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Orienting versus avoidance/defense behavior: A layer-specific descending projection from the auditory cortex to the superior colliculus

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Introduction

The superior colliculus (SC) is located in the midbrain and is divided into dorsal, intermediate, and deep layers (for review see Stein 1998 and King 2004). The dorsal layers of the SC process predominately visual information, while the intermediate and the deep layers of the SC process sensory information from different modalities. Manipulations of the SC in rodents using unilateral pharmacological block, glutamate infusion, electrical stimulation, or optogenetic manipulations in the intermediate and deep layers of the SC indicate that these layers of the SC can initiate different behaviors such as: 1) orienting behavior and 2) avoidance/defense behavior (Redgrave, Dean et al. 1981; McHaffie and Stein 1982; Sahibzada, Dean et al. 1986; Liang, Xiong et al. 2015; Zingg, Chou et al. 2017).

The SC receives ascending auditory information from the inferior colliculus (King, Jiang et al. 1998; Nodal, Doubell et al. 2005). However, it is also established that the auditory cortex (AC) can directly modulate the response of the SC neurons via descending corticofugal projections (Diamond, Jones et al. 1969; Wallace, Meredith et al. 1993); for review see Malmierca and Ryugo (2011); Bajo and King (2012); Stebbings, Lesicko et al. (2014). Recently, we investigated the projection from the AC to the SC in mice using a combination of retrograde and anterograde tracing, electrophysiological recordings, and anatomical approaches (doi.org/10.1093/cercor/bhx161). Overall, we describe two layer-specific sub-classes of projection neurons to the SC that may serve separate functions in cortico-collicular circuits and that may be engaged differently during defense-like and/or orienting behavior.

Results

In initial experiments to identify cortico-superior-collicular pyramidal neurons in the auditory cortex, adult C57BL/6 mice (4-6 weeks old) were used for injections of a retrograde tracer into the superior colliculus. The same basic surgical procedures were followed as previously described (Rock and Apicella 2015; Rock, Zurita et al. 2016; Rock, Zurita et al. 2017). Briefly, ~20 nl of a retrograde tracer (RetroBeads, Lumafluor) were injected into the superior colliculus by stereotaxically (4.05 mm posterior to bregma, 0.835 mm lateral to midline, at a depth of 1.8 mm ventral to bregma (Kopf instruments model 1900) pressure injecting (Nanoject II, Drummond Scientific) a

