These data accord with earlier studies demonstrating regulation of RGS4 expression by dopamine[4] and specifically by COMT genotype.[8] Lipska and colleagues showed significantly decreased RGS4 mRNA expression in the DLPFC of Val/Val subjects, relative to Met-carriers. These changes suggest that variation in cortical dopamine may alter RGS4-dependent signaling by regulating its transcription. Alternatively or in addition, genetic variation in RGS4 may become more manifest in COMT Val/Val subjects, whose cortical synaptic dopamine levels are lower. Our findings support earlier statistical genetic and neuropathological evidence for epistasis between COMT and RGS4 by demonstrating an interaction between these two putative risk genes on an *in vivo* measure of prefrontal function. These results bolster the notion that COMT val158met genetic background mediates the impact of other schizophrenia susceptibility genes.

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JW Buckholtz[1,2], S Sust[1,2], HY Tan[1,2], VS Mattay[1,3], RE Straub[1], A Meyer-Lindenberg[1,3], DR Weinberger[1] and JH Callicott[1,2]

[1]*Clinical Brain Disorders Branch, Genes, Cognition, and Psychosis Program, Division of Intramural Research, Department of Health and Human Services, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA;* [2]*Unit on Dynamic Imaging Genetics, Division of Intramural Research, Department of Health and Human Services, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA and* [3]*Neuroimaging Core Facility, Division of Intramural Research, Department of Health and Human Services, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA*

E-mail: callicottj@mail.nih.gov

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Activated p38 MAPK is associated with decreased CSF 5-HIAA and increased maternal rejection during infancy in rhesus monkeys

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Recent data indicate that activation of the p38 mitogen-activated protein kinase (MAPK) signaling cascade by cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF)-α increases the expression and activity of the serotonin transporter (SERT). Herein, we report that increased p38 activity, as manifested by an increased percentage of peripheral blood monocytes staining positive for intracellular phosphorylated p38 (p-p38), was associated with decreased cerebrospinal fluid (CSF) concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and increased maternal rejection in 17 rhesus monkeys, 8 of whom were exposed to poor maternal care as infants. These data provide the first evidence of an *in vivo* relationship between p38 MAPK activation and brain serotonin metabolism in an animal model of early life stress and indicate that activation of p38 MAPK signaling pathways may participate in the contribution of early life stress to psychiatric morbidity.

Early life stress including physical/sexual abuse as well as neglect has been associated with a number of adverse health outcomes including increased anxiety and depression.[1] We recently reported that adolescent rhesus monkeys (*Macaca mulatta*) exposed to physical abuse and high levels of maternal rejection as infants exhibit increased distress and anxiety, delayed social development and reduced exploration, compared to non-abused animals.[2,3] This early exposure to poor maternal care was also associated with reduced brain serotonergic function as reflected by decreased CSF concentrations of serotonin (5-HT) and the serotonin metabolite, 5-HIAA, that were in turn correlated with increased anxiety-like behavior during adolescence.[4]

Data indicate that stress, including early life stress, also can be associated with activation of innate immune responses including release of proinflammatory cytokines and activation of proinflammatory cytokine signaling cascades.[5,6] Relevant to the impact of early life stress on serotonin metabolism, the cytokine signaling pathway, p38 MAPK, has been found to increase expression and activity of the SERT in both a rat embryonic raphe cell line

