様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月　 ４ 日現在

機関番号：８２４０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１５２１２

研究課題名（和文）Neural mechanism underlying cognitive operations in emotional learning systems

研究課題名（英文）Neural mechanism underlying cognitive operations in emotional learning systems

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交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：mPFCのニューロンのカルシウム活動を検出した結果、これらのニューロンが直接的な嫌悪記憶と推論による嫌悪記憶の両方に応答することがわかりました。オプトジェネティクスを使用してmPFCから扁桃体への投射を抑制し、その投射が推論による嫌悪記憶の想起時には必要であるが、元の記憶の想起時には必要でないことを発見しました。扁桃体に投射するmPFCニューロンのカルシウム活動も、推論による嫌悪記憶の想起中に特に増加した感覚応答を示しました。さらに、推論による嫌悪記憶の消去は、推論による嫌悪記憶の防御行動にのみ影響を与え、mPFCニューロンのカルシウム活動もこれに一貫した証拠を提供します。

研究成果の学術的意義や社会的意義
Because there are emerging evidences indicating the importance of cognitive system in human anxiety disorders (e.g. PTSD etc.), this study can provide novel information about the mechanism and inspirations for the development of new medical therapy to cure these debilitating syndromes in future.

研究成果の概要（英文）：In the three years, I adapted an animal behavioral paradigm to detect the expression of aversive inference. I detected the Calcium activity of neurons in mPFC and found that neurons are responsive to both direct aversive memory and inferred aversive memory. I used optogenetics to inhibit the projections from mPFC to amygdala and found that the projection is necessary during the recall of inferred averisve memory but not the original memory. The calcium activity of mPFC neurons projecting to amygdala also specifically show increased sensory responses for inferred aversive memory during recall. Moreover, the extinction of inferred aversive memory only affect the defensive behavior for inferred aversive memory and independent from original aversive memory and the calcium activity of mPFC neurons also provide consistent evidence. The results are presented in JNS conferences of 2021, 2022 and 2023. Recently, the results were summited to Nature and now the manuscript is in revision.

研究分野：neuroscience

キーワード：Inference　Cognition　Emotion　mPFC　Rats

## １．研究開始当初の背景

Emotions are a central aspect of human experience and coordinate bodily physiology and behavior to enhance survival. Aversive experiences mobilize emotion systems in the brain to produce defensive responses and prevent harm. Previous studies have identified the brain circuits for forming simple associations between sensory stimuli and aversive events and revealed the neural mechanisms mediating memory recall of these emotional experiences. In many cases, however, appropriate emotional responses in uncertain situations must be inferred based on prior, indirectly related experiences. Influential psychological theories suggest that higher-order emotions involving inference arise from internal models in the brain which interpret incoming sensory and interceptive information in the context of our past experiences and current state. Nevertheless, the neural mechanisms underlying higher-order emotional associations remain largely unexplored. Internal models have been studied in sensory, motor and decision-making systems and findings from this work suggests that they represent experience dependent statistical relationships in the environment which can be used to infer sensory structure or novel solutions in uncertain conditions. Several studies examining aversive and reward related behaviors have identified brain regions which participate in encoding models of the associative relationships between sensory stimuli, independent of whether they were associated with salient events (i.e. a sensory model). However, whether and, if so, how the brain encodes models of emotionally relevant associations and whether emotional models are distinct from internal sensory models has not been examined.
A candidate brain region for encoding emotional internal models is the dorsomedial prefrontal cortex (dmPFC) which has been investigated for its role in cognitive processes that require the integration of sensory, mnemonic and decision information. Related to aversive emotional learning, the dmPFC has been implicated in simple forms of aversive memory expression, sensory-generalization of emotional responses and avoidance. Furthermore, the mPFC maintains robust anatomical and functional connectivity with the amygdala, a brain structure involved in the learning and storage of directly associated aversive memories. This circuit could allow dmPFC to coordinate higher-order emotional processing by mobilizing subcortical defensive response systems.

