

Neural Correlates of Neon Color Spreading Illusion in Mouse Visual Cortex

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Alireza Saeedi
aus Mashhad/Iran

Tübingen
2023

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der
Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:

05.09.2024

Dekan:

Prof. Dr. Thilo Stehle

1. Berichterstatter/-in:

Prof. Dr. Andreas Bartels

2. Berichterstatter/-in:

Prof. Dr. Nikos Logothetis

3. Berichterstatter/-in:

Assistant Prof. Matthew McGinley

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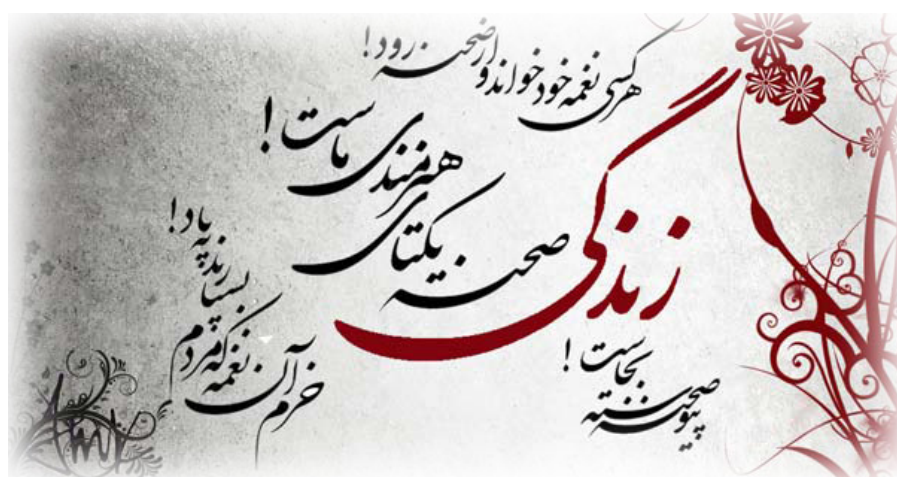
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Dedicated to the **women of Afghanistan** who have been restricted from pursuing education and to the **Hazara people** who have suffered from systematic genocide. Your courage and fortitude against enormous oppression are an inspiration to all of us. May this dedication remind us that education is a fundamental human right that must be accessible to all and that justice and equality must be upheld for every individual. Let us continue working towards equal opportunity for everyone to educate and live without fear of violence or discrimination.



Translation:

Life is but our unique performance stage, where we sing our hearts.

As we depart, the stage endures...

May our songs be ones of delight,

That echo through the halls of time.

— Poem by Zhale Esfehani

(Subjectively translated by Alireza Saeedi)

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PREFACE

Underlying mechanisms of cognitive brain functions (such as perception) have occupied scientists' minds for a considerable time. Despite the considerable efforts to reveal those mechanisms (particularly at the neuronal level), human knowledge in this field still needs to be completed. This is mainly due to several challenges; first, there are limited human single-neuron data, and on the other hand, drawing conclusions about a cognitive function such as perception from animal single-neuron databases is challenging.

The study of optical illusions allows us to dissociate the neural correlate of direct sensory stimulation (outside reality) and visual perception. Hence, they can be used to discover how the brain can shape visual perception using ambiguous visual cues. Among the extensive database concerning visual illusions, "neon color spreading" (NCS) reveals the exquisite beauty of our visual perceptual system. NCS is the combination of different illusory sub-components, including diffusion of color and filling-in, illusory brightness, illusory contours, transparency, glowing, and depth. Therefore, the NCS illusion can be used to investigate different perceptual qualities.

This thesis is an effort to reveal some aspects of visual perception at the single-cell level. Here, we present the results of a series of experiments designed to investigate the neuronal correlates of illusory brightness using the NCS illusion in mice. We introduce an achromatic version of the NCS illusion, which produces illusory drifting grating, and explore its single-neuron correlates in mouse cortical visual areas. The findings of this research demonstrate that V1 single units respond to illusory brightness and that higher lateral visual areas, particularly LM, have an essential role in processing visual illusions through feedback signaling to V1.

Furthermore, we discuss the limitations of the research and acknowledge the potential weaknesses in the methodology or findings and our conclusion. Overall, this thesis provides insights into the neuronal mechanisms underlying the processing of illusory qualities, which can have implications for understanding the brain's hierarchical organization and perception.

SUMMARY

Illusions are a powerful tool for studying the single-neuron correlates of perception as they are the dissociation point of the sensory information processing and the perceptual signal in the brain. In this thesis, in chapter 1, I provide a background introduction to the neon color spreading illusion. I discuss the illusory perceptual qualities of this illusion and its potential implications for understanding perception. I also review the literature regarding this illusion and the related exciting findings. Furthermore, by discussing what needs more examinations, I elaborate on the motivation for this endeavor.

Chapter 2 presents the results from our first experiment on the mouse visual system. We introduce an achromatic version of the neon color spreading (NCS) illusion and report the neuronal correlates of illusory brightness, which has been studied using human fMRI. We designed a novel NCS paradigm to generate an illusory drifting grating, and in a series of experiments, we recorded nearly six thousand single units in mouse cortical visual areas. For this experiment, we recorded 636 single units in the mouse primary visual cortex (V1). A substantial proportion of V1 single units (60.5%) responded to illusory gratings. Moreover, by presenting luminance-defined gratings (LDG) with a 180° phase shift relative to NCS gratings, we show that spatial phase tuning shifted 180° for most single units. This finding conclusively demonstrates that V1 single units respond to illusory brightness. Using this novel mouse paradigm, we show that responses to illusory gratings have a lower magnitude and are delayed relative to physical gratings. We determined what types of V1 single units contribute more to the illusion processing mechanism in V1. We found that NCS-preferring cells are more likely to receive surround suppression, to be classified as fast-spiking putative interneurons, and to have complex receptive field properties. In chapter 3, I present the results of two other experiments in which we expanded our search for the neuronal correlates of NCS to higher lateral visual areas of the mouse. We recorded 5058 single units from four areas, namely V1, LM (lateromedial), LI (lateroinfermediate), and LL (laterolateral), simultaneously. We found that LM has an essential role in illusion processing as the NCS-evoked responses in LM were stronger and appeared earlier than in other visual areas. Lastly, optogenetics inhibition of higher visual areas reduced the V1 single unit response

to the NCS stimulus but not the LDG stimulus, implicating top-down feedback specifically in processing visual illusions by V1 neurons in mice. These findings reveal a hierarchical organization in which higher-level neurons are pivotal to processing illusory qualities, such as brightness.

Finally, chapter 4 provides the general conclusion, where I discuss all our findings in a more unified framework. I highlight the significance of our findings and the limitations in our methodologies and results. Moreover, I suggest possible future studies to overcome those limitations and broaden our knowledge of visual perception.

Part I

BACKGROUND

BACKGROUND

1.1 NEON COLOR SPREADING ILLUSION

Our brain continuously infers our perception from noisy sensory information. Hence, a principal goal of visual science is to explain how a distinct visual perception can be made of an ambiguous visual stimulus through visual information processing. One of the most profitable methods to tackle this problem is to use optical illusions, which are the dissociation point of direct sensory stimulation (outside reality) and visual perception. Therefore, vision scientists have accumulated a considerable database concerning visual illusions and the paradoxical connection between visual images and visual perception (some reviews: [50, 11, 107, 23]).

Among all the optical illusions, "neon color spreading" discovered in 1971 [114] by Varin¹, which exposes the exquisite beauty of our perception and provides the opportunity to understand visual perception. This effect can happen in a line drawing if some segments of lines are replaced by colored segments (or segments with different brightness) (see Figure 1.1 as an example). The neon-like glow of colored segments leaves the borders and fills the surrounding area until the boundaries of a transparent figure. The figure has a subtle bluish hue that emits a weak light, and it is illusory because a pixel-by-pixel spectrum of the surrounding white background does not describe the bluish figure.

1.2 PERCEPTUAL QUALITIES OF NEON COLOR SPREADING: PHENOMENOLOGY AND PSYCHOPHYSICS

Neon color spreading has drawn considerable attention as it combines different perceptual phenomena, including illusory color, illusory brightness, diffusion of color and filling in, illusory contours, transparency, glowing, and depth. Numerous scientists have tried to describe the perceptual qualities of the illu-

¹ Varin's work "chromatic contrast and diffusion" was written in Italian and did not receive much international circulation. In 1971, Harrie van Tuijl rediscovered this phenomenon and named it neon-like color spreading [112]

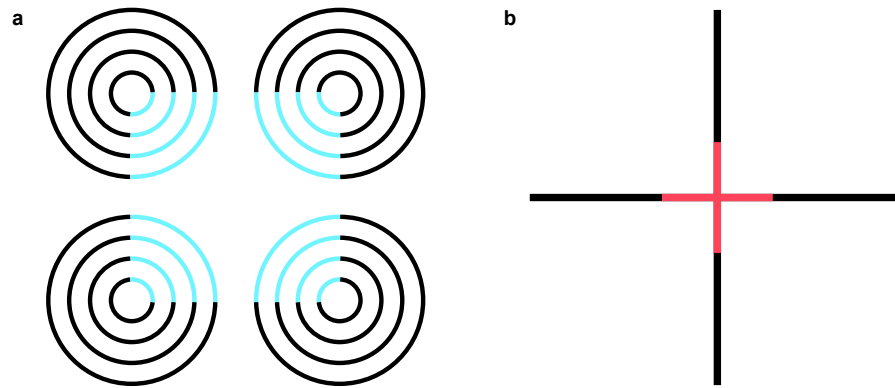


Figure 1.1: Neon color spreading illusion.

- a.** Only a quarter of each concentric circle is colored blue. The blue color leaves the colored segments and spreads into the illusory area. This neon color is constrained by illusory contours at the edges of the illusory square.
- b.** The inner parts of the lines are colored red, which produces an illusory transparent red figure.

sion itself and its relation to other illusions and investigated the human brain's perceptual mechanism using this illusion. In this section, I will review those efforts and point out what is puzzling in this line of research.

1.2.1 *Illusory color/illusory brightness*

The bluish/reddish flank in Figure 1.1 is illusory as a pixel-by-pixel spectrum of the figure would not show any changes corresponding to the color. This illusory color originates from the colored segments, spreads into the surrounding area, and produces a "transparent" and "glowing" surface. However, the spreading of color decreases to a narrow stripe if the length of colored segments increases. With a further increase in the length of the colored segments, the illusory figure becomes even smaller and can be perceived only at the junctions of black lines and the segments. This local effect at the junctions has been named neon flanks [94].

The illusion is most apparent when lines and segments are collinear, continuous, and with the same thickness but progressively weakened as they become more and more separated along these dimensions. In fact, many bi-colored drawings can lead to neon color spreading with proper arrangement of lines and colored segments, and the effect is robust against amputation, deformation, twisting, and other disturbances of the line and segment arrangements [93]. Bressan has indicated that color spreading occurs as

long as the colored segments and lines can be seen as a whole structure [21].

Although a considerable part of the beauty of neon color spreading is due to the hue, the colored segment does not need to have a hue to form the neon color spreading phenomenon [22]. For instance, gray segments embedded in a black line drawing give rise to an achromatic version of the illusion. In this case, neon-like illusory brightness spreads throughout the illusory area (Figure 1.2a). Similarly, gray segments in white lines on a black background give rise to the perception of an illusory "blacker than black" area (Figure 1.2b).

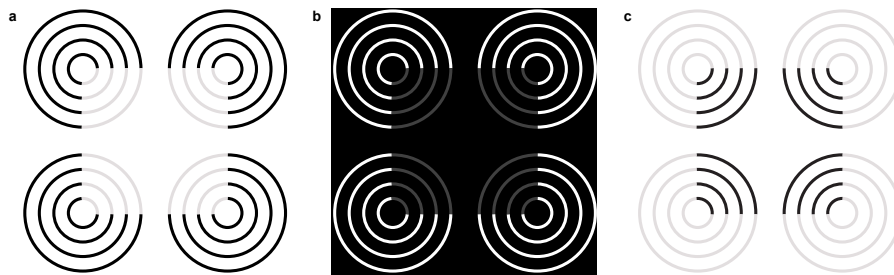


Figure 1.2: Achromatic version neon color spreading illusion.

a. Gray segments of black concentric circles give rise to illusory brightness spreading in the surrounding area. **b.** Gray segments of impeded in white concentric circles on a black background produce an illusory "blacker than black" figure. **c.** Black segments embedded in gray lines on a white background can also give rise to the neon color spreading illusion.

The achromatic version of neon color spreading has the advantage of simplicity and has been systematically investigated before the chromatic version. First, a simple hierarchy has been suggested as a basic rule in the achromatic version in which the luminance of the segment has to be between the background luminance and the luminance of the lines [113, 77]. Later, Bresnan showed that neon spreading could be generated with other luminance hierarchies (Figure 1.2c). She claimed that neon spreading luminance conditions represent the requirements for perceiving a transparent subjective figure [22].

In the chromatic version, neon spreading also depends on the hues of the segments and embedding lines as well as their contrasts [34]. For instance, red components embedded in orange lines have a weak neon color spreading, but red segments with bluish-green lines give rise to a more vibrant neon color spreading. In general, the effect appears to be most vivid with complementary colors in the segments and lines [34]. Therefore, studies on the

chromatic version of the neon color spreading illusion must provide explanations that refer not only to spatial structure and luminance hierarchies but also incorporate the theories of color perception and contrasts.

1.2.2 *Diffusion of color and filling-in effect*

Diffusion of color, also known as color spreading or color bleed, is a visual phenomenon where the color appears to spread beyond its physical boundaries and blend into adjacent colors. This effect can be found in various visual stimuli, including paintings, photographs, and optical illusions. The diffusion of color does not necessarily lead to color spreading or the perception of a continuous illusory surface. For instance, in "assimilation," the diffusion of color exists, but there is no perception of an illusory surface. Filling-in is a perceptual phenomenon in which the brain fills in missing or incomplete information in a visual stimulus. Filling-in can occur in various contexts, including where part of an object is occluded or where a stimulus contains a blank area or a gap. In these cases, the brain fills in the missing information based on contextual cues and prior knowledge to create a complete and seamless perception. In the neon color spreading illusion, illusory color leaves the colored segments and "diffuses" from the colored elements into the surrounding area and "fills in" the entire illusory area equally to give rise to the perception of a continuous illusory surface. Therefore, the diffusion and filling-in effects are essential mechanisms that contribute to the perception of the continuous surface in the neon color spreading illusion. These effects help the brain to create a coherent and complete representation of the visual scene, even when the actual stimulus is incomplete or ambiguous. Understanding the neural mechanisms underlying these effects can help us better understand how the brain processes and interprets visual information.

1.2.3 *Illusory contours*

Illusory contours are edges or boundaries the visual system perceives even though they are not physically present in the visual stimulus. These contours are created through a process known as perceptual completion, which involves completing missing information in visual stimuli based on context and prior knowledge. One of the main illusory components of the neon color spreading illusion is the perception of illusory contours. These contours are

perceived to be present despite no physical boundary or edge. In the neon color spreading illusion, illusory contours are generated by the interaction between the color, the black or white pattern of inducers, and the background color, which produces a boundary not present in the visual stimulus. In neon color spreading illusion, color can diffuse and fill the entire surrounding area up to the illusory contours. Therefore, illusory contours alongside the "filling-in" and "color diffusion" are necessary to perceive the illusory surface.

1.2.4 *Transparency*

Transparency describes the perception of multiple surfaces or objects as transparent, allowing one to see through them. In visual illusions, transparency effects refer to the perception of overlapping surfaces as distinct, transparent layers rather than a single, mixed surface. In the neon color spreading illusion, the transparency effect refers to the perception of the overlapping colors as transparent rather than mixed. This illusory surface generated in the neon color spreading illusion appears transparent, and the neon-like color overlays on the original colors rather than a mixture of the two colors of inducers. This transparency effect is due to the creation of illusory contours, which separate the colors into distinct surfaces.

Overall, the transparency in the neon color spreading is an intriguing example of visual processing in the brain and the perception of complex visual information. The illusion provides insight into the mechanisms of contour perception and the creation of illusory surfaces and may have broader implications for our understanding of visual perception.

1.2.5 *Depth*

Depth perception is the ability to perceive the spatial relationships between objects in three-dimensional (3D) space. It allows us to distinguish between near and far objects and perceive their relative size, shape, and distance. In neon color spreading, while the illusory surface appears flat and transparent, the brain can also perceive it as a solid object occupying a particular volume in space. The perception of depth in the neon color spreading illusion emerges from a combination of illusory aspects, such as the filling-in effect and the illusory contours. When the brain perceives a transparent surface, it also fills in the surface with the color of the surrounding area, creating the

perception of a solid object. The illusory contours further enhance this perception by creating the impression of a well-defined, volumetric shape with distinct edges and borders.

1.3 UNDERLYING NEURONAL MECHANISM OF NEON COLOR SPREADING

As the neon color spreading illusion is combined with different illusory qualities, a complex interaction of various neuronal circuits in the brain would be necessary for the neon color spreading illusion. Research suggests that processing different illusory attributes, such as illusory contours, filling-in, and transparency, requires integrating information from multiple levels of visual processing.

Although neon color spreading has contributed to our understanding of specific visual processes; however, its neural basis is not fully comprehended. Here, I focus on the available neurophysiological data regarding the main existing illusory elements of neon color spreading, such as illusory contours, apparent contrast reduction, color diffusion, and illusory brightness/darkness.

1.3.1 *Illusory contours*

Illusory contours are among the most well-studied phenomena to probe the neural correlates of perception, visual awareness, and consciousness. Researchers have used illusory contours to explore how the brain processes visual information and constructs objects' perceptions and boundaries [48, 73, 79, 90]. It has been shown that a subset of neurons in the macaque and cat early visual cortices, namely primary visual area (V1) and secondary visual area (V2), respond to illusory contours in the Kanizsa square [79]. The Kanizsa square is a classic example of an illusory contour, where four Pac-Man-like shapes positioned with their "mouths" point inwards, creating the perception of an illusory square despite no physical square being present (Figure 1.3) [48].

These studies have helped to reveal the neural mechanisms that underlie the perception of illusory contours in primates. For instance, Lee and Nguyen (2000) examined how the visual areas V1 and V2 process Kanizsa figures [60]. They found that the response to illusory contours in V1 emerges 100ms after stimulus onset in the superficial layers and 120-190ms in the deep lay-

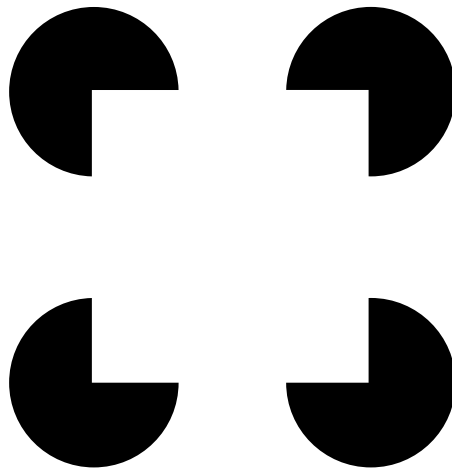


Figure 1.3: Kanizsa figures.

a. The arrangement of four Pac-Man-like shapes with their "mouths" pointing inward causes the perception of an illusory square that is not present in the stimulus. Illusory borders make this illusory square seem brighter than the white background. However, there are no differences in the brightness of pixels within the square and the surrounding area.

ers, whereas in V2, it initiates earlier at 70ms in the superficial layers and 95ms in the deep layers. Their results suggest that the computation of illusory contours involves intercortical interaction and that early perceptual organization will likely be an interactive process. Based on the differences in response latencies, they conclude that V2 neurons are responsible for integrating more global cues and computation of more abstract information with their big receptive field (twice the receptive field of V1 neurons). However, intercortical feedback of global contour information from V2 to V1 is necessary to construct spatially precise and complete contours with input from the global scene context. Although response latency is an independent measure of hierarchy [56], it is not strong evidence for top-down modulation, and their conclusion remains speculative. Therefore, causal manipulation is required to infer the actual neuronal circuit of the illusion processing. Recently, it has been discovered that mice can also perceive illusory subjective contours despite their simpler visual system [82, 86]. Moreover, the mouse's primary visual cortex also responds to illusory contours at the population level. Importantly, it has been shown that optogenetics inhibition of mouse lateromedial cortex (LM), which is analogous to primate V2, weakens the V1 responses to illusory contours [86]. This suggests that the neural mechanisms underlying the perception of illusory contours are likely preserved across species, even those with different visual systems and levels of complexity. Overall,

research on illusory contours provides valuable insights into the neural correlates of perception and helps to unravel the mysteries of visual awareness and consciousness.

1.3.2 Filling-in

Perceptual filling-in refers to completing or filling in the missing information in the visual field. Filling-in can occur instantly and independently of the stimulus configuration, as is the case when filling-in occurs at the blind spot or retinal scotomas. It can be instant and dependent on the stimulus configuration, as with illusory contour or surface perception, where the visual system fills in missing information to create the perception of a continuous edge or surface. Moreover, it can also be delayed as it occurs in the case of Troxler fading [111], Artificial scotomas [91], Motion-induced blindness [16] where it is stimulus-independent, or in the case of Stabilized retinal images [33] where it depends on the stimulus configuration. Many studies have tried describing or using it to explain other phenomena. The filling-in effect can be categorized using different taxonomies. A filling-in phenomenon can be classified based on perceptual qualities, psychophysical, or phenomenological and psychophysical characteristics. Here, using the current data, I discuss the neural basis of the instant filling-in effect.

