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Translational Molecular Psychiatry

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INVOLVEMENT OF THE WNT-SIGNALING IN METHYLPHENIDATE (RITALIN) TREATMENT OF ADHD

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BACKGROUND

Attention-Deficit Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders in children and adolescents affecting over 5% of the population worldwide and characterized by functional and structural brain maturation delays [1]. Genetic studies indicate a possible involvement of the Wnt-signaling pathway, known to play a fundamental role in essential cell processes, such as proliferation, differentiation, and maturation during neurodevelopment [2,3]. Methylphenidate (MPH), the first line treatment for ADHD, seems to ameliorate brain maturational delays in patients, even though molecular mechanisms involved on this effect are not fully elucidated. To assess patient-specific phenotypes and mechanisms underlying ADHD at the cellular and molecular levels, the use of induced pluripotent stem cells (iPSCs)-derived neural stem cells (NSCs) and forebrain cortical neurons (FCNs) is a promising model for the generation of a functional Central Nervous System microenvironment *in vitro*.

AIM OF THE STUDY

Our aim is to test functional alterations in the Wnt-signaling in ADHD and its possible association with affected neurodevelopment seen in patients. Concomitantly, we will investigate whether MPH modulates this pathway, restoring ADHD-related phenotypes.

METHODS

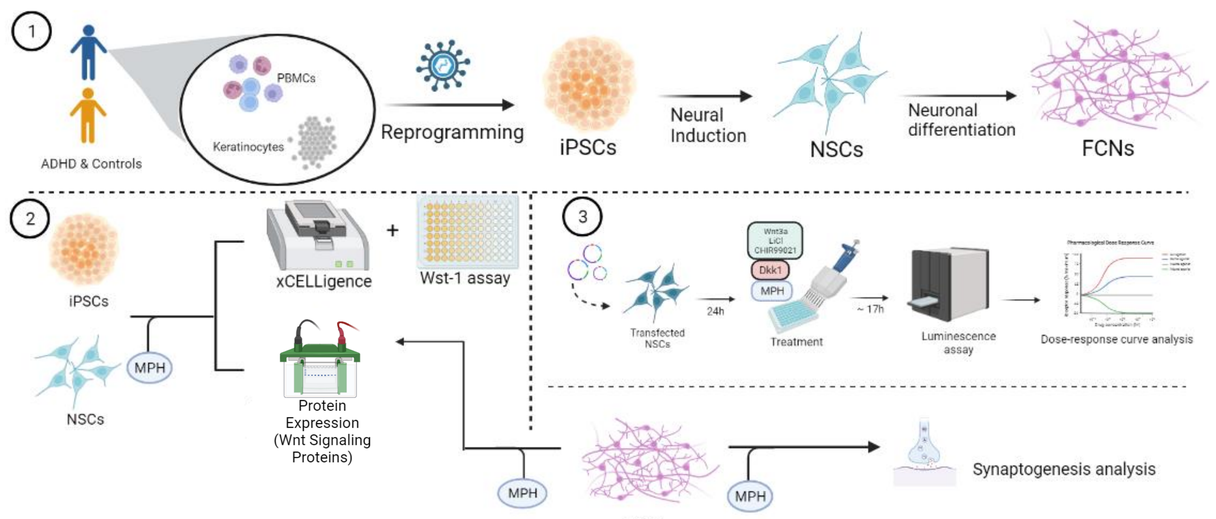

Figure 1. General experimental design of the project. Figure created on Biorender.com

Table 1. Cell lines included in this study [4,5,6].

Control	ADHD
K001 i6 / i9	MR001 x3 / x15
K005 z12 / z13	MR010 c3 /c18
K011 c6 / c10	MR013 c3 /c13
K015 c1 / c9	MR014 c12 / c27
K013 c20 / c39 ♀	MR030 c1 / c2 ♀

♀ Female participants are highlighted in purple.
Two clones per individual are considered.

RESULTS

Patient-derived iPSCs, NSCs and FCNs were generated and biologically characterized (Fig. 2) [4,5,6].

