IDENTIFYING THE NEURAL MECHANISMS OF APPROACH BEHAVIOR: STUDYING THE ROLE OF SUPERIOR COLLICULUS DURING PREY-CAPTURE BEHAVIOR IN THE MOUSE

by

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Cris Niell

In mammalian brains, there are two areas that process information important for image formation and goal directed visual behavior: primary visual cortex (V1), and the superior colliculus (SC). However, it is unclear how these regions support visually driven orienting and approach behaviors towards naturally rewarding stimuli. In this study, we seek to identify how the SC directs visual behavior using a mouse model of prey-capture behavior. Here, we investigate whether natural prey-capture behavior in mice is affected when regions of SC are silenced through injections of the $GABA_A$-R agonist, muscimol, and through the use of pharmacogenetics known as DREADDs. We found that inhibition of the SC decreases the accuracy of approaches to prey and increases time to capture. Our studies so far indicate that inhibition of SC impairs ethological prey-capture behavior in mice. An understanding of the specific circuitry underlying visually guided behaviors directed towards rewarding stimuli will give insight into neurological disorders such as PTSD and addiction, where processes of orienting and approach are affected.

Acknowledgements

I would like to thank Dr. Niell and Dr. Hoy for guiding and teaching me throughout this project so that I can gain a full understanding of the work done and also for giving me this opportunity to contribute to research. Along with them, I would like to thank Emily Galletta and Hannah Bishop for contributing a substantial amount to the project and the rest of the Niell lab for being an amazing group to work with. Of course, none of this could be done without the support of my friends and family, which I am grateful for. So, thank you for all the help and support!

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Introduction

While recognizing a puddle of water on a rainy day and avoiding it seems to come naturally to us, scientists still have much to learn about how our visual systems recognize such objects and automatically formulate the appropriate behavioral response. Our visual systems first receive information from the outside world in the form of photons of light that hit the retina. Our brains then encode and transform that information through a series of hierarchically organized neural circuits in order to guide behavior (Niell 2011). The first figure provides a general idea of the pathway visual information can take starting at the eye, through the lateral geniculate nucleus (LGN), and eventually to the primary visual cortex, also called the striate visual cortex (V1) (Rowe 2008). A second pathway is shown as well where retinal information is sent directly to superior colliculus (SC) for rapid motor responses (Morris, Ohman, and Dolan 1999). Since we do not fully understand how the brain processes visual information, we are currently unable to address disorders where people inappropriately respond to environmental stimuli with pathological behavior. For example, certain visual stimuli may trigger inappropriate generalized fear in people with PTSD (Steuwe et al. 2014) or may stimulate automatic and overpowering approach responses to cues that signal access to addictive drugs despite the understood negative consequences (Rapaka, Schnur, and Shurtleff 2008). In these cases, the brain sees a normal situation and tells you to do the wrong thing. These miscommunications between a stimulus and the brain could prove to be dangerous or debilitating. Therefore, understanding how the brain translates visual information and subsequently directs behavioral outputs would be a step forward in addressing neurological disorders.

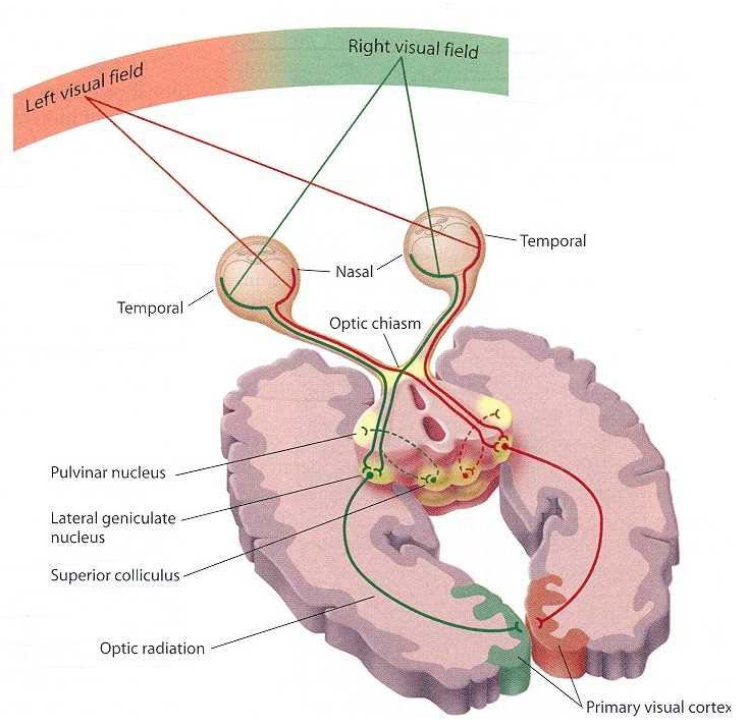

Figure 1: Bottom view of the brain highlighting the anatomy of visual pathways

The general flow of visual information through the brain is shown here. Photons of light first enter the retina. In higher order mammals such as primate, retinal information is sent mainly through the LGN to the V1. Information from V1 is next sent to higher order visual areas in cortex and elsewhere to ultimately yield appropriate behavioral/ motor responses. In addition, a subcortical pathway takes retinal information and sends it, in parallel, directly to the SC (Hannula, Simons, and Cohen 2005). This pathway drives rapid orienting to natural, highly salient visual information. Figure from Hannula, Simons, and Cohen (2005).

In order to analyze how the brain processes visual inputs to direct behavioral outputs, we must study a robust, visually driven behavior. We must also understand which specific visual features are responsible for triggering the consistent and quantifiable behavior so that we may localize the brain regions that link the stimulus to an appropriate behavior response. In addition, we will learn the most if we study this process in an animal model where we can trace how a specific stimulus is encoded and transformed through the brain at the neural level. Towards this end, we have chosen to

study visually driven prey-capture behavior in the common house mouse, *Mus musculus*, as our behavior to quantify and cricket prey as the visual input. The mouse model will also allow us to determine which regions of the brain allow it to express this behavior. We can ultimately pinpoint the specific sets of neurons and their connectivity that lead to the expression of an important behavior.

In general, prey-capture is an ethological behavior that serves as an effective model to study how the brain processes visual stimuli in order to drive appropriate behavioral responses towards naturally rewarding stimuli. When an animal demonstrates successful prey-capture behavior, its brain has to see the prey and be able to tell the body to pounce accurately and quickly. If this process is broken, the animal could react aversively to prey or miss an opportunity for a highly rewarding meal severely impacting its ability to survive and successfully reproduce. Thus, the basic ability to use visual systems to accurately detect, localize, identify, and physically react to prey items is highly conserved throughout the animal kingdom, which is why this behavior is studied in mice. In human terms, our most basic abilities to rapidly orient and respond to stimuli that naturally capture our attention may rely on the same neural mechanisms engaged during prey-capture in other animals such as mice. Therefore, a detailed understanding of this behavior in mice may reveal key insights into how our own brains are wired to produce important orienting behaviors.

Importantly, studying ethological, visually driven approach behavior such as prey-capture in the mouse is a novel area of study, because prior studies have focused almost exclusively on the neural circuitry of avoidance behaviors and fear responses in mice (Shang et al. 2015). Here, we study how mice hunt crickets (*Acheta domestica*),

because mice produce robust prey-capture behavior towards these insects within 12 hours of first exposure. In addition, the Niell lab has already shown that rapid detection and accurate localization of these prey are visually driven (Hoy et al. 2016). This finding provides a basis for more visually driven studies on prey-capture behavior.

