Monkey neurophysiology to clinical neuroscience and back again

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Gustav Theodor Fechner defined the field of psychophysics as the study of the relationship between the physical properties of stimuli and the internal responses or perceptions they evoke. Since the mid-19th century, psychophysics has evolved into a very powerful field of study exploring the inner workings of the mind. Carefully designed and well-controlled psychophysical experiments have revealed some of the most fascinating aspects of our brains. Think about visual illusions. How is it that we can "see" things that are not there? How is it that we can look at one image and in one moment it looks like a vase and the next it looks like two faces? Experiments using different visual stimuli and measuring the time it takes to detect or discriminate something in the image provide clues as to how our brains work. This understanding is not a one-way street. The real power of psychophysics depends on fundamental knowledge gained by physiological experiments performed in animals, particularly monkeys, when inferences about the inner workings of the human mind are made. The paper by Hall and Colby in PNAS (1) shows how critically important basic research in monkeys is for our understanding of the human brain and how it goes awry in disease.

Psychophysics investigates the relationship between sensory input and behavior. The sensory input studied is often visual, because humans rely heavily on this sense. Eye movements lend themselves to study as a behavioral read-out because of the ease with which they are measured and, the relationship between vision and eye movements remains a pillar of psychophysical investigation. A central structure in the brain involved in visual processing and eye movement control is the superior colliculus (SC). The SC is a structure in the midbrain that has been the focus of studies in psychophysics and neuroscience for over 40 y. Based on this work, we know that the SC plays a crucial role in converting sensory signals, particularly visual signals, into commands to orient toward those sensory signals. In lower mammals, orienting takes the form of whole-body movements toward the stimulus. In monkeys, as in humans, it is often just the eyes that move to realign the fovea—the high-acuity portion of the retina—on the visual object of interest.

Our visual sense can be decomposed into many domains and subsystems. One broad domain is color vision. The retina of humans and monkeys contains three types of cone receptors providing us with our daylight and color vision; they are the S-, M-, and L-cones, defined by the short-, medium-, and long-wavelength stimuli that activate them maximally. The S-cone system exists in nearly all mammals and is evolutionarily older than the M- and L-cone systems. Interestingly, the S-cone system remains segregated from the L- and M-cone systems from the retina to visual cortex. Human studies indicate that the S-cone system contributes to color perception and not very strongly to luminance perception. It has low spatial frequency sensitivity so it is not useful for fine visual discriminations. The segregated and special nature of the S-cone system has made it an area of active investigation, with an entire recent issue of *Visual Neuroscience* devoted to the topic (2).

Monkey Neurophysiology to Human Psychophysics

By the 1970s it was well known that retinal ganglion cells provide inputs directly to the SC, but a new result at that time showed that there were relatively few projections to the SC from the S-cone system (3, 4). In fact, visual inputs to the SC seemed to contain only luminance information. This observation led to the idea that the SC is colorblind and participates in vision for action, rather than vision for perception. It is the retinal projections to the lateral geniculate nucleus of the thalamus and then to the cerebral cortex that serve visual perception. The S-cone signals are conveyed to the cerebral cortex, where they contribute to color perception together with the M- and L-cone signals.

This accepted view, that SC lacks S-cone input, led to the clever idea of using S-cone–specific stimuli to activate the cerebral cortical visual system independently of the subcortical visual system. This approach was pioneered by Sumner et al. (5), who developed a psychophysical method in humans to define stimuli that activate only S-cones. Sumner and his colleagues used these calibrated S-cone stimuli in

human behavioral experiments with the expectation that S-cones would not activate subcortical visual structures, but only activate cerebral cortical structures. They reasoned that this stimulus specificity would allow them to tease apart the roles of cerebral cortical and subcortical areas in perception and higher mental processing. The ability to isolate specific neuronal pathways using targeted physical stimuli is important because it would allow noninvasive interrogation of the functioning of sites of damage in patients with visual deficits. A broad range of phenomena have been tested for dependence on the SC using this kind of approach, including the neural mechanisms of blindsight (6) and interhemispheric transfer of information in patients without a corpus callosum (7). This influential technique is currently being used in studies of face perception (8) and visual development (9). Indeed, a full review article promoting this research strategy in humans recently appeared in the literature (10).

