ONTOGENY OF COGNITIVE IMPAIRMENT FOLLOWING PRENATAL INFECTION.

J. Richetto[1], U. Meyer[2], F. Calabrese[1], G. Racagni[1], J. Feldon[3] and M. A. Riva[1]

[1] Center of Neuropharmacology, Dept. Pharmacological Sciences, University of Milan, via Balzaretti, 9, 20133 Milan (Italy). [2]Physiology and Behavior Laboratory, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland. [3]Laboratory of Behavioral Neurobiology, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland

INTRODUCTION

Various retrospective and prospective epidemiological studies, as well as experimental rodent models, highlight a significant association between prenatal maternal infection and elevated risk of developing neuropsychiatric and neurological diseases. On these bases, various animal models have been used to investigate how prenatal infection could actually cause transient or lasting changes in CNS function, and what behavioural and cognitive alterations this determines in the offspring. However, little is know about the putative roles of maternal postnatal factors in triggering and modulating the emergence of psychopathology following prenatal immunological manipulations. In particular, the ontogeny of the behavioural alterations, and specifically of the cognitive impairment observed in the offspring, still remains unknown. Thus, objective of our study was to dissect the relative contributions of prenatal inflammatory events and postnatal maternal factors in precipitating cognitive abnormalities observed in the resulting offspring, using a cross-fostering design. For the cognitive phenotyping of our animals we focused on working memory (WM), a core feature of the cognitive impairments found in schizophrenia. In particular we used two tests, the *Dry Maze* and the *Y-Maze*. Moreover, we also investigated locomotor response to amphetamine challenge, as amphetamine sensitivity can be seen as a marker of the positive symptoms of schizophrenia. We thus investigated the possible molecular mechanisms underlying these abnormalities, focusing on the GABA system, which is pivotal for correct working memory functions. In particular, we analyzed the gene expression levels of two important GABAergic markers: Reelin (RELN) and GAD67.

MATERIALS and METHODS

ANIMALS & TREATMENT

Pregnant mice were injected on gestation day (GD) 17 with PolyI:C (5 mg/kg, i.v.) or vehicle (saline). On the day of birth [postnatal day (PND) 0], all the offspring were cross-fostered to surrogate rearing mothers. Half of a given litter was placed with a PolyI:C-treated surrogate rearing mother and the other half with a vehicle-treated rearing mother. Each surrogate mother thus simultaneously fostered pups originating from both prenatal treatment conditions, but not its own. The cross-fostering resulted in four experimental treatment groups: (1) offspring subjected to prenatal vehicle exposure and raised by a vehicle-treated surrogate mother (SAL-SAL), (2) offspring subjected to prenatal vehicle exposure and raised by a PolyI:C-treated surrogate mother (SAL-POL), (3) offspring subjected to prenatal PolyI:C exposure and raised by a vehicle-treated surrogate mother (POL-SAL), and (4) offspring subjected to prenatal PolyI:C exposure and raised by a PolyI:C- treated surrogate mother (POL-POL). The offspring were then subjected to behavioural and cognitive testing in adolescence (PND35-PND42) and adulthood (PND70-PND77).

ANALYSIS OF RNA AND PROTEIN LEVELS

The brain areas of interest (Prefrontal Cortex and Hippocampus) were dissected and used for the isolation of total RNA. The analysis of RELN and GAD67 mRNA levels was performed by Real-Time Quantitative PCR.

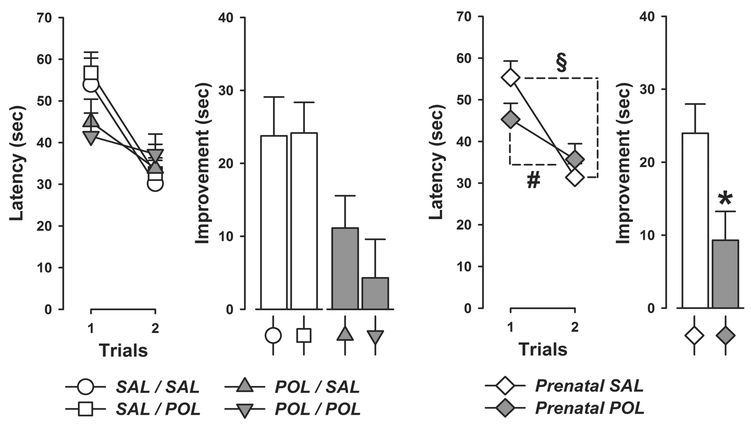
STATISTICAL ANALYSIS OF DATA

Statistical analysis of the molecular data was performed by two-way ANOVA with SCPHT, while the behavioural results were analized with parametric ANOVA, followed by Fisher's LSD post-hoc comparisons or restricted ANOVAS whenever appropriate. Data has been expressed as percentage versus SAL/SAL control group (100%). Significance for all tests was assumed at least at $p<0.05$.