Many past studies provide insightful evidence that suggest where in the brain to look for neural mechanisms that underlie successful prey-capture. The superior colliculus (SC), a subcortical region in the mammalian brain, mediates rapid visual orientation responses relevant to prey-capture behavior as well as defensive behaviors in many mammalian species (Westby et al. 1990). By understanding how this structure helps generate these behaviors and facilitates prey-capture, we can understand important fundamental aspects of visual processing during the behavior. In our studies, I worked with others in Cris Niell's lab to help create a more detailed understanding of how specific neural circuits in the brain produce the correct response according to distinct features of visual stimuli. Specifically, I studied how impairing regions of the SC changes prey-capture behavior in mice as they hunt crickets. Moreover, I have begun experiments to determine which specific cells types and circuits embedded within the superior colliculus are specially required for the approach behaviors we see during prey-capture. Together, our findings will contribute to a more detailed understanding of the brain mechanisms required to produce this important, visually guided approach behavior. Ultimately, this is the first step towards revealing novel neural-circuit level mechanisms underlying orienting and approach behavior that can only as of yet be uncovered in the mouse.

Background

Model of Research

The important question of how the brain produces the correct response to a visual stimulus remains unanswered because neuroscientists have not had the proper tools to label and manipulate the specific neural circuits in the brains of mammals. Historically, vision studies have been carried out on humans, non-human primates, and cats. While non-human primates such as macaques have similar visual ability and processing to humans, their visual systems are very complex and the techniques available to dissect their neural circuit functions are limited (Huberman and Niell 2011). Understanding the function of basic visual system and behavior in mice is a more tractable problem. Mice have simpler visual systems, which allow scientists to isolate and modulate specific cell types and neural circuits in a way that is not currently possible in other mammalian subjects because of advanced genetic techniques (Huberman and Niell 2011). Having access to specific cell types consequently allows scientists access to manipulate neural circuitry in the brain and modulate activity of the cells and circuits. Thus, despite having poorer visual resolution relative to primates, the mouse is an exemplary model of study because of the tools applicable to it and the simplified neural system.

The advanced tools available for mouse studies, the simplicity of the mouse's brain, and the lack of many complex, visually guided behaviors might imply that findings in the mouse would be hard to relate to the complexity of a human brain. One big difference between the visual capabilities of mice and monkeys is that monkeys have evolved retinas that enable them to encode the visual scene at a higher resolution.

In contrast, mice may only resolve objects on a larger scale leading them to observe a blurrier version of a visual scene relative to a primate (Huberman and Niell 2011). Regardless of this difference, Huberman and Niell argue that even though visual acuity differs vastly between a mouse and a monkey and they express many different visual behaviors, the basic aspects of visual processing related to image formation are highly conserved between the two species (Huberman and Niell 2011). Therefore, it is advantageous to study the simple neural system of mice in order to understand the more basic aspects of human vision that is conserved across species.

Prior work has shown that basic visual behavior such as avoiding naturally threatening stimuli and approaching rewarding stimuli is a conserved behavior across species. The anuran, an amphibian, innately and visually recognizes predator and prey through its pretectal structures (Sewards and Sewards 2002). Innate visual recognition of predator and prey is shown in avian species as well as rodents, which indicate homology of behavior and potential neural structure across species (Sewards and Sewards 2002). Also, in the 2016 paper published by *Current Biology*, Hoy et al. successfully demonstrate that mice use their eyesight to capture prey (Hoy et al. 2016). Through a series of experiments, we show that mice are more successful at capturing prey when they can see. In no-light conditions, the time to capture crickets is significantly longer (Hoy et al. 2016). Despite mice's poor visual acuity, the conclusions from Hoy et al. show that mice do use vision during prey-capture. Therefore, prey-capture behavior is accessible as a natural behavior, is consistent across species, and utilizes vision. These findings support our ongoing study of prey-capture behavior in mice and neural circuitry of visual processing of approach behavior. My

studies will directly work towards this goal of studying prey-capture behavior and the neural circuits involved.

Superior colliculus (SC)

While it remains unclear how different visual areas successfully integrate information to drive the appropriate behavior, we are beginning to understand the basics of how innate visual responses are processed in the SC by studying rodents including mice. The superficial layer of SC is necessary for processing multimodal sensory input and regulating spatially targeted motor output (Wang and Burkhalter 2013). Specifically, the superficial layer of SC is interesting to our study because of its ability to rapidly detect visual stimuli, direct reorientation, and direct approach and avoidance behaviors (Sewards and Sewards 2002). In particular, different regions of SC are found to differentially mediate pursuit behaviors versus avoidance behavior in rats (Westby et al. 1990). The approach and avoidance behavior are seen in rats to be directed by lateral and medial regions of the SC. Westby et al. reveal that projections from medial colliculus, which represent information from the upper visual field, mediate defensive and "explosive, escape-like behavior" (Westby et al. 1990).

Contrasting this, the lateral SC in rats processes information from lower visual fields and directs approach responses since rewarding prey usually comes from the ground (Westby et al. 1990). However, the studies are unclear at indicating the role of different cell-types in SC. Within these sub-regions of SC, there are different cell-types, which are differentially required for behavior (Gale and Murphy 2014). Thus, the SC represents a good and large candidate to study, because it is known to process visual information, redirect head orientation, and direct approach and avoidance behaviors.

Furthermore, the regions and cell-types in these regions of SC potentially contain the neural-circuitry that directs approach behavior. These findings provide a basic idea of where in the brain of mice to study prey-capture behavior.

Preliminary Experiments and Results

The first set of experiments we performed tested whether a laboratory species of mice would produce prey-capture behavior towards crickets. I worked with Dr. Hoy and Dr. Niell to systematically demonstrate the sensory conditions under which the mouse would naturally pursue and consume crickets. By first placing a cricket in the home cage of a strain of mice known as C57BL/6J overnight and finding that cricket eaten within 24 hours, we demonstrated that common laboratory strains of mice perform prey-capture. Overall, 96.5% of the mice we tested ate crickets through prey-capture (Hoy et al. 2016). This finding allowed us to continue research into which sensory input is used by the mouse to perform prey-capture, which we started by habituating mice to arena and cricket hunting (fig. 1).

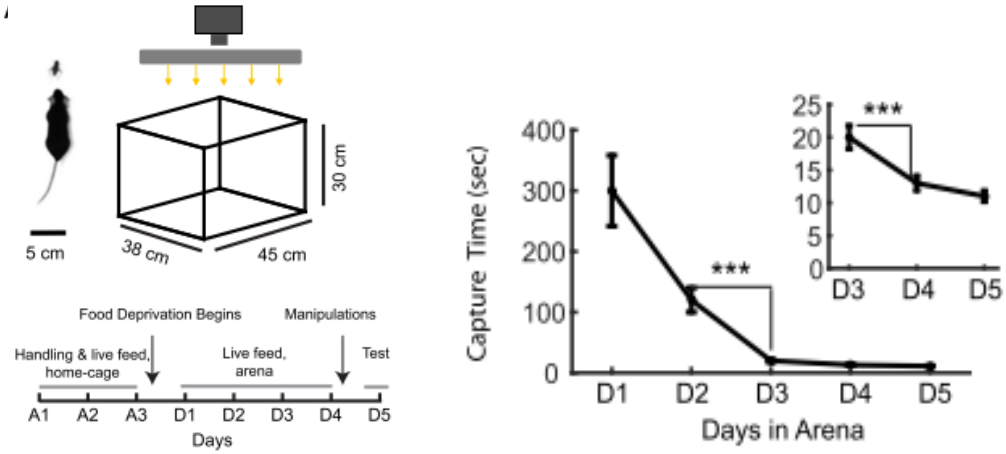

Figure 2: Experimental set up and habituation timeline

Left top, the arena where mice perform prey-capture. Left bottom, the protocol we use to condition the mice to hunt under circumstances where recordings of behavior are more systematic. Right, the charts indicate that the time to capture crickets in the arena decreases quickly over the subsequent days until capture time is reliably less than 30 seconds. This shows that mice needed a few days to habituate to testing conditions before consistently performing prey-capture. Figure from Hoy et al. (2016).

