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

平成　２９　年　　８　月　１５　日現在

機関番号：　８２４０１
研究種目：　基盤研究(B)（一般）
研究期間：　2014～2016
課題番号：　２６２９００１１

研究課題名（和文）Optogenetic control of visual perception

研究課題名（英文）Optogenetic countrol of visual perception

研究代表者

ベヌッチ　アンドレア（Benucci, Andrea）

国立研究開発法人理化学研究所・脳科学総合研究センター・チームリーダー

研究者番号：５０７２２３５２

交付決定額（研究期間全体）：（直接経費）　　13,000,000円

研究成果の概要（和文）：我々は、マウスの行動訓練のための新規プラットフォームを開発した。当該プラットフォームは、全自動かつハイスループットであって、またマウスが自発的に自らの頭部を固定するという点に特徴がある。我々は、これを用いることで、マウスが知覚に基づいた概念を利用して意思決定課題を遂行することを見出した。この能力は、マウスにおいてはこれまで示されていなかった高度な認知能力である。また、我々は、大脳皮質ネットワークの光イメージングと単一神経細胞レベルでの光遺伝学的活動操作を組み合わせるための手法を開発した。現在、当該手法を用いて、成熟マウスの大脳皮質における機能的可塑性の原理を探るための研究を遂行中である。

研究成果の概要（英文）：We have developed a new platform for mouse behavioral training which is high-throughput, fully automated, and features voluntary head fixation. We have used several of these setups to provide evidence mice can use perceptual concepts in sensory-based decision making tasks, a complex cognitive skill so far undemonstrated in this mammalian species. We have also developed a technology for simultaneous imaging and optogenetic perturbations of cortical networks at near single cell resolution. With this technology we are studying the in-vivo rules of functional plasticity in the cortex of adult mice.

研究分野：　neural circuits

キーワード：　neural circuits　behavioral training　decision-making　cortex　optogenetics　two-photon imaging

１．研究開始当初の背景

In this report we outline the overall technical and scientific achievements made possible by the awarded Kakenhi grant (文基 B, #26290011) during fiscal years FY2014-FY2016. The overall aim was to advance our understanding of the computational principles of cortical processing for visual perception and sensory-based decision-making. We are glad to report we have met and exceeded all goals and expectations outlined in the original proposal.

２．研究の目的

The purpose of the research can be subdivided into two main goals: (1) to setup and optimize a high throughput technology for mouse behavioral training featuring voluntary head fixation; (2) to setup and optimize a technology for the interrogation of neural circuits with simultaneous recording and optogenetic stimulation, and (3) to use these technologies in combination for the modification of a sensory percept via direct perturbations of the cortical dynamics in animals performing in a sensory-based decision making task. Below we detail the achievements in these aims.

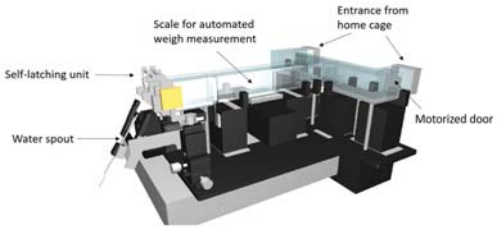
３．研究の方法

Details of the research methods will be outlined in the result section. Briefly here, they consisted on a behavioral setup for automated training of mice with voluntary head fixation, neural recording methods based on two-photon imaging of GCaMP signals from large neuronal populations, and optogenetic tools for perturbations of the neural dynamics at near single-cell resolution.

４．研究成果

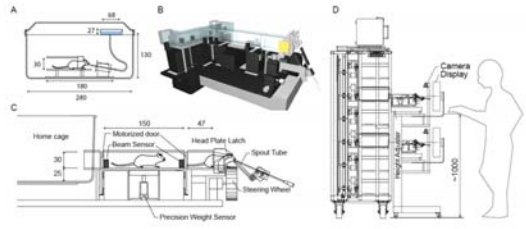
(1) High throughput technology for mouse behavioral training, featuring voluntary head fixation

We developed a platform for behavioral training, fully-automated, with voluntary head fixation, and high-throughput capacity. Its modularity allows for behavioral training of mice based on diverse sensory modalities, and it readily integrates with virtually any physiology setup for neural circuit and cellular level analysis. Moreover, its remote accessibility and web based design make it ideal for large scale implementations. To demonstrate the optimality of the system for the integration of complex behavioral assays with physiology setups, we used the platform to train mice in sensory-based decision making behavioral tasks. We did so



compatibly with cellular level imaging stability as demonstrated below by two-photon GCaMP recordings and optogenetic perturbations in trained animals.