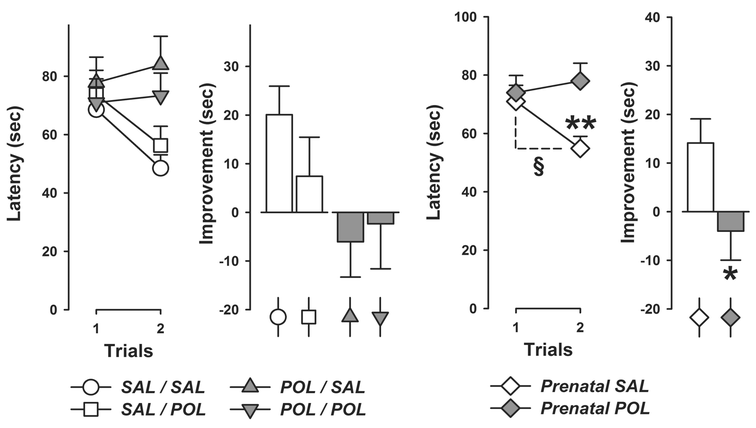
BEHAVIOURAL ANALYSES

Spatial Working Memory (Dry Maze)

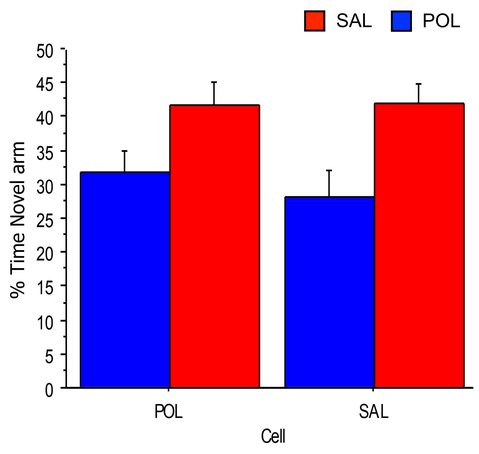
Adolescence



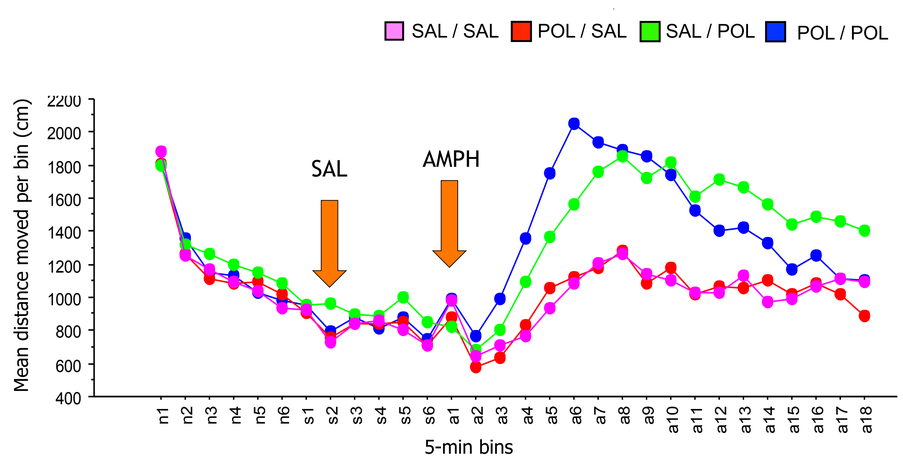
Adulthood



Spatial Working Memory (Y-Maze)



