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Sexually dimorphic role of BNST vasopressin cells in sickness and social behavior in male and female mice

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ABSTRACT

Circumstantial evidence supports the hypothesis that the sexually dimorphic vasopressin (AVP) innervation of the brain tempers sickness behavior in males. Here we test this hypothesis directly, by comparing sickness behavior in animals with or without ablations of BNST AVP cells, a major source of sexually dimorphic AVP in the brain. We treated male and female AVP-iCre+ and AVP-iCre− mice that had been injected with viral Cre-dependent caspase-3 executioner construct into the BNST with lipopolysaccharide (LPS) or sterile saline, followed by behavioral analysis. In all groups, LPS treatment reliably reduced motor behavior, increased anxiety-related behavior, and reduced sucrose preference and consumption. Male mice, whose BNST AVP cells had been ablated (AVP-iCre+), displayed only minor reductions in LPS-induced sickness behavior, whereas their female counterparts displayed, if anything, an increase in sickness behaviors. All saline-treated mice with BNST AVP cell ablations consumed more sucrose than did control mice, and males, but not females, with BNST AVP cell ablations showed reduced preference for novel conspecifics compared to control mice. These data confirm that BNST AVP cells control social behavior in a sexually dimorphic way, but do not play a critical role in altering sickness behavior.

1. Introduction

Most animals experience pathogen-induced sickness during their lifetime. While each pathogen brings its own set of inflammatory responses and other symptoms, sickness often causes general behavioral changes such as lethargy, reduced ingestive behavior, and social withdrawal (Kelley et al., 2003). These behavioral changes are generally thought to complement physiological responses, such as fever, in speeding up recovery (Hart, 1988). While such behavioral changes may be beneficial for survival, long-term or inappropriate inflammation may contribute to mental health conditions such as depression (Miller and Raison, 2016). Consequently, understanding the ways in which inflammation alters behavior may help treat such conditions.

The physiological basis of sickness behavior involves multiple pathways that relay information about peripheral inflammation, such as vagus nerve activity and humoral immune signaling (D'Mello and Swain, 2017). Ultimately, peripheral inflammation activates brain regions such as the paraventricular hypothalamus (PVH), medial amygdala (MeA), bed nucleus of the stria terminalis (BNST), and preoptic area (Goehler et al., 2000; Sagar et al., 1995), all of which have been associated with behavioral profiles altered during sickness (Konsman et al., 2008; Lacosta et al., 1999; Taylor et al., 2012).

Some of these areas, such as the posterior BNST and MeA, contain arginine vasopressin (AVP) cells that have been indirectly implicated in regulating fever and sickness behavior (Pittman et al., 1998a,b; Sens et al., 2017). For example, in rats, fever increases BNST neuronal activity (Mathieson et al., 1989), and electrical stimulation of the BNST reduces fever (Naylor et al., 1988). This may be due to effects of AVP in the septum, a target of BNST/MeA AVP projections, as septal AVP administration also reduces fever. This effect is testosterone-dependent, and is found in males but not in females (Pittman et al., 1998b, 1988), mirroring the sex differences in BNST AVP expression, which is more pronounced in males than in females (De Vries and Panzica, 2006), which suggest that BNST AVP cells modulate the fever response.

The same cells may also regulate sickness behavior. For example, in male rats intracerebroventricular injections of AVP reduce sickness behavior, whereas AVP antagonism exacerbates sickness behavior (Dantzer et al., 1991). These effects are also testosterone-dependent; effects of inflammation and AVP administration are more pronounced in castrated animals, which cease to produce AVP in the BNST (Dantzer

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et al., 1991; De Vries and Panzica, 2006). However, whether BNST AVP cells modulate sickness behavior has not been directly tested. We do so here by selectively ablating AVP cells in the BNST of male and female AVP-iCre mice via injections of viral vector containing a Cre-dependent cell death construct (caspase-3/Tev) and testing effects of ablation on sickness behavior. We induced sickness behavior via intraperitoneal injections of lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, commonly used as a proxy for bacterial infections (Dantzer et al., 1998), followed by tests for behaviors altered in sickness. We predicted that BNST AVP cell ablation would intensify sickness behavior, more so in males than in females.

2. Material and methods

2.1. Animals

All mice were maintained at 22 °C on a 12:12 reverse light cycle with food and water available *ad libitum*, housed in individually ventilated cages (Animal Care Systems), and provided with corncob bedding, a nestlet square, and a housing tube. All animal procedures were approved by the Georgia State University Institutional Animal Care and Use Committee (IACUC) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Founding AVP-iCre mice were obtained from Dr. Michihiro Mieda (Kanazawa University, Japan). These mice were generated using a bacterial artificial chromosome that expressed codon-improved Cre recombinase (Shimshek et al., 2002) under the transcriptional control of the AVP promoter (AVP-iCre mice). In these animals, iCre expression is found in the bed nucleus of the stria terminalis and the medial amygdala, as well as in hypothalamic areas (Mieda et al., 2015). Subjects were derived by crossing heterozygous iCre+ mutants to wildtype C57Bl/6J mice and genotyped (ear punch) by polymerase chain reaction (PCR) at 21–24 days of age (Transnetyx). Both iCre+ and iCre− littermates were used in behavioral experiments. All subjects were used in a prior experiment (Rigney et al., 2019) and all surgical procedures described below were conducted as part of that study. Stimulus animals for the three-chamber test were adult C57B6/J mice of both sexes, group housed in the same room conditions as the experimental animals.