We predicted that prey-capture would use vision and/or hearing to mediate quick prey detection and rapid re-orientating towards the target. Therefore, we investigated whether vision and hearing were necessary for successful prey-capture performance in mice. We compare the prey-capture behavior under three conditions: a well-lit condition with no sensory manipulation, total darkness (eliminating visual cues), a well-lit but ears-plugged condition (eliminating auditory cues), and a total darkness with ears-plugged (Fig. 3). Overall, when visual cues are eliminated, the mouse has more difficulty detecting the cricket and wanders more (Fig. 3A). The quantified results in figure 3B reveal that mice are three-fold faster in time to capture a cricket in the well-lit condition compared to the dark condition (Light: 11 ± 2 s, Dark: 36 ± 9 s). This strongly implicates vision as a key sense in mediating successful prey-capture. In the ear-plugged and light condition, the time to capture is similar to the light condition (ear-plugged: 15 ± 3 s), suggesting that hearing offers minimal improvement to behavior when the mice can see. However, mice perform significantly worse when their ears are also plugged while hunting in the dark (ear-plugged, dark: 230 ± 56 s). This suggests that hearing might aid in prey-capture behavior when vision is eliminated. Importantly, when we analyze how mice oriented their heads relative to the target over

the prey-capture sessions, we find that mice maintain a bearing of 0˚ only under well-lit conditions (fig. 4). This tendency for mice to directly focus and orient towards prey when they could see would explain the rapid reduction in capture time relative to other sensory conditions. Overall, these data show that vision is required for accurate, precise, and fast orientating behavior during an approach, but mice might rely on other senses if vision is restricted (Hoy et al. 2016).

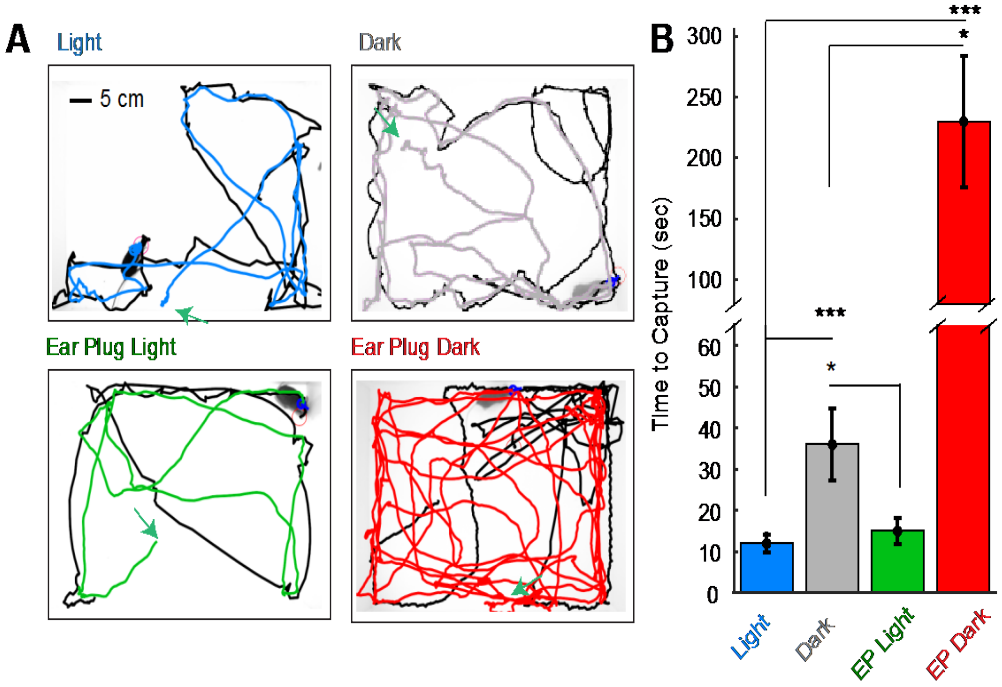

Figure 3. The relative performance of mice participating in prey-capture under different sensory conditions

Mice are tested under four conditions: light, dark, ear-plugged light, and ear-plugged dark. (A) Shows the paths mouse took towards cricket. The path the mouse takes to find the cricket is short and efficient in the light and ear-plugged light conditions. In the dark and ear-plugged dark conditions, the mouse searches over more area before successfully finding the cricket. (B) The time to capture the cricket in each condition shows that mice took three times longer to capture crickets in dark conditions. The capture time is significantly increased in ear-plugged dark conditions. Figure from Hoy et al. (2016).

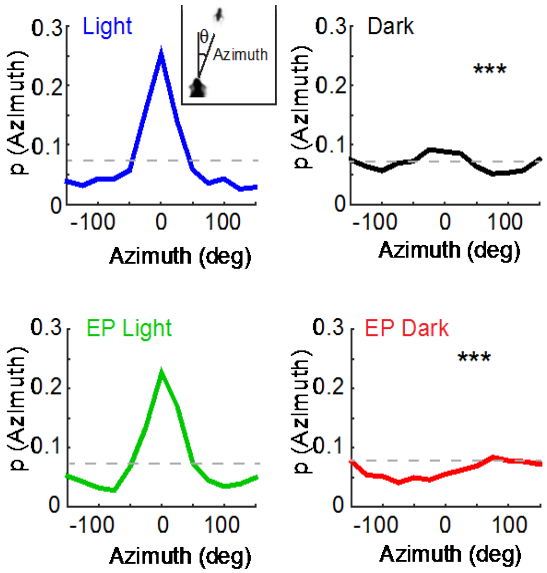

Figure 4. The probability distributions of azimuth

The probability distribution of the angular position of the cricket relative to the bearing of the mouse's head, termed "azimuth," across each sensory condition. When the mouse's head is facing directly towards the cricket, the azimuth is zero. In light and ear-plugged light conditions, it is more probable that the mouse is directly facing the cricket. In dark and ear-plugged dark conditions, the peak disappears. It shows that head angles towards crickets are randomized under conditions where the mice cannot see. Figure from Hoy et al. (2016).

Next, we find that vision is sufficient for mice to detect and orient towards prey. In this set of experiments we limit the cricket movement to a 1-dimensional path (behind a Plexiglas wall) and analyze the approach behavior of mice towards the target in light and dark conditions (Fig. 5). This simplification restricts sensory cues to vision. We see, in light conditions, that the mouse approaches the cricket directly to investigate 93 ± 5% of all contacts. In dark conditions, the mouse moves randomly and successful contact is made 14 ± 6% of all contacts with the Plexiglas barrier. However, it is unclear if the contacts are direct approaches towards the cricket or if the mouse randomly touches the wall with the cricket when placed in dark conditions. These results indicate that visual cues predominantly drove accurate approaches towards targets (Hoy et al. 2016).

