Type III neurons' relationship with type II neurons (GABAergic neurons)

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ABSTRACT

Dopamine is the neurotransmitter known as a hormone. As it communicates messages between nerve cells, it plays an essential role in the reward prediction error (RPE), which measures the discrepancy between reality and expectation. In the past experiments conducted by Cohen et al., the researchers developed a system of three types of neurons that play significant roles in motivation and reward. The three neuron types are neuron type I, neuron type II, and neuron type III. Though type I and II neurons are known as Dopaminergic neurons and GABAergic neurons, we still do not know the identity of neuron type III. Therefore, in this proposal, I aim to focus on type III neurons and reveal their identity. I developed two aims, caveats, and contingencies for the research plan in this proposal. In the first aim, I identified the type III neurons by recording the firing rate of all neurons and identifying the transcriptomes of the other two types of neurons. Then, I identified the specific neurotransmitter and promoter to type III neurons. In the second aim, I investigated the function of type III neurons within the VTA by using the CRISPR engineering technique. The result of this proposal might contribute to the field of neuroscience as it reveals more information about type III neurons. By knowing this information, more researchers can conduct further experiments on these three types of neurons.

Keywords: type III neurons, type II neurons (GABAergic neurons), ventral tegmental area (VTA), reward prediction error (RPE), channelrhodopsin-2 (ChR2)

1. INTRODUCTION

1.1 Background

Neurons (Figure 1), also known as neuronal cells, are the most fundamental structural and functional units of the nervous system. It is divided into two parts: cell body and protrusion. The cell body is composed of the nucleus, cell membrane, and cytoplasm and connects and integrates input information and transmitting information. Dopamine plays a significant role in reward and punishment. Dopaminergic neurons, the neurotransmitter dopamine in the ventral tegmental area (VTA), indicate the difference between expected and received rewards, which is known as reward prediction errors (RPE). RPE plays a vital role in fundamental mechanisms of reward-based learning and motivates individuals to pursue further rewards, hence conferring an advantageous characteristic from an evolutionary standpoint. Rewards facilitate the process of acquiring knowledge and skills. One of the classical experiments supporting this concept involves Pavlov's dog. However, how dopaminergic neurons compute such signals remains unexplored. In a study by Jeremiah Y Cohen et al., researchers investigated the firing rate of neurons in the VTA and established neuron-type-specific signals for reward and punishment (2012). Specifically, they used punishments such as an air puff and rewards such as water on mice. The researchers labeled dopaminergic and GABAergic neurons in mice with the light-sensitive protein channelrhodopsin-2(ChR2). Subsequently, dopaminergic and GABAergic neurons were distinguished by their respective reactions to optical stimulation throughout the recording process. As a result, the researchers categorized three types of neurons with distinct activity. They named the neurons type I (Dopaminergic), type II neurons (GABAergic), and type III neurons. Furthermore, results indicated an inverse relationship between the firing activity of type II neurons and type I neurons during experiments on punishments and rewards. Like type II neurons, type III neurons are sensitive to conditioning stimuli (CS) and display persistent firing activity during the delay between stimulus and outcome. However, type III neurons were reactive towards punishments, whereas type II neurons were reactive towards rewards.

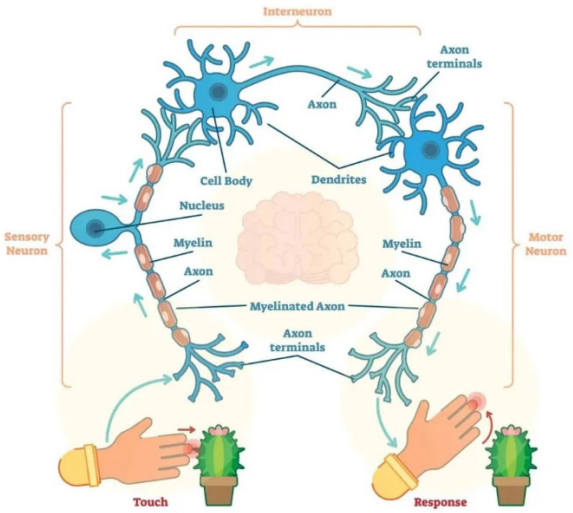

Figure 1 Common types of neurons

1.2 Rationale

Even though Cohen et al. discovered the neurotransmitters of type I and type II neurons, there remains little information regarding type III neurons in the process of RPE. We aim to investigate type III neurons and their relationship with type II neurons (GABAergic). We will explore the type III neurons and uncover their neurotransmitter, function, and mechanism of action within the VTA. Type III neurons may be able to inhibit or activate, or they have no potential relationship with Type II neurons. The results of our experiment will help clarify the biological and physiological role of type III neurons in RPE.

