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研究課題名（和文）Neural circuits regulating fear learning and anxiety disorders

研究課題名（英文）Neural circuits regulating fear learning and anxiety disorders

研究代表者

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研究成果の概要（和文）：我々は、嫌悪的ショックが予測される場合にショックの処理を抑制する、負のフィードバック神経回路を同定した。さらに、この回路が学習の程度や漸近を定める機能を持つことを示した。光遺伝学によりこの回路を抑制すると、恐怖記憶を貯蔵する扁桃体外側核において予測誤差の抑制が解かれ、恐怖学習の程度が強められた。以上の成果により、予測誤差表現と恐怖記憶の強度設定の回路機構を明らかにした。予測誤差は報酬・運動学習など多くの学習回路にみられる普遍的なコーディングである。本成果は、神経系による予測誤差表現の一般特性を示唆する。また、この回路の攪乱は、過度な嫌悪学習により特徴づけられる不安障害の原因となりうるだろう。

研究成果の概要（英文）：In our work on this grant we've identified a specific neural circuit which provides negative feedback to inhibit aversive shock processing when it is predicted by other sensory cues in the environment. Furthermore, we demonstrated function for this circuit in setting behavioral learning levels or asymptotes. Optogenetically inhibiting this feedback circuit disinhibited prediction error coding in lateral amygdala neurons which store fear memories resulting in exaggerated fear learning levels. Together this work reveals a distributed circuit mechanism for setting prediction error coding and fear memory strength. Prediction error coding is a ubiquitous neural coding mechanism in many learning circuits including those involved in reward and motor learning. This could represent a general feature of how prediction errors are encoded in the nervous system. Disruption of this feedback circuit could underlie anxiety disorders that are characterized by exaggerated aversive learning.

研究分野：　Neuroscience

キーワード：　amygdala　prediction error　fear conditioning

１．研究開始当初の背景: Aversive experiences powerfully regulate memory formation and produce learned changes in behavior which facilitate survival. However, excessive aversive learning can lead to psychological anxiety disorders such as post-traumatic stress disorder (PTSD). Fear conditioning is an ideal model system for studying how neural circuits are adaptively changed by aversive experiences. Importantly, aversive fear learning and PTSD occur through changes in common neural circuits suggesting that pathological changes in the neural circuits of fear mediate this anxiety disorder. During auditory fear conditioning a neutral auditory tone (conditioned stimulus, CS) is paired temporally with an aversive event (unconditioned stimulus, US, usually electric shock). Following learning, presentation of the auditory CS alone produces a constellation of behavioral (such as behavioral freezing) and visceral responses that provide a measure of emotional memory. Importantly, a site of associative plasticity mediating fear memory formation has been identified in the lateral nucleus of the amygdala (LA). As a result of fear conditioning the strength of auditory CS inputs to LA neurons is enhanced (***Fig. 1A***). We have shown recently that depolarization of LA pyramidal neurons evoked by the aversive US triggers enhancement of coactive auditory CS inputs to the same neurons resulting in fear conditioning Thus depolarization of LA neurons by aversive experiences engages neural plasticity in LA and fear memory formation. Following fear conditioning, auditory CS inputs in

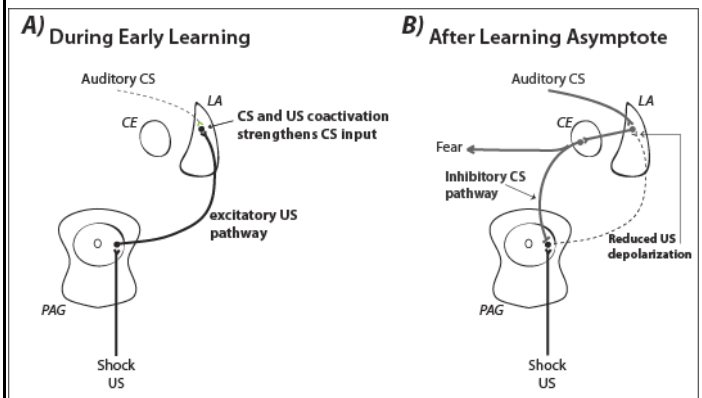

Figure 1: **Hypothetical circuit regulating fear learning asymptotes**

the LA are strong enough to activate output circuits in the central nucleus of the amygdala (CE) which produce emotional fear responses (***Fig. 1B***).

