The Complex Role of Neuromodulatory Sensors in the Social Transfer of Pain

By Maggie Hou

Abstract:

The social transfer of pain is recognized as feeling others' pain as your own, often emotionally. While commonly known as empathy, this paper explores the scientific implications behind this universal idea. Through a series of experiments featuring laboratory mice, due to their pure genetic background in a controlled environment, scientists and researchers can gain a more complete knowledge of exactly how and why the social transfer of pain functions as it does in the brain. My ultimate goal for this project was to help deepen this journey and contribute to the findings of monumental research. I hypothesized that specific neural pathways involving neuromodulatory inputs—particularly the signaling molecule vasopressin—were responsible for activating the social transfer of pain. To test this hypothesis, I used engineered fluorescent proteins and Adeno-Associated Viruses (AAV) to map and trace vasopressin activity in neurons. Fluorescence microscopy enabled the observation of active regions, such as the PVT, where neuronal activity was quantified. Additionally, tracing experiments confirmed a connection between the suprachiasmatic nucleus (SCN), a key source of vasopressin, and the PVT, highlighting important inter-regional communication. Ultimately, the social transfer of pain, while an emotional experience, is governed by intricate molecular mechanisms that can transform the understanding of cognitive and emotional regulation. The goal of this research is to contribute to solving larger challenges by providing insight into how disruptions in neuron communication might help to broaden knowledge on neurodevelopmental disorders like autism and schizophrenia.

Introduction

Across cultures, empathy has been described as feeling the troubles of others as your own. However, as research methods develop along with societal advancements, scientists have strived for a more scientific approach towards this holistic virtue. The project focuses on the neural basis of the social transfer of pain. The social transfer of pain encapsulates the idea that witnessing the suffering of other individuals results in the pain of oneself (Langford et al. 2006, Smith et al. 2021). For example, seeing or hearing other people in pain transfers the sensation of pain to the observer. As a high school student, dealing with empathy and the social transfer of pain is a part of everyday life. In turn, the question of how exactly the process works arose. With over 170 billion cells making up a singular human brain (National Institutes of Health), the question developed into which cells in which regions of the brain contributed to the social transfer of pain.

Looking through Google Scholar's field of neuroscience, and considering different factors such as proximity and research, I found Professor Xiaoke Chen's lab at Stanford University. Professor Chen's research focuses on the negative consequences of social interactions which are indirectly linked to the social transfer of pain as well (Zhu et al., Nature, 2016, Keyes et al Neuron 2020, Yang et al Nature 2023). To understand which part of the brain is important in these social behaviors, the Chen lab uses brain activity mapping by measuring the expression of a gene called c-fos, which is a gene that is selectively expressed in highly activated neurons. Through mapping, the paraventricular thalamus (PVT) stands out as a highly activated region, in agreement with the previous understanding that PVT is important for higher-order emotional and cognitive functions (Zhu et al, Science 2019 Keyes et al Neuron2020). The PVT region is in the middle of the brain, and is often implicated in psychiatric disorders such as addiction. PVT

receives a large amount of inputs from other brain regions that regulate its function. Among these inputs, many are neuromodulatory inputs, which are signaling molecules like dopamine, serotonin, and vasopressin. These molecules are sent from one neuron to another and modify the function and activity of the receiver neuron. One neuromodulator, vasopressin, is known to be important for social behaviors based on previous studies surrounding humans. However, we have a limited understanding of vasopressin's role in other aspects of behaviors such as pain and which brain regions it acts upon. Therefore, I hypothesized that neuromodulatory sensors play a pivotal role in activating the social transfer of pain. Work done in the Chen lab shows that vasopressin is important in the social transfer of pain paradigm and a potential region that vasopressin acts on is the PVT. Therefore, it is vital to monitor the effect of vasopressin on PVT neurons, leading to my research question.

Results:

During my time at the Chen lab, I prepared two sensors for vasopressin activities. One sensor is an engineered fluorescent protein that fluoresces upon binding with vasopressin. Once these sensors are expressed in the PVT neurons, we could monitor the changes in fluorescent intensity as a read-out for vasopressin activity and release into the PVT. To prepare the sensors, I cloned the vasopressin reporter gene into a plasmid that targets neurons. The DNA sequence of the vasopressin reported is fused with the plasmid backbone through DNA ligase, and then the engineered plasmid is transfected into the bacteria, *E. coli*, for amplification. After being amplified, the plasmids are extracted and purified. Later, these plasmids were made into adeno-associated viruses (AAV) (**Figure 1**), which delivered the gene into specific types of neurons in the mouse brain (**Figure 2**). The other sensor is also an engineered protein to label cells that receive vasopressin input. The same process is then repeated with the other sensor.

