Assessment of spine density and brain morphological phenotype in three rodent models of neurodevelopmental psychiatric disorders, poly(I:C); two-hit poly(I:C); and prenatal MAM rat model

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Introduction

Prenatal infection and postnatal exposure to traumatizing experience are involved in the pathogenic processes of various neurodevelopmental psychiatric disorders such as schizophrenia. Dendritic spine number undergoes substantial changes during development. Spine-density loss and cortical thinning is observed in patients with schizophrenia during adolescence. Excessive spine pruning is thought to underlie the reduced cortical gray matter volume in schizophrenia (fig A). The aim of the present study was to compare spine density and neuroinflammation of 3 animals models. These data provide a way to differentiate these three prenatal perturbation models of schizophrenia in terms of their impact on synaptic density and cortical thickness.

Aim: to find efficacy model that measures spine density reduction to support our internal programs. Our goal for this program is to inhibit the loss of spine density observed in the cortical layers of schizophrenic patients (fig B).

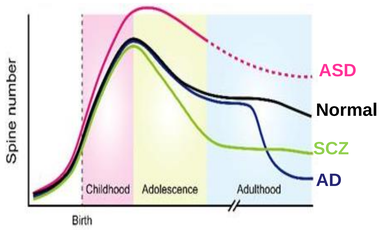


FIG A: Trajectory of spine number during life spam in different brain disease

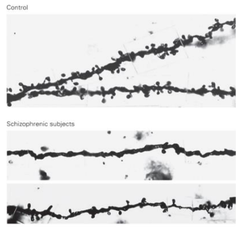


FIG B: Golgi-impregnated basilar dendrites and spines from healthy subject (top) and 2 schizophrenic patients (bottom)

Cytokines and chemokines mRNA expression

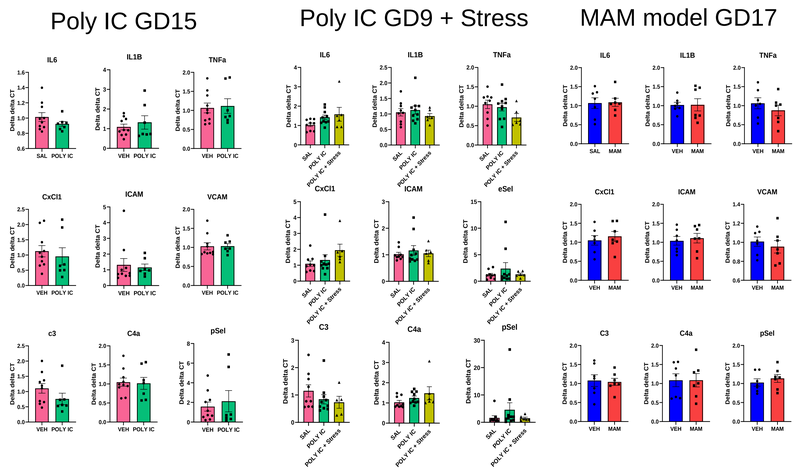


Fig 1: mRNA expression of cytokines (IL6, IL1β, TNFα), chemokines (Cxcl1, C3a, C4a) and adhesion molecules (pselectin, VACM, ICAM) in the prefrontal cortex of mice and rats from the different models. No difference in any of the evaluated mRNA were founded between VEH and treated animals.

Spine density

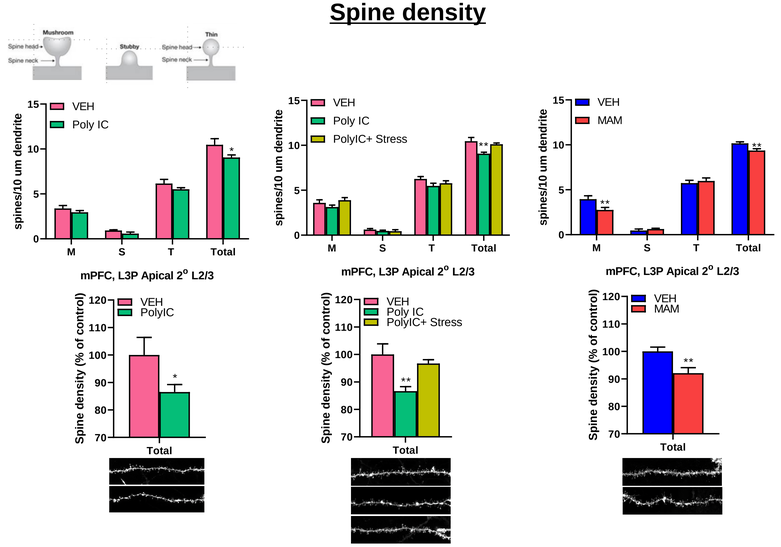
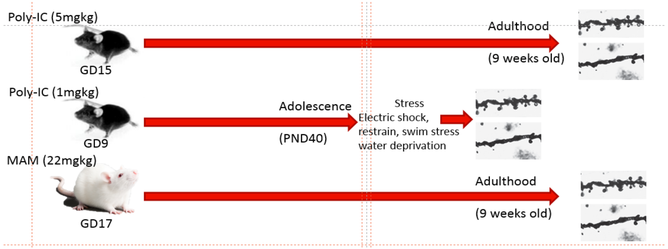


