Competition driven by retinal waves promotes the morphological and functional synaptic development of neurons in the superior colliculus

Running head: Retinal waves promote synapse development

Moran Furman¹, Hong-Ping Xu¹, Michael C. Crair¹,²,³

Departments of Neurobiology¹, Ophthalmology and Visual Science², Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven CT, 06510

Corresponding author: Michael Crair, michael.crair@yale.edu

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ABSTRACT

Prior to eye opening, waves of spontaneous activity sweep across the developing retina. These “retinal waves”, together with genetically encoded molecular mechanisms, mediate the formation of visual maps in the brain. However, the specific role of wave activity in synapse development in retino-recipient brain regions is unclear. Here, we compare the functional development of synapses and the morphological development of neurons in the superior colliculus (SC) of wild-type mice (WT) and transgenic mice (β2-TG) in which retinal wave propagation is spatially truncated (Xu et al., 2011). We use two recently developed brain slice preparations to examine neurons and synapses in the binocular vs. mainly-monocular SC. We find that retinocollicular synaptic strength is reduced whereas the number of retinal inputs is increased in the binocular SC of β2-TG mice in comparison to WT mice. In contrast, in the mainly-monocular SC, the number of retinal inputs is normal in β2-TG mice, but transiently, synapses are abnormally strong, possibly due to enhanced activity-dependent competition between local, ‘small’ retinal wave domains. These findings demonstrate that retinal wave size plays an instructive role in the synaptic and morphological development of SC neurons, possibly through a competitive process among retinofugal axons.
**INTRODUCTION**

Construction kits typically arrive with instruction manuals. The brain, in contrast, largely relies on internal cues for the preliminary construction of its neural circuitry. In the visual system, for instance, the overall layout of visual maps is well established before eye opening and the onset of external sensory experience (Rakic, 1981, Simon and O'leary, 1992, Crair et al., 1998), suggesting that genetically encoded molecular cues play a prominent role in visual map development (McLaughlin et al., 2003a, Huberman et al., 2008, Huberman et al., 2010, Triplett et al., 2012). In addition, spontaneously generated patterns of neural activity complement molecular mechanisms and support the refinement of developing neural circuits (Grubb et al., 2003, McLaughlin et al., 2003b, Stafford et al., 2009, Triplett et al., 2009, Xu et al., 2011, Ackman et al., 2012).

During the first postnatal week in mice, spontaneous “waves” of neural activity sweep across the retina (Feller et al., 1996, Wong, 1999, Stafford et al., 2009, Hennig et al., 2011). Propagation of these waves is mediated by nicotinic acetylcholine receptors containing the β2 subunit (β2-nAChR). Normally, nAChR-mediated waves propagate across relatively long distances, covering large fractions of the retina (Ackman et al., 2012). At a local level, retinal waves correlate the spiking activity of neighboring retinal ganglion cells (RGCs). These local correlations are thought to “report” to the developing visual maps in the brain about neighborhood relationships in the retina, thus supporting the fine-
scale spatial refinement of visual maps. In contrast, long-range retinal wave propagation may be essential for segregating input between the eyes, but dispensable for the local refinement of retinotopy. The large temporal offset in neural activity between distant RGCs may help to eliminate grossly mistargeted axon branches and synapses (Stafford et al., 2009). It is also possible that, at least at the synaptic/functional level, long-range propagation of retinal waves is simply epiphenomenal, with little significance for synapse development.

Determining the specific role of spontaneous neural activity during brain development is challenging because most manipulations that interfere with these activity patterns also affect the overall level of spontaneous neural activity. A recent study from our lab addressed this caveat using conditional transgenic mice (β2-TG). This genetic manipulation interferes with the long range propagation of β2-nAChR mediated retinal waves without changing the firing properties of individual RGCs (Xu et al., 2011). Anatomically, in β2-TG mice, retinotopic refinement and eye-specific segregation are impaired in the antero-medial crescent of the superior colliculus (SC), which receives binocular input. In contrast, retinotopic refinement is normal in the remaining parts of the SC, which receives predominantly contralateral monocular input and in the antero-medial crescent of the SC after monocular enucleation (a similar phenotype was observed in the lateral geniculate nucleus, LGN).
These experiments suggest that β2-TG mice are a useful model system for examining the role of long-range retinal wave propagation in visual map development. Furthermore, two recently developed brain slice preparations allow us to selectively probe synapse development in the medial (binocular) vs. the lateral (mainly-monocular) SC (Furman and Crair, 2012). We utilized anatomical and whole-cell patch-clamp physiological techniques in β2-TG and wild-type mice to address the following questions: (1) What role, if any, does long-range retinal wave propagation play during the formation of synaptic contacts between RGCs and their postsynaptic partners in the brain? (2) Do these roles differ during formation of binocular vs. monocular maps?