Amphetamine Sensitivity

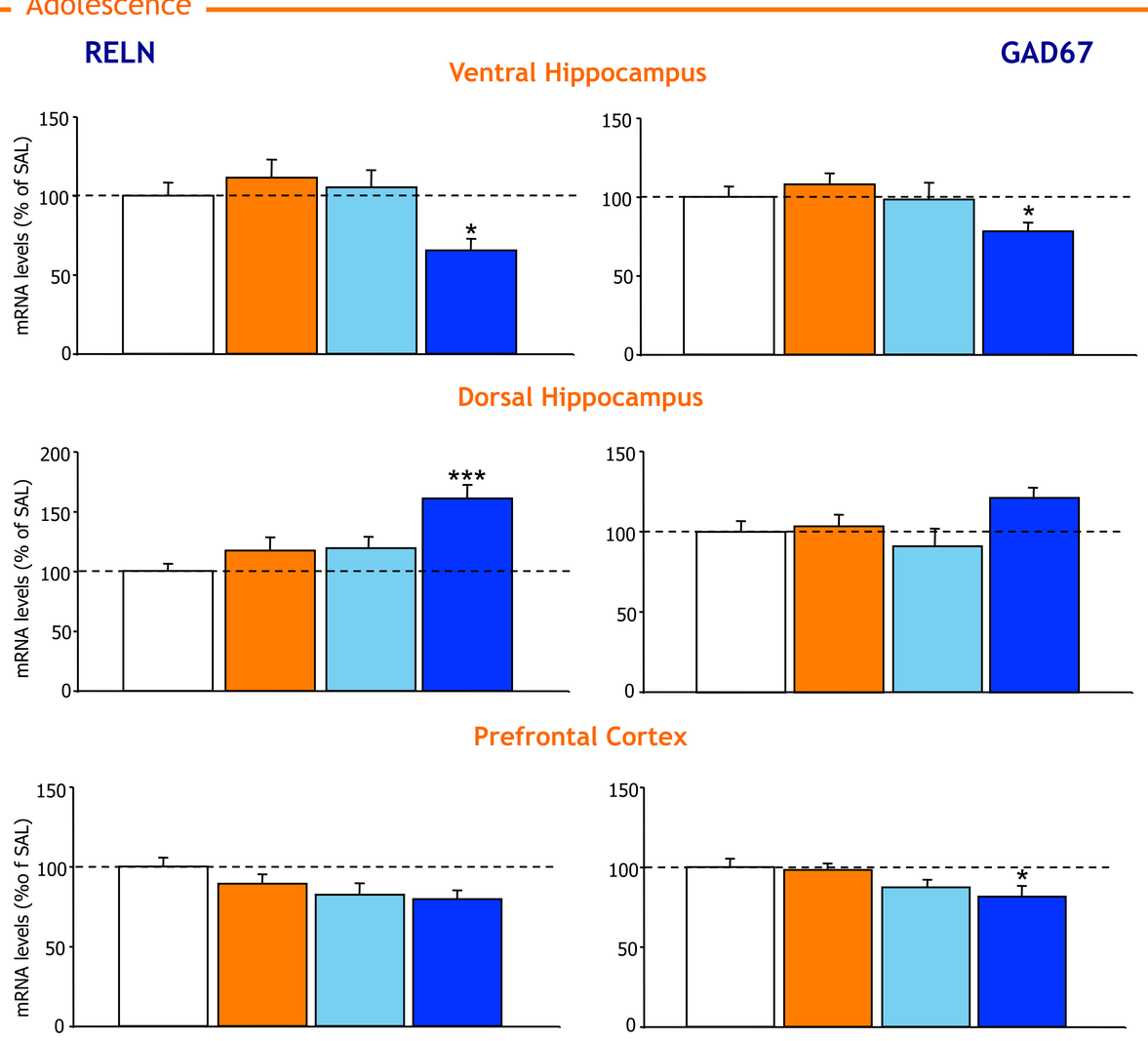


Results

- The cognitive abnormalities emerging in the offspring are independent from the postnatal adoption procedure, and are manifest **only** in animals subjected to **prenatal PolyI:C.**
- The cognitive impairment is present both in adolescent and adult animals.
- On the other hand, response to amphetamine challenge was influenced, in adolescence, by postnatal maternal factors, while in adulthood the effect extended to all animals except the offspring subjected to prenatal vehicle exposure and raised by a vehicle-treated surrogate mother.