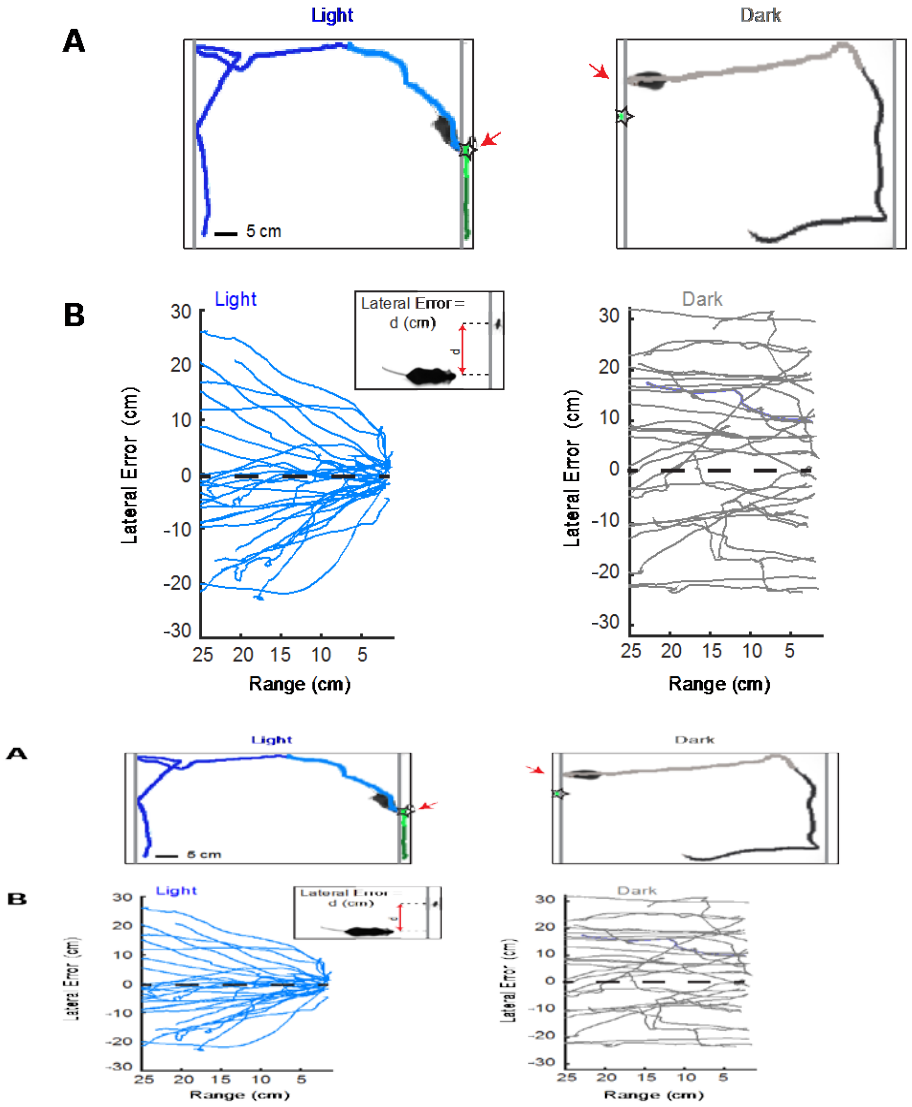

Figure 5: Vision is sufficient for prey detection shown with 1-D restrained cricket

(A) Mouse approach paths towards a cricket are isolated in light and dark conditions. The light blue and gray parts of the each path are further isolated to calculate approach accuracy. (B) The approach paths (light blue and gray) are tracked in each mouse and plotted on a graph showing horizontal range from the cricket (0 cm is on cricket) and lateral error (vertical distance between mouse's head and cricket). (C) This graph shows the probability of the lateral error being a certain value. (D) The absolute lateral error of light (blue) and dark (black) conditions. The shading indicates standard error. In the dark condition, mice had to be closer to target to see and head directly towards it (Hoy et al. 2016).

As one can imagine, it would be difficult to systematically and precisely control for cricket size, movement, color, and even activeness in experiments with actual live crickets. Yet, this is what we need to achieve in order to properly assess whether vision is sufficient to drive approach behavior in mice and to determine which specific features naturally evoke approach behavior towards prey. Thus, we require a better control for the presentation of cricket stimuli. Therefore, we replace the actual cricket with computer generated, virtual stimuli with simple features that are cricket-like. In our simplified virtual stimulus, we use solid, black ellipses that are sized proportionally to the length and height of a real cricket, which limits sensory information to vision only. Initial experiments with virtual stimuli assess the likelihood that mice would approach virtual stimuli of different sizes (Fig. 6). We hypothesized that when stimuli were significantly larger than their prey, they would avoid the stimuli more than approach. Moreover, we include smaller sized stimuli in order to determine the furthest distance the mice could detect the stimulus with their vision. This information was previously unknown. Results show that mice trained to hunt crickets approach the virtual stimuli with a preference towards sizes equivalent to real cricket proportions. Any size smaller yields fewer approaches and stimuli four times the size of crickets has fewer direct approaches as well. The size preference is a good indicator that mice will approach the virtual stimulus as if it is a cricket. From these experiments, we observe that mice can approach virtual stimuli (making future experiments more straightforward), and they also have a general preference for cricket-sized stimuli. Our studies from here on will utilize the virtual stimulus to measure controlled approaches by mice.

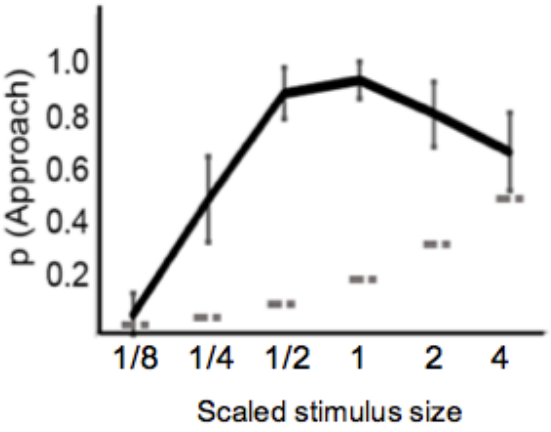

Figure 6: Probability of approaches towards virtual stimuli of varying sizes

The graph shows the probability of the mouse approaching a stimulus of a particular size. All sizes are proportional to the average sized cricket (2 cm). At 1, the stimulus is the size of a normal cricket (2 cm) and at 2, it is twice the size of a cricket (4 cm).

Overall, I am able to contribute to work in the Niell lab where we demonstrate that lab mice not only participate in prey-capture behavior, they do so with vision as their main sensory input (Hoy et al. 2016). Furthermore, we discover that mice also approach virtual stimuli with a preference towards cricket-sized stimuli. These basic findings allows for more in-depth studies on neural circuitry in the brain that mediates visual processing of approach behavior. I expand on these findings to determine the role of the SC in mediating prey-capture behavior, because it is still unclear which neurons and neural circuits mediate approach behavior in mammals.

Research Question and hypothesis

For this project, I work to understand the neural circuitry underling approach behaviors in mice. It will take the form of two main questions. With the common laboratory mouse as our model, we first set out to determine if the SC is required for approach behavior during prey-capture. Then, if the SC is found to be relevant to the behavior, then the next step is finding which cells within the structure are required for the behavior.

Specifically, we will focus on the lateral part of the SC as it encodes the ventral and nasal portions of the visual field in the mouse. Since the prey is likely to appear in the lower visual space, lateral SC is predicted to be the most relevant region in SC to prey-capture behavior in mice, because mice would aim their head and possibly their eyes towards prey. In order to determine its relevance, we will directly turn off this region of the mouse's brain. Inhibition of lateral SC is predicted to decrease prey-capture performance noticeably.

If SC is found to be important for prey-capture behavior, the next study will determine which specific cell types within the structure are required for the behavior. A previous study reveals the existence of a specific transgenic mouse, which we can use to isolate and manipulate a subset of cells in SC (Gale and Murphy 2014). Thus, this mouse line, Ntsr1-cre transgenic mouse, expresses cre protein in cells that are poised to mediate important visual processing of prey-capture. We hypothesize that inhibiting the Ntsr1-cre positive cells, using cre-dependent modulators of neural activity, would perturb orienting and approach behaviors of mice during prey-capture.