MATERIALS AND METHODS

Mice. All procedures were carried out in compliance with Yale IACUC, US Department of Health and Human Services and Institution guidelines. C57BL/6J wild-type (WT) mice were obtained from Jackson Laboratory (Bar Harbor, ME). β2-nAChR transgenic (β2-TG) mice were generated using a Tet-off transgenic system and genotyped using genomic PCR as previously described (Picciotto et al., 1995, King et al., 2003, Xu et al., 2011). In this transgenic system, β2-::TetOP-β2+::NSEtTA+ mice have retina-specific expression of β2-nAChRs (Xu et al., 2011), whereas absence of TetOp-β2 or NSEtTA abolishes retinal expression of β2-nAChRs. β2-TG mice were backcrossed for at least 8 generations onto a C57BL/6J background.
For control WT data, we included results from our previous publication (Furman and Crair, 2012), which provided a quantitative description and comparison of ‘binocular’ (medial) vs. ‘monocular’ (lateral) retinocollicular synapses in WT mice. The WT control experiments in Furman and Crair (2012) were done with identical methodology and conditions as the β2-TG experiments, with over 80% of the WT experiments performed during the same time period as the β2-TG experiments reported here, in a temporally interleaved fashion. Since the production of β2-TG mice relies on the combined expression of two transgenes on a β2-/- background, the generation of littermate β2-TG and WT (control) mice proceeds with very low yield. However, we performed additional control experiments to verify that our WT (C57BL/6J) data was an appropriate comparison group for the data derived from the β2-TG mice and to control for potential effects of breeding lineages. In these experiments, we directly crossed WT (C57BL/6J) mice with β2-TG mice, and through second-generation cross-breeding obtained β2+::TetOP-β2-::NSEtTA- mice as well as β2-::TetOP-β2-::NSEtTA+ and β2+::TetOP-β2+::NSEtTA- mice. The data from these new control experiments are included here, and are consistent with our original WT data (Furman and Crair, 2012). These additional WT experiments were performed during the same time period, and in an interleaved fashion, as the β2-TG experiments, but after the WT experiments included in our previous publication (Furman and Crair, 2012).
The number of WT control data points from our prior publication (Furman and Crair, 2012) included in the current figures and tables is as follows: Fig. 2C: 9/10, 11/14, 8/10 for P3-4, P6-7 and P12-13 respectively; Fig. 2D: 7/9, 7/11, 5/7; Fig. 3C,E: 7/8, 14/15, 7/9; Fig. 3D,F: 5/6, 11/13, 5/8; Fig. 4E-G, medial slice: 10/12; lateral slice: 8/10; Fig. 5B-D, medial slice: 10/12, lateral slice: 9/11; Table 3: WT medial slice: 16/17, 28/33, 15/19, WT lateral 12/15, 18/26, 10/15, for P3-4, P6-7 and P12-13 respectively. The P6-7 WT data from the above is also summarized and compared to β2-TG data in Tables 1 and 2. Additionally, the following experimental sample traces were reproduced from Furman and Crair (2012): Fig. 2A: WT P7; Fig. 2B: WT, P4 and WT, P12; Fig. 4A-B; and Fig. 5: WT P6-7 lateral slice, bottom.