Blind spot: The blind spot refers to the area of the retina where the optic nerve leaves the eye. Due to the lack of photoreceptors, it cannot convey visual information from the corresponding region in the visual field.

In the literature, two primary models exist to explain the mechanism of perceptual filling-in. The first model suggests that neural filling-in does not occur. The structures of the visual system ignore the absence of information in the blind spot and allocate more of the same information as surrounding information to that region. This model, called the symbolic or cognitive theory, also proposes that there would be no retinotopic representation of the filled-in surface in earlier visual areas. However, based on symbolic theory, the filling-in-related activities might be found in higher visual areas representing objects [32, 71, 83]. In contrast, the second model, known as the isomorphic model, proposes an active process for some types of perceptual filling-in, which involves point-for-point representations of filled-in regions in the retinotopic cortex. Based on isomorphic theory, the filling-in may be mediated by lateral propagation of neural signals or passive remapping of receptive fields. Substantial evidence is accumulating for this model, with studies suggesting that the spread of activation across the retinotopic map from the border to

the interior surface of the filled-in figure may be responsible for the neural mechanism underlying filling-in [36, 67, 109].

Based on electrophysiological studies, the immediate content-independent filling-in occurs mainly in the early stage of visual processing, where they have found single units responding at the retinotopic representation of the blind spot in the primary visual cortex, and some of those single units had large receptive field [36, 67, 54]. Moreover, fMRI studies have revealed a weak responsive cortical area in the primary visual cortex that corresponds to the cortical representation of the blind spot, which no longer exists in the secondary visual area [109, 110].

Altogether, these investigations propose that the perceptual filling-in phenomenon at the blind spot may reflect an active mechanism, including lateral propagation signals between neighboring neurons in the primary visual cortex, and may also entail feedback signals from extrastriate regions. In this process, captured visual information by the retina is projected to the V1 and processed in a retinotopic map. When there is a scotoma in the visual field, neighboring neurons with similar receptive fields fill the gap through lateral interactions through horizontal connections in V1, where the representation of the surround overlaps the representation of the blind spot. This mechanism enables the visual system to maintain a continuous perceptual experience in the presence of visual gaps. In contrast, content-dependent filling-in (mainly for surface and contour perception) involves feedback processing from higher visual areas to lower ones [101, 79]. The retinal information projected to the primary visual cortex and the extrastriate cortex is processed in parallel pathways. The dorsal pathway processes spatial information, motion, and action, while the ventral pathway processes object recognition and form. The ventral pathway, particularly the lateral occipital cortex (LOC), is involved in the filling-in [100]. When there is missing information, such as in the Kanizsa illusion or the watercolor illusion, the feedback from the LOC to the V1 enables the neural circuit in V1 to construct spatially precise and complete contours with information from the global scene context. The feedback from higher visual areas might facilitate the horizontal connections in V1, leading to more accurate filling-in of missing information.

Despite all the efforts and advances in the investigation toward understanding the filling-in effect, this effect needs more exploration, especially for surface filling-in, where the literature is puzzling, and there is contradictory evi-

dence on what fills in and what does not. I will discuss them in the following sessions.

1.3.3 *Illusory brightness*

Brightness is the perceived luminance and intensity of light. Brightness perception can be affected by various factors, such as light source intensity, observer distance, the reflective properties of the surfaces, and, importantly, the brightness of the surrounding environment. Illusory brightness perception refers to the perception of brightness not present in the visual scene, or the brain perceives an object or area to be brighter or darker than it is due to various factors such as contrast, context, and cognitive biases. Illusory brightness is an illusory component of optical illusions such as neon color spreading and Craik-Cornsweet-O'Brien illusion [29, 30, 84]. A straightforward example of an illusory brightness perception happens when the brightness of an object appears to change based on the brightness of the surrounding objects. For example, a gray square on a black background appears brighter than the same gray square on a white background, even though the physical luminance of the square remains the same. However, illusory brightness perception is a complex process that involves interactions between sensory input and higher-level cognitive processes. Understanding how the brain processes illusory brightness can provide insights into the neural mechanisms that underlie visual perception and may have applications in fields such as psychology and neuroscience.

By performing electrophysiological recording, researchers have shown that V1 single units in the primary visual cortex of non-human primates and cats respond to physical brightness [52, 64, 87, 97, 98]. However, their response to illusory brightness is poorly understood, leaving uncertainty about how V1 neurons respond to this type of visual input [45, 95]. In addition, studies using fMRI to investigate the response of the V1 region in humans to illusory brightness have yielded conflicting results. Some studies have found a correlation between the BOLD signal in the V1 area and the perception of illusory brightness, while others have found no such correlation. These inconsistencies suggest that the V1 neuronal response to illusory brightness is poorly understood. The lack of knowledge about how V1 neurons respond to illusory brightness in humans and animals highlights the need for further research.

1.3.4 *Illusory surface*

The representation of surfaces in the visual system is a complex process that relies on the interactions between different neural circuits and various visual cues [27, 26, 42, 89]. One important aspect of surface representation is the interaction between brightness and contour-processing circuits. A perceived surface has a different brightness and color than the background and should be bounded between some edges. Therefore, brightness-responsive cells and edge detective neurons should work together to create a coherent representation of the surface properties of objects in the visual field. Depth, color, and motion are additional visual cues that contribute to surface perception and help the brain construct a complete and accurate representation of the environment. In the case of illusory surface perception, the filling-in effect and its corresponding neuronal circuit also need to contribute to this collective process.

Although the neural correlates of subjective contours have been considerably studied, and substantial evidence has been accumulated on how the brain processes them (discussed in section 1.3.1), the neural mechanisms underlying filling-in and subjective surface perception still need to be better understood. It remains a debate whether these neurons play a direct role in illusory surface perception.

Human fMRI studies have presented contradictory findings regarding surface perception. For instance, Sasaki and Watanabe examined how the brain reconstructs an illusory surface by measuring functional MRI signals corresponding to "neon color spreading." They found that activity for the filling-in exists only in the primary visual cortex with controlled attention, while multiple visual areas show correlated activity for illusory contours. They suggest that the brain processes the surface information by multiple rather than single processing and challenges the idea that V1 activity is solely due to feedback from midlevel vision [101]. Moreover, Meng et al. explored the neural mechanisms underlying the filling-in of visual phantoms, which are illusory moving grating. Typically, when an orthogonal gap separates a low-contrast moving grating with low contrast, observers perceive a fainter version of the dynamic pattern in the surrounding region, appearing to continue across the blank gap. They found that visual phantoms enhanced activity in early visual areas (V1 and V2) corresponding to the blank illusory gap. This effect persisted regardless of where the subject attended [69]. On the contrary, Perna et al. found

no selective response for surface brightness in the primary visual cortex. Instead, they discovered two associative areas of the dorsal pathway, located in the caudal region of the intraparietal sulcus and the lateral occipital sulcus, respond specifically to the Craik-O'Brien-Cornsweet illusion generated by high-pass filtered edges. They suggested that the primary visual cortex does not explicitly encode illusory brightness [88]. Moreover, Cornelissen et al. investigate whether perceived brightness and color are represented in the fMRI signals in cortical visual areas to look for neural surface filling-in signals. They found no local modulation in the primary visual cortex (or other nearby retinotopic maps) corresponding to perceived brightness changes induced by modulating a surrounding field rather than the surface itself. They argue that the observed extended edge responses in V1 and V2 may be responsible for the surface brightness and that the characterization of neurons previously identified as brightness-responsive may need to be revised. They conclude that human V1 and V2 visual areas do not contain filled-in, topographical representations of surface brightness and color [28]. However, high-resolution fMRI showed that illusory surface brightness changes modulate the brain responses in the lateral geniculate, challenging these conclusions [7].

Such a discrepancy also exists in the literature regarding electrophysiological data in cats and monkeys. Some research has shown that V1 neurons respond selectively to the contrast of brightness surfaces [52, 64, 87, 97, 98]. However, contradictory findings suggest that V1 may not directly affect surface perception. For example, some research has failed to locate such neurons in the primary visual cortex, indicating that other brain regions may also be involved in surface perception [38, 95].

Several possible scenarios might explain the discrepancy in this literature related to surface representation in V1. Firstly, this discrepancy may be due to a genuine difference between species regarding the role of the primary visual cortex in complex visual functions, such as the reconstruction of the perceptual brightness of surfaces. In less complex visual systems with less functional segregation, the primary visual cortex needs to process more complex information and even multimodal inputs [37, 49, 47, 46], whereas, in humans, these complex processes can occur at a higher level of the visual processing pathway. These inter-species differences may account for some variation in previous research on the neural mechanisms underlying surface perception across different species. Another possible scenario is that the distinctive experimental paradigms in various studies may have led to inconsistent find-

ings. Furthermore, the neural mechanisms underlying surface perception are highly complex; therefore, isolating and interpreting the specific contribution of V1 neurons while there are differences in the experimental design (e.g., differences in stimulus properties, task demands, or analysis methods) is challenging.

Overall, the neural mechanisms underlying surface representation and illusory surface perception are complex and still need to be fully understood. Future research could utilize more standardized methods and paradigms to directly compare the responses of V1 neurons across various experimental conditions. Additionally, new advances in neuroimaging techniques and computational modeling may provide a better understanding of surface representation in the brain and the contribution of different neural units to this process.

1.4 OVERVIEW OF THE THESIS

In this thesis, our primary goal is to probe the neural correlates of neon color spreading illusion at the single unit level. We use an achromatic version of neon color spreading because it provides the opportunity to address and discuss the filling-in effect underlying illusory brightness and surface perception without getting involved with complications of the visual color system. Recently it has been shown that mice can perceive illusory contours in Kanizsa square [86, 82]. Thus, it is likely that mice can also perceive neon color spreading since it contains illusory contours; moreover, the underlying mechanism of the illusory contour and illusory surface (filling-in) shares common features. Therefore, we use the mouse as a well-established model to study vision with the advantage of causal manipulation. As for the first step, we use electrophysiological recordings to examine whether there are any responses to the neon color spreading illusion and its illusory brightness in the mouse's primary visual cortex. Then, we probe those responses further to check the types of illusion-responsive neurons and try to infer the V1 neuronal circuits processing this illusory information of brightness and filling-in effect. The result of this V1 recording is presented in chapter 2. Next, we expand our search for neural correlates of neon color spreading illusion to higher visual areas. Electrophysiological recordings from different visual areas help us to have a broader view of the perception of illusions. Recent indications suggest that rodents' visual cortex resembles the human ventral

and dorsal stream [119, 120], which processes sensory input's shape and motion information. The ventral-like stream is mainly located at the lateral extrastriate cortex of rodents [108]. Using a long laminar silicon probe, we record higher lateral visual areas simultaneously with V1. Similar to monkey's studies on the perception of illusory contour perception [60], measuring and comparing the neural response latencies to visual stimulation as an independent measure of the hierarchy in different visual areas can reveal the source of perception related to neon color spreading illusion, which can be the key feature of the neural circuitry of illusion perception. Importantly we found that illusion-evoked responses in the anteromedial area appeared earlier than other recorded visual areas (i.e., V1, laterointermediate area (LI), laterolateral (LL)). We present these results in chapter 3. After finding the area which triggers the neural processing of neon color spreading, we test our finding with causal manipulation. In this regard, the ability to inhibit or activate a specific cell type in specific brain regions, provided by optogenetics, comes in handy. We perform simultaneous electrophysiology and optogenetics inhibition using a silicon probe combined with optical fiber to deliver the light. Therefore, we can inhibit multiple areas (e.g., LM and LI) and record neural responses to the illusion from the other areas to infer the causal circuit of darker-than-bleak perception. We present the result of this experiment in chapter 3.

Part II

MAIN RESULTS

NEURAL CORRELATES OF ILLUSORY DRIFTING GRATING IN MOUSE V1

2.1 INTRODUCTION

The study of optical illusions allows us to dissociate between the neural correlates of direct sensory stimulation (outside reality) and visual perception. This means optical illusion can be used to discover how the brain can shape visual perception using ambiguous visual cues. Among the extensive database concerning visual illusions, "neon color spreading" (NCS) [112] reveals the exquisite beauty of our visual perceptual system [11, 50, 107]. This effect can occur in a line drawing by changing the brightness or color in some segments of lines (Figure 2.1a, Supplementary Movie 1). NCS combines various illusory sub-components, including the diffusion of color and filling-in, illusory contours, transparency, glowing, and depth. Therefore, NCS illusions can be used to investigate different perceptual qualities [23].

A well-studied illusory aspect of neon color spreading is the illusory contours at the edges of illusory areas (i.e., the edges of the illusory grating in Figure 2.1a). Illusory contours, which exist in different illusions, are among the most thoroughly studied phenomena to probe the neural correlates of perception, visual awareness, and consciousness [42, 48, 73, 79, 90]. It has been shown that a subset of neurons in the macaque and cat early visual areas (V1 and V2) responds to illusory contours in the Kanizsa square [41, 60, 105, 116, 117]. Moreover, mice can perceive these illusory subjective contours, and their V1 neurons respond to illusory contours at the population level [82, 86].

The "filling-in" effect is an illusory sub-component of NCS and a perceptual phenomenon that can transpire in different situations [54]. It is a crucial aspect of the surface perception mechanism [53]. In NCS, color (e.g., dusky gray in Figure 2.1a) diffuses from colored segments and "fills in" the illusory area, resulting in an illusory grating that can be perceived as blacker than the black background (Figure 2.1a). Surface representation can be a result of interactions between the filling-in and contour processing units as

well as depth, brightness, color, and motion [26, 27, 42, 89]. Therefore, NCS has been used extensively to study the mechanism of surface perception [6, 42, 76, 77, 101, 121]. However, much less is known about the neural correlates of NCS at the single unit level.

In the present study, using in vivo electrophysiology in awake mice, we show that single neurons in the mouse primary visual cortex (V1) respond to an illusory "darker than black" in neon color spreading. Furthermore, we provide evidence of the association between neural correlates of NCS and different cellular properties. Our findings suggest that neurons with a more definite preference for NCS may have a higher functional or physiological hierarchical level within V1.

2.2 RESULTS

We recorded single-unit responses of the primary visual cortex (V1) in awake mice that were head-fixed on a disk and passively viewed the visual stimuli on the screen. The electrophysiological experiment included three recording sessions (see methods section for detailed explanation). First, a receptive field (RF) mapping session with a duration of 20 minutes was conducted to capture the receptive field location of neurons around the probe. For this session, we recorded the electrical activities in response to small black squares (15 degrees of visual angle) presented in random locations on the screen. A quick multi-unit analysis of neuronal responses followed the RF mapping to estimate the overall receptive field of nearby neurons. Second, we recorded neural activities in response to circular patches of sinusoidal moving grating with different sizes (2.5-45 degrees) presented at the multi-unit receptive field. In the last session, we presented three stimulus types: 1) Illusory motion grating in neon color spreading (NCS; Figure 2.1a, Supplementary Movie 1), in which the intersection of concentric circles and a virtual moving grating is replaced with gray segments. 2) A diffusion-blocked control (DBC) condition with the exact temporal change, but two static circles constrain each concentric circle (Figure 2.1c, Supplementary Movie 2). 3) Luminance-defined motion grating (LDG) above concentric circles (Figure 2.1d, Supplementary Movie 3). These three stimulus types were presented in 8 different moving directions in a pseudo-randomized sequence.

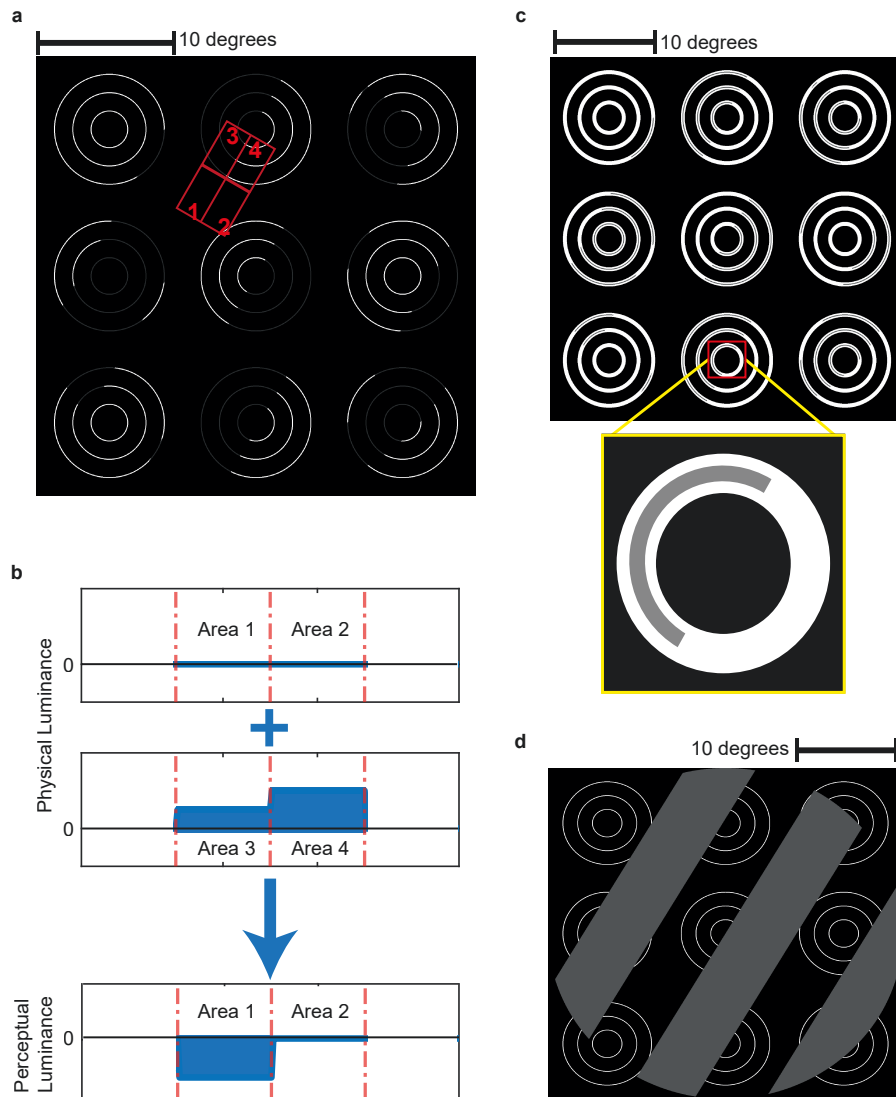


Figure 2.1: Designed visual stimuli to study neural correlates of illusory brightness.

(a) Neon color spreading. The intersection of concentric circles and a square wave grating is colored gray, resulting in the "darker than black" illusory grating. **(b)** Physical and perceptual luminance in four example areas that have been marked in (a) to compare what physically exists in the stimulus and what can be seen by a human observer (note here that the luminance values are arbitrary values only with the purpose of demonstration as reporting a perceived luminance requires a comprehensive study in human). Luminance differences in areas 3 and 4 make a perceptual luminance difference between areas 1 and 2. **(c)** Diffusion blocked control condition in which steady blocking circles constrain the concentric circles to block the spreading of dusky gray. **(d)** Luminance-defined grating over a background compound of a steady concentric circle. This stimulus is designed as a control stimulus to contrast neuronal responses of illusory grating with an ordinary moving grating.

2.2.1 Mice V1 single units respond to the neon color spreading illusion

Compared to other brain recording methods, single-unit recordings provide high specificity and accuracy in determining the neural circuits responsible for processing specific sensory information. It has been shown that V1 neurons in mice respond to illusory contours in the Kanizsa square [86]. We analyzed the response profile of 520 visually responsive single units (out of 636 recorded neurons) to different stimulus types (Figure 2.2). Here, for the first time, we report that mouse V1 single units respond to the neon color spreading illusion. Example responses to NCS from simple and complex neurons¹ are depicted in Figure 2.2, where simple cells have a solid phased-locked response to the temporal frequency of drifting grating.

We obtained the preferred angle of neurons to examine neuronal responses to different stimulus conditions (i.e., NCS, LDG, and DBC). Notably, We showed that the preferred angle remains invariant for a majority of neurons (Figure 2.3a). Hereafter, we present and discuss the responses for the preferred angle of individual neurons. We found that a substantial subset (57.2 %) of visually-evoked neurons in mouse V1 responds to both NCS and LDG (Figure 2.3b). Population analysis shows that the magnitude of responses to NCS is significantly smaller than LDG for excited cells (Figure 2.3c-e).

To verify that the V1 response is due to the neon color spreading illusion but not the physical changes within RF, we presented a diffusion-blocked stimulus as the control condition with confined inducers but the same dynamical changes as NCS (Figure 2.1c). As shown in Figure 2.3d and e, the control condition is not evoking neurons despite its exact pixel-wise changes compared to the NCS stimulus. This result demonstrates that the NCS response is not due to the local physical changes.

