HHS Public Access

Author manuscript

J Intellect Disabil Res. Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

J Intellect Disabil Res. 2018 December ; 62(12): 1008–1017. doi:10.1111/jir.12558.

Self-injurious behaviors in rhesus macaques: potential glial mechanisms

Joseph Ramsey, BS[1], **Elizabeth C. Martin, PhD**[2], **Olivia M. Purcell, MS**[1], **Kim M. Lee, MD, PhD**[3,4], and **Andrew G. MacLean, PhD**[1,3,4,5,6,*]

[1]Tulane Program in Neuroscience, Tulane University, New Orleans, LA 70112

[2]Center for Stem Cell Research and Regenerative Medicine, School of Medicine, Tulane University, New Orleans, LA 70112

[3]Tulane National Primate Research Center, Covington, LA 70433

[4]Tulane Program in Biomedical Science, Tulane Medical School, New Orleans, LA 70112

[5]Department of Microbiology & Immunology, Tulane Medical School, New Orleans, LA 70112

[6]Tulane Center for Aging, Tulane University New Orleans, LA 70112

Abstract

Background: Self-injurious behaviour (SIB) can be classified as intentional, direct injuring of body tissue usually without suicidal intent. In its non-suicidal form it is commonly seen as a clinical sign of borderline personality disorder, autism, PTSD, depression, and anxiety affecting a wide range of ages and conditions. In rhesus macaques SIB is most commonly manifested through hair plucking, self-biting, self-hitting, and head banging. SIB in the form of self-biting is observed in approximately 5–15% of individually housed monkeys. Recently, glial cells are becoming recognised as key players in regulating behaviors.

Method: The goal of this study was to determine the role of glial activation, including astrocytes, in macaques that had displayed SIB. To this end, we performed immunohistochemistry and next generation sequence of brain tissues from rhesus macaques with self-injurious behaviours.

Results: Our studies showed increased vimentin, but not nestin, expression on astrocytes of macaques displaying SIB. Initial RNA Seq analyses indicate activation of pathways involved in tissue remodeling, neuroinflammation and cAMP signaling.

Conclusions: Glia are most probably activated in primates with self-injury, and are therefore potential novel targets for therapeutics.

*Corresponding author. amaclean@tulane.edu.

Current affiliations: Joseph Ramsey – National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, Elizabeth Martin – Department of Biological and Agricultural Engineering, Louisiana State University, Baton Rouge, LA, Olivia Purcell – Louisiana State University Health Science Center, Kim Lee – Department of Neurology, Vanderbilt University Medical Center

Conflicts of Interest.

The authors declare they have no conflicts of interest.

Keywords

self-injury; transcriptional control; glia; RNA Seq; astrocyte

Introduction.

Self-Injurious Behaviour (SIB), which has been defined as intentional, direct injuring of body tissue without suicidal intent (Baguelin-Pinaud et al., 2009), is a serious global health problem occurring in several high risk populations with neuropsychiatric diseases / disorders (including borderline personality disorder, PTSD, depression, autism spectrum disorders) and in other neurodevelopmental disorders (Zlotnick et al., 1997, Mork et al., 2013, Al-Sharqi et al., 2012). Of particular concern is its lack of effective treatment (Navines et al., 2013, Novak et al., 2014) and growth in children and adolescence, most commonly in cases of depression or neurodevelopment (Jacobson and Gould, 2007). Macaques have been noted to spontaneously develop SIB (Novak, 2003), an important distinguishing factor from other animal models (namely rodents) which tend to be induced (with some exceptions (Bechard et al., 2017), making the macaque model a closer match with the human analog (Novak et al., 2014, Tiefenbacher et al., 2005, Davenport et al., 2008). This fact is especially important when considering the prevalence of SIB in populations with neurodevelopmental disorders. Such similarities with human SIB phenotypes suggest that a molecular investigation of SIB macaques could provide beneficial insight into the human pathology of SIB.

The use of macaques in research appears to represent the best compromise between phylogenetic and physiological relatedness to humans, cost efficiency, life-span, resources, expertise in animal husbandry practices, and adaptability for translation of results to humans. Aspects of research studies that utilise primarily rhesus macaques include neurobiology, anatomy, cognition and behavior (Urbanski and Sorwell, 2012, Voytko and Tinkler, 2004, Peters, 2002, Bailey et al., 2011, Schultz et al., 2001), reproductive senescence (Atsalis and Margulis, 2008), and immune senescence (Asquith et al., 2012, Messaoudi et al., 2011, Vaccari and Franchini, 2010). The rhesus macaque has been considered the “gold standard” model for human research, has been in use since the 1960’s, and has proven to have very high translational validity with respect to neurologic and behavioral assessments of infants (Nelson and Winslow, 2009). Equally important is the striking similarity between the innate immune systems of rhesus monkeys and humans (Evans and Silvestri, 2013). Due to this similarity, rhesus monkeys are susceptible to infection by many pathogens that result in disease states almost identical to those found in humans (a noteworthy example being Simian Immunodeficiency Virus, which induces an AIDS-like disease, including CNS compliactions, in rhesus monkeys (Lackner and Veazey, 2007). Importantly, the immune system of rhesus monkeys is more similar to humans than the immune systems of rodents, making monkeys the best choice for evaluating and correlating immunologic responses and CNS disease. Indeed, recent studies have linked severe stress to specific immune activation (Beurel and Lowell, 2017) and glial activation (Nijs et al., 2017).