Unlike the first sensor, the second sensor isn't fluorescent. Upon binding with vasopressin, it will trigger the gene expression of another fluorescent molecule in the cell, therefore making the cell that receives vasopressin input fluoresce as red (**Figure 2**). Using these prepared plasmids, they were later made into viruses and injected into the mouse brain. Two weeks later, the brains were dissected so the neurons could be identified for those who had received vasopressin (**Figure 2)**. As a result, it is possible to study the effects of PVT in vivo, furthering the project.

In addition to working with these two sensors, I was also interested in a tracing experiment to map out downstream targets for the suprachiasmatic nucleus (SCN). SCN is a major source of vasopressin. It strongly connects with the PVT. To verify this connection, I injected viruses that expressed a fluorescent molecule into the SCN and waited for the red fluorescent molecule in the virus to express. The red fluorescent molecule is known as mScarlett. After mScarlett is expressed throughout the infected cells, it was easy to identify where SCN projected to (**Figures 3, 4, 5 and 6**). I helped with preparing the brain sections as well as staining the sections with a nuclear marker to distinguish what was a cell and what was not. Later on, we were able to examine the projection of neurons in the SCN. I was able to verify the projection from SCN to PVT, demonstrating the connection between the two.

Conclusion and Discussion:

These two projects are crucial for understanding the social transfer of pain because people don't have a deepened knowledge of the molecular mechanism behind empathetic behaviors. The discovery of fluorescent proteins revolutionized neuroscience research and was heavily applied to this experiment. This type of social behavior is based on the communication between neurons. The tools used to study this type of communication are through the pivotal fluorescent proteins and the virus-mediated delivery of the proteins. The question of how the

messages between the neurons communicate across different brain regions, such as the PVT and SCN are extremely important in understanding people who have defects in neuron-to-neuron communications, failing in social pain transfer. From this research, it is possible to further the journey to fully understand certain neurodivergent diseases, particularly relating to the connection between neuron communication and the social transfer of pain.

Figures

Figure 1: A diagram shows the AAV plasmid consisting of the Internal Terminal Repeat (ITR), hSyn promoter, and m-Scarlett (a fluorescent protein).

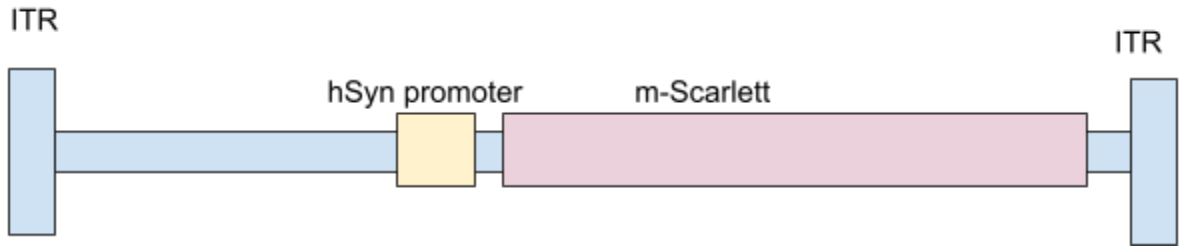


Figure 2: Experiment process showing preparing the plasmids, AAV production, AAV injection, harvesting of the mouse brain, sectioning the brain and observing under a fluorescence microscope.

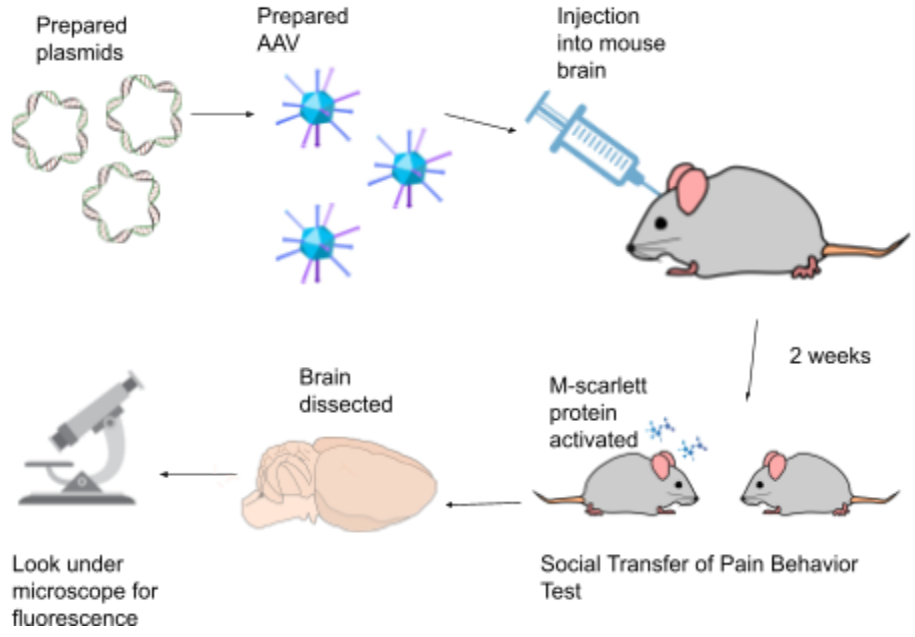


Figure 3: This figure depicts an SCN labeled cell. AAV m-Scarlett 3 were injected into the SCN. Two weeks later, The brain sections were perfused then dehydrated with 30% sucrose solution, then sectioned on a cryostat to 50 micrometer (μm) thick sections. They were then stained with DAPI prior to being imaged on a BioRad slide scanner to produce this image.

