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研究課題名（和文）Investigation of nucleus accumbens subpopulations controlling reward and aversive learning

研究課題名（英文）Investigation of nucleus accumbens subpopulations controlling reward and aversive learning

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研究成果の概要（和文）：このプロジェクトでは、シグナル伝達と報酬と嫌悪に寄与し、報酬と嫌悪に関連する行動を制御できる側坐核細胞の新しい亜集団を特定するために、単一細胞解像度の in-vivo イメージングと光遺伝学を使用することに成功しました。 これらのエキサイティングな発見の結果として、私は現在、影響力の大きいジャーナルに掲載することを意図して、プロジェクトをさらに拡大しています. このプロジェクトは、Nature Comms に掲載された 1 つの論文や Front. Neurosci に掲載された別の論文など、現在提出中または公開済みのいくつかの科学論文のデータにも直接貢献しています。

研究成果の学術的意義や社会的意義
Given that dysfunction of the NAc is associated with several psychiatric disorders charactorized by abnormal reward and aversion learning (including depression), these findings may provide new therapeutic targets for treatments of such conditions.

研究成果の概要（英文）：During this project, I was successful in using single-cell resolution in-vivo imaging and optogenetics to identify new subpopulations of nucleus accumbens cells that contribute to the signaling and reward and aversion, and can control reward and aversion-related behaviors. As a result of these exciting findings, i have now expanded the project further with the intention of publishing in a high-impact journal. This project has also directly contributed to data in several scientific papers that are now in submission or have been published, including one paper published in Nature Communications and another paper published in Frontiers in Neuroscience.

研究分野：Neuroscience

キーワード：Reward　Aversion　Learning　Nucleus Accumbens　Basal Ganglia　In-vivo imaging　Optogenetics

Background at the beginning of the research:

The nucleus accumbens (NAc) of the basal ganglia can be divided into two cellular populations, dopamine D1 or D2 receptor-expressing medium spiny neurons (D1/D2-MSNs). Previously, our group had demonstrated D1- and D2-MSNs to be critically involved in reward and aversive learning, respectively (Hikida et al., 2010, *Neuron, 66(6):896-907*; Hikida, et al.., 3013, *PNAS,* 110 (1) 342-347; Macpherson et al., 2016, *Learn. Mem,* 23(7): 359-364; Macpherson et al., 2018, *Front. Neurosci,* 12:418). However, recent single-cell RNA sequencing studies have indicated there to be a large degree of heterogeneity within NAc D1- and D2-MSNs, and studies utilizing optogenetic and chemicogenetic modulation of the activity of NAc D1-/D2-MSNs have often produced contradictory results, with stimulation of either neuron type reported to produce rewarding/motivational, aversive, or no apparent effects (Lobo et al., 2010, *Science*, 330(6002) 385-390; Gallo et al. 2018, *Nat Commun,*(9) 1086; Soares-Cunha et al., 2019, *Molecular Psychiatry;* Cole et al., 2018, *Plos One,* 13(11)). These studies indicate that there may be far more complex control of reward and aversion signaling within the NAc than had originally been appreciated.

Purpose of research:

Surprisingly, the roles of NAc D1- and D2-MSNs had yet to be investigated at the single-cell (ensemble) level (rather than the population level) during the signaling of rewarding or aversive stimuli. Therefore, I set out to investigate how the activity of ensembles of D1- and D2-MSNs within the NAc were modulated by exposure to rewarding or aversive stimuli. Investigating these processes will allow a better understanding of limbic processing within the brain and may help to identify novel cellular targets for the treatment of clinical conditions associated with abnormal reward and aversion learning, including addiction, PTSD, depression, and schizophrenia.

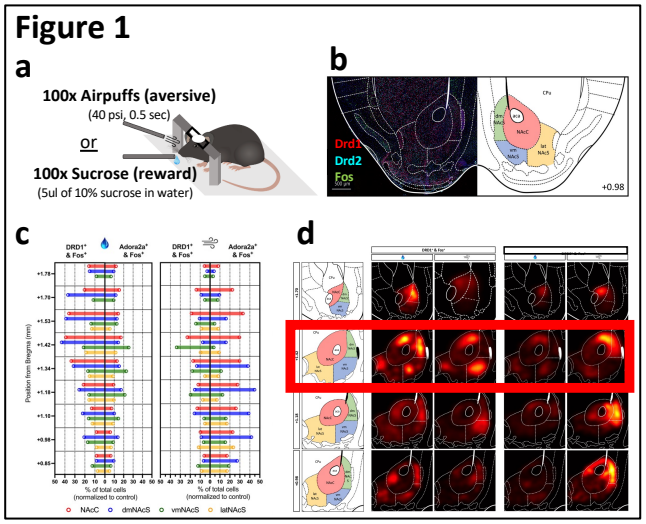
Method of research:

