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研究課題名（和文）Optogenetic identification and manipulation of distinct midbrain dopaminergic projection pathways encoding fear and reward learning

研究課題名（英文）Optogenetic identification and manipulation of distinct midbrain dopaminergic projection pathways encoding fear and reward learning

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研究成果の概要（和文）：腹側被蓋野ドーパミン神経は報酬行動に重要であることが知られているが恐怖やその消去にも関係していることが示唆されている。ドーパミン神経の消去学習への関与を明らかにするため、我々は恐怖記憶の消去学習中において嫌悪刺激がくる時間帯のみいくつかの脳部位でのドーパミン神経軸索末端を光遺伝学を用いて抑制した。側坐核での軸索末端を抑制すると消去学習の長期記憶が阻害され、一方前頭前野の軸索末端を抑制すると消去学習を促進した。これらの結果から、腹側被蓋野ドーパミン神経は投射部位特異的に恐怖記憶の消去に関与していることが明らかとなった。

研究成果の概要（英文）：Ventral tegmental area (VTA) dopamine neurons are thought to code for reward behaviors, but recent work has demonstrated their importance in fear and fear extinction processes. Can traumatic stress, as modelled by fear conditioning, and posttraumatic disorders, as exemplified by inability to extinguish fear memories, also be modulated or triggered by the midbrain dopamine system? We optogenetically manipulated VTP dopamine cells during the shock period of fear learning and during the expected (but omitted) period of fear extinction learning, and saw that dopamine cells that project to different areas of the brain have differential effect on fear-related memories. We found that inhibition of dopamine projections to nucleus accumbens abolishes extinction memory retrieval whereas inhibition of dopamine projections of infralimbic area of prefrontal cortex enhances extinction memory. Together this suggests midbrain dopamine projections differentially affects the learned recovery from fear.

研究分野：　neuroscience

キーワード：　dopamine　VTA　IL　fear　extinction　learning　amygdala

１．研究開始当初の背景

Although the role of ventral tegmental area (VTA) dopamine neurons in appetitive learning is well established, its role in aversive learning such as fear conditioning has been ambiguous. A subset of putative midbrain dopamine cells in monkeys have been found to be excited by both rewarding and aversive stimuli (Matsumoto & Hikosaka, 2009), but they show little response to outcome omission contrary to the prediction error models. A study in mice showed that VTA GABAergic cells are excited by aversive air puffs, and respond in tonic fashion after CS onset, but that different dopamine cells may be excited or inhibited by aversive stimuli (Cohen et al., 2012). However, from these studies it is not clear how dopamine neurons are encoding aversive experiences, i.e. as an attentional signal or a signed prediction error. It is also not known whether dopamine neurons differentially encode aversive stimuli depending on their projection patterns.

Although learning to fear and predict danger is critical to survival, reversing or extinguishing fear memories is important when threats are no longer present. The neural signals which detect when fear responses should be disengaged are not known. During fear conditioning, animals learn that an auditory stimulus (conditioned stimulus CS) predicts the occurrence of an aversive shock (unconditioned stimulus US) and learn to freeze to the CS alone. If the CS is presented over many trials in the absence of shock, animals learn to reduce their freezing behavior, a process termed fear extinction. Prior work has shown that midbrain dopamine neurons in the VTA are activated by better than expected outcomes including rewards and the omission of expected aversive events. This suggests dopamine neurons may provide a signal to initiate extinction learning when aversive outcomes are no longer present. Studies have shown that nucleus accumbens (NAc) dopamine receptor activation is required for extinction learning (Holtzman-Assif et al, 2010), suggesting that VTA projections to NAc may be critical for fear extinction learning in addition to its role in fear learning.

２．研究の目的

Midbrain dopamine cells are activated by reward and stimuli that predict reward. Recent evidence indicates that dopamine cells also respond to aversive outcomes, outcomes that better than expected, or worse than expected, but little is known about how they regulate behaviors through projections to distinct brain areas. Dopamine cells that code for reward or aversiveness are thought to innervate the nucleus accumbens and/or medial prefrontal cortex. A novel way of using optogenetics is to trace ventral tegmental area dopamine outputs to regions like the nucleus accumbens and medial prefrontal cortex, and manipulate both fear learning and extinction of fear. In this project, we used optogenetics to dissect the functional role of midbrain dopamine cell projections in fear and extinction learning.

