PROJECT SUMMARY (See instructions):

Brain Circuit Function and Locus Coeruleus

How the brain processes information about the environment strongly depends on the behavioral state. Sleep, neutral rest during wakefulness, physical activity, attentive states, reward, stress and emotions are associated with varying intensities of arousal, mainly controlled by the neuromodulators orexin, acetylcholine and norepinephrine (NE). Locus coeruleus (LC) is the central source for delivering NE throughout the forebrain and cerebellum. Cellular and circuit mechanisms how neuromodulators influence activity in the brain during awake behavior is not well understood. The arguably most controlled experimental approach to investigate circuit activity with cellular resolution dependent on awake behavioral states is the head-fixed mouse locomotion paradigm. Locomotion can be easily monitored and it is unambiguously distinguishable from the resting state. Recent experimental evidence from us and others implicates NE and acetylcholine in mediating locomotion-induced changes in astroglial and neuronal activity. We found that locomotion induced an NE-dependent transient global activation of astroglia throughout the brain and cerebellum, lasting up to 10 s. To achieve a comprehensive understanding of the contribution of noradrenergic signaling to behavioral state-dependent circuit function we need to manipulate LC activity independently from monitoring select cell types' activity in the brain. Specifically, we want to be able to inhibit LC activity by means of optogenetics while simultaneously monitor Ca^{2+} dynamics in astrocytes or interneurons in the target areas. Our preliminary data indicate that the dopamine-beta-hydroxylase (Dbh)-BAC-Cre mouse enables noradrenergic neuron-specific Cre recombination. In order to preserve cell type specificity while manipulating two cell types simultaneously and at the same time capitalizing on the established Cre-loxP based GCaMP tools available, we propose to develop NE neuron-selective manipulation based on the Tet-OFF system. We will create a Dbh-BAC-tTA mouse line. Our expected result is that injection of AAV5-TRE-Arch-mCherry in the vicinity of LC will lead to noradrenergic neuron-specific expression of the inhibitory optogenetic proton pump Arch. Combining optogenetic inhibition of LC with in vivo two-photon Ca^{2+} imaging of vasointestinal peptide $(VIP)^{+}$ interneurons, which experience acetylcholine-dependent locomotion-induced Ca^{2+} elevations, will enable us to test if locomotion-induced cholinergic signaling depends on LC activity. We have recently also found a tight covariability of NE-mediated Ca^{2+} responses in visual cortex astrocytes and cerebellar Bergmann glia. This suggests that global astroglia Ca^{2+} dynamics can serve as indicator of LC activation. To capitalize on this possibility for understanding trial-to-trial variability in locomotion-induced neuronal Ca^{2+} dynamics we will create the GLAST-BAC-RCaMP mouse line. We expect that we will be able to image red fluorescence signals of astrocyte Ca^{2+} dynamics and simultaneously green GCaMP6 signals of VIP interneuron Ca^{2+} dynamics. If locomotion-induced cholinergic activation of VIP interneurons depends on LC activation, we expect a significant trial-to-trial covariability between VIP interneuron and astrocyte Ca^{2+} elevations.

RELEVANCE (See instructions):

The neuromodulators NE and acetylcholine are released from separate terminals throughout large areas of the cerebral cortex and adapt the functional state of local circuits to the behavioral state. We are proposing to create novel transgenic mouse lines to facilitate our understanding of cellular mechanisms underlying cortical circuit adaptations and how neuromodulatory pathways may interact. Specifically, we want to test the hypothesis that LC plays a master regulator role in behavioral state-dependent circuit adaptations. NE is critical for arousal, synaptic plasticity, attentional shifts and reward. An imbalance in noradrenergic signaling is implicated in a wide range of neuro-psychiatric diseases, such as attention deficit hyperactivity syndrome (ADHS), schizophrenia, post-traumatic stress disorder (PTSD), Parkinson's and Alzheimer's disease. Our studies of the role of NE in behavioral state-dependent brain circuit function will help build the foundation for devising new therapeutic strategies to tackle these devastating diseases. Drs. Paukert and Bhat have complementary and non-overlapping expertise in the key technologies for studying brain circuit function in awake mice with cellular resolution and specificity, in vivo two-photon microscopy and mouse transgenesis.