Studies in Rhesus Monkeys Reveal that 5-HTTP S-Carriers have Multiple Stress Associated Neural Vulnerabilities.

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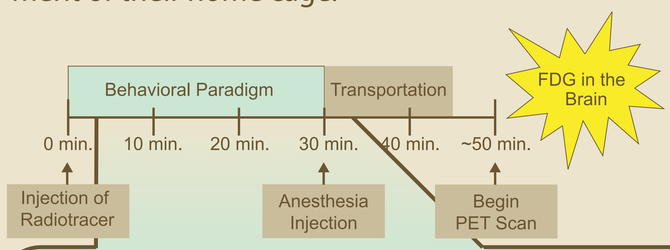
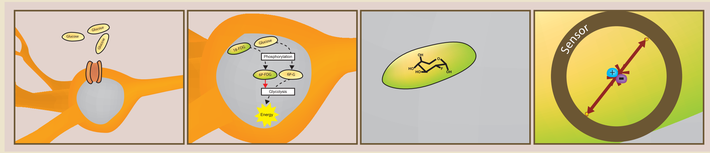
A polymorphism in the promoter region of the serotonin transporter gene, the s allele, is associated with increased vulnerability to develop anxiety-related traits and depression. Furthermore, fMRI studies reveal that s carriers have increased amygdala reactivity in response to aversive stimuli which is thought to be an intermediate phenotype mediating the influences of the s allele on emotionality. Rhesus monkeys provide an excellent model to understand mechanisms underlying human anxiety and [18F]fluoro-2-deoxy-D-glucose (FDG) microPET allows for the assessment of brain activity associated with naturalistic environments outside of the scanner. During FDG uptake, monkeys were exposed to different ethologically relevant stressful situations (relocation and threat) as well as to the less stressful familiar environment of their home cage.

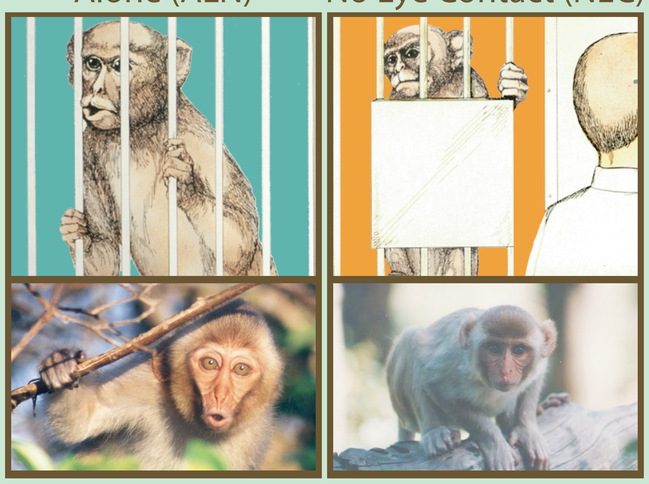




Each animal received an intravenous injection of 10 mCi [18F]-flouro-2-deoxyglucose (FDG) prior to the 30-minute behavioral test, the optimum time for FDG uptake. After the FDG injection, animals were exposed to one of the three behavioral test conditions.

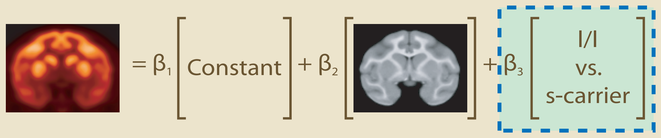
Differential Stress Paradigms:

Alone (ALN) | No Eye Contact (NEC)



We scanned 30 monkeys in 3 different conditions. During FDG uptake, monkeys were exposed to different ethologically relevant stressful situations (relocation and threat) as well as the less stressful familiar environment of their home cage.

During the home cage (BSLN) condition the animals were alone without their cage mate in their home cage for 30 minutes. During the alone (ALN) condition the subjects were separated from their cage mates and relocated to a test cage in the testing room where they remained alone for 30 minutes. The no eye contact condition (NEC) involved relocating the subject to the test cage in the test room. Then, a human intruded into the test room and stood completely still and presented her profile to the test subject. These conditions were chosen because of their ecological validity, and their ability to elicit different stress related behaviors. Specifically, the ALN and NEC conditions have been shown to reliably elicit Coo vocalizations and freezing behavior respectively.



