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研究課題名（和文）先天的恐怖誘発機構の解明および精神疾患との関連

研究課題名（英文）Elucidate the molecular mechanisms of innate fear and its relation to mental disorders

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研究成果の概要（和文）：恐怖感情制御の分子機構を解明するため、フォワード・ジェネティクスにより捕食者の匂いによって誘発される恐怖反応に関する2つの変異を同定した。一方は先天的恐怖反応の減少を導くTrpa1遺伝子の劣性変異、他方は異常な恐怖反応（ジャンプを行う表現型：ポップコーン変異）を導く優性変異である。また、生化学、細胞学など様々な手法により、Trpa1が捕食者の匂いに関する化学センサーとして機能することを明らかにし、Trpa1を発現する三叉神経節ニューロンは、捕食者の匂いによって誘発される先天性の恐怖行動に重要な役割を担うことを実証した。ポップコーン変異はマウスに統合失調症のような行動を引き起こすことも分かった。

研究成果の学術的意義や社会的意義
We established the first forward genetics screen to uncover the molecular mechanism of fear, a basic emotion that is inextricably linked with mental health. The results have the potential to contribute greatly to developing medicines and therapeutic interventions for fear-related mental disorders.

研究成果の概要（英文）：Irrational fears are linked to a variety of mental illnesses. However, the molecular mechanisms of fear remain largely unknown. To this end, we conducted a novel predator odor-based “innate fear” forward genetic screen of ethylnitrosourea (ENU)-mutagenized mice, and identified a recessive mutation in Trpa1 gene that led to diminished innate fear responses and a dominant mutation that led to an abnormal fear response, jumping phenotype (hence named Popcorn mutant), upon predator odor exposure. By using a host of biochemical, cellular, neuroscience and behavior assays, we demonstrated that Trpa1 functions as a novel chemosensor for predator odors and Trpa1-expressing trigeminal ganglion (TG) neurons contribute critically to predator odor-evoked innate fear/defense behaviors. We found that Popcorn mutation causes schizophrenia-like behaviors in mice.

研究分野：動物生命科学

キーワード：先天的恐怖　恐怖感情　精神疾患

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Fear is a basic emotion that triggers characteristic defensive behaviors and physiological responses to promote survival in dangerous situations. Irrational fears are linked to a variety of mental illnesses, such as phobia, anxiety, depression, post-traumatic stress disorder (PTSD). Fear can be induced by both innate and learned mechanisms. Innate fear (e.g., predator induced fear) is genetically encoded, whereas learned fear is acquired through experience. Both innate and learned fears are involved in the pathogenesis and symptoms of mental disorders. Although rapid progress has been made in mapping the neural circuitries of innate and learned fear, the molecular circuits underlying fear, particularly the core signaling pathways that mediate various fear/defensive responses, remain poorly understood.

To tackle this problem, we selected 2-methyl-2-thiazoline (2MT), a potent analog of fox odorant 2,4,5-trimethyl-3-thiazoline (TMT), to develop a simple and highly robust innate fear assay suitable for high-throughput mouse screening. Using Freezeframe to quantify freezing as a readout of fear, we performed both recessive and dominant genetics screens of randomly mutagenized mice to identify core fear genes. The screening identified a mutant that exhibited low freezing rate "fearless" phenotype and a mutant that exhibited jumping phenotype, hence termed Popcorn, upon 2MT exposure. Further analysis indicated that the "fearless" phenotype was linked to a homozygous mutation in the *Trpa1* gene, and the jumping phenotype was linked to a point mutation at the 3' splice site for an intron of *Popcorn* gene (Fig. 1).