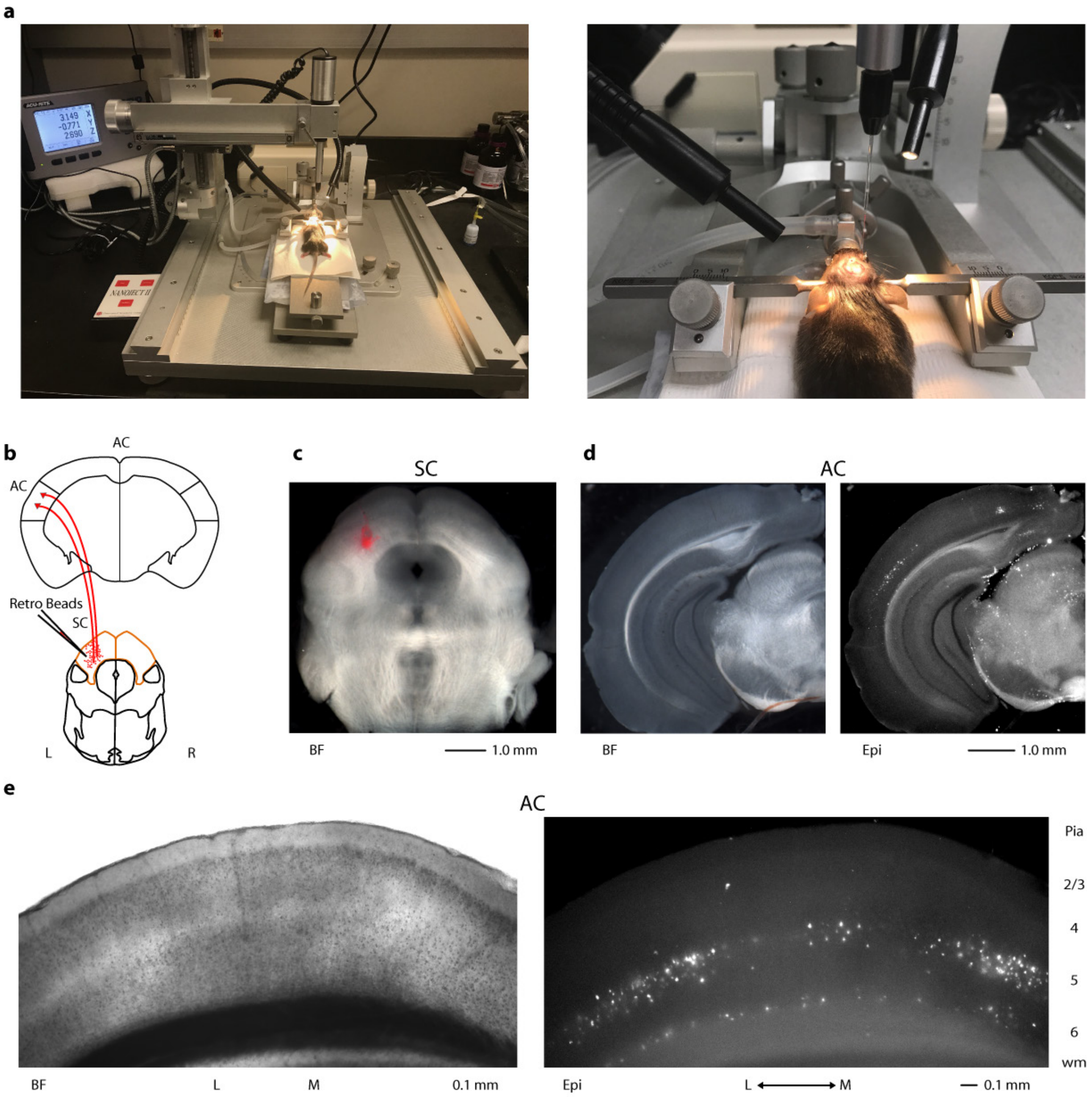

Figure 1. Auditory corticofugal projections to the superior colliculus.

(a) Left and Right: Images of the Kopf stereotaxic apparatus model 1900 used for the injection of red RetroBeads to identify cortico-superior-collicular pyramidal neurons by anatomical retrograde labeling. The same apparatus is used for the delivery of the AAV-viruses.

(b) Schematic depicting the injection of red RetroBeads to identify cortico-superior-collicular pyramidal neurons by anatomical retrograde labeling.

(c) Bright-field image of a slice containing the superior colliculus injection site of red RetroBeads.

(d) Lower magnification bright-field (left) and epifluorescence (right) images of a slice indicating the location in the auditory cortex of the retrograde labeled cortico-superior-collicular pyramidal neurons.

(e) Higher magnification bright-field (left) and epifluorescence (right) images of the laminar distribution of cortico-superior-collicular pyramidal neurons identified by anatomical retrograde labeling. Note that the long-range projecting pyramidal neurons are located in layer 5 and layer 6 of the auditory cortex.

retrograde tracer in the left superior colliculus. The tracer was delivered over a time span of three to five minutes with the glass pipette remaining in place for an additional five to ten minutes before being withdrawn. Using this method, we found that cortico-superior-collicular neurons were located in layers 5 and 6 of the AC in the hemisphere ipsilateral to the injection site (**Figure 1**).

Next, to visualize axons from cortico-superior-collicular pyramidal neurons in the superior colliculus (SC), adult Sim1 KJ18-Cre mice (17-18 weeks old) were injected in the auditory cortex (AC) with a combination of Adeno Associated Virus such as AAV.GFP and AAV.tdTomato.Flex (AAV2/1.CAG.Flex.tdTomato.WPRE.bGH, UNC Vector Core). In these mice, Cre recombinase, an enzyme derived from the P1 bacteriophage, is expressed in layer 5 projecting-type (PT-type) pyramidal neurons. These injections, because of the selectivity of the recombination events between the Cre recombinase and the AAV-Flex virus, resulted in non-specific transfection of pyramidal neurons with GreenFluorescentProtein and targeted transfection of layer 5 PT-type pyramidal neurons with tdTomato. These projections (auditory cortex → superior colliculus) were visualize by stereotaxically (2.5 mm posterior from bregma, 4.25 mm lateral from midline; Kopf instruments model 1900 **Figure 1**) pressure injecting (Nanoject II, Drummond Scientific) the viruses in the auditory cortex (AC).

Two to three weeks following injection, these mice were deeply anesthetized with 5% isoflurane and transcardially perfused with phosphate-buffered saline solution (PBS), followed by 10% neutral buffered formalin. The brain was carefully removed and fixed for several hours at room-temperature. The fixed brain was then sectioned into 100 µm thick slices on a vibratome. After washing in PBS, the slices were mounted on microscope slides with Fluoromount-G mounting medium containing DAPI (4’,6-diamidino-2-phenylindole) (Southern Biotech).

