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研究課題名（和文）Neural circuit and molecular mechanisms for noradrenergic modulation of fear learning and memory

研究課題名（英文）Neural circuit and molecular mechanisms for noradrenergic modulation of fear learning and memory

研究代表者

Ｔａｎ　ＢａｏＺｈｅｎ（Tan, Bao Zhen）

国立研究開発法人理化学研究所・脳神経科学研究センター・研究員

研究者番号：８０７５６７８４

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研究成果の概要（和文）：外側扁桃体（LA）のノルアドレナリンはβアドレナリン作動性（b-AR）受容体を介してHebbian可塑性機構を調節して形成を促進する聴覚恐怖の思い出我々は、特に嫌悪体験中のＬＡにおけるＬＣ活性およびＬＣ末端の活性化が、恐怖記憶形成に必要であり、それを促進することを示した。また、LAのLCターミナルからのNAの解放は、CRTC1に対する行動を通じて恐怖条件付けを調整することもわかった。恐怖学習は、ＬＡニューロンにおけるＣＲＴＣ１核蓄積の有意な増加をもたらし、そしてＣＲＴＣ１のドミナントネガティブ形態の発現は、長期の恐怖記憶形成を減少させた。

研究成果の学術的意義や社会的意義
We have examined both the circuit and molecular mechanism behind aversive memory formation and potentially identified a therapeutic strategy for excessive fear memories and anxiety disorders.

研究成果の概要（英文）：Noradrenaline in the lateral amygdala (LA) modulates Hebbian plasticity mechanisms via beta-adrenergic (b-AR) receptors to facilitate formation of auditory fear memories. We have showed that LC activity and activation of LC terminals in LA specifically during aversive experience is required for and facilitates fear memory formation. We have also found that NA release from the LC terminals in LA regulates fear conditioning through actions on CRTC1. Fear learning produced a significant increase in CRTC1 nuclear accumulation in LA neurons and expression of dominant negative form of CRTC1 reduced long term fear memory formation, implicating CRTC1 as a potential mechanism for transducing noradrenergic signaling in LA neurons into long term fear memories.

研究分野：Neuroscience

キーワード：Fear learning and memory　Noradrenaline　Optogenetics　Consolidation　Amygdala

１．研究開始当初の背景

Fear conditioning, a powerful paradigm to investigate the neuronal circuit and molecular mechanisms of associative learning and memory, involves the pairing of an aversive unconditioned stimulus (US) such as an electric foot shock with a neutral conditioned stimulus (CS) such as an auditory tone. The tone acquires aversive properties during this acquisition phase, and on subsequent exposure (memory test phase), elicits fear responses such as freezing. Pathways transmitting the CS and US converge in the lateral amygdala (LA), and behavioral and *in vivo* electrophysiological evidence indicate that synaptic plasticity and concomitant activation of neuromodulatory receptors to the LA underlies the acquisition of fear conditioning (Fig. 1; Johansen et al., 2011, 2014; Bush et al., 2010). However, the mechanisms through which neuromodulators regulate this plasticity in the LA during aversive experiences are not completely understood. Hebbian plasticity suggests that memories are formed through strengthening of synaptic connections between neurons with correlated activity (Hebb, 1949). Recent studies found that activation of neuromodulatory systems is necessary to co-regulate Hebbian processes and synaptic strengthening during memory formation (Johansen et al., 2011, 2014). One such neuromodulator is noradrenaline (NA). Emotional arousal can lead to activation of the NA cells located in the locus coeruleus (LC) and other brainstem areas and cause the subsequent release of NA in the brain and modulate memory storage. The LC is a prominent source of NA to the LA and LC neurons and NA release in the LA are activated by aversive events (Sara et al., 2009).

Recent studies suggest that β-adrenergic receptor activation (β-AR, an important NA receptor) in the LA is involved in auditory fear memory formation (Bush et al., 2010). Other work showed that optogenetic activation of LA cells can serve as an US in fear conditioning (Johansen et al., 2010). Importantly, it was found that this effect is potentiated when β-ARs are coactivated during the US, suggesting that β-AR activation directly modulates Hebbian/calcium dependent mechanisms in the LA mediating fear memory formation (Johansen et al., 2014). However, it is unclear if LC is the source of NA to the LA for fear conditioning and, the precise temporal aspects of LC signaling during fear acquisition are not known. Furthermore, the intracellular mechanisms by which NA and β-AR activation potentiates LA synaptic plasticity to produce fear memory are also unclear. A recent study has suggested that CREB-regulated transcriptional coactivator-1 (CRTC1), a well-known coincidence detector of calcium and cAMP-mediated intracellular signaling pathways, in the LA is necessary for fear memory consolidation (Sekeres et al., 2012; Nonaka et al., 2014). However, we do not know whether these intracellular signaling cascades (from neuromodulatory input signals) modulate CRTC1 activity in the LA neurons during fear conditioning, or if altered CRTC1 activity in LA neurons projecting to specific targets affect fear learning and memory. The central amygdala (CEA, principal output structure of the amydaloid complex) projects to downstream outputs such as the periaqueductal gray (PAG, controls behavioral freezing) and outputs from LA to CEA could be important for fear learning and memory.