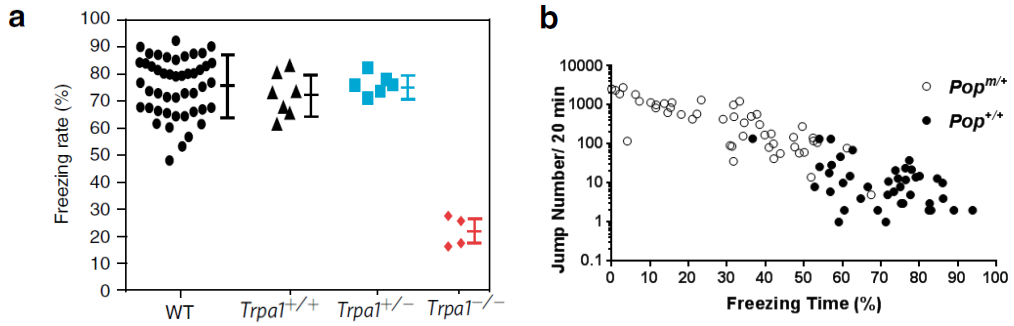

Fig. 1. **a)** A graph of the *fearless* mutant pedigree consisting of seven *Trpa1*$^{+/+}$, six *Trpa1*$^{+/-}$, and four *Trpa1*$^{-/-}$ individuals. A separate group of wild-type (WT) mice were included as controls. **b)** Plot of freezing rate vs jump number in mice of *Popcorn* pedigree.

２．研究の目的

In this study we aimed to: (1) elucidate the role of *Trpa1* gene in predator odor-evoked innate fear/defensive behaviors; (2) determine whether *Popcorn* mutant gene causes mental disorders by comprehensive behavioral test. These studies will substantiate the utility of a forward genetics approach to investigate the molecular mechanism of innate fear and fear-related mental disorders.

３．研究の方法

(1) Elucidate the role of *Trpa1* gene in predator odor-evoked innate fear/defensive behaviors

① Examine whether loss of Trpa1 leads to malfunction of the olfactory system by conducting olfactory habituation-dishabituation test using 2MT in Trpa1$^{+/-}$ and Trpa1$^{-/-}$ male mice.

② Examine whether loss of Trpa1 causes impairment in generating fear responses or fear-related learning by conducting learned fear assay and other fear-related assays, such as open field and elevated plus maze in Trpa1$^{+/-}$ and Trpa1$^{-/-}$ male mice.

③ Identify neural regions that are activated in response to 2MT/TMT by conducting c-fos mapping experiment.

④ Investigate biochemical functions of *Trpa1* gene product by performing Ca^{2+} imaging in Trpa1-expressing HEK293T cells upon 2MT/TMT exposure. Because TMT is a pungent odor, we hypothesized that Trpa1, a well-known pungency/irritancy receptor, may function as a chemosensor for TMT-like thiazolines.

⑤ Identify Trpa1^{+} neurons that are critical for 2MT-evoked fear/defensive behaviors by carrying out classic tissue lesion experiments in wild-type mice and by ectopic expression of Trpa1 in Trpa1$^{-/-}$ mice.

(2) Determine whether *Popcorn* mutant gene causes mental disorders

A host of behavior tests, such as open field, elevated plus maze, light-dark box, Y maze, nesting and sucrose preference, were performed in *Pop*$^{m/+}$ mice and their wild-type littermates to determine whether *Popcorn* mutant mice can be established as an good genetic model of some mental disorder.

4．研究成果

(1) Elucidate the role of *Trpa1* gene in predator odor-evoked innate fear/defensive Behaviors

Trpa1$^{-/-}$ mice can smell and learn to fear 2MT/TMT

We found that homozygous *Trpa1*$^{-/-}$ mice were healthy and fertile, and exhibited normal anxiety behaviors in the open field and elevated-plus maze tests. Habituation-dishabituation test showed that *Trpa1*$^{-/-}$ mice exhibited the same level of detection threshold for 2MT as *Trpa1*$^{+/-}$ mice. Moreover, *Trpa1*$^{-/-}$ mice were proficient in dual odor-based and sound-based fear conditioning assays, suggesting that they could distinguish different odors and exhibited normal learned fear responses. Importantly, *Trpa1*$^{-/-}$ mice could be further trained to fear 2MT by pairing it with electric footshocks. These findings suggest that *Trpa1*$^{-/-}$ mice have a normal sense of smell and can learn to fear 2MT.

Trpa1 is required for activation of known fear/stress centers in the mouse brain upon 2MT exposure

Compared to *Trpa1*$^{+/-}$ mice, 2MT-evoked c-fos induction in *Trpa1*$^{-/-}$ brains was greatly diminished in the central nucleus of amygdala (CeA), the ventral periaqueductal gray (vPAG), and the paraventricular nucleus (PVN) of hypothalamus in *Trpa1*$^{-/-}$ brains relative to *Trpa1*$^{+/-}$ brains (Fig. 2), which are brain regions mediating response to fearful/stressful situations.

