HHS Public Access

Author manuscript

Proc Int Soc Magn Reson Med Sci Meet Exhib Int Soc Magn Reson Med Sci Meet Exhib. Author manuscript; available in PMC 2021 January 12.

Published in final edited form as:

Proc Int Soc Magn Reson Med Sci Meet Exhib Int Soc Magn Reson Med Sci Meet Exhib. 2019 May ; 27: .

Imaging the evolution acute fear: Longitudinal whole brain imaging in living mice of neural activity with MEMRI

Elaine L. Bearer[1,2], **Daniel Barto**[1], **Russell E. Jacobs**[3]

[1]Pathology University of New Mexico Health Sciences Center, Albuquerque, NM, United States

[2]California Institute of Technology, Pasadena, CA, USA

[3]Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, CA, United States

Introduction:

Life-threatening events cause extreme fear, which evolves in vulnerable people into a debilitating mental illness--post-traumatic stress disorder (PTSD), affecting 6.8% of all Americans[1]. Those who progress to PTSD display both anxiety-related (hyper-arousal) and depressive (avoidance) symptoms. PTSD is accompanied by changes in brain functional anatomy. Some experience relief from selective serotonin reuptake inhibitors (SSRI), some from a1 adrenergic receptor inhibitors, and some respond to combined inhibition of both noradrenergic and serotonergic transporters (NET and SERT), SNRI. Cross-talk between serotonergic and noradrenergic systems (SS and NS) is well known. Both are disturbed in PTSD[2–4]. Early life adversity is a known risk factor for PTSD. There are no predictive or diagnostic tests, and no reliably effective prevention or intervention for PTSD. Using manganese-enhanced MRI (MEMRI) tract-tracing, we reported that SERT-KO mice have abnormal circuitry in the prelimbic system[5]. To determine whether early life adversity affects this circuit and the behavioral and neural response by fear, SERT-KO and WT mice raised either normally or with maternal deprivation, were exposed to predator stress (PS) in young adulthood, a known naturalistic provocateur of persistent fear in rodents. We use the light-dark box to quantify behavior and Mn^{2+}-enhanced MRI (MEMRI) to witness behavior and brain activity. Mn^{2+} increases the relaxation rate of protons in water in T_1-weighted pulse sequences, and thus produces a hyper-intense signal in T_1-weighted MRI. Mn^{2+} is a metabolic contrast agent that reports on neural activity by entering active neurons through voltage-gated Ca^{2+} channels[6], and is transported along axons tracing multi-synaptic circuitry when injected locally[7,8].

Methods:

WT littermates and SERT KO mice (12 each) were either exposed to fragmented care during post-natal day P2–10 or raised normally and exposed to PS at 10 weeks of age. For PS, we used a synthetic fox odor, 2,3,5-Trimethyl-3-thiazoline (TMT). Behavior was recorded by Noldus video system in the light-dark box. Mice were imaged in an 11.7 T vertical bore Bruker MR scanner prior to and then after intraperitoneal (IP) injection of Mn^{2+} (0.3 mM/kg) and re-scanned 24hr later. Immediately following the 24hr scan, mice were exposed first to saline and then to PS in a light-dark box, their behavior recorded, and another MR

image obtained. 9d later, behavior was again recorded and mice were re-scanned, injected IP with Mn^{2+} and scanned 24hr later to test for persistence of neural activity. Whole head images of living mice were skull-stripped and aligned[8,9]. Using statistical parametric mapping (SPM) tools we compared images by paired T-tests. Automated segmentation based on our In Vivo MR Atlas[10] (Figure 1) identifies sub-regions of brain activated by PS and in SERT-KO and ELS-exposed mice at 9d. We confirmed intensity changes by ROI analysis of locations detected by SPM as statistically different, and localized neural activation by c-Fos staining.

Results and Discussion:

We detected strong signals in the "resting" brain, prior to PS (Figure 2). This signal had maximal increase at 24hr and dissipated over 13d. After PS, signal increased specifically in amygdala, hypothalamus, hippocampus and reticular activating system, with more increase in ELS-exposed mice and SERT-KO than wild-type-normally raised animals. Both fearful behavior and brain activity in ELS and in SERT-KO mice was prolonged after PS. Comparisons of the 9d post PS detected statistically significant changes ($p<0.001$ *uncorr*) in 6 regions in SERT KO mice compared to WT by ANOVA ($p<0.0001$ uncorr). An ROI analysis demonstrated that the extent of difference in voxel intensity, after normalization, between groups at the 10d time-point was 2–6%. Activation of these brain regions was confirmed by c-Fos staining of the same mice. MEMRI tract-tracing of the prelimbic circuit was also affected by SERT KO, NET KO, PS and ELS. Hence SERT KO mice exhibited both sustained behavioral response to PS as well as prolonged neural activity in anxiety-associated brain regions. Interestingly locations of activity evolve over time.