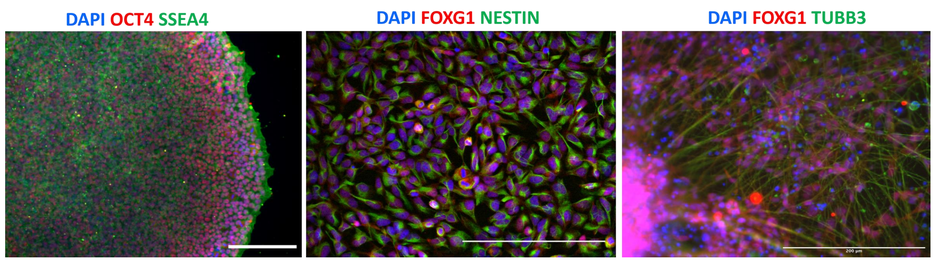

Figure 2. Exemplary immunocytochemistry images of iPSCs, NSCs and FCNs. The images show positive protein expression of pluripotency markers OCT4 and SSEA4 in iPSCs, NSC markers (Nestin and FOXG1) and FOXG1 and TUBB3 in FCNs. Scale bar: 200 µm.

Reduced growth rates of NSCs from ADHD patients (Fig. 3) [7].

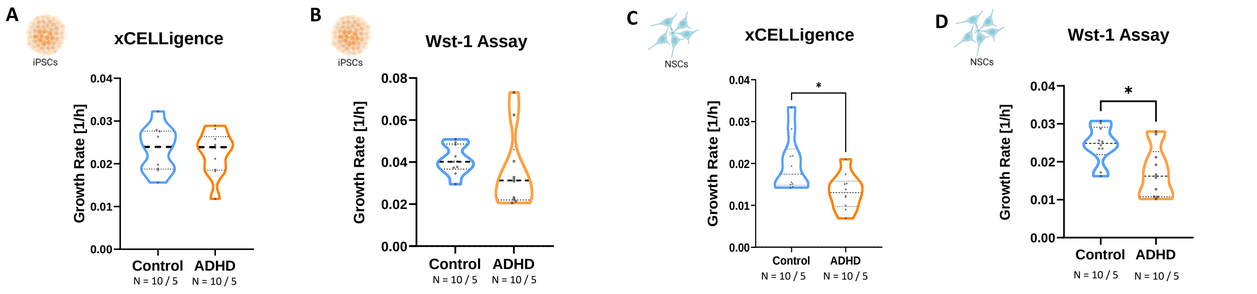

Figure 3. Growth rate analysis by xCELLigence and Wst-1 assays in iPSCs (A,B) and NSCs (C,D). In iPSCs, no differences in growth rates were only found in both methods. In NSCs, growth is significantly higher in controls compared to the ADHD group. For xCELLigence and Wst-1 in NSCs, Mann-Whitney (**p*=0.0147) and Welch's t tests were applied (**p*=0.0124), respectively.

Slight increase in growth rates observed after MPH treatment of ADHD NSCs in a Wnt-dependent manner (Fig. 4).

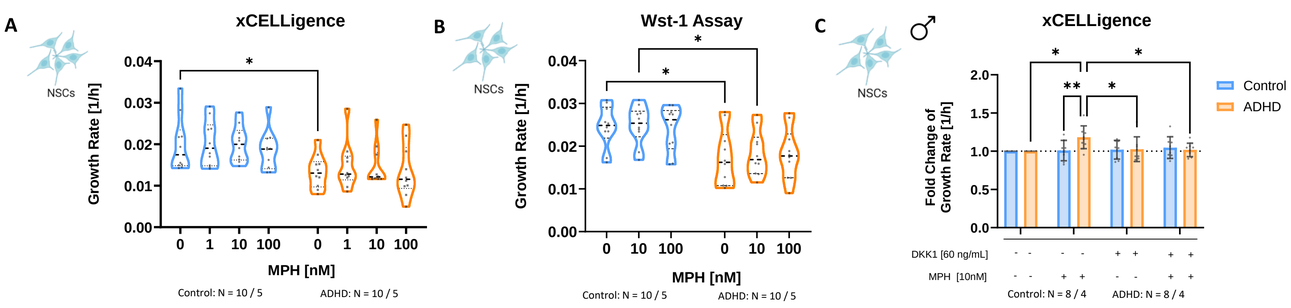

Figure 4. Growth rate analysis by xCELLigence (A) and Wst-1 assays (B) in NSCs, before and after chronic MPH treatment. Ordinary Two-Way ANOVA, * 0.01 ≤ p < 0.05, ** 0.001 ≤ p < 0.01. C) Wnt signaling blockade by DKK1 60 ng/mL prevents restoration effects of MPH in ADHD.

Wnt activity is increased in ADHD NSCs, while MPH modulates it at 10 nM and 100 µM (Fig. 5).