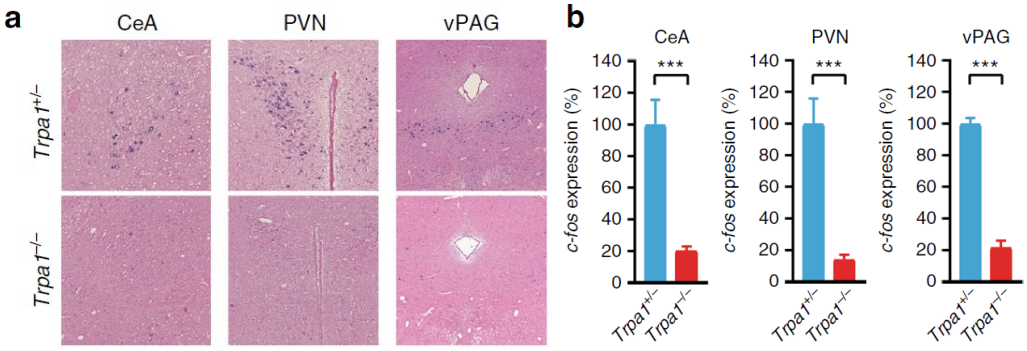

Fig. 2. **a)** c-fos in situ hybridization. **b)** Quantitative analysis of c-fos-positive neurons. ***P < 0.001.

Trpa1 acts as a chemosensor for TMT/2MT

2MT, but not 2-methyl-2-oxazoline (2MO), a structurally related non-fear inducing odorant, evoked Ca^{2+} transients in the Trpa1-expressing HEK293T cells in a dose-dependent manner. Substitution of a number of cysteines with serines in mouse Trpa1 protein abolished or reduced 2MT/TMT-evoked Ca^{2+} responses in transfected HEK293T cells (Fig. 3).

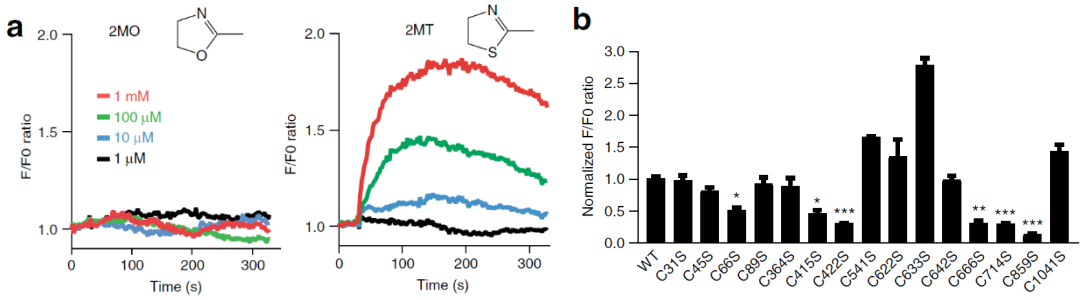

Fig.3. **a)** Ca2+ imaging in HEK293T cells upon exposure to 1, 10, 100, 1000 µM of 2MO, 2MT. **b)** Quantitative analysis of wild-type (WT) and mutant Trpa1 activities in response to 2MT (100 µM). *P < 0.05, **P < 0.01, ***P < 0.001.

Trpa1^{+} TG neurons are critical for 2MT-evoked freezing

2MT exposure induced equivalent levels of c-fos expression in the olfactory bulbs (OB) of *Trpa1*$^{+/-}$ and *Trpa1*$^{-/-}$ mice, but elicited strong nuclear c-Fos signals in specific TG neurons of *Trpa1*$^{+/-}$, but not *Trpa1*$^{-/-}$ mice. Unilateral loss of the trigeminal input by TG lesion in wild-type mice led to significantly attenuate 2MT-evoked innate freezing behavior. On the other hand, Ectopic expression of Trpa1 in the TG by bilateral injection of adeno-associated virus expressing Trpa1 (AAV-Trpa1) into the TG of *Trpa1*$^{-/-}$ mice could significantly rescue 2MT-evoked innate freezing as compared to AAV-GFP-injected mice (Fig. 4).