To investigate how ensembles of NAc D1- and D2-MSNs control reward and aversion signaling, I used a combination of genetic, molecular, imaging, and behavioral techniques. Specifically, I first used in-situ hybridization to establish regions of the NAc in which D1- or D2-MSNs were activated following exposure to rewarding or aversive stimuli. Then, a combination of transgenic mice, a virally-expressed calcium biosensor, and a miniature microscope was used to perform in-vivo imaging of the activity of NAc D1- and D2-MSNs during the performance of reward and aversion learning tasks.

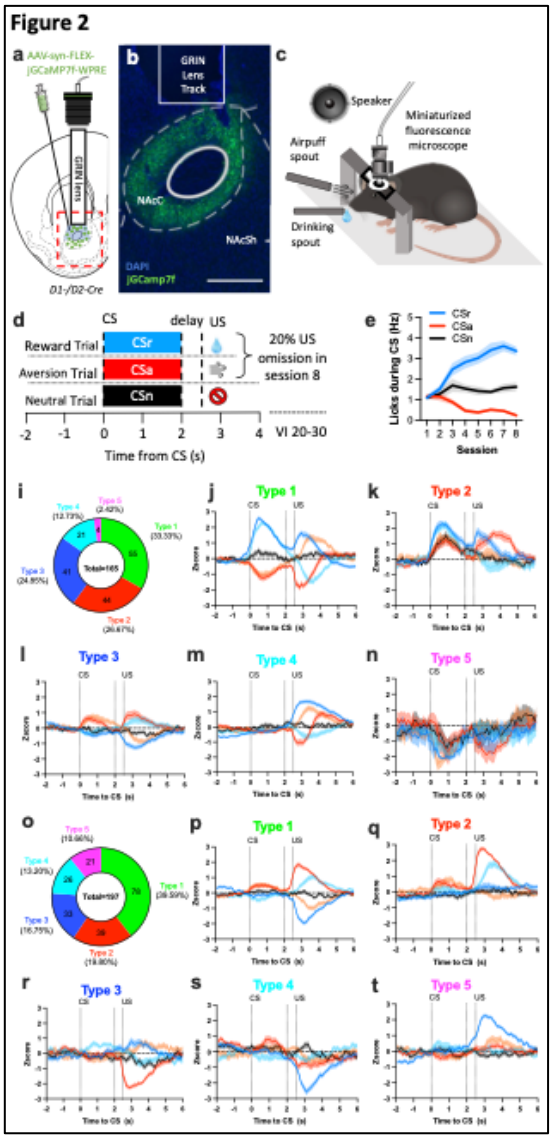
Research results:

In the first year of the project, I began by investigating the distribution of D1- and D2-MSNs within various subregions of the NAc that were activated by rewarding and aversive stimuli. This was achieved by taking headfixed wildtype mice and exposing them to repeated 100 presentations of a rewarding sucrose stimulus (10% sucrose in water) or an aversive stimulus (airpuff to the face) (Fig. 1A). 30-minutes later, brains were dissected, the entirety of the NAc sectioned into 10uM slices, and slices subjected to RNAscope in-situ hybridization using probes for dopamine D1 and D2 receptors (markers for D1- and D2-MSNs, respectively) and the early immediate gene activity marker c-fos. Automated cell counting software (HALO, Indica Labs, USA) was then used to count the amount of cells double-positive for c-fos and either D1- or D2- within the NAc core, lateral shell, dorsomedial shell, and ventromedial shell

(Fig. 1b). The amount of D1- or D2-MSNs activated by rewarding or aversive stimuli was then quantified (see Fig 1B) and the resulting spatial information used to plot heatmaps of activated neurons in the NAc (see Fig. 1c). It was identified that a region of the dorsal NAc core demonstrated D1- and D2-MSNs that were activated by both rewarding and aversive stimuli, indicating this area as a region of interest for further investigation (Fig. 1d).

