and 14 control rats (saline-treated: n=8; memantine-treated: n=6).

As expected, we detected no statistically significant effects of saline treatment on any of the behavioral measures (Figure 1A). Similarly, however, behavioral measures, exposing compulsivity in quinpirole-injected rats, were not significantly altered after memantine treatment in control and quinpirole-injected rats. Correspondingly, we also did not find any statistically significant effects of the memantine treatment on functional and structural connectivity within the frontostriatal system (Figure 2A and 2B, respectively). We could not reliably measure interhemispheric structural connectivity between the left and right frontal cortex, because tractography results were inconsistent due to close distance of the two frontal regions. On the other hand, the body weight of control and quinpirole-injected adolescent rats significantly increased in the seven days between pre- and post-treatment with saline (Figure 1B), but not in rats that were treated with memantine.

In conclusion, our study did not reveal beneficial effects of memantine treatment for compulsive behavior in the quinpirole-induced adolescent rat model of compulsive checking behavior. In deliverable D02.02, we showed that acute memantine induced widespread brain activation in the frontal cortex in control rats, but not in rats that received repeated quinpirole injections. Our latest experiments demonstrated that this memantine-induced brain activation is also absent in rats that received only a single quinpirole injection. Together, these findings suggest that the absence of treatment effects of memantine may be at least partly explained by model-treatment interactions between the dopaminergic and glutamatergic system and suggest that anti-glutamatergic drugs may not be effective in individuals with altered dopaminergic neurotransmission. Future studies applying this adolescent rat model should carefully consider possible interactions between quinpirole and pharmacological treatments.

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3. Tables and other supporting documents where applicable and necessary

