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Early gene activation initiates neuroinflammation prior to VSV neuroinvasion: Impact on antiviral responses and sleep

Richard P. Ciavarra [a,*], Patric Lundberg [a], Mayumi Machida [b], Marta A. Ambrozewicz [b], Laurie L Wellman [b], Kimberly Breving [a], Christina Steel [a,1], Larry D. Sanford [b]

[a] Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, 700 West Olney Road, Norfolk, VA 23501, United States
[b] Sleep Research Laboratory, Department of Pathology and Anatomy, Eastern Virginia Medical School, 700 West Olney Road, Norfolk, VA 23501, United States

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ABSTRACT

Rapid eye movement (REM) sleep is rapidly and persistently suppressed during vesicular stomatitis virus (VSV) encephalitis in C57Bl/6J (B6) mice. REM sleep suppression was associated with a complex global brain chemokine/cytokine response with bimodal kinetics although regionally distinct cytokine profiles were readily identified. Cytokine mRNA was translated either immediately or suppressed until the pathogen was cleared from the CNS. Innate signaling pathway (TLRs, RIG-I) activation occurred rapidly and sequentially prior to VSV neuroinvasion suggesting that antiviral states are quickly established in the CNS in advance of viral pathogen penetration. Il1β suppressed REM sleep mimicking aspects of VSV-induced sleep alterations whereas some robustly induced chemokines may be protective of REM. Thus, multiple brain chemokines may mediate sleep across VSV encephalitis via differential somnogenic effects.

1. Introduction

VSV is a member of the *Vesiculovirus* genus in the Rhabdoviridae family, one of the four families in the Order Mononegavirales, and is the prototypic virus of this family that includes rabies virus. The VSV genome is relatively simple, containing only five major proteins (Kang and Prevec, 1969), but is sufficient to highjack the cellular machinery to produce productive infections in a broad range of host cells, including human (Whelan et al., 2004). Administered intranasally, VSV produces a well-characterized model of viral encephalitis in mice. It initially infects and replicates in olfactory receptor neurons and then subsequently infects the deeper layers and surrounding cells of the nasal mucosa (Reiss et al., 1998). VSV protein can be detected in the olfactory bulb (OB) on day 1 post-infection (PI) and then over the course of the next 6 to 7 days, it travels caudally to infect regions and tracts extending into the brain stem. Major structures that show VSV antigen include the septal region, the amygdala, bed nucleus of the stria terminalis, hypothalamus, thalamus and hippocampus, and the dorsal pons including the noradrenergic locus coeruleus (LC) and the serotonergic dorsal raphe nucleus (DRN) (Huneycutt et al., 1994). By day 12 after inoculation, the virus has cleared the brain (Christian et al., 1996) and animals typically recover from infection.

Infectious diseases are commonly accompanied by excessive sleepiness, fatigue and fever. Interactions between sleep, immune responses and disease processes have been amply demonstrated and sleep has been hypothesized to be a component of the acute phase response to infection, to function in host defense (Toth et al., 1993), and to promote recovery (Imeri and Opp, 2009). While changes in sleep are relatively well characterized for the acute phase of infection, much less is known about the long-term alterations in sleep and arousal that can occur with certain infections. Our work with VSV encephalitis in C57Bl/6J (B6) mice demonstrates that it produces increases in non-rapid eye movement (NREM) sleep and decreases in rapid eye movement (REM) sleep and activity during the acute phase response similar to those reported for various infectious agents. However, with VSV infection, decreases in REM (and activity) can begin as early as day 1 PI and can persist after recovery from encephalitis and after viral clearance from the brain (Machida et al., 2014). Long-lasting alterations in behavior have also been reported. For example, infant rats that survive a transient VSV infection show a permanent reduction in serotonin and permanent behavioral alterations, including hyperactivity and poor

Abbreviations: REM sleep, rapid eye movement; NREM sleep, non-rapid eye movement sleep; IFN-I, type I interferon; PI, post infection; TLRs, Toll like receptors; RIG-I, retinoic acid inducible gene-I; ICV, intracerebroventricular; NLRP3, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) pyrin-containing protein 3; OB, olfactory bulb; VP, ventral pons; DP, dorsal pons; Hypo, hypothalamus; BF, basal forebrain; B6, C57Bl/6J; VSV, vesicular stomatitis virus; DRN, dorsal raphe nucleus; IPA, Ingenuity Pathway Analysis; UR, Upstream Regulators; TG, Top Expressed Genes.

* Corresponding author at: Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501, United States.
E-mail address: ciavarrp@evms.edu (R.P. Ciavarra).
[1] Present address Department of Biology, Old Dominion University, Norfolk, VA 23529.

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performance on the Morris water maze (Mohammed et al., 1992), which can be observed in adulthood long after the virus has been eliminated by the immune system. Thus, VSV can be a valuable model for examining how transient viral infections of the brain can lead to persisting alterations in neural function.

Similar to several other neurotropic viruses, VSV neuroinvasion induces an inflammatory response characterized by a potent mixed cellular infiltrate of neutrophils, macrophages, DCs, T cells and eventually B cells (Bi et al., 1995; Steel et al., 2009). This response most likely reflects the early and abundant production of chemokines and proinflammatory cytokines (Ireland and Reiss, 2006) which act to regulate host defense (Johnston et al., 2009). They are also major mediators of the sleepiness and fatigue that occur during the acute phase response (Dinarello, 1988; Ebadi et al., 1997; Moldofsky, 1994). Interestingly, recent studies from Bevan's group identified a population of infiltrating T cells, termed tissue resident T memory (Trm) cells, that remain in the brain following VSV clearance and express a distinctive phenotype and genetic program that may allow them to become permanent residents in the brain (Wakim et al., 2010, 2012). Importantly, Trm cells continue to express elevated RNA levels for a number of chemokines and inflammatory cytokines that may have long-term consequences for sleep and behavior even after the virus is cleared. Thus, while there is the potential for virus-induced damage to impact sleep, as suggested by the classic work of von Economo on encephalitis lethargica (Economo, 1931), changes associated with VSV infection could involve the production of chemokines and cytokines both before the virus enters the brain and after its clearance.

