Acute stress and corticosterone increase the readily releasable pool of glutamatergic vesicles in rat synaptosomes of prefrontal/frontal cortex via mineralocorticoid and glucocorticoid receptors

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Stress and its mediators were shown to cause short- and long-lasting functional/structural changes in the brain, which may trigger neuropsychatric disorders. In this regard, changes in glutamate release/transmission were shown to play a primary role in the stress response (Popoli et al., 2012). We have previously found that Footshock (FS)-stress induces a marked increase of serum corticosterone (CORT) and of depolarization-dependent glutamate release from synaptosomes of prefrontal/frontal cortex (PC/FC), via activation of glucocorticoid receptor (GR) and SNARE complexes accumulation in synaptic membranes. The increase of glutamate release was prevented by chronic antidepressants (Musazzi et al., 2010). Main aim of the present work was to verify if the changes induced by stress on glutamate neurotransmission were mediated by a synaptic (non-genomic) effect of corticosterone (CORT), comparing the effects of acute stress *in vivo* with that of *in vitro* application of CORT on purified synaptosomes.

We found that FS-stress increases the size of the readily releasable pool (RRP) of vesicles, measured as hypertonic sucrose-evoked glutamate release from PFC/FC synaptosomes. This effect was abolished by selective antagonists for mineralcorticoid receptor (MR) or GR (spironolactone (SPIR) and RU486 respectively). In order to verify if acute stress was able to change the distribution of vesicles in excitatory synaptic terminals of medial PFC (mPFC), we used a stereological approach for synaptic vesicles quantification. We counted the number of total vesicles and the number of vesicles with their membrane overlapping with the presynaptic, in excitatory, perforated and non-perforated mPFC synapses from control and FS-stressed rats. We found that acute stress induced a significant increase in the number of docked vesicles, selectively in perforated synapses but not in non-perforated ones; these data are in line with our results with hypertonic sucrose-evoked release, and suggests that acute stress selectively affects the RRP size in perforated synapses. To investigate whether acute synaptic exposure to the stress hormone CORT is able to directly and rapidly modulate glutamate release/transmission as observed after acute stress, we first investigated the effects of CORT on glutamate release in the absence of stimulus. We superfused synaptosomes with CORT for 10 min and we found a slightly increased spontaneous glutamate release. Then, we measured the effect of acute CORT application by evoking glutamate release in vitro using depolarizing stimulus or hypertonic sucrose and contrary to what previously observed after acute stress, acute CORT affected only sucrose-evoked glutamate release via activation of MR and GR, but did not alter neither depolarization-dependent glutamate release from PFC/FC synaptosomes, nor changes evoked postsynaptic currents in medial PFC slices, suggesting that, despite the CORT-induced increase of RRP size, the increase of glutamatergic transmission implies also other mechanisms. Moreover, experiments using total internal reflection microscopy (TIRFM) demonstrated that CORT increases vesicle mobilization toward the RRP, via activation of presynaptic MR and GR. Finally we found that both stress and acute CORT increase the phosphorylation of synapsin I, selectively at site 1 in presynaptic membranes. The increase of phosphorylation of synapsin I was blocked by SPIR and RU486, suggesting that this effect is downstream of MR and GR activation.

The present results suggest that, despite CORT plays a key role in the modulation of glutamatergic neurotransmission induced by acute stress in PFC/FC, its local non-genomic action on synaptic receptors is necessary but not sufficient for increasing glutamate transmission.

Popoli et al. (2012). *Nat Rev Neurosci.* 13, 22-37.
Musazzi et al. (2010). PloS ONE. 5, e8566.