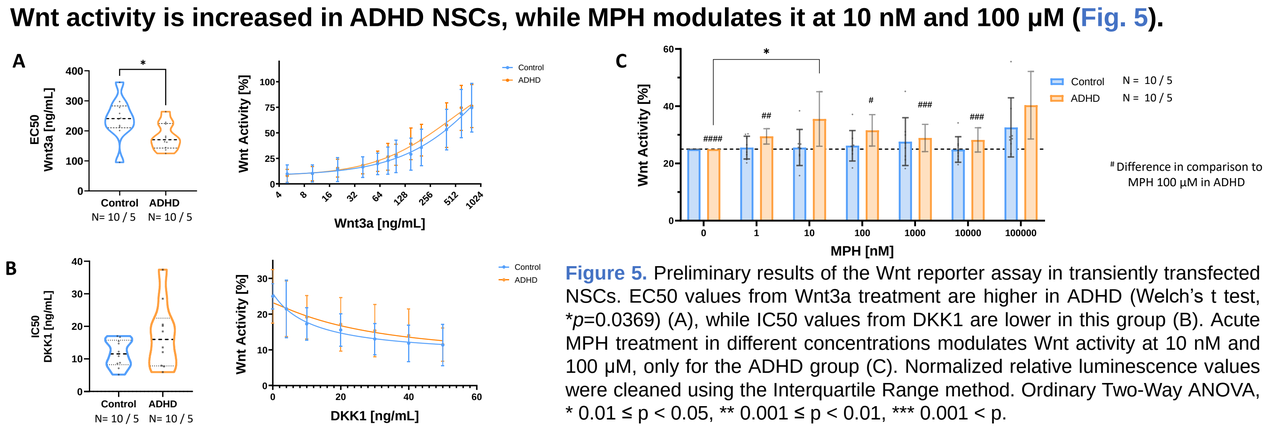

Figure 5. Preliminary results of the Wnt reporter assay in transiently transfected NSCs. EC50 values from Wnt3a treatment are higher in ADHD (Welch's t test, **p*=0.0369) (A), while IC50 values from DKK1 are lower in this group (B). Acute MPH treatment in different concentrations modulates Wnt activity at 10 nM and 100 µM, only for the ADHD group (C). Normalized relative luminescence values were cleaned using the Interquartile Range method. Ordinary Two-Way ANOVA, * 0.01 ≤ p < 0.05, ** 0.001 ≤ p < 0.01, *** 0.001 < p.

Proteomic expression of Wnt-related proteins tends to vary throughout neural differentiation and indicate alterations in NSCs (Fig. 6).

