A0764 The insula, a key brain area for bladder pain control, is modulated by stress

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Introduction & Objectives: Stress may cause or aggravate Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) symptoms. Thus, stress models are being refined to be used as systemic in counterpoint to bladder centric animal models We hypothesised that different stress insults modulate the insula activity diversely.

Materials & Methods: Two models were used in female Wistar rats, Maternal Deprivation Model (MDM, stress applied to new-borns and studies done at 6M) and Water Avoidance Stress test (WAS, stress is applied to 6M rats for 1h every 10 days and studies carried afterwards). Mechanical pain threshold was evaluated in L6/S1 dermatomes which share the spinal cord segments of bladder sensory innervation. Bladder function was evaluated by cystometry. Then Insulas were removed. One was immersed in RNAlater, homogenized, RNA extracted, and expression of different biomarkers assessed by real-time PCR. The other insula was immunoreacted against microglia markers Iba1 and Cd68. The analysis of positive cells used LASAF and ImageJ software. Morphometric analysis and 3D reconstructions used IMARIS software.

Results: MDM decreased mechanical (p=0.0457) and thermal (p=0.0273) pain threshold in L6-S1 dermatomes. Bladder hyperactivity was present in 2/3 of the rats. C-fos mRNA expression decreased (p=0.0036), while there were no changes in the expression of Ccr2, Pdyn, Olig2, GFAP, Iba1, cd68, Sst and TNFa mRNA (p>0.05). The morphological analysis of the anterior insular cortex revealed a decrease in the number of microglia cells, that presented less ramification (sholl analysis, $p < 0.0001$) although with a maintained length (p>0.05). The microglia cells presented an increase in CD68 immunoreactivity (p=0.0074). WAS decreased mechanical (p=0.0055) and thermal (p=0.0390) pain threshold in L6/S1 dermatomes. All animals presented bladder hyperactivity ($p = 0.0079$). An increased expression of Ccr2 mRNA ($p = 0.0274$) and a decreased expression of Pdyn ($p = 0.0213$), Olig2 ($p = 0.0005$) and GFAP ($p = 0.0350$) mRNA occurred. There were no changes in the expression of Iba1, cd68, c-fos, Sst and TNFa mRNA ($p > 0.05$). Microglia cells increased in number ($p = 0.0138$) and had a bigger soma size ($p = 0.0021$) with less ramifications (sholl analysis, $p < 0.0001$) but preserved branch length (p>0.05). Microglia cells did not present changes in CD68 immunoreactivity (p>0.05).

Conclusions: Stress-induced changes in the female rat insula depend on the moment it occurs. MDM induce long lasting changes in the insula by decreasing neuronal activity and increasing microglia phagocytic activity, as demonstrated by the increased CD68 staining. WAS induces neuroinflammation and changes in neuronal and glial cells. These findings should be taken in consideration when choosing a stress models as systemic (non-bladder centric) animal models to study BPS/IC-like symptoms.