The purpose of the current study was to characterize the neuroimmune response to VSV neuroinvasion during the acute and recovery phases, identify innate recognition molecules and signaling pathways triggered by this neurotropic viral pathogen and to determine the potential of highly expressed chemokines and proinflammatory cytokines for producing alterations in sleep. We first characterized global (whole brain) cytokine/chemokine profiles and then regional responses in brain regions associated with sleep regulation. We then identified innate antiviral signaling pathways activated in the CNS either prior to or coincident with VSV neuroinvasion. Based on the resulting cytokine/chemokine mRNA profiles, we also assessed the ability of selected, robustly induced chemokines (Ccl7, Ccl25, Cxcl10) and two cytokines (interleukin 1β (Il1β), osteopontin (OPN)) to alter sleep to determine whether they could be important modulators of the changes in sleep seen during VSV encephalitis. The results of our studies are the subject of this report.

2. Methods

2.1. Subjects

All studies were performed in male B6 mice purchased from Jackson Laboratories (Bar Harbor, ME). After arrival, they were housed in sterile microisolator cages and maintained in our satellite animal biosafety level 2 facility. Sterile food and water were given ad libitum. The colony room was kept on a 12:12 light-dark cycle and ambient temperature was maintained at 24 °C ± 0.5 °C. Throughout the experiments, measures were taken to avoid unnecessary pain and discomfort of the animals. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by Eastern Virginia Medical School's Institutional Animal Care and Use Committee.

2.2. Virus infection and brain cytokine profiles

Wild-type VSV-Indiana strain, provided by Dr. Philip Marcus, University of Connecticut, was grown and assayed as previously described (Marvaldi et al., 1977). Virus was grown in confluent monolayers of Vero cells and virus titers determined by standard plaque assays (Sekellick and Marcus, 1986). VSV was introduced into the brain via intranasal application of 5 ul/nostril containing 5×10^4 PFU VSV (Barna et al., 1996). At select time points, mice were euthanized, perfused with cold PBS and either whole brains or discrete brain regions isolated for subsequent analysis. RNA was purified from homogenized brain tissue using the QuickGene RNA tissue kit SII (RT-S2) from FUJIFILM Corporation, pooled and cytokine profiles characterized by real-time PCR using a commercial inflammatory cytokine array (Qiagen, catalogue number PAMM-0011). Brains from uninfected mice given intranasal PBS were used as controls (mock infected) to calculate fold differences in experimental groups. Innate antiviral responses were characterized in a similar fashion but using a qPCR array focused on several innate signaling pathways (Qiagen, catalogue no. PAMM-122ZD). To validate that cytokine mRNA was translated, protein levels were determined in individual mouse brain homogenates using cytokine-specific ELISAs (R&D Systems, Minneapolis, MN).

2.3. Surgery

The subjects for the sleep studies were 18 male C57Bl/6J mice weighing 20 to 25 g at the time of surgery. All animals were maintained in the colony room as described above. After 1 week of acclimation to the colony room, the mice were implanted intraperitoneally with telemetry transmitters (ETA10-F20, DataSciences, St. Paul, MN) for recording EEG, activity and core body temperature in order to monitor sleep and wakefulness and potential febrile responses. The mice were also provided with a guide cannula aimed into a lateral cerebral ventricle for microinjections. EEG leads from the transmitter body were led subcutaneously to the head, and the free ends were placed into holes (one in the left anterior quadrant and one in the right posterior quadrant near Bregma) drilled in the dorsal skull to allow recording cortical EEG. Aseptic techniques were used for all surgical procedures. Prior to surgery, the animals received potassium penicillin (100 IU/g weight), dexamethasone (0.005 mg/g weight), and gentamicin (0.005 mg/g weight) subcutaneously. All surgery was conducted under isofluorane (as inhalant: 5% induction; 2% maintenance) anesthesia. Ibuprofen (30 mg/kg, oral) was continuously available in each animal's drinking water for 24 to 48 h preoperatively and for a minimum of 72 h postoperatively to alleviate potential postoperative pain. The animals were kept undisturbed except for weekly bedding changes for at least 2 weeks for post-surgery recovery before used in the study.

2.4. Chemokines and microinjection procedures

Ccl7, Ccl25 and Cxcl10 were purchased from Creative Biomart (Shirley, NY) and Il1β and OPN were purchased from R&D Systems (Minneapolis, MN). Each was freshly prepared in pyrogen-free saline on the day of microinjection and was given ICV in a volume of 0.6 μl administered over 2 min. All microinjections were performed during the second hour of the light period. Prior to receiving microinjections, the animals were habituated to the 5 min handling procedure required for administering the microinjections over 2 consecutive days to minimize stress. The injection cannula were left in place for 1 min post-injection to allow maximal absorption of the solution. Mice were returned to their home cages and recording began at the beginning of the third hour for 10 h. For control experiments, an equal amount of vehicle only (pyrogen-free saline) was microinjected using identical procedures. These control recordings were obtained from the same animal; thus, a within-subject design was used. Four to six animals were used to test each chemokine/cytokine at two different doses (20 ng; 100 ng). Some mice were used for testing multiple chemokines, but all comparisons were to vehicle controls specific to each microinjection series. Treatments were separated by at least 4 days.