名古屋大学
NAGOYA UNIVERSITY

August 12, 2020

A screening method to identify factors regulating neurons important in stress response has been developed

Prof. Akihiro Yamanaka and his colleagues have developed a screening method to identify factors regulating neurons involved in stress response (corticotropin-releasing factor (CRF)-producing neurons in the paraventricular nucleus of the hypothalamus, or PVN-CRF neurons) in collaboration with Prof. Keiichi Itoi of Tohoku University. They revealed that 15 bioactive substances actually regulated the activity of PVN-CRF neurons.

When stressed, the body defends itself and tries to maintain the homeostasis through various physiological functions called stress response. PVN-CRF neurons are responsible for initiating the stress response by being activated by stressors. It has been technically difficult to examine all of the potential factors that regulate the activity of the PVN-CRF neurons.

In this study, we introduced a method to measure several tens of neuronal activities at once. We developed a screening method to examine the effect of each candidate factor on PVN-CRF neuronal activity. In this study, we examined 63 bioactive substances as candidate factors. As a result, we confirmed that PVN-CRF neurons were activated by 12 substances and inhibited by 3 substances.

The newly developed screening method is applicable not only to PVN-CRF neurons but also to all types of neurons. It is expected that this screening method will be used to elucidate the factors that regulate various types of neuronal activity.

The study has been published in Scientific Reports on August 12, 2020.

This research was carried out with support from the Japan Society for the Promotion of Science.

【Points】

- Development of a screening method to identify factors regulating the activity of neurons important in stress response (PVN-CRF neurons)
- Fifteen bioactive substances confirmed to regulate the activity of PVN-CRF neurons
- Three bioactive substances (Cholecystokinin (CCK8S, CCK4) and tyramine) have been newly found to activate PVN-CRF neurons
- Three bioactive substances (Angiotensin II, histamine and carbachol) were found to strongly activate some PVN-CRF neurons

【Background and result】

When stressed, the body defends itself and tries to maintain the homeostasis through various physiological functions called stress response. PVN-CRF neurons (Glossary 1) are responsible for initiating the stress response by being activated by stressors. Therefore, identifying factors that regulate the activity of PVN-CRF neurons is important in understanding the initiation of a stress response. However, it has been technically difficult to examine all of the potential factors that regulate the activity of the PVN-CRF neurons.

In this study, we used a method called "calcium imaging" (Glossary 2), which can measure several tens of neuronal activities at once. This method visualizes calcium ion (Ca^{2+}) concentration, which is one of the indicators of neural activity. Yellow cameleon-Nano50 (YC) (Glossary 3), a fluorescent calcium-indicating protein, was used for visualization. We produced mice that express YC only in the PVN-CRF neurons, by injecting adeno-associated virus vector (AAV) (Glossary 5) into the paraventricular nucleus of the hpoyhalamus (PVN) of transgenic mouse (*CRF-iCre* mouse, created by Prof. Keiichi Itoi of Tohoku University. Glossary 4) (**Figure 1**).

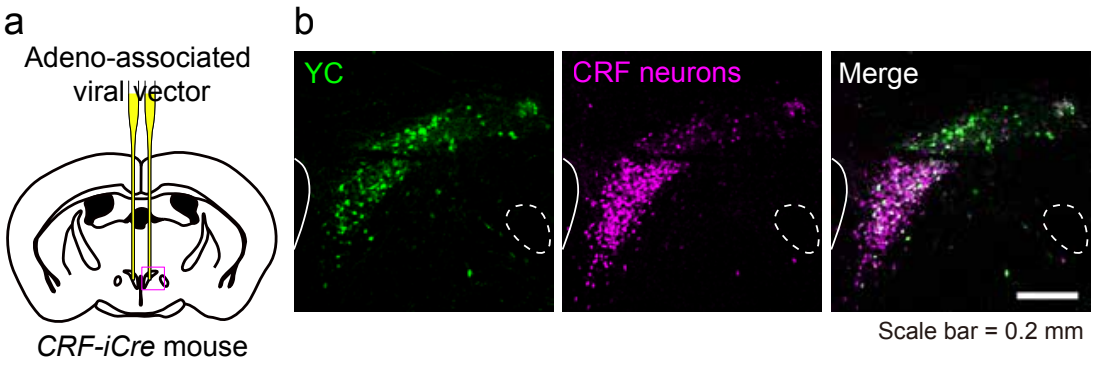

Figure 1. (a) Schematic diagram of the AAV injection site (coronal section of the mouse brain). (b) Expression of YC in the PVN-CRF neurons.