Slice electrophysiology. Electrophysiological experiments were performed as previously described (Shah and Crair, 2008, Furman and Crair, 2012). Mice pups of either sex were anesthetized on ice (P3-P4) or through isoflurane inhalation (P7-P13) and decapitated. The brain was quickly removed and transferred to ice cold (4°C) sucrose-based cutting solution containing, in mM: 190 Sucrose, 25 Glucose, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 CaCl, 5 MgCl, 4 Na-pyruvate, and 0.6 Na-ascorbate, and saturated in 95% O₂ and 5% CO₂. Brains were mounted and sectioned into 350μm-thick slices using a Leica VT1200 microtome (Leica Microsystems, Germany). Medial (“binocular”) slices were cut parasagittally with a 20° tilt towards the coronal plane. Lateral (“monocular”) slices were cut 40° from the parasagittal towards the horizontal plane. After sectioning, slices were
transferred to room temperature bubbled ACSF and allowed 30-60min recovery before recording. Experiments were performed at room temperature with bubbled ACSF perfused at a rate of 2-3 ml/min. ACSF contained (in mM): 124 NaCl, 5 KCl, 1.25 NaH$_2$PO$_4$, 1.3 MgSO$_4$, 2 CaCl$_2$, 26 NaHCO$_3$, and 11 glucose, pH 7.2, 290-310 mOsm. In all experiments, bath ACSF contained 10μM bicuculline (Tocris, Ellisville, MO) to eliminate GABAR-mediated currents. Only one cell per slice and one slice per animal was used. Input resistance of neurons in both the medial and lateral slices gradually declined with age, and was no different between WT and β2-TG mice at any given age (Table 3).

Whole-cell recording electrodes (2-5MΩ) were pulled using a Sutter P-97 puller (Sutter Instruments, CA). Internal solution contained (in mM): 100 cesium-gluconate, 17.5 CsCl, 8 NaCl, 10 HEPES, 0.2 EGTA, 4 Mg-ATP, 0.3 GTP, 7 phosphocreatine, and 10 BAPTA; pH 7.2-7.5, 290-310 mOsm. Whole-cell voltage-clamp recordings were performed on cells in the SGS layer of the SC. Evoked responses were considered monosynaptic if they exhibited short and constant latency (3–5 ms).

Stimuli (1-5 0.1msec-long pulses of 30–150μA at 0.1msec inter-stimulus interval) were delivered every 10-15s through bipolar stimulating electrodes made from a pair of stainless-steel or tungsten electrodes (FHC, Bowdoinham, ME). The evoked responses in these recordings are predominantly of retinal origin (see Furman and Crair, 2012 for details). Data was collected and analyzed using
custom programs written in IgorPro (WaveMetrics, Lake Oswego, OR) and Matlab (Mathworks, MA). Input and series resistances were measured continuously to monitor the health of the cell, and data were discarded from analysis if these parameters drifted >20% over the course of the experiment.

Monocular enucleations. P0 mice were anesthetized on ice. After cutting the eye lid and the conjunctiva, the eyeball was displaced from the socket, the extraocular muscles were sectioned and the eyeball was removed. Antibiotic ointment was applied to the eye.

AMPA/NMDA ratios. After recording a stable monosynaptic evoked AMPA response at -70mV holding potential, AMPA receptors were blocked with 10μM NBQX (Tocris), and the holding potential was switched to 40mV to record NMDA receptor-mediated currents at the same stimulation strength. AMPA/NMDA ratios were computed based on the average peak current amplitudes of 10-20 sweeps at each holding potential.

Strontium evoked AMPA miniature events. Stable monosynaptic evoked AMPA responses were recorded at -70mV holding potential. Extracellular Ca\(^{2+}\) (2mM) in the bath solution was then replaced with 3mM Sr\(^{2+}\) to desynchronize synaptic vesicle release (Xu-Friedman and Regehr, 1999). Evoked miniature events were recorded in 1s epochs every 10s. Miniature events were analyzed offline using Mini Analysis software (Synaptosoft, Decatur, GA). Amplitude thresholds were
set at 2.5 times root-mean-square noise, and at least 50 events were used for analysis in each cell.

**Graded stimulation experiments.** Evoked AMPA responses were recorded at -70mV holding potential. AMPA receptors were then blocked with 10μM NBQX (Tocris), and the holding potential was switched to 40mV to record NMDA receptor-mediated currents. Stimulation strength was adjusted until a mixture of subthreshold (failures) and suprathreshold (successes) responses were observed. Stimulation strength was then gradually increased to recruit more inputs until a saturating current response was achieved. Success responses were defined as those with peak above 6pA at the minimal stimulation strength. Success responses and saturation responses were averaged overall relevant trials. The number of inputs was estimated as the mean saturating response divided by the mean single-fiber response.