While we have identified depolarization as a trigger for initial learning, it is not clear how the amount of fear conditioning that occurs during aversive learning is set. During fear conditioning, learning occurs up to a specific level called the learning asymptote beyond which no further learning occurs even with continued training (***Fig. 2***). Importantly, this asymptote is not the result of a standard, set biological ceiling, but is instead directly proportional to the intensity of the aversive experience. Thus, at learning

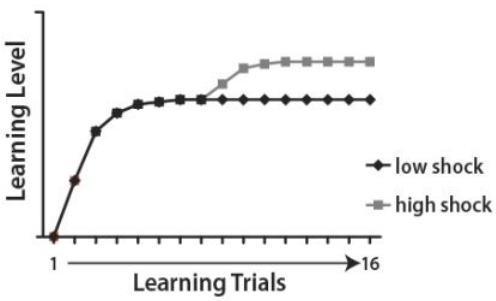

Figure 2: **Learning asymptotes are dependent on shock US magnitude**

asymptote, further CS-US pairings don't produce learning unless the shock US intensity is increased (***Fig. 1B***). While

there are many factors which could contribute to setting learning asymptotes, conceptual models have suggested that a negative feedback circuit from the central nucleus of the amygdala (CE) to the midbrain periaqueductal gray (PAG, a part of the US pathway to the amygdala) may limit shock evoked responding in LA neurons as learning progresses to set learning levels (Fig. 1). This learning dependent reduction in aversive US processing has been termed prediction error coding and is shared across many learning circuits including those responsible for reward, motor and vocal learning. Supporting this, we have found that as fear learning progresses there is a corresponding reduction in the magnitude of the aversive US-evoked responses in LA neurons. Furthermore, we have demonstrated that depolarization of LA neurons is important in triggering fear learning. Finally, we have discovered that the PAG is part of a neural pathway which relays aversive US information to the LA and, as in LA neurons, US-evoked responses in PAG neurons are reduced during learning (Johansen et al. 2010b). These data suggest the hypothesis that depolarization of LA pyramidal neurons by aversive experiences and negative feedback from the CE to the PAG aversive US pathway function to set fear learning levels (as described in ***Fig. 1B***).

２．研究の目的: Based on this previous research we proposed the following Specific Aims:

Specific Aim 1: We examined whether a negative feedback pathway from the CE to the PAG is activated by predictive auditory CSs to inhibit aversive shock-evoked responding in LA neurons and setting behavioral fear learning levels (see model in ***Fig. 1B***).

Specific Aim 2: We tested whether this fear learning induced reduction in aversive shock responding in LA neurons functioned to set behavioral fear learning asymptotes (***Fig. 1B***).

３．研究の方法: To perform these experiments we used developed a behavioral learning asymptote task in which animals were trained on day 1, tested on day 2 to measure their freezing behavior, overtrained on day 3 and tested again on Day 4. No change in freezing levels were apparent on from test 1 on day 2 to test 2 on day 4 if shock intensity was held constant, demonstrating that day 3 overtraining was purely asymptotic learning. We used this behavioral task in combination with optogenetic manipulations of various brain circuits along with in-vivo electrophysiology to examine the effect of the circuit manipulations on neural activity in different parts of the brain (LA and PAG).

４．研究成果:

1) We first examined whether the CE-PAG pathway provided inhibition of aversive responding when the shock was predicted (i.e. prediction error coding) in LA and PAG neurons. To do this we trained animals to learning asymptote and then presented shocks that were predicted by auditory CSs or unexpected shocks. Replicating our previous results we found that unpredicted shocks produced larger activation of LA neurons than predicted shocks. However, when we optogenetically CE axonal terminals in the PAG shock responses in PAG and in the LA were significantly disinhibited (Fig. 3).

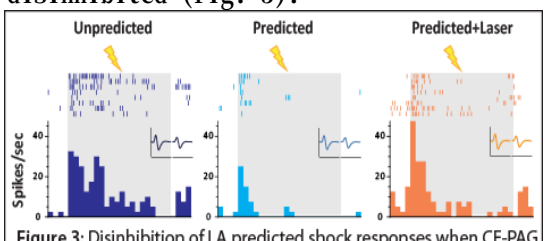

Figure 3: Disinhibition of LA predicted shock responses when CE-PAG pathway is inhibited

2) We next examined the function of this pathway to behavior. For this experiment we trained animals to asymptote tested their memory once (Test 1) overtrained them (asymptotic learning) and tested their memory again (Test 2). If the shock intensity was held constant there was no change in freezing responses to the CS from test 1 to test 2, showing that animals were at asymptote. However, if the CE-PAG pathway was inhibited, during overtraining animals froze more to the CS on Test 2 (Fig. 4), demonstrating that learning asymptotes were increased and that the CE-PAG pathway is integral in setting learning asymptotes.

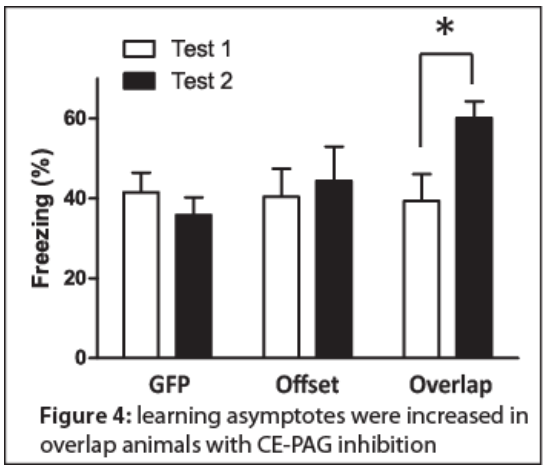

Figure 4: learning asymptotes were increased in overlap animals with CE-PAG inhibition