Conclusions:

Persistent fear in SERT-KO animals at 9–24d after PS includes avoidance and hyper-arousal. Heightened activation of brain regions continued for 9d after PS in SERT-KO and ELS. Changes in location of signal at 9d suggest that prolonged fear responses in the brain evolve. Thus, longitudinal MEMRI provides an experimental system to explore the dynamics of whole brain biological mechanisms that result in fear persistence, with possible clues for diagnosis and interventions. These results show interactions between genotype, early life experience and context, suggesting a 2 or even 3-hit model for human PTSD.

References:

1. Harvard Medical School. National Comorbidity Survey (NCS). in Data Table 2: 12-month prevalence DSM-IV/WMH-CIDI disorders by sex and cohort. (Harvard Medical School, Boston, MA, 2017).
2. Hall FS et al. The effects of DAT, SERT and NET gene knockout on behavior in animal models of depression. Neuropsychopharmacology 31, S155–S156 (2006).
3. Perona MTG et al. Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behavioural Pharmacology 19, 566–574 (2008). [PubMed: 18690111]

4. Vizi ES, Zsilla G, Caron MG & Kiss JP Uptake and release of norepinephrine by serotonergic terminals in norepinephrine transporter knock-out mice: Implications for the action of selective serotonin reuptake inhibitors. Journal of Neuroscience 24, 7888–7894 (2004). [PubMed: 15356201]

5. Bearer EL, Zhang X, Janvelyan D, Boulat B & Jacobs RE Reward circuitry is perturbed in the absence of the serotonin transporter. Neuroimage 46, 1091–104 (2009). [PubMed: 19306930]

6. Malkova NV, Gallagher JJ, Yu CZ, Jacobs RE & Patterson PH Manganese-enhanced magnetic resonance imaging reveals increased DOI-induced brain activity in a mouse model of schizophrenia. Proc Natl Acad Sci U S A 111, E2492–500 (2014). [PubMed: 24889602]

7. Bearer EL et al. Role of neuronal activity and kinesin on tract tracing by manganese-enhanced MRI (MEMRI). Neuroimage 37 Suppl 1, S37–46 (2007). [PubMed: 17600729]

8. Medina CS, Manifold-Wheeler B, Gonzales A & Bearer EL Automated Computational Processing of 3-D MR Images of Mouse Brain for Phenotyping of Living Animals. Curr Protoc Mol Biol 119, 29A 5 1–29A 5 38 (2017).

9. Delora A et al. A simple rapid process for semi-automated brain extraction from magnetic resonance images of the whole mouse head. J Neurosci Methods 257, 185–93 (2016). [PubMed: 26455644]

10. MacKenzie-Graham A et al. A multimodal, multidimensional atlas of the C57BL/6J mouse brain. J Anat 204, 93–102 (2004). [PubMed: 15032916]