**Morphological reconstructions.** For cell staining, 60μM Alexa Fluor 594 hydrazide was included in the internal solution. After recordings, slices were fixed in 4% PFA, mounted on slides, and imaged using a Zeiss AxioImager Z2 microscope (Carl Zeiss, Germany). Reconstructions were carried out using Neurolucida and NeuroExplorer (MBF Bioscience, VT). Fractal dimension was calculated using the box counting method. The fractal dimension reflects the dendritic tree’s space-filling properties as one gradually zooms down to progressively finer scales. We chose to use the fractal dimension because it
characterizes branching and dendritic complexity locally. This contrasts with Sholl’s analysis, which is based on counting the intersections of neuronal processes with concentric circles of increasing radii and therefore strongly depends on the overall size of the dendritic tree.

Statistical analysis. Data values are presented as mean ± SE. Differences in means were tested for significance as follows. Effects of age were tested using one-way ANOVA followed by pairwise comparisons using Tukey's honestly significant difference (HSD) criterion for multiple-comparisons. Differences between β2-TG and WT mice at each age were tested using Student’s t test with false discovery rate (FDR) procedure for multiple-comparisons (Curran-Everett, 2000, Curran-Everett and Benos, 2004). Results at P6-7 (Figs. 4,5) were tested using one-way ANOVA with pairwise adjusted post-hoc tests. Statistical analysis was done in Matlab (Mathworks, MA).

RESULTS

We examined the synaptic basis of visual map development prior to eye opening in the superior colliculus of wild-type and β2-TG mice. For this purpose, we performed patch-clamp recordings in acute retinocollicular brain slices dissected from neonatal mice at postnatal days P3-P13. As previously reported, β2-TG mice (“small wave mice”) have retina-specific expression of β2-nAChRs that is largely
limited to the ganglion cell layer (Xu et al., 2011). Because retinal wave propagation relies on β2-nAChRs in amacrine cells in the inner nuclear layer (Butts et al., 1999, Zheng et al., 2006, Blankenship and Feller, 2009, Ford et al., 2012), the spatial spread of retinal waves is “choked off” in β2-TG mice, resulting in local patches of correlated activity (Fig. 1A). Importantly, however, firing properties of individual RGCs are normal in β2-TG mice (Table 1 in Xu et al., 2011).

As previously reported (Xu et al., 2011), focal dye injections into the retina of β2-TG mice reveal a surprising map phenotype. RGC projections from the ventro-temporal retina, which map to the antero-medial SC, have an enlarged target zone, reflecting poor retinotopic refinement in this part of the map (Fig. 1B). In contrast, projections from other regions of the retina have normally-refined target zones. Interestingly, the antero-medial crescent of the SC (shaded region in Fig. 1B), where retinotopic refinement is poor, represents the binocular visual field and accordingly receives input from both eyes, whereas the lateral/posterior SC is dominated by monocular, contralateral retinal input (Hofbauer and Dräger, 1985).

This intriguing anatomical mapping phenotype prompted us to design two novel brain slice preparations in order to record selectively from neurons in the medial vs. lateral SC. These slices, described in detail in Furman and Crair (2012), differ in their cutting orientations in 3D, but preserve similar circuitry along the retinocollicular pathway (Fig. 1B). In particular, both slices allow us to record retinocollicular synaptic currents by electrically stimulating retinal axons while...
performing patch-clamp recordings from neurons in the retinorecipient SGS layer of the SC (Grantyn et al., 2004, Mize and Salt, 2004).

Early in development, both the medial and lateral SC receive a substantial ipsilateral and contralateral retinal input. Towards the end of the first postnatal week, a clear distinction between the binocular and monocular region of the SC emerges as retinocollicular axon arbors refine to focal target zones (Fig. 1C, Dhande et al., 2011). The monocular (lateral) SC also differs to some extent between β2-TG and WT mice. In both mouse strains, the ipsilateral projection initially extends laterally, beyond the antero-medial crescent, and also dorsally into the SGS layer. During normal development, the ipsilateral projection retracts to small clusters located ventrally to the SGS layer and confined to the antero-medial SC. In β2-TG mice, like in other transgenic mouse lines in which eye-segregation is impaired, the ipsilateral input remains somewhat more widespread laterally than in WT mice, and also persists in the SGS layer beyond the first postnatal week. For these reasons, we label the two slices based on their anatomical location (medial/lateral), appreciating that the medial slice is largely binocular, while the lateral slice is predominantly monocular in its innervation.