While once controversial, the concept of glia regulating complex behaviours is rapidly gaining ground. While much work focusses on schizophrenia (see review by Xia and colleagues (Xia et al., 2016)), the basic physiology of abnormal astrocyte activation would be conserved. The very recent study by the Goldman group definitively showed astrocytes are responsible for behavioral traits (Windrem et al., 2017, Han et al., 2013). We have shown that astrocytes of macaques with SIB have a distinct morphological atrophy, and that the glial activation may be a component of the behaviors (Lee et al., 2013b, Lee et al., 2015a).

Support for such potential findings is grounded in the neuroinflammatory response of Spontaneous SIB Macaques. Such subjects demonstrated astrocyte activation, which has been demonstrated by the upregulation of intermediate filaments (IF), most specifically glial fibrillary acidic protein (GFAP), vimentin, and nestin. Furthermore, such responses have been linked to the dysregulation of downstream gene expression, suggesting neuroinflammation in SIB macaques can also be studied at the molecular level. To this end, we examined brain tissues from macaques with SIB, and compared them with matched tissues from animals without reported abnormal behaviors.

Methods.

Ethics statement, Animal housing and selection of tissues

Animals were maintained in Animal Biosafety Level 2 housing with a 12:12-hour light:dark cycle, relative humidity 30% to 70%, and a temperature of 17.8 to 28.9°C. Water was available *ad libitum*, and a standard commercially formulated nonhuman primate diet (Lab Fiber Plus Monkey DT, 5K63, PMI Nutrition International, St. Louis, MO) was provided twice daily and supplemented daily with fresh fruit and/or forage material as part of the environmental enrichment program. All animals at Tulane National Primate Research Center (TNPRC) received environmental enrichment, widely used to improve welfare in captive macaques. Over the course of their life times, all subjects experienced some pair or group housing as well as periods of single housing. Each cage (Allentown, Inc., Allentown, NJ) measured 36 inches (91.4 centimeters) in height with 4.3– 8.6 square feet (0.4–0.8 square meters) of floor space and contained a perch, a portable enrichment toy, a mirror, and a forage board for feeding enrichment. Practices in the housing and care of animals conformed to the regulations and standards of the PHS Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals. The Tulane National Primate Research Center (Animal Welfare Assurance # A4499–01) is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care-International. All animals are routinely cared for according to the guidelines prescribed by the NIH Guide to Laboratory Animal Care. The TNPRC conducts all research in accordance with the recommendations of the Weatherall report – “The use of non-human primates in research.” The Institutional Animal Care and Use Committee (IACUC) of the Tulane National Primate Research Center approved all animal-related protocols, including any treatments used with nonhuman primates. All animal procedures were overseen by veterinarians and their staff.

Historically, 15–20 animals per year (from a colony of approximately 5,000) are identified as exhibiting self-biting behavior and/or self-wounding at the TNPRC. Records of self-

wounding and clinical intervention are maintained in the animal records system. All such individuals receive enhanced monitoring and implementation of inanimate enrichment.

Animals were humanely euthanised by the veterinary staff at the TNPRC in accordance with endpoint policies. Euthanasia was conducted by anesthesia with ketamine hydrochloride (10 mg/kg) followed by an overdose with sodium pentobarbital and immediate necropsy. This method was consistent with the recommendation of the American Veterinary Medical Association guidelines (Lee et al., 2013b). Three brain regions approximately 1cm thick are routinely collected during necropsy of colony animals at TNPRC representing frontal lobe, parietal & temporal lobe /thalamus/ basal ganglia, and cerebellum / occipital lobe. The frontal area corresponds to a section through Brodmann area 8, between 32 and 33, and ending around Brodmann 12. All tissues are fixed at routine necropsy by immersion in 10% neutral buffered formalin with zinc modification for 48 hours before trimming and paraffin embedding.

For this retrospective study, tissues were selected solely on their availability in the TNPRC tissue archive: no animals were euthanised for this study. All study subjects had been euthanised when clinical or research-related endpoints were reached. For this reason, we were not able to examine regional differences in protein or gene expression. None of the macaques had been used for infectious or pharmacological studies, nor had any received medication for the SIB. Tissue taken from the frontal lobe from 9 control, and 6 SIB rhesus macaques (*Macaca mulatta*) were used for this and previous studies (Lee et al., 2015b, Lee et al., 2013b), for a total of 15 animals (Table 1).

Immunohistochemistry was performed as standard in the Division of Comparative pathology at TNPRC using pre-conjugated GFAP-Cy3 and Alexa 488- conjugated secondary antibodies against TLR2, vimentin or nestin. Slides were images on a Nikon fluorescent microscope, and proportions of double-labeled astrocytes were determined. Statistical significance was determined using Student’s t-test of proportions of double-postitive (GFAP and either vimentin or nestin) cells, as is routine in the MacLean lab (Snook et al., 2014, Robillard et al., 2016, Lee et al., 2016, Lee et al., 2015b, Lee et al., 2014, Lee et al., 2013b, Lee et al., 2013a, Inglis et al., 2016).

To determine the underlying mechanisms, Total RNA was isolated from five sequentially-cut 6μm paraffin sections. Next Generation Sequencing (NGS) sequencing was performed by Vaccine and Gene Therapy Institute, Port St. Lucie, FL on an Illumina HiSeq platform. To remove ribosomal RNA, exome capture was utilized to probe for coding RNA sequences where the exome probe set covers coding RNA sequences. Exome library was then generated and aligned against NCBI37/hp19 human reference genome with 91% homology for target regions. Results were aligned in the fastq format and transcript annotation was aligned to ensemble rhesus macaque, Macaca mulatta (Mmul 1.74). Quality control measures were as follows: masking of abundant transcripts (tRNA/rRNA), read distribution in each sample, maintaining Q-score of 20 or greater, and removal/trimming of adaptors. Mapped reads were determined through maximum 2 misaligned bases mapped to the annotation exons. Alignments were made with the STAR aligner (Dobin et al., 2013). Counts were determined from the number of reads mapped to each aligned read and

genomic feature. Differential gene expression tests were performed using EdgeR software and fold change in gene expression were calculated using counts. Normalization factors were calculated by using the raw library size with the TMM (Trimmed Mean of M-values). Pathway analysis was performed using the Pathway Interaction Database (Schaefer et al., 2009). FDR was calculated with Bonferroni correction, however due to decrease in detection of genes FDR below 0.05 was not detected for most genes in each sample set. For pathway analysis threshold p-value scores generated by edgeR set at 0.05 or less was used. Fold change and p-value for all detectable genes can be found in supplemental file S1.

