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Naltrexone treatment reverses astrocyte atrophy and immune dysfunction in self-harming macaques

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ABSTRACT

The role of glia in the development and treatment of behavioral abnormalities is understudied. Recent reports have observed glial activation in several disorders, including depression, autism spectrum disorders and self-injurious behaviors (SIB). In the current study, we examined SIB in the physiologically and anatomically relevant nonhuman primate (NHP) model. At the Tulane National Primate Research Center (TNPRC), approximately 5% of singly housed macaques develop symptoms of SIB. We have previously demonstrated that naltrexone hydrochloride can be effective in reducing SIB. We have also demonstrated that the astrocytes of animals with SIB are distinctly atrophic and display heightened innate immune activation compared with control animals. We have added a third group of animals (five macaques identified with SIB and treated with oral naltrexone at a dose of 3.2 mg/kg) to the previous cohort (six macaques with a history of SIB but not treated, and nine animals with no history of SIB) for this study. Gray and white matter astrocytes from frontal cortical tissue were examined following necropsy. Innate immune activation of astrocytes, which was increased in SIB animals, was markedly decreased in animals receiving naltrexone, as was atrophy of both grey and white matter astrocytes. This was concomitant with improved behavioral correlates. Preventing astrocyte activation in select areas of the brain to reduce injurious behavior is an innovative concept with implications for mental health studies. Differences in multiple areas of primate brain would help determine how self-injurious behavior develops. These studies suggest a stronger role for astrocytes in the cellular events associated with self-injurious behaviors.

1. Introduction

Self-injurious behavior (SIB) is recognized as a significant public health problem in the US (Jacobson and Gould, 2007). SIB is frequently associated with intellectual disabilities, genetic disease, anxiety and depressive disorders (American Psychiatric Association, 2000; Norton et al., 2008). The rhesus monkey model of SIB is informative about some types of human SIB, not only because it is a primate model but also because it arises spontaneously. We have recently initiated studies with a nonhuman primate model for self-harm: self-injurious behavior (SIB) in rhesus macaques (Lee et al., 2013b). In rhesus macaques, SIB usually takes the form of self-directed biting, hair pulling, and head banging (Ribka and Baker, 2004). These animals respond well to treatments with naltrexone and fluoxetine, further indicating the usefulness of this model (Kempf et al., 2012; Ribka and Baker, 2004).

It is known that stress can affect the plasticity of glial cells, including astrocytes (Czeh et al., 2006; Lee et al., 2013b). There are two distinct mechanisms whereby astrocytes can be activated in psychiatric disorders. First, gap junction proteins are down-regulated (Sun et al., 2012) restricting the overall syncytia of astrocytes. Reductions in the number of connecting proteins also alter the morphology of the astrocytes including the number and nature of synapses they can form with neurons and the blood–brain barrier (Czeh et al., 2006). Alternatively, dysregulation in the immune function of glial cells have been implicated in the pathogenesis of psychiatric disorders (Leonard, 2010). Our studies showed that both systems are involved (Lee et al., 2013b), unifying these areas of research.

In humans, SIB comprises of maladaptive behaviors that include skin cutting, scratching, hair-pulling, and injury to body parts and can occur alongside depression and other psychiatric disorders

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(Kerr et al., 2010; Langbehn and Pfohl, 1993; Nagamitsu, 1993). Critically, rhesus macaques also develop SIB, and at the Tulane National Primate Research Center (TNPRC), approximately 5% of singly-housed animals historically have exhibited this potentially debilitating syndrome (Novak, 2003). In rhesus monkeys, this pathology results in self-biting that, on occasion, can result in tissue damage and mutilation (Ribka and Baker, 2004). Although the neurobiological mechanisms of SIB are poorly characterized, we have recently demonstrated that astrocytes in the frontal cortex of macaques with SIB show significant differences in arbor, cell body size and number of processes, as well as increased expression of Toll-like receptor 2 (TLR2), when compared with control animals (Lee et al., 2013b).