**Synapse development in β2-TG mice**

To characterize the development of individual retinocollicular synapses, we used whole-cell patch clamp electrophysiological techniques to record miniature evoked synaptic currents (“AMPA-minis”). In these experiments, we first isolated
synchronous evoked AMPA receptor-mediated responses by clamping the holding potential at -70mV and stimulating retinal axons. At this holding potential, NMDA receptors are largely blocked by magnesium ions contained in the extracellular solution. We then replaced the extracellular Ca$^{2+}$ with Sr$^{2+}$ to desynchronize vesicle release at stimulated axon terminals and obtain AMPA-minis that presumably reflect events at single synapses (Fig 2A,B; Xu-Friedman and Regehr, 1999). Because $\beta_2$-nAChR-mediated retinal waves span approximately the first postnatal week, manipulating these waves is likely to have little effect on synapse development just after birth. Indeed, at P3-4, we observed no significant differences in AMPA-mini amplitudes between $\beta_2$-TG and WT mice (Fig 2C,D). At P6-7, however, interesting differences between these two mouse populations emerged. In the medial slice, AMPA-mini amplitudes were smaller in $\beta_2$-TG than WT mice. This difference did not persist to P12-13, as AMPA-mini amplitudes in $\beta_2$-TG “caught up”, to some extent, with the developmental increase observed in WT mice. In contrast, in the lateral slice, AMPA-mini amplitudes increased significantly between P3-4 and P6-7 in $\beta_2$-TG but not in WT mice, resulting in dramatically larger AMPA-mini amplitudes in $\beta_2$-TGs at P6-7. Again, this difference was transient, and did not persist to P12-13. These findings suggest that manipulating wave propagation during the first postnatal week affects not only the anatomy of the retinocollicular map but also its synaptic and functional basis at P6-7.

The production of $\beta_2$-TG mice relies on the combined expression of two transgenes on a $\beta_2^{-/-}$ background, thus greatly complicating the generation of
littermate β2-TG and WT mice. The WT control experiments in Figures 2-5 include (see Methods and figure captions for numbers): (1) WT data from our previous publication (Furman and Crair, 2012); importantly, these experiments were performed under identical methodology as the β2-TG experiments and were temporally interleaved; (2) additional WT experiments on mice that have been re-crossed to β2-TG mice, to control for potential effects of breeding lineages (see Methods). In each experimental condition (age, slice, and protocol), we tested for differences between β2-TG mice and the two categories of WT mice described above. We found no difference between the two WT groups (p>0.05 in all cases). Furthermore, when synaptic properties of β2-TG differed from the pooled group of all WT data, those differences were also statistically significant between β2-TG and each of the two WT sub-groups (one-way ANOVA followed by pairwise comparisons using Tukey's criterion for multiple-comparisons). Accordingly, in Figures 2-5 we pooled the WT data into one group. It should be noted, as previously mentioned, that the genetic manipulation in β2-TG mice had opposite effects on synapse development in the lateral vs. medial SC. In particular, we observed weaker than normal synapses in the medial SC, and stronger than normal synapses in the lateral SC. These findings further substantiate the use of WT data as relevant control data for the β2-TG experiments, as they argue against the possibility of a global, non-selective defect in synapse development in β2-TG mice.
To further investigate retinocollicular synaptic properties in β2-TG mice, in a different pool of cells, after recording a stable synchronous AMPA response at -70mV holding potential we switched to a holding potential of +40mV and recorded NMDA mediated currents while pharmacologically blocking AMPA-mediated transmission (Fig. 3A,B). Maturing synapses are often characterized by shortening of the NMDA decay time constant τNMDA, and an increase in the AMPA/NMDA ratio (Hestrin, 1992, Crair and Malenka, 1995, Shi et al., 2000, Lu et al., 2001, Shi et al., 2001, Malinow and Malenka, 2002, Lu et al., 2003, Takahashi et al., 2003, Shah and Crair, 2008). Overall, τNMDA and AMPA/NMDA ratios indeed followed these developmental trends (Fig. 3C-F). One exception is AMPA/NMDA ratios in the lateral slice of WT mice, which remained similar from P3-4 to P12-13 (see also Furman and Crair, 2012). In contrast to AMPA-minis, τNMDA and AMPA/NMDA ratios were similar between β2-TG and WT mice at P6-7. τNMDA was slightly, but not significantly shorter in β2-TGs in the lateral slice. At P12-13 synaptic properties were mostly similar in β2-TG and WT mice, with one interesting exception: in the lateral slice, AMPA/NMDA ratios were larger in β2-TG than in WT mice (see Discussion). We also observed, as expected, a gradual decrease in the input resistance of SC neurons over development that was similar in β2-TG and WT mice (see Table 3 and Materials and Methods).

