Input clustering in the normal and learned circuits of adult barn owls

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ABSTRACT

Experience-dependent formation of synaptic input clusters can occur in juvenile brains. Whether this also occurs in adults is largely unknown. We previously reconstructed the normal and learned circuits of prism-adapted barn owls and found that changes in clustering of axo-dendritic contacts (putative synapses) predicted functional circuit strength. Here we asked whether comparable changes occurred in normal and prism-removed adults. Across all anatomical zones, no systematic differences in the primary metrics for within-branch or between-branch clustering were observed: 95–99% of contacts resided within clusters (<10–20 μm from nearest neighbor) regardless of circuit strength. Bouton volumes, a proxy measure of synaptic strength, were on average larger in the functionally strong zones, indicating that changes in synaptic efficacy contributed to the differences in circuit strength. Bootstrap analysis showed that the distribution of inter-contact distances strongly deviated from random not in the functionally strong zones but in those that had been strong during the sensitive period (60–250 d), indicating that clusters formed early in life were preserved regardless of current value. While cluster formation in juveniles appeared to require the production of new synapses, cluster formation in adults did not. In total, these results support a model in which high cluster dynamics in juveniles sculpt a potential connectivity map that is refined in adulthood. We propose that preservation of clusters in functionally weak adult circuits provides a storage mechanism for disused but potentially useful pathways.

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1. Introduction

Encoding of learned skills is thought to involve a combination of changes in synaptic connectivity, synaptic weights and the integrative properties of neurons. The relative contribution of each mechanism may differ across circuits and over time due to physical differences between developing, juvenile and adult brains. One unifying model is the *input clustering hypothesis* (Mel, 1992; Poirazi & Mel, 2001), also referred to as *synaptic clustering* or the *clustered plasticity model* (reviewed in Branco & Hausser, 2010; DeBello, 2008; Govindarajan, Kelleher, & Tonegawa, 2006; Larkum & Nevian, 2008; Magee, 2011; Winnubst & Lohmann, 2012). It states that learned information can be stored through spatial clustering of functionally related synaptic inputs on individual branches of dendrite, enabling supralinear summation that strengthens the postsynaptic response. Tests of this idea using diverse techniques and brain systems have recently emerged with heavy emphasis on juvenile brains (reviewed in DeBello, McBride, Nichols, Pannoni, Sanculi, & Totten, 2014). Yet the microstructural

changes needed to drive the formation or dissolution of input clusters are observed in both juveniles and adults (Holtmaat & Svoboda, 2009), raising the question of whether such dynamics are harnessed to modify clustering patterns in adults.

The barn owl auditory localization pathway is well suited for investigating this issue. Owls reared wearing prismatic spectacles (Knudsen & Brainard, 1991) develop a new, learned microcircuit (DeBello, Feldman, & Knudsen, 2001) that drives adaptive auditory orienting behavior. At the same time, the normal microcircuit becomes functionally weak, yet is preserved anatomically – the neural trace of a now dormant skill (Knudsen, 2002). The axonal inputs and target dendrites of each circuit can be identified on the basis of topographic position within the external nucleus of the inferior colliculus (ICX). This provides a before and after snapshot of learning within the same block of tissue (Fig. 1).

We previously used *in vivo* electrophysiological mapping and retrospective confocal microscopy to measure circuit strength and input clustering in two experimental groups: normal juveniles and prism-adapted owls up through the age of sexual maturity (Fig. 2). In all topographic zones and across both groups, the pattern of input clustering mirrored functional circuit strength (McBride, Rodriguez-Contreras, Trinh, Bailey, & Debello, 2008). These results are consistent with the model and provided the first

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direct demonstration that clustering patterns can adjust in accordance with behaviorally relevant learning signals.

Here we extend this analysis to normal and prism-removed adults (Fig. 2). When prisms are removed from owls that have continuously adapted throughout the juvenile sensitive period and beyond, auditory tuning reverts to normal (Brainard & Knudsen, 1998). We hypothesized that weakening of the prism-induced learned circuit is associated with decreased input clustering, and strengthening of the dormant normal circuit with increased clustering. The latter was observed; the former was not. Close spacing of contacts was present across all topographic zones in both experimental groups. These results are not entirely consistent with the idea that changes in clustering drive refinement of procedural skills in the adult brain. However, the fact that the brain preserves even maladaptive clusters supports the idea that they have long-term adaptive value.

2. Materials and methods

2.1. Animals

15 barn owls (*Tyto alba*) of both sexes were used in this study. Animals were housed in aviaries and cared for in accordance with the UC Davis animal care policy.