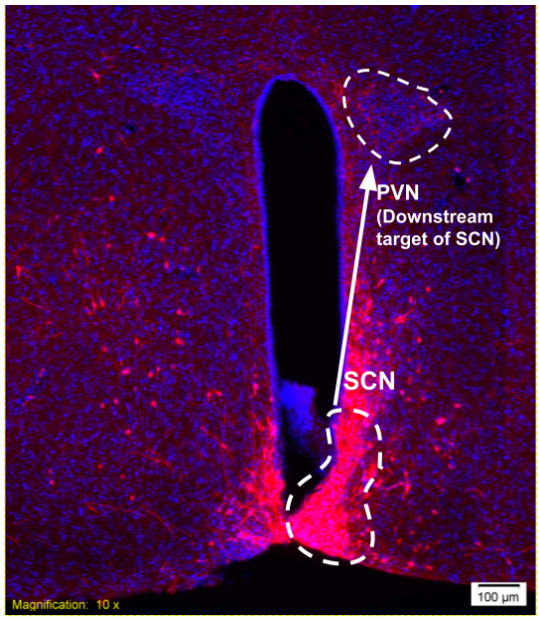


Figure 4: This figure depicts the SCN Projection Mapping of the red fluorescent molecule known as mScarlett that is a fluorophore expressed in the virus. The virus was injected with Scarlett to identify where SCN projected to. The brain was then sectioned and imaged to produce this image.

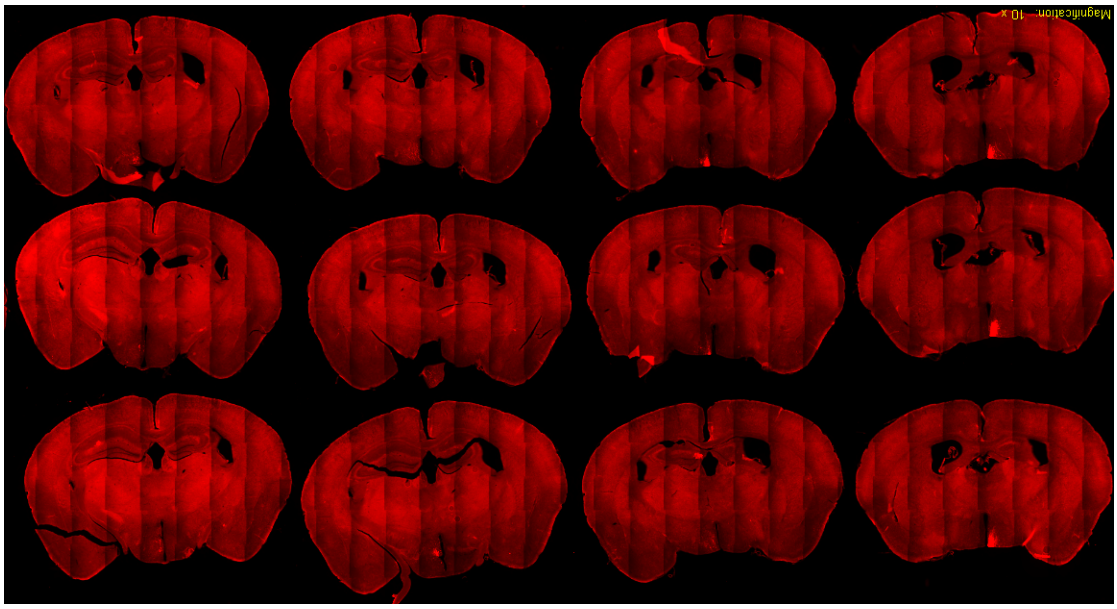


Figure 5: This figure depicts the SCN Projection Mapping of DAPI, a blue fluorescent dye that stains double-stranded DNA in cells.