Fig 3: Spine density evaluation by Afraxis. We choose to measure spine density in the layer 2/3 of medial prefrontal cortex (mPFC). 3 different type of spines were measure (mushroom, stubby and thin). PolyIC alone in the adult mice (left panel) or in the adolescent mice (middle panel) decreased spine density by approximately 15%. MAM-treated rats, also showed reduction of spine density by 10%. *p<0.05, **p<0.01vs VEH treated animals

Material and methods

3 animal models were assessed: 1. C57BL/6J mice born from dams infected with polyriboinosinic-polyribocytidylic acid (poly I:C; 5 mg/kg ip on gestational day 15), 2. C57BL/6J mice born from dams infected with a subthreshold exposure of polyriboinosinic-polyribocytidylic acid (1 mg/kg iv gestational day 9) that were subsequently exposed to unpredictable stress, and 3. Sprague-Dawley rats born rom dams infected with methylazoxymethanol acetate (MAM) on gestational Day 17. Cohort 2 was exposed to a sub-chronic unpredictable stress between post-natal day (PND) 35 and PND 43, a period which corresponds to the peripubertal period. Rats of cohort 3 and mice of the cohort 1 were sacrificed at 9 weeks of age; mice of the cohort 2 were sacrificed at 6 weeks of age. The brains and blood were harvested and used for several analyses: 1) Spine density, 2) Markers of inflammation (TNF-alfa, Il1b, IL6, C3, C4).



Sub-chronic unpredictable stress

Days	Stressors	Description
1	Electric foot shock	12 min 3 shocks
3	Restrain stress	45 min
5	Food deprivation	20h from 12 pm to 8 am
7	Forced swimming test	18° C water two 1-min, 2 min apart
9	Repeated changing of home cage	5 changes irregular interval

Cytokines and chemokines in plasma

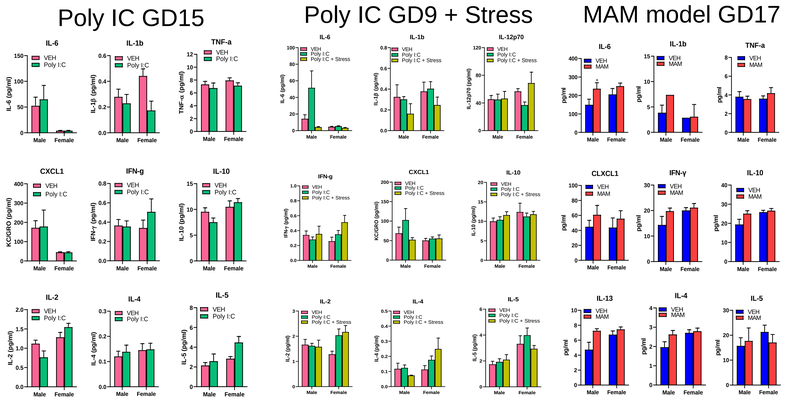


Fig 2: plasma concentration of cytokines (IL6, IL1β, TNFα, INFγ, IL10, IL2, IL4 and IL5), chemokines (Cxcl1) in mice and rats from the different models. No difference in any of the evaluated mRNA were founded between VEH and treated animals.

Conclusion:

- **These experiments revealed the possibility to use these models to study synaptic pruning**
- **All 3 of them showed a reduction in spine density induced by the maternal insult.**

Further analysis and future approaches:

- **Study time course of spine density reduction will be our next step**
- **We are planning to analyze the microbiome of these treated animals with Holobiome to asses whether any of the interventions have an impact on both microbiome community structure and function, which may provide targets for future studies or biomarkers that can be comparable with human data**

Acknowledgment

Holobiome

afraxis