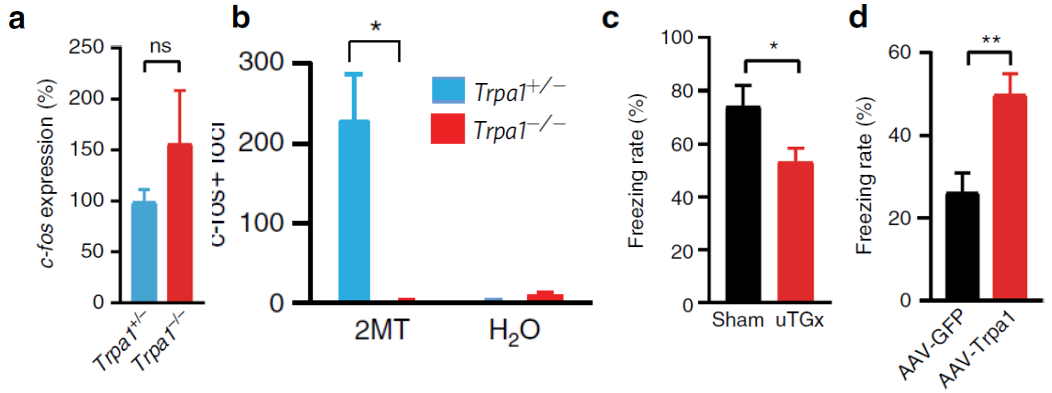

Fig. 4. Quantitative analysis of **a)** c-fos-positive neurons in the OB of mice after 2MT exposure. **b)** c-Fos-expressing TG neurons of mice after H_2O or 2MT exposure. **c)** 2MT-evoked freezing behavior in the sham and uITGx mice. **d)** 2MT-evoked freezing behavior in the AAV-GFP and AAV-Trpa1-injected mice. *P < 0.05, **P < 0.01, ns not significant.

In summary, Trpa1 acts as a chemosensor for 2MT/TMT, and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. Our results, coupled with previous studies, establish that both the trigeminal and olfactory systems play important roles in predator odor-evoked innate fear/defensive behaviors.

(2) Behavior analysis of *Pop*$^{m/+}$ mice

Behavioral analysis showed that *Pop*$^{m/+}$ mice exhibited significant increased locomotor activity (open field test) and risk-taking behaviors (elevated plus maze test and light/dark box test (LDB)), diminished motivation (nesting test), impaired working memory (Y maze test) and anhedonia (sucrose preference test, SPT) (Fig. 5). Together with the blunted fear response to predator odor, these results suggest that Pop$^{m/+}$ mutation causes schizophrenia-like behaviors.

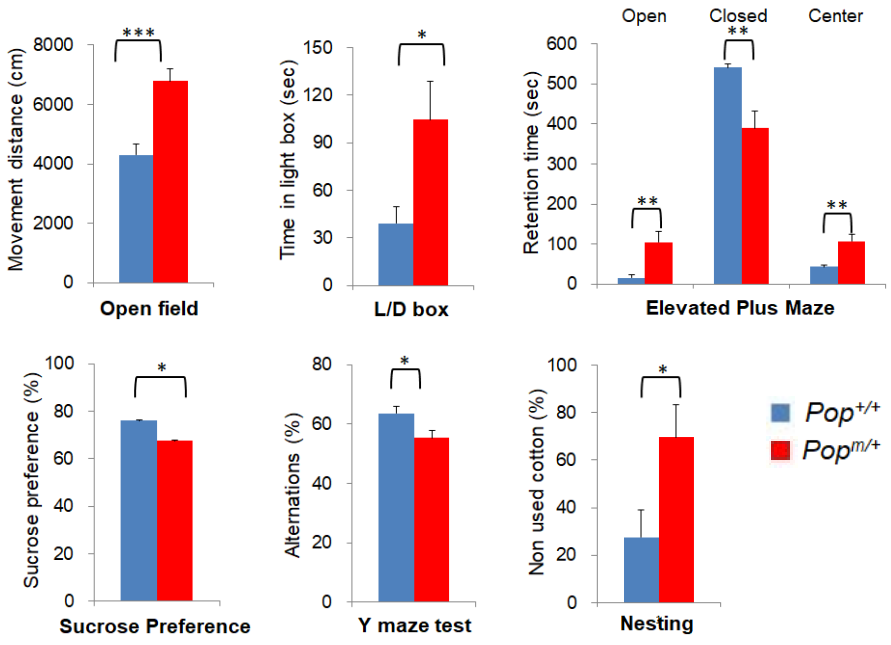

Fig. 5. Schizophrenia-like phenotypes in *Pop*$^{m/+}$ mice. *P < 0.05, **P < 0.01, ***P < 0.001.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　1件）

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掲載論文のDOI（デジタルオブジェクト識別子） DOI: 10.1038/s41467-018-04324-3	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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