In the experiment, the brains were removed and sliced. Ca^{2+} signals visualized by YC were recorded under a microscope. A total of 63 bioactive substances (6 amine, 3 amino acids, 1 choline, 2 lipids, 2 nucleic acids and 49 peptides) were applied as candidate factors involved in regulation of the activity. Changes in Ca^{2+} signals due to each substance were measured (**Figure 2**).

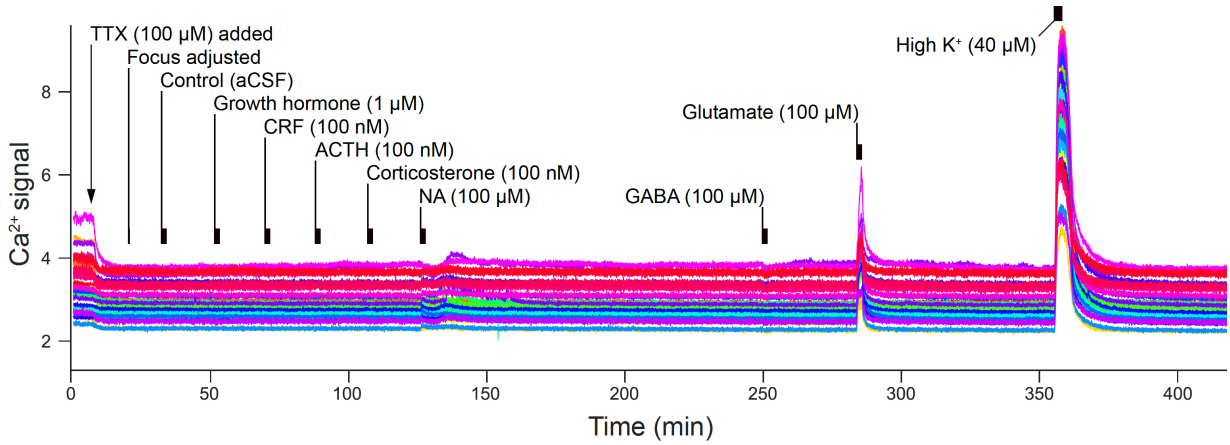

Figure 2. Representative Ca^{2+} signal changes in screening experiments

Each colored graph represents a single neuronal signal. At the timing of the black bar (■), the candidate substance was applied for 2 minutes each.

As a result of the screening, we confirmed that PVN-CRF neurons are activated by 12 substances and suppressed by 3 substances (**Figure 3**).

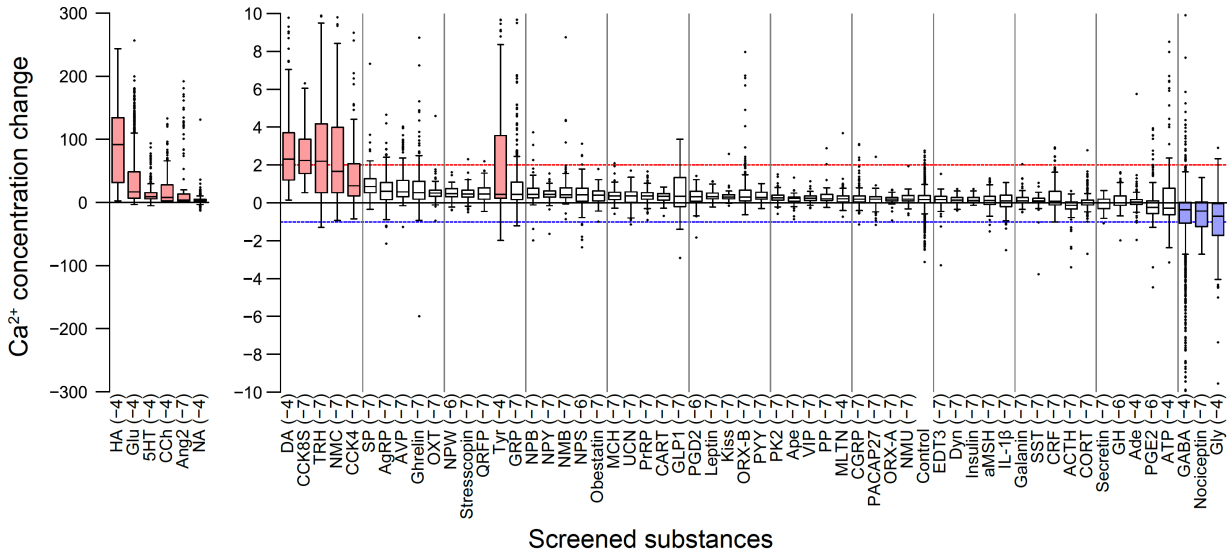

Figure 3. Results of Screening Experiments

Box plots show the amount of change in Ca^{2+} concentration in cells (rise and fall) when each screening agent is applied. The substances filled with red or blue are activated or suppressed the activity of PVN-CRF neurons, respectively.