As an independent measure of visual computations in the brain, neural response latency has been used extensively to infer functional neuronal circuits. To investigate the temporal dynamics of responses, we have calculated the response latency of V1 neurons for the different stimulus conditions [68](see Methods). We have also excluded simple neurons since the phase-locked response to the drifting grating strongly affects their estimated latency, and it

¹ Hubel and Wiesel discovered two types of receptive fields in the visual cortex of cats: simple and complex cells. Simple cells have smaller receptive fields that respond to a specific orientation of a line or edge, while complex cells have larger circular receptive fields that respond to more complex patterns of light and dark, such as moving edges or gratings. Both simple and complex cells extract features from visual scenes and work together to process visual information.

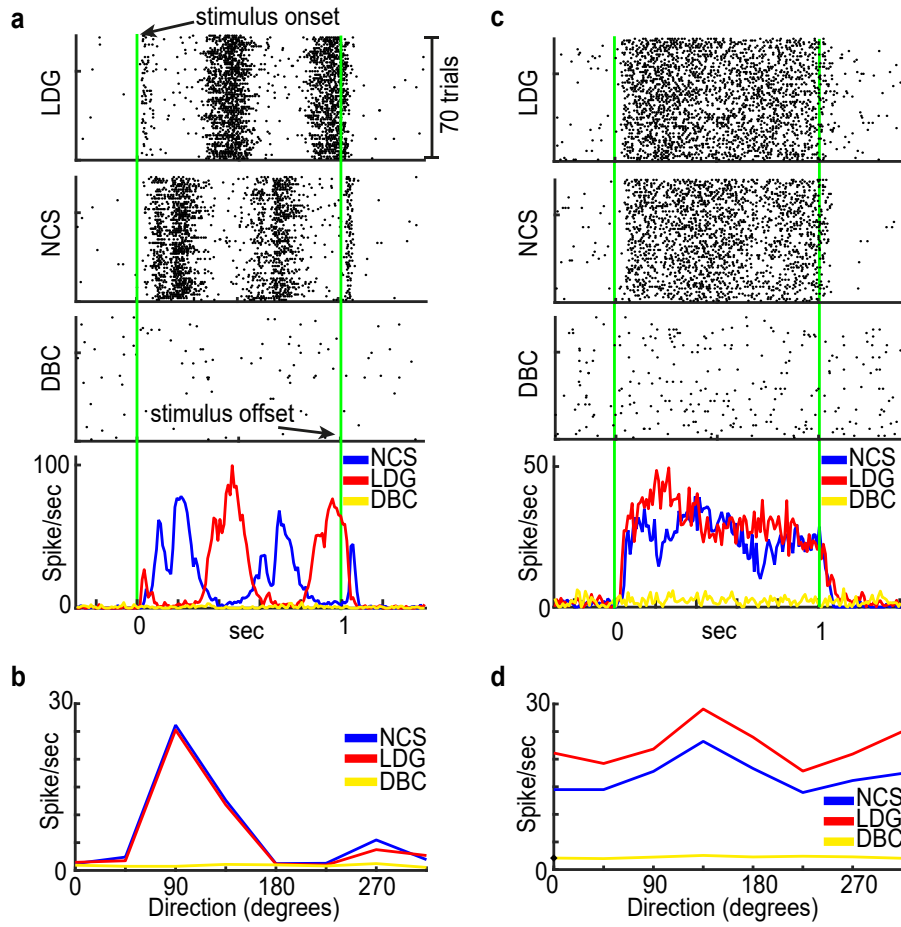


Figure 2.2: Example of single-unit responses to different stimulus types.

(a) Raster and peristimulus time histogram (PSTH; smoothed with a moving average window of 10 ms) of a V1 simple cell responding to different stimulus types at its preferred direction (90 degrees), green lines show the stimulus onset and offset. **(b)** Direction tuning for the example neuron in (a). The response in each direction is the average firing rate of the neuron during the whole stimulus presentation (i.e., one second). **(c)** Raster plot and PSTH of a V1 complex cell in response to different stimulus types at the preferred direction of the cell (135 degrees). The plot conventions are identical to panel (a). **(d)** Direction tuning of the example single-unit in (c). The plot conventions are identical to those of the panel (b).

is not possible to estimate a genuine response latency for those neurons using drifting grating. A latency comparison shows that responses to NCS are more delayed than responses to LDG (Figure 2.3f; mean $\pm$ SEM, LDG latency: 65.74 ± 0.17 ms, NCS latency = 99.18 ± 0.33 ms, $p = 5.75e-16$). This relative delay in response time is likely due to the complex nature of the illusory stimulus, which requires additional information processing by the neuronal circuitry.

2.2.2 V1 neurons respond to "illusory brightness" in the NCS illusion.

Due to the diffusion of dusky gray in NSC, the illusory area is perceived as "darker than black." In order to study this illusory brightness in the achromatic neon color spreading illusion, we presented NSC and LDG stimuli with an inverted phase. Figure 2.4a shows the perceived luminance of moving grating at the onset time for NCS and LDG. Since the illusory moving grating in NCS seems darker than black, it has 180 degrees of luminance phase shift compared to LDG (Figure 2.4a down). If neurons respond to the illusory brightness, their responses to NCS and LDG should have a phase shift of around 180 degrees. As is shown in the raster and peristimulus time histogram (PSTH) of an example neuron in Figure 2.2 a and b, the NCS response is shifted compared to the LDG response. We calculated the phase shift of the neuronal response's first harmonic (F1 component) to different stimulus conditions to quantify the effect. In order to ensure the accuracy of the calculated phase, this analysis was restricted to a subset of neurons where the F1 frequency component was the dominant frequency (i.e., the F1 component was the most vital non-zero component). The frequency profile of an example neuron with a dominant F1 component is shown in Figure 2.3b. The distribution of the phase shift between the NCS response and LDG response (Figure 2.4c) shows a clear phase shift around 180 degrees.

2.2.3 Neural correlate of NCS within the V1 hierarchy: surround modulation

We showed that the V1 responses to NCS are delayed compared to the LDG responses, suggesting a more time-consuming process of illusory content in the brain. Therefore, we hypothesize that NCS-responding neurons might belong to a higher visual hierarchy within V1. In order to test this hypothesis, we examined different neuronal properties and compared them with the

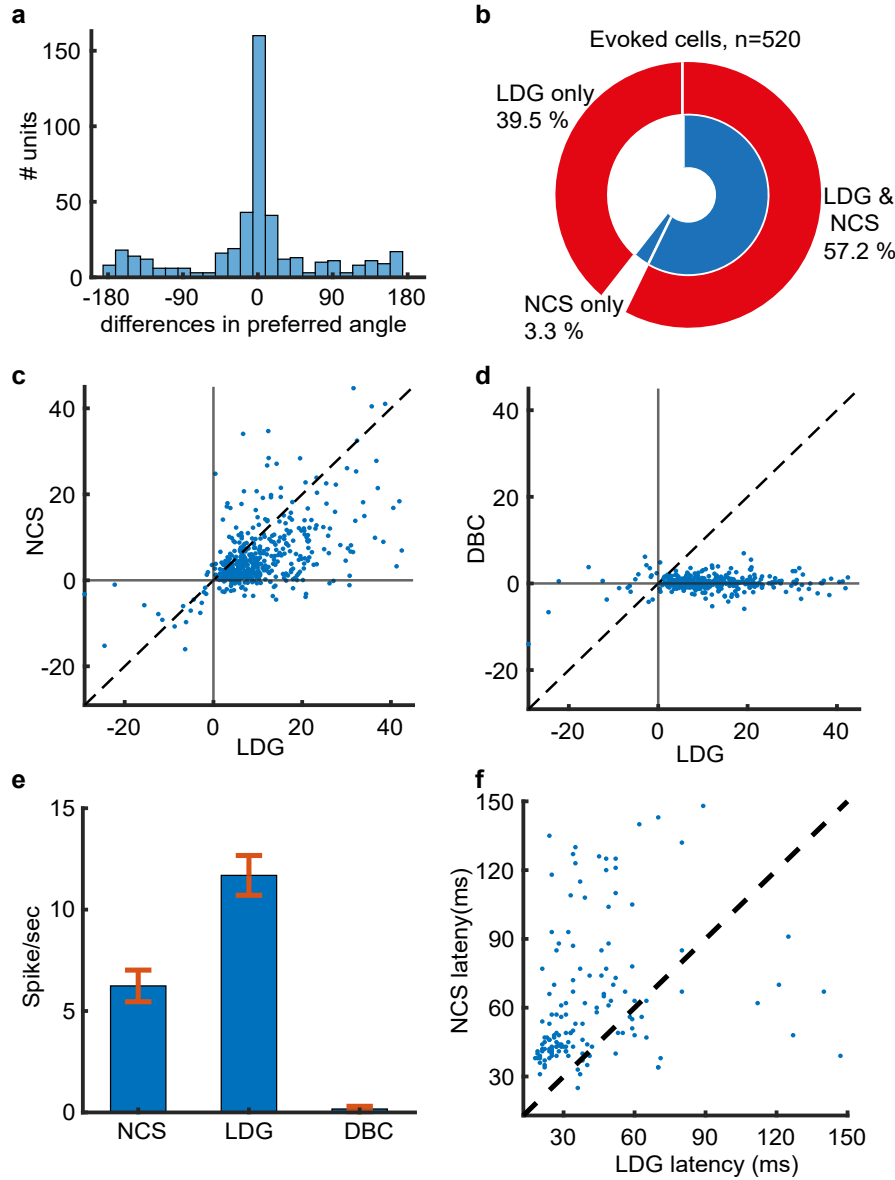


Figure 2.3: Population analysis of neuronal responses to different stimuli.

(a) Histogram of the differences in preferred angle in response to NCS and LDG shows that the preferred angle remains invariant in most neurons. **(b)** Population of evoked neurons for different conditions. **(c and d)** Scatter plots show the average response of cells over the duration of stimulus presentation (spike/sec) for different stimulus types. Dashed lines represent the identity lines. **(e)** The bar plot summarizes the average responses of all neurons to different stimuli ($\text{mean} \pm \text{SEM}$). **(f)** The neural latencies in response to NCS and LDG. The dashed line represents the identity line.

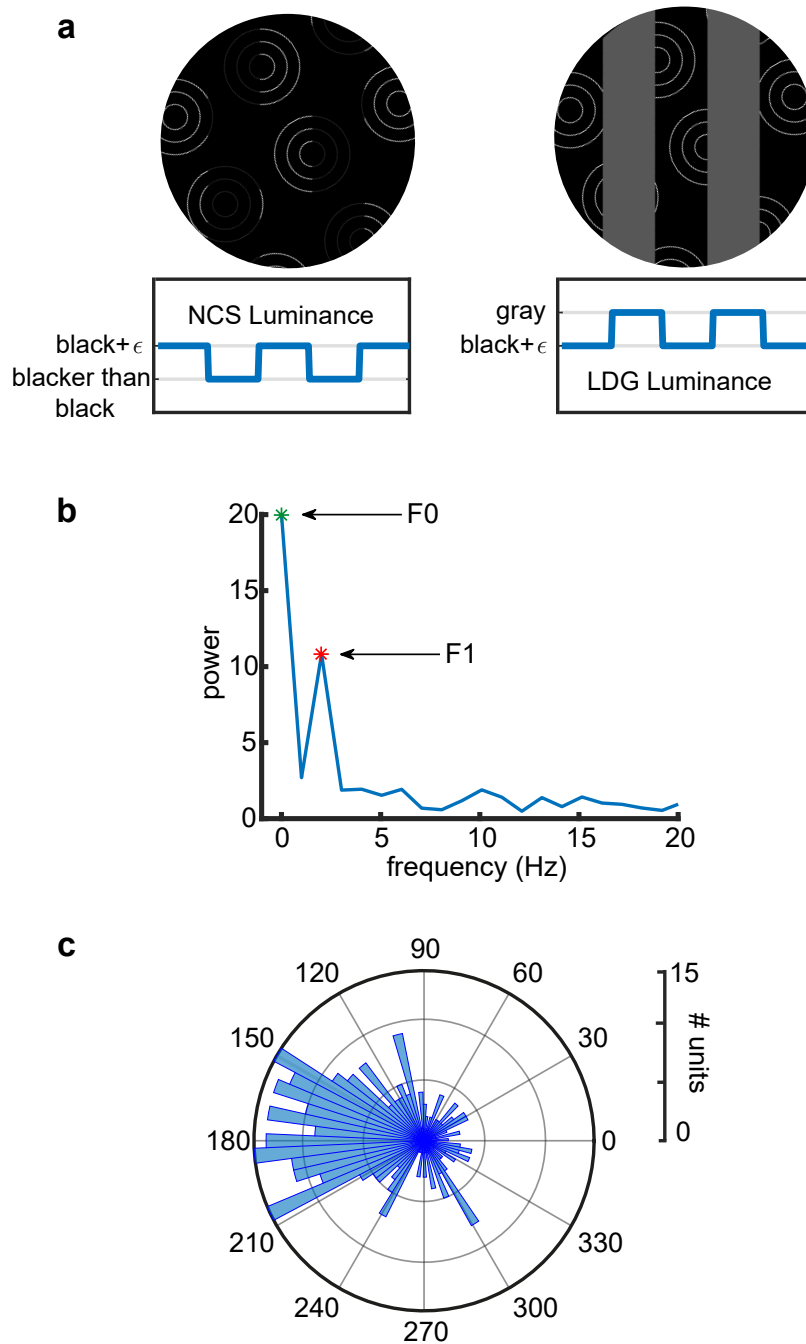


Figure 2.4: V1 neurons respond to illusory darker than black.

(a) Stimulus phase shift. As NCS seems to be blacker than black, there is a 180-degree phase shift between the luminance of NCS and LDG. **(b)** Frequency profile of a complex neuron with a dominant F1 component. **(c)** The phase shift of the response to NCS and LDG is around 180° for most v1 neurons. This is a piece of strong evidence that V1 cells respond to the illusory blacker than black grating.

NCS response of the neurons. As a first neuronal property, we investigated the surround modulation. It has been shown that presenting stimuli in the surrounding extra-classical receptive field (eCRF) can modulate the neurons' response to stimuli simultaneously presented in the classical RF [4, 43, 61]. This surround modulation (SM) is an active property of neurons during visual information processing [8, 92]. It has been suggested that SM in V1 cells results from intra-V1 horizontal connections [2, 17, 102] as well as intracortical feedback/feedforward connections [8, 9].

One can classify neurons into three groups based on their SM effect: suppressible, plateau, and facilitative cells [31, 63, 118](Figure 2.5a-c). In suppressible cells, the stimulation of eCRF attenuates the neuronal response to a stimulus in the CRF (i.e., by increasing the stimulus size, the neuronal response will decrease) (Figure 2.5a). In plateau cells, surround modulation does not exist; therefore, the cell response will be maximized for a specific stimulus size and remains unchanged for larger stimuli (Figure 2.5b). For facilitative cells, surround modulation has an excitatory effect, and the neuron's response increases for bigger stimulus sizes (Figure 2.5c). To examine the association between SM and the neural correlate of neon color spreading, we presented motion gratings of varying sizes (from 2.5 to 45 degrees) to the mice. We introduce the following SM index:

$$SMI = \frac{\max(R(< 30^\circ)) - R(45^\circ)}{\max(R(< 30^\circ))} \quad (2.1)$$

Where R is the average response of the neuron to the stimulus over the presentation period. The SM index is negative for facilitative cells and positive for suppressible cells. We also defined and calculated the illusory grating response (IGR) index, which is the neuron's relative response to NCS and LDG:

$$IGR = \frac{R(NCS) - R(LDG)}{R(NCS) + R(LDG)} \quad (2.2)$$

A positive IGR represents a higher response to NCS and vice versa. Figure 2.5d shows that IGR and SMI are correlated ($r=0.26$, $p=4.6e-8$), and neurons with a higher response to NCS have a stronger suppressive surround modulation. In order to perform statistical analysis, we have divided neurons into two groups of LDG and NCS-preferring cells based on their IGR. The distribution of SM for these two groups shows that NCS-preferring cells are more suppressible ($p=3.03e-13$), while LDG-preferring cells can be facilitative, plateau, or suppressible (Figure 2.5e).

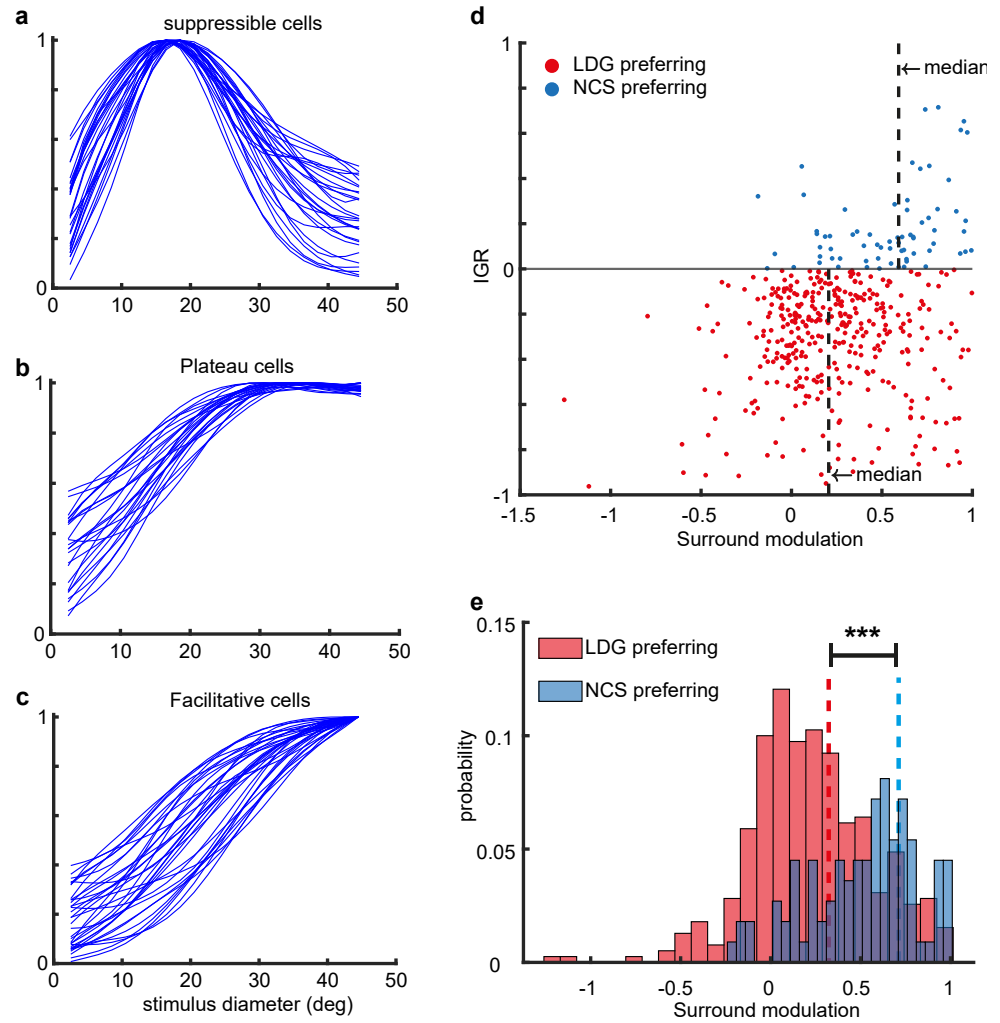


Figure 2.5: Neurons with more surround suppression have a higher response to NCS.

(a-c) Three types of eCRF modulation. (a) In suppressible cells, the effect of eCRF stimulation is suppressive. (b) A neural firing rate will be saturated as the stimulus size increases if there is no modulatory effect. (c) eCRF modulation can also facilitate the neuronal response to the stimuli. **(d)** The scatter plot shows the illusory grating response (equation 2.1) against surround modulation for two groups of cells (LDG-preferring neurons with higher responses to LDG and NCS-preferring neurons with higher responses to the NCS stimulus). Dashed lines represent the median of each population. **(e)** Distribution of surround modulation for two groups of NCS- and LDG-preferring cells. NCS-preferring cells are more suppressible ($p=3.03e-13$). Dashed lines show the median of each population. *** represents $p < 0.0001$.

2.2.4 Neural correlate of NCS within the V1 hierarchy: complex-simple modulation

Another well-known property of visual neurons related to the functional hierarchy is their complex/simple receptive field [44, 72]. Many studies have suggested that complex cells receive bottom-up inputs from simple cells [1, 5, 39, 66, 72]. Therefore, complex cells might belong to a higher hierarchical level in V1. We have calculated the complex-simple modulation ($CSM = \frac{F1-F0}{F1+F0}$) from neuronal responses to LDG and used it as an index to investigate the neural correlates of illusory brightness processing in this framework. Since we found no significant correlation ($r=0.01$, $p=0.8$) between CSM and IGR (Supplementary Figure 2.1a), we focused on the F1 component and calculated the relative amplitude of the F1 responses as in the following,

$$IGR_{F1} = \frac{R_{F1}(NCS) - R_{F1}(LDG)}{R_{F1}(NCS) + R_{F1}(LDG)} \quad (2.3)$$

Where $R_{F1}(NCS)$ and $R_{F1}(LDG)$ are the amplitude of the F1 component of the response to NCS and LDG, respectively. As depicted in Figure 2.4a, IGR_{F1} is negatively correlated with complex-simple modulation ($r=-0.302$, $p=2.4e-11$). To perform a statistical test, we divided cells into three groups of cells based on their CSM (Figure 2.6a): simple cells ($CSM > 0$, $n=178$), slightly complex ($-0.39 < CSM < 0$, $n=230$), and highly complex cells ($CSM < -0.39$, $n=230$). We found that the F1 response of highly complex neurons to neon color spreading illusion is higher than their F1 response to that of the luminance-defined grating (Figure 2.6b). The response of two highly-complex cells is shown in Supplementary Figure 2.2. This result is evidence of the contribution of highly-complex cells in the neural correlate of NCS.