(RN46A) and mouse midbrain and striatal synaptosomes.[7] We therefore endeavored to examine the relationship between activation (phosphorylation) of p38 MAPK in peripheral blood monocytes and central serotonin function as assessed by CSF serotonin metabolites in adolescent rhesus monkeys approximately half of whom had been exposed to physical abuse and neglect as infants. A significant negative correlation was found between the percentage of monocytes staining positive for intracellular p-p38 and CSF concentrations of 5-HIAA ($r=-0.53$, $P=0.027$) (Figure 1a). Because our previous data indicated a significant relationship between CSF 5-HIAA concentrations and rates of maternal rejection early in life,[8] we also examined the relationship between intracellular p-p38 and maternal rejection. A significant positive correlation was found between the rate of rejection and the percentage of monocytes staining positive for p-p38 ($r=0.54$, $P=0.025$) (Figure 1b). Entering gender as a covariate in these correlational analyses did not alter the significance of the results. Of note, although no statistically significant mean differences in p-p38 were found between abused and non-abused monkeys, abused animals exhibited a trend for a higher percentage of monocytes positive for intracellular p-p38 (33.0 versus 25.1, $t=1.88$, d.f. = 15, $P=0.079$). No correlations were found between p-p38 and CSF concentrations of 5-HT, possibly owing to a weaker effect of early life stress on CSF 5-HT versus 5-HIAA, consistent with previous reports in non-human primates.[9] These data provide evidence of an *in vivo* relationship between activation of p38 MAPK signaling pathways and CNS serotonin function/metabolism. By increasing SERT expression/activity, activation of p-38 pathways would be expected to decrease synaptic availability of serotonin and reduce serotonin metabolites as was found in this study. Of note, proinflammatory cytokines, including IL-1 and TNF-alpha, are also capable of influencing the activity of the enzyme indolamine 2,3 dioxygenase, which metabolizes tryptophan (TRP) to kynurenine and quinolinic acid, thereby shunting TRP from the synthesis of serotonin.[6] Thus, in addition to directly influencing the expression of the SERT, proinflammatory cytokines may influence serotonin metabolism by altering the availability of TRP, the primary precursor of serotonin. Taken together, the data suggest that increased activity in p-38 MAPK pathways as a function of early abuse/neglect may represent a novel mechanism by which early life stress is translated into risk for illness. Moreover, p38 pathways may serve as a unique translational target for reversing the impact of early

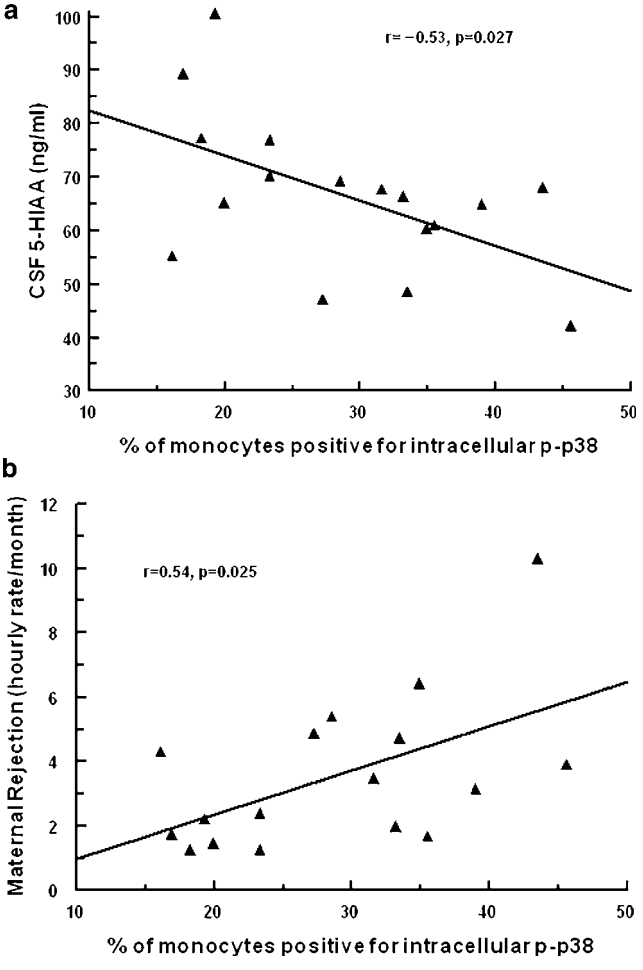

Figure 1 Relationship between activated p38 MAPK and CSF 5-HIAA and maternal rejection during infancy in adolescent rhesus monkeys. Percentage of peripheral blood monocytes positive for intracellular phosphorylated (activated) p38 (p-p38) MAPK was inversely correlated with CSF concentrations of the 5-HT metabolite, 5-HIAA (**a**) and positively correlated with maternal rejection during infancy (hourly rate per month, across the first 6 months of life) (**b**). Note: physical abuse and high rates of maternal rejection were highly co-morbid in our study population, as previously reported.[2,8] *Methods*: CSF and peripheral blood samples were obtained under resting conditions following general anesthesia (5 mg/kg BW telazol, i.m.) from eight abused (four males, four females) and nine non-abused (four males, five females) rhesus monkeys, aged 3–4 years. CSF samples were collected from the *cisterna magna* as previously described,[4,8] and concentrations of serotonin and its metabolite (5-HIAA) were assessed using reverse-phase, ion-pair high-performance liquid chromatography. Whole blood was obtained in EDTA-coated tubes, and red blood cells were lysed using FACS lysing solution (BD Biosciences, San Jose, CA, USA). Cell pellets were then washed, fixed in 2% paraformaldehyde and incubated with anti-human antibodies directed to CD14 or a relevant isotype control antibody (to identify monocytes) (BD Biosciences). Cells were then permeabilized with 90% methanol at 4°C for 30 min, stained with mouse anti-phospho-p38 MAPK (T180/Y182) phycoerythrin-conjugated monoclonal antibody (BD Biosciences) (1 h at room temperature), washed and resuspended in 2% paraformaldehyde for flow cytometric assessment (FACScalibur, BD Biosciences). Data were analyzed using Flojo software (Tree Star Inc., Ashland, OR, USA). CSF, cerebrospinal fluid.

