Chronic stress changes expression of the axonal membrane glycoprotein M6a in the brain

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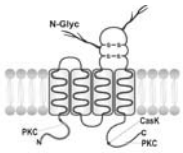
Introduction

Stress can induce psychiatric diseases and animal models of stress are used to study central nervous processes that may occur in the brains of depressed patients. Alterations in dendrites and spines of neurons in hippocampus and medial prefrontal cortex have been observed after stress in several species. These stress effects may change the integrity of neurons and impair information transfer between brain regions.

Here we report that stress also affects neuronal axons. We investigated expression of M6a, a membrane glycoprotein encoded by a gene which is associated with a form of depression detected in schizophrenic patients GPM6A (1). Our previous studies have shown that M6a expression is downregulated by stress in the hippocampus and that an antidepressant treatment prevents its downregulation (2, 3).

We provide data that M6a is specifically expressed in axons of glutamatergic neurons, and that stress reduces M6a expression in the hippocampus but not in the prefrontal cortex (4, 5).



The membrane glycoprotein M6a is a modulator of neurite outgrowth and spine formation in neurons. We here demonstrate that this glycoprotein is specifically expressed in axons of glutamatergic neurons (see Results).
N-Glyc, glycosylation residues; PKC, site of phosphorylation by PKC; CasK, site of phosphorylation by casein kinase.

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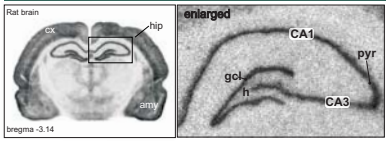
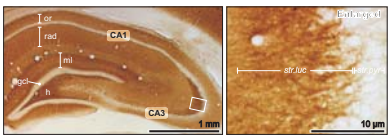
Results

M6a mRNA in limbic regions



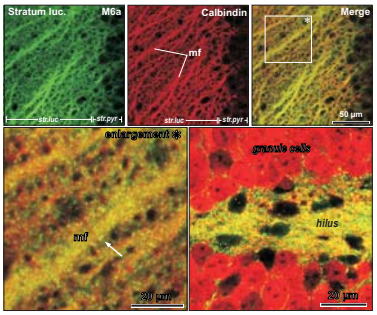
In situ hybridization shows that M6a mRNA is strongly expressed in the hippocampal formation (hip), in other limbic regions such as amygdala (amy), and in cortical regions (cx).
gcl, granule cells; h, hilus; pyr, pyramidal cells



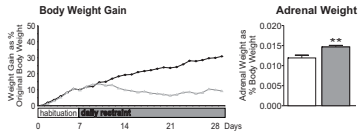
Immunocytochemistry shows that within the hippocampal formation, M6a protein is present in fibers, e.g. in the stratum lucidum (str. luc) where the mossy fiber axons terminate.
gcl, granule cells; h, hilus; ml, molecular layer; or, stratum oriens; rad, stratum radiatum;

Methods
Adult male Sprague Dawley rats were submitted to daily restraint stress in a plastic tube (6 h/day) during the dark phase, for 3 weeks (6).
M6a mRNA expression was localized in the brain by in situ hybridization. A rat monoclonal antibody against M6a (from MBL) was used for immuno-cytochemistry. Quantitative real time PCR with cyclophilin as reference gene was performed to quantify M6a mRNA.

M6a in mossy fiber axons

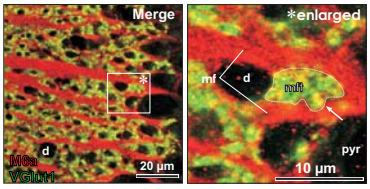


Double immunofluorescence shows that M6a (green) colocalizes with calbindin (red) within the hippocampal formation. The mossy fiber axons (mf) in the stratum lucidum strongly express the glycoprotein that is present in the axonal membrane.

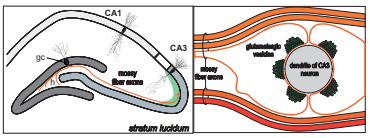


Daily restraint stress (6 h/day during dark phase) reduces body weight gain. The stress-induced increase in adrenal weight reflects the activation of the HPA axis.

M6a in glutamatergic axons

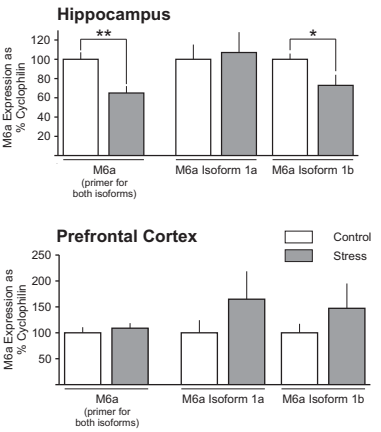


Double immunofluorescence shows that M6a (red) is present in the mossy fiber axons and partially colocalizes with the vesicular glutamate transporter 1 (VGlut1, green) that is exclusively present in glutamatergic nerve terminals. The arrow indicates a site of colocalization, probably where the synaptic vesicles (green) fuse with the terminal membrane that contains M6a (red).



The glutamatergic mossy fiber axons (red, left) terminate in the stratum lucidum (green area) where they form giant synapses with the dendrites of the CA3 pyramidal neurons (right).

Stress downregulates M6a



Quantitative real-time PCR shows that chronic stress reduces expression of M6a isoform 1b in the hippocampal formation, but not in the prefrontal cortex.

Conclusions

The present study shows for the first time that chronic stress reduces expression of a glycoprotein that is located in the axonal membrane of glutamatergic neurons.
Reduced M6a expression might in particular affect the integrity of the projections from the granule cells to the CA3 pyramidal neurons. This coincides with previous findings showing that chronic stress changes the morphology of the giant mossy fiber terminals.
The observed changes may contribute to the inhibition of long-term potentiation that has been recorded in the hippocampus after stress.

References

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