2.2.5 Putative inhibitory/excitatory cells and neural correlates of NCS

Studying interneurons can provide a more detailed understanding of the complex neural circuits that underlie visual processing since they are involved in various functions, such as modulating the activity of sensory neurons, forming receptive fields, and shaping visual information processing pathways [10, 58, 122, 62, 35, 40]. Therefore, they may play a critical role in illusory visual processing. The physiological identity of cells was determined from their extracellular activity to probe the neural correlate of NCS in relation to different types of physiologically defined cells. We captured cells' trough-to-peak

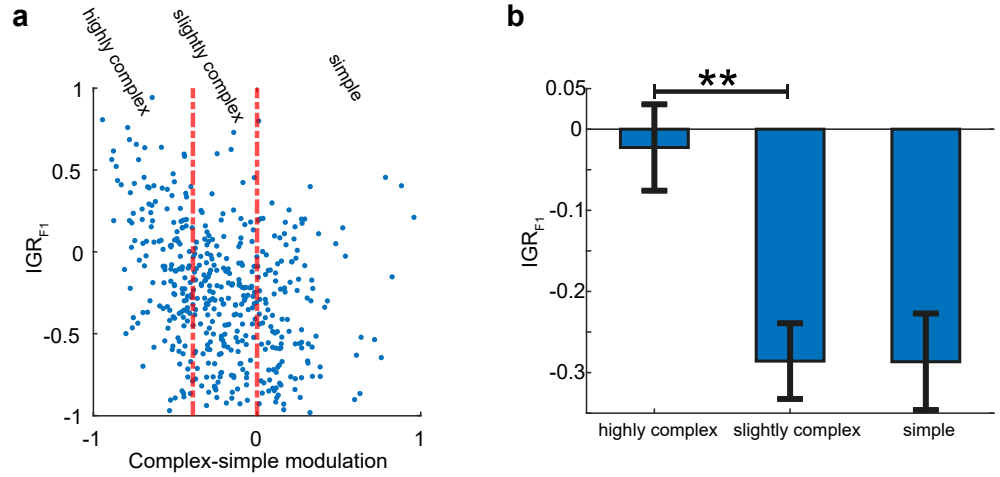


Figure 2.6: Highly complex cells have a stronger F1 response to NCS.

(a) The F1 component of illusory grating responses (Equation 2.3) against complex-simple modulation. Dashed lines determine the grouping of the neurons into simple, complex, and highly complex cells. **(b)** Population average of IGR_{F1} for three groups of cells. Error bars show the 95% confident intervals of the mean.

latency (TPL) from their unfiltered waveform [12, 70, 80]. The distribution of TPL exhibited a bimodal behavior, yielding two classes of narrow-waveform cells (putative inhibitory interneurons, $n=129$ I cell) and wide-waveform cells (putative principal neuron, $n=338$ E cell; Figure 2.7a). AS figure 2.7b shows, the number of evoked cells in response to the NCS is higher for the putative inhibitory population. Moreover, we found that the magnitude of the NCS response of putative interneurons is larger than that of putative excitatory neurons ($p=1.2e-4$, Figure 2.7c). We have also analyzed the F1 component, which replicated the tendency whereby the relative F1 response of putative I cells to NCS is higher than the F1 response of putative E cells. This result indicates a key role for putative inhibitory cells in the neural correlate of neon color spreading.

Furthermore, we compared the phase shift for all F1 dominant cells within putative I/E cells. Figure 2.7e shows the phase shift distribution of putative E cells ($n=147$) localized around 180 degrees, while such a clear phase shift cannot be seen for putative I cells (Figure 2.7f, $n=59$). The response of two putative inhibitory cells with a dominant F1 component is illustrated in Supplementary Figure 2.3, which does not show the 180 degrees of phase shift. However, putative I cells, which are F1 dominant, have a relatively more negative IGR ($IGR=-0.18\pm0.0025$, $n=84$) than other I cells ($IGR=-0.13\pm0.0048$,

$n=45$). Although this difference is not significant ($p=0.321$), it suggests that putative I cells without F1 dominant frequency might have a more outstanding contribution to the neural correlate of NCS. The weaker phase shift in the response of putative inhibitory cells might seem to be contradictory with their higher responses to the NCS illusion. Therefore, more studies are required to address their role in illusory information processing.

2.3 DISCUSSION

In this chapter, we reported single cells responsive to achromatic neon color spreading in the mouse primary visual cortex, indicating the neuronal correlate of illusory brightness in V1. Using electrophysiological recordings, we found that while V1 cells are responding to the illusion (Figure 2.1a), the DBC control condition (Figure 2.1c, the diffusion of dusky gray is blocked by constraining each inducer within two static white circles) does not evoke neuronal responses (Figure 2.3d) despite the exact pixel-wise changes in those stimuli. This finding demonstrates that the response to NCS stimulus cannot be a simple response caused by the local physical changes in the receptive field of V1 neurons but a result of global information processing in the neuronal network. From a phenomenological viewpoint, what can be perceived by a human is that the diffusion of dusky gray into the illusory area is blocked by added circles (i.e., actual contours) in the control condition. Our results consistently show the corresponding neural activities to the decrease in the control condition.

While the NSC has never been studied in an electrophysiological paradigm, the neural correlate of the Kanizsa illusory figure [48] has been extensively studied [41, 60, 86, 105, 116, 117]. However, these studies have focused only on the illusory contours, and the neural correlates of the illusory surface in the Kanizsa figure (which can be perceived as "whiter than white") remain unknown. We analyzed the F1 phase and found that mouse V1 cells respond to the illusory "blackier than black" area in the NCS illusion. These results are consistent with a recent neuroimaging study showing the human primary visual cortex responds to the illusory surface in NCS [101].

Consistent with the previous studies on humans [74], macaques [60], and mice [86], we found that the neuronal response to the illusion is delayed compared to the luminance-defined grating. This result suggests that the process

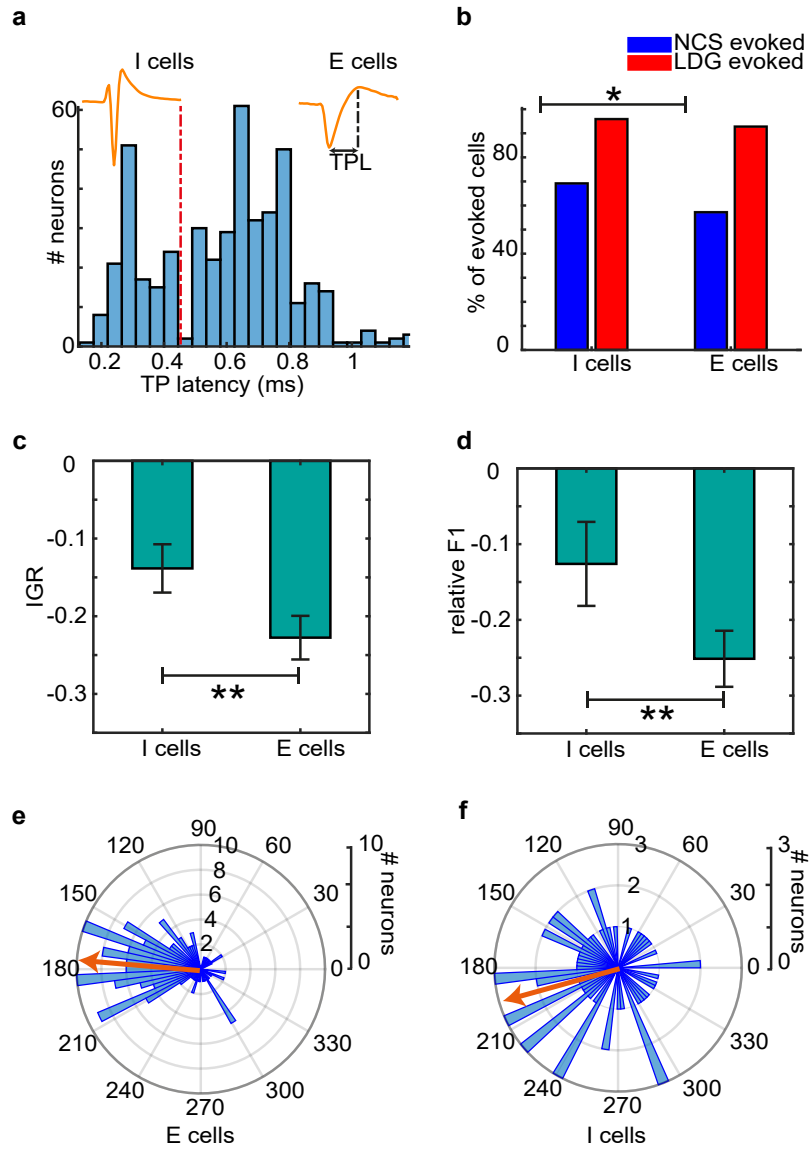


Figure 2.7: Putative inhibitory/excitatory cells and neural correlate of NCS.

(a) The distribution of extracellular waveform TPL values for all recorded units. The dashed red line on the bimodal distribution shows the intersection of two distinct Gaussian distributions. Example spike waveforms are shown for one example unit from each distribution. Those with short TPL are putative interneurons, and those with long TPL are putative pyramidal neurons. **(b)**, The percent of E cells and I cells with visually evoked responses to NCS and LDG stimuli. * indicates $p < 0.05$, chi-squared test. **(c and d)**, The mean IGR and IGR_{F1} magnitude of I cells and E cells (error bar = 95% Confidence Interval). ** indicates $p < 0.0005$ Wilcoxon rank-sum test. **(e)** The phase shift distribution in the NCS responses for putative pyramidal cells with a dominant F1 component. The red arrow shows the circular mean of the data **(f)** The phase shift distribution in the NCS responses for putative interneurons with a dominant F1 frequency.

of NCS illusion requires a more elaborated computation in the brain compared to the corresponding visual processing for LDG.

What types of neurons and connections contribute to the network activity in mouse V1 to process illusory information? Since the local changes in the control condition cannot evoke the neuronal network, one can argue that the response to NCS is not solely due to direct bottom-up activations and that a more global activity within the network is required. This is in line with the previous neuroimaging study on NCS, which suggests the contribution of long-range interactions to process filling-in information in NCS [101]. Moreover, complex cells that receive input from simple cells within V1 and other visual areas [1, 5, 39, 44, 66, 72] have a higher F1 response to the illusory grating. This finding supports the contribution of intra-V1 horizontal connections to the brain's illusory information process.

It has been shown that GABAergic interneurons have a crucial role in the visual gain response, feature selectivity, and visual perception [10, 58, 24, 59, 104, 122]. Moreover, interneurons in V1 are the target of top-down cortical modulations [40, 51, 124]. For instance, while somatostatin-positive GABAergic neurons receive direct inputs from the cingulate (Cg) region of the mouse frontal cortex and contribute to the surround suppression, vasoactive intestinal peptide-positive interneurons contribute to center facilitation [124]. We found a more robust illusory modulation in putative fast-spiking interneurons, which indicates the substantial role of putative interneurons in illusory visual information. However, we found that the small portion of putative inhibitory cells with a dominant F1 response ($n=61$) do not show a clear phase shift to BTB, which remains an enigma that needs to be solved by further investigation. Moreover, we have classified I/E cells based on their electrophysiological properties (i.e., spike waveforms); therefore, the specific role of different types of interneurons and their cortical connections in the perception of NCS remains to be determined. Therefore, further studies are required to address this future question.

The neural mechanism of surround modulation is under debate. Many studies have shown the existence of surround suppression in the lateral geniculate nucleus (LGN), suggesting that some suppression is inherited from LGN through feedforward connections [3, 15, 103, 106]. Nevertheless, optogenetic studies have shown that SM in V1 is not solely due to the LGN afferents, but intra-V1 horizontal connections [2, 102] and feedback connections [124, 81] contribute to the SM in V1. We found that V1 neurons with higher surround

suppression are more activated during illusory information processing, supporting the role of top-down modulation in SM.

2.4 METHODS

We performed experiments on head-fixed adult mice on a disc. The local authorities approved all animal procedures. We collected the data via 32 electrode penetrations in both hemispheres of two C57BL/6 mice (male) and four PV-Cre mice (three males, one female; homozygous for the PV-Cre genes, B6;129P2-Pvalbtm1(cre)Arbr/J).

2.4.1 *Surgical Preparation*

Mice were induced by 2.5% of isoflurane during surgery and held at 1-2%. Also, Atropine (Atropinsulfat B. Braun, 0.3mg/kg) and Buprenorphine (0.1 mg/kg) were administered via subcutaneous injections to relieve bronchial secretions and as analgesics, respectively. The scalp was sterilized and opened to uncover the lambda and bregma sutures. We installed a lightweight head-post onto the skull by applying an adhesive primer and dental cement (Opti-Bond FL primer and adhesive, Kerr dental, Tetric EvoFlow dental cement, Ivoclar Vivadent). A small well was constructed around the uncovered area using dental cement. Two silver wires were embedded between the dura and skull over the frontal lobe as ground references for electrophysiology. Then the uncovered area on the skull was then covered with Kwik-Cast (WPI). The post-surgery analgesic (Flunixin, 4mg/kg) continued to be injected every 12 hours for three days, and antibiotics (Baytril, 5mg/kg) were injected for five days. The five days of recovery were followed by three days of habituation period (0.5 hours/day), where animals were adapted to head fixation and placed on the recording setup in front of the screen.

On the fourth day, a small craniotomy (1 mm²) was created above the V1 at 2.5 mm laterally and 1.1 mm anterior of the transverse sinus [119] under general anesthesia. Electrophysiological recordings were started one day after craniotomy surgery and continued on consecutive days for as long as the unit isolation remained high quality. After each recording, we covered the craniotomy with Kwik-Cast and returned the mouse to the animal facility.

2.4.2 *Electrophysiological Recordings*

Mice were head-fixed on a disc and allowed to sit or run in a dark, electromagnetic isolated room. A 32-channel linear silicon probe (Neuronexus, A1x32-5mm-25-177-A32) was inserted with a perpendicular angle to the brain surface with a depth of 900 μm below the brain surface. We recorded data by amplifying the electrical signals and digitized at 30 kHz by the Cerebus data acquisition system (Blackrock Microsystems LLC) or RHD recording system (Intan Technologies). A photo-diode signal was recorded by attaching it to the screen's lower right corner to capture the exact stimulus onset from a white square synchronized to the presented stimulus.

2.4.3 *Visual Stimulation and Experiment Design*

Stimuli were projected onto a gamma-corrected LED monitor (Dell U2412M, 24 inches, 60 Hz) placed 15 cm before the animal's eye. Visual stimuli were programmed and generated in MATLAB (MathWorks, Inc.) and Psychophysics Toolbox Version 3 (PTB-3). To obtain the receptive field (RF) map of targeted neurons, black squares (15 degrees widths) were presented on a gray background with a duration of 100 ms and an interval of 100 ms in different locations of 8 by 13 grids. The duration of the RF mapping session was 20 minutes. After this section, the response to each square was extracted by analyzing multiunit activity (MUA). Then, the center of the MUA receptive field was estimated by the best fit of a two-dimensional Gaussian to the MUA activities. Subsequent target stimuli were presented on a gray background at the estimated RF center.

To obtain the size-tuning curve, circular patches of moving grating (spatial frequency 0.05 cycles/degree, temporal frequency 3 Hz) with different sizes (2.5, 5, 10, 15, ..., 45 degrees) and two moving directions (rightward and upward) were presented. The duration of the stimulus presentation was 666.7 ms with a 500 ms interval, and the whole session was 25 minutes. For the neon color session, moving grating stimuli under three different conditions were defined as follows: (1) Achromatic neon color spreading (NCS) illusory grating consists of nine patches of white concentric circles (0.1-degree thickness) as inducers (each patch had three circles), arranged on a three by three virtual grid on a black square (35 degrees width). The diameter of the inducers was 3, 6, and 9 degrees, respectively. At each frame, the inter-

section of concentric circles and a moving grating (spatial frequency = 0.05 cycle/degree and temporal frequency = 2 Hz) was replaced with gray segments, resulting in the "blacker than black" illusory grating (Figure 2.1a, Supplementary Movie 1). (2) Diffusion-blocked darkness is a control condition with precisely the same pixel-wise changes as NCS, while each inducer circle is sandwiched by two white circles (0.4 thickness). The added circles constrain the gray filling-in and reduce the illusory effect (Figure 2.1c, Supplementary Movie 2). (3) Luminance-defined grating (LDG) is defined as gray grating, moving on top of inducers (Figure 2.1d, Supplementary Movie 3). These three types of moving grating were generated in eight directions, making 24 conditions. These stimuli were presented with a duration of 1 second for 70 trials in a pseudo-randomized order.

2.4.4 Data analysis

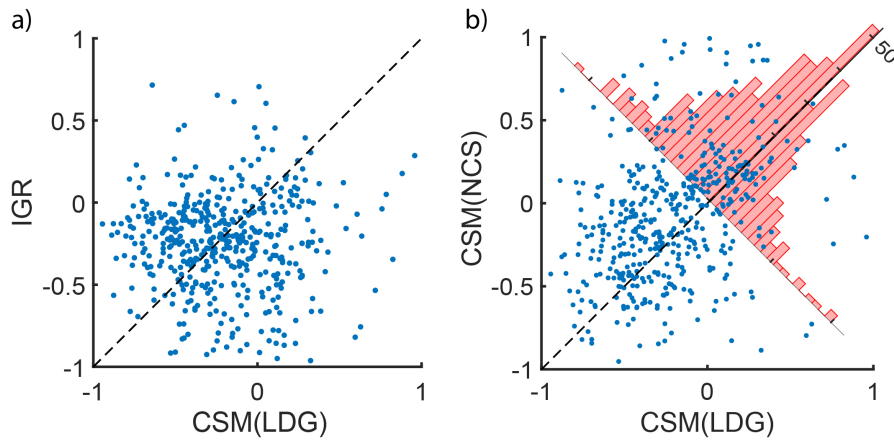
We concatenated the recorded data for spike sorting and clustering in all three experiment sessions (i.e., RF mapping, size tuning, and neon color). We then used the Kilosort algorithm, a template-matching algorithm written in MATLAB for spike sorting, with the default parameters [85]. After this, we conducted a manual clustering for further merging, splitting, and choosing isolated clusters using template-gui [96]. We accomplished all further analyses using built-in functions or in-house written code in MATLAB. The peristimulus time histogram (PSTH) was initially calculated with a resolution of one ms and smoothed by a moving average window of 2 ms. To extract visually evoked neurons and calculate their onset latency, we assumed that the spontaneous neuronal firing rate before stimulus presentation follows a Poisson distribution [68]. Therefore, we fitted a Poisson distribution to 300 ms before stimulus onset and estimated the spontaneous firing rate (λ). If the spiking activity after stimulus onset differs from the background Poisson distribution to a particular level in three successive bins (a probability of $p < 0.01$ for the first two bins and $p < 0.05$ for the third bin), we considered it as an evoked neuron, and the corresponding time for the first bin was regarded as a response latency of the neuron [68]. We defined the preferred angle of cells as the stimulus direction with the maximum response.

To calculate changes in the preferred angle of neurons in NCS and LDG, first, we estimated the direction-tuning curve of the neuron by taking the average response of cells during the stimulus presentation (one second). Then

we interpolated the tuning curve with the spline method to better estimate the preferred angle. To extract the temporal profile of the neuronal responses, we implemented the Fast Fourier Transform (FFT) on the calculated PSTH. We defined the F1 component of the response as the FFT value for 3 Hz, the same as the stimulus's temporal frequency, and the F0 component as the average response.

