A PACAP analogue prevents LHb dysfunction and motivational deficits following mild traumatic brain injury in mice

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INTRODUCTION

- Many mTBI patients exhibit stress-related disorders which have potentially life-threatening impact on quality of life and perceived symptom severity.
- The lateral habenula (LHb) is a critical brain region involved in the pathophysiology of psychiatric illnesses including depression and anxiety.
- Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous neuropeptide and playa an important role in neuromodulatory regulation of behavioral and endocrine responses to stress.
- Enhancing PACAP in the brain is shown to exert neuroprotective and neurotrophic effects in pathological conditions associated with PACAP deficiency, such as traumatic brain injury.
- In this study, we tested the preventive efficacy of a novel PACAP agonist on long-term negative effects of mTBI on motivated behaviors through regulation of LHb activity in male mice.

METHODS

- **mTBI model:** Five closed-head impacts delivered approximately to bregma via Controlled Cortical Impact device separated by 24-h intervals under isoflurane anesthesia. SHAM surgery consisted of identical procedures without delivery of impact (Flerlage et al., 2023).
- **Early PACAP Agonist Delivery:** PACAP agonist (10mg/kg) was delivered through intraperitoneal (IP) route 30-min after each closed head-impact. VEH delivery consisted of identical delivery using saline.
- **Sucrose Splash Test:** Conducted at 17-days following final mTBI. Following a 10-min acclimation period to the test arena mice were sprayed twice with a 10% sucrose solution on the dorsal coat, then monitored for an additional 5-min. Delay to initiate grooming after splash and total grooming was scored by a blinded observer.
- **Late PACAP Agonist Delivery:** PACAP agonist (10mg/kg) was delivered through intraperitoneal (IP) route 24-h before sucrose splash test or sacrifice for ex vivo slice electrophysiology.
- **Ex vivo slice electrophysiology:** ~30 days post-TBI, cell-attached and whole cell current clamp recordings were made using a potassium gluconate-based internal solution to determine firing pattern of LHb neurons, and classified into three categories (tonic, bursting, silent), as well as to assess of action potential generation in response to depolarization.
- **Immunohistochemistry:** LHb sections from sham and mTBI were immunostained with anti-GFAP and anti-Iba1.

RESULTS

LHb hyperactivity following mTBI

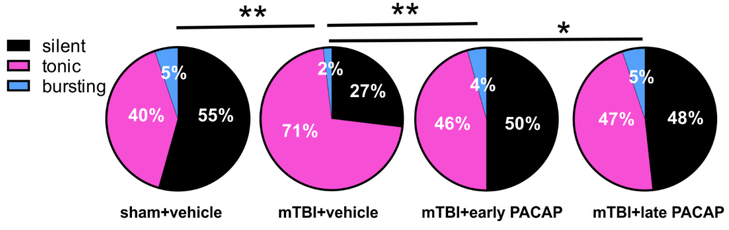

Figure 1. LHb spontaneous activity following mTBI. mTBI increases LHb tonic activity while decreasing bursting activity in male mouse LHb. Both early and late interventions with the PACAP agonist normalizes mTBI-induced LHb hyperactivity to sham levels.

LHb hyperexcitability following mTBI

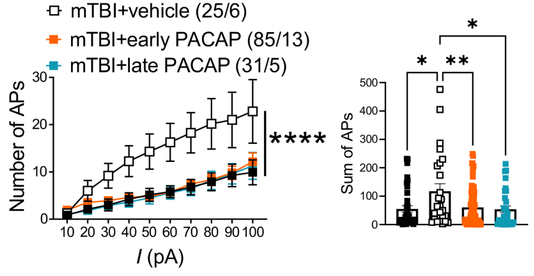

Figure 2. LHb excitability following mTBI. mTBI increases LHb excitability in intact synaptic transmission in male mouse LHb. Both early and late interventions with the PACAP agonist normalizes mTBI-induced LHb hyperexcitability to sham levels.

Persistent neuroinflammation within the LHb following mTBI

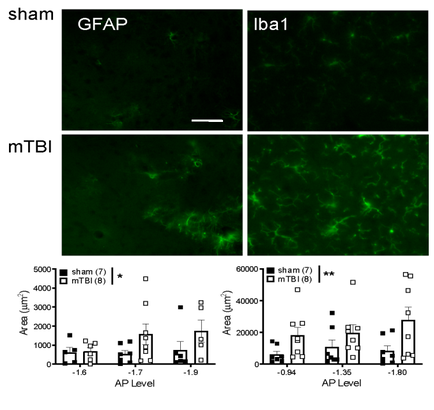

Figure 5. Representative images showing immunostaining for the astrocyte marker (Glial fibrillary acidic protein, GFAP) and microglial marker (Ionized calcium-binding adaptor molecule 1, Iba-1) (green) in the LHb of sham and mTBI mice at ~4 weeks post-injury. mTBI increased both neuroinflammatory markers within the LHb. Scale Bar represents 50 µm.

Motivational deficits following mTBI

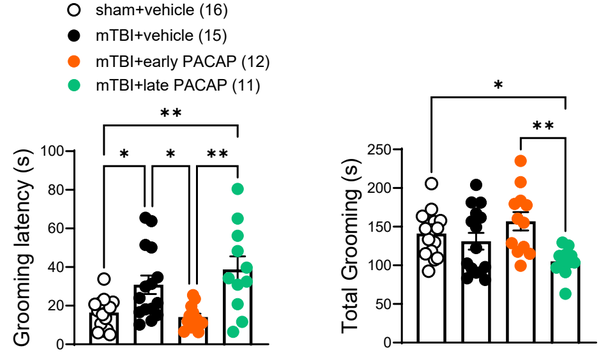

Figure 4. Latency to initiate grooming and total grooming time after 10% sucrose splash 17 days post-mTBI. Male mice have an increased latency to initiate grooming following mTBI. Early PACAP agonist treatment reverses the effect of mTBI on grooming latency. Late PACAP agonist treatment does not alter the effect of mTBI on grooming latency. Late PACAP-treated mice have a decreased total grooming time when compared to sham and early PACAP-treated mice.

DISCUSSION

- Our results show potential efficacy of the PACAP analogue in prevention of mTBI-induced LHb hyperexcitability and motivational deficits in self-care grooming behavior in sucrose splash test.
- We also demonstrated chronic neuroinflammation within the LHb following mTBI utilizing astrocyte and microglial markers.
- Future studies will determine the effects of late PACAP agonist treatment on total grooming time and intrinsic properties of LHb neurons.
- Future studies will also determine the effects of early and late PACAP on chronic neuroinflammation within the LHb.

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