Three substances (Cholecystokinin (CCK8S, CCK4) and tyramine) were novel, which had not been reported (**Figure 4**).

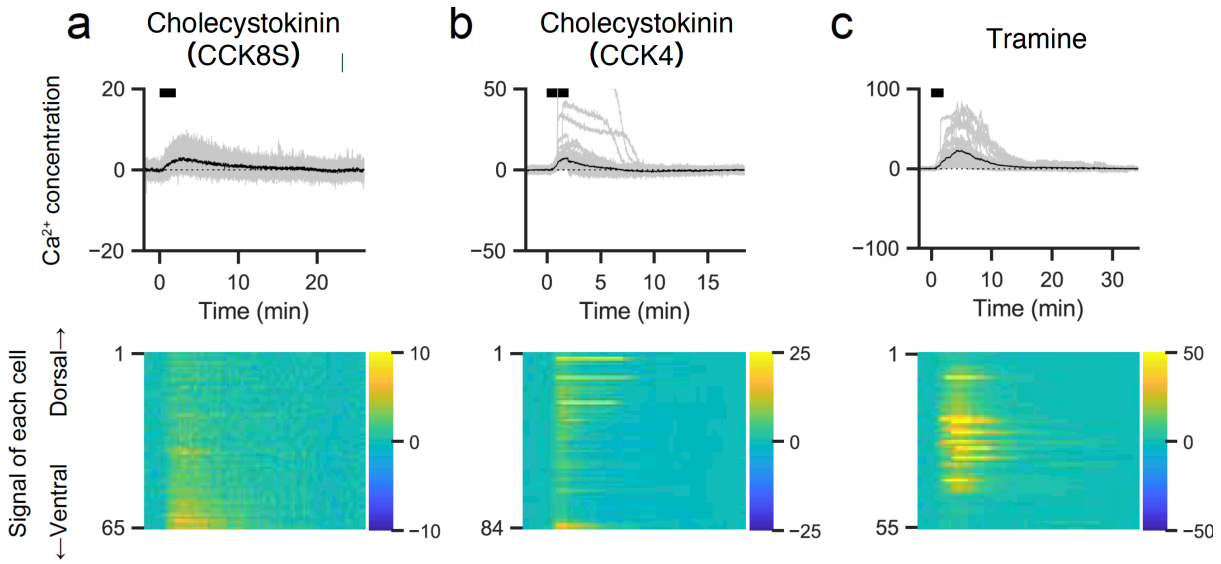

Figure 4. Representative Ca^{2+} signal changes induced by novel 3 substances

The substance was applied for 2 minutes at the timing of the black bar (■) above the graph. The bottom graph shows the signals per cell in the colors shown on the right side of the graph.

In addition, three other substances (Angiotensin II, histamine and carbachol) showed different levels of activation between the dorsal and ventral portions of PVN-CRF neurons (**Figure 5**).