Human Psychophysics to Monkey Neurophysiology

In parallel with the studies in humans, Hall and Colby hoped to use this behavioral approach in monkeys. If the approach worked, the SC could be temporarily "lesioned" on a trial-by-trial basis without any actual damage to the brain. The strategy is to present either a luminance or an S-cone stimulus on separate trials of a visual-oculomotor task. Because the SC is involved in visual-oculomotor behavior, Hall and Colby reasoned that if performance of monkeys differed in response to the S-cone stimulus compared with the luminance stimulus, they could conclude that there was a dependence on the SC. The argument is simple: If the SC cannot detect the S-cone stimulus, it cannot generate a behavior that depends on it.

Hall and Colby began by testing explicitly whether the SC is blind to S-cone stimuli. They did so in three significant steps, culminating in the work described in PNAS (1). The first step was to invent a psychophysical method in monkeys for identifying visual stimuli that selectively activate S-cones (11). They used saccadic eye movements as the behavioral read-out of whether or not the monkey detected a stimulus—if it could look at the stimulus, it could see the stimulus. Both S-cones and macular pigment are distributed very nonuniformly across the retina, so the stimuli have to be calibrated for each monkey and each retinal location where a stimulus would appear. Calibrated visual stimuli that activate only S-cones were then used for behavioral and physiological studies in monkeys.

The next step was to record neuronal responses in the SC while monkeys performed visual and oculomotor tasks. Others previously showed that SC neurons in monkeys have color sensitivity (12). The essential test moving forward was to determine whether SC neurons would discharge action potentials in response to S-cone isolating stimuli. Hall and Colby (13) found that, indeed, SC neurons responded strongly to S-cone stimuli and that these responses were sensitive to the contrast of the S-cone stimuli. This finding is dispositive because it shows that SC neurons are not blind to S-cone isolating stimuli.

The final step was to use S-cone stimuli to test a behavior that is known to require the SC, namely, express saccades. This is the critical step because it is a behavioral measure, as are the measures used in human psychophysical experiments. Express saccades are a particular class of saccadic eye movement that occurs with very short reaction times. The SC is required for the generation of express saccades. If the SC is removed, monkeys

can still make regular latency saccades (less accurately and with a lower velocity than normal) but they can no longer make express saccades (14). Recent work in the slice preparation provides support for an exclusive role of the SC in express saccade generation, because there are direct, excitatory projections from the sensory layers of the SC to the motor layers of the SC whose neurons project to brainstem areas that drive the extraocular motoneurons (15). Hall and Colby reasoned that if monkeys can generate express saccades to S-cone stimuli, then the SC must be able to see S-cone stimuli. In PNAS, Hall and Colby (1) demonstrate that monkeys make express saccades to stimuli that activate only S-cones. A further confirmation of the role of the SC is their finding that neuronal response strength correlates with the

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latency of express saccades. This result shows solidly that that the SC can see stimuli that activate only S-cones.

So, how can there be responses to S-cone stimuli in the SC if there are no retinal S-cone projections to SC? It is important to note that the work of Hall and Colby does not address how these signals get to the SC, only that they do get there and they are used by the saccadic system. The SC is important for visually guided action rather than conscious perception, and the current consensus on express saccades is that they rely on information provided by corticotectal projections (16), a pathway that could underlie the findings of Hall and Colby.

Another important point to make clear is that just because SC neurons discharge in relation to S-cone stimuli does not mean that they play a role in color vision. In other words, the results of Hall and Colby do not imply that the SC participates in our perception of color. SC neurons are not color-opponent and are not selective for specific colors. The results of Hall and Colby show that SC neurons are sensitive to relative changes in retinal S-cone excitation, independent of excitation in L- and M-cones and sensitivity to cone input is not equivalent to color perception.

The experiment of Hall and Colby in PNAS (1) is an illuminating example of the rich interplay between animal, and in particular monkey, and human research. The original physiological and anatomical studies were conducted in monkeys. These findings led to an idea for psychophysical and clinical investigations in humans. Hall and Colby then tested the assumptions of this psychophysical and clinical approach. They replicated the best psychophysical techniques and paired them with simultaneous neuronal recording in alert, behaving monkeys. Their behavioral and physiological studies rule out the possibility of using S-cone stimuli as a way of discriminating between cerebral cortical and subcortical visual mechanisms.

Neuroscience and psychophysics aim to discover how the human brain works. Understanding neural mechanisms in healthy and diseased brain states requires careful study of human subjects. This research must be based on solid assumptions grounded in physiological data collected in animals. In turn, human behavioral methodology can be verified and expanded through the use of monkey models. The demonstration that S-cone stimuli can drive a behavior that is dependent upon the SC in monkeys demands reinterpretation of previous work in humans using S-cone stimuli. The present study by Hall and Colby (1) performed in monkeys advances human studies by demonstrating the need for a change in methodological direction.

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