Methods

Habituation and training

All studies were carried out under approved protocols of University of Oregon Institutional Animal Care and Use Committees, in accord with National Institutes of Health Guidelines for the care and use of experimental animals.

Before beginning experimental measurements, mice are handled and habituated to the arena. The handling process follows figure 2. Mice are handled for three days and introduced to crickets in the home cage after each day. Next, mice are placed in the arena with a cricket in order to habituate to a novel environment. This is continued daily until mice can capture crickets consistently under 30 seconds, although they typically capture crickets in less than 20 seconds. Then, mice are considered proficient hunters.

Muscimol inactivation of SC

Muscimol is a psychoactive compound from the mushroom, *Amanita muscaria* (Chandra et al. 2010). Muscimol affects neurons by acting as an agonist for γ-Aminobutyric acid type A receptors ($GABA_A$-R). The receptor is generally known to promote a cascade of reactions to inhibit regional neural activity. $GABA_A$-R is activated by the ligand, GABA, which causes hyperpolarization of the neuron (Chandra et al. 2010). We inject mice with muscimol directly into the superficial and intermediate layers of SC, bilaterally. They are given a 30-40 minute recovery period. Once actively moving, the mouse will be placed in the arena and will go through four trials, each trial with a live cricket or the optimal, cricket-sized virtual-stimulus. The behavioral trials are recorded with a camera. Trials are converted into videos with ImageJ and

subsequently tracked with MATLAB. After a full set of behavior is recorded and tracked, we further analyze the data with custom written program scripts in MATLAB

After we recorded the behavior, the animals are perfused with para-formaldehyde to preserve the brains. Then, the brains are extracted, sectioned at 50μM, mounted with DAPI, and imaged for localization of our injection or other manipulation sites.

DREADDs Inactivation of SC

Expressing DREADD

The Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) system is a genetic system where we may express the mutant form of a G-protein coupled receptor, hM4Di (the Designer Receptor) that inhibits neuronal activity exclusively in the presence of clozapine N-oxide (CNO). CNO is the designer drug that is normally inert in mice, but in mice with cre-dependent hM4Di receptors, CNO inhibits neuronal activity through hyperpolarization (Roth 2016). In our case, the genetic material coding for the hM4Di receptor and a fluorescent tag is virally inserted into the tissue of our interest, the SC, and it is under the control of cre expression. The virus that we injected into the SC contains DNA for the hM4Di receptor, and the same DNA ensures that the receptor is expressed only in cells making cre-protein. Because the receptor is cre-dependent, it is important that we inject the virus into a transgenic mouse that only expresses cre in specific cells of the SC. That is the Ntsr1-GN209 cre positive mouse. This line of transgenic mice is injected with the cre-dependent virus and allowed approximately two weeks for expression.

The cre-lox system is a powerful tool available to mice in order to control gene expression to specific cell-types. We use it here to direct hM4Di to our cells of interest here in SC. It has been found previously that wide-field (WF) neurons express cre in the Ntsr1-GN209 mice (fig. 7) (Gale and Murphy 2014). There are other transgenic mice that show distinct positive cre expression patterns like GRP-cre, which narrow-field neurons expresses cre (Gale and Murphy 2014). For this study, we use the Ntsr1-cre mice to inhibit wide-field cells with DREADDs.

Figure 7: Cre expression patterns in the Ntsr1-GN209 cre mouse

Left, the red-brown colors represent cre expression. The image shows the cre expression pattern of the Ntsr1-cre mice with dense cre expression in the superior colliculus and the lateral posterior section. Right, morphological image of a wide-field neuron.

Assessment of Behavior after SC inactivation

During the time for expression, the mice are handled and trained to hunt crickets. After approximately one week, the mice can successfully hunt in less than 30 seconds. The mice are food deprived the night before the behavioral experiment. The next day, the mice are given an intraperitoneal injection (IP), of CNO, the drug that stimulates the inhibitory receptor expressed in specific cells in SC, causing inhibition of activity in those cells. We recorded the prey-capture behavior to both live and virtual stimuli between 2-60 minutes after injection. For the final comparison of behavior

across conditions, we use trials recorded 60 minutes after CNO injection to ensure CNO properly took full effect. The time to capture is noted from mice hunting live insects and the measures of lateral error are taken as mice approach virtual targets.

After the recordings, the brain is extracted from the mice and fixed. The next day, the brain is sliced at 50 μM sections, mounted on a slide with DAPI, and imaged for fluorescence to localize and quantify the cells that are expressing the inhibitory DREADD. In this case, the fluorescence for the inhibitory DREADD is red as the fluorescent protein is mCherry. The recordings are tracked with MATLAB and subsequently analyzed with custom scripts written in MATLAB.

Results

Muscimol inactivation of SC

The bilateral inactivation of SC with muscimol successfully targets the lateral and superficial regions of SC in mice (Fig. 8, left). Our histology shows that the spread of muscimol are mostly localized to the injection site. Muscimol did not diffuse beyond 900 μM. Overall, most injections successfully target both hemispheres of the brain, but there are some variability in the anterior and posterior parts of SC. We confirm that all of the injections are restricted to lateral regions of the SC and the extreme ends of anterior and posterior SC are avoided.

Figure 8: Example of muscimol injection into one hemisphere of SC

Most injections are performed bilaterally. Left, the picture shows a coronal section of one hemisphere of the SC from a mouse injected bilaterally with the muscimol. The red fluorescence is the site of muscimol injection. Right, is an image obtained from an atlas of the mouse brain, highlighting the region depicted on the left with a black box.

Based on observations of live-cricket hunting, the mice injected with muscimol or fluorescent dye along (control) both show some unsteady movement after the injection surgery. This indicates some small motor changes after the injection due to the treatment procedure. Therefore, we compare results of post muscimol-injected mice to post fluorescent dye-injected controls in order to accurately determine the effects

specific to muscimol. The muscimol-injected mice exhibit difficulty hunting as they overall have trouble re-orientating towards crickets after a cricket escapes as compared to the control mice. In addition, the muscimol-injected mice would also approach the cricket and freeze, before attempting to catch it compared to the control. Finally, the average time of successful capture increases four-fold with the muscimol injection as compared with the control (Fig. 9).

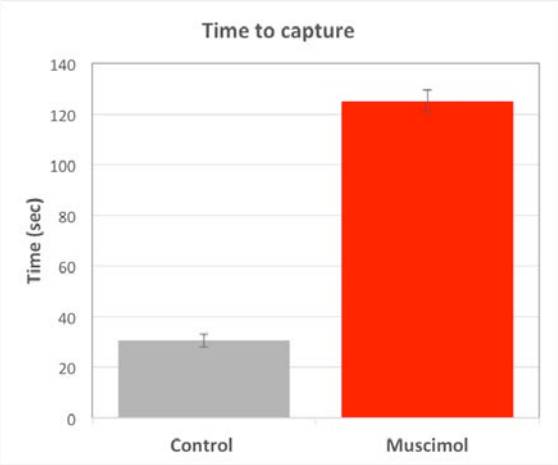

Figure 9: Time to capture live crickets

The average time to capture live crickets by mice injected with muscimol (red) versus fluorescent dye (grey). The control mice had a capture time of 30.5 s and the muscimol mice had a capture time of 125 s.

