New evidence of specific synaptic remodelling by corticosterone-treated astrocytes conditioned medium

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Introduction

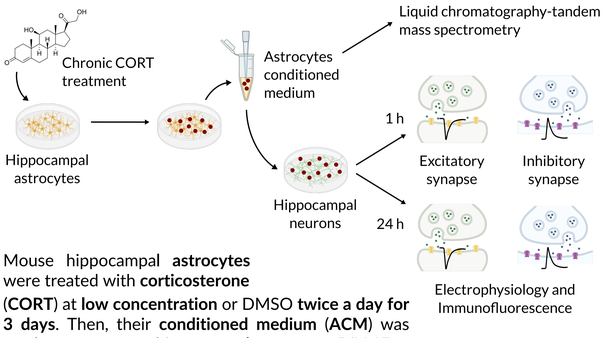
Major depressive disorder (**MDD**) is a debilitating multifactorial neuropsychiatric syndrome, representing the leading cause of disability worldwide with severe social and economic consequences. Stress exposure has been recognized as the main risk factor and more in detail, an individual's ability to cope with stress in an adaptive way can determine their resilience or vulnerability to MDD development.
MDD is characterized by many alterations among which, in most patients, **hyperactivity of the hypothalamic-pituitary-adrenal** (**HPA**) **axis** and **glucocorticoid resistance**. In patients with severe depression, increased levels of serum and salivary cortisol have been found.

Astrocytes are glial cells fundamental for the central nervous system, functioning as neuronal support and participating in the regulation of ion homeostasis, neurotransmission, synaptic plasticity and neuroinflammation. Evidence from both human post-mortem brains and animal models indicate the involvement of astrocytes in MDD pathophysiology.

Aim

To study new ways in which **glucocorticoids** and **astrocytes** participate in **MDD pathophysiology,** investigating how astrocytes exposed chronically to corticosterone can impact on **neuronal function** and **structure.**

Project layout



Mouse hippocampal **astrocytes** were treated with **corticosterone** (**CORT**) at **low concentration** or DMSO **twice a day for 3 days**. Then, their **conditioned medium** (**ACM**) was used to treat mouse hippocampal **neurons** at DIV 17 for either **1** or **24 hours**. Finally, excitatory and inhibitory synaptic function and structure were studied by (i) recording **miniature post-synaptic currents**, and by (ii) performing **immunofluorescence** experiments targeting pre-synaptic markers.

