Program#/Poster#: 93.07/HHH23

Presentation Title: Optogenetic silencing of prelimbic inputs to paraventricular thalamus impairs long-term fear retrieval

Location: Halls B-H

Presentation time: Saturday, Nov 09, 2013, 3:00 PM - 4:00 PM

Topic: ++F.02.x. Fear and aversive learning and memory: Extinction

Authors: ***F. H. DO MONTE**, G. J. QUIRK;
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Abstract: We recently reported that the dorsal midline thalamus (dMT) is necessary for retrieval of a well-consolidated auditory fear memory (Padilla-Coreano et al., 2012). Pharmacological inactivation of dMT, including the paraventricular thalamus (PV) and the mediodorsal thalamus (MD), impaired fear retrieval when performed 24 hours after conditioning, but not 8 h after. Last year, we reported that tone-induced Fos expression in PV, but not in MD, was increased 7 days after conditioning, but not 6 h after (Do Monte & Quirk, SFN 2012), suggesting that PV is gradually recruited into the fear circuit. The prelimbic prefrontal cortex (PL), a region essential for fear retrieval (Sierra-Mercado et al, 2011), sends dense projections to PV (Hoover and Vertes, 2007; Li and Kirouac, 2012). Unlike PV, inactivation of PL at 6 h and 7 d reduced fear, and Fos in PL was increased at both timepoints. Thus, PL projections to PV may be necessary for recruiting PV into the fear circuit. We tested this hypothesis by using an optogenetic approach to specifically inactivate PL somata or their terminals in PV at 6 h and 7 d after conditioning. Rats were infused in PL with an adeno-associated virus encoding for a light sensitive protein (halorhodopsin) and a promoter specific to glutamatergic neurons. Eight weeks later, rats were conditioned to a tone and then tested for fear retrieval. Laser illumination of PL somata during tone presentation significantly impaired freezing at both 6 h (45% vs. 82 %, $p < 0.01$) and 7 d (53 % vs. 84 %, $p < 0.01$). In contrast, illumination of PL efferents in PV significantly impaired fear retrieval at 7 d (49 % vs. 81 %, $p < 0.01$), but not at 6 h (77 % vs. 73 %, $p = 0.52$). Taken together, these results suggest that a time-dependent recruitment of PL to PV synapses is necessary for retrieval of consolidated fear memory.

Program#/Poster#: 93.08/HHH24

Presentation Title:	The infralimbic cortex is critical for avoidance extinction, but not avoidance expression
Location:	Halls B-H
Presentation time:	Saturday, Nov 09, 2013, 4:00 PM - 5:00 PM
Topic:	++F.02.x. Fear and aversive learning and memory: Extinction
Authors:	***C. BRAVO-RIVERA**, C. ROMAN-ORTIZ, E. BRIGNONI-PEREZ, H. BRAVO-RIVERA, G. J. QUIRK; Depts. of Psychiatry and Anat. & Neurobio., Univ. Puerto Rico Sch. Med., San Juan, Puerto Rico
Abstract:	Using a platform-avoidance (PA) task in which rats avoid a tone-signaled footshock by stepping onto a nearby platform, we found that inactivation of prelimbic cortex (PL) or basolateral amygdala (BLA) blocked expression of PA (Bravo-Rivera et al., SfN, 2012). Here, we examined extinction of PA, focusing on the infralimbic cortex (IL), an area necessary for extinction of Pavlovian fear conditioning (Sierra-Mercado et al., 2011). A recent study using signaled shuttle avoidance found that IL inactivation increased freezing to the tone, and blocked avoidance (Moscarello et al., 2013). In our PA task, muscimol inactivation of IL also increased freezing to the tone (MUS 56%; SAL 27%, $p<0.01$), but had no effect on avoidance expression (MUS 93%; SAL 90%, $p=0.78$). Furthermore, it significantly impaired the recall of extinction the following day (Freezing: MUS 85%; SAL 46%, $p<0.05$, Bar-press suppression: MUS 74%; SAL 38%, $p<0.01$). In a separate group, we found that ~20% of rats persist in their avoidance of the tone, even after two days of PA extinction. To explore regions mediating persistence of avoidance, we measured cFos positive cells (activity marker) in the prefrontal cortex and amygdala of persistent and non-persistent rats, immediately following an avoidance test 24 hours after extinction training. Compared to non-persistent rats, persistent rats showed fewer cFos cells in IL ($p<0.05$), and more cFos cells in PL ($p<0.001$), amygdala CeM ($p<0.05$), and BLA ($p<0.05$). Taken together, these results suggest that IL is necessary for avoidance extinction, but not avoidance expression. Moreover, overactivity in PL, BLA, and CeM could underlie persistent avoidance.
Program#/Poster#:	93.09/HHH25
Presentation Title:	Deep brain stimulation of the ventral striatum increases BDNF in the fear extinction circuit