The effect of inhibiting all cells in lateral SC on visual processing and behavior is quantified by measuring the difference between a mouse's head location along the y-axis and the location of the virtual stimulus (lateral error). The lateral error is plotted as a function of the distance the mouse is to the target along the x-axis (Fig. 10). The measure of lateral error as a function of distance indicates how accurate the mice are when they make contact with the stimulus and the distance where the mice are able to detect the cricket. When detection happens, it is quantified as a change in behavior where the mouse modifies its approach to minimize lateral error. The control mice

exhibit direct and accurate approach paths towards the stimulus (Fig. 10, left). In contrast, the mice with their SC inactivated ultimately approach the Plexiglas and the stimulus, but the accuracy of the approach decreases, which is seen as an increase in lateral error (Fig. 10, right). From the preliminary analysis of the approach paths, the muscimol inactivation of SC seems to decrease the accuracy of the mouse's approach behavior.

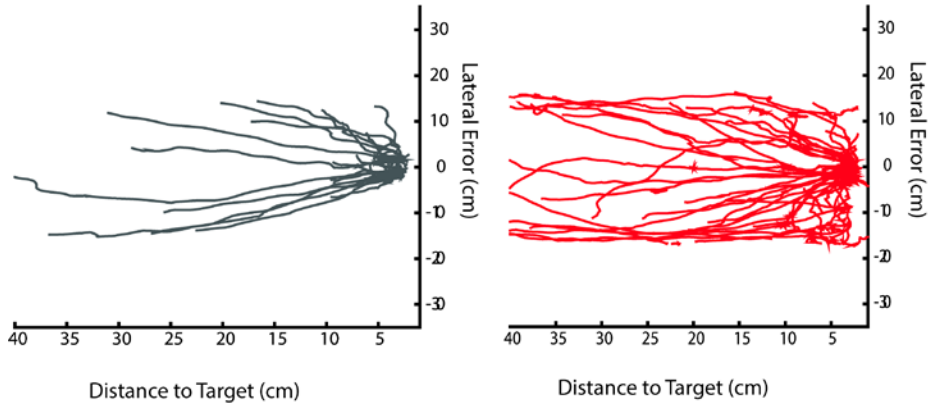

Figure 10: Lateral Error of the muscimol injected mice and control during approach

The left figure shows the approach paths of control mice with inert fluorescent dye injection (grey paths). The right figure shows the lateral error of mice with muscimol injections in superior colliculus as they approach the target. The paths are a measure of the lateral error (cm) between the mouse's head and the virtual target location as a function of distance (cm) from the target.

Next, the lateral error of all the approach paths, for each group, is averaged at as a function of distance from target (Fig. 11). The red plot represents the muscimol-injected mice, which shows an inflection point around 10 cm. In comparison, the control mice had an inflection point around 20 cm. The inflection point indicates the average distance where the mouse detects the target, changes its behavior, and starts to systematically reduce its lateral error as they approach the behavior. On average,

muscimol inhibition of superficial and lateral SC decreases the distance where mice can detect cricket and reorient itself towards the cricket. Finally, observations of SC inhibited mice show that these mice uniquely paused in an alert manner in front of the stimulus before touching the Plexiglas barrier, relative to controls. This behavior will be interesting to study when we apply more precise manipulations of SC.

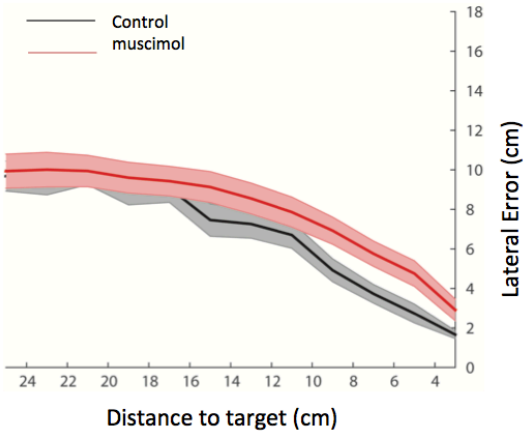

Figure 11: Absolute average lateral error of muscimol and control mice's approach paths

The red plot represents the mice injected with muscimol in the superior colliculus averaged together, which has an inflection at 10 cm. The black plot is the averaged data from the control mice with an inflection around 20 cm. The colored shading indicates standard error.

Overall, the muscimol inhibition of the SC shows obvious effects on prey-capture behavior in mice. The mice with superficial and lateral regions SC inhibited by muscimol have a four-fold increase in time to capture cricket. The SC inhibited mice also exhibit a tendency to freeze before the target and a decrease in accuracy and ability to reorient its head towards the target.

DREADD inactivation of genetically identified cells in SC

The inhibitory version of the DREADD system is utilized to selectively impair specific cell types in order to precisely identify specific neural circuits in the SC required for prey-capture. In this study, Ntsr1-GN209-cre transgenic mice encode the hM4Di inhibitory receptor in WF cells in the SC. (Fig. 12, left). The expression of hM4Di receptor is found mainly in the intermediate layer of SC with projections from the superficial layer. The data here are summarized from a preliminary study consisting of one mouse per condition: no DREADD, unilateral expression of DREADD, and bilateral expression of DREADD.

Figure 12: Inhibitory DREADD expression (red cells) in the superior colliculus of Ntsr1-cre transgenic mouse.

Left, a coronal section of brain tissue collected from the superior colliculus of an Ntsr1-cre transgenic mouse that is successfully infected with a virus that delivered Cre-dependent inhibitory DREADD. The right figure shows a coronal view of an anatomically annotated mouse's brain at the location where we obtained our fluorescent tissue. The area that is shown in the left is indicated by the black box shown on top of the section from the mouse brain atlas on the right.

Based on observations of the prey-capture behavior in the presence of live crickets, the unilateral mouse and the bilateral mouse exhibit trouble reorienting its head

towards the cricket when the cricket escapes from the mouse. If the cricket is at a close distance directly in front of the mouse, the mouse would approach as normal compared with the control. All mice in this study do not display unsteady movement as we observe after muscimol injection in mice. Furthermore, if the unilateral and bilateral mouse and cricket are on opposite sides of the arena, both mice do not make straight, non-stop approaches towards the cricket. Only at a closer distance does the mouse seem to pursue the cricket without stopping. Therefore, the unilateral and bilateral DREADD affected mice have WF cells inhibited, and they do not exhibit unsteady movement especially compared to the muscimol experiments, but they show a specific decrease in ability to reorient their head accurately towards the cricket from long distances.

The capture times of when mice are presented with live prey are recorded. The average capture times are shown for the three mice in this study (Fig. 13). The control mouse does not exhibit any concrete DREADD expression when injected with CNO, however a few cells expressed DREADD. Therefore, the control mouse has minimal WF cells inhibited and performs prey-capture very well (capture in less than 9 s) despite having CNO in its system. This is a strong indicator that CNO has an inert effect on mice. The next mouse tested shows unilateral expression of DREADD upon CNO exposure. With half of the hemisphere of the brain's WF cells inhibited, the unilateral mouse exhibits a slower prey-capture time compared to the control (16.4 s). Finally, the mouse expressing DREADD bilaterally has both hemispheres of the brain's WF cells inhibited, and the bilateral mouse takes significantly longer to capture prey compared to the other two mice (capture time 27 s). Overall, there is an approximate two-fold increase in prey-capture time when a hemisphere of SC is inhibited. The data also show

a stepwise increase in capture time as more of the WF neurons in SC are inhibited due to DREADD.

