

REGIONAL FMRI ACTIVATION IN RESPONSE TO VISCERAL STIMULATION IN RATS WITH AMYGDALA IMPLANTS OF CORTICOSTERONE

J. Lazovic¹, A. C. Johnson², B. Myers², R. Towner³, and B. Greenwood-Van Meerveld²

¹Biology, California Institute of Technology, Pasadena, CA, United States, ²University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, ³Oklahoma Medical Research Foundation, Oklahoma City, OK, United States

INTRODUCTION: Visceral pain is clinically one of the most common complaints. Irritable bowel syndrome (IBS) is characterized by visceral pain and irregular bowel movements. It is estimated that IBS affects 10-22% adults, with a high female to male ratio. Frequently, episodes of visceral pain coincide with specific stressful event(s) or increased anxiety suggesting parallel activation of higher cognitive areas with pain processing circuits. The amygdala is thought to play a crucial role in the generation and propagation of fear and anxiety [1]. Furthermore, the central nucleus of the amygdala has been shown to facilitate activation of hypothalamic-pituitary-adrenal axis (HPA) in response to stress and increase corticotrophin release factor (CRF) [2]. The aim of this work was to examine brain activation in response to visceral pain and increased anxiety as modeled by corticosteroid implants delivered bilaterally to amygdale.

METHODS: Male Fisher rats 2-3 months old (250-310 g) were used for all experiments. Rats were anesthetized with ketamine/xylazine mixture (100 mg/kg/ 10mg/kg *ip*) prior to stereotaxic surgery. A 25-gauge cannula was used to deliver 30 µg micropellet of corticosterone (CORT, N=6 rats), and 30 µg of cholesterol (controls) (CHOL, N=5 rats) to the dorsal margin of amygdale coordinates (-2.5 mm AP, 4.2 and -4.2 mm ML (bilateral), -7.0 mm DV). Animals were then allowed 7 days to recover. Prior to imaging rats were anesthetized with chloral hydrate (400 mg/kg *ip*). Respiration was monitored during the imaging, and when necessary additional anesthesia was delivered (via *ip* catheter). A 5-cm long latex balloon was inserted into the colon and secured with surgical tape around the tail. The fMRI studies were performed on 7T Bruker System, with S116 gradient coil, and combination of volume coil for excitation and quadrature receive-only surface coil. Functional images were acquired using T_2^* -weighted gradient echo sequence in axial plane to minimize signal loss due to susceptibility artifact (TR/TE:160/14 ms, 64X64 matrix, 4X4 cm² FOV, 8 slices, 1.2 mm slice thickness, 1 average, 10 s time for 1 scan). Anatomical T_2 -weighted images were acquired using RARE sequence (TR/TE_{eff}:2500/50.78 ms, matrix size 128X128) with the same slice thickness and position as gradient echo images. Colorectal distension (CRD) was induced by inflating the balloon using a constant pressure barostat (Model IIR, G&J Electronics, Toronto, Canada) synchronized with fMRI acquisition. The stimulation paradigm consisted of 8 cycles of 90 s baseline period (9 scans) with balloon deflated (0 mmHg), followed by a 30s activation period (3 scans) with the balloon inflated to 40 mmHg and 60 mmHg pressures. The fMRI data post processing and analysis were performed using the CCHIPS software [3]. Image co-registration and motion corrections were done with a pyramid co-registration algorithm [4]. Images were discarded in the case of observed gross motion. Statistical analysis was done with cross-correlation ($r=0.4$ lower threshold and $r=1.0$ upper threshold). Fisher's exact test was used for between group comparison.