Results – 1 hour

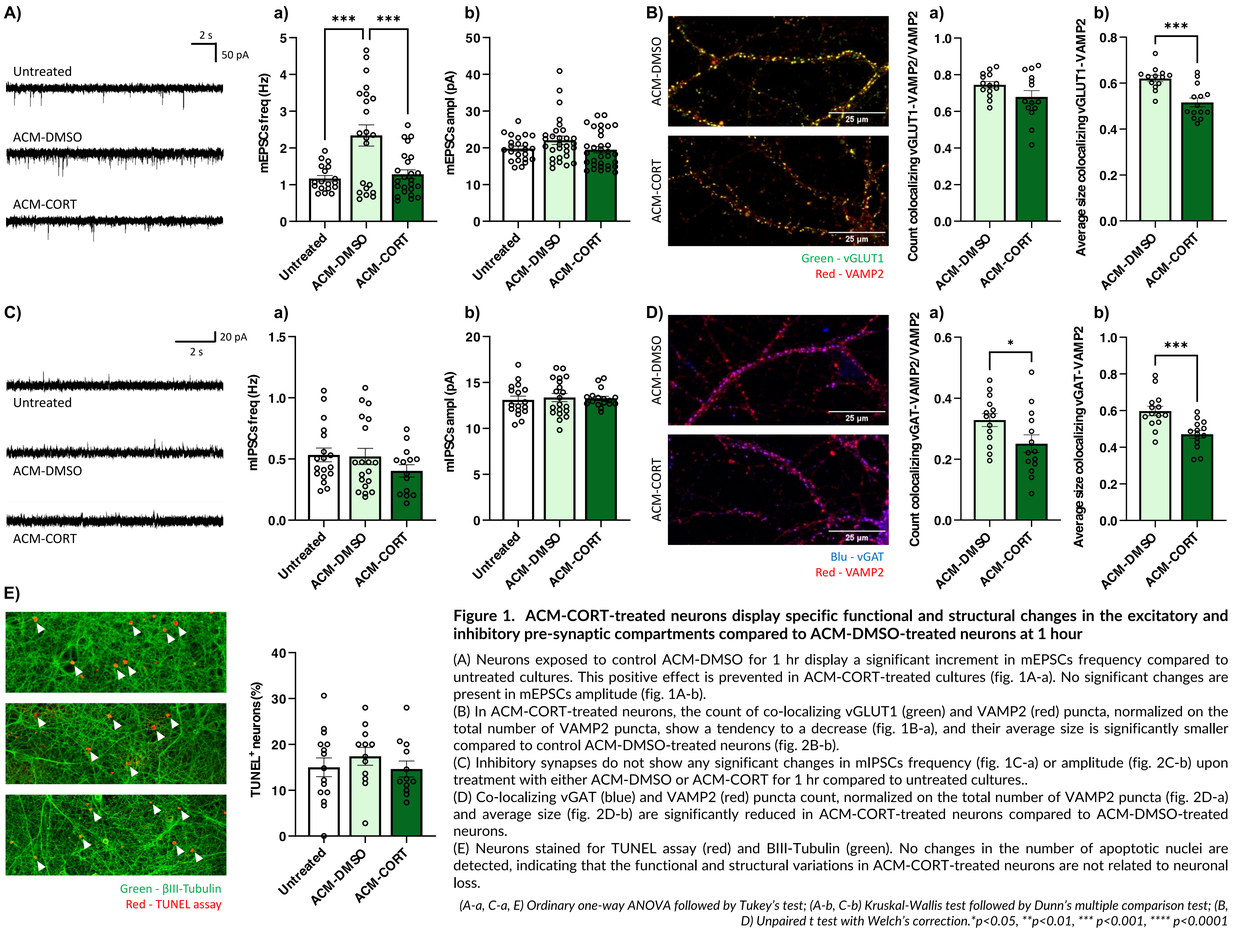


Figure 1. ACM-CORT-treated neurons display specific functional and structural changes in the excitatory and inhibitory pre-synaptic compartments compared to ACM-DMSO-treated neurons at 1 hour

(A) Neurons exposed to control ACM-DMSO for 1 hr display a significant increment in mEPSCs frequency compared to untreated cultures. This positive effect is prevented in ACM-CORT-treated cultures (fig. 1A-a). No significant changes are present in mEPSCs amplitude (fig. 1A-b).
(B) In ACM-CORT-treated neurons, the count of co-localizing vGLUT1 (green) and VAMP2 (red) puncta, normalized on the total number of VAMP2 puncta, show a tendency to a decrease (fig. 1B-a), and their average size is significantly smaller compared to control ACM-DMSO-treated neurons (fig. 2B-b).
(C) Inhibitory synapses do not show any significant changes in mIPSCs frequency (fig. 1C-a) or amplitude (fig. 2C-b) upon treatment with either ACM-DMSO or ACM-CORT for 1 hr compared to untreated cultures..
(D) Co-localizing vGAT (blue) and VAMP2 (red) puncta count, normalized on the total number of VAMP2 puncta (fig. 2D-a) and average size (fig. 2D-b) are significantly reduced in ACM-CORT-treated neurons compared to ACM-DMSO-treated neurons.
(E) Neurons stained for TUNEL assay (red) and BIII-Tubulin (green). No changes in the number of apoptotic nuclei are detected, indicating that the functional and structural variations in ACM-CORT-treated neurons are not related to neuronal loss.

*(A-a, C-a, E) Ordinary one-way ANOVA followed by Tukey's test; (A-b, C-b) Kruskal-Wallis test followed by Dunn's multiple comparison test; (B, D) Unpaired t test with Welch's correction.*p<0.05, **p<0.01, *** p<0.001, **** p<0.0001*

Results – 24 hours

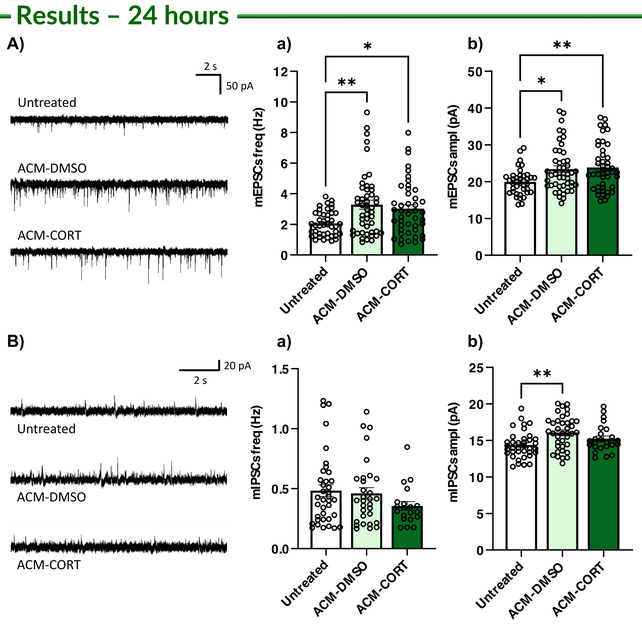
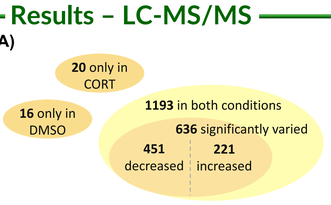