Results.

In control macaques, vimentin expression was limited to endothelial cells (Figure 1A). In macaques with SIB, we noted that astrocytes had increased levels of vimentin (Figure 1B), that reached significance in white matter astroctyes only (1C). We were very surprised to note that there was no increase in nestin expression, at least as measured as the proportion of GFAP / nestin double positive cells (1D).

As a first step to determine the underlying mechanisms of cell activation in animals with SIB, we performed RNA Seq analysis. We filtered data to determine genes that were either up-regulated or down-regulated to the highest degree. The top ten genes that were up- or down-regulated are presented in Table 2. Seven of the top ten genes that were either up- or down-regulated have antimicrobial functions (noted by *), confirming our previous studies of immune activation in these animals (Lee et al., 2013b, Lee et al., 2015b). Further, two genes were directly associated with astrocyte activation or maturation (§), and eight were linked to behavioral abnormalities and/or intellectual disability (#). Of the top 20 genes up or down regulated, eight were previously associated with behavioral or developmental disabilities, and seven with inflammation.

To determine the molecular mechanisms underlying this differential activation of glial cells, we have begun analyses of the RNA Seq data. Comparing total genes up and down regulated, there were 437 genes down regulated, and 526 up regulated. At the molecular level, we have noted there were multiple pathways activated linked to tissue remodeling and inflammation (Figure 2A). When we imported genes either up- or down-regulated into String software, to explore how these gene changes were inter-related.

We examined vimentin upregulation by tracking other molecular components associated with IF upregulation, which may be part of a mechanism of altered glial cell function in SIB. While vimentin protein was clearly increased (Figure 1A), there was no increase in vimentin at the mRNA level (from RNA seq data). However, several other genes important for vimentin expression were differentially regulated (Figure 2B). Importantly, within this pathway, *TPM3* (1.6 fold), *TNNT2* (3.3-fold), *CASP7* (4.5-fold), *NTRK2* (3.1-fold) and *MYH14* (5.3-fold) were upregulated, whereas *MYL2* (9.1-fold) was downregulated. In summary, one could reasonably explain increased vimentin within astrocytes.

Separately, we noted that multiple genes associated with *Notch* and *Wnt* signaling pathways were also upregulated (Figure 2C). Of note, *HES4* (4.3-fold), *DLL1* (2.7-fold), *PSEN2* (3.3-

fold), *WNT2B* (3.2-fold), *TCF* (2.4-fold) and *CTBP2* (3.6-fold) were all upregulated. These genes have roles in neurogenesis and neuronal differentiation (*HES*, *PSEN2*, *TCF4* and *CTBP2*)(Kageyama et al., 2005), further strengthening the potential for altered neuronal plasticity in macaques with SIB.

Discussion.

We have previously reported that animals with SIB exhibited shorter and less complex astrocytes that were more likely to be activated with regards to TLR2 (Lee et al., 2013b) expression. In this study, we aimed to better understand the molecular basis for spontaneously occurring SIB in rhesus macaques, and by inference, self-injury in humans. As we had previously showed innate immune activation and altered expression patterns of GFAP in astrocytes, we were interested in examining other glial activation markers and underlying molecular changes in frontal cortex of macaques with SIB. That vimentin, GFAP and TLR2 were altered, but not nestin, indicates at least a degree of specificity to the astrocyte activation (Duan et al., 2015). Molecular analyses of differentially-regulated genes were linked to cellular differentiation, innate immune activation and vascular remodeling.

Both Biocarta and the NCI-Nature curated pathway analysis tools indicate that the *Rho* pathway was most upregulated in the SIB macaques. RhoGTPases have been identified as potential targets in rodent models of Rett syndrome (De Filippis et al., 2012). While astrocytic degeneration in animals and humans with depressed phenotypes could point to *Rho* pathway activation, this study provides further evidence in primates. Upregulation of *HES4* could lead to extensive remodeling in key brain areas (Bai et al., 2015) through activation of the *rho* and *rac* (Hall and Lalli, 2010), as well as the *wnt* pathways. Further, activation of the *wnt* pathway can increase expression of vimentin (Knoll et al., 2014), leading to further remodeling.

Astrocytes are glial cells important in maintaining homeostasis and the blood brain barrier, regulating neurogenesis and supporting metabolism at synapses (Lee and MacLean, 2015). There are several reports of GFAP-low/negative astrocytes, including in depressive disorders (Fatemi et al., 2004, Torres-Platas et al., 2015, Tynan et al., 2013, Miguel-Hidalgo et al., 2010). Altered expression of GFAP, vimentin (Al-Ahmad et al., 2011), and nestin (Strong et al., 2004, Pekny et al., 1998) is linked to altered vesicle motility in astrocytes (Potokar et al., 2007). Therefore IF changes could logically be linked with altered secretion of pro- or anti-inflammatory molecules as well as with morphological changes. This hypothesis is supported by several lines of evidence: astrocytes show altered morphology concomitant with inflammation (Xing et al., 2008), associated with altered expression of IFs.