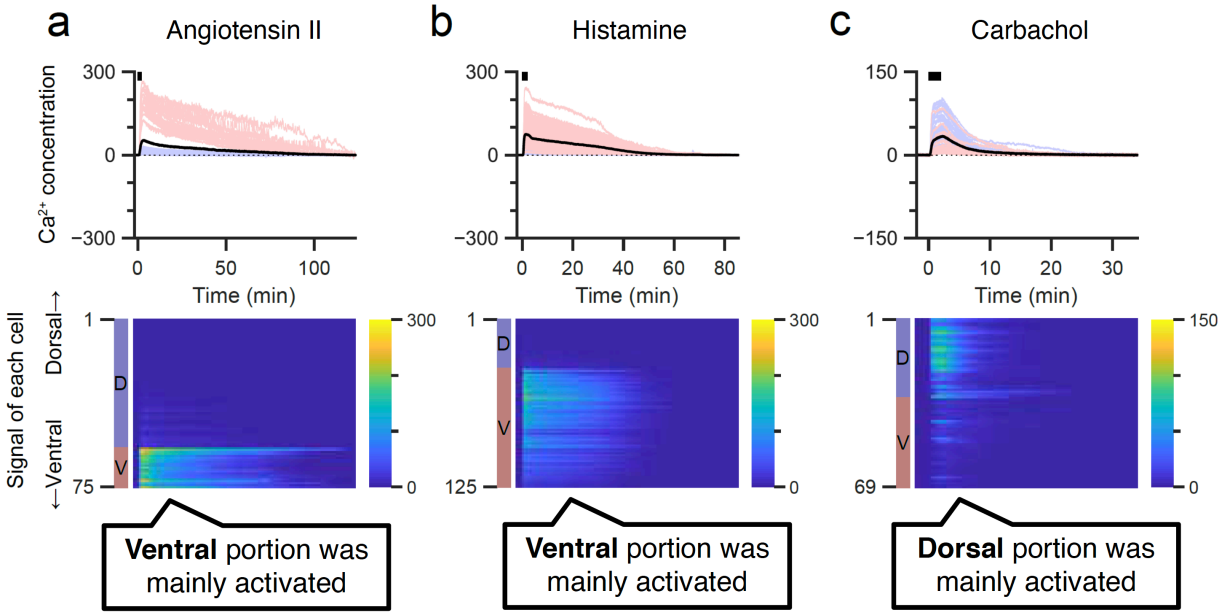

Figure 5. Different activation levels between the dorsal and ventral portions

The substance was applied for 2 minutes at the timing of the black bar (■) above the graph. The bottom graph shows the signals per cell in the colors shown on the right side of the graph. The top is dorsal and the bottom is ventral. Angiotensin II (a) and histamine (b) activated mainly the ventral portion, whereas carbachol (c) activated mainly the dorsal portion.

【Significance of the results】

This study established a precise and reliable screening method to assess the effect of factors regulating the activity of PVN-CRF neurons. The screening showed that 15 substances regulate the activity of PVN-CRF neurons. These results are expected to provide clues to elucidate the physiological functions of each substance. In the future, it is expected that this will lead to the elucidation of the mechanism of stress response in living organisms and the development of new drugs and treatments.

The newly developed screening method can be applied not only to PVN-CRF neurons, but also to all types of neurons. It is expected that this screening method will be used to elucidate the factors that regulate various types of neuronal activity.

【Glossary】

1. **PVN-CRF neurons** (Return to the main text)
   Neurons that produce corticotropin-releasing factor (CRF) in the hypothalamic paraventricular nucleus (PVN). When the body is stressed, the PVN-CRF neurons release CRF into a blood vessel called the pituitary portal vein. CRF then acts on cells in the anterior pituitary gland that produce adrenocorticotropic hormone (ACTH) to stimulate the synthesis and release of ACTH, which travels through the bloodstream to the adrenal cortex. ACTH then stimulates the synthesis and secretion of glucocorticoids in the adrenal cortex, which in turn trigger a stress response. The hypothalamic (CRF) → pituitary (ACTH) → adrenal (glucocorticoid) axis, called the hypothalamic-pituitary-adrenal (HPA) axis, is known to be important in stress responses.
2. **Calcium imaging** (Return to the main text)
   A method to visualize the concentration of calcium ions (Ca^{2+}) in cells using a substance (compounds or calcium-indicator proteins) whose brightness changes with the concentration of Ca^{2+}. Generally, when a neuron is activated or suppressed, the concentration of Ca^{2+} in the neuron increases or decreases, respectively.
3. **Yellow cameleon-Nano50（YC）** (Return to the main text)
   One of calcium-indicator proteins used in calcium imaging. YC contains yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) domains connected by a protein domain called calmodulin (CaM), which changes its conformation when bound to Ca^{2+} (Figure 6). Excitation of YC by blue light results in emission of cyan and yellow fluorescence. When Ca^{2+} binds to the CaM domain, the structure changes, and the intensity of YFP and CFP fluorescence changes. Ca^{2+} concentration is measured by the ratio of the intensity of fluorescence of two colors.