Location:	Halls B-H
Presentation time:	Saturday, Nov 09, 2013, 1:00 PM - 2:00 PM
Topic:	++F.02.x. Fear and aversive learning and memory: Extinction
Authors:	***L. E. ROSAS-VIDAL**, F. H. DO MONTE, J. RODRÍGUEZ-ROMAGUERA, G. J. QUIRK; Psychiatry and Anat. & Neurobio., Univ. of Puerto Rico, San Juan, Puerto Rico
Abstract:	Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) is used clinically for the management of treatment-resistant obsessive compulsive disorder (OCD) (Greenberg et al., 2010), and improves patients' response to extinction-based therapies (Denys et al., 2010). We recently reported that DBS of the dorsal portion of the VS (dorsal-VS) enhanced fear extinction memory, whereas DBS of the ventral-VS impaired extinction (Rodriguez-Romaguera et al, 2012). Moreover, DBS of dorsal-VS increased pERK (plasticity marker) in the intercalated cell masses and centrolateral nucleus of the amygdala (ITC/CeL), as well as the prelimbic (PL) and infralimbic (IL) cortices, areas important for fear extinction. Brain-derived neurotrophic factor (BDNF) in IL and its inputs is necessary for extinction learning (Peters et al., 2010; Soliman et al., 2010; Rosas-Vidal et al., SFN 2012;). Thus, we hypothesized that dorsal-VS DBS may be enhancing extinction by increasing BDNF in IL. To address this question, we used immunocytochemistry to measure levels of cFos (activity marker) and neuronal BDNF in dorsal-VS or ventral-VS sites following 3 h of DBS (130Hz). We found that DBS of dorsal-VS increased cFos and BDNF levels in both IL ($p<0.05$) and PL ($p<0.05$). In addition, DBS increased cFos ($p<0.05$), but not BDNF, in CeL. In contrast, ventral-VS DBS had no effect on cFos or BDNF levels in IL and PL, whereas it increased cFos ($p<0.05$), but not BDNF, in CeL. These data suggest that DBS of dorsal-VS may enhance extinction by increasing BDNF in IL neurons, leading to increased activation of inhibitory neurons in ITC/CeL.
Program#/Poster#:	93.10/HHH26
Presentation Title:	Extinguishing fear: Revisiting the infralimbic cortex with an optogenetic approach
Location:	Halls B-H

Presentation time: Saturday, Nov 09, 2013, 2:00 PM - 3:00 PM

Topic: ++F.02.x. Fear and aversive learning and memory: Extinction

Authors: ***G. MANZANO-NIEVES**, F. H. DO MONTE, G. J. QUIRK; Psychiatry and Anat. & Neurobio., Univ. of Puerto Rico, San Juan, Puerto Rico

