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Research report

Effects of early eye removal on the morphology of a multisensory neuron in the chicken optic tectum

Katharina Lischka *, Jiamin Yan, Stefan Weigel, Harald Luksch

Technical University of Munich, Chair of Zoology, 85354 Freising, Germany

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A B S T R A C T

The midbrain is a subcortical area involved in central functions such as integrating sensory modalities, movement initiation and bottom-up and top-down attention. In chicken, the midbrain roof is termed optic tectum (TeO) and consists of 15 layers with distinct in- and output regions. Visual input targets the superficial layers, while auditory input terminates in deeper layers. It has been shown that ablation of sensory epithelia leads to changes in the cellular patterning and structural organization of the sensory pathways. For the tectum, ablation of the eye *anlagen* was shown to affect retinorecipient neurons. While the gross morphology remained intact after enucleation, the shape of dendritic endings was changed presumably due to missing presynaptic input during synaptic pruning.

We investigated the effect of deafferentation in a multisensory cell type, the Shepherd's crook neuron (SCN) in the TeO. SCNs have distinct dendritic branches in retinorecipient layers (superficial layers 1 to 5 and 7) and in layers where auditory input terminates. To assess whether removal of a single sensory input only affects the dendrites recipient for that input, we removed the eye *anlagen* and retrogradely labeled SCNs later in embryogenesis to visualize the morphology in lesioned and non-lesioned embryos. We found no changes in the gross morphology or in the basal dendrites, but an altered growth of the fine structures at the apical dendrite of SCNs in the retinorecipient layers. Our data indicate that the neuronal morphology of SCNs is mostly predefined before retinal innervation affect the fine structure.

1. Introduction

The development of the nervous system in vertebrates is dependent on a complex ensemble of cellular and molecular mechanisms (Nakamura and Sugiyama, 2004). Interrupting this precisely timed interplay leads to malformation or death of the embryo during its development (Barkovich et al., 2009). In the precocial chicken, the sensory pathways have to be established precisely during development (Mey and Thanos, 2000; Rubel and Fritzsch, 2002). The tectum is involved in integrating sensory information, interfacing these to both premotor networks and higher brain regions (Luksch, 2003). The layered structure of the TeO with distinct sensory input regions allows to analyze the influence of sensory information on single neurons. Thus, the chicken optic tectum (TeO, counterpart to the superior colliculus in mammals) is an ideal model to study the influence of sensory input during development. The TeO is organized in 15 layers. Retinal ganglion cells terminate in the retinorecipient layers (3 to 5 and 7, Yamagata et al., 2006). Early unilateral enucleation of the eye *anlagen* leads to an undeveloped eye in the older embryo (Kelly and Cowan (1972)). This retinal deafferentation also affects the fine structure of retinorecipient neurons in the tectum. In the chicken, Luksch and Poll (2002) investigated the influence of retinal deafferentation on a particular neuron type in the *stratum griseum centrale* (SGC). This neuron has large dendritic fields with specialized dendritic endings (termed bottlebrush endings) in retinorecipient layers. The gross morphology of SGCs was unaltered, but the bottlebrush endings were significantly deformed and covered a much larger area compared to control animals. While unimodal neurons such as the SGC neurons will thus be affected in their recipient dendritic structures, we were interested in how the removal of only one sensory input affects the development of a multimodal cell. We thus investigated the tectal Shepherd's crook neuron (SCN) that has two distinct areas that receive different sensory input. This neuronal cell type, which is located in layer 10, has a unique morphology as the apical dendrite terminates in the retinorecipient superficial layers (Wang et al., 2006), whereas the basal dendrites ramify in the deeper layers, where auditory input was shown to terminate (Niederleitner et al., 2016). We examined the alterations of the optic tectum after

* Corresponding author at: Technical University of Munich, Chair of Zoology, Liesel-Beckmann-Str. 4, 85354 Freising, Germany.

E-mail addresses: katharina.lischka@tum.de (K. Lischka), jiamin.yan@tum.de (J. Yan), stefan.weigel@wzw.tum.de (S. Weigel), harald.luksch@mytum.de (H. Luksch).

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early eye *anlagen* removal and focused on the morphology of Shepherd's crook neurons, in particular the fine dendritic structures. By investigating a cell type with dendrites in two different input regions, we could analyze whether removal of one sensory input affects only the particular dendritic area or the morphology in general.

2. Results

2.1. Significant reduction of the superficial layers

To investigate whether the lack of retinal innervation influences the growth of visually responsive neurons, we first analyzed the

