understanding of the underlying neural circuits, evidence is accumulating that cephalopods are capable of high-level congitive and affective processing of sensory inputs. In recent studies, we have demonstrated the presence of nociceptors in the peripheral nervous system of cephalopods, which bear physiological similarities to those of other invertebrates and vertebrates, but are more vertebrate-like in their patterns of long-term plasticity after tissue injury. We have also demonstrated behavioral plasticity that suggests pain-like states are experienced by cephalopods. These ongoing studies raise important and novel questions about the evolution of complex brains and behaviors in diverse animals, and shed light on underlying, sharred organizing principles of pain-associated neural circuits that are common to large-brained animals widely separated over evolutionary time. I will also disucss the ethical implications of neurobiological research on cephalopods in light of growing evidence for potential suffering during invasive procedures.

Declaration of Interest Statement

None

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PARALLEL SYMPOSIUM
PARALLEL SYMPOSIUM 34: HOW DO ORGANISMS ADAPT? LESSONS FROM EVOLUTION, PROPRIOCEPTION, AND RNA EDITING
13-09-2023 10:05 - 12:05

HIGH-LEVEL MRNA RECODING IN THE CEPHALOPOD NERVOUS SYSTEM

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Within the nervous systems of poikilotherms, temperature changes challenge the integration of physiological function. Within the complex nervous systems of the behaviorally sophisticated coleoid cephalopods, these problems are magnified. RNA editing by adenosine deamination is a well-positioned mechanism for environmental acclimation. By converting specific adenosines to inosines, a biological mimic for guanosine during translation, the ADAR enzymes can recode messages. In previous studies, our group has shown that the cephalopod neural proteome undergoes massive reconfigurations due to A>I RNA editing. In fact, the majority of neural messages are altered, and editing is particularly enriched in messages encoding the machineries for excitability and synaptic transmission. Editing frequencies at thousands of sites are dependent on temperature, with editing stronger in the cold. For two highly temperature sensitive examples, recoding tunes protein function. For synaptotagmin, a key component of Ca^{2+}-dependent neurotransmitter release, crystal structures and supporting experiments show that editing alters Ca^{2+} binding. For kinesin-1, a motor protein driving axonal transport, editing regulates transport velocity down microtubules. Seasonally sampled wild-caught octopus specimens indicate that temperature-dependent editing occurs in the field as well. These data indicate that temperature has a profound effect on the encoding of the neural proteome. Cephalopod genomes encode two catalytically active ADARs, orthologous to vertebrate ADAR1 and ADAR2. We next asked which one is responsible for the high-level recoding activity. Using the squid *Euprymna berryi*, we knocked out the ADAR1 ortholog using CRISPR-Cas9. Adar1$^{-/-}$ animals died immediately before hatching, but heterozygotes could be bred through multiple generations. Transcriptome-wide editing analyses of pre-hatchling brains indicated that the vast majority of recoding sites are edited by ADAR1. In mammals, ADAR2 is the principal recoder. Thus it appears that ADAR1 in cephalopods has independently evolved into a potent message recoder.

Declaration of Interest Statement

None

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PARALLEL SYMPOSIUM
PARALLEL SYMPOSIUM 35: NEUROBIOLOGY OF STRESS: SEX DIMORPHISMS, STRESS RESILIENCE AND NEUROCHEMICAL AND NEUROIMMUNE MECHANISMS
13-09-2023 10:05 - 12:05

SITE-SPECIFIC REGULATION OF STRESS RESPONSES ALONG THE ROSTROCAUDAL AXIS OF THE INSULAR CORTEX IN RATS: ROLE OF LOCAL CORTICOTROPIN-RELEASING FACTOR (CRF) NEUROTRANSMISSION

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The insular cortex (IC) is implicated in responses to stressful stimuli, and a functional dissociation along the rostrocaudalaxis of the IC has been reported in this control. Terminals containing the corticotropin-releasing factor (CRF) and neurons expressing the CRF1 receptor were identified in the IC. Thus, we investigated the role of CRF neurotransmission, acting via local CRF1 receptors, in different sites along the rostrocaudal axis of the IC in anxiogenic-like and cardiovascular responses evoked by acute restraint stress. For this, male Wistar rats had cannula-guide bilaterally implanted within the anterior (aIC), rostral posterior (prIC) or caudal posterior (cpIC) subregions of the IC. The restraint stress was realized by placing the animals in a cylindrical plastic tube for 60 minutes. Anxiogenic-like effect was investigated by evaluating the behavior in the elevated plus maze (EPM) five minutes after ending the restraint session, whereas mean arterial pressure (MAP) and heart rate (HR) were recorded throughout the restraint period. Independent sets of animals received the selective CRF1 receptor antagonist CP376395 (5nmol/100nL), CRF (0.07nmol/100nL) or vehicle (100nL) into either the aIC, prIC or cpIC 10 minutes before the restraint onset. Restraint stress caused an anxiogenic-like effect in the EPM and increased both MAP and HR. Bilateral microinjection of either CP376395 into the cpIC ($P<0.05$) or CRF into the rpIC ($P<0.05$) inhibited the restraint-evoked anxiogenic-like effect. Administration of CP376395 into the rpIC decreased selectively the MAP increase during restraint stress ($P<0.05$), whereas the same treatment in the cpIC reduced both the pressor ($P<0.05$) and tachycardiac ($P<0.05$) responses. Pharmacological treatments in the aIC did not affect the behavioral and cardiovascular changes evoked by restraint