There is significant evidence that highlights glial dysfunction in the pathology of mood and psychiatric disorders. Post-mortem histopathological studies have observed reduced glial cell numbers in various frontolimbic areas of depressed patients (Hercher et al., 2009). Changes in cortical astrocyte morphology have been reported in suicidal patients (Torres-Platas et al., 2011). Recently, studies examining the effects of chronic stress on astrocyte morphology and expression of astrocyte markers, GFAP and S100β, observed atrophy in astrocyte process length, branching, and volume (Tynan et al., 2013). Along with our previous studies, these results suggest that significant remodeling in the astrocyte network occurs in the setting of mental disorders. Because of morphological findings showing less GFAP in astrocytes between patients with depression (Fatemi et al., 2004; Torres-Platas et al., 2015) and monkeys with SIB, we postulate that there was a potential pathological and behavioral phenotype associated with these brain changes that may shed light on cellular mechanisms of psychiatric disorders. The mechanisms by which astrocytes are activated in self-injury and how these disrupts trophic and immune responses to neurons and endothelial cells, however, remain to be clarified in more detail.

We have recently shown that SIB macaques treated with two intramuscular injections of 20 mg/kg extended-release naltrexone (separated by 4 weeks) had no reversion to SIB phenotype during a 7 months follow up period (Kempf et al., 2012). Rhesus macaques with SIB also exhibit persistent dysfunction in opioid and stress response systems (Tiefenbacher et al., 2005). We were therefore naturally interested in the underlying impact of this opioid inhibitor on astrocyte activation and morphology.

One hypothesis of SIB implicates the opioid system in the pathophysiology of symptoms (Roy et al., 2014; Stanley et al., 2010). In earlier studies at Tulane National Primate Research Center, we found that injectable naltrexone treatment produced a lasting decrease in SIB that persisted after the end of the treatment period. As naltrexone is effective for addressing SIB in macaques, we examined archival tissues from animals diagnosed with SIB that received oral naltrexone. We were interested in examining effects of naltrexone therapy on glial cell activation. Here we demonstrate that astrocytes of SIB macaques treated with oral naltrexone have a phenotype more similar to control macaques than those displaying the abnormal behavior. We further postulate that activation of astrocytes and concomitant retraction of processes could be a key component in the development of abnormal behaviors.

2. Materials and methods

2.1. Ethics statement and animal housing

Animals were maintained in Animal Biosafety Level 2 housing with a 12:12-h light:dark cycle, relative humidity 30–70%, and a temperature of 17.8–28.9 °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 cm) 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.

2.1.1. Animals were humanely euthanized 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 1 cm thick are routinely collected during necropsy of colony animals at TNPRC representing frontal lobe, parietal & temporal lobe/thalamus/basal ganglia, and cerebellum/occipital lobe. All tissues are fixed at routine necropsy by immersion in 10% neutral buffered formalin with zinc modification for 48 h before trimming and paraffin embedding.

2.1.2. Selection of tissues

For this retrospective study, tissues were selected solely on their availability in the TNPRC tissue archive. All study subjects had been euthanized when clinical or research-related endpoints were reached. For this reason, we were not able to examine regional differences in cell morphology. None of the macaques had been used for infectious or pharmacological studies. Tissue taken from the prefrontal cortex from 9 control, 6 SIB and 5 SIB receiving naltrexone rhesus macaques (*Macaca mulatta*) were used for this study, for a total of 20 animals (Table 1).

Subjects in the current study were drawn from three populations: (1) The control population which did not exhibit SIB, (2) animals flagged for SIB but not treated pharmacologically, and (3) naltrexone-treated animals flagged for SIB. Populations could not be matched for rearing, age at first single housing, duration of lifetime single housing, or severity of self-biting or self-wounding, frequency of relocation, whether they were breeding colony or research animals, or intensity of research procedures, because these factor, as risk factors for SIB, influenced the population to which subjects were appropriately assigned (Gottlieb et al., 2013; Lutz et al., 2003; Novak, 2003; Rommeck et al., 2009) as well as feasibility of obtaining tissue.