In this study statistical analyses were performed on a voxelwise basis across the whole brain correcting for anatomical differences as measured by standard voxel-based morphometric techniques, as previously described in Oakes et al. (2007).

Genotyping of serotonin transporter polymorphism was performed by amplifying the polymorphic repeat unit region of the rhesus monkey serotonin transporter promoter (Lesch et al. 1997) using the following primers: Forward-5'cagcacctaacccctaatgtccctg3' and Reverse-5'gattctggtgccacctagacgccag3'. The genotyping revealed the following frequencies of the s and l alleles in the 30 rhesus monkeys tested: [l/l=20; unrelated s-carriers=10; (s/s = 2, s/l = 8)].

T-tests of the group difference in FDG uptake during the BSLN condition, as well as interactions between genotype and stressor type: s (ALN-BSLN) vs. l /l (ALN-BSLN) or s (NEC-BSLN) vs. l/l (NEC-BSLN) were performed.

NEC - BSLN = NEC - BSLN

ALN - BSLN = ALN - BSLN

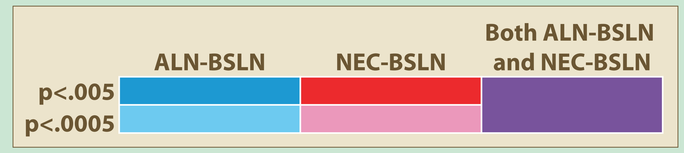
BSLN comparison:
In comparing the l/l to the s individuals when administered FDG in the home cage, no significant differences in metabolic activity were found in the amygdala, BNST, (NAcc), insula, or OFC (using p<.005 (uncorrected) as a threshold). The only differences observed when animals were tested in the home cage were that s carriers had increased activity bilaterally in the cerebellum (t=4.19) and decreased activity bilaterally in somatosensory cortex (t=-4.07) and in left visual cortex (V1) (t=-3.77).

ALN vs. BSLN comparison:
In response to the relocation stress the s-carriers, compared to the l/l monkeys, significantly increased their metabolic activity within multiple brain regions with local maxima within the amygdala (peak = [-9.975,-3.75,-8.75]; t = 4.21; Fig 1a), anterior insula (peak = [-15.575,7.55,2.55]; t = 4.88) and area 11 of OFC (peak = [7.525,22.55,6.25]; t = 5.24; Fig 2) (p < .005, two-tailed uncorrected).

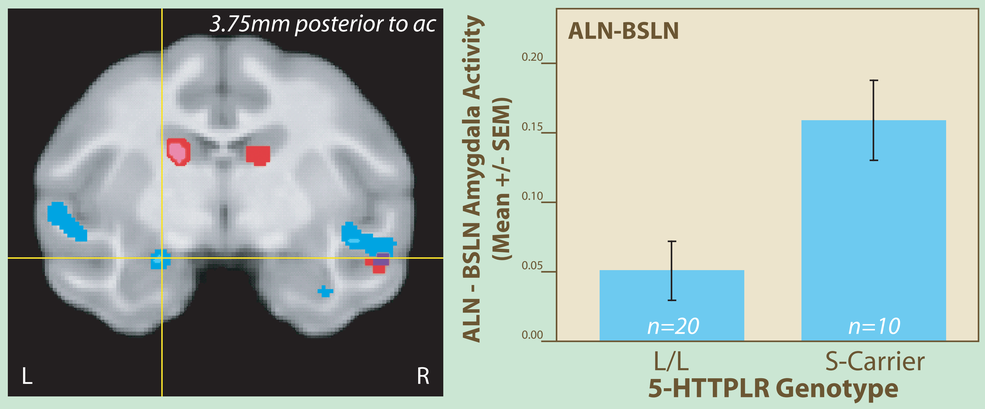
NEC vs. BSLN comparison:
In response to the introduction of a threat, the s-carriers significantly increased their metabolic activity in various brain regions including the striatum with local maxima in a region that borders the BNST and NAcc (BNST/NAcc) (peak = [-13.775,7.55,2.55]; t = 5.11; Fig 1b), the anterior insula (peak = [-13.775,7.55,2.55]; t = 4.58) and area 11of OFC (peak = [3.725,20.05,3.75]; t = 3.72; Fig 2) (p < .005, two-tailed uncorrected).