Self-injurious behaviour in macaques is a spontaneously occurring phenomenon, affecting approximately 5% of the captive population. The underlying molecular mechanisms have remained somewhat a mystery. As such, these studies expand upon those of induced depressive-like behaviors in rodents that have shown glial activation and neuroinflammation as probable mechanisms (Tynan et al., 2013, Hinwood et al., 2013). We acknowledge that ideally tissues would be collected prospectively, allowing simultaneous collection of CSF and plasma for cytokine studies, and deeper studies of links between mRNA and protein

expression. Notable in this study was increased vimentin expression at the protein, but not the mRNA levels. *In vitro* studies could dissect this and other disconnects between protein and mRNA expression. Further, as indicated in Figure 2C, several genes associated with *vim* expression were differentially expressed, potentially facilitating increased protein expression. Future studies will validate the top hits by PCR, and provide pathway targets for potential behavior modifying medicaments.

An important caveat in these studies is the retrospective nature of the studies. It would be very interesting to repeat these studies using an induced depressive behaviour model (Li et al., 2013) with age and social hierarchy matched animals with no history of self-injury. Further investigation is needed to determine precisely which cells are activated, and if treatment with antidepressants reverse all the components of the activation observed (Lee et al., 2015a).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements.

This work was supported by PHS grants MH113517 (AGM), NS104016 (AGM) OD11104, which funds the TNPRC (AGM, KML), and U54 GM104940 from NIGMS, which funds the Louisiana Clinical and Translational Science Center (ECM). Supplemental bridge funding was provided by from Tulane University School of Medicine and Tulane Neuroscience Program (AGM). Dr. Lee was the inaugural TNPRC Biomedical Sciences Research Fellow. Ms. Purcell and Mr. Ramsey were supported by the Tulane Program in Neuroscience. AGM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References Cited:

AL-AHMAD AJ, LEE B, SAINI M & BIX GJ 2011 Perlecan domain V modulates astrogliosis In vitro and after focal cerebral ischemia through multiple receptors and increased nerve growth factor release. Glia, 59, 1822–40. [PubMed: 21850672]

AL-SHARQI AM, SHERRA KS, AL-HABEEB AA & QURESHI NA 2012 Suicidal and self-injurious behavior among patients with alcohol and drug abuse. Subst Abuse Rehabil, 3, 91–9. [PubMed: 24474869]

ASQUITH M, HABERTHUR K, BROWN M, ENGELMANN F, MURPHY A, AL-MAHDI Z & MESSAOUDI I 2012 Age-dependent changes in innate immune phenotype and function in rhesus macaques (Macaca mulatta). Pathobiol Aging Age Relat Dis, 2.

ATSALIS S & MARGULIS S 2008 Primate reproductive aging: from lemurs to humans. Interdiscip Top Gerontol, 36, 186–94. [PubMed: 18523379]

BAGUELIN-PINAUD A, SEGUY C & THIBAUT F 2009 [Self-mutilating behaviour: a study on 30 inpatients]. Encephale, 35, 538–43. [PubMed: 20004284]

BAI G, CHEUNG I, SHULHA HP, COELHO JE, LI P, DONG X, JAKOVCEVSKI M, WANG Y, GRIGORENKO A, JIANG Y, HOSS A, PATEL K, ZHENG M, ROGAEV E, MYERS RH, WENG Z, AKBARIAN S & CHEN JF 2015 Epigenetic dysregulation of hairy and enhancer of split 4 (HES4) is associated with striatal degeneration in postmortem Huntington brains. Hum Mol Genet, 24, 1441–56. [PubMed: 25480889]

BAILEY ME, WANG AC, HAO J, JANSSEN WG, HARA Y, DUMITRIU D, HOF PR & MORRISON JH 2011 Interactive effects of age and estrogen on cortical neurons: implications for cognitive aging. Neuroscience, 191, 148–58. [PubMed: 21664255]

BECHARD AR, BLIZNYUK N & LEWIS MH 2017 The development of repetitive motor behaviors in deer mice: Effects of environmental enrichment, repeated testing, and differential mediation by indirect basal ganglia pathway activation. Dev Psychobiol, 59, 390–399. [PubMed: 28181216]

BEUREL E & LOWELL JA 2017 Th17 cells in depression. Brain Behav Immun.

DAVENPORT MD, LUTZ CK, TIEFENBACHER S, NOVAK MA & MEYER JS 2008 A rhesus monkey model of self-injury: effects of relocation stress on behavior and neuroendocrine function. Biol Psychiatry, 63, 990–6. [PubMed: 18164279]

DE FILIPPIS B, FABBRI A, SIMONE D, CANESE R, RICCERI L, MALCHIODI-ALBEDI F, LAVIOLA G & FIORENTINI C 2012 Modulation of RhoGTPases improves the behavioral phenotype and reverses astrocytic deficits in a mouse model of Rett syndrome. Neuropsychopharmacology, 37, 1152–63. [PubMed: 22157810]

DOBIN A, DAVIS CA, SCHLESINGER F, DRENKOW J, ZALESKI C, JHA S, BATUT P, CHAISSON M & GINGERAS TR 2013 STAR: ultrafast universal RNA-seq aligner. Bioinformatics, 29, 15–21. [PubMed: 23104886]

DUAN CL, LIU CW, SHEN SW, YU Z, MO JL, CHEN XH & SUN FY 2015 Striatal astrocytes transdifferentiate into functional mature neurons following ischemic brain injury. Glia, 63, 1660–70. [PubMed: 26031629]

EVANS DT & SILVESTRI G 2013 Nonhuman primate models in AIDS research. Curr Opin HIV AIDS, 8, 255–61. [PubMed: 23615116]