2.2. Prism rearing

Owls were hatched in captivity or collected from the wild at young ages (<40 d). Five normal adults (NA) and six prism-removed owls (PR) completed the multi-year experimental paradigm. They were raised together in large flight rooms from the age of fledging, ∼60–70 d. At this age, PR owls received fresnel lenses with 19° lateral displacement (prisms), reversibly attached to headgear surgically mounted on the skull. Details of the surgical procedure can be found in (Swofford & DeBello, 2007). In the flight rooms all owls hunted live mice, flew to perches and interacted with each other, natural experience that drives both normal development and adaptive adjustments to prisms. The PR owls were first mapped in young adulthood to confirm auditory tuning shifts. Prisms were removed at various times in adulthood. After 1–17 months without prisms, individuals were re-mapped to confirm re-expression of normal auditory tuning and then sacrificed for clustering analysis. The schematic and timeline presented in Figs. 2 and 3 include experiments presented in this study and those from a previous study that focused on normal juvenile (NJ) and prism-adapted (PA) owls, all of substantially younger ages.

2.3. Electrophysiology

For mapping, prisms were temporarily removed and the owls secured in a stereotax located within an anechoic chamber. Auditory stimuli were presented through a free-field speaker array (4° separation), and visual stimuli by hand-held projection onto a co-aligned tangent screen made of acoustically transparent cloth. Neural responses were recorded using platinum–iridium electrodes of 1–5 Mohm impedance (Thomas Recording, Germany) and digitized using Spike2 software (CED, Cambridge, England). Recordings were focused on the deep layers of the optic tectum (OT), where individual neurons exhibit sharp spatial tuning for both auditory and visual stimuli. The auditory map in deep OT is inherited from monosynaptic connections originating in the external nucleus of the inferior colliculus (ICX), the main site of plasticity. For each recording site in prism-adapted and prism-removed owls, adaptive shift was calculated as the (weighted average of auditory spatial tuning in microseconds) × 2.5 − (center of the visual receptive field in degrees).

2.4. In vitro anterograde tracing and immunohistochemistry (IHC)

Axons were labeled by focal injection of anterograde tracer into the lateral shell of the central nucleus of the inferior colliculus (ICCls). Injections were performed in acute brain slices to facilitate
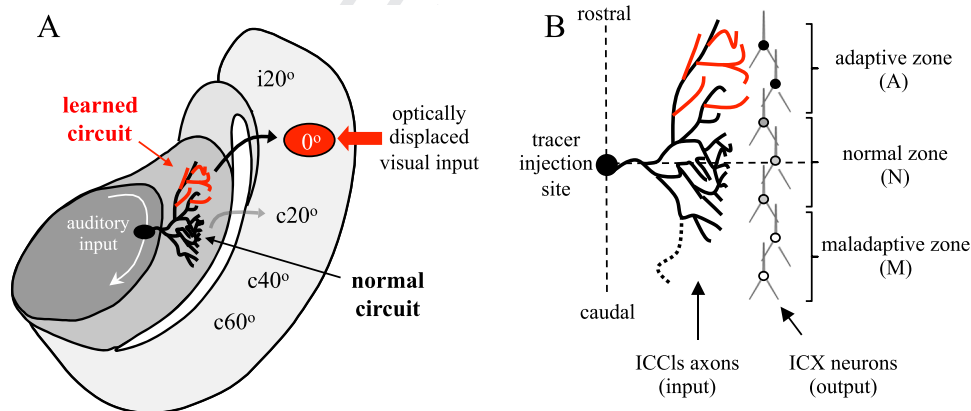

Fig. 1. Experimental design. A. Diagram of horizontal section through the R midbrain of a prism-adapted owl. Neurons in the central nucleus of the inferior colliculus (ICC) are tuned to distinct values of interaural time difference (ITD) and arranged topographically to form a map, indicated by the curved arrow located in the lateral shell of the ICC (ICCls). ITDs corresponding to ipsilateral space are represented in the rostral pole and ITDs corresponding to progressively more contralateral space toward the caudal pole. ICCls neurons project to the external nucleus of the inferior colliculus (ICX) where a complete map of auditory space is assembled. The axonal projection labeled by focal injection of anterograde tracer at c50 μs ITD is depicted. Black and red lines depict the normal and learned circuits. Major postsynaptic targets of these axons are CaMKII+ space-specific neurons in ICX (depicted in B). Curved arrows indicate the projection of CaMKII+ neurons to the OT where the auditory space map aligns with a visual space map. An ITD of 50 μs corresponds to 20° azimuth in visual space. In both ICX and OT, ipsilateral space is represented in the rostral pole and contralateral space progressively toward the caudal pole. Red arrow indicates the location of optically displaced visual input arising from a stimulus located 20° to the owl's left (for illustration; actual prisms used were 19°). After full adaptation, depicted here, the learned circuit drives strong responses in ICX which are conveyed to the appropriate location in OT (black arrow), whereas the persistent normal circuit drives weak responses in ICX which are conveyed to the non-displaced location in OT (grey arrow). Following prism removal, the normal responses are re-expressed and the learned responses suppressed (see Figs. 2 and 4). B. Microanatomical analysis in a prism-adapted owl. Contacts between tracer-labeled axons and CaMKII+ dendrites are distributed across the entire rostrocaudal extent of the axonal arbor, ∼2 mm, and with similar bulk density within the adaptive and normal zones. Nonetheless, in response to auditory stimulus and co-activation of these synapses, the postsynaptic cells located in the adaptive zone respond most strongly. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)