Abstract: Failure to extinguish a fear memory results in a prolonged high fear state, which is a characteristic of anxiety disorders such as post-traumatic stress disorder. In auditory fear conditioning, pharmacological inactivation of infralimbic cortex (IL) impairs extinction of freezing (Sierra-Mercado et al, 2011), and electrical stimulation of IL reduces freezing to the tone (Milad et al, 2004). However, the techniques used in these studies lacked temporal precision and neural specificity to determine when IL projection neurons modulate fear expression and fear extinction processes. For example, muscimol inactivation lasts several hours, affecting both extinction acquisition and consolidation. Here, we used an optogenetic approach which permitted control of specific glutamatergic transmission in a millisecond temporal scale. Rats were bilaterally injected into IL with an adeno-associated virus encoding for one of two light sensitive proteins: halorhodopsin (eNpHR) or channelrhodopsin (ChR2). Protein expression was controlled by a promoter specific for glutamatergic neurons (CaMKII). Rats were fear conditioned to a 30 s tone and then tested the next day for fear retrieval and extinction. IL-neurons expressing eNpHR or ChR2 were, respectively, inhibited by yellow laser illumination or activated by blue laser illumination. We found that IL inactivation during extinction tones did not affect extinction learning, but significantly impaired extinction recall the next day (64 % vs. 25 %, $P < 0.01$), suggesting that IL activity during extinction tones only is necessary for subsequent extinction recall. When IL was activated during the entire 30 s of the tone, freezing was reduced (66 % vs. 3 %, $p < 0.01$), but not when activation was limited to the initial 5 s (61 % vs. 60 %, $p = 0.96$), suggesting that suppression of tone-induced freezing requires sustained activity in IL. Taken together, these results suggest that activity of IL projection neurons during extinction tones is critical for modulation of both fear expression and extinction memory.

Program#/Poster#: 583.05/MMM3

Presentation Title: Deep brain stimulation of the ventral striatum impairs extinction of morphine-induced conditioned place preference

Location: Halls B-H

Presentation time: Tuesday, Nov 12, 2013, 8:00 AM - 9:00 AM

Topic: ++F.03.i. Motivation and emotions: Reward

Authors: ***F. J. MARTINEZ**[1], J. RODRÍGUEZ-ROMAGUERA[2], F. H. DO MONTE[2], O. A. MUÑIZ-SEDA[1], G. J. QUIRK[2], J. L. BARRETO-ESTRADA[1];
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Abstract: Deep brain stimulation (DBS) is a neurosurgical procedure used to treat refractory neurological and psychiatric disorders. Recent studies have suggested that DBS of the ventral striatum may be a potential target for treating addictive disorders (Luigjes et al., 2011). We recently showed in rats that DBS of the dorsal portion of ventral striatum (dorsal-VS) reduced fear expression and enhanced fear extinction (Rodríguez-Romaguera et al., 2012). Here, we examined whether DBS of dorsal-VS could also reduce the expression of morphine-induced conditioned place preference (CPP), and enhance its extinction learning. Male Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes aimed at dorsal-VS (−6.5 mm DV, ±2.0 mm ML, and +1.2 mm AP). Using a two-compartment CPP box, rats were conditioned across 8 days to prefer the side paired with morphine (5 mg/kg, s.c.). Subsequently, rats expressing morphine-CPP received 6 extinction sessions on 6 consecutive days, together with dorsal-VS DBS (130 Hz, 0.1 ms pulse, 100 µA, 60 min) or sham stimulation. DBS did not reduce the expression of morphine-CPP, as indicated by equivalent % time spent in the morphine paired side (Sham: 70%, DBS: 68%). Surprisingly, DBS impaired extinction of CPP, as indicated by a high % of time spent in the morphine paired side throughout extinction (Day 6 - Sham: 52%, DBS: 74%, ANOVA repeated-measures between group; $p<0.05$). Additional experiments showed that the DBS itself did not induce CPP, or alter rats' locomotion. These results suggest that ventral striatum DBS may have opposite effects on fear- vs. reward-extinction. Furthermore, it suggests that the dorsal-VS site may not be a promising target for treating addictive disorders with high-frequency DBS.