This anterograde viral tracing approach allowed us to visualize the contribution of both layer 5 and 6 (GFP-expressing axons) and layer 5 alone (tdTomato-expressing axons) long-range projections originating in the cortex and terminating in the superior colliculus (**Figure 2**). We further investigated the SC target layers for these axonal projections by dividing the SC into three main layers (superficial, intermediate, and deep) and examining the relative fluorescence levels in each. The boundaries for these layers were approximated based on bright-field image landmarks and comparison to the Allen Institute for Brain Science coronal mouse atlas. On the average, both GFP and tdTomato expression was highest in intermediate layers of the SC (**Figure 2**). It is important to note that this approach does not exclude the possibility that layer 6 cortico-superior-collicular pyramidal neurons may target isolated layers in the SC. These results demonstrate that layer 5 cortico-superior-collicular pyramidal neurons as well as non-specifically labeled pyramidal neurons, including layer 6 cortico-superior-collicular pyramidal neurons, in the AC innervate both the intermediate and the deep layers of the SC.

Conclusions

Auditory cortico-superior-collicular pyramidal neurons: from circuits to behavior

Lesions of the AC in the ferret produce deficits in orienting behavior (Nodal, Kacelnik et al. 2010). These findings may highlight an important point; that changes in the corticofugal projections from the AC to the SC could disrupt normal sound-driven orienting behavior. Our findings, that cortico-superior-collicular neurons are located in both layers 5 and 6 of the AC suggest these two sub-classes of projection neurons could be involved in different functions. Previous studies have shown that layer 5 pyramidal neurons have differ-