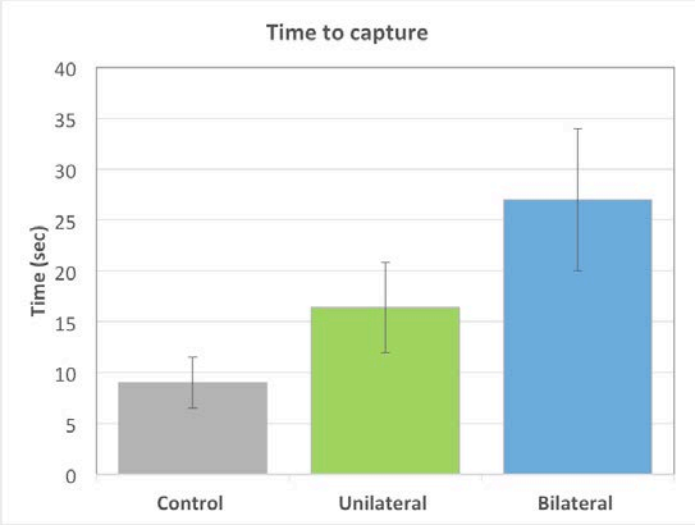

Figure 13: Time to capture live crickets

The control mouse (gray) has no DREADD expression and has a capture time of 9 s. The unilateral mouse (blue) exhibits a capture time of 16.4 s. The bilateral mouse (green) exhibits a capture tie of 27 s. As more WF neurons are inhibited with more DREADD expression, there is a stepwise increase in time to capture prey.

We also analyze approach behavior towards virtual stimuli to further assess the effect of DREADD mediated inhibition of WF neurons in SC. The mouse infected bilaterally with inhibitory DREADD shows less accurate approach behavior when injected with CNO (Fig. 14, right). Furthermore, more of its approaches begin from closer distances relative to the target. Almost half the approaches start from 20 cm away in the bilateral mouse, but all the approaches made by the control mouse start from distances greater than 20 cm.

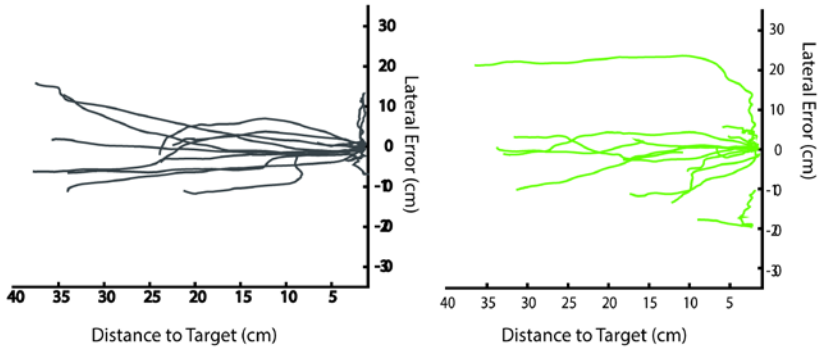

Figure 14: Lateral error of the bilateral mouse and control mouse during approach

The left figure represents the lateral error as a function of distance during approaches to virtual targets made by a control mouse with no DREADD expression in SC, 60 minutes after CNO injection. The right figure shows the same kind of "paths" generated by a mouse with bilateral inhibitory DREADD expression in the WF cells of the SC, 60 minutes after CNO injection.

The average lateral error as a function of approach distance of the bilateral and the control mice are plotted in figure 15. There is a slight inflection point for the bilateral mouse at 9 cm from the target, which is similar to the muscimol-inhibited SC in mice. In the control mouse, there is an inflection point around 14 cm. This indicates that inhibition of WF cells in SC decreases the mouse's ability to detect prey and reorient its approach towards prey. From the DREADD study, inhibition of a WF cells in SC changes orientation and efficiency of approaches in prey-capture behavior. Inhibition of WF cells increases time to capture live cricket and it creates difficulty for the mouse to reorient its head towards an escaped cricket. Interestingly, the mouse does not exhibit freezing behavior similar to the muscimol study.

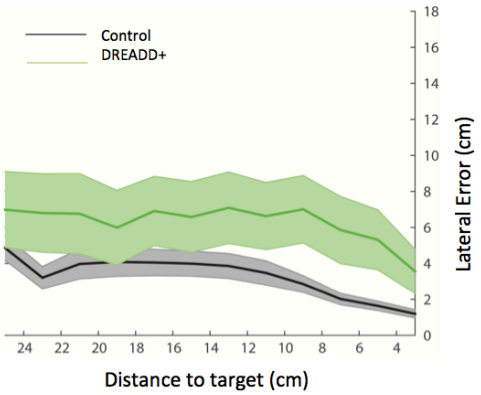

Figure 15: Absolute average lateral error of the bilateral mouse and control mouse

Lateral error (cm) is averaged as a function of the range of approach (cm) for the bilateral mouse (green) and DREADD negative mouse (gray) regardless of whether they approached from the left or right. All data were obtained 60 minutes after CNO injections into both mice.

When we measure the head bearing of the mouse relative to the target during the approaches, we find that there is a reduction in the probability that the mouse's head is directly aimed at the target (prey azimuth is 0˚) in both the muscimol inhibited SC of mice (Fig. 16, left) and the DREADD inhibited WF cells of the mouse (Fig. 16, right) relative to their controls (gray lines). Overall, my findings suggest that inhibition of a specific cell type located in the intermediate layer of SC is sufficient to impair both visually-mediated orienting behaviors towards virtual prey targets and capture of live prey.

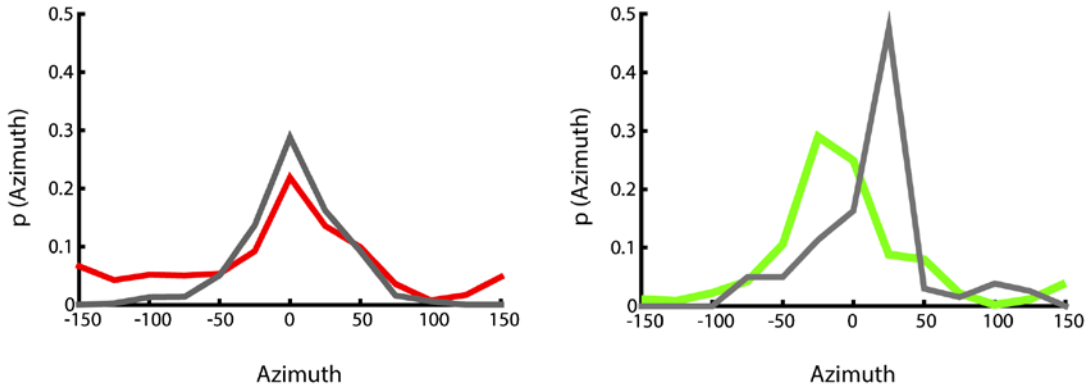

Figure 16: Accuracy of approach behavior between the muscimol and the DREADD studies Left, the probability of a certain degree of azimuth occurring is plotted for muscimol-injected mice (red) and the fluorescent-dye injected mice (gray). Right, the same probability is plotted for mice positive for inhibitory DREADD (green) and the mice negative for inhibitory DREADD (gray).

Discussion

Summary

From our studies focused on the inhibition of SC, it is evident that the lateral portion of SC is necessary for accurate prey-capture behavior. The inhibition of whole lateral region of SC with muscimol and the inhibition of a specific cell type in the SC with DREADDs both effected accurate prey-capture. This is seen in increased time to capture prey and increased lateral error as mice approach virtual targets. The experimental mice from both studies also exhibit difficulty reorienting their heads in order to track crickets.

The bilateral muscimol injection into the SC completely inhibited all the cells within the site of injection. Thus, the array of deficits observed under these conditions are likely to be associated with many of the specific neural circuits and behaviors known to be mediated by the SC. Overall, the muscimol study is important to establish whether the SC is required for prey-capture behavior at all in the mouse. It is designed to get a maximal effect on prey-capture and to give us clues of the different ways that SC might regulate prey-capture behavior.