RESULTS: The fMRI activation was examined in N=6 rats with CORT implants, and N=5 rats with CHOL implants in response to 40 and 60 mmHg balloon pressure as a noxious stimulus. For both groups there was a pressure-dependent progressive increase in the total pixels activated (CHOL: 40 mmHg=188, 60 mmHg=1042; CORT 40 mmHg=1277, 60 mmHg=1962). Regional analysis of brain activation revealed increased activity in the regions involved in the processing of emotion and pain. In CORT implanted animals exposed to a 40 mmHg visceral stimulus, significant activation was observed in amygdala, insular cortex, cingulated cortex, hippocampus and associated cortical areas (including perirhinal, ectorhinal and entorhinal cortices). Increased activation was also found in the somatosensory cortex, thalamus and sensory processing areas (inferior and superior colliculi, piriform cortex). Additionally, brain regions that form descending efferent pathways such as the motor cortex, basal ganglia, and cerebellum were also activated. Compared to 40 mmHg, CORT implanted animals exposed to 60 mmHg stimulus showed an increase in activated areas including additional limbic structures (hypothalamus and orbitofrontal cortex). Furthermore, specialized nuclei (serotonergic raphe nucleus, and dopaminergic/cholinergic tegmental area) were also only activated at 60 mmHg, **Fig. 1**. In contrast most CHOL implanted animals did not show any activation in response to 40 mmHg. Similarly, there was also significantly less activation associated with 60 mmHg pressure compared to CORT implanted animals in amygdala, somatosensory cortex, hippocampus, entorhinal and ectorhinal cortices, thalamus and cerebellum ($p<0.01$, Fisher's exact test).

DISCUSSION: Presented data suggest corticosterone delivery to amygdale results in differential regional brain activation in response to noxious visceral stimulus in anesthetized rats. We propose that processing of visceral pain in higher order cognitive areas (amygdala, entorhinal and perirhinal cortices and hippocampus) via relay nuclei (thalamus, raphe nucleus and tegmental area) is enhanced with corticosterone delivery, which results in the observed pattern of activation. These results support the hypothesis that stress and anxiety modulate visceral pain perception. In conclusion, the use of CORT implants may provide further insights into the relationship between stress/anxiety and IBS.

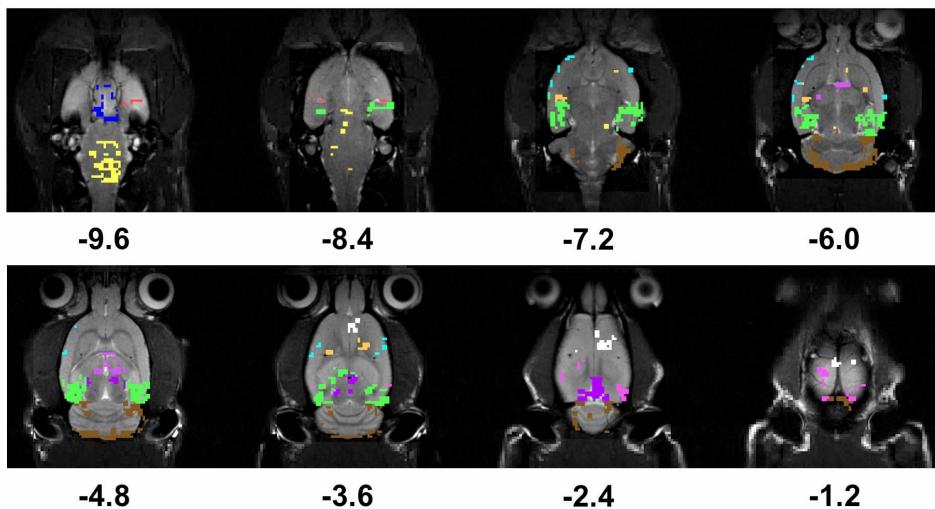


Figure 1. Specific brain/brain stem nuclei activated in response to 60 mmHg CRD in CORT implanted rats. Each brain region is color coded differently. The colored areas represent "averaged" activated pixels for all rats treated with CORT. Amygdala (red), reticular, tegmental and raphe nucleus (yellow), hypothalamus (blue), hippocampus and associated cortical areas (green), basal ganglia (orange), thalamus (violet), insular and somatosensory cortex (turquoise), inferior and superior colliculi (purple), cingulated, motor and orbitofrontal cortex (white), visual cortex (pink) and cerebellum (brown).

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