## ２．研究の目的

We try to determine the neural pathways which convey aversive information to the amygdala to trigger fear memories and posit a novel solution to the problem of how aversive memories form in the presence or absence of pain. We propose that the neural pathway which instructs emotional memories integrates aversive information from the peripheral world (e.g. pain, loud noises, etc) as well as our internal bodily reactions to these experiences to trigger neural plasticity in the LA and fear memories in the presence or absence of pain. Furthermore, we hypothesize that in conditions of higher stress, our

bodily reaction to aversive events and subsequent activation of the instructive neural circuit is enhanced leading to heightened emotional learning. Thus we will elucidate a brain mechanism for forming aversive emotional memories both in the presence and absence of painful experiences and determine how stress produces pathological fear memories through dysregulation of this brain system.

## ３．研究の方法

We firstly hypothesized that dmPFC encodes a flexible internal model which is used to infer associations between sensory stimuli which were indirectly associated with an emotion inducing experience and that the recall of this inferred memory occurs through dmPFC projections to subcortical emotion processing systems in the amygdala. We tested this hypothesis using an inference based, aversive associative learning task in male and female rats, coupled with longitudinal, *in-vivo* calcium imaging and optogenetic approaches to track populations of single neurons across days while they first formed sensory and then emotional models and manipulate activity to test their functional role in behavior. We found that dmPFC population activity uses existing sensory models to encode an internal model of emotionally relevant associations and their relationship with related aversive experiences. While dmPFC population activity does not encode a sensory model prior to aversive experiences, sensory-sensory learning tags and stabilizes specific dmPFC cells which are then associated with aversive events during learning to form representations of inferred aversive memories. dmPFC associative representations are flexible and individual elements can be selectively updated if one component of the representation is devalued. In contrast to the larger dmPFC neuronal population, calcium imaging and optogenetic experiments from dmPFC neurons which project to the amygdala revealed that these cells specifically encode inferred emotional memories and that inhibition of this projection selectively abolished inferred emotional responding. These findings reveal how a flexible internal model of aversive emotional associations is generated and encoded in dmPFC and demonstrate how this information is conveyed to subcortical emotional control systems to facilitate the expression of inferred emotional memories.

## ４．研究成果

Results show that the dmPFC encodes a flexible model of emotional associations and reveal how this information is incorporated in subcortically projecting cell populations to guide inferred emotional processing. While the dmPFC population representation does not change following sensory-sensory learning, specific auditory-visual co-responsive cells become tagged and associated with aversive events during emotional learning to support inferred memory recall. Despite the binding of the sensory-aversive representations, individual elements of this representation are flexible, and extinguishing the inferred stimulus selectively reverses its associative relationship with

the representation of the aversive event. In contrast to the larger dmPFC population, aversive learning selectively strengthens and enhances the inferred memory representation in amygdala projecting dmPFC neurons, but they do not encode learning induced changes in stimuli that were directly paired with aversive experiences. Moreover, this subcortical projection is required specifically for expressing inferred aversive memories.

Our results suggest that sensory-sensory models encoded elsewhere are integrated in associative networks in the dmPFC only when they are associated with salient experiences. Furthermore, the extinction learning findings show that this associative representation is flexible and that individual elements can be modified by subsequent experience. This segregation of sensory and emotional models allows for flexibility in circumstances requiring pure sensory or contextual processing as well as context dependent mapping of sensory models to multiple, distinct outcomes. Moreover, the flexibility of this representation in conditions such as extinction allows for the maintenance of the relevant sensory associative information, but excision of the connection between the sensory stimulus which has been devalued and the representation of the aversive experience it was associated with. Several potential regions which are anatomically connected with the dmPFC have been implicated in processing sensory-sensory or contextual models mediating appetitive and aversive learning including perirhinal, anterior cingulate and orbitofrontal cortices as well as the hippocampus. Further studies are required to determine how these regions interact to build sensory and value-based internal models and how more complex models involving multiple aversive and reward-based associations are encoded in dmPFC.