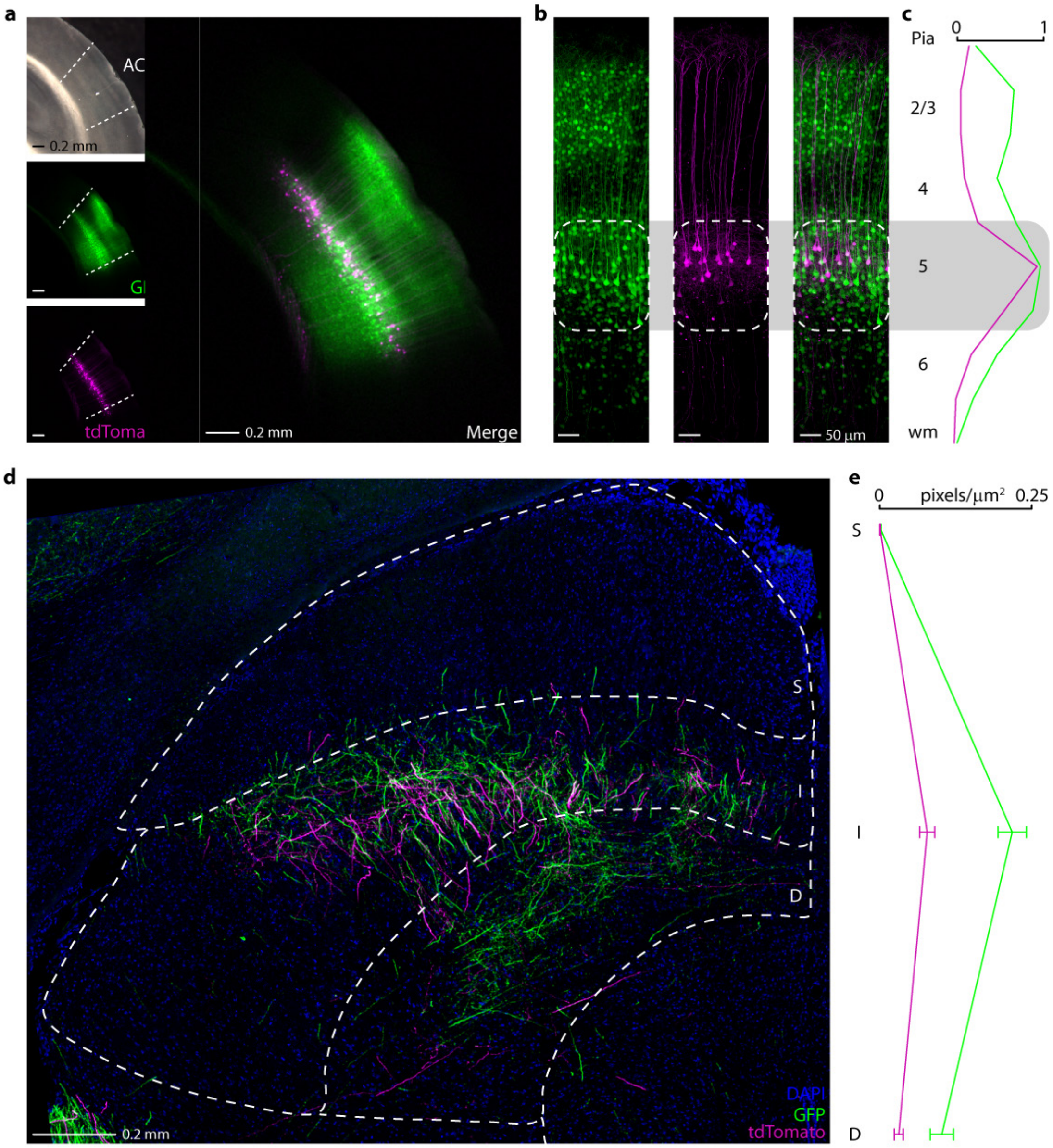

Figure 2. Layers distribution of auditory corticofugal projections to the superior colliculus.

(a) Top, left: Bright-field image of a slice containing the auditory cortex injection site of AAV.GFP (pseudo-colored green in subsequent images) and AAV.tdTomato.Flex (pseudo-colored magenta in subsequent images) in the Sim1 KJ18-Cre transgenic mouse line. Middle, left: GFP fluorescence in the injection site. Bottom, left: tdTomato fluorescence in the injection site. Right: overlay of GFP and tdTomato images.

(b) Left: Higher magnification image of GFP-expressing pyramidal neurons in the auditory cortex near the injection site. Middle: Higher magnification image of tdTomato-expressing layer 5 pyramidal neurons in the auditory cortex near the injection site. Right: Overlay of GFP and tdTomato images. The dashed box indicates the approximate layer 5 boundaries.

(c) Normalized distribution of GFP and tdTomato expression in the auditory cortex. Fluorescence was calculated in 100 µm bins.

(d) Higher magnification images of GFP and tdTomato fluorescent axons in the intermediate and deep layers of the superior colliculus with the approximate boundaries of superficial (S), intermediate (I), and deep (D) layers of the superior colliculus indicated by dashed lines.

(e) Normalized distribution of GFP and tdTomato expression in the superficial, intermediate, and deep layers of the superior colliculus.

ent morphology, connections (for review see, Winer, Larue et al. 1998; Winer 2006), and exhibit different responses to auditory stimuli compared to layer 6 pyramidal neurons (Sugimoto, Sakurada et al. 1997; Hromadka, Deweese et al. 2008; Atencio and Schreiner 2016; for review see Linden and Schreiner 2003; Wu, Tao et al. 2011). Recently, Sun, et.al, (2013) suggested that intrinsic-bursting pyramidal neurons (such as layer 5 cortico-superior-collicular-like pyramidal neurons) have spectrally and temporally broader synaptic integration than regular spiking pyramidal neurons (such as layer 6 cortico-superior-collicular-like pyramidal neurons). These and our results (doi.org/10.1093/cercor/bhx161) suggest that layer 5 cortico-superior-collicular pyramidal neurons may contribute to the sustained response of layer 5 intrinsic-bursting neurons to auditory stimuli (Sun, Kim et al. 2013). Moreover, given that corticofugal projections promote sound localization learning by shaping the responses of the inferior collicular neurons (Bajo, Nodal et al. 2010), invites speculation that sustained activity in the layer 5 cortico-superior-collicular pathway may play an important role in inducing learning of acoustically-driven orientation behavior.

Anatomical studies have revealed three main classes of pyramidal neurons located in layer 6 of the neocortex (for review see Thomson 2010): 1) Corticothalamic pyramidal neurons are estimated to constitute ~50% (Ojima 1994; Prieto and Winer 1999; Rouiller and Welker 2000; Winer, Miller et al. 2005; Takayanagi and Ojima 2006; Llano and Sherman 2008); 2) corticocortical pyramidal neurons that are typically classified as ~30-40%; and 3) GABAergic neurons that are ~15% of the entire layer 6 neuronal population (Gilbert and Kelly 1975; Zhang and Deschenes 1997; Kumar and Ohana 2008). Our results (doi.org/10.1093/cercor/bhx161) reveal that there exists another class of layer 6 pyramidal neurons, projecting directly to the superior colliculus, which also contributes to descending auditory corticofugal projections. A recent study in rodents Zhou et al. (2010) revealed that the majority of layer 6 pyramidal neurons do not respond to sound stimulation while a smaller fraction of layer 6 pyramidal neurons respond with a robust sound-evoked spike response. It is yet to be determined whether layer 6 cortico-superior-collicular pyramidal neurons will belong to the class of layer 6 pyramidal neurons which do or do not respond to sound stimuli in vivo. However, our results demonstrate that layer 6 cortico-superior-collicular pyramidal neurons have spike frequency adaptation and a broad action potential (doi.org/10.1093/cercor/bhx161), suggesting that these neurons by responding more effectively to new acoustic stimulation, could preferentially trigger behaviors related to novelty such as flight behavior (Liang, Xiong et al. 2015; Xiong, Liang et al. 2015; Zingg, Chou et al. 2017).

