Investigating the effects of olanzapine, risperidone and haloperidol using an induced pluripotent stem cell based model of human hippocampal neurogenesis

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Background

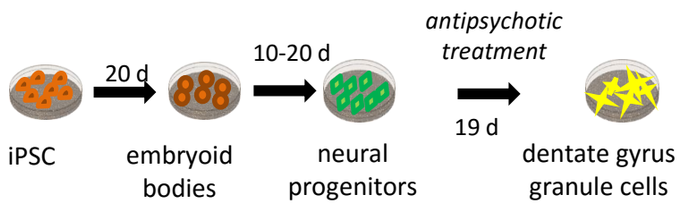
- Induced pluripotent stem cells (iPSCs) derived from postmitotic somatic cells and differentiated into neural lineage provide a unique opportunity to study
  - neural differentiation,
  - neuropsychiatric disorders,
  - current and novel pharmacological agents.
- Animal and postmortem studies suggest that antipsychotics influence memory functions, neuro- and gliogenesis, and the neural gene expression in various, poorly understood ways.
- Hippocampal dentate gyrus is one of the two regions where adult neurogenesis occurs in the human brain. Developing granule cells are crucial for learning and cognitive functioning.

Aims

1. Targeted differentiation of PROX1 expressing dentate gyrus granule cells from human iPSCs using an established protocol.
2. Investigation of the effects of antipsychotic agents (risperidone, olanzapine and haloperidol) on the gene expression of differentiating neurons.

Methods

1. Human iPS cells 6/2/F were differentiated into PROX1 positive dentate gyrus granule cells using the protocol of Yu et al. (2014).



2. During the 19-day-long differentiation, developing dentate gyrus granule cells were treated with antipsychotics.

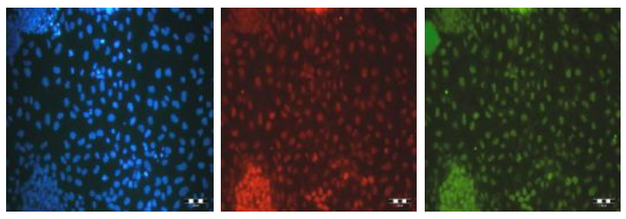
treatment	group	conc.
haloperidol	HL_{low}	0,003 µM
	HL_{high}	0,03 µM
olanzapine	OL_{low}	0,16 µM
	OL_{high}	1,6 µM
risperidone	RP_{low}	0,24 µM
	RP_{high}	2,4 µM
DMSO	CNT	0,2 µl/ml

3. Cell characterization
   - iPSC cultures were investigated by fluorescence activated cell sorting (FACS) on the base of SSEA4 positivity and immunocitochemistry (ICC) for stem cell markers NANOG and OCT3/4.
   - mature neurons were evaluated by ICC for neural marker MAP2 and dentate gyrus granule cell specific PROX1.
4. RNA isolation with Trizol → reverse transcription using random hexamer primers → TaqMan qPCR
5. Data analysis
   - gene expression alterations were calculated by the ΔΔCt method
   - A gene was considered differentially expressed if $|\Delta\Delta Ct| \geq 0.3785$ and *p*-value ≤0.05. (Student's t-test, Bonferroni and Holms correction)

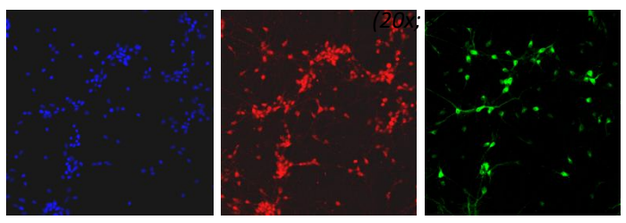
Cell Characterisation

The **iPSCs** showed stem cell like phenotype. FACS and ICC revealed that more than 95% of the cells expressed stem cell surface antigen SSEA4 and the colonies consisted of **OCT3/4** and **NANOG** positive cells. The pluripotency of the hiPSCs was also tested *in vitro* when we induced free-floating EB formation .

After 19-21 days, the differentiation protocol resulted in morphologically mature **neurons** expressing neural marker **MAP2** and **Prox1**. The latter is specific and essential for the survival and commitment of dentate gyrus neuronal progenitors and descendent granule cells .