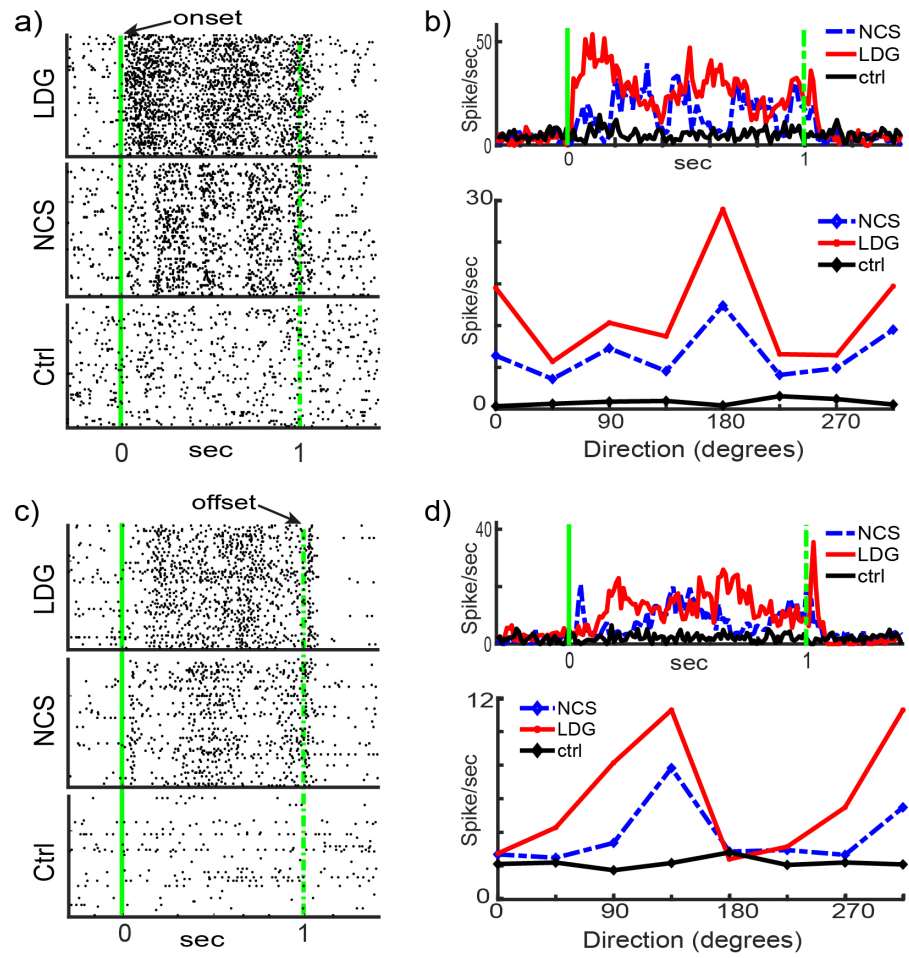
To classify cells into two groups of putative fast-spiking interneurons and excitatory pyramidal cells, we first calculated each neuron's trough-to-peak latency of the spike waveform. Then, we fitted two Gaussian functions to the histogram of TPL, which shows a bimodal distribution. The intersection point of two Gaussian curves was selected as a threshold to classify putative I/E cells (Supplementary Figure 2.4). We used the two-sided Wilcoxon rank sum test for the statistical analysis.

2.5 SUPPLEMENTARY INFORMATION



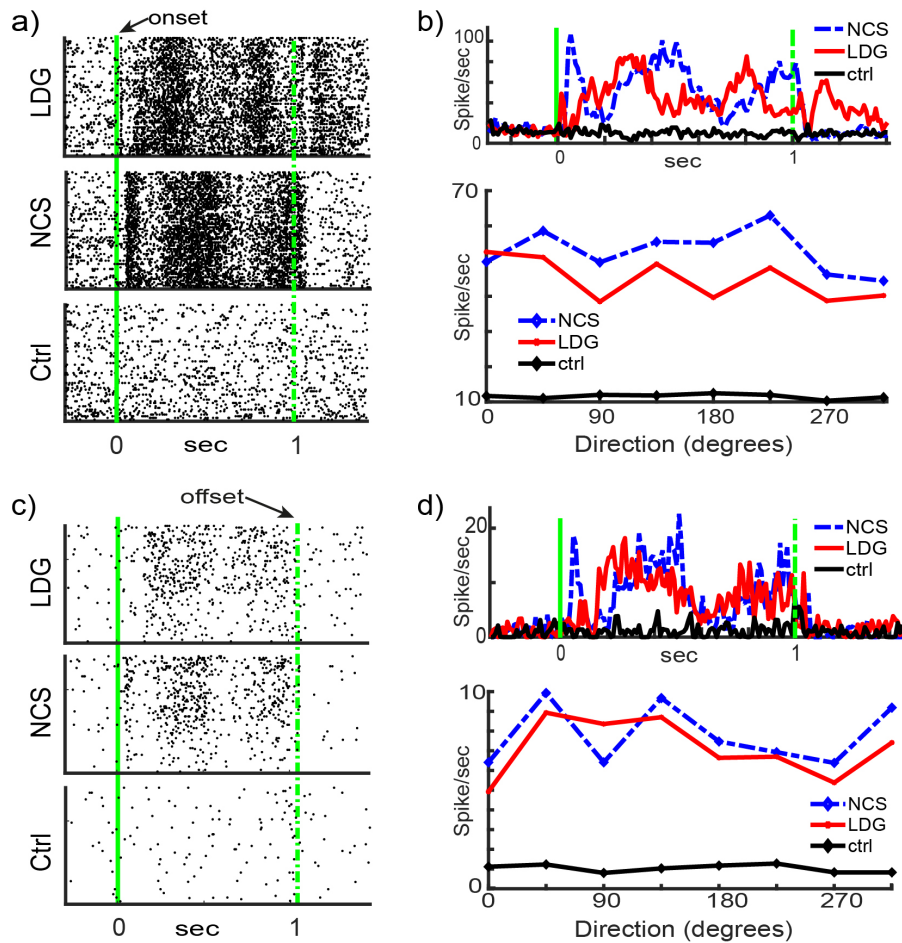
Supplementary Figure 2.1: Complex-simple modulation

(a) illusory grating response versus complex simple modulation (CMS). There is no significant correlation ($r=0.01$, $p=0.8$) between CSM and IGR. the dashed line represents the identity line **(b)** CMS for LDG and NCS, The histogram shows the distribution of scattered data around the identity line.



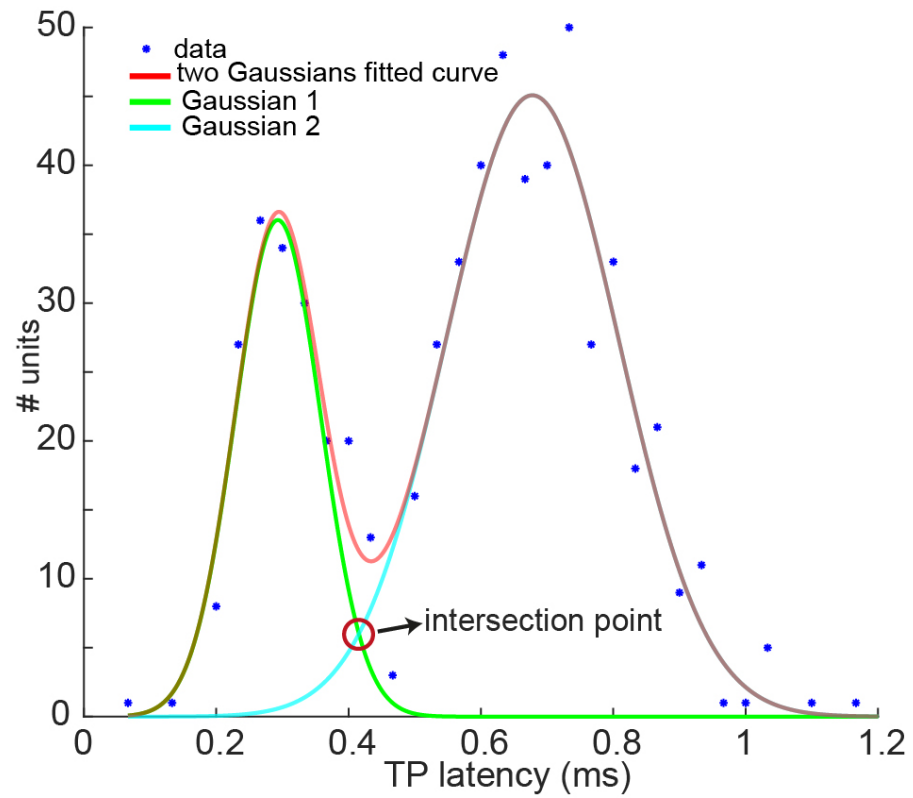
Supplementary Figure 2.2: Example of complex cell responses

(a) Raster plot **(b, top)** PSTH, **(down)** direction tuning for unit Nr. 1067. The green lines represent the onset and offset responses. The CSM index for this neuron is -0.702 in response to LDG and -0.331 in response to NCS, resulting from a bigger F1 response to NCS. **(a)** Raster plot **(b top)** PSTH, **(down)** direction tuning for unit Nr. 61. The CSM index for this neuron is -0.621 in response to LDG and -0.131 in response to NCS. The plot conventions are identical to (a) and (b).



Supplementary Figure 2.3: Example of putative inhibitory cell responses

(a) Raster plot **(b, top)** PSTH, **(down)** direction tuning for unit Nr. 49. The green lines represent the onset and offset responses. **(a)** Raster plot **(b top)** PSTH, **(down)** direction tuning for unit Nr. 159. The green lines represent the onset and offset responses.



Supplementary Figure 2.4: Classification of putative I/E cells

Two Gaussian functions are fitted to the distribution of trough-to-peak latency (TPL), and the intersection point is selected as a threshold. Cells with a TPL shorter than the threshold are considered as narrow-waveform inhibitory cells. Other cells are considered wide-waveform principal cells (i.e., putative excitatory cells).

UNDERLYING CIRCUIT OF NEON COLOR SPREADING IN MOUSE HIGHER VISUAL AREAS

3.1 INTRODUCTION

Optical illusions help study perception by providing a way to manipulate and control the stimuli presented to the observer. By manipulating specific features of an image or visual display, we can selectively activate or suppress different aspects of neural processing and then observe the resulting changes in perception or behavior. This allows for a more precise and controlled investigation of the neural correlates of perception, compared to naturalistic or complex visual scenes that are difficult to control.

In the previous section, I presented data from mouse electrophysiology, where I showed that mouse V1 neurons respond to illusory brightness (which we called "darker than black") and found different characteristics of illusion-responsive cells in mouse V1. However, those findings were limited to the primary visual area, and to obtain a broader view of the neuronal representation of illusory brightness, we need to study higher visual areas, too. Studying illusion perception in mice higher visual areas can provide valuable insights into the underlying neural mechanisms of perception in humans.

Moreover, higher visual areas of the brain are involved in processing complex visual stimuli, such as object recognition and face perception. Understanding the neural correlates of perception in these areas can help us understand how the brain encodes and processes visual information. Furthermore, studying the neural correlates of illusory brightness in mice can provide insights into the development of visual perception. Mouse models allow for precise optogenetic manipulations, which can be used to investigate the role of specific visual areas in the underlying mechanism of illusion processing and can have implications for understanding perception in humans and developing treatments for visual disorders.

In this part of the results, I present a new set of experiments where we expand our investigation to lateral higher visual areas. We improve some stimulus features with more controllability to isolate neuronal response to the

illusory brightness. We show that the illusion-evoked responses in the latero-medial visual area appear earlier than the primary visual cortex, suggesting a critical role in illusion processing and the possibility of giving feedback to V1 and/or other visual areas during illusion processing. Therefore we have tested this possibility and confirmed that the optogenetic inhibition of LM reduces the V1 responses to illusory darker-than-black grating spreading illusion and has no effect on V1 responses to a luminance-defined grating.

3.2 RESULTS

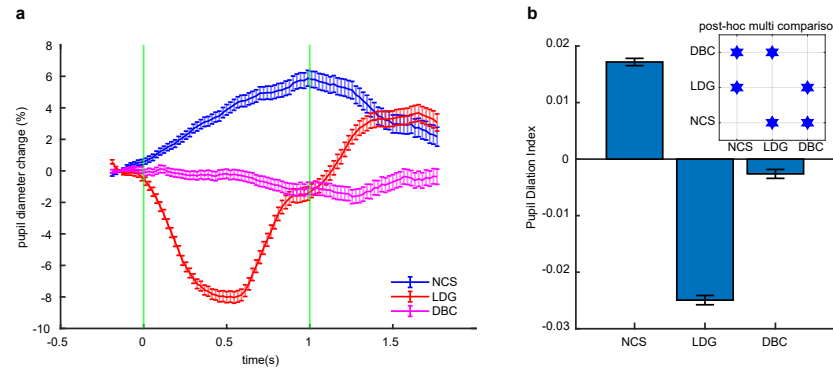


Figure 3.1: Mouse pupil responds to illusory brightness.

(a) Percentage of average pupils' diameter changes in response to different stimuli. (b) Mean pupil dilation index ($\frac{D(stim) - D(pre)}{D(stim) + D(pre)}$) in three conditions. A positive value shows pupil dilation and a negative value represents pupil constriction. **Inset;** Post hoc multi-comparison of dilation index in different conditions. Stars represent the significant difference of $p < 0.05$. The number of mice = 6.

3.2.1 Pupillary responses to illusory brightness as a putative measure of perception in mice.

We previously demonstrated single-cell V1 correlates during the processing of illusory brightness in mice. However, assessing whether these neuronal responses drive "perception" of the illusion in mice is challenging. To address this issue, we investigated the pupillary light response (PLR) as an indirect and putative measure of illusory brightness perception in mice. We utilized the reflexive response of the pupil to light, which has been shown to be modulated by the subjective perception of brightness and darkness in humans

[14, 55, 75, 123]. For instance, Binda et al. [14] have shown pupil constriction in response to a picture of the sun compared to a control picture with the same luminance in which the perception of the sun is eradicated. Furthermore, recent work has shown that visual illusions that humans perceive as subjectively bright can also evoke pupil constriction in rats [115].

We anticipated observing pupil dilation in response to the illusory brightness in the achromatic neon color spreading, which humans perceive as "darker than black" gratings relative to a control stimulus with luminance-defined gratings. Importantly, the pupil should not dilate in response to diffusion-blocked control stimulus since a human observer does not perceive illusory grating in this control condition. In a group of six mice, we observed pupil dilation in response to NCS stimuli, whereas it constricted in response to LDG stimuli (Figure 3.1a). This finding is consistent with the opposing perceived brightness changes in humans viewing these stimuli. We calculated a pupil dilation index ($\frac{D(stim)-D(pre)}{D(stim)+D(pre)}$) and found a significantly different PLR in the NCS compared to the diffusion-blocked control (DBC) stimulus despite the exact temporal luminance changes in both conditions (Figure 3.1b)). Our results suggest that the opposing responses of the mouse pupil to the NCS and LDG stimuli provide a reflexive behavioral report that supports the perception of the NCS illusory grating by mice. These findings demonstrate the usefulness of the PLR as an indirect measure of perception in mice and its potential to study visual illusions.

3.2.2 *Different visual areas respond to illusory brightness.*

We have performed electrophysiology on head-fixed mice on a disk where they could move or stay immobile (Figure 3.2a). We used 64 channels electrode array with an oblique insertion angle into the cortex to simultaneously record from four lateral visual areas, including V1, lateromedial (LM), laterointermediate (LI), and laterolateral (LL; see Figure 3.2b). We recorded 5058 single units from 13 awake mice passively viewing visual stimulation on the monitor. The experimental design has been thoroughly explained in the methods section. Briefly, we started the recording with a receptive field (RF) mapping section, followed by the analysis of multi-unit activities where we identified borders of different lateral visual areas relative to the recording channels along the electrode (Figure 3.2c and d. After receptive field mapping and detection of lateral visual areas, we started the main experiment, where we

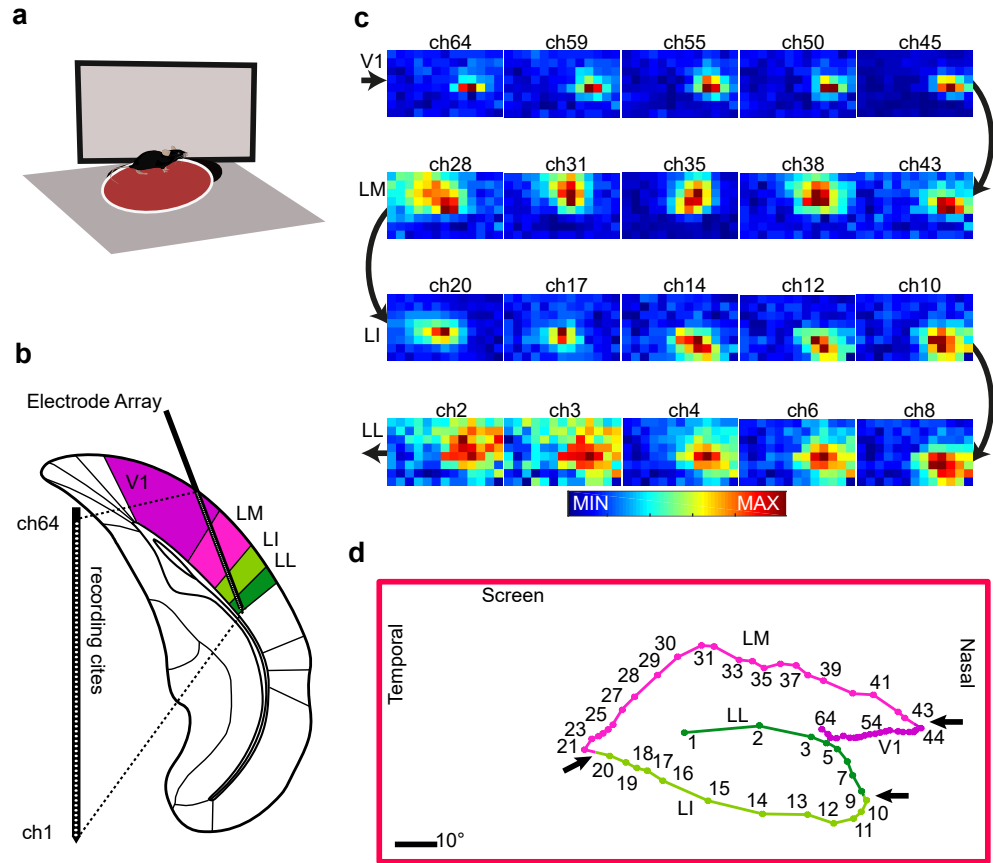


Figure 3.2: Simultaneous multi-area recording in mice.

(a) Recording setup where the mouse is head-fixed on a disk and observes the presented stimuli on the screen. (b) Oblique insertion of the laminar electrode into the visual cortices for multi-area recording. (c) Example of multi-unit responses to the presented squares in different locations on the screen. Each panel shows the multi-unit response of one recording site along the electrode. (d) Center of the multi-unit receptive field on each recording channel on the screen. Black arrows show the RF centers' reversal points, indicating borders of visual areas.

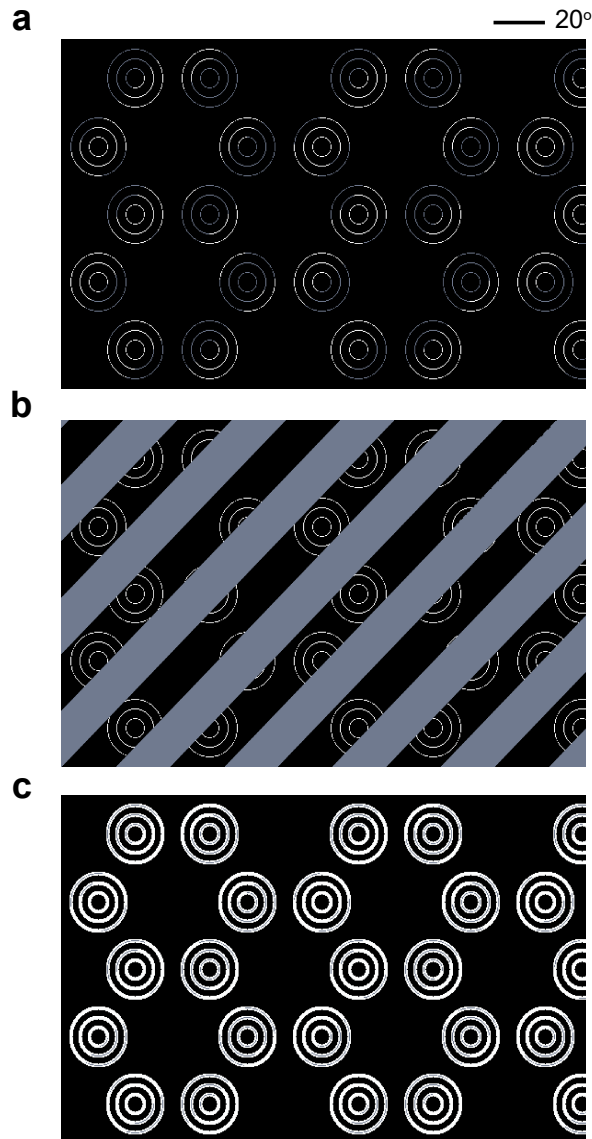


Figure 3.3: Stimulus design.

(a) Achromatic neon color spreading (NCS), designed to generate illusory drifting grating, which seems to be darker than black to a human observer. **(b)** Luminance defined grating (LDG) stimulus presented to the mice as a control condition to contrast the illusion-evoked responses with the responses to a physical stimulus. **(c)** Diffusion-blocked control (DBC) is designed to block the filling-in effect and weaken the illusory grating.

recorded the response of the neurons to three stimulus types, as explained in the previous chapter. However, we made a few changes in the stimulus to tailor it for multi-area electrophysiology (Figure 3.3, Supplementary Movies 4-6). First, we used a full-screen version of the stimuli to stimulate neuronal responses in a broader region of the visual cortices. Second, we arranged the white concentric circles on a honeycomb structure and positioned them at further distances compared to the stimuli in the previous study. Third, we have decreased the thickness of blocking circles in the diffusion-blocked control (DBC) condition compared to the one used in the previous experiment presented in the last chapter. Thinner blocking circles are not capable of completely blocking the diffusion of dusky gray into the illusory area but weaken the effect (Supplementary Movie 6). These three changes bring much more freedom to analyze and explore the neuronal correlate of illusion processing in the brain, which I will present in the following sections. We presented these three types of stimuli to the mouse in a pseudorandom sequence.