２．研究の目的

Noradrenaline (NA), released by locus coeruleus (LC) neurons during periods of anxiety and stress, is important for learning and synaptic plasticity. Pharmacological studies suggest that NA modulates cued fear memory via beta-adrenergic receptors (β-ARs) in the lateral amygdala (LA), but it is unclear if LC NA projections to the LA modulate learning, and what intracellular changes are triggered by NA release from LA and β-AR activation. I will use optogenetic and viral approaches to dissect the functional roles of LC NA projections to LA and altered CRTC1 (a calcium- and cAMP-sensitive coincidence detector) activity in LA neurons in fear memory.

３．研究の方法

I inject a retrograde transducing CAV2-Cre virus into the LA in rats to retrogradely express Cre recombinase in neurons that project to LA, and locally inject a Cre-dependent virus containing floxed ChR2 or Arch with tdtomato on a CAG promoter in the LC to specifically target LA-projecting LC NA neurons. I have injected CAV2-Cre virus and Arch using AAV virus into LA and LC respectively, and saw widespread expression in LC cell bodies. To trace ChR2 and Arch expression from LC to LA, I look for terminal expression 6 weeks after virus injection, as demonstrated in study using CAV2-Cre (Gore

et al., 2013). Because tyrosine hydroxylase (TH) is only expressed in adrenergic, NA and dopaminergic neurons in the brain, I determine that the LC neurons projecting to LA are noradrenergic with immunohistochemical techniques.

４．研究成果

Project 1: To determine whether the locus coeruleus (LC) is the source of noradrenaline (NA) to the LA and to define the specific temporal epochs during fear conditioning in which these inputs are important. I used a retrogradely transported canine adenoviral viral vector encoding Cre-recombinase (CAV2-Cre) and adeno-associated viral (AAV) vector encoding Cre-dependent inhibitory opsin ArchT (a green light-activated outward proton pump used to inhibit neural activity) to limit viral expression in LC-NA neurons that project to the LA. 6 weeks after viral expression, animals were trained with 3 pairings of a neutral conditioning stimulus, an auditory tone (CS) terminating with noxious unconditioning stimulus, a foot shock (US). Lasers are delivered during the US presentation into the LA to specifically inhibit the LC-NA projections. We found that inhibition of these projections, specifically during US presentation, blocks fear memory formation and consolidation 24 hours later (Fig 1).

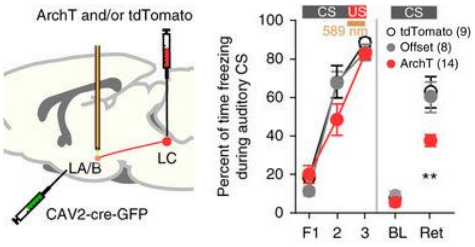

Fig. 1: (Left) Schematic of CAV2-Cre injection into LA, combined with Cre-dependent inhibitory opsins into LC. Optic fibers are located above LA. (Right) Optical inhibition of LC-NA terminals in the LA during strong US significantly decreases fear learning.

Project 2: To determine if NA from LC facilitates fear learning by activation of CRTC1 and CREB-dependent gene expression. We examined nuclear expression of CRTC1 in the LA neurons of animals immediately and 1 hour after fear conditioning, and found a significant increase in nuclear translocation of CRTC1 in LA neurons (immunohistochemical sections) 1 hour after fear conditioning (Fig 2).

