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A pilot study of the role of the claustrum in attention and seizures in rats

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ABSTRACT

Objective: The claustrum has been implicated in consciousness, and MRIs of patients with status epilepticus have shown increased claustral signal intensity. In an attempt to investigate the role of claustrum in cognition and seizures, we (1) assessed the effect of high-frequency stimulation (HFS) of the claustrum on performance in the operant chamber; (2) studied interclaustral and claustrohippocampal connectivity through cerebro-cerebral evoked potentials (CCEPs); and (3) investigated the role of claustrum in kainate-induced (KA) seizures.

Methods: Adult male Sprague-Dawley rats were trained in operant conditioning and implanted with electrodes in bilateral claustra and hippocampi. Claustrum HFS (50 Hz) was delivered bilaterally and unilaterally with increasing intensities from 50 to 1000 μA, and performance scores were assessed. CCEPs were studied by averaging the responses to bipolar stimulations, 1-ms wide pulses at 0.1 Hz to the claustrum. KA seizures were analyzed on video-EEG recordings.

Results: Generalized Estimating Equations analysis revealed that claustral stimulation reduced task performance scores relative to rest sessions (bilateral: −15.8 percentage points, $p < 0.0001$; unilateral: −15.2, $p < 0.0001$). With some stimulations, the rats showed a stimulus-locked decrease in attentiveness and, occasionally, an inability to complete the operant task. CCEPs demonstrated interclaustral and claustrohippocampal connectivity. Some KA seizures appeared to originate from the claustrum.

Conclusions: Findings from the operant conditioning task suggest stimulation of the claustrum can alter attention or awareness. CCEPs demonstrated connectivity between the two claustra and between the claustrum and the hippocampi. Such connectivity may be part of the circuitry that underlies the alteration of awareness in limbic seizures. Lastly, KA seizures showed early involvement of the claustrum, a finding that also supports a possible role of the claustrum in the alteration of consciousness that accompanies dyscognitive seizures.

1. Introduction

The claustrum is a thin region of grey matter located between the external and extreme capsules, and is conserved across mammalian species (Jakubowska-Sadowska et al., 1998). This region is bidirectionally connected to the prefrontal cortex, visual, auditory, sensory, and motor regions, among others (Remedios et al., 2010; Sloniewski et al., 1986; Smith et al., 2012). Although its cytoarchitecture and connectivity have been studied, the function of the claustrum remains unclear. While some have proposed roles such as attention (Goll et al., 2015), sensory integration and possibly, consciousness (Crick and Koch, 2005), little experimental evidence is available to support these suggestions. In cats, high frequency stimulation (HFS) of the claustrum can alter motor activity, induce autonomic changes, and precipitate an "inactivation syndrome", characterized by "decreased awareness" (Gabor and Peele, 1964). Similarly, in humans, one report described complete arrest of volitional behavior, unresponsiveness, and amnesia upon stimulation of the left claustrum (Koubeissi et al., 2014). Furthermore, brain lesions circumscribed to the claustrum have shown impairments of memory (Kozlowski et al., 1997; Yamamoto et al., 2007; Smith et al., 2008; Kalaitzakis et al., 2009), cognition (Sperner et al., 1996), and consciousness (Chau et al., 2015), and increased claustral signal intensity in MRI has been associated with status epilepticus by a number of investigators (Silva et al., 2017; Meletti et al., 2015).

Alteration of consciousness is a hallmark feature of focal dyscognitive seizures. These seizures, which typically originate in the limbic system, are associated with transient loss of consciousness, motor

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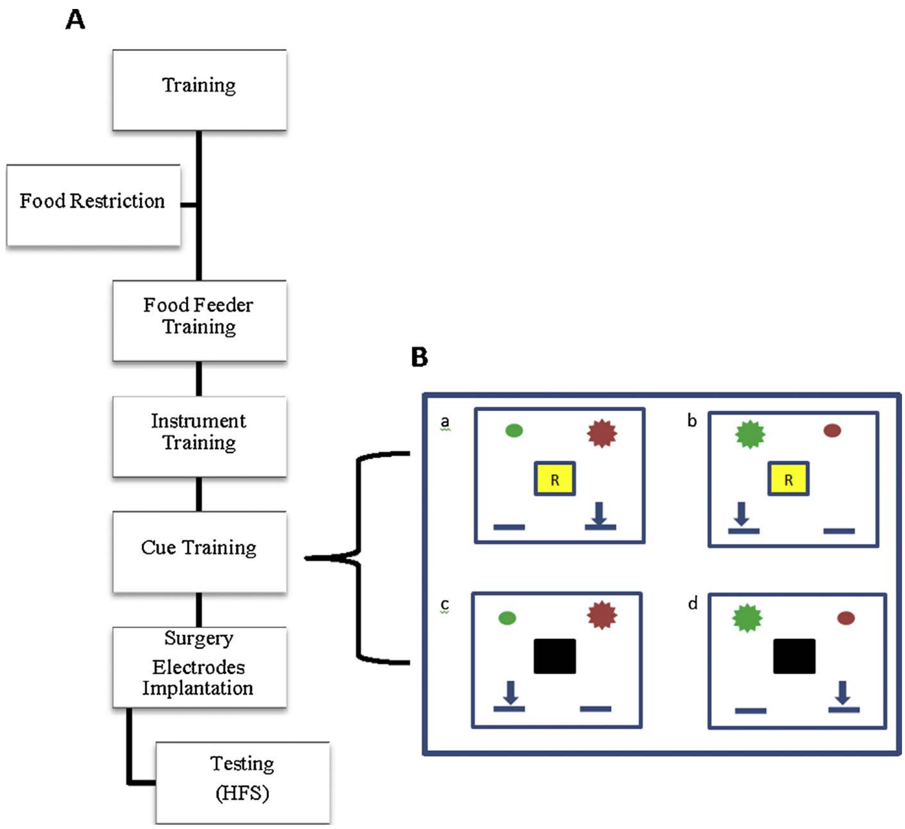