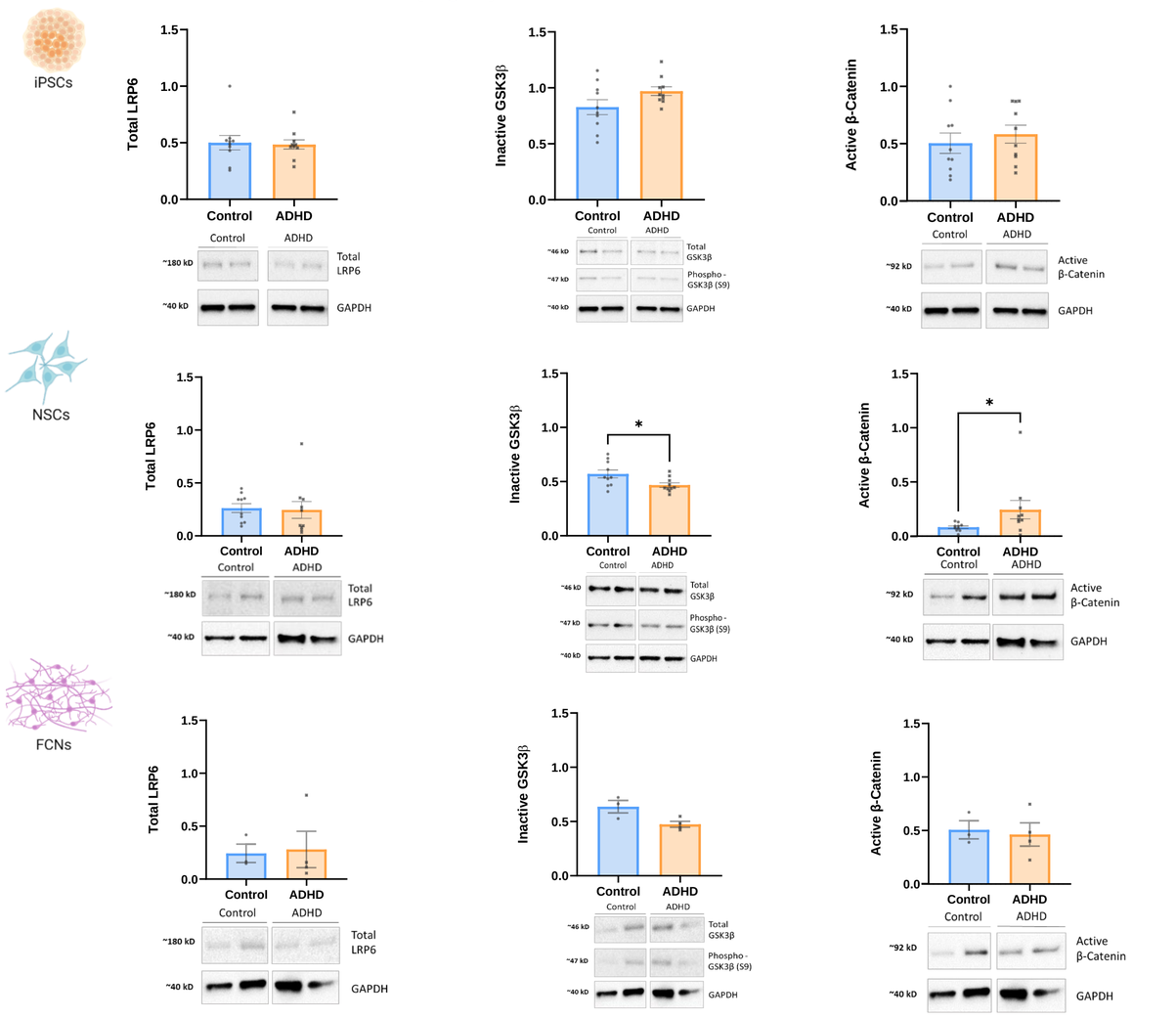

Figure 6. Protein expression of Wnt signaling components in iPSCs, NSCs and neurons. Significantly higher expression of active β-catenin (Mann-Whitney, **p*=0.0185) and lower inactive GSK-3β levels (Welch t test, **p*=0.0286) are seen in ADHD NSCs. For iPSCs and NSCs, N=10 / 5 for both control and ADHD groups, while N = 3 and 4 for controls and ADHD FCNs, respectively.

Preliminary findings of decreased synapses in ADHD (Fig. 7).

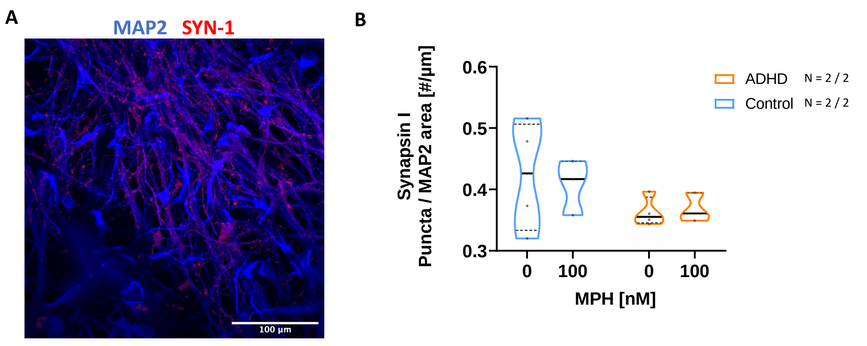

Figure 7. Synaptogenesis analysis with FCNs. A) The picture shows the exemplary line MR014 c12, after a 14-day treatment with MPH 100 nM and labelled with MAP2 (blue) and Synapsin-1 (red). Scale bar: 100 µm. B) After counting MAP2 and SYN-1 colocalizations, the analysis show a tendency of decreased number of synapses in ADHD, before and after MPH treatment.

CONCLUSION

- Proteomic analysis suggests altered **Wnt signaling activity in ADHD NSCs**
- **Functionally, ADHD NSCs seem to have a higher Wnt activity than controls**
- **ADHD NSC lines grow significantly less than controls**
- **MPH treatment restores proliferation in ADHD NSCs by modulating the Wnt signaling pathway**
- **Synapses might be decreased in ADHD**, compared to controls
- These unbalances might be associated with **impaired neurodevelopment in ADHD**

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