life stress on relevant pathophysiologic end points including anxiety and depression.

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MM Sanchez[1,2], O Alagbe[1], JC Felger[1], J Zhang[1], AE Graff[2], AP Grand[2,3], D Maestripieri[2,4] and AH Miller[1]

[1]*Department of Psychiatry and Behavioral Science, Emory University, Atlanta, GA, USA;* [2]*Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA;* [3]*Department of Psychology, University of Georgia, Athens, GA, USA and* [4]*Department of Comparative Human Development, University of Chicago, Chicago, IL, USA*

E-mail: amill02@emory.edu

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Evidence that many of the DISC1 isoforms in C57BL/6J mice are also expressed in 129S6/SvEv mice

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Recently, Koike *et al.*[1] identified a 25-bp deletion in a coding exon of the *Disrupted-In-Schizophrenia (DISC1)* gene in the 129S6/SvEv strain, which was also confirmed at the genomic level for all extant 129 mouse inbred substrains.[2] This mutation could interfere with the production of the full-length DISC1 protein. When the 129S6/SvEv-derived *DISC1* gene was transferred to C57BL/6J genetic background, the resultant mice displayed a subtle behavioral abnormality in working memory, without any other major deficits in behavior and brain anatomy.[1] Several lines of evidence from animal models with RNA interference to DISC1, as well as studies in patient-derived lymphoblasts, suggest that loss of DISC1 function may be involved in the abnormalities underlying the pathophysiology of major mental conditions.[3] The minor behavioral abnormality resulting from the 25-bp deletion is in contrast to the results of three independent groups who have generated partial loss-of-function models by expressing dominant-negative DISC1 constructs and obtained substantial changes in behavior, including deficits in prepulse inhibition, latent inhibition and working memory.[4] Moreover, the absence of obvious anatomical changes in the mutant mice[1] conflicts with cellular models based on the knockdown of DISC1 or expression of dominant-negative DISC1, which disrupts developmental processes critical for normal cortical architecture.[5] Thus, if the 25-bp deletion completely abolishes the full-length DISC1 protein that is crucial for proper neurodevelopment, why are there such small phenotypic changes in mice with this mutation?

To address this question, our collaborative group examined expression of DISC1 protein in the 129S6/SvEv strain, and systematically compared expression with that in the C57BL/6J strain by using antibodies against more than 10 independent epitopes that were generated from eight independent groups, including commercially available antibodies (Figure 1a). Although DISC1 has several isoforms, we focused our analyses of immunoreactivity on the one that corresponded to full-length DISC1. Interestingly, all the antibodies except the one that was a generous gift from Dr Joseph A Gogos[1] showed almost identical immunoreactivity to DISC1 at this molecular weight band, or at least immunoreactivity that was indistinguishable at the detection levels, between these two strains (Figure 1b and Supplementary Figure 1). The specificity of these antibodies against DISC1 was tested by western blotting with extracts from monkey COS7 cells expressing full-length C57BL/6J-derived mouse DISC1 or with mock transfection (Supplementary Figure 2). The specificity of the mExon3 antibody was also confirmed by preabsorption tests by both western blotting and immunohistochemistry (Supplementary Figure 3). D27 is the best-characterized antibody against mouse DISC1 in both western blotting and immunohistochemistry. D27 recognizes two distinct bands at 100–110 kDa, corresponding in size to full-length DISC1.[6] We conducted immunoprecipitation (IP) with mouse brain extracts by using the mExon3 antibody, which was followed by western blotting with the D27 antibody, and found that the molecule corresponding to the lower band detected by D27 was specifically precipitated by mExon3. Consistent with the results of western blotting, we failed to observe any difference in IP between the 129S6/SvEv and the C57BL/6J strains.