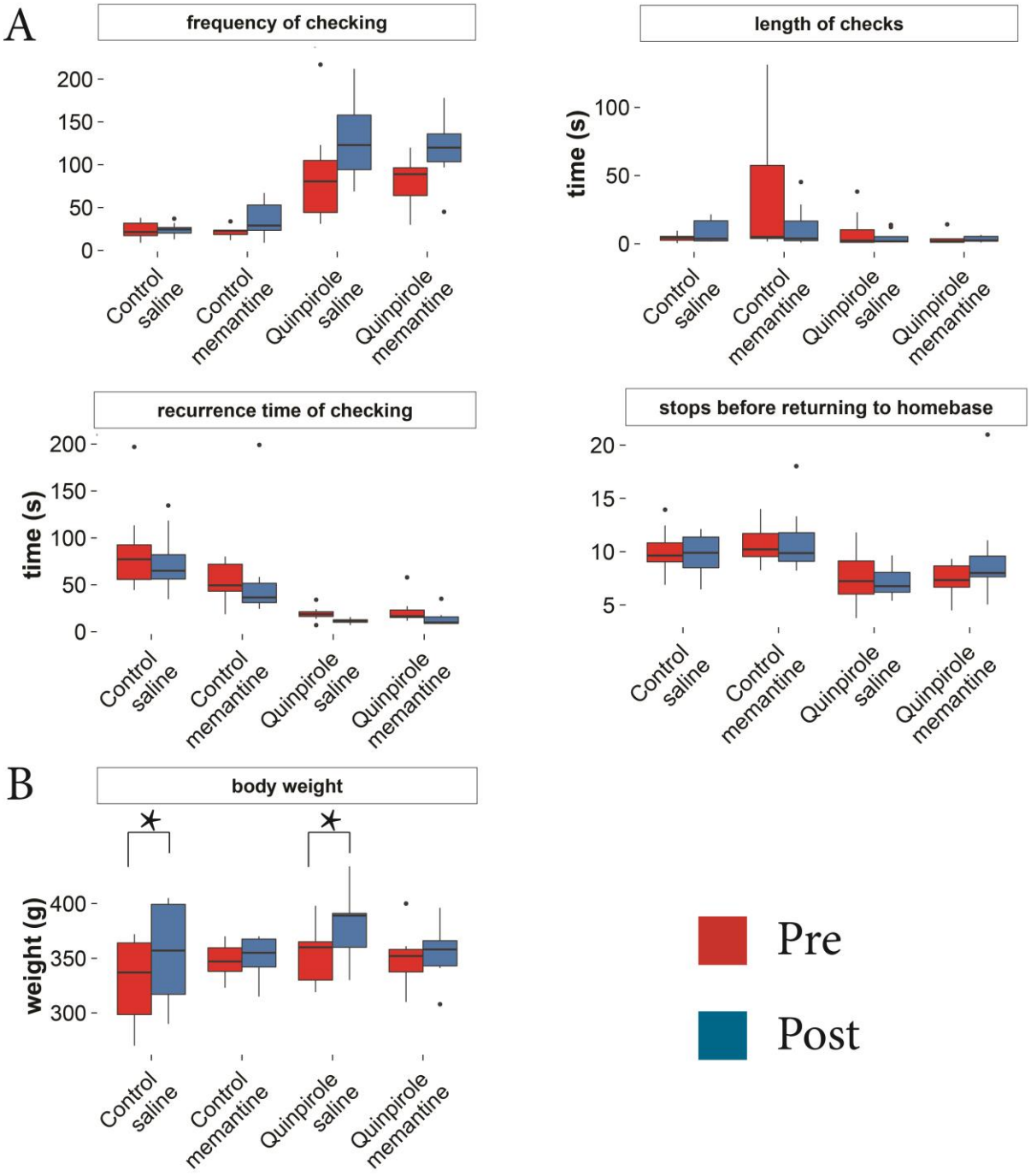

Figure 1: Behavioral measures of compulsivity and body weight, before and after saline/memantine treatment in control and quinpirole-injected rats. Compulsive behavior (frequency of checking (total number of visits at the home-base during 30 min observation), length of checks (average time (s) spent at the home-base), recurrence time of checking (average time (s) before returning to the home-base), stops before returning to the home-base (average number of zones visited in between two visits of the home-base)) (A) and body weight (g) (B) before (Pre; red) and after (Post: blue) 7 days of daily saline/memantine treatment. * $p<0.05$. Error bars represent 1.5 × interquartile range and dots represent outliers.

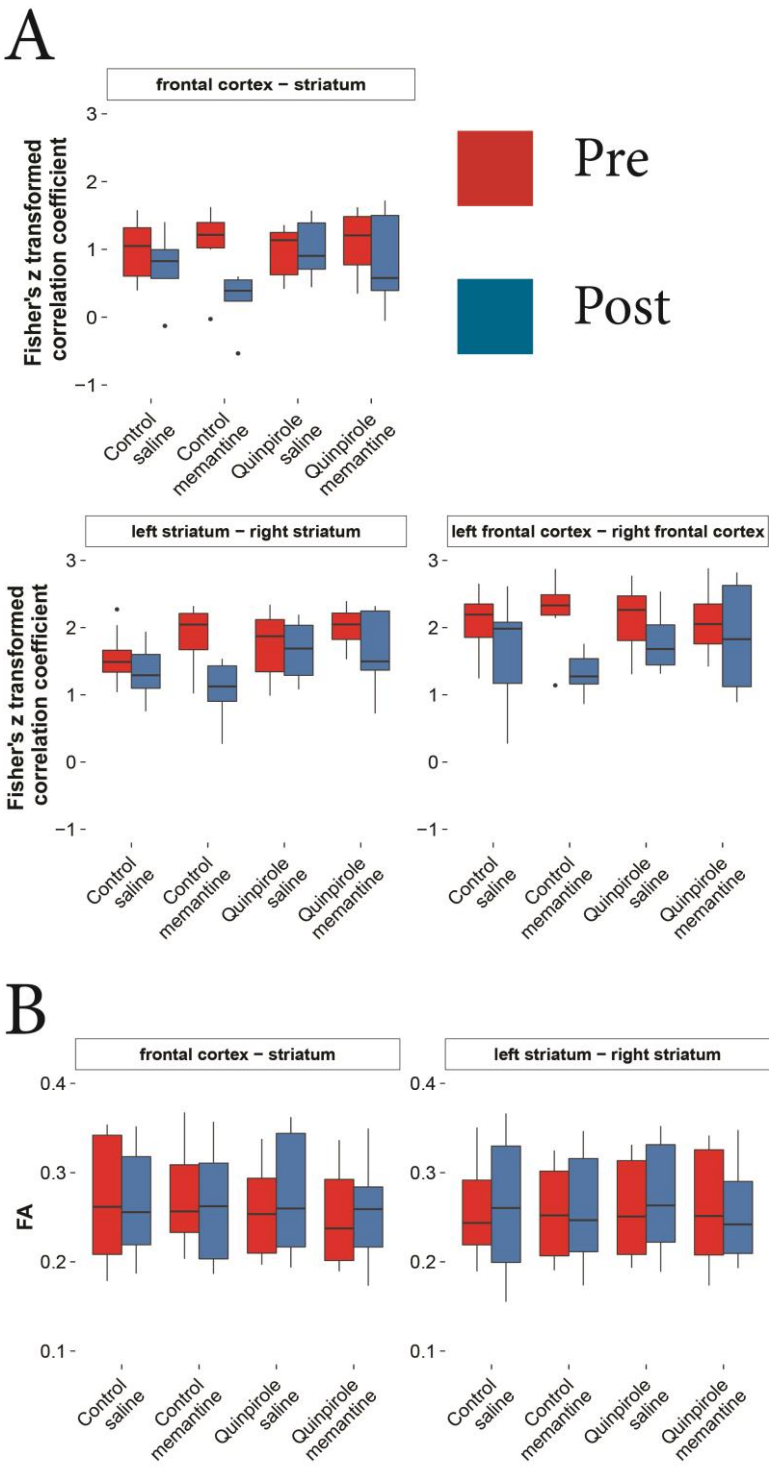

Figure 2: Structural and functional connectivity in the frontostriatal system before and after saline/memantine treatment in control and quinpirole-injected rats. Bar graphs of functional connectivity (Fisher's Z transformed correlation coefficient) (A) and structural connectivity (median fractional anisotropy (FA)) (B) of interhemispheric homologous and intrahemispheric connections within the frontostriatal system before (Pre; red) and after (Post; blue) 7 days of daily saline/memantine treatment. Structural connectivity between the left and right frontal cortex could not be determined because of unreliable tractography results. Error bars represent 1.5 × interquartile range and dots represent outliers. FA: fractional anisotropy.

4. Acknowledgement and Disclaimer

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