The very definition of separated visual areas is based on the fact that each area covers a full retinotopic map of the visual field. In species with well-developed vision (e.g., primates), these areas show distinct functional properties in response to visual stimulation and more clear structural differences than rodents. Such a functional difference also can be found in mice [65]. Here, we took advantage of the functional definition of visual areas to detect them. We used the receptive mapping session data to detect the visual areas to which each neuron belongs. First, we captured the multi-unit receptive field location for every recording site. As shown in Figure 3.2c and d, the receptive location on the screen shifts back and forth along the electrode. Every time the receptive field location on the screen shifts in the opposite direction, it indicates that the electrode sites enter another visual area. Therefore, we used the reversal points of the receptive field location on the screen as the indicator to identify recorded visual areas along the probe (Figure 3.2d).

We found that all four recorded visual areas, namely, V1, LM, LI, and LL, respond to neon color spreading stimuli and illusory brightness. Some example neurons are shown in Figure 3.4. We recorded 1807 single units from V1, where 1522 single units had stimulus-evoked activities. Among those visually evoked cells, 93.1% responded to LDG, 66.6% responded to NCS, and 12.6% responded to DBC stimuli. In LM, 2012 single cells out of 2401 recorded single cells were visually responsive, in which 92.6% responded to LDG, 70.4% responded to NCS, and 10.2% responded to the DBC. From

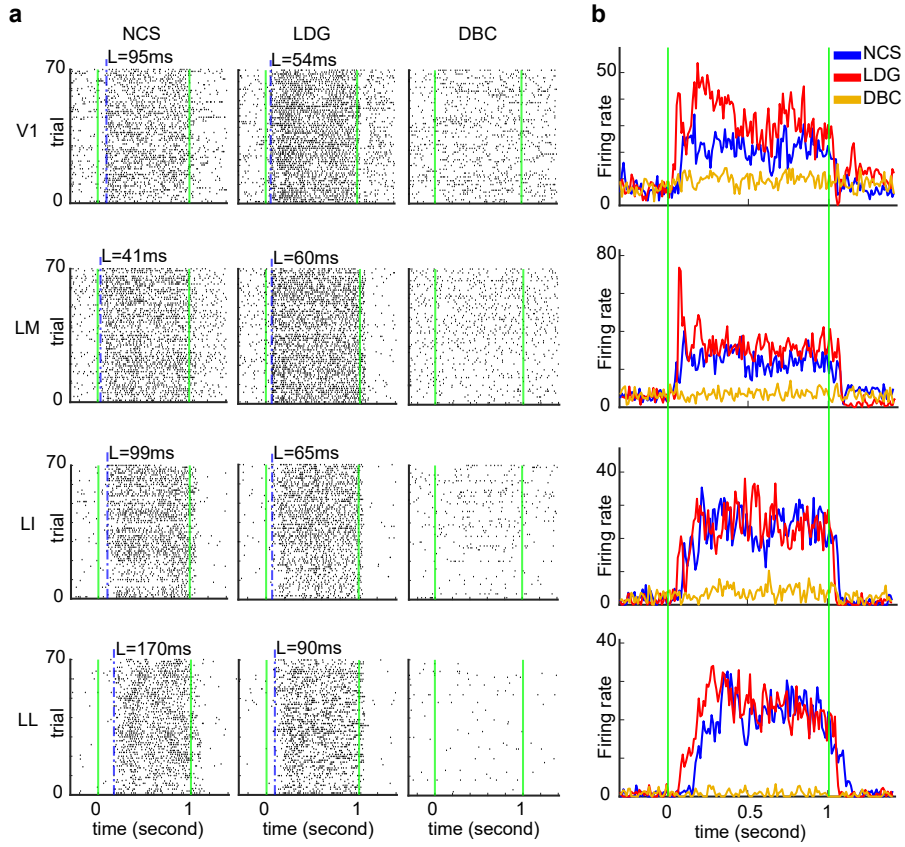


Figure 3.4: Responses of example neurons from different visual areas.

- (a)** Raster plots show the response of four example neurons from four visual areas to the three types of stimuli (NCS, LDG, and DBC). Green lines represent the stimulus onset and offset. The dashed blue lines show the estimated response latency of neurons to the corresponding stimuli.
- (b)** The peristimulus time histogram (PSTH) of the neurons shown in (a). The green lines show the stimulus onset and offset.

683 recorded single units in the LI, 512 single units had stimulus-evoked responses, where 91.7%, 56.4%, and 2.5% were responding to LDG, NCS, and DBC stimuli, respectively. We also recorded 168 single units from LL, and 95 of them were visually evoked, in which 91.5%, 52.6%, and 7.3% responded to LDG, NCS, and DBC stimuli, respectively. The percentage of evoked responses to the neon color spreading illusion in different visual areas exhibit inter-area differences, while such differences can not be seen in the case of LDG-evoked cells. Using the bootstrap method and Kruskal-Wallis test, we have statistically tested the differences in the percentage of NCS-evoked populations in the recorded visual areas ($H(3)=155.5$, $p = 1.7e-23$, see Supplementary Figure 3.1). We found that the percentage of the NCS-evoked population in LM is higher than in V1, and V1 has a larger population of NCS-responding cells compared to the LI and LL. However, we did not observe a significant difference in the percentage of NCS evoked population between LI and LL (Figure 3.5a). This finding suggests the potential role of LM neurons in illusory brightness processing.

The DBC stimuli in the previous study totally blocked the filling-in effect, and therefore, we observed no DBC-evoked responses. However, in this study, we used a narrower blocking ring in the DBC stimulus, which only weakens the precept of illusory grating to a human observer (Supplementary Movie 6). We found that a small proportion of recorded neurons in different visual areas respond to the DBC stimuli. We use the DBC-evoked activities as a filter and consider those DBC-evoked neurons as the most sensitive neurons to the small temporal changes in their receptive field and exclude them from later analysis (Figure 3.5a). We also checked the direction selectivity of recorded neurons. We found that the majority of the neurons preserve their preferred angle in response to NCS compared to LDG (Figure 3.5b). For a more detailed graph, please see Supplementary Figure 3.2, where we have plotted the changes in preferred angles for each area separately. This is consistent with our findings in the previous chapter.

Moreover, as the average population PSTHs in Figure 3.5a-f show, the magnitude of NCS responses compared to the LDG responses in LM and LL is higher than other areas. We have confirmed this by calculating the correlations responses to LDG and NCS and found that LL has the higher NCS-LDG correlation (Pearson correlation, $r = 0.72$), and LM had a correlation of $r = 0.62$, while the correlations in V1 and LI were 0.49 and 0.41, respectively (Figure 3.6). This

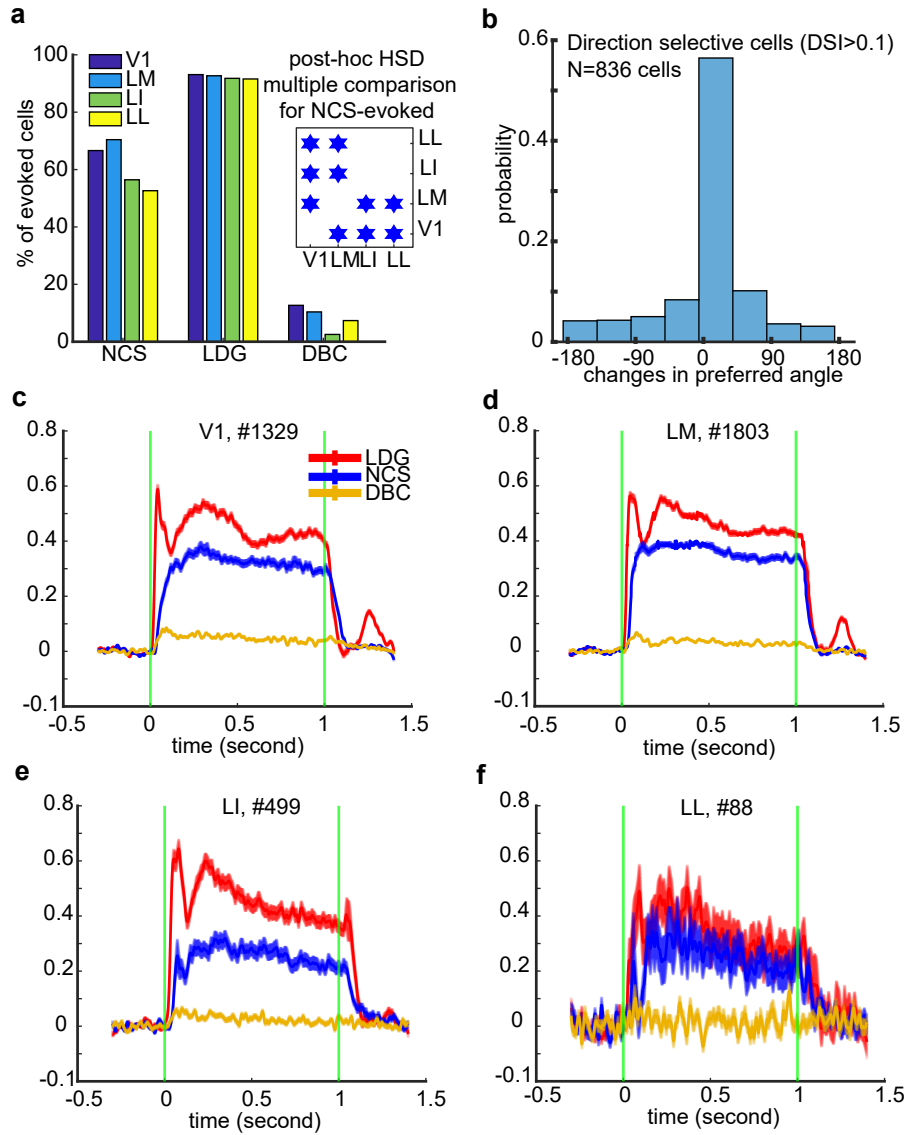


Figure 3.5: Population responses of different visual areas.

(a) The bar plot shows the percentage of stimulus-evoked cells in different visual areas and stimuli. The inset plot shows the result of post hoc analysis on the percentage of NCS-evoked cells in different areas. Stars represent $p < 0.05$ **(b)** Bar plot shows the distribution of changes in the preferred angle of neurons in response to LDG and NCS. **(c-f)** Averaged population PSTH of neuronal responses in different areas. All PSTHs have been normalized by the max value of the LDG responses. The green lines represent the stimulus onset and offset. DBC-evoked neurons have been excluded.

correlation comparison of the response amplitudes highlights the role of LM and LI in illusory brightness processing.

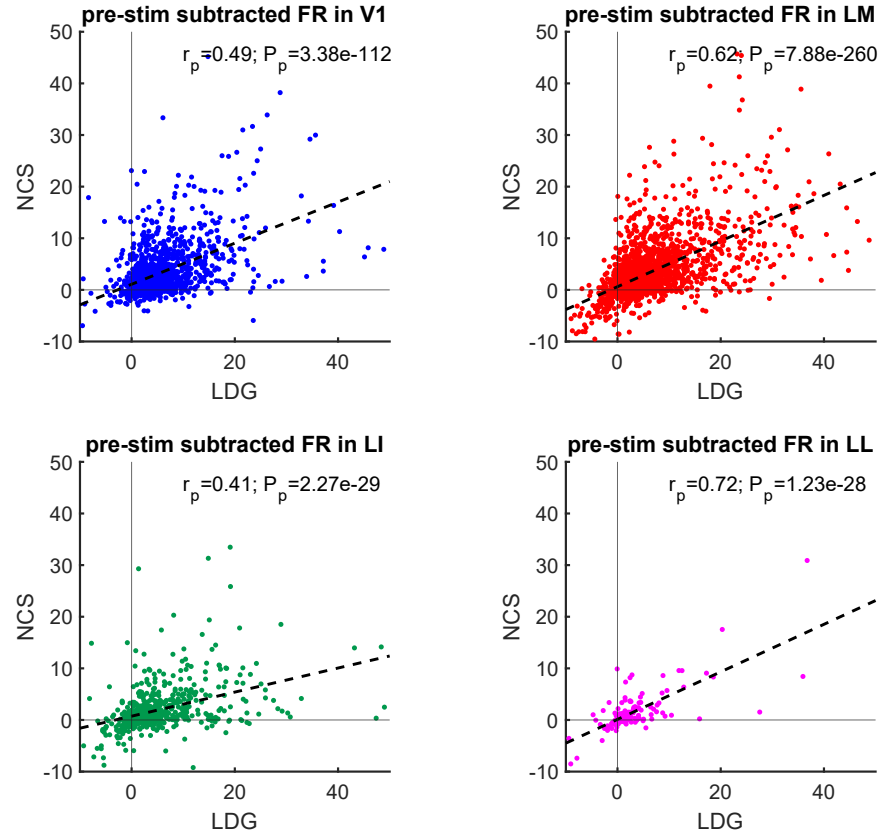


Figure 3.6: population responses to NCS against LDG stimuli.

The scatter plots show the pre-stimulus normalized spiking activities of recorded neurons in four visual areas. The black dashed lines represent a least-square fit on the data.

3.2.3 Functional hierarchies of illusion processing circuits in mouse visual areas

To infer the functional neural circuit that processes the illusory information (in our case, it can be the illusory brightness of the illusory surface), we need

to have an estimation of the hierarchical structure of the circuit alongside the population contributing in each area. Moreover, in the previous chapter, we showed that NCS response latencies are delayed compared to LDG response latency. This indicates the demand for more brain computation to process such ambiguous scenes to extract a clear picture of the illusory grating. Therefore, we further examined the functional hierarchy of the NSC processing circuit using analysis of response latencies, which has been considered one of the primary indications of functional hierarchies [56]. We have calculated the response latency of neurons to each stimulus type using their preferred angle responses, which was determined as the angle with maximal LDG responses. Moreover, we have excluded simple cells as calculating their response latency for a drifting grating is not viable due to their sensitivity to the stimulus phase.

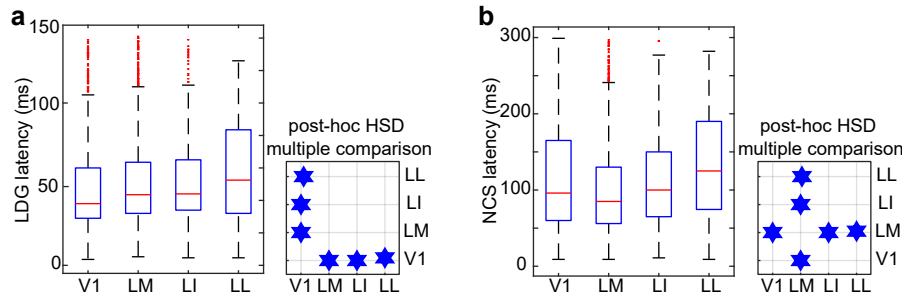


Figure 3.7: Response latencies reveal the hierarchical organization of illusion processing.

(a) LDG response latency for the neurons in different visual areas (Left) and the results of post hoc Tukey-Kramer's honest significant difference (HSD) multi-comparison test (right). Stars show a significant level of $p < 0.05$ **(b)** NCS response latencies in different visual areas and related statistical test results. Plot conventions are identical to (a)

As we have shown in Figure 3.7a, we found that in the case of response to luminance-defined grating, V1 neurons are faster than other areas (Kruskal-Wallis test $H(3) = 33.22, p = 2.89e-7$, Supplementary table 1) and as we go to higher visual areas the average response latency increases, which we expected and is consistent with our previous knowledge. Importantly, we observed a different hierarchical organization in response to NCS. We found that the average response latency of neurons in LM is shorter compared to other visual areas (Kruskal-Wallis test $H(3) = 22.69, p = 4.67e-5$, Supplementary table 2). Our findings suggest that LM neurons may trigger the NSC responses and

send feedback to V1 neurons during the illusory information processing in the mouse brain.

3.2.4 *Filling-in signal in the mouse visual cortices*

In the previous chapter, we showed that V1 neurons respond to illusory brightness in the achromatic neon color spreading illusion, but no stimulus-evoked was observed for the DBC condition despite the exact temporal changes in the NCS stimuli. Therefore, we concluded that those small physical changes in the stimulus alone could not trigger neuronal activities in the NCS-evoked responses. However, to study the filling-in mechanism, we need to examine a subset of neurons whose receptive field is not directly stimulated by the physical changes of the neon color spreading illusion. However, the stimulus we used in the previous experiment was not large enough (remember that the stimulus width was 35 degrees while the average diameter of mouse V1 neurons was 20 degrees) to capture such neurons. In the current study, we have used a fullscreen stimulus in which the diameter of the illusory area between the inducers was 39 degrees. Thus, we could find non-intersecting neurons in which the receptive field did not overlap with the inducer circles. An example of a non-intersecting neuron is shown in Figure 3.8 (for more examples from other visual areas, please see Supplementary Figures 3.3, 3.4, and 3.5).

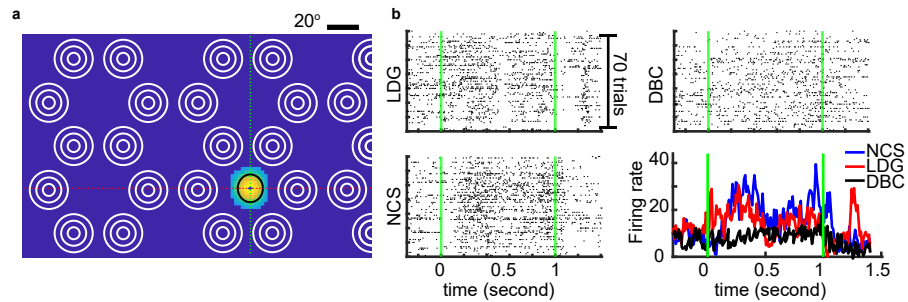


Figure 3.8: Example of non-intersecting RF/inducer neuron in V1.

(a) Receptive field location of a non-intersecting cell relative to the inducer circles. The black ellipse shows half width at half height for a two-dimensional Gaussian curve fitted on the receptive field. **(b)** Raster and PSTH in response to different stimuli for the neuron showed in (a). the green lines show the stimulus onset and offset.

We isolated 714 non-intersecting single units (234 single units in V1, 286 single units in LM, 97 single units in LI, and 21 single units in LL). As we have

shown in Figure 3.9a, the population of LDG-evoked responses of these neurons was not different in four visual areas (94%), while the NCS-evoked population in LM was substantially higher than other visual areas (50.4% in V1, in LM, 50.5% in LI, and 38% in LL were responding to NSC). Moreover, these neurons respond to the illusory drifting grating with the same preferred angle as LDG drifting grating. The comparison of the NSC versus LDG response intensity in different visual areas highlights the role of LM and LL as NCS-evoked response in those two areas are higher than LI and V1 (Figure 3.9). Interestingly, in the sub-population of non-intersecting neurons with dominant F1 frequency (2 Hz; the F1 frequency component is the corresponding frequency of the PSTH to the temporal frequency of drifting grating), we observed anti-phase activity in V1 but not in LM (Figure 3.9d). In other words, the NSC-evoked response of V1 neurons is out of phase compared to LDG responses, which is strong evidence for the V1 neurons responding to the illusory brightness (i.e., "darker than black") and is further confirmation of our claim in the previous chapter (Figure 3.9d, top). However, the phase shift for LM neurons has a bimodal distribution in which one mode exhibits no phase shift, and the other mode shows the phase shift (Figure 3.9d, bottom).

3.2.5 *Top-down modulation contributes to the illusory brightness processing in V1.*

So far, we have shown that NCS-evoked neuronal responses in LM appear earlier than in other visual areas, and a larger population of neurons in LM respond to the illusory grating. Moreover, the NCS response amplitude in LM and LL was more correlated to the LDG response amplitude. These findings propose the existence of top-down feedback from higher lateral visual areas to V1 during the illusory brightness processing. Additionally, it has been shown that top-down modulation from LM contributes to subjective contour representation in the mouse V1 [86].