(P > 0.05). Thus, our findings indicate that CRF neurotransmission within the IC is involved in anxiogenic-like and cardiovascular responses caused by stress, and this control is site-specific along the rostrocaudal axis of the IC.

Declaration of Interest Statement

None

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PARALLEL SYMPOSIUM
PARALLEL SYMPOSIUM 35: NEUROBIOLOGY OF STRESS: SEX DIMORPHISMS, STRESS RESILIENCE AND NEUROCHEMICAL AND NEUROIMMUNE MECHANISMS
13-09-2023 10:05 - 12:05

NEUROIMMUNE TARGETS TO ENHANCE RESILIENCE TO A HYPERVIGILANT PHENOTYPE IN FEMALES

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Stress-related disorders such as post-traumatic stress disorder are prevalent world-wide and disproportionately affect females. One hallmark symptom of this disorder is hyperarousal and hypervigilance, regulated in part by an exaggerated locus coeruleus (LC)-norepinephrine system. Recent evidence points towards heightened neuroimmune signaling in response to stress as a mechanistic factor underlying this behavioral dysfunction. Previous experiments in our lab determined that social stress, specifically vicarious witness stress (WS), leads to development of a hypervigilant phenotype and increased neuroimmune signaling within the LC-norepinephrine system of females. Importantly, proinflammatory cytokines activate LC neurons, ultimately promoting downstream norepinephrine release. We have identified that decreasing IL-1β expression via microglial cell knockdown in the LC blocks the hypervigilant behavioral and autonomic response to WS. Taken together, these studies determined if increased neuroimmune signaling may be a critical factor eliciting increased susceptibility to social stress-evoked behavioral dysfunction. This study used a chemogenetic DREADDs techniques with a virus engineered to target microglia (AAV-CD68-Gi) to evaluate the functional and behavioral impact of transiently blocking microglial activity within the LC during WS exposure on subsequent hypervigilance-related outcomes. First studies aimed at validating the selectivity and efficacy of the DREADD virus to inhibit microglia were conducted. Next, rats were injected with either CNO to activate the DREADDs virus or vehicle one hour prior to stress. These studies concluded that DREADD-mediated microglial inhibition prevents the emergence of the hypervigilant burying behaviors both during stress and in response to the stress context. Further, as predicted, the DREADD-mediated inhibition of microglial cells was confirmed to suppress neuronal activity of noradrenergic cells in the LC as confirmed with cfos immunohistochemistry. In all, these experiments identified stress-evoked LC microglial activation as a key factor mediating hypervigilant burying behavior in females and may provide a novel treatment target for the prevention of stress-related pathologies

Declaration of Interest Statement

None

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PARALLEL SYMPOSIUM
PARALLEL SYMPOSIUM 35: NEUROBIOLOGY OF STRESS: SEX DIMORPHISMS, STRESS RESILIENCE AND NEUROCHEMICAL AND NEUROIMMUNE MECHANISMS
13-09-2023 10:05 - 12:05

HIPPOCAMPUS SELECTIVE INACTIVATION THROUGH DAUN02 METHOD IN MALE AND FEMALE C-FOS TRANSGENIC RATS AND STRESS-INDUCED ADAPTATION RESPONSES

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Aim

Present work used Daun02 inactivation method in c-Fos lacZ transgenic rat in order to evaluate the effect of ventral hippocampus (VH) on behavioral responses related to repeated chronic stress (CRS) in male and female rats. Daun02 is substrate of β-galactosidase that catalyses Daun02 into daunomycin, causing neuronal inactivation. Methods: Guide cannulas were implanted into the VH of male and female c-Fos LacZ transgenic rats. We submitted rats to acute restraint stress (RS, 2 h/day for 1 day) or CRS (2 h/day for 21 days) and studied the effect of vehicle or Daun02 microinjection into the VH on RS or CRS-induced anxiety- (elevated plus maze-EPM and open field-OF) and depressive-like (forced swimming test-FST and splash test-ST) responses observed, respectively, 24 and 48 h after the last stress session.

Results

RS or CRS caused no anxiety-related effect observed in EPM and OF tests in both gender of lacZ animals. However, bilateral microinjection of Daun02 into the VH promoted an anxiogenic-like effect observed in EPM in chronically stressed female rats. Furthermore, CRS but not RS caused an increase in grooming time in both gender