1.3 Significance of the study

The results of this study will help readers access further information about type III neurons, such as their neurotransmitters, their role within the VTA, and their impact on type II neurons (GABAergic). From a big-picture perspective, researchers can update their understanding of the three types of neurons and develop more studies on the topic of RPE, Parkinson's disease, blood pressure, and brain functions. Dopaminergic neurons are also closely associated with Parkinson's disease, which is one of the most common neurological disorders of human beings (Chinta et al., 2004). GABAergic neurons are important to many physiological aspects, such as blood pressure and brain function. The intricate network of neural connections, which are tightly connected to brain maturation, plays a crucial role in creating memories and integrating new knowledge with prior learning. Learning has been a topic of heated debate and fascination in neuroscience. The fundamental relationship between RPE and learning promises that the more we discover about RPE, the farther we sail on the ocean of learning.

2. RESEARCH PLAN

2.1 Aim 1: To identify the neurotransmitter of Type III neurons.

To identify the type III neurons, we will record the activity of all neurons in the VTA of twenty laboratory mice (10 male mice and ten female mice) and confirm the presence and consistent activity of all three kinds of neurons. We used ten male mice and ten female mice so that the experiment had a proper sample size and was not influenced by the sexuality of the mice. In addition, the mice were housed on a 12-h dark/12-h light cycle (dark from 06:00 to 18:00). Each mouse participated in the experiment at the same time of the day, between 08:00 and 18:00. By mimicking the natural day and night, we reduce the environmental factors that might influence the result. We will isolate the VTA so that the DNA of all neurons is accessible. Then, we will extract and sequence the DNA of all neurons within the VTA. We will identify the transcriptome of type I, type II, and type III neurons by cross-checking with publicly available datasets. We can confirm that we extracted the type III neurons by using media databases on websites such as press pages. This exploratory investigation may help us identify neurotransmitters and promoters specific to neurotransmitters of type III neurons.

2.2 Caveats and Contingencies:

We may be unable to find neurotransmitters of type III neurons due to the biological nature of neurons. However, we may be able to distinguish type III neurons from other types of neurons regarding cofactors or co-secreted enzymes. Cofactors refer to non-protein chemical substances that facilitate an enzyme's function as a catalyst.

It is also possible that type III neurons also secrete dopaminergic or GABAergic neurotransmitters similar to type I and type II neurons, but they might secrete a different type of these enzymes or a different splice variant. For example, it might secrete a different variant of dopaminergic neurotransmitters.

Last, we may find more than one relevant neurotransmitter. In this situation, we will pick and choose one of the neurotransmitters and conduct experiments.

2.3 Aim 2: To identify the functional role of Type III neurons within VTA

To generate mice with type III neurons that are reactive to blue light stimulation, we will apply CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

engineering to knock in the Chr2 genes under Type III neurotransmitter-specific promoters. We use the CRISPR engineering technique because it can help us selectively modify the DNA of living organisms, especially human DNA. After that, we will utilize blue light pulses to trigger those mice's Type III neurons, which express the ChR2 genes, and record the firing rate of all three different types of neurons. Following the activation of the Type III neurons in the mice, we will compare the responses of all three neurons to the blue light pulses and the patterns of spontaneous spikes. In our expectation, type III neurons may activate type II neurons if the spontaneous spikes are similar to those induced by blue light pulses. Because of the graph constructed in the paper by Cohen et al., we are led to believe that Type III neurons have a more intimate connection with Type II neurons than with Type I neurons. Thus, we can conclude that there is a potential relationship between type III neurons and type II neurons.

2.4 Caveats and Contingencies:

If activating Type III neurons in the brain of mice causes an apparent rise in the line of Type II neurons in the graph, then we can presume that Type III neurons are involved in activating Type II neurons. On the other hand, if Type II neurons present decreasing activity when we stimulate the Type III neurons, we can assume that the Type III neurons inhibit the firing rate of the Type II neurons. Last but not least, if the graphs show almost no consistency between the firing rates of type II and type III neurons, this may suggest that there is almost no connection between the two types of neurons.

The drawback of CRISPR includes drawbacks such as a lack of on-target editing efficiency. If CRISPR does not effectively target type III neurons, we can use Adenovirus with the ChR2 gene as a backup plan. Adenovirus is a group of viruses that cause infections such as cold-like symptoms.

3. CONCLUSION

This research proposal aims to reveal the role of type III neurons within the VTA. Through two experiments, a deeper understanding of style III neurons' neurotransmitters and their relationship with type II neurons will be explored. The research results might aid future studies not only on the circuitry of RPE but also on more broad topics such as brain functions and specific neurological disorders such as Parkinson's disease.

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