MOLECULAR ANALYSES

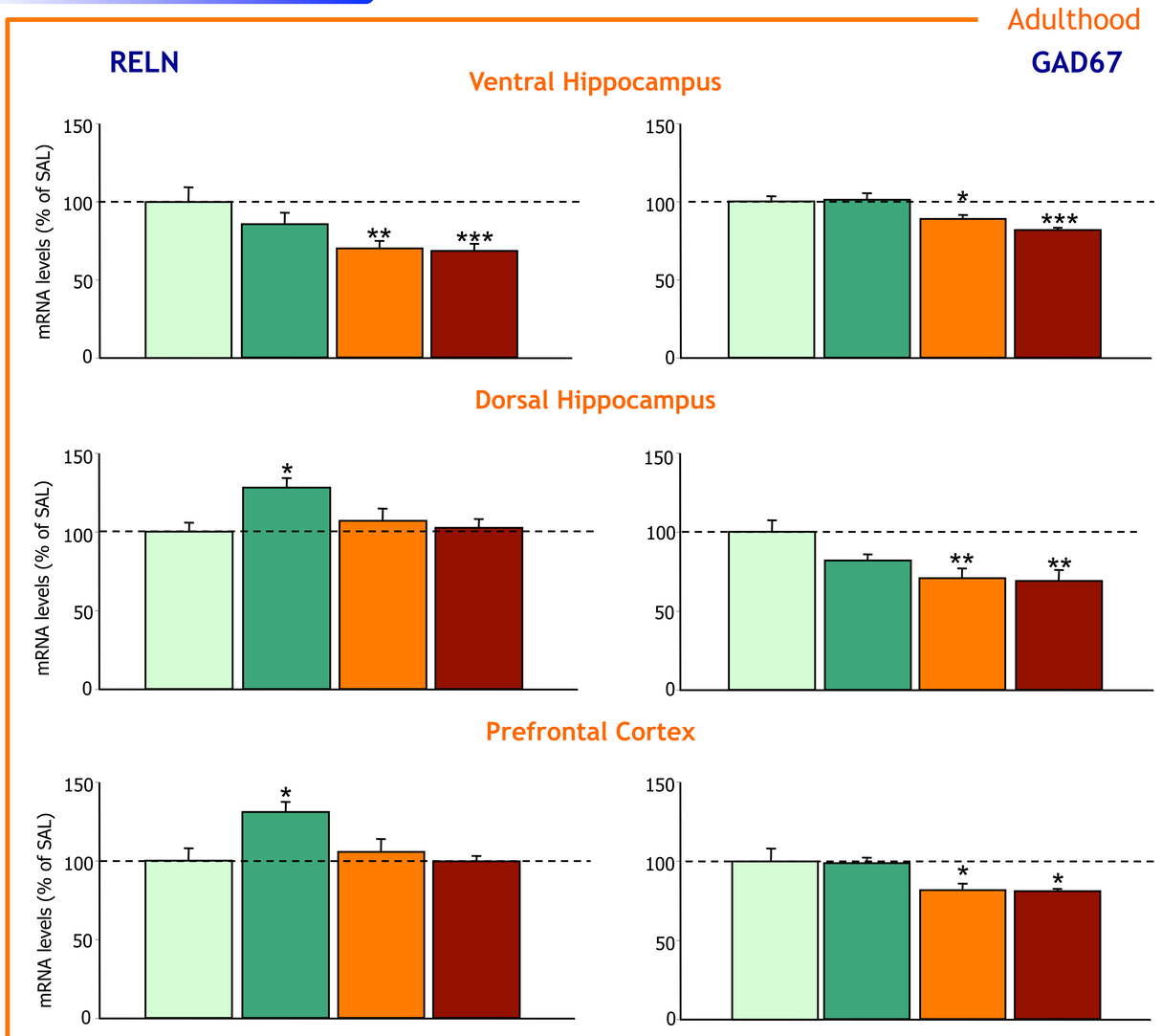
Adolescence



RELN Gene expression levels are reduced in the HV and in the PFC (in this area the effect does not reach statistical significance) of the POL/POL group, and increased in the HD of these same animals. **GAD67 Gene expression levels** are also reduced in the HV and PFC of the POL/POL animals.

The data represent the average ± sem of at least 10 animals per group expressed as % of vehicle injected mice
*p<0,05 vs SAL/SAL, ***p<0,001 vs SAL/SAL

Adulthood



RELN Gene expression levels are reduced in the HV of the POL/SAL and POL/POL group, and increased in the HD and PFC of the SAL/POL group. **GAD67 Gene expression levels** are reduced in all the three brain regions of the POL/SAL and POL/POL animals.

The data represent the average ± sem of at least 10 animals per group expressed as % of vehicle injected mice
*p<0,05 vs SAL/SAL, **p<0,01 vs SAL/SAL

Results

- The cognitive abnormalities are paralleled by concomitant alterations in the gene expression levels of GABA markers, result in line with studies conducted in schizophrenic patients.
- These alterations are present already in adolescence, in particular in the POL/POL group, and persist into adulthood, where we see a worsening of the effect.

CONCLUSIONS

Prenatal immunological manipulation is responsible for the impaired cognition found in our animals, while AMPH sensitivity is affected by postnatal factors, suggesting that different components of schizophrenia are influenced by alternative factors. The cognitive deficits are paralleled by concomitant **reduction in the expression of GABA markers**, in line with clinical results. All the effects emerge already in adolescence

No potential conflict of interest
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