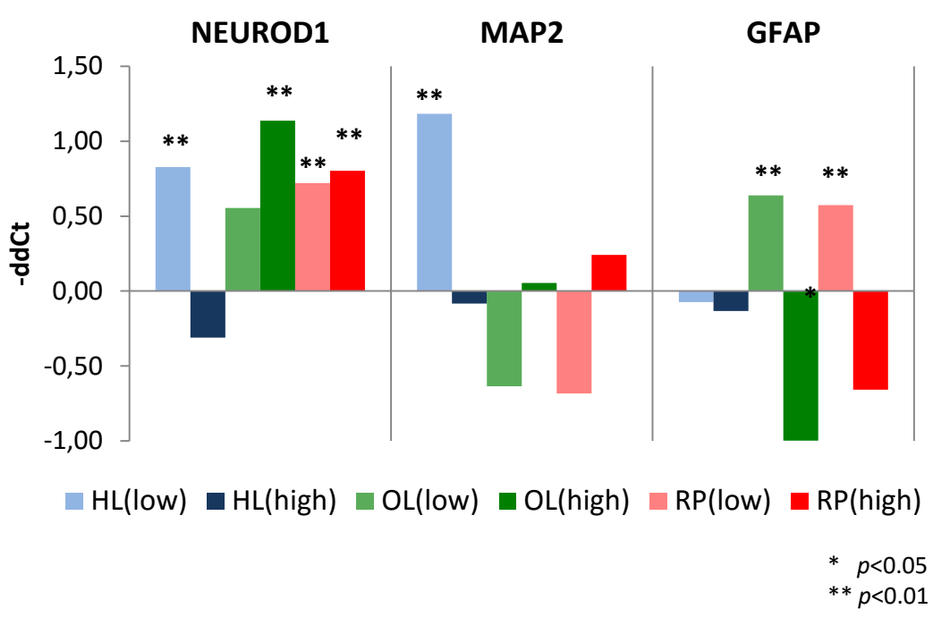
DAPI, OCT3/4, and NANOG staining of iPSC cell cultures.



DAPI, PROX1, and MAP2 staining of differentiated cultures (20x; scale bar 100 µm)

Antipsychotic-induced gene expression alterations

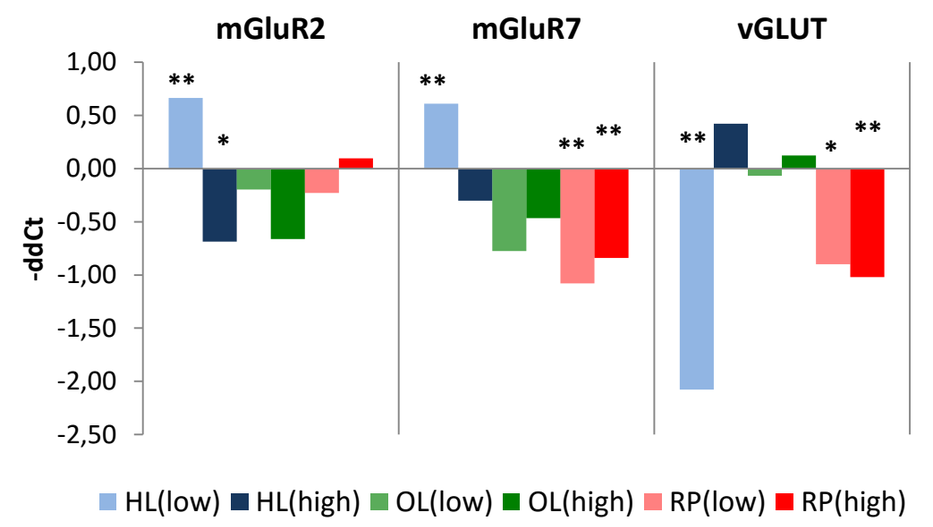
NEUROD1 was overexpressed after risperidone, high dosage olanzapine and low concentration haloperidol treatment.
MAP2 was modulated only by haloperidol at low concentration.
Risperidone and olanzapine exerted inverse effects on the ***GFAP*** gene at different concentrations (RP_{high} *p=0,091*).



* *p*<0.05
** *p*<0.01

NEUROD1 is essential for the differentiation and survival of dentate gyrus granule cells; thus our results might suggest – aligned with the previous literature – that antipsychotics facilitate cell survival only at certain concentrations. Haloperidol-induced **MAP2** overexpression might refer to active synaptic dynamics, functional and/or structural changes in the dendritic tree.
Elevated **GFAP** levels can refer either to astrocyte activation or increased number of glial elements. Given that the effect mechanism of antipsychotics on glial cells is understudied and thought to be human and region specific, hiPSC-derived cell lineages might be promising research model systems.

mGluR2 and ***mGluR7*** were both upregulated by haloperidol at low concentration and underexpressed in the risperidone and olanzapine treated cells.
vGLUT transcription was decreased by risperidone and low dosage haloperidol but not affected by olanzapine.



Our results – consistent with previous human and animal studies – suggest that risperidone and olanzapine might downregulate **mGluR2**, a potential new therapeutic target in schizophrenia, anxiety and depression. The atypical antipsychotics exerted similar effect on **mGluR7** - implicated in psychiatric genetics and modulation of GABAerg and dopaminerg circuits.
vGLUT1 orchestrates the synaptic vesicle cargos and influences directly the glutamate release, synaptic plasticity and postnatal neurodevelopment; however, our understanding on its psychopathological significance and interplay with current antipsychotic medication is highly deficient.

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