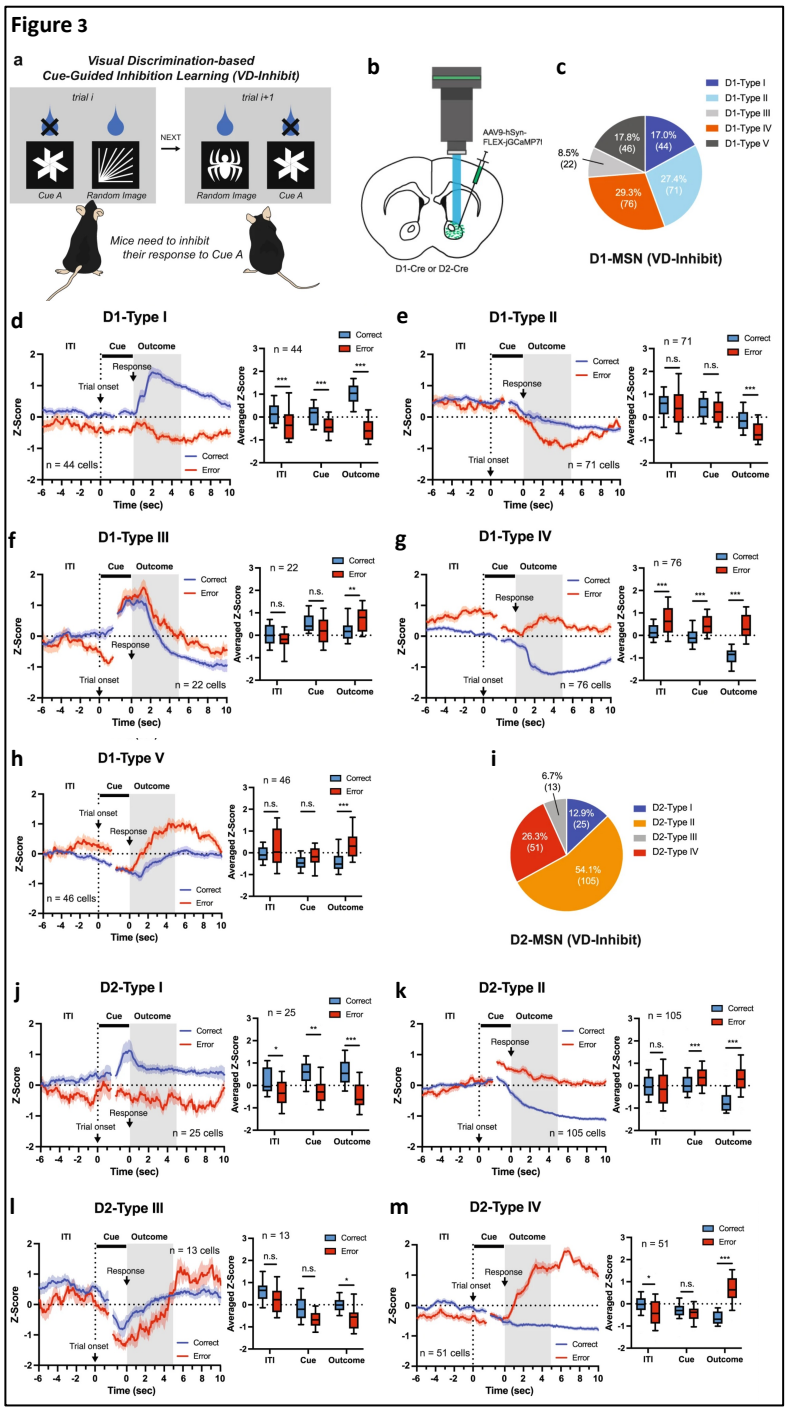


Based upon the results of the in-situ hybridization it was still unclear whether the same D1- and D2-MSNs that were activated by rewarding stimuli were also activated by aversive stimuli. Therefore, in the next stage of the project we perform in-vivo neural imaging of the activity of the NAc neurons during a reward and aversive learning task.



Specifically, D1- and D2-Cre transgenic mice were infused with an adeno-associated virus (AAV) expressing the calcium biosensor jGCaMP7f in a cre-dependent manner into the NAc core, above which a gradient index lens attached to a miniature microscope was placed (Fig. 2a&b). Then, these mice were headfixed to a platform and exposed to a Pavlovian conditioning task in which rewarding, aversive, or neutral stimuli were paired with the presentation of different auditory tones to induce Pavlovian associative learning (Fig. 2c&d). Anticipatory licking responses to these reward/aversion/neutral conditioned stimuli (CS) were then measured via a capacitance sensor attached to the drinking spout in order to confirm reward and aversion learning (Fig. 2e). Finally, following the completion of training, the neural activity of D1- and D2-MSNs was measured and cells were clustered into groups demonstrating similar physiological responses. In total 5 types of D1-MSN (Fig. 2i-n) and 5 types of D2-MSNs (Fig. 2o-t) were identified. Interestingly, a wide degree of heterogeneity was observed between these groups, with groups of both D1- and D2-MSNs demonstrating activation to rewarding (Fig. 2j,k,m,&t) or aversive (Fig. 2l,p&q) stimuli. These data suggest complicated functional organization of reward and aversive signaling within the NAc core that likely involved ensembles of both D1- and D2-MSNs.