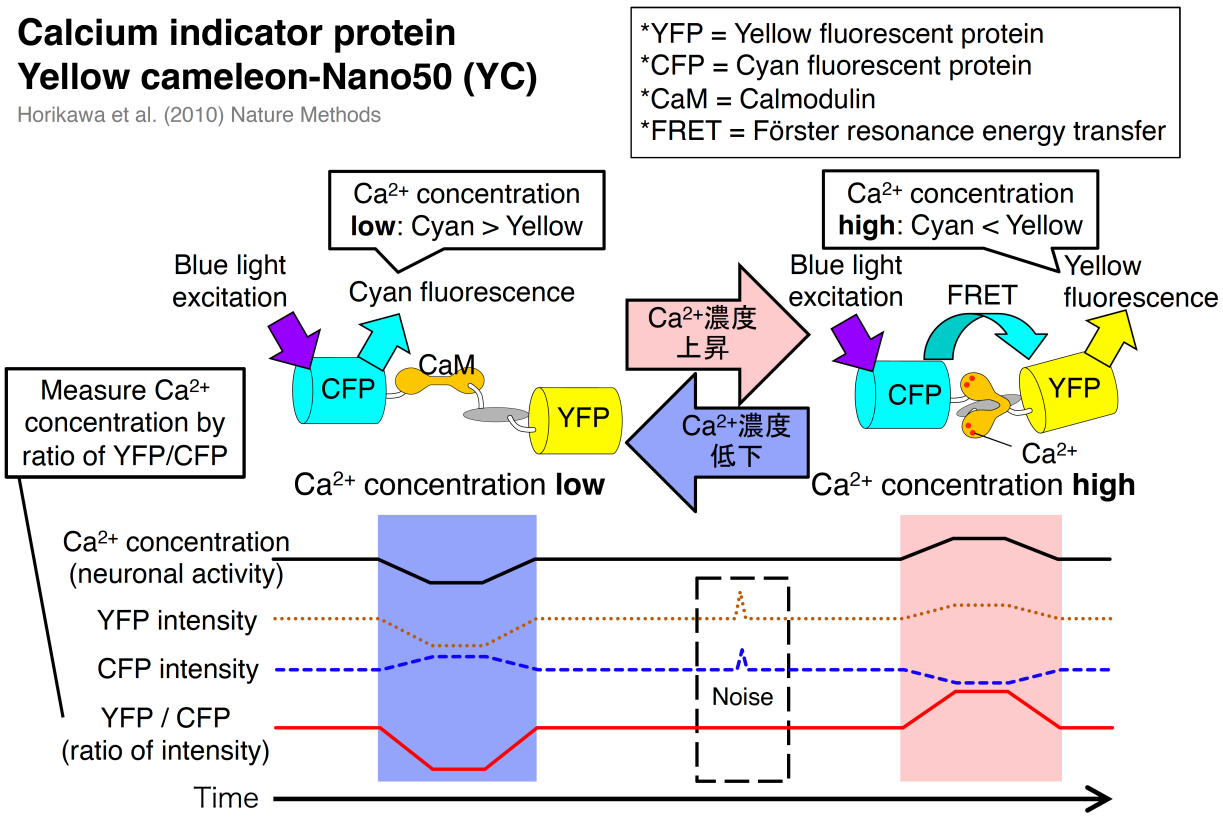

Figure 6. Schematic diagram of Yellow cameleon-Nano50 (YC)

4. CRF-iCre mouse (Return to the main text)

A mouse strain genetically modified to make a protein called "iCre" in the neurons that produce CRF (CRF neurons). The "iCre" is a type of protein called recombinant enzyme that recognizes a specific sequence of DNA and can replace two DNA sequences. This property can be used in combination with adeno-associated viral vectors (Glossary 5) to express a gene of interest exclusively in CRF neurons.

5. Adeno-associated viral vector（AAV） (Return to the main text)

A nonpathogenic virus used to artificially express a gene of interest. When the virus carries the gene of interest and infects cells, the infected cells can express the gene of interest. In this study, YC genes were introduced into infected cells to make YC proteins. YC can only be produced if the DNA sequence is altered by the recombinant enzyme iCre (Glossary 4). Therefore, in this study, we were able to produce YC exclusively in CRF neurons.

【Information of the study】

Journal: Scientific reports
Title: Identification of substances which regulate activity of corticotropin-releasing factor-producing neurons in the paraventricular nucleus of the hypothalamus
Authors: Yasutaka Mukai, Ayako Nagayama, Keiichi Itoi & Akihiro Yamanaka
DOI: 10.1038/s41598-020-70481-5

【Contact】

Research Institute of Environmental Medicine, Nagoya University
Nagoya University Graduate School of Medicine
Professor, Akihiro Yamanaka
TEL：+81-52-789-3864　　FAX：+81-52-789-3889
E-mail: yamank@riem.nagoya-u.ac.jp