Future Directions

An important question for future studies, in order to better understand the effect of cortico-superior-collicular pathways on the SC, will be to distinguish how each cell-type may serve separate functions in cortico-collicular circuits which may be engaged differently during defense-like and/or orienting behavior. To address this question we will combine optogenetics and behavior. Before surgical placement of the fiber implant, it is important to decide the best location for optical stimulation of channelrhodopsin2 in the experiment. In general, placing the fiber implant in the same region as the viral injection, so that illumination targets channelrhodopsin2-expressing cell bodies, will lead to the most robust activation of channelrhodopsin2. However, if the cells which express channelrhodopsin2 project to different regions of the brain, all of these pathways will be activated by this illumination. On the other hand, placement of the fiber implant over the target region of interest which contains only channelrhodopsin2-expressing axon terminals will lead to activation of a specific pathway, but this will also depend on the

Figure 3. Auditory corticofugal projections to the superior colliculus: fiber implant.

(a) Images of the Kopf stereotaxic frame model 1900 used for the fiber implant.

(b) Images of an adult Sim1 KJ18-Cre mouse implanted with fiber optics in both left and right superior colliculus.

(c) Images of the Kopf dual cannula insertion tool (model 1973) used for precisely implanting the fiber optics in the mouse superior colliculus.

density of axons in the target region of interest. It is also important to consider that activation of axon terminals can lead to backpropagation of the action potential which can then affect the cell body of the transfected neuron. Control experiments may be performed in which the cell bodies are pharmaceutically silenced with muscimol during optogenetic activation of axonal terminals in order to minimize the stimulation of multiple pathways.

Our fiber optic implants are made in-house using a technique similar to Sparta, Stamatakis et al. (2011). Briefly, a length of multimode optical fiber was threaded through a ceramic zirconia ferrule and fixed in place with heat-curable epoxy. The interface side of the implant was polished and the side to be implanted was cut to the desired length based on coordinates in Paxinos and Watson (2012) and targeting experiments performed previously in our lab. Fiber implants were tested for the loss of power before implantation (in general, fibers with >20% power loss were discarded).

Adult mice of either gender will be used for this study. Mice will be anesthetized in an induction chamber with isoflurane (5%, 2L/min O2 flow rate) until they no longer respond to a toe pinch. Preparation for the surgery will be conducted apart from the Kopf stereotaxic frame (Model 1900; **Figure 1 and 3**) to keep it free of hair trimmings. Mice will be returned to the induction chamber to reestablish anesthesia, and then mounted on the stereotaxic frame using non-rupture ear bars. The isoflurane level will be reduced to 1-2% for maintenance of anesthesia for the rest of the surgery.

The optical fibers, by stereotaxic coordinates (4.05 mm posterior to bregma, 0.835 mm lateral to midline, at a depth of 1.8 mm ventral to bregma) will be inserted in both left and right superior colliculi using a Kopf dual cannula insertion tool (model 1973) and a Kopf frame (model 1900) (**Figure 3**). In order to allow optogenetic photoactivation of cortico-superior-collicular projections, during the same procedure, the animals also will be injected with channelrhodopsin2 (AAV1.CAGGS.ChR2-tdTomato.WPRE.SV40) into the left and right auditory cortex (AC).

This method will allow us to distinguish how optogenetic manipulation of layer 5 and layer 6 cortico-superior-collicular pyramidal neurons may provide separate functions in cortico-collicular circuits that may be engaged differently during defense-like and/or orienting behavior.

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Biography

Alfonso junior Apicella completed his Ph.D. at the University of California at San Diego in conjunction with The Second University of Naples, Italy. He did postdoctoral studies at the University of California, San Diego, the Cold Spring Harbor Laboratory, the University of California, Berkeley, and the Northwestern University, Chicago. The goal of his laboratory is to reveal the neural basis of perception and specifically, to understand how cortical microcircuits process sensory information to drive behavior.