Figure 2. ACM-CORT-treated neurons display specific functional changes in the inhibitory pre- and post-synaptic compartment compared to ACM-DMSO-treated neurons at 24 hours

(A) Neurons treated with ACM-DMSO for 24 hrs still show significant increased mEPSCs frequency compared to untreated cultures. In this case, the same effect is present also in ACM-CORT-treated neurons (fig. 2A-a). A significant increase of mEPSCs amplitude is present upon both ACM treatments compared to untreated neurons (fig. 2A-b).
(B) After 24 hrs, ACM-CORT-treated neurons mIPSCs frequency show a tendence to decrease compared to both untreated and ACMD-DMSO-treated neurons (fig. 2B-a). Moreover, ACM-DMSO-treated neurons showed a significant increase in mIPSCs amplitude compared to untreated neurons which was not present in ACM-CORT-treated neurons (fig. 2B-b).

*(A-b, B-b) Ordinary one-way ANOVA followed by Tukey's test; (A-a, B-a) Kruskal-Wallis test followed by Dunn's multiple comparison test. *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001*

Results – LC-MS/MS



B)

Gene	CORT-DMSO	Student's T-test p-value
Igfbp3	3.252	0.000199
Ogn	2.857	3.46E-06
Serpina3n	2.743	9.24E-08
Ctsb	2.186	5.05E-06
Htra1	2.019	0.000187
Adamts1	1.839	8.52E-05
Vwa1	1.798	0.007528
Nbl1	1.703	0.00561
Lox	1.612	0.00015
Zp2	1.562	0.000495
Pdgfrl	-1.526	2.10E-05
Pla2g7	-1.568	0.000102
Xylt1	-1.579	1.18E-05
Reln	-1.598	4.69E-06
Metrn	-1.626	0.000561
Galnt16	-1.715	0.000286
Fgfrl1	-1.914	0.006875
Chadl	-1.992	2.80E-06
Ptn	-2.407	1.66E-05
Cdhr1	-3.415	1.07E-05
Gene	CORT-DMSO	Student's T-test p-value

C)

Ingenuity Canonical Pathways	-log(p-value)	z-score
Coronavirus Replication Pathway	4.81E+00	-2.449
cAMP-mediated signaling	1.62E+00	-2.449
Sertoli Cell-Sertoli Cell Junction Signaling	2.56E+00	-2.333
Ephrin Receptor Signaling	1.45E+00	-2.000
Amyotrophic Lateral Sclerosis Signaling	1.29E+00	-2.000
fMLP Signaling in Neutrophils	1.14E+00	-2.000
Gαi Signaling	1.06E+00	-2.000
Endocannabinoid Neuronal Synapse Pathway	9.81E-01	-2.000
Gαq Signaling	8.30E-01	-2.000
Signaling by Rho Family GTPases	6.62E-01	-2.000
Senescence Pathway	3.16E-01	-2.000

Figure 3. Results of liquid chromatography-tandem mass spectrometry (LC-MS/MS) with label-free quantification on ACM-DMSO and ACM-CORT

(A) Eulero-Venn diagram of proteins identified in ACMs.
(B) 10 significantly most up-regulated and down-regulated proteins in ACM-CORT compared to ACM-DMSO.
(C) Most down-regulated pathways in ACM-CORT compared to ACM-DMSO highlighted by ingenuity pathway analysis.

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Conclusions & Future perspectives

The **medium conditioned** by **astrocytes** treated **chronically** with **CORT** has a **toxic effect** on **synapses function** and **structure** and differentially affects the excitatory and inhibitory pre- and post-synaptic compartment at 1 hour and 24 hours.

Understanding exactly which **factors** released from CORT-treated astrocytes are impacting on neurons will allow to better understand how astrocytes and glucocorticoids are involved in **MDD pathophysiology** and potentially be targeted to discover new treatments

References

"Depression." World Health Organization
M. H. Han and E. J. Nestler 2017, Neurother.
X. Zhou et al. 2019 Front. Mol. Neurosci.
L. S. Nandam et al. 2020, Front. Psychiatry