To test whether feedback from higher visual areas contributes to the neuronal representation of illusory brightness in V1, we performed optogenetics experiments simultaneously with electrophysiological recording in awake mice. To inhibit HVAs, we activated the parvalbumin-positive (PV+) interneurons in LM and LI, which is analogous to the "ventral" pathway in primate [108]. We injected adeno-associated virus (AAV5) expressing Channelrhodopsin-2 (CHR2) gene into HVAs of a knock-in line of PV-Cre mouse (see Methods)

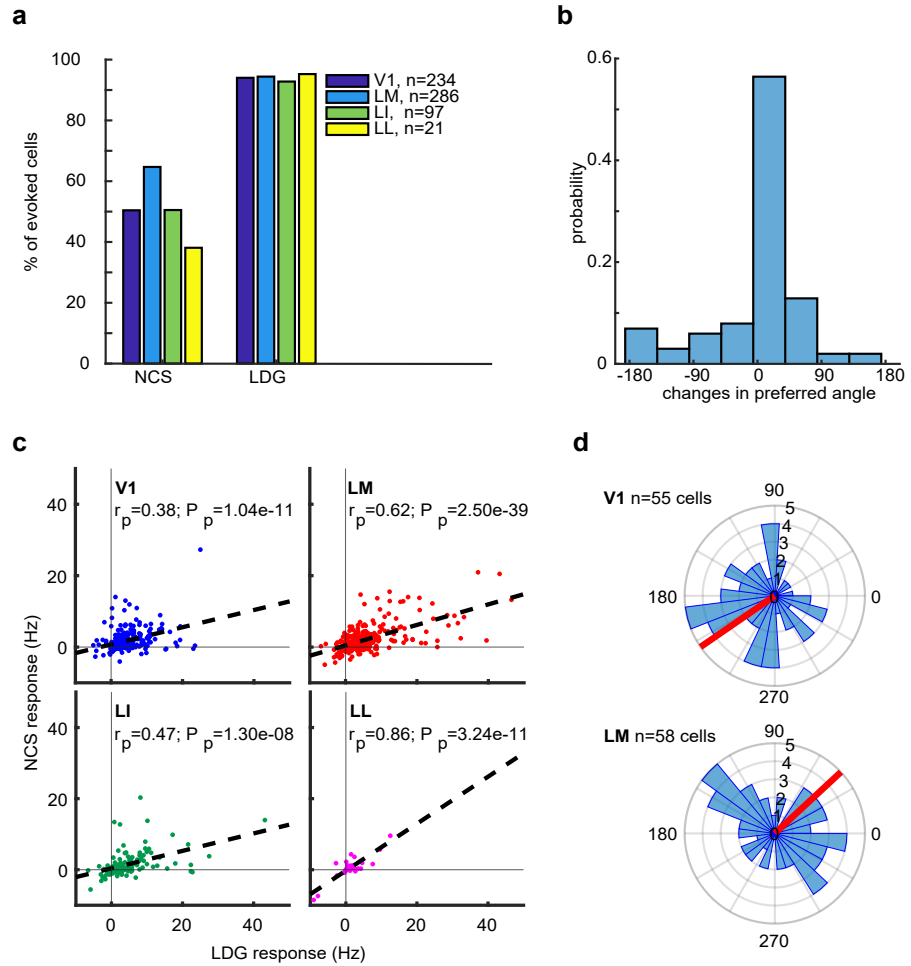


Figure 3.9: Response properties of non-intersecting RF/inducer neurons.

(a) The bar plot shows the percentage of non-intersecting stimulus-evoked cells in different visual areas and stimuli. (b) Bar plot shows the distribution of changes in the preferred angle of neurons in response to LDG and NCS. (c) The scatter plots show the response firing rate of non-intersecting neurons to LDG and NCS stimuli. The dashed lines represent a least-square fit on the data. (d) The polar histograms show the circular distribution of the F1 phase shift between LDG and NCS responses. The red line shows the circular mean of the data

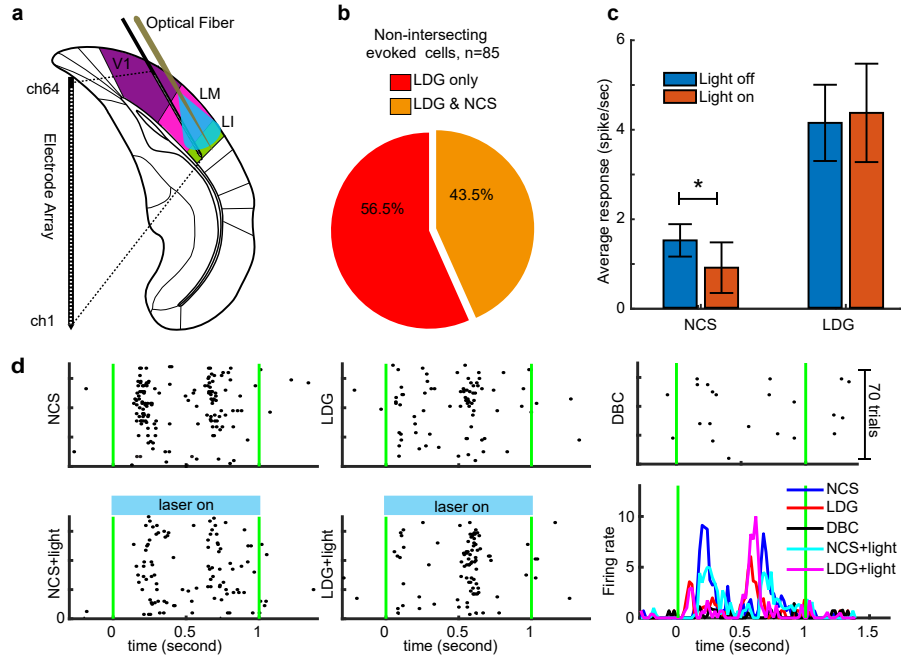


Figure 3.10: Contribution of top-down modulation to illusion processing in Mouse V1.

(a) Oblique insertion of a single-shank electrode array combined with optical fiber, targeting mouse lateral visual areas for simultaneous electrophysiological recording and optogenetics manipulation. The silicon electrode array contained 64 recording channels spanning 1275 μm . The optical fiber had a 1.5 mm tapered tip and 200-core diameter. **(b)** The pie chart shows the percent of non-intersecting RF cells ($n=86$) that responded to the various stimuli. **(c)** The bar plot shows the average firing rate (baseline subtracted) across four conditions. (error bars show the 95% confidence interval of the mean). * indicates $p<0.005$ Wilcoxon rank-sum test. **(d)** Raster and PSTH of one example neuron from V1 in different condition. the green vertical lines show the stimulus onset and offset. Number of mice = 6.

[59, 25]. Four weeks after the injection of the virus, we performed electrophysiological recording using a 64-channel silicon probe combined with a tapered fiber implanted with an angle about 30 degrees into the cortex, covering V1, LM, and LI (Figure 3.10a). After receptive field mapping and identifying the borders of visual areas, we optogenetically inhibited higher visual areas. The combined tapered fiber enabled site-selective light manipulation [57], where we set the upper bound of light to limit the light leakage onto V1 and ensured that activity recorded from channels located in V1 was not affected directly by the light. We recorded 653 single units from V1, 921 single units from LM, and 241 single units from LI in six PV-Cre mice. As shown in Supplementary Figure 3.6, LDG neuronal responses in LM and LI have been suppressed by optogenetics inhibition. We found 85 non-intersecting neurons in V1, of which 43.5% (n=27) responded to the NCS illusion (Figure 3.10b). Importantly, we found that inhibiting higher lateral visual areas (LM and LI) weakens the V1 cell's responses to illusory brightness (Wilcoxon rank-sum test: $Z=3.19$, $p=0.0014$) but has no significant effect on the physical luminance responses (Figure 3.10c). The raster and PSTH of an example V1 neuron are plotted in Figure 3.10d. These results indicate the partial contribution of LM and LI to the illusory brightness representation in V1 through a top-down feedback signal.

3.3 DISCUSSION

Our study provides novel insights into the neural mechanisms underlying illusory brightness processing. We found that single neurons in higher lateral visual areas and V1 can respond to illusory drifting gratings and brightness, even when no direct receptive field stimulation exists. This suggests that these areas play an active role in generating and integrating visual information to produce the subjective surface representation in the brain.

Nevertheless, based on only neuronal responses, we can not directly claim that the mice can perceive the illusory brightness of NCS as humans perceive. Therefore, we have analyzed pupil responses as a putative measure of perception in rodents [115], similar to human pupil responses during brightness perception [14, 55, 75, 123]. We found that mouse pupils dilate in response to the illusory drifting grating, which seems to be darker than black, and there are no pupil responses to the DBC stimuli, although both NCS and DBC stimuli had exactly similar temporal dynamics (Figure 3.1). This finding

supports the perception of illusory brightness in mice. However, a behavioral task where the mouse can actively report the perception is required to have solid evidence for the perception of illusory drifting grating in mice, similar to humans. It can be done in a go/no-go paradigm in which the mouse observes and reports the drifting direction of stimuli.

Interestingly, we observed that the percentage of NSC-responsive neurons was higher in LM than in other visual areas (Figure 3.5), and the illusion responses appeared earlier in LM (Figure 3.7). This suggests that LM may be essential for processing illusory visual stimuli and integrating information from multiple sources to generate the illusion. Given that Mouse LM is analogous to primate to the secondary visual area (V2), our findings related to the illusory brightness response latency are consistent with monkey studies on illusory contours where they found V2 responds to the subjective contours earlier than V1. On the other hand, LM neurons did not show an overall phase shift corresponding to the inverted phase NCS stimulus, while the phase shift distribution for V1 neurons was well localized around 180 degrees (Figure 3.9). Putting these findings together, we suggest a feedback mechanism for the illusory brightness processing in the mouse visual cortex where LM neurons gather information on a larger scale on the visual scene and feed the V1 neurons with that information. This feedback signal to V1 is required to increase the resolution and complete the inverted brightness illusion.

Furthermore, our optogenetic inhibition experiments showed that inhibiting LM and LI weakened the neuronal responses to the illusion but did not affect the physical stimulus (Figure 3.10). This finding confirms the existence of the feedback signal and indicates that LM and LI actively integrate the illusory cues rather than simply responding passively to visual input. These findings provide additional evidence for the importance of these areas in processing visual illusions. However, the inhibition of LM and LI did not eliminate the NCS-evoked responses in V1, suggesting that either the integration of illusory cues happens in V1 or lower visual areas (e.g., LGN) or the integration happens through feedback from other visual areas around V1.

At last, we found that the correlation between the magnitude of NCS and LDG responses in LL was higher than in other visual areas (Figure 3.6 and 3.9c), but the visually evoked responses were delayed. We expected this as LL is considered to be down on the ventral pathway; the information LL processes are more abstract, and therefore, there should be little difference in their response to NCS or LDG. However, any conclusion about LL remains

speculative due to the very small number of recorded neurons in LL, and further studies are required to test it.

Overall, our results contribute to a better understanding of the neural basis of illusory perception, highlighting the importance of higher lateral visual areas, particularly LM, in generating and integrating visual information to produce the perceptual experience of illusory brightness. Our findings pave the way for further investigations into the neural circuitry underlying visual illusions and will have implications for understanding perception in general.v

3.4 METHODS

We performed experiments on awake head-fixed adult mice on a disc while they could run upon their will. The local authorities approved all animal procedures in compliance with EU Directive 2010/63/EU (European Community Guidelines for the Care and Use of Laboratory Animals). We conducted data acquisition through 69 electrode penetrations in both hemispheres of 3 C57BL/6 mice (male) and 10 PV-Cre mice (4 males, six female; homozygous for the PV-Cre genes, B6;129P2-Pvalbtm1(cre)Arbr/J).

3.4.1 *Surgical operation*

The surgical preparation for the mice involved inducing them with 2.5% isoflurane to anesthetize the animal. Subcutaneous injections of Atropine (Atropin-sulfat B. Braun, 0.3mg/kg) and Buprenorphine (0.1 mg/kg) were administered to minimize bronchial secretions and provide analgesia, respectively. The mouse was kept in Anastasia during the operation by maintaining isoflurane at 1-2%. The scalp was sterilized, shaved, and opened to expose the lambda and bregma sutures. The skull was cleaned by removing the remaining tissues. To provide protection against microleakage and protect the skull against exposure, Optibond primer and adhesive were applied to the open area of the skull(OptiBond FL primer and adhesive). A lightweight head-post was attached to the skull using dental cement (Tetric EvoFlow dental cement, Ivoclar Vivadent). Dental cement was used to create a small well around the exposed area. At the end of the surgical operation, the skull was covered with Kwik-Cast (WPI), and the animals were administered post-surgery analgesic (Flunixin, 4mg/kg) every 12 hours for three days and antibiotics (Baytril, 5mg/kg) for five days. After recovery, the animal was habituated to the exper-

imental setup and head fixation for three days (0.5 hours/day). On the last habituation day, a small craniotomy (1.2 mm²) was drilled above the V1 and LM at 3 mm laterally and 1.1 mm anterior to the transverse sinus under general anesthesia [119]. Electrophysiological recordings began one day after craniotomy surgery and continued consecutively for as long as the neuron isolation remained of high quality.

3.4.2 *Electrophysiology*

Electrophysiological recordings were conducted on head-fixed mice placed on a disc, which were allowed to either sit or run in a dark and electromagnetically isolated room. A 64-channel laminar silicon probe (CambridgeNeuroTech, H9 or H3) was inserted with an oblique angle (30 degrees) penetration distance of approximately 1400 μ m). The captured electrical signals at each site were amplified and digitized at 30 kHz using either the Cerebus data acquisition system (Blackrock Microsystems LLC) or the RHD recording system (Intan Technologies). The recorded raw data with the RHD system was stored in openephys format using OpenEphys software. The recorded raw data with the Blackrock system was stored in NS6 format for postprocessing. Moreover, a photodiode was attached to the lower right corner of the screen to capture the exact stimulus onset from a white square synchronized with the presented stimulus. The photodiode signal was transferred to the acquisition system and stored with a sampling frequency of 30 kHz. Following each recording, the craniotomy and the skull were sealed with Kwik-Cast, and the animal was sent back to the animal facility.

3.4.3 *Pupillometry data acquisition*

Using near-infrared illumination (Thor Labs LED, M850L3 and Thor Labs Collimation optics, COP4-B), videos were captured from the mouse eye at a rate of 45 frames per second. The frames were captured using a near-infrared camera (Allied Vision, G-046B) with a variable zoom lens, fixed 3.3x zoom lens, and 0.25x zoom lens attachment (Polytec, 1-60135, 1-415 62831, 6044). The image acquisition was carried out via a GigE connection using the MATLAB image processing toolbox. With each video frame, the camera provided a TTL pulse recorded directly into the neurophysiology system to be used to align pupillometry data with stimulus presentation in further analysis.

3.4.4 *Experimental design*

To present the stimuli to the head-fixed mouse, we used a gamma-corrected LED monitor (Dell U2412M, 24 inches, 60 Hz) positioned 12 cm before the animal's eye (covering 130.7 degrees of the visual field). We generated the visual stimuli using MATLAB (MathWorks, Inc.) and Psychophysics Toolbox Version 3 (PTB-3). After electrode insertion, we start the recording with a receptive field mapping session, which is necessary to locate the receptive field location of the recorded neurons. The receptive field mapping was done by presenting black squares (15-degree widths) on a gray background in various locations of an 8-by-13 grid. The receptive field mapping session lasted for 20 minutes, during which the response to each square was recorded and was followed by an analysis of multi-unit activity (MUA) to quickly confirm the proper depth and location of the electrode as well as the quality of signal in each recording site. A two-dimensional Gaussian was fitted to the MUA activities to estimate the center of the MUA receptive field in different recording sites. We used the receptive field center of the MUA response to locate the corresponding visual area for each channel. We identified borders of distinct visual areas by finding reversal points of MUA RF centers on the screen (Figure 3.2).

After receptive field mapping, we started the main experiment and recorded neuronal responses to three drifting grating stimuli to study the achromatic version of the neon color spreading illusion. The first stimulus type was Neon Color Spreading (NCS), consisting of white concentric circles (inducers) arranged as a honeycomb structure with a fullscreen size. The thickness of the inducers was 0.4 degrees, and their diameter was 8, 16, and 24 degrees, respectively. The arrangement of concentric inducers with a honeycomb structure provided a maximal illusory area between each patch of inducers with a diameter of 39 degrees in diameter. At each frame, the intersection of concentric circles and a drifting grating (spatial frequency = 0.05 cycle/degree and temporal frequency = 2 Hz) was replaced with gray segments, resulting in the "darker than black" illusory grating. The second type was the diffusion-blocked stimulus, which served as a control condition with the same pixel-wise changes as NCS but with each inducer circle sandwiched by two white circles (0.8 degrees of thickness), constraining the gray filling-in and reducing the illusory effect. The third type was the Luminance-Defined Grating (LDG), defined as a gray grating moving on top of inducers. These three types of

drifting grating were generated in eight directions, resulting in 24 conditions. These stimuli were presented with a duration of 1 second for 70 trials in a pseudo-randomized order.

3.4.5 *Viral injection and optogenetics manipulation*

For viral injection, a small opening in the skull was made above the LM region (from bregma, AP: -4 mm, LM: $\pm$ 4.1 mm). A glass micropipette containing undiluted Cre-inducible DIO-AAV4 (AAV-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-pA) was used to perform the injections at a rate of 6 nl/sec using Nanoject III (Drummond Scientific). The injections were carried out at four depths, 0.2, 0.4, 0.6, and 0.8 mm below the cortical surface, with 40 nl of the virus injected at each depth. The injection was started with the deepest spot, and after each injection, the glass pipette was left in place for 5 minutes before being pulled up. We observed that both concentrations of the virus resulted in a localized injection. The craniotomy was covered with Kwik-Cast (WPI). The animals typically regained consciousness within 30 minutes following the surgery and were monitored for three days afterward.

To inhibit LM and LI, we expressed the Channelrhodopsin-2 (ChR2) gene in parvalbumin-positive (PV+) interneurons with blue light (473-nm laser, Laser-glow Technologies) to stimulate the region. The laser was attached to an optomechanical tool to deliver spatial selective light with tapered fibers (ThetaStation OptogeniX). The manual operation of a micrometric screw executes the selection of the emitting sub-region of a Lambda fiber. For simultaneous optogenetics manipulation and electrophysiology, we used a silicon probe combined with tapered fiber (H3 probe, Cambridge NeuroTech; lambda-b fiber 200 core, 0.66 NA, emitting length 1.5 mm, Optogenix). We calibrated the power level for each depth at 100 μ W by adjusting the power at the end of the patch cord (before fiber entry). We adjusted the depth of light emission after implantation, and the receptive mapping session (where we identified the borders of visual areas), to prevent direct suppression of V1 spontaneous activities. The light stimulation consisted of a constant one-second pulse delivered synchronously with the visual stimulus presentation. The optical setup was controlled by Psychophysics Toolbox Version 3 (PTB-3) and custom code in Matlab, which communicated with a modified sound card using a TTL signal.

3.4.6 Data analysis

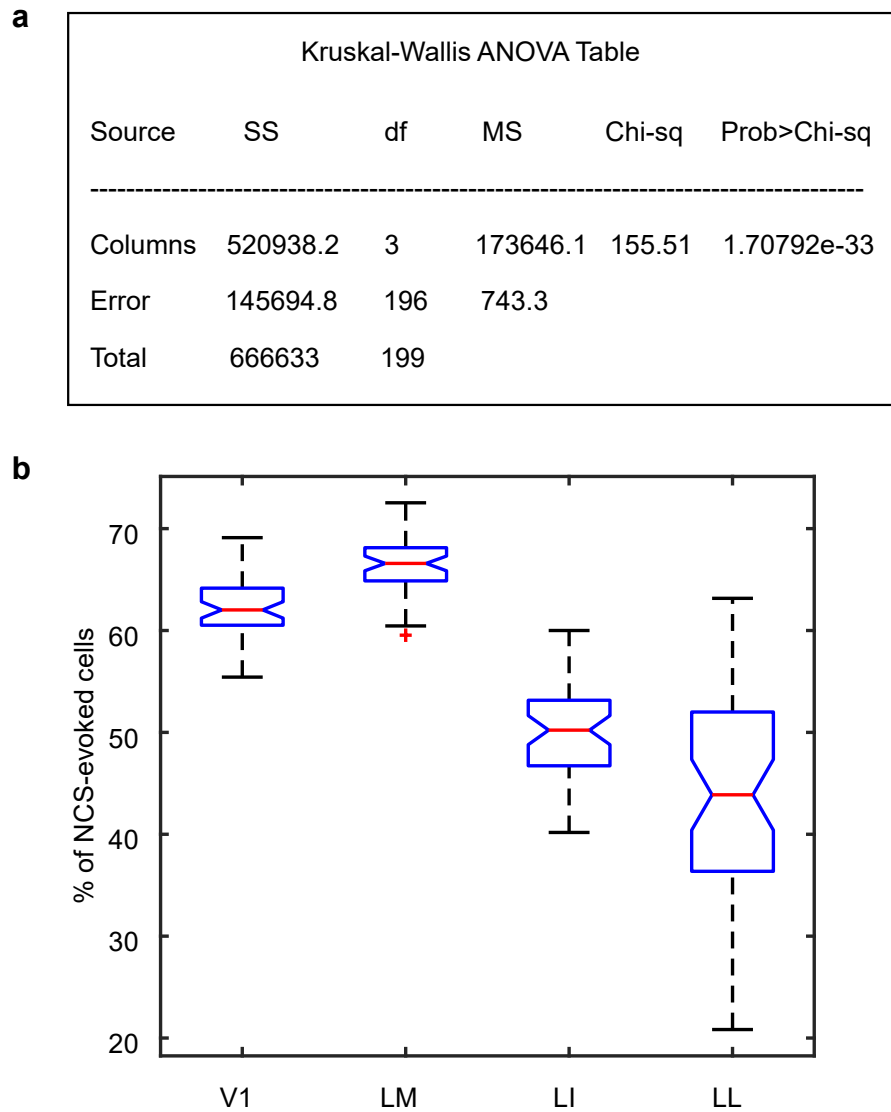
To detect and cluster spiking activities, the recorded data from two experiment sessions (receptive field mapping, size tuning, and neon color) were concatenated, and the Kilosort algorithm, a template matching algorithm implemented in MATLAB, was utilized with default parameters [85]. Manual clustering was then performed using template-gui to merge, split, and choose isolated clusters. All subsequent analyses were conducted in MATLAB using built-in functions and the CircStat Toolbox [13].