FATEMI SH, LAURENCE JA, ARAGHI-NIKNAM M, STARY JM, SCHULZ SC, LEE S & GOTTESMAN II 2004 Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophr Res, 69, 317–23. [PubMed: 15469203]

HALL A & LALLI G 2010 Rho and Ras GTPases in axon growth, guidance, and branching. Cold Spring Harb Perspect Biol, 2, a001818. [PubMed: 20182621]

HAN X, CHEN M, WANG F, WINDREM M, WANG S, SHANZ S, XU Q, OBERHEIM NA, BEKAR L, BETSTADT S, SILVA AJ, TAKANO T, GOLDMAN SA & NEDERGAARD M 2013 Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell, 12, 342–53. [PubMed: 23472873]

HINWOOD M, TYNAN RJ, CHARNLEY JL, BEYNON SB, DAY TA & WALKER FR 2013 Chronic stress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline. Cereb Cortex, 23, 1784–97. [PubMed: 22710611]

INGLIS FM, LEE KM, CHIU KB, PURCELL OM, DIDIER PJ, RUSSELL-LODRIGUE K, WEAVER SC, ROY CJ & MACLEAN AG 2016 Neuropathogenesis of Chikungunya infection: astrogliosis and innate immune activation. J Neurovirol, 22, 140–8. [PubMed: 26419894]

JACOBSON CM & GOULD M 2007 The epidemiology and phenomenology of non-suicidal self-injurious behavior among adolescents: a critical review of the literature. Arch Suicide Res, 11, 129–47. [PubMed: 17453692]

KAGEYAMA R, OHTSUKA T, HATAKEYAMA J & OHSAWA R 2005 Roles of bHLH genes in neural stem cell differentiation. Exp Cell Res, 306, 343–8. [PubMed: 15925590]

KNOLL S, FURST K, KOWTHARAPU B, SCHMITZ U, MARQUARDT S, WOLKENHAUER O, MARTIN H & PUTZER BM 2014 E2F1 induces miR-224/452 expression to drive EMT through TXNIP downregulation. EMBO Rep, 15, 1315–29. [PubMed: 25341426]

LACKNER AA & VEAZEY RS 2007 Current concepts in AIDS pathogenesis: insights from the SIV/macaque model. Annu Rev Med, 58, 461–76. [PubMed: 17217334]

LEE KM, CHIU KB, DIDIER PJ, BAKER KC & MACLEAN AG 2015a Naltrexone treatment reverses astrocyte atrophy and immune dysfunction in self-harming macaques. Brain Behav Immun, In Press.

LEE KM, CHIU KB, DIDIER PJ, BAKER KC & MACLEAN AG 2015b Naltrexone treatment reverses astrocyte atrophy and immune dysfunction in self-harming macaques. Brain Behav Immun, 50, 288–97. [PubMed: 26191654]

LEE KM, CHIU KB, RENNER NA, SANSING HA, DIDIER PJ & MACLEAN AG 2014 Form follows function: astrocyte morphology and immune dysfunction in SIV neuroAIDS. J Neurovirol, 20, 474–84. [PubMed: 24970236]

LEE KM, CHIU KB, SANSING HA, DIDIER PJ, FICHT TA, ARENAS-GAMBOA AM, ROY CJ & MACLEAN AG 2013a Aerosol-induced brucellosis increases TLR-2 expression and increased complexity in the microanatomy of astroglia in rhesus macaques. Front Cell Infect Microbiol, 3, 86. [PubMed: 24350061]

LEE KM, CHIU KB, SANSING HA, DIDIER PJ, LACKNER AA & MACLEAN AG 2016 The flavivirus dengue induces hypertrophy of white matter astrocytes. J Neurovirol, 22, 831–39. [PubMed: 27273075]

LEE KM, CHIU KB, SANSING HA, INGLIS FM, BAKER KC & MACLEAN AG 2013b Astrocyte atrophy and immune dysfunction in self-harming macaques. PLoS One, 8, e69980. [PubMed: 23922882]

LEE KM & MACLEAN AG 2015 New advances on glial activation in health and disease. World J Virol, 4, 42–55. [PubMed: 25964871]

LI X, XU F, XIE L, JI Y, CHENG K, ZHOU Q, WANG T, SHIVELY C, WU Q, GONG W, FANG L, ZHAN Q, MELGIRI ND & XIE P 2013 Depression-Like Behavioral Phenotypes by Social and Social Plus Visual Isolation in the Adult Female Macaca fascicularis. PLoS One, 8, e73293. [PubMed: 24023857]

MESSAOUDI I, ESTEP R, ROBINSON B & WONG SW 2011 Nonhuman primate models of human immunology. Antioxid Redox Signal, 14, 261–73. [PubMed: 20524846]

MIGUEL-HIDALGO JJ, WALTZER R, WHITTOM AA, AUSTIN MC, RAJKOWSKA G & STOCKMEIER CA 2010 Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. J Affect Disord, 127, 230–40. [PubMed: 20580095]

MORK E, WALBY FA, HARKAVY-FRIEDMAN JM, BARRETT EA, STEEN NE, LORENTZEN S, ANDREASSEN OA, MELLE I & MEHLUM L 2013 Clinical characteristics in schizophrenia patients with or without suicide attempts and non-suicidal self-harm--a cross-sectional study. BMC Psychiatry, 13, 255. [PubMed: 24106884]

NAVINES R, GUTIERREZ F, ARRANZ B, MORENO-ESPANA J, LUISA IMAZM, SOLER V, VAZQUEZ M, CARLOS PASCUALJ, MARTIN-SANTOS R & KAHN DA 2013 Long-term and bizarre self-injurious behavior: an approach to underlying psychological mechanisms and management. J Psychiatr Pract, 19, 65–71. [PubMed: 23334681]