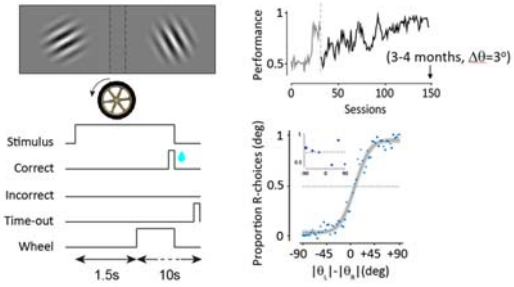
The highlights of the platform are depicted in the figure above. The setup is connected to two mouse cages housed in a standard rack. Only one animal at a time can access the setup, made possible by computer-controlled motorized entrance doors. Once inside the setup, the animal walks down a Plexiglas corridor reaching a scale-platform for automated weight measurement. After obtaining a stable reading, a door opens and allows the animal to reach the self-latching unit, where a water spout is located. The animal self-latches and the behavioral task begins for ~20 min. At the end of the session, a servomotor actuator releases the animal and the mouse can return to the home cage.



The figure above details specific features of this high-throughput setup for voluntary head-fixation and of the pre-training devices for habituation to self-latching. Briefly: (A) Head-restraining habituation system. Depiction a mouse inside a Plexiglas tube drinking water from a water spout connected to a water tank (blue box). Narrowing rails on the sides of the tube progressively restrain the head-post (dotted lines on the side of the tube). There is no latching mechanism for the head-post, hence mice can back-out of the tube at any time. The containing box represents a standard mouse cage with an air-filtering lid and a metal grid for mouse containment (horizontal solid line under the lid) as in standard individually-ventilated cages. Over the course of a couple of days, mice routinely self-restrain to drink water. (B) 3D view of the main dual-cage setup. (C) Side view of setup and labeling of its main components. (D) Side view of two setups (training capability of a

single setup is 4 mice/day) housed in a standard mouse rack. Several setups can be accommodated in the same rack. The current capacity is 12 platforms, **48 mice/day**, **~12,000 trials/day**.

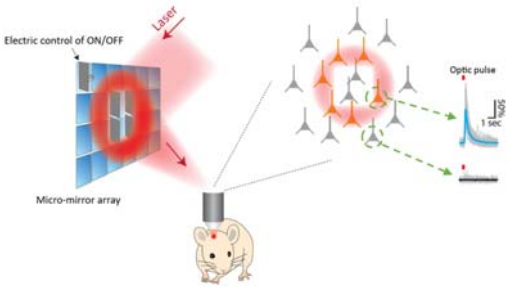
We achieved this high-throughput capacity earlier than expected, allowing us to readily test the ability of mice to perform in complex cognitive tasks. In the Figure below we designed a task to probe the ability of mice to use a *perceptual concept* in a two-alterative forced choice orientation discrimination task. Briefly, two oriented stimuli were shown on a screen in front of the head-fixed animal. The mouse had to decide which of the two was "more" vertical, with neither of them being vertical. The mouse signaled his choice by moving the chosen stimulus to the middle of the screen. He did so by using the front paws to rotate a small wheel in real-time, close-loop with the stimuli. The hypothesis we tested was that to solve the task the animal had to form a perceptual concept of *verticality* and compare the non-vertical stimuli against this internal concept in order to make a correct comparison of the stimuli's verticality.



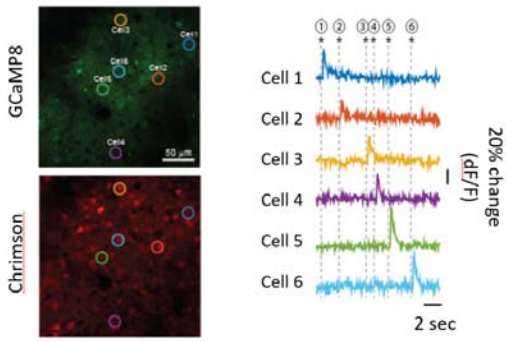
Not all mice learned the task, and for those who did it took an average of 3-4 months to become experts. We calculated that the training time to obtain an expert mouse when all our 12 setups were used for this task was just about two hours. This yield would be unachievable if animals were trained by one or two lab members. The first part of this work regarding the high-throughput setup has been submitted for publication. Instead, the demonstration of conceptual reasoning, together with simultaneous cortical recordings (GCaMP indicators) establish the mouse as the mammalian species with the smallest brain featuring this cognitive skills, and furthermore examine the underlying neural architectures. This work is currently being finalized.

(2) Interrogation of neural circuits with simultaneous recording and optogenetic stimulation

In this project we aimed to understand the neural computations underlying visual processing and vision-based decision making by using perturbative optogenetic methods in combination with large-scale dynamical network models of the cortical dynamics. Specifically, we examined solutions to important technical challenges related to in-vivo recordings from a large population of cortical neurons while simultaneously performing optogenetic perturbations at near single-cell resolution. In the figure below we show the successful use of a digital-micromirror device in combination with a two-photon microscope for in-vivo recordings of fluorescence signal (GCaMP) together with patterned optogenetic perturbations. The figure shows an array of mirrors (the DMD) controlled by an electric field which projects an illumination pattern (a red ring in this example) onto a cartoon representation of a cortical network.