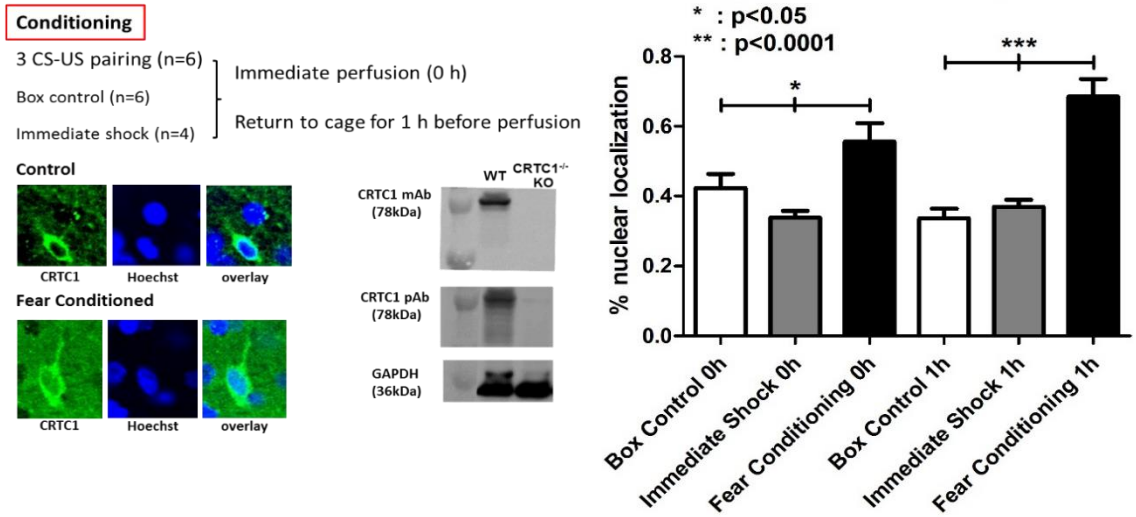

Fig. 2: CRTC1 nuclear translocation occurs with fear conditioning and is associative. *(Left, top)* Experimental design. *(Left, bottom)* CRTC1 and Hoechst, a nuclear marker, immunolabeling in LA sections, and specificity of CRTC1 antibodies, immunoblotting of whole brain lysates. *(Right)* Percentage CRTC1 nuclear localization in LA sections of fear conditioned experimental animals and box control and immediate shock control animals at 0 h and 1 h after behavioral training.

To test if CRTC1 in the LA is necessary for behavioral fear conditioning, we virally infected the LA neurons with shRNAs targeting CRTC1 before training. We found that LTM is significantly reduced in animals with down-regulated CRTC1 expression in LA, and that CRTC1 in LA is necessary for fear memory consolidation (Fig 3).

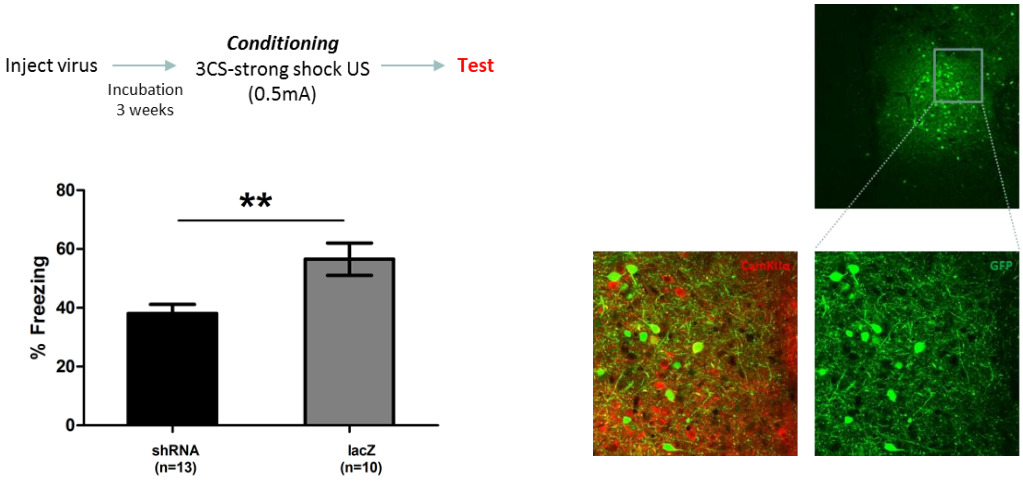

Fig. 3: CRTC1 in LA is necessary for fear memory consolidation. *(Left, top)* Experimental design. *(Left, bottom)* Percentage freezing (percent of total duration of a 20-s CS, y-axis) at the LTM time point. *(Right)* Decreased expression of CRTC1 in shRNA animals, immunoblotting of amygdala lysates, CaMKIIα, a marker for LA pyramidal neurons, and GFP for virally infected LA neurons, immunolabeling in LA sections.

５．主な発表論文等

〔学会発表〕

1. Tan Bao Zhen, Japan Neuroscience Meeting 2018, Noradrenergic modulation of aversive memory reconsolidation - from circuits to molecules.
2. Tan Bao Zhen, FENS-EMCCS 2016, Neural circuit and molecular mechanisms for noradrenergic modulation of memory formation.

６．研究組織

(1)研究分担者

(2)研究協力者