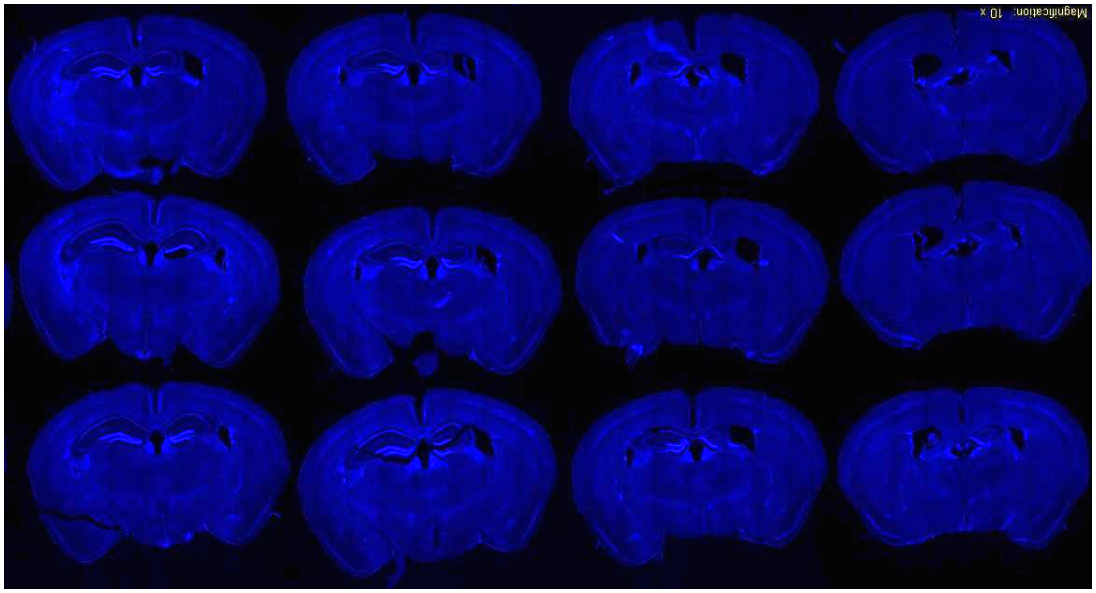
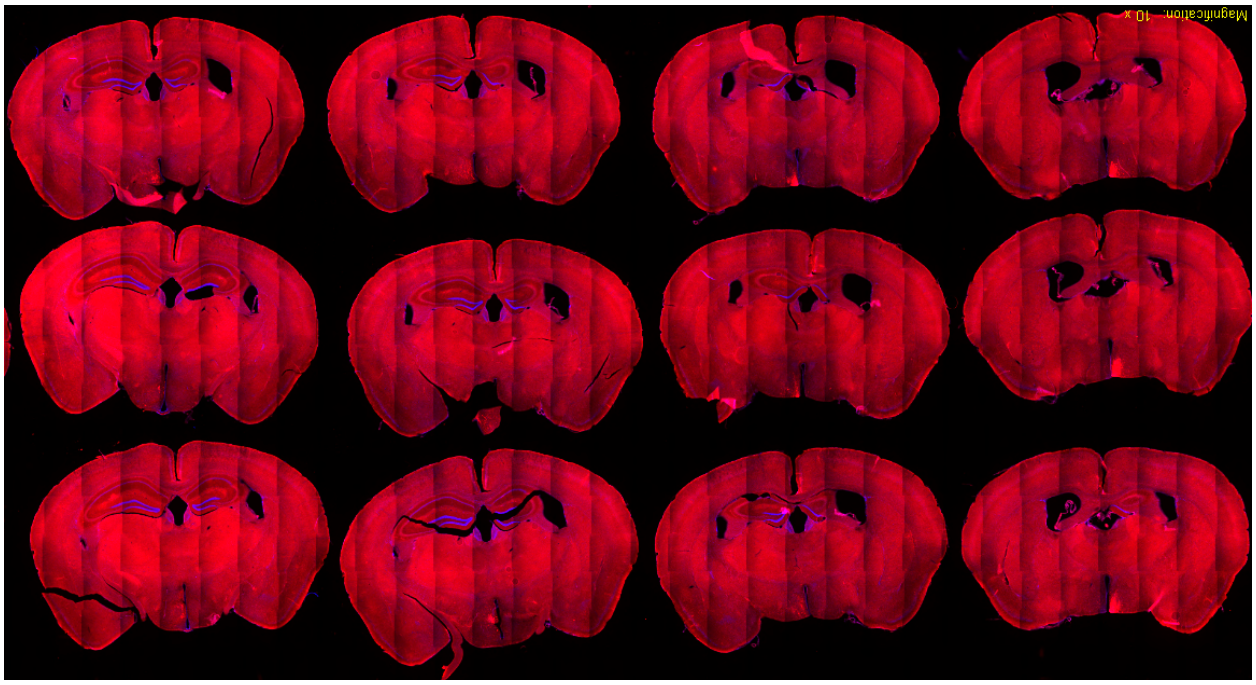


Figure 6: This figure depicts the overlapped channels of both the DAPI and m-Scarlett mapping.



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