In total, 45 animals with confirmed BNST AVP cell ablation (Rigney et al., 2019) were used for the behavioral testing described below: 11 Cre− males, 13 Cre − females, 13 Cre+ males, and 8 Cre+ females. Two female subjects (1 Cre+ and 1 Cre−) did not recover from initial LPS treatment and were euthanized and removed from all analyses. The remaining 43 animals were tested on all behavioral measures described below. Video recording error forced removal of 1 Cre+ female from the tail suspension test, and removal of 1 Cre+ and 1 Cre− female from the three-chamber social test analyses. Bottle failure and fluid leakage forced the removal of 6 females (5 Cre−, 1 Cre+) from the sucrose preference analysis.

2.2. Viral vectors

BNST neurons with AVP promoter-driven Cre-expression were ablated using an adeno-associated virus (AAV-flex-taCasp3-TEVp) (University of North Carolina at Chapel Hill Vector Core) that encodes Cre-dependent pro-caspase-3. This enzyme activates an apoptotic signaling cascade, cleaving multiple structural and regulatory proteins critical for cell survival and maintenance (Unger et al., 2015; Yang et al., 2013) and thereby inducing far less inflammation than other lesion approaches (Morgan et al., 2014). High titer AAV of serotype 2/1 (3×10^{12} IU/mL) was purchased from the University of North Carolina at Chapel Hill Vector Core (Rigney et al., 2019).

2.3. Stereotaxic surgery

All surgeries were carried out using 1.5–3% isoflurane gas anesthesia in 100% oxygen; 3 mg/kg of carprofen was given before surgery to reduce pain. Mice were positioned in a stereotaxic frame (David Kopf Instruments) with ear and incisor bars holding bregma and lambda level. After a midline scalp incision, a hand operated drill was used to make holes in the skull, exposing the dura. For all subjects, 500 nl of AAV-flex-taCasp3-TEVp was delivered bilaterally to the BNST (coordinates: AP −0.01 mm; ML ± 0.75 mm; DV 4.8 mm (Paxinos and Franklin, 2012) at a rate of 100 nl/min using a 5 µl Hamilton syringe with a 30-gauge beveled needle mounted on a stereotaxic injector. Following virus delivery, the syringe was left in place for 15 min and slowly withdrawn from the brain (Rigney et al., 2019).

2.4. Experimental procedure

All behavior tests were done in the dark phase under red lighting, and animals were acclimated to the behavior testing suite for at least one hour before testing. At least one week after tests for social and communicative behavior described in (Rigney et al., 2019) and seven weeks after viral ablation, subjects were weighed and injected intraperitoneally with either 1 mg/kg LPS (from *E. coli* 0111:B4, Millipore-Sigma) or sterile saline one hour before dark phase (ZT11). While the LPS dose used is highly variable across previous studies, inflammation and sickness behavior after LPS administration have generally been reported to occur from 2 h to over 24 h post-injection (Biesmans et al., 2013; Dantzer et al., 2008), and the dose of LPS used in this study reliably induces sickness behavior (Lacosta et al., 1999). Therefore, the open field test (OFT) was conducted three hours following LPS or saline injections, and the elevated zero maze (EZM) test was conducted immediately following the OFT. Sucrose preference was then assessed in the home cage over a 20-hour period, starting 5 h after LPS or saline injections). Immediately following this (25 h post-injection), animals were tested in the three-chamber social interaction test and tail suspension test (TST). This entire sequence was repeated one week later, with animals that first received LPS now receiving saline and vice versa as indicated in Fig. 1. An interval of one week between treatments was chosen, because LPS causes sickness for only up to four days post-injection (Weiland et al., 2007). To make sure there were no residual effects of the initial LPS treatment results were compared across treatment order.

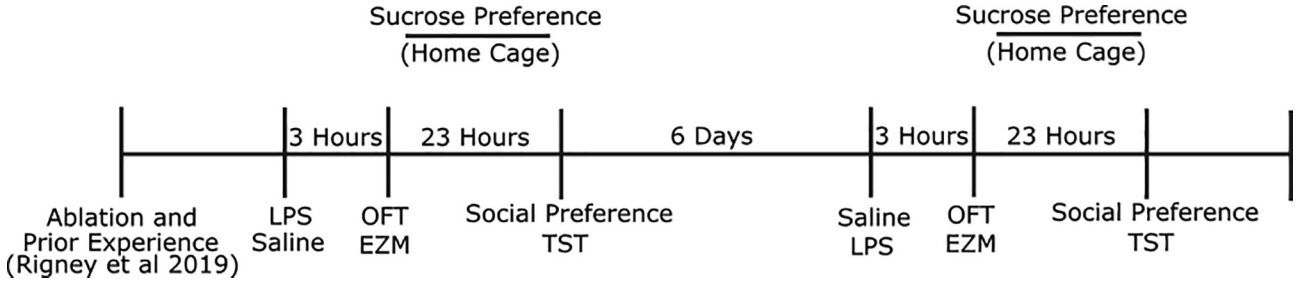

Fig. 1. Experimental timeline.