３．研究の方法

Light-activated proteins halorhodopsin (NpHR) and ArchT were expressed in VTA dopamine cell bodies and terminals in tyrosine hydroxylase (TH)-cre rats. Animals learned to associate a tone cue with a foot shock, and subsequent training extinquished the response Light inactivation of dopamine terminals in the nucleus accumbens (NAc) and medial prefrontal cortex (infralimbic IL) were used

during the shock period of fear learning, and during the expected but omitted period in extinction learning to manipulate behavioral freezing in subsequent test sessions. We also recorded in the VTA during and after fear conditioning, as well as dopamine voltammetry signals in the NAc in attempting to dissect these dopamine projections.

We used BAC-transgenic rats from the Deisseroth lab that express Cre recombinase only in TH+ neurons to specifically target dopamine cells. A Cre-dependent virus containing floxed NpHR with eYFP on an EF1a promoter, or ArchT with eGFP on a CAG promoter were used in different experiments, each injected into the VTA under isoflurane anaesthesia. Upon getting expression in 3-4 weeks (for cell body) or 10-12 weeks (for terminals), we trained animals in classical conditioning task in a sound-proof chamber, where CS is a 20 second 1hz tone, and US is a 1 second footshock. In fear manipulation experiments, we inhibited VTA or VTA terminals using NpHR or ArchT during the shock period. In fear extinction studies, we inhibited during the 3 seconds after termination of the last (20th) tone.

After behavioral experiments, animals were perfused with 4% paraformaldehyde in PBS, and 40um slices were cut and mounted in a cryostat. For antibody statining we used either mouse or rabbit anti-TH and anti-GFP antibodies from Millipore, and secondaries of the same species for Alexa Fluor 488 (GFP) and 555 (TH). In MAP kinase studies, we used a pMAPK rabbit antibody and a ABC kit for rabbit followed by application of DAB for visualization.

4．研究成果

First we investigated whether optogenetically manipulating entire dopamine cell populations in VTA affects fear and extinction learning. When we inhibited VTA dopamine cells during the shock period of fear learning, fear learning was potentiated, suggesting that dopamine cells work to suppress learning of fear.

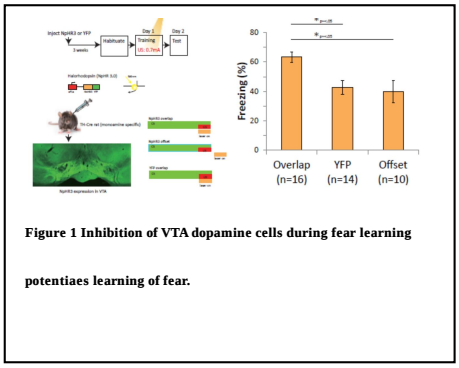

Figure 1 Inhibition of VTA dopamine cells during fear learning potentiaes learning of fear.

Next we examined whether VTA dopamine cells contribute to fear extinction learning. We presented animals with 22 trials of CS tones only, and inhibited dopamine cells in a 3 second period after termination of pips, and found that inhibition of these cells prevented extinction learning, suggesting that VTA dopamine triggers extinction learning.

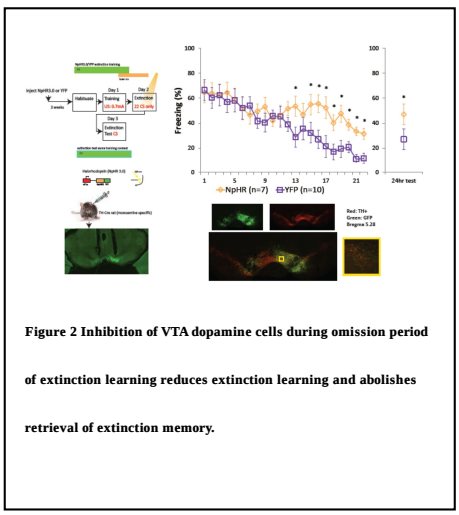

Figure 2 Inhibition of VTA dopamine cells during omission period of extinction learning reduces extinction learning and abolishes retrieval of extinction memory.