We are grateful to H. Van Valkenberg, T. Johnson, W. Shelledy, A. Converse, the staff at the Harlow Center for Biological Psychology and the Wisconsin National Primate Research Center at the University of Wisconsin (RR000167), and R. Garcia of the Southwest Foundation for Biomedical Research for their technical support. This work was supported by grants MH046729, MH052354, MH069315, The HealthEmotions Research Institute and Meriter Hospital.

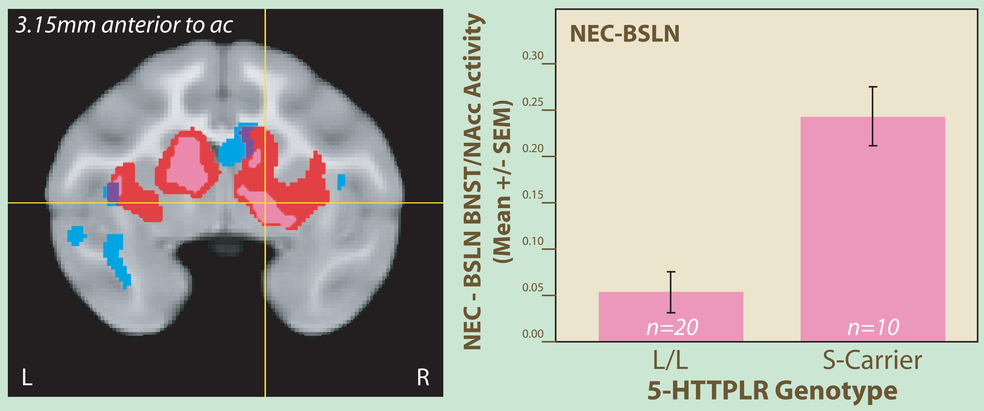
Fig 1



a) Amygdala



b) Bed Nucleus of the Stria Terminalis/Nucleus Accumbens



Serotonin transporter promoter s-carriers compared to l/l individuals have greater increased amygdala metabolic activity in response to ALN; and a greater increase in BNST/NAcc activity in response to NEC. Brain pictures represent the significant voxels (p<.005 and p<.0005, two-tailed uncorrected) for the interaction between genotype and stressor type: l/l(ALN-BSLN) vs. s(ALN-BSLN) in blue, and l/l(NEC-BSLN) vs. s(NEC-BSLN) in red (overlap between the two tests in purple) overlaid on a rhesus monkey MRI template. Graphs represent the peak voxels from clusters within the Amygdala and BNST/NAcc highlighted by the yellow crosses overlaid on the brain pictures.

Subsequent analyses examined the effect of genotype on AMYG and BNST activity between the two stressors; s (NEC-ALN) vs. l/l (NEC-ALN). The NEC vs. ALN comparison showed that during ALN the s-carriers had significantly greater amygdala activity (peak = [-8.775,-4.35,-8.75]; t = 2.8) (p < .05, two-tailed uncorrected), whereas during NEC the s-carriers displayed significantly greater BNST/NAcc activity (peak = [7.525, 3.15,-3.15]; t = 2.66) (p < .05, two-tailed, uncorrected).

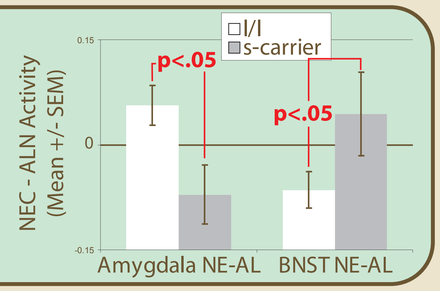
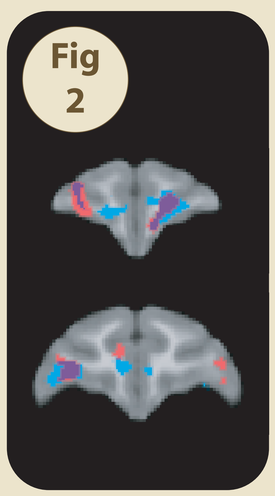


Fig 2



The s-carriers displayed increased orbitofrontal cortex activity in response to both relocation and threat. However, during relocation they displayed increased amygdala reactivity and in response to threat they displayed increased reactivity of the bed nucleus of the stria terminalis. No increase in the activity of any of these regions occurred when the animals were administered FDG in their home cages. These findings demonstrate context-dependent intermediate phenotypes in s-carriers which provide a framework for understanding the mechanisms underlying the vulnerabilities of s-allele carriers exposed to different types of stressors.