3) We next examined prediction error coding in LA neurons was responsible for setting learning asymptotes. First we performed the same behavioral learning asymptote experiment described above with optogenetic inhibition of the CE-PAG pathway, except that animals received either muscimol (a GABA-receptor agonist) or saline (PBS) into the LA. Muscimol inhibits neural activity allowing us to determine whether the disinhibition of prediction error coding we saw was in LA neurons with CE-PAG inhibition was responsible for the increase in learning asymptote. Consistent with this we saw that muscimol inactivation blocked the effect of the CE-PAG inhibition (Fig. 5a). We then used channelrhodopsin expression in LA neurons to optogenetically mimic prediction error coding during the shock period (overlap) of overtraining. When we did this learning asymptotes were increased which did not happen in controls (eYFP, Offset, Fig. 6).

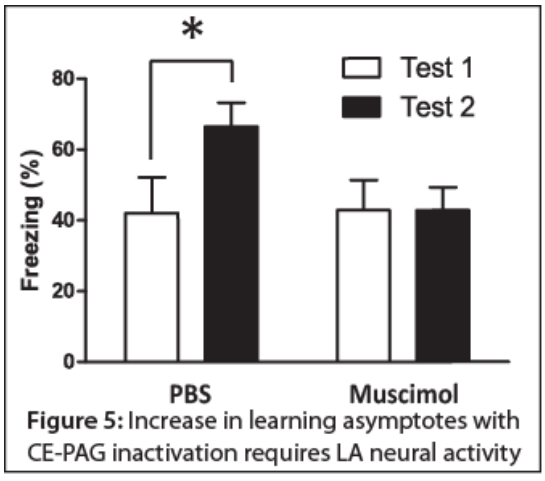

Figure 5: Increase in learning asymptotes with CE-PAG inactivation requires LA neural activity

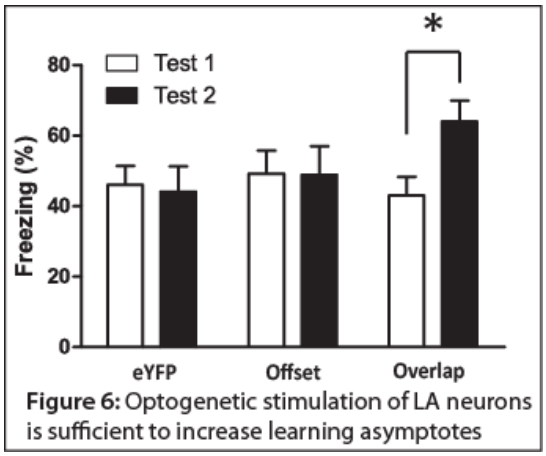

Figure 6: Optogenetic stimulation of LA neurons is sufficient to increase learning asymptotes

Summary: in the experiments funded by this grant we ' ve identified a specific neural circuit which provides negative feedback (blue) on the aversive shock circuit (red) and set prediction error coding (Fig. 7). Behaviorally this is important in setting learning asymptotes through modulation of prediction error coding in LA neurons.

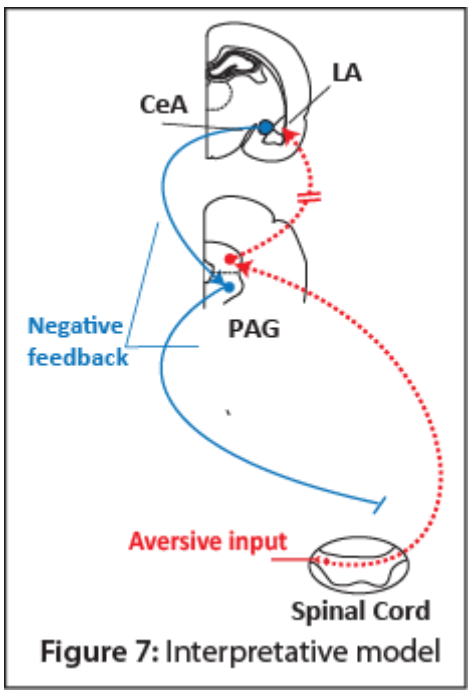

Figure 7: Interpretative model

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　3　件）

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③ Joshua P. Johansen, Bidirectional control of fear memories through distinct functional populations of locus coeruleus noradrenaline neurons, Tohoku Forum for Creativity: Memory and Mind,Sendai, September29, 2015 Japan

④ Joshua P. Johansen, Neural circuit mechanisms for triggering, regulating and reducing fear memories, Mount Sinai School of Medicine, New York,August11, 2015, USA

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〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）

名称：
発明者：
権利者：
種類：
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出願年月日：
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○取得状況（計　　件）

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発明者：
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番号：
取得年月日：
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〔その他〕
ホームページ等

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