Finally, to investigate how ensembles of NAc core D1- and D2-MSNs control reward and aversion related behaviors we performed a free-moving cognitive-behavioral task in mice.



A novel task was designed in which mice had to use aversive (reward omission) learning to guide goal (reward)- directed behavior, enabling the visualization of neural responses to both reward and aversion learning (Fig. 3a). Specifically, mice were required to inhibit responses at a touch window that was signaled by a consistently presented visual cue (Cue A) and make a response at a touch window signaled by a random visual cue to receive a reward. As previous, D1- and D2-Cre mice were infused with a cre-dependent calcium biosensor into the NAc core, and neural responses of groups of D1- or D2-MSNs were observed via a miniature microscope (Fig. 3b). In this task too, we observed a broad degree of heterogeneity within populations of D1- and D2-MSNs (Fig. 3c&i). Indeed, while some groups of D1-MSNs were activated by correct choices (reward) (Fig 3d,f&j), others were activated by incorrect choices (reward omission/aversion) (Fig. 3f,g,,h,,k &m).

Altogether, these studies indicate complex functional organization within ensembles of NAc core D1- and D2-MSNs. As a result of these interesting findings, I have now decided to extend this project and investigate the effect of optogenetic activation or inhibition of these subtypes of NAc D1- and D2-MSNs.

５．主な発表論文等

〔雑誌論文〕　計8件（うち査読付論文　8件／うち国際共著　8件／うちオープンアクセス　8件）

１．著者名 Nishioka Tadaaki、Attachaipanich Suthinee、Hamaguchi Kosuke、Lazarus Michael、de Kerchove d' Exaerde Alban、Macpherson Tom、Hikida Takatoshi	４．巻 14
２．論文標題 Error-related signaling in nucleus accumbens D2 receptor-expressing neurons guides inhibition-based choice behavior in mice	５．発行年 2023年
３．雑誌名 Nature Communications	６．最初と最後の頁 1-15
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-023-38025-3	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 該当する

１．著者名 Macpherson Tom、Kim Ji Yoon、Hikida Takatoshi	４．巻 16
２．論文標題 Nucleus Accumbens Core Dopamine D2 Receptor-Expressing Neurons Control Reversal Learning but Not Set-Shifting in Behavioral Flexibility in Male Mice	５．発行年 2022年
３．雑誌名 Frontiers in Neuroscience	６．最初と最後の頁 1-12
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fnins.2022.885380	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 該当する

１．著者名 Attachaipanich Suthinee、Ozawa Takaaki、Macpherson Tom、Hikida Takatoshi	４．巻 1
２．論文標題 Dual roles for nucleus accumbens core dopamine D1-expressing neurons projecting to the substantia nigra pars reticulata in limbic and motor control in male mice	５．発行年 2023年
３．雑誌名 eNeuro	６．最初と最後の頁 1-28
掲載論文のDOI（デジタルオブジェクト識別子） 10.1523/ENEURO.0082-23.2023	査読の有無 有
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〔学会発表〕　計7件（うち招待講演　7件／うち国際学会　1件）

１．発表者名 Tom Macpherson
２．発表標題 Neural Mechanisms in Reward and Aversion
３．学会等名 Osaka University Institute for Protein Research Symposium（招待講演）
４．発表年 2021年

１．発表者名
Tom Macpherson

２．発表標題
Cell-type-specific control of reward and aversive signaling in the Nucleus Accumbens

３．学会等名
The Physiological Society of Japan Meeting（招待講演）

４．発表年
2023年

１．発表者名
Tom Macpherson

２．発表標題
Striatal mechanisms of discrimination learning and their dysfunction in schizophrenia model mice

３．学会等名
52nd Annual Meeting of the Society for Neuroscience (SfN)（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
Tom Macpherson

２．発表標題
Neural mechanisms in reward and aversion signaling

３．学会等名
National Institute of Physiological Science (NIPS) Emotion Conference（招待講演）

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2022年

１．発表者名
Tom Macpherson

２．発表標題
Neural mechanisms in reward and aversion signaling

３．学会等名
45th Annual Meeting of the Japan Neuroscience Society (JNS)（招待講演）

４．発表年
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 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

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