Studies using ex-vivo and in-vivo experimental approaches have revealed detailed mechanisms of synaptic plasticity and linked these mechanisms to behavioral associative memory formation. However, the plasticity mechanisms mediating the formation of internal models in the brain are not well understood. Specifically, it is unclear how the larger internal model can be altered when only a portion of the network is engaged during a specific experience. This is also an important question in recurrent neural networks where artificial, supervised 'backpropagation' is commonly used for large-scale network updates but is computationally expensive. For the instantiation of internal models of emotional associations, our results suggest a multi-process model involving cellular tagging and stabilization of specific multi-sensory cell populations followed by a form of Hebbian plasticity in which aversive events recruit these cell populations into the long-term memory trace. In recurrent neural networks, a tag-and-capture mechanism could reduce computational cost by allowing previous experience to focus the effects of subsequent connectivity updates to specific units in the network. Cellular tagging has been demonstrated in mPFC for selection of neurons which will be used for storing remote memories in the cortex. Furthermore, increases in excitability underlies memory allocation and linking memories that occur close in time. We show that increases in basal neuronal responsiveness can be engaged as a latent mechanism for longer term tagging of cells representing sensory associations to be recruited by

subsequent emotionally relevant experiences, merging sensory and aversive representations in dmPFC to form an emotional internal model. However, in addition to the co-responsive neurons which are tagged by the original sensory learning, the final neural representation at memory recall is expanded and recruits new, untagged co-responsive and auditory-selective cells. This suggests that additional mechanisms such as off-line replay following aversive learning, but perhaps recruited through the tagging mechanism we describe here, may act to expand the memory trace.

Our results also suggest a hierarchical model of emotional processing involving distributed interactions between dmPFC and amygdala. LA/B neurons store directly associated sensory-aversive outcome associations through changes in their synaptic connectivity with thalamic and cortical sensory regions. Furthermore, the representation of sensory cues directly associated with shock merges with the representation of the shock itself in LA/B neurons. While higher order associations which are modulated by inference are reflected in LA/B neural activity, this information could be inherited from dmPFC. Supporting this, our results show that dmPFC encodes an emotional internal model and that information specifically about inferred aversive associations is sent to the LA/B and required for inferred emotional memory. LA/B may support the formation of emotional models through bottom-up projections to dmPFC during associative learning, an idea which is consistent with recent theories on hierarchical and distributed emotional processing. It will be important in future work to understand whether LA/B neurons encode inferred associations and how the dmPFC contributes to this encoding. Furthermore, how LA/B contributes to the formation of internal models in dmPFC is an important question for future study.

Rodent experiments have elucidated the detailed neural circuit and synaptic plasticity mechanisms underlying simple forms of emotional associative learning and brain imaging studies have largely validated the existence of similar neural systems in humans. Despite this considerable progress on understanding the neural underpinnings of these simple forms of emotional learning, this has produced significant advances in the treatment of anxiety and trauma related disorders. One shortcoming could be that the cause of dysfunction in these patients arises more from dysregulation of brain systems involved in the interpretation of threatening situations in the context of our individual experiences through internal models, resulting in inappropriate evaluations of threat. The present results demonstrate a critical role for the dmPFC in processing internal models of emotion and outline a basic research framework for understanding the neural basis of higher order emotion and its dysfunction in rodent models. Future studies can build on this framework in animal models and through cross-species collaborations with researchers studying human emotion to extend our understanding of emotional processing in the brain and improve treatment options for anxiety and trauma related disorders.

5．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　0件）

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|---|
| 1．発表者名<br>Xiaowei Gu |
| 2．発表標題<br>Prefrontal cortex encoding and control of aversive emotional inference |
| 3．学会等名<br>the 46th Annual Meeting of the Japan Neuroscience Society |
| 4．発表年<br>2023年 |

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|---|
| 1．発表者名<br>Xiaowei Gu |
| 2．発表標題<br>Brain circuits of cognitive control in emotional inference |
| 3．学会等名<br>the 45th Annual Meeting of the Japan Neuroscience Society |
| 4．発表年<br>2022年～2023年 |

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| 1．発表者名<br>Xiaowei Gu |
| 2．発表標題<br>Neural mechanism underlying cognitive operations in fear inference |
| 3．学会等名<br>the 44th Annual Meeting of the Japan Neuroscience Society/ the 1st CJK International Meeting |
| 4．発表年<br>2021年～2022年 |

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
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7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
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