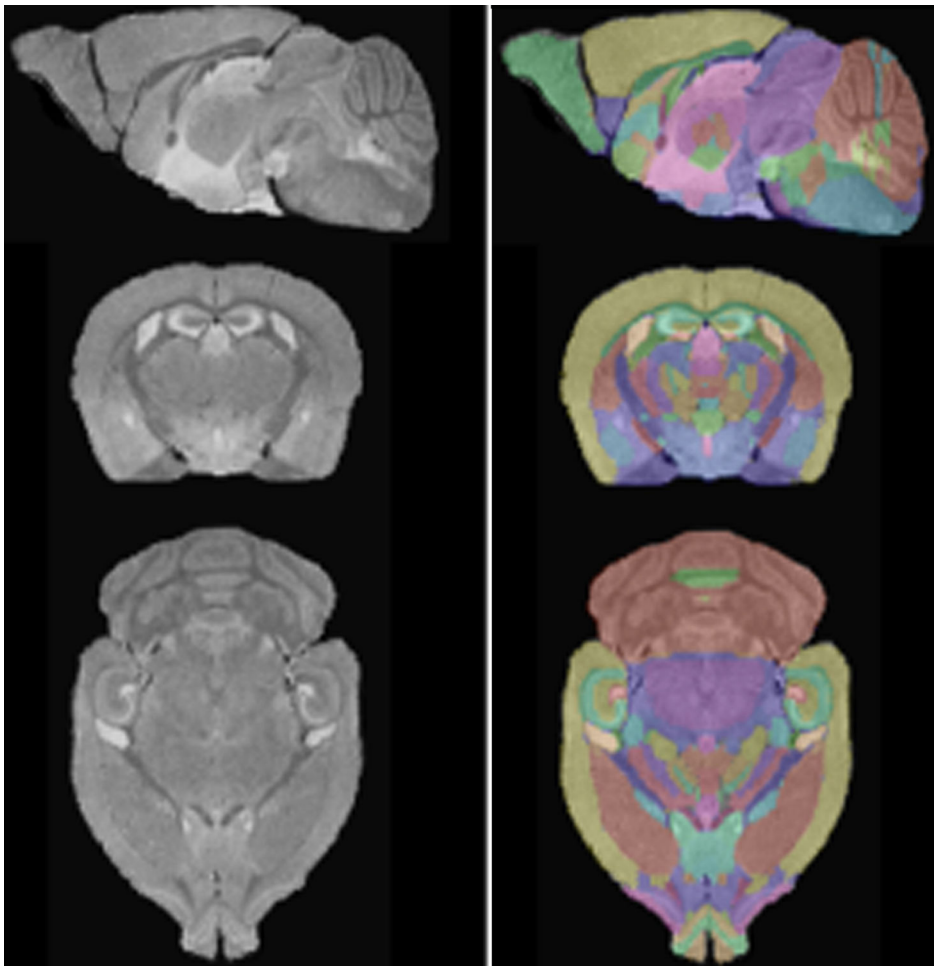

Figure 1:
In Vivo atlas segments 87 brain regions and aligns with our experimental dataset.

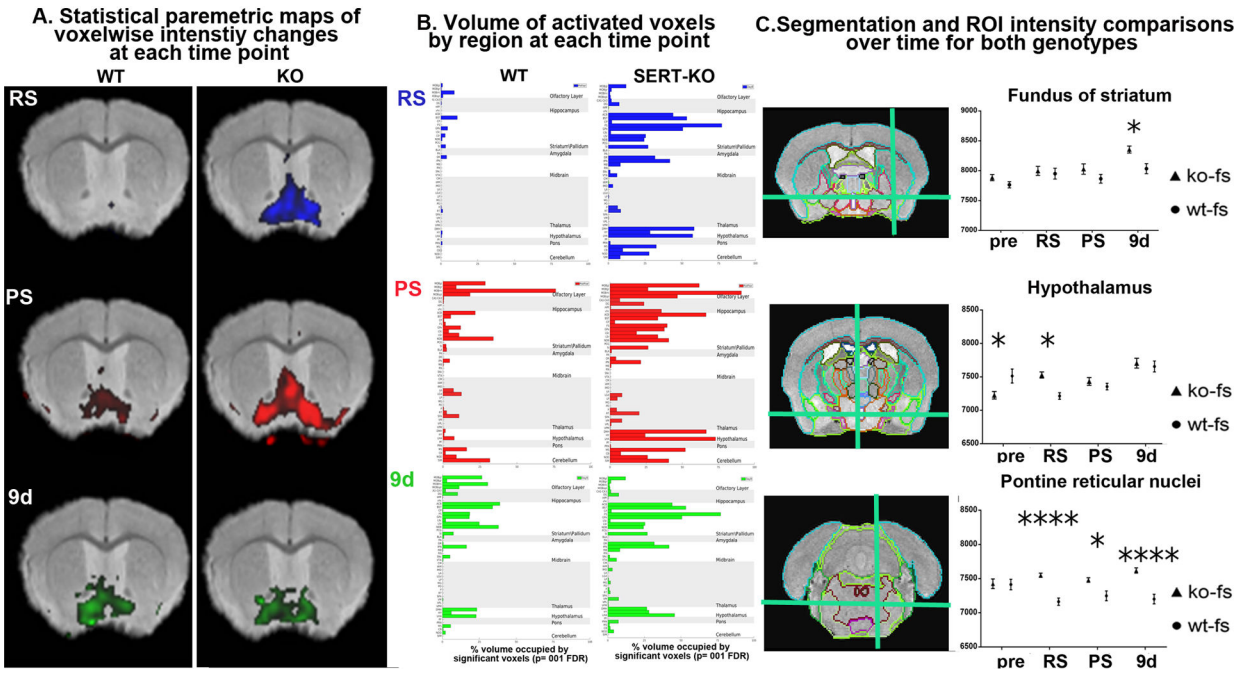

Figure 2:

Transition from acute innate fear to persistent fear in WT and SERT KO mice. A. SPM maps at each time point, RS, resting state; PS, immediately after predator stress; 9d, 9 days after PS; B, Relative volumes of 50 activated regions in WT and SERT KO mice; C. Segmentation and ROI measurements with statistical analysis of regions activated at 9 days. In a 11.7T Bruker Biospin scanner with a FLASH imaging sequence: 8 averages, TR/TE_{eff}=25 ms/5 ms; matrix size of 200×124×82 and FOV 20.0 mm×12.4 mm×8.2 mm, yielding 100 μm isotropic voxels with 34m scan time.