Title: Characterizing affective dysfunction across rodent models of pulmonary hypertension

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Background: Pulmonary hypertension (PH) is a progressive cardiopulmonary disease characterized by pulmonary vascular remodeling and right ventricular hypertrophy, leading to right heart failure. PH is also associated with increased rates of affective disorders, such as anxiety and depression, that cannot be fully explained by disease burden. The monocrotaline rat model of PH also exhibits anxiety- and depressive-like behaviors, suggesting that a neurobiological mechanism may underlie this dysfunction. Despite these high rates, less than half of PH patients with affective disorders are treated with psychotropic medication. Neuroimaging studies in PH patients have revealed reduced gray matter volume in multiple brain regions involved in affective function, including the amygdala and hippocampus. Recent evidence from our lab and others indicates neuroinflammation in affective regions, such as the hypothalamus, and implicates microglial activation in increased PH severity. Microglia, the resident immune cells of the brain, are responsible for a variety of roles, including modulation of neural circuitry through synaptic pruning. Whether microglia-dependent synaptic pruning is dysfunctional in PH is unknown. First, we hypothesize that affective dysfunction is an intrinsic feature of PH pathophysiology rather than a consequence of monocrotaline toxicity and is therefore reproducible across other PH models. We further hypothesize that aberrant microglia-dependent synaptic pruning drives synaptic reorganization that underlies affective dysfunction in PH. Methods: Animals were subjected to affective behavioral assays, including the elevated plus maze and open field test, at an early and late stage of disease development to assess anxiety-like behavior in multiple rodent models of PH. We selected the chronic hypoxia mouse model (n=18), the Sugen-hypoxia mouse model (n=10), and the Sugen-hypoxia rat model (n=12), which most closely recapitulates human PH. Right ventricular systolic pressure (RVSP) was measured via right heart catheterization, and brains were collected for immunohistochemical analysis. Results: Mice exposed to the chronic hypoxia model of PH displayed no changes in anxiety-like behavior, despite a significant but modest elevation of RVSP (p=0.01). Similar results were observed in the Sugen-hypoxia (SuHx) mouse model (p=0.01), suggesting that mice do not develop anxiety-like behavior in these models of PH. In the Sugen-hypoxia rat model, however, SuHx rats exhibited a significantly greater decrease in the proportion of open arm entries in the elevated plus maze (-35%, p=0.04), indicating increased anxiety-like behavior over time. Furthermore, in SuHx rats, change in open arm duration and proportion of open arm entries had strong negative correlations with RVSP (r=-0.73, p=0.03; r=-0.71, p=0.03), suggesting that anxiety-like behavior worsens with increasing disease severity. Conclusions: These findings demonstrate that behavioral dysfunction is not limited to a single model of PH and suggest a potential connection between disease severity and affective dysfunction. Future studies will investigate whether altered microglia-dependent synaptic pruning is associated with anxiety-like behavior across these preclinical models of PH.