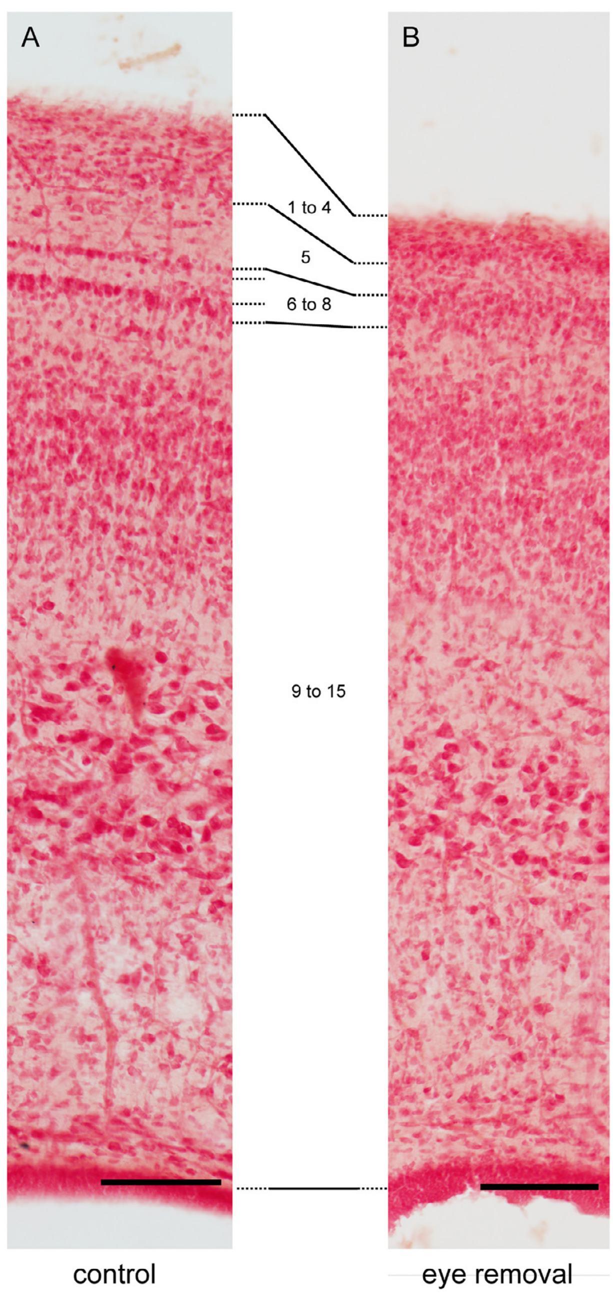

Fig. 1. The optic tectum is reduced after unilateral removal of the eye *anlagen*. A. The retinorecipient layers of the optic tectum. Layer 6 and 8 are clearly visible as single-cell (layer 6) and multiple-cell (layer 8) band. (N = 11, n = 60). B. The early eye removal has an impact on the retinorecipient layers. (N = 11, n = 60). Scale bar: 200 μm.

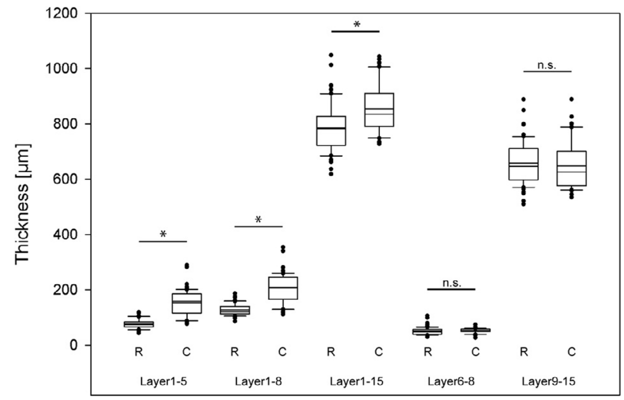

Fig. 2. The overall reduction of the optic tectum of embryos with unilaterally removed eye *anlagen* is a result of the significant reduction of the retinorecipient layers. The layers with no input from the retina is unaffected. R: early eye removal. N = 11, n = 60. C: control. N = 11, n = 60 *: significant. n.s.: not significant.

influence of unilateral lesion on the TeO by analyzing the layer thickness in removed eye *anlagen* and normal developed embryos (Fig. 1). None of the lesioned embryos developed an eye after early eye enucleation. . In general, the thickness of the TeO of lesioned embryos was lower and the stratification between layer 6–8 appeared diffuse.

We compared the thickness of layer 1–5, layer 1–8, layer 1–15 and layer 6–8 (Fig. 2 and Table 1). Without retinal innervation, the retinorecipient layers of the TeO (layer 1–8) are strongly reduced by 42% ($p < 0.001$; early eye removal: 121.56 ± 16.63 μm, N = 11, n = 60; control: 208.96 ± 42.23 μm, N = 11, n = 60). Albeit the thickness of layer 9–15 was not changed ($p = 0.142$; early eye removal: 647.54 ± 61.53 μm, N = 11, n = 60; control: 626.52 ± 67.85 μm, N = 11, n = 60). A closer look at the retinorecipient layers revealed the loss of precise stratification of layer 6–8. In normally developed tecta, layer 6 is a one-cell layer, layer 7 contains no somata and layer 8 is again a densely packed cell layer. After early eye removal, this separation is lost, and these three layers are in a diffuse arrangement leading to a slight but not significant thinner layer 6–8 in enucleated embryos ($p = 0.091$; enucleated: 48.56 ± 10.61 μm; control: 53.70 ± 6.38 μm, each N = 11, n = 60). Layer 1–5 are considerably thinner when retinal innervation is lacking ($p < 0.001$; early eye removal: 74.09 ± 13.02 μm, N = 11, n = 60; control: 158.09 ± 37.62 μm, N = 11, n = 60). It seems that the reduction of these layers is crucial for the overall reduction of retinorecipient layers when retinal innervation is missing. To further parse the reduction in these layers, we did an immunostaining against calbindin (Fig. 3) which is known to be expressed in layer 5 neurons. The calbindin-positive cells in layer 5 are horizontal cells with multipolar dendrites. The dendrites are running parallel to the surface of the optic tectum (Luksch and Golz, 2003).

In the control optic tectum, we found somata and neurites of calbindin-positive cells in layer 5 ($p < 0.001$; enucleated: 11.77 ± 1.51 μm, control: 42.92 ± 4.96 μm, each N = 2, n = 3; Table 1). Both, the amount of cells and fine projections are reduced by enucleation (Fig. 3). However, enucleation also remarkably affects the size of layer 1–4 ($p < 0.001$; enucleated: 65.98 ± 1.98 μm, control: 162.18 ± 16.58 μm; each N = 2, n = 3).

2.2. Influence of early eye removal on a multimodal cell type

Furthermore, we analyzed the influence of the lack of retinal innervation on Shepherd's crook neurons by measuring different morphometrical parameters. The somata of SCNs are located in layer 10 with an apical dendrite extending to the retinorecipient