To provide a more refined understanding, the DREADD study is performed and it limits the focus to a specific cell type in SC, WF cells, in the intermediate layer of SC. The DREADD study controls for the potential effects of the invasive procedure from the muscimol study and it also controls for how muscimol might spread and affect other parts of the brain and behavior. Accordingly, the DREADD study shows a narrower range of prey-capture time and visually mediated orienting behavior compared to the muscimol study. Interestingly, the muscimol-injected mice exhibit freezing behavior

while facing the prey, which is not seen in the DREADD mouse. This could indicate a behavior associated with a specific cell type not affected by DREADD, which could prove to be an interesting future study as well. While the two studies act in different manners, they both work towards the answer of how SC participates in prey-capture behavior. The experiments starts with Muscimol, which elicits a broad range of effects, and narrows to the DREADD study to hone in on behavior outputs at the level of specific cell types.

Potential problems and future directions

The muscimol study shows a distinct difference in prey-capture behavior. However it is unclear whether visual processing is affected or motor outputs are affected. The answer will come in part by the histology to see where the injection site hit and other potential sources of damage for the invasive procedure. Otherwise, we will need more controls in the experiment to understand if vision or motor is affected by muscimol. One possible control to test for the muscimol affect on visual processing is placing the mouse in the dark setting, similar to preliminary studies. Once vision is taken away, if approach accuracy stays the same, then it is mainly a motor problem. If approach accuracy worsens, then the muscimol affected mainly vision. It's a basic study that would need more refinement for controls. Overall, the data from the muscimol study provide a good start by supporting the importance of SC to prey-capture behavior.

The DREADD data hold promise for studying changes in behavior. However, it is unclear whether the WF neurons in the SC are actually being inhibited by the DREADD activation. The fluorescence reveals the presence of the receptor, but it does not indicate the receptor's activity. The small behavior changes are a good indicator that

neurons are inactivated. However, to firmly address this, we will perform cell recordings for activity level in future studies. Furthermore, our initial study of DREADD consisted of n = 1 mouse for each condition: one control, one unilateral expressing, and one bilateral expression of DREADD. For clear patterns of behavior to appear, we will need to increase batch size in the future. Therefore, our immediate goal is to increase batch size (n) of the DREADD study.

More future studies will include a more detailed analysis of the data gathered so far. This includes an investigation of the mouse's head angles as they hunt, which will provide a clearer insight on how SC outputs to head orientation and an analysis of the speed of approaches in mice during the muscimol and DREADDs behavioral study. This would provide more insight on the approach since it is generally patterned that a mouse would increase its speed as it actively pursues prey. These future analytical plans will provide clearer insight into what aspect of prey-capture is changed under each study.

Future studies well beyond this thesis will work to identify characteristics of stimuli that would elicit strong behavioral response. This includes identifying how movement and elevation of stimulus affects approach behavior. We expect movement would mimic more prey-like signals and would induce more direct and fast approaches. Finally, a long-term project for the future will study how different cell types in SC, besides the WF cells in the Ntsr1-cre positive mice, may play a role in prey-capture behavior.

Connection to the human brain

Studying the SC and vision processing in the mouse model gives a lot of insights, especially in how it translates to avoidance behavior. The knowledge gained from animal studies can be applied to an array of concepts, including facial and object recognition in technology and health-related neurological disorders.

A study in 2014 correlates the innate alarm response in humans with PTSD to a subcortical pathway involving the SC and periaqueductal gray region of the brain (PAG) (Steuwe et al. 2014). The innate alarm system detects potential threats and the authors use eye contact with virtual, human characters as a stimulus for the innate alarm response. They found in patients diagnosed with a childhood PTSD an increase in activity in the SC and PAG region when eye-contact is made (fig. 18) (Steuwe et al. 2014). It is a broad study, but this is a good indicator of the importance of SC in directing avoidance behavior in humans.

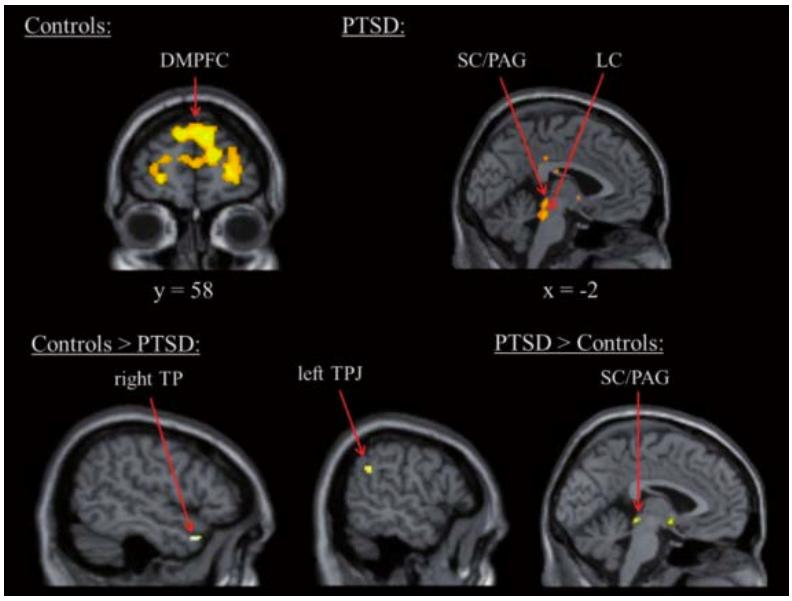

Figure 16: fMRIs after eye-contact recording is played

Patients with PTSD exhibit higher activity in the SC/PAG region after a recording is played where a virtual person makes eye contact with the subject. The control group consist of subjects with no PTSD (Figure from Steuwe et al. 2014).

Overall, this paper provides another example of the behavioral response the SC can provide. Our focus relates the SC to approach behavior. However, the SC is also commonly cited to elicit escape and other fear responses across many species. It will be interesting to see how the approach and avoidance behavior in SC are connected and how studies on SC translate to human behavior.

Basic Glossary

Agonist: A compound that binds a receptor and activates the receptor

Anterior: towards the nose or frontal part of the brain

Azimuth: angle, in degrees, between the position of mouse's head and prey-target

Bilateral: both hemispheres of the brain

Clozapine N-oxide (CNO): Designer drug that binds to the hM4Di receptor

Coronal: a plane that divides the brain in the dorsal and ventral parts. So it looks at the brain if it is sliced from top down.

DAPI: is a fluorescent stain that binds to A-T nucleotide rich regions of DNA.

Fix: preserve tissue

Hyperpolarization: Effectively making the neuron more negative in charge so no signal is fired from neuron to the next neuron.

Intraperitoneal (IP): injection through the abdominal cavity of the animal

Lateral: towards the sides of the brain

Medial: towards the center of the brain

Mounted: to cover and protect tissues with a fluorescent stain

Muscimol: agonist for $GABA_A$-R . Muscimol activates the GABA receptor to inhibit neurons.

Perfusion: A technique that essentially preserves animal tissue. The heart is utilized to pump paraformaldehyde throughout the body and eventually to the brain.

Posterior: towards the back of the head

Prey-capture: describes hunting behavior of animals

Transgenic: genetically modified

γ-Aminobutyric acid (GABA): molecule released from the inhibitor neuron. It attaches to GABA receptors in the active neuron, which inhibits the neuron by hyperpolarization

Aminobutyric acid type A receptors ($GABA_A$-R): receptor that acts to inhibit neurons through hyperpolarization upon binding of GABA

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