Notice also that cell populations we manipulated appear to express specifically

TH+, so indeed we are inhibiting dopamine cells only in the VTA.

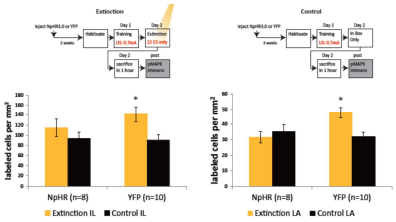

Figure 3 Inhibition of VTA dopamine cells during omission period of extinction ablishes the increase in MAPK expression in mPFC (IL) and LA following extinction training.

In order to see the effect of these cell body manipulations in other regions in the brain, we used antibody for pMAPK to see how light manipulation affects the increased MAP kinase expression that accompanies fear extinction learning. We found that the increase in MAPK phosphorylation found in mPFC (IL) and lateral amygdala following extinction learning is abolished by inhibition of dopamine activity during the omission period of extinction learning, suggesting that projections of VTA dopamine to these areas are affecting extinction learning behaviorally.

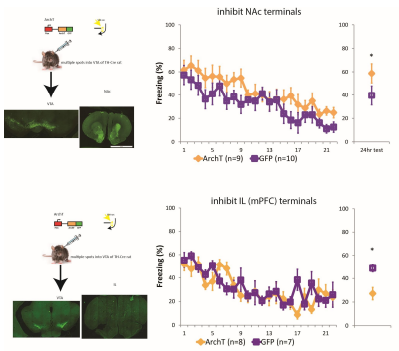

Figure 4 Inhibtion of terminals of VTA dopamine cells in NAc abolishes extinction learning, while inhibition of terminals of VTA dopamine cells in IL (mPFC) enhances extinction learning.

Given this regional effect of dopamine manipulation, we decided to injected animals with a more readily terminal expressing contruct (ArchT in AAV9) and manipulated the terminals of dopamine cells originating in VTA. We thus inhibited terminals in NAc and in infralimbic (IL) of mPFC during omitted periods of fear extinction. We found that inhibition of dopamine terminals in NAc abolishes extinction learning, suggesting that the VTA cell body effects are indeed undertaken by projections of these cells to the NAc, a sign of classic reward prediction error signals. On the other hand, inhibition of dopamine terminals in IL (mPFC) facilitates extinction learning, suggesting that projections from VTA to IL actually opposes extinction learning, a case that agrees with recent studies regarding the possible heterogeneity of dopamine neurons in VTA (Lammel et al., 2012).

Together these results suggest that dopamine projects to different areas of the brain have different effects on the ability of animals to learn to extinguish fear. In particular, NAc projections appear to work like classic reward prediction error cells, in which better than expected outcomes (lack of shock) trigger the learning of extinction. IL projections, however, appear to work through a different mechanism which serves to promote fear and oppose extinction learning.

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　0　件）

〔学会発表〕（計　4　件）

1. Ray Luo, *Jenny Koivumaa*, *Lindsay Preston*, *Yanq*iu Tao, Joshua P. Johansen. “Ventral tegmental area dopamine neurons trigger fear extinction learning.” 2015-12-05 7th International Symposium on Optogenetics, Tokyo Medical Dental University, Tokyo Japan.

2. Ray Luo, Jenny Koivumaa, Lindsay Preston, Yanqiu Tao, Joshua P. Johansen. “Ventral tegmental area dopamine neurons trigger fear extinction learning.” 2015-10-18 45th Society for Neuroscience meeting, McCormick Center, Chicago, Illinois USA.

3. Ray Luo, Luca Aquili, Jenny Koivumaa, Lindsay Preston, Yanqiu Tao, Joshua P. Johansen. “Midbrain dopamine neurons suppress fear and trigger extinction memory formation.” 2015-07-29 38th Japan Neuroscience Society annual meeting, Kobe International Conference Center, Kobe Japan.

4. Ray Luo, Luca Aquili, Jennifer Takahashi Cutler, Joshua P. Johansen. “VTA dopamine neuron inactivation during aversive outcomes enhances fear learning.”2013-11-21 RIKEN Brain Science Institute annual retreat, Karuizawa Prince Hotel West, Karuizawa Japan.

〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）

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〔その他〕
ホームページ等

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