Fig. 1. Schematic illustration of operant task process (A) and cue training (B): A correct response to flashing the red light (a) or green light (b) activates the correct operandum and releases the reinforcement (R). Incorrect response to the flashing red light (c) or green light (d), i.e. an activation of the incorrect operandum, leads to time out and no reinforcement. 30 correct responses within 30 min marked the end of the task, otherwise the task was stopped after 30 min, regardless of the performance.

automatisms, and impairment of memory. While some components of the networks mediating the alteration of awareness accompanying focal dyscognitive seizures have been described, we are far from complete identification of the neural correlates of consciousness. The claustrum may play a role in processes such as sustained attention and consciousness. Attention is a cognitive task recruited in the early processing of information before transfer to higher cognitive functions (Arnold et al., 2003). Establishing the role of the claustrum in attention could help clarify its plausible role in consciousness, and eventually, its implication in focal dyscognitive seizures. While tractography (Milardi et al., 2015; Arrigo et al., 2015) illustrates extensive anatomical connectivity between the claustrum and wide cortical areas, evidence regarding effective connectivity (defined as measureable responses evoked by applying a stimulus to the brain) of these connections remains minimal. Previous works have shown the importance of the claustrum in seizure generation and spread in kindling models (Zhang et al., 2001, Sheerin et al., 2004, Paul Mohapel et al., 2001, Mohapel et al., 2000, Wada and Tsuchimochi 1997). However, we are unsure if claustrum engagement in the kainic acid (KA) model may aid in clarifying its role in the alteration of awareness accompanying dyscognitive seizures. For example, we are unaware of whether ictal propagation to the claustrum contributes to dyscognitive seizures or whether the claustrum itself can be an epileptogenic zone. To address these knowledge gaps, we conducted this study to test the following hypotheses: 1) HFS of the claustrum alters performance in tasks requiring sustained attention; 2) there is connectivity between the claustra and the hippocampi, which can be demonstrated using cerebro-cerebral evoked potentials (CCEP), and 3) the claustrum has a role in seizure generation and semiological manifestations in the KA model in rats.

2. Materials and methods

2.1. Operant conditioning task

For this study, 10 adult male Sprague-Dawley rats weighing 250–390 g (Hilltop, Scottdale, PA) were used. All animal procedures were conducted in accordance with the NIH guide for the care and use of Laboratory animals (NIH Publications No. 8023) and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of George Washington University. Upon arrival, rats were pair-housed in conventional plastic animal cages. They were allowed to acclimate for 7 days before experimentation began. The cages and experimental rooms were climate controlled and illuminated on a standard light-dark cycle (light on from 7:00 A.M. to 7:00 P.M.).

To assess the effect of claustrum stimulation on sustained attention, we used an operant conditioning task, modified from prior reports (Arnold et al., 2003; West et al., 2011; West et al., 2012). We used the software package Graphic State (Version 4.0, Coulborn Instruments) to collect and record the rats' responses in the operant box. The operant conditioning task was utilized because it requires sustained attention, among other functions such as perception and execution. We defined sustained attention as "undisturbed signal detection and discrimination performance" throughout a testing session (Arnold et al., 2003).

The operant chamber (Coulbourn Instruments) was equipped with two nose poke operanda each with a red light and a motion sensor located inside. The chamber was also equipped with a houselight, a food dispenser, and a panel light (2.8 W) above the food feeder which has a red light on the left side and a green light on the right. During all test sessions, 70 dB white noise was provided. As detailed in supplemental materials, the operant conditioning task consisted of 4 steps: food habituation, instrument training, cue training, and testing phase.

Rats were trained until they were able to correctly poke their nose into the active, i.e. flashing, operandum. A correct response resulted in a pellet delivery, whereas an incorrect nose-poke or a failure to respond