The peristimulus time histogram (PSTH) was calculated with a resolution of 1 ms and smoothed using a moving average window of 2 ms. To identify visually evoked neurons, we have compared the spiking activities of each neuron with their prestimulus spiking using Wilcoxon rank-sum, and a p -value=0.001 was chosen as the significant level for being evoked. The F1 dominant neurons were identified by applying Fast Fourier (FFT) on the PSTH. If the power of the F1 frequency (2 Hz) was the largest non-zero frequency, that neuron was chosen as F1 dominant.

To estimate neuronal onset latency, a Poisson distribution was assumed for the spontaneous spiking activity preceding stimulus presentation [68]. The background firing rate λ was estimated by fitting a Poisson distribution to the 300 ms preceding stimulus onset. If the spiking activity after stimulus onset deviated from the background Poisson distribution to a particular level in three consecutive bins (with a probability of $p < 0.01$ for the first two bins and $p < 0.05$ for the third bin), the corresponding time for the first bin was considered the neuron's response latency [68]. The stimulus direction that elicited the maximum response was determined as the preferred angle of the cells. Wherever two groups of data have been compared, we used the Wilcoxon rank-sum test, and for multi-group statistical analysis, we implemented the Kruskal-Wallis test with Tukey-Kramer correction.

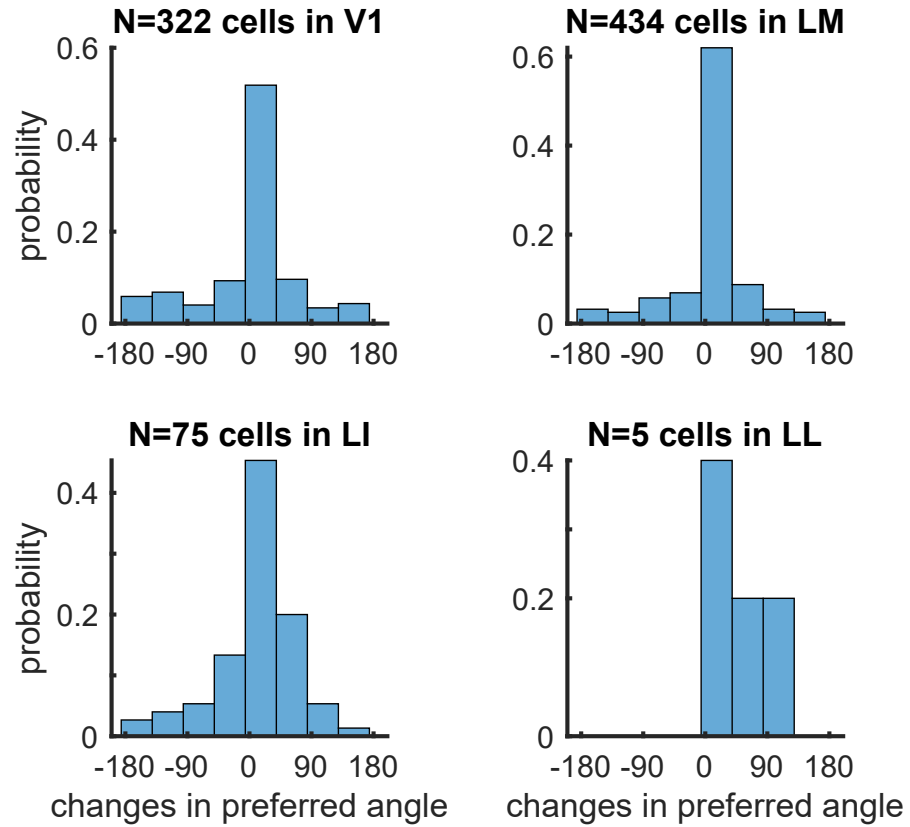
We have used DeepLabCut to analyze the pupillometry data. The training dataset was prepared by manually labeling and specifying the pupil on 150 randomly chosen frames of recorded videos from different mice. We used the "imgaug" option for the data augmentation in the training dataset, and we used a trained "efficient net-b0" network with 500000 iterations to analyze captured videos and extract pupil size. This was followed by the outlier extraction of the output results to improve the output result quality [78].

3.5 SUPPLEMENTARY INFORMATION

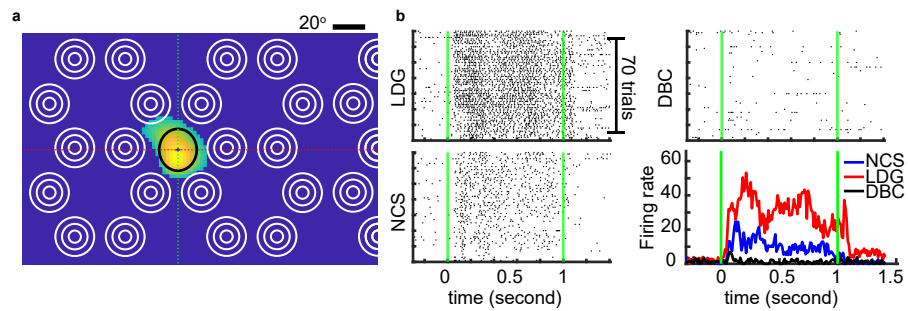


Supplementary Figure 3.1: Statistical test on the percentage of NCS-evoked cells.

(a) Shows the Kruskal-Wallis ANOVA Table for the percentage of NCS-evoked cells. **(b)** Box plot shows the distribution NCS-evoked percentage for the bootstrapped populations.

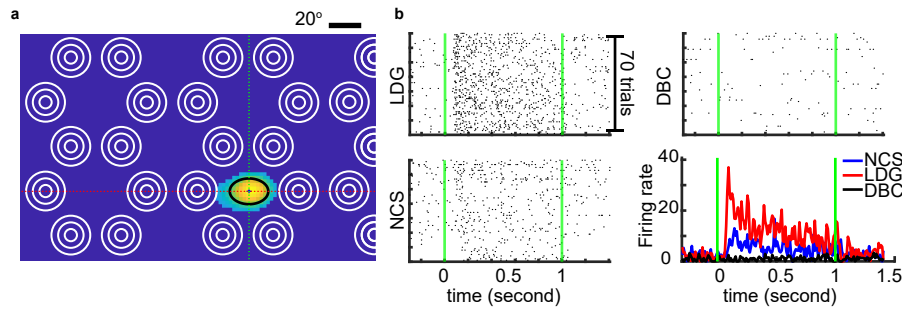


Supplementary Figure 3.2: Histograms show the differences between NCS and LDG preferred angles in four visual areas.



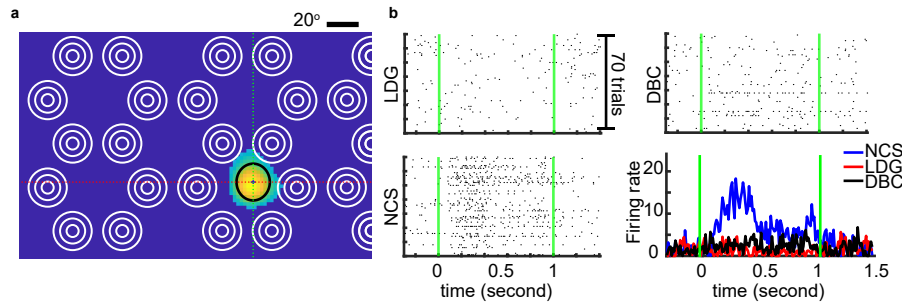
Supplementary Figure 3.3: Example of non-intersecting RF/inducer neuron in LM.

(a) Receptive field location of a non-intersecting cell relative to the inducer circles. The black ellipse shows half width at half height for a two-dimensional Gaussian curve fitted on the receptive field. **(b)** Raster and PSTH in response to different stimuli for the neuron showed in (a). Green lines show the stimulus onset and offset.



Supplementary Figure 3.4: Example of non-intersecting RF/inducer neuron in LI.

(a) Receptive field location of a non-intersecting cell relative to the inducer circles. The black ellipse shows half width at half height for a two-dimensional Gaussian curve fitted on the receptive field. **(b)** Raster and PSTH in response to different stimuli for the neuron showed in (a). Green lines show the stimulus onset and offset.

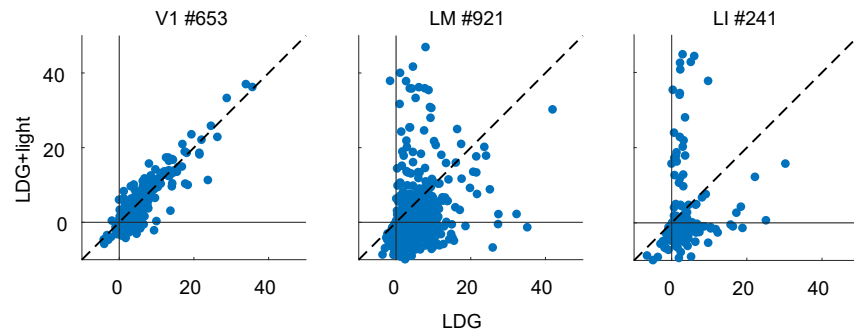


Supplementary Figure 3.5: Example of non-intersecting RF/inducer neuron in LL.

(a) Receptive field location of a non-intersecting cell relative to the inducer circles. The black ellipse shows half width at half height for a two-dimensional Gaussian curve fitted on the receptive field. **(b)** Raster and PSTH in response to different stimuli for the neuron showed in (a). Green lines show the stimulus onset and offset.

Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Groups	1.98e+07	3	6.62e+6	33.21	2.89e-7
Error	1.58e+9	2675	5.91e+5		
Total	1.60e+9	2678			

Supplementary Table 1: Kruskal-Wallis ANOVA table for the LDG response latencies in four visual areas



Supplementary Figure 3.6: Inhibition of LM and LI.

Scatter Plots show the neuronal responses to LDG stimulus against LDG+ light in three different areas. The dashed lines represent the identity line.

Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Groups	7.75e+06	3	2.58e+6	22.69	4.67e-5
Error	6.84e+8	2021	3.38e+5		
Total	6.91e+8	2024			

Supplementary Table 2: Kruskal-Wallis ANOVA table for the NCS response latencies in four visual areas

Part III

OUTLOOK

GENERAL CONCLUSION

This thesis introduces a novel approach to studying the neuronal correlates of visual illusions in a mouse model. By designing an achromatic version of the neon color spreading (NCS) illusion, we have shown that illusions can be practical tools for investigating the single-neuron basis of perception. Our findings in chapter 2 reveal that mouse V1 single units process the illusory brightness, which has an influential contribution to the field as it addresses the last lingering debate on the contribution of V1 in the brightness and surface processing in the brain. Some human fMRI studies reported correlated V1 activities in response to illusory or physical brightness [101, 19, 20], but others failed to find V1 such a correlation in fMRI signal [88, 18, 28]. However, it is challenging to interpret the results due to the nature of the fMRI signal and those experimental setups. The more straightforward method is to use electrophysiological data and directly record single neurons in animals where it has been shown that V1 single units in cats and monkeys respond to brightness. Yet here, the challenge is translating those results and claims that the perception mechanism in different species is similar. For instance, it has been discussed that V1 single-unit responses to the brightness in the simpler animals are due to the fact that in the less complex brains (e.g., cat and mouse), the visual system is not well developed; therefore, lower visual areas need to process such information while in human brain functional processing is done through a more hierarchical and well-segregated network in which lower visual areas like V1 process the primary information of the scene and higher visual areas are responsible for brightness processing and surface perception [88].

Nonetheless, by presenting the NSC and LDG stimulus in opposite temporal phases, we showed that single units in mouse V1 respond to "illusory" brightness for the first time, which opens up new opportunities to study the neural mechanism of perception in this framework. Using pupillary light responses (PLR, pupil dilation, and constriction in response to the luminance changes), we showed that the mouse pupil responded to the brightness of illusory moving grating similar to the human pupil as a putative measure of perception [14, 55, 75, 123]. However, PLR does not provide any information

on the perceived direction of the illusory drifting grating. To conclude, if mice similarly perceive the illusory drifting grating as physical grating (as we have seen the invariance preferred angle in most single units), one should design a task in mouse has to report the direction of illusory drifting grating actively. Future research could build upon the current study by addressing this issue.

We found that the V1 responses to illusory gratings are delayed more than physical gratings, suggesting the demand for more elaborated computation and synaptic interactions for illusions to be processed. Therefore, we examined what cell types contribute to the time-consuming computations of NCS. We discovered that NCS-preferring cells in V1 are more likely to receive surround suppression, to be classified as fast-spiking putative interneurons, and to have complex receptive field properties. The cell type specification of NCS-evoked reported here is valuable to the field as it can reveal within the V1 hierarchical processing of the illusory grating. For instance, it has been known that complex cells receive bottom-up input from lower-level simple cells, and therefore, they can capture more global information in the visual stimulus. However, more investigations are needed to fully discover optical illusions' hierarchical processing. One limitation of our experiment was the lack of high-resolution receptive field structure as well as information about on- and off-pathway since we used only black square for the receptive field mapping session. By presenting both black and white squares, one can extract a more detailed spatial structure of neurons' receptive field and detect the on and off sub-regions in the RF. Such a detailed RF structure would help better examine NCS-evoked response relative to the RF structure. It also can provide information about on- and off-pathways and see how those pathways are processing the "darker than black" grating versus luminance-defined grating.

Beyond V1, we have expanded our search for the neural correlates of NCS to higher visual areas in mice, including LM, LI, and LL. Our results demonstrate that LM plays a pivotal role in processing the illusion of brightness, as NCS-evoked responses in LM were stronger and appeared earlier than in other visual areas. Moreover, a higher percentage of neurons in LM showed NCS-evoked responses. On the other hand, LM neurons showed less phase shift compared to V1. These findings suggest that the feedforward/feedback mechanism where LM captures the visual cues more globally and provides that information to V1 in order to process the illusory brightness (presented with the opposite phase).

We have tested the above-mentioned hypothesis by inhibiting higher lateral visual areas. We found that inhibition of LM and LI reduces the NCS-evoked responses in V1 but has no significant effect on the LDG-evoked responses. Our optogenetics experiments suggest the explicit contribution of top-down feedback to the processing of NCS illusion in V1. Although we controlled the upper bound of light emission to prevent direct opto-stimulation of V1, we did not contain the lower bound light emission. Therefore, our results only suggest the existence of a feedback signal to the V1 from higher lateral visual areas. Still, we can not explicitly specify which area contributes more to the feedback signal. Future research can determine the origin of such feedback to V1 by a more localized opto-stimulation. Moreover, our optogenetics and electrophysiological recordings in this thesis are limited to only V1 and lateral visual areas, and as we showed, the inhibition of LM and LI partially reduced the V1 NCS-evoked responses. Therefore, the role of other higher visual areas (i.e., medial or anterior visual areas) remains undiscovered, and we hope future studies will reveal their contribution to illusory brightness and surface perception.

Overall, this thesis provides significant insights into the neural foundation of illusory brightness and hence offers essential knowledge for understanding surface perception. It also demonstrates the power of the NCS illusion to investigate neural mechanisms of perception. Through our work, we have shown that the NCS illusion helps uncover the complex neural circuits underlying the brightness processing, shedding light on the underlying mechanisms of visual perception and paving the way for future research in this field.

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LIST OF FIGURES

- Figure 1.1 Neon color spreading illusion. **a.** Only a quarter of each concentric circle is colored blue. The blue color leaves the colored segments and spreads into the illusory area. This neon color is constrained by illusory contours at the edges of the illusory square. **b.** The inner parts of the lines are colored red, which produces an illusory transparent red figure. 4
- Figure 1.2 Achromatic version neon color spreading illusion. **a.** Gray segments of black concentric circles give rise to illusory brightness spreading in the surrounding area. **b.** Gray segments of impeded in white concentric circles on a black background produce an illusory "blackier than black" figure. **c.** Black segments embedded in gray lines on a white background can also give rise to the neon color spreading illusion. 5
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ACRONYMS

NCS Neon Color Spreading

LDG Luminance Defined Grating

IGR Illusory Grating Response

DBC Diffusion Blocked Control

SM Surround Modulation

CSM Complex-Simple modulation

LM Lateromedial

LI Laterointermediate

LL Laterolateral

FFT Fast Fourier Transform

PSTH Peristimulus Time Histogram

RF Receptive Field

CHR2 Channelrhodopsin-2

PV+ Parvalbumin-positive

FR Firing Rate

fMRI Functional Magnetic Resonance Imaging

STATEMENT OF CONTRIBUTIONS

The work presented in this thesis is primarily conducted by Alireza Saeedi (A. S.) under the supervision of Prof. Dr. Masataka Watanabe (M. W.), Prof. Dr. Nikos K. Logothetis (N. K. L.), and Prof. Dr. Andreas Bartels (A. B.). Other collaborators include Nelson K. Totah (N. K. T.), Kun Wang (K. W.), and Ghazaleh Nikpourian (G. N.).

Currently, the contents in Chapter 2 have been published elsewhere [99], while the contents in Chapter 3 are in preparation for submission as manuscripts. The contributions to each chapter are elaborated below:

CONTRIBUTION OF CHAPTER 2

- Conceptualization: M. W., A.S.
- Methodology: A. S., M. W., N. K. T.
- Data acquisition: A. S., K. W.
- Data curation: A. S., G. N.
- Formal analysis: A. S.
- Visualization: A. S.
- Writing - original draft preparation: A. S.
- Writing - review: A. S., M. W.
- Supervision: M. W., A. B., N. K. L.
- Funding acquisition: N. K. L.

The remaining chapters of this thesis, including conceptualization, data acquisition, formal analysis, visualization, and writing, were solely conducted by Alireza Saeedi (A. S.) under the supervision of Prof. Dr. Masataka Watanabe (M. W.), Prof. Dr. Nikos K. Logothetis (N. K. L.), and Prof. Dr. Andreas Bartels (A. B.).

ACKNOWLEDGMENT

Completing this thesis would not have been possible without the support and encouragement of many people. First and foremost, I would like to express my gratitude to my supervisors, Prof. Masataka Watanabe, Prof. Nikos Logothetis, and Prof. Andreas Bartels, for all their support, invaluable input, and feedback on this project. Their insightful comments and suggestions have helped shape this work and have been essential to its success. Specifically, I thank Prof. Watanabe for his unwavering guidance throughout my Ph.D. His insight, expertise, and encouragement have been invaluable, and I am deeply grateful for his mentorship.

I sincerely thank Prof. Nikos Logothetis, Dr. Oxana Eschenko, and their son, Feodor Logothetis, for their support and kindness, which have been invaluable to me. I consider them to be like my second family.

I want to express my profound appreciation to our IT specialist, Joachim Werner, for his tireless efforts in providing technical support and troubleshooting around the clock. Additionally, I want to thank Conchy Moya for her excellent administrative support in handling various matters related to the project. I also want to thank the animal caretakers who ensured the welfare and comfort of the mice used in this study. Their dedication and commitment to the ethical treatment of animals are deeply appreciated.

Furthermore, I extend my appreciation to the master's and Ph.D. students at MPI, Hao Mei, Jiatong Liu, Kaidi Shao, Kun Wang, Mingyu Yang, Morteza Soltanpour, Ryo Iwai, Shanqian Ma, Shervin Safavi, Weiyi Xiao, Xin Sui, and Yan Ma, and as well as my postdoc colleagues Ernesto Duran, Leonid Fedorov, Rui Kimura, Vahid S. Bokharaie, and Vishal Kapoor, not only for their technical assistance, support, and insightful discussions throughout this project but also for being friends whom I could spend time, have fun and rely on them in difficult situations.

I would like to express my heartfelt gratitude to my family for being on my side in all situations and to my close friends, Ehsan Amirzadeh, Ferdinand Sörensen, Fereshteh Rabbani, Hosein Hshemi, Maria Paz Contreras Santander, Mohammad Khalili, Morteza Rahmati, Mostafa Jannesari, Pourya Toranj, Setareh Fadavi Toodeh, Shayan Mohamadhoseini, and Soroush Karimi for being my pillars of strength. Their support has helped me to overcome

many obstacles.

Finally, I would like to apologize to those whose names have slipped my mind but who were an essential part of this journey. I am incredibly grateful to all those mentioned above and countless others who have supported me. Thank you for your invaluable contributions and support throughout my Ph.D. journey.

Lastly, I would like to express my deepest gratitude to my wife, Nasibeh Amini, for her unwavering love, patience, and encouragement throughout this journey. Her belief in me, even during the most challenging times, has been a constant source of strength and motivation. Her support allowed me to focus on my work, and for that, I am eternally grateful. This thesis is as much hers as it is mine.

COLOPHON

This document was typeset using the typographical look-and-feel `classicthesis` developed by André Miede, and modified by Alireza Saeedi for the purpose of this thesis. The style was inspired by Robert Bringhurst's seminal book on typography, "*The Elements of Typographic Style*". `classicthesis` is available for both $\text{\LaTeX}$ and $\text{\LyX}$:

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