NELSON EE & WINSLOW JT 2009 Non-human primates: model animals for developmental psychopathology. Neuropsychopharmacology, 34, 90–105. [PubMed: 18800061]

NIJS J, LOGGIA ML, POLLI A, MOENS M, HUYSMANS E, GOUDMAN L, MEEUS M, VANDERWEEËN L, ICKMANS K & CLAUW D 2017 Sleep disturbances and severe stress as glial activators: key targets for treating central sensitization in chronic pain patients? Expert Opin Ther Targets, 21, 817–826. [PubMed: 28685641]

NOVAK MA 2003 Self-injurious behavior in rhesus monkeys: new insights into its etiology, physiology, and treatment. Am J Primatol, 59, 3–19. [PubMed: 12526035]

NOVAK MA, EL-MALLAH SN & MENARD MT 2014 Use of the cross-translational model to study self-injurious behavior in human and nonhuman primates. ILAR J, 55, 274–83. [PubMed: 25225306]

PEKNY M, ELIASSON C, CHIEN CL, KINDBLOM LG, LIEM R, HAMBERGER A & BETSHOLTZ C 1998 GFAP-deficient astrocytes are capable of stellation in vitro when cocultured with neurons and exhibit a reduced amount of intermediate filaments and an increased cell saturation density. Exp Cell Res, 239, 332–43. [PubMed: 9521851]

PETERS A 2002 Structural changes in the normally aging cerebral cortex of primates. Prog Brain Res, 136, 455–65. [PubMed: 12143402]

POTOKAR M, KREFT M, LI L, DANIEL ANDERSSON,J, PANGRSIC T, CHOWDHURY HH, PEKNY M &ZOREC R 2007 Cytoskeleton and vesicle mobility in astrocytes. Traffic, 8, 12–20. [PubMed: 17229312]

ROBILLARD KN, LEE KM, CHIU KB & MACLEAN AG 2016 Glial cell morphological and density changes through the lifespan of rhesus macaques. Brain Behav Immun, 55, 60–9. [PubMed: 26851132]

SCHAEFER CF, ANTHONY K, KRUPA S, BUCHOFF J, DAY M, HANNAY T & BUETOW KH 2009 PID: the Pathway Interaction Database. Nucleic Acids Res, 37, D674–9. [PubMed: 18832364]

SCHULTZ C, DICK EJ, COX AB, HUBBARD GB, BRAAK E & BRAAK H 2001 Expression of stress proteins alpha B-crystallin, ubiquitin, and hsp27 in pallido-nigral spheroids of aged rhesus monkeys. Neurobiol Aging, 22, 677–82. [PubMed: 11445268]

SNOOK ER, FISHER-PERKINS JM, SANSING HA, LEE KM, ALVAREZ X, MACLEAN AG, PETERSON KE, LACKNER AA & BUNNELL BA 2014 Innate immune activation in the pathogenesis of a murine model of globoid cell leukodystrophy. Am J Pathol, 184, 382–96. [PubMed: 24316110]

STRONG MJ, LEYSTRA-LANTZ C & GE WW 2004 Intermediate filament steady-state mRNA levels in amyotrophic lateral sclerosis. Biochem Biophys Res Commun, 316, 317–22. [PubMed: 15020220]

TIEFENBACHER S, NOVAK MA, LUTZ CK & MEYER JS 2005 The physiology and neurochemistry of self-injurious behavior: a nonhuman primate model. Front Biosci, 10, 1–11. [PubMed: 15576335]

TORRES-PLATAS SG, NAGY C, WAKID M, TURECKI G & MECHAWAR N 2015 Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. Mol Psychiatry, 21, 509–15. [PubMed: 26033239]

TYNAN RJ, BEYNON SB, HINWOOD M, JOHNSON SJ, NILSSON M, WOODS JJ & WALKER FR 2013 Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol, 126, 75–91. [PubMed: 23512378]

URBANSKI HF & SORWELL KG 2012 Age-related changes in neuroendocrine rhythmic function in the rhesus macaque. Age (Dordr), 34, 1111–21. [PubMed: 22198672]

VACCARI M & FRANCHINI G 2010 Memory T cells in Rhesus macaques. Adv Exp Med Biol, 684, 126–44. [PubMed: 20795545]

VOYTKO ML & TINKLER GP 2004 Cognitive function and its neural mechanisms in nonhuman primate models of aging, Alzheimer disease, and menopause. Front Biosci, 9, 1899–914. [PubMed: 14977596]

WINDREM MS, OSIPOVITCH M, LIU Z, BATES J, CHANDLER-MILITELLO D, ZOU L, MUNIR J, SCHANZ S, MCCOY K, MILLER RH, WANG S, NEDERGAARD M, FINDLING RL, TESAR PJ & GOLDMAN SA 2017 Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell, 21, 195–208.e6. [PubMed: 28736215]

XIA M, ABAZYAN S, JOUROUKHIN Y & PLETNIKOV M 2016 Behavioral sequelae of astrocyte dysfunction: focus on animal models of schizophrenia. Schizophr Res, 176, 72–82. [PubMed: 25468180]

XING HQ, MORI K, SUGIMOTO C, ONO F, IZUMO K, KUBODA R & IZUMO S 2008 Impaired astrocytes and diffuse activation of microglia in the cerebral cortex in simian immunodeficiency virus-infected Macaques without simian immunodeficiency virus encephalitis. J Neuropathol Exp Neurol, 67, 600–11. [PubMed: 18520778]

ZLOTNICK C, DONALDSON D, SPIRITO A & PEARLSTEIN T 1997 Affect regulation and suicide attempts in adolescent inpatients. J Am Acad Child Adolesc Psychiatry, 36, 793–8. [PubMed: 9183134]