As depicted in the figure above an optic pulse can produce optogenetic excitation in cells illuminated by the ring pattern, but not outside the ring. The use of a red-shifted variant of channelrhodopsin allows to obtain spectral separability relative to the GCaMP indicator in order to avoid cross-over excitation. In the figure below we demonstrate the use of the DMD with spatial-temporal light patterns where a small disk of light, large enough to excite the soma of only one cell, is flashed across the cortex in a random temporal sequence. Neighboring cells, as close as 30-40um (e.g. cell 5 and 6), can be individually excited without spatial-excitation overlap.



With this technology we are now addressing a fundamental scientific questions: how do neuronal networks learn and adapt in order for an organism to thrive in a constantly changing environment? To address this problem we are

using the DMD technology to study the plasticity of in-vivo, adult cortical networks. We are using plasticity rules akin to Hebbian plasticity to induce changes in the connectivity of these networks in a functional specific way, and at near single-cell spatial resolution. We are particularly interested in studying the spatial extent of the circuits and the neural machinery needed to engage such plasticity. Findings in this field will have profound impact not only on our understanding of how neural networks learn, but also on how they can recover computational power when damaged or altered because of traumas, injuries, or degenerative diseases.

(3) Alteration of a sensory percept via direct perturbations of the cortical dynamics

Finally, we are now combining results from aim 1 and 2 to investigate the fundamental computations of cortical processing for the emergence of a sensory percept. We do so using a causal approach based on DMD optogentic excitation of large neuronal populations in the visual cortex of mice at single-cell resolution. We aim to induce a functionally-targeted perturbation as the animal is engaged in an orientation discrimination task. We do so by optogenetically exciting cells with the DMD based on the cells' tuning for stimulus orientations. Then, we use psychophysical quantifications to evaluate the specificity and the efficacy of the optogenetic stimulation for the perturbation of the visual percept. We are currently collecting preliminary data and we are confident significant results will soon be available.

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 1 件）

Pisauro, M.A., Benucci, A., Carandini, M.、Local and global contributions to hemodynamic activity in mouse cortex、J Neurophysiol、査読有、115（6）巻、2016、2931-6
DOI：10.1152/jn.00125.2016

〔学会発表〕（計 11 件）

①Andrea Benucci、Plastic changes of neuronal network dynamics in the visual cortex of adult mice、Invited talk、17 Mar. 2017、Normal University, Beijing, China

②Tadashi Tsubota、Sculpting the dynamics of neuronal networks in the mouse cortex with optogenetic tools、Poster presentation、12 Nov. 2016、San Diego Convention Center, San Diego, US

③Ryo Aoki、Invariant and abstract perceptual representations in mouse decision-making、Poster presentation、12 Nov. 2016、San Diego Convention Center, San Diego, US

④ Andrea Benucci 、Evidence of Perceptual concepts in mouse decision-making、invited talk、28 Dep. 2016、SNU&KSBNS, Seoul, Korea

⑤ Andrea Benucci 、Linking neural-circuit dynamics to computation 、Japan Neuroscience Society Annual Meeting、21 Jul. 2016 、Yokohama Conference Center, Yokohama, JP

⑥Andrea Benucci、Perceptual decision-making in mice、Invited Talk、29 Apr. 2016、NYU Shanghai, Shanghai, China

⑦Ryo Aoki、Fully automated training fixed mice、Annual Meeting of Society for Neuroscience 2015、21 Oct. 2015、Chicago, USA

⑧Andrea Benucci、Studying perceptual learning in mice with a fully-automated training system for voluntary head fixation、Sanford University School of Medicine (invited)、17 Sep. 2015 、Sanford University School of Medicine, USA

⑨Andrea Benucci、Adaptation in the visual cortex equalizes population responses、Japan Neuroscience Society Annual Meeting、29 Jul. 2015、Kobe international conference center, Kobe, JP

⑩Andrea Benucci、Adaptation in the visual cortex equalizes population responses 、Invited talk、22 Jan. 2015、Weizmann Institute of Science

⑪ Andrea Benucci 、Neural Correlates of Perceptual decision-making in the mouse visual cortex、Invited talk、01/08/2015、UCL Institute of Ophthalmology, UCL London, UK

〔図書〕（計 0 件）

〔産業財産権〕

○出願状況（計 1 件）

名称 : Fully-automated, high-throuput system for voluntary had fixation in mice
発明者：Andrea Benucci
権利者：Andrea benucci
種類：特許
番号：JP2016-129406
出願年月日：June 29 2016

国内外の別： 国内

○取得状況（計 0 件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織
(1)研究代表者
ベヌッチ　アンドレア（Benucci Andrea）
国立研究開発法人理化学研究所・脳科学総合研究センター・チームリーダー
研究者番号：50722352

(2)研究分担者
（　　）

研究者番号：

(3)連携研究者
（　　）

研究者番号：

(4)研究協力者
坪田　匡史（Tadashi Tsubota）
青木　亮（Ryo Aoki）