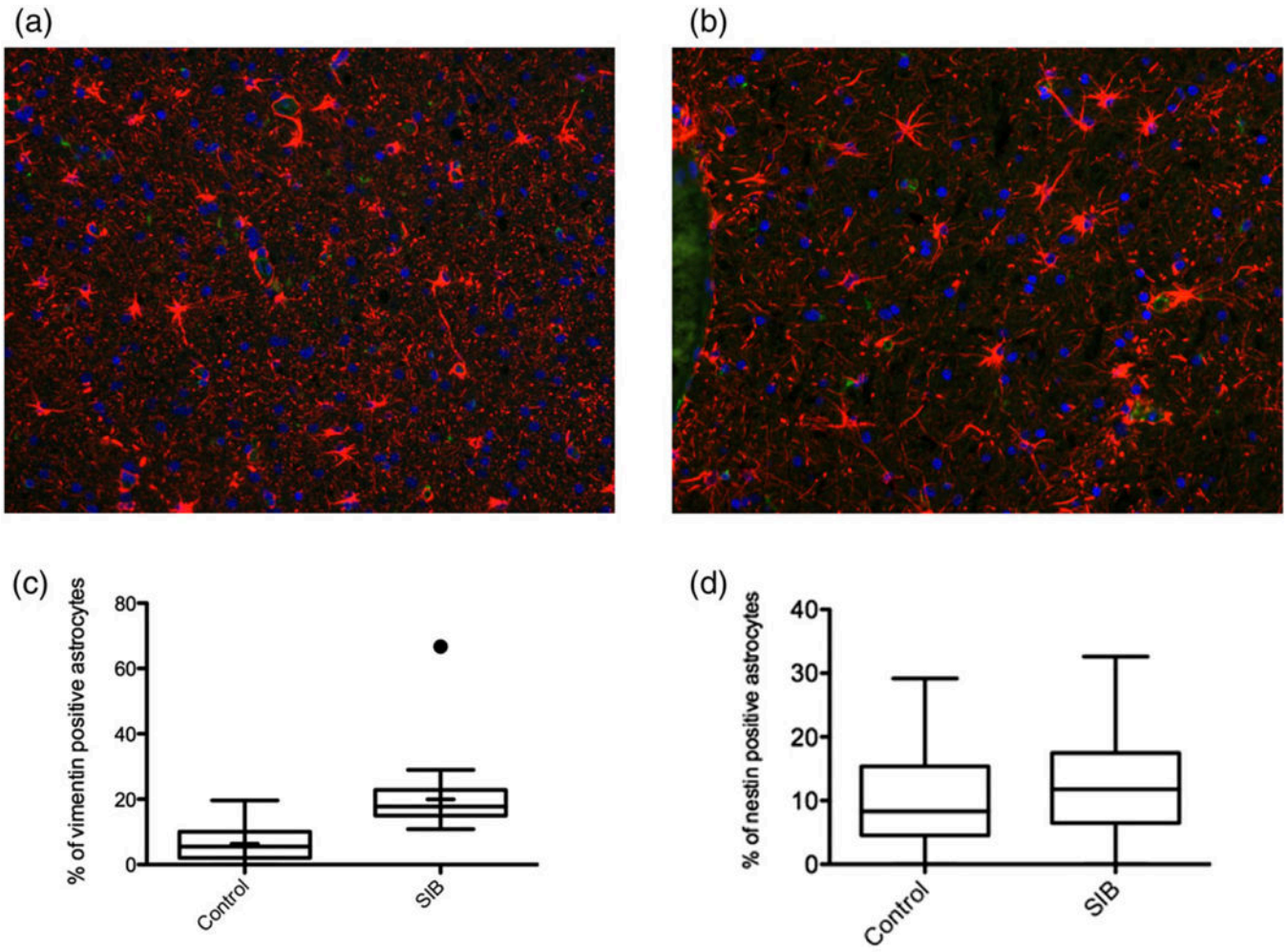

Figure 1. Astrocytes in macaques with SIB have increased vimentin, but not nestin, expression.
Control macaques have very low expression of vimentin (A) in white matter of frontal lobes. There was increased vimentin expression in macaques flagged for SIB (B), which was statistically significant, increasing from 4% to 16% (C). There was no significant change in the proportion of nestin positive astrocytes in animals with SIB (D).

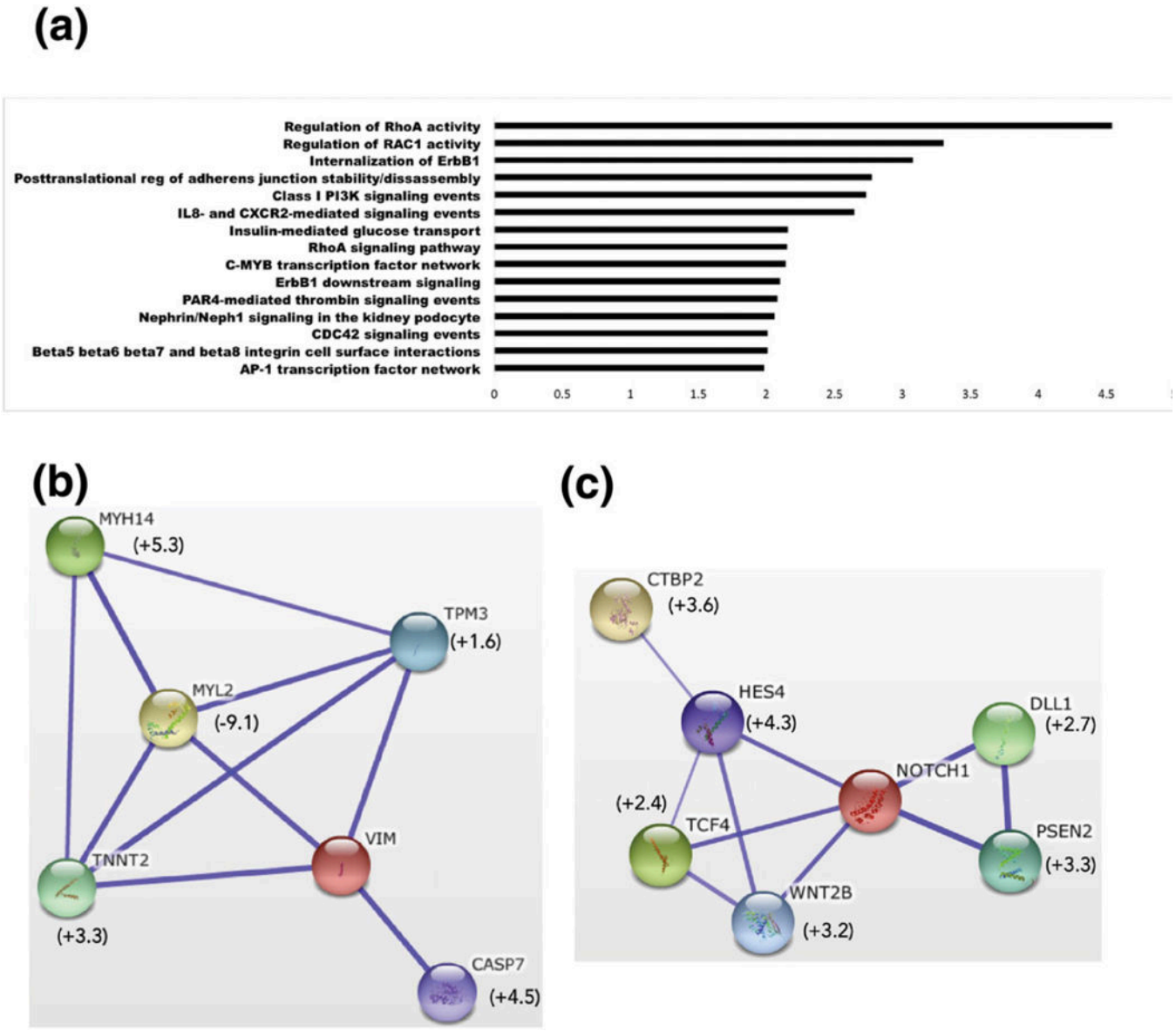

Figure 2. Gene expression analyses of monkeys with self-injury.

Graphical representation for pathways altered following self injury. Results represent –log (p-value) where p-value determined by number of genes in each pathway, relevance of genes to each pathway and number of molecules in database. Data represents probability that altered genes are involved in a particular pathway. Analysis was performed using the Pathway Interaction Database (performed on July 21, 2015). Analyses of interconnected genes were performed using String software. It was noted that genes linked to vimentin expression were differentially-regulated (B). Genes associated with *wnt* and *notch* were also differentially-regulated (C).

Table 1.

Animals, treatment groups, behavioral status and neuropathologic findings, Characteristics of animals on this study.

Animal #	Necropsy #	SIB	Age (years)	Sex	Neuropathologic findings (NSL = no significant lesions)
CN59	08A690	YES	19.52	M	Spongiosis
GB61	11A128	YES	5.65	M	NSL
N061	10A556	YES	18.31	M	Lipofuscin
A999	91A083	YES	12.52	M	Data not available
N539	01A315	YES	8.87	F	Data not available
EH70	06A143	YES	3.00	F	NSL
EI93	08A523	NO	5.31	F	NSL
HT22	11A238	NO	2.91	M	NSL
HM63	11A263	NO	3.04	M	NSL
HN64	11A280	NO	3.03	M	NSL
HP24	11A299	NO	3.03	M	NSL
EB20	06A146	NO	3.82	F	NSL
AV71	08A520	NO	18.42	M	NSL
N142	11A297	NO	18.98	M	NSL
J650	11A560	NO	22.26	M	NSL

Table 2.

Top ten genes up- or down-regulated.

Gene Name	logFC	logCPM	LR	PValue
#HAL	10.429	4.225	23.362	0.000
*BPIFA2	9.244	2.254	9.128	0.003
*MUC7	8.825	1.423	9.773	0.002
HOXD4	8.677	1.552	8.043	0.005
*PI3	8.280	0.968	8.204	0.004
#HBB	7.947	0.665	7.564	0.006
BEX5	7.731	1.146	8.613	0.003
*HRG	7.627	1.706	6.586	0.010
§NKX6–1	7.553	0.374	6.816	0.009
#SLC2A2	7.493	0.393	6.952	0.008
*RNASE7	−11.303	4.921	9.056	0.003
#CPS1	−10.711	4.400	10.196	0.001
#INVS	−10.293	4.069	9.828	0.002
#CA3	−9.957	3.453	7.743	0.005
*MYPN	−9.804	3.564	11.549	0.001
#SNORD115	−9.769	4.854	11.732	0.001
*§FSHR	−9.646	3.192	5.777	0.016
#GALE	−9.505	3.099	8.456	0.004
ACTG2	−9.469	3.302	12.627	0.000
CD28	−9.246	2.978	7.350	0.007

*(*)*The ten genes most up- or down-regulated in SIB are shown. Note that seven of these 20 genes are associated inflammation,

*(#)*eight are associated with behavioral or intellectual disabilities,

*(§)*and two are directly linked to astrocyte activation / maturation .