Because AMPA-mini amplitudes differed between β2-TG and WT mice in both slices at P6-7, and because this is the time point where altered retinal activity in
β2-TGs is more likely to exert its influence on synapse development, we focused for the following analyses on this age group. Anatomically, by end of the first postnatal week map refinement is impaired in the antero-medial crescent of β2-TG mice (Xu et al., 2011). The smaller AMPA-mini amplitudes are consistent with the anatomical data in a model where poor map refinement is linked to reduced strengthening of synaptic contacts. This model further suggests that the presence of mistargeted retinal axons should be accompanied by an abnormally large number of retinal inputs converging onto postsynaptic neurons. To examine this prediction, we used a graded stimulation protocol (Chen and Regehr, 2000, Hooks and Chen, 2006, Chandrasekaran et al., 2007) that allowed us to estimate (1) the response amplitude to single retinal axon stimulation; and (2) the number of RGC inputs to individual SC neurons.

In this protocol, after detecting a stable NMDA response at +40mV holding potential, we lowered the stimulus intensity to obtain a mixture of failures and successes. The mean amplitude of successful responses is an estimate of single-fiber response. We then increased the stimulation strength until a saturating response level was achieved (Fig. 4A-D). To estimate the number of inputs, we divided the saturating response by the single-fiber response. Importantly, because AMPA currents are blocked, a wide range of stimulation strengths can be examined without the complication of intra-collicular polysynaptic responses. Notably, in the medial slice, the number of inputs was significantly larger in β2-TG than in WT mice (Fig. 4E, red and orange). Although we cannot identify the
specific spatial origin of these inputs, it is possible that the abnormally large number of functional inputs to neurons in the medial SC of β2-TG mice at P6-7 reflects synaptic contacts of mis-targeted retinal axons in this region of the SC in β2-TG mice. In the lateral slice, in contrast, the number of inputs in β2-TG mice was no different than in WT mice (Fig. 4E, blue and green), which is consistent with the normal retinotopic refinement of RGC afferents in this part of the map.

We previously reported that in WT mice, the single-fiber response amplitude is larger in the medial than in the lateral SC due to binocular interactions that are more prominent in the medial SC. Here, we observed that the single-fiber response amplitude in the medial SC was comparable in β2-TG mice and WT mice. However, the single-fiber response in the lateral slice was dramatically larger in β2-TG mice, both compared to WT and to the medial slice (Fig. 4F). This result complements the large AMPA-mini amplitudes observed in the lateral slice of β2-TG mice.

To summarize the physiological findings at P6-7: in the medial (“binocular”) SC, we observed that AMPA-minis amplitudes were reduced in β2-TG mice but the number of retinal inputs per SC neuron was abnormally large (Table 1). In contrast, in the lateral (predominantly monocular) SC, AMPA-minis and single-fiber response were large in β2-TGs, whereas the number of inputs was normal (Table 2). The findings from the medial slice of β2-TG mice are consistent with their impaired anatomical map in this sub-region of the SC (Xu et al., 2011).
electrophysiological findings from the lateral slice are surprising, and may reflect
enhanced synapse strengthening in response to axonal competition due to
breakdown of retinal activity into small de-correlated patches or domains (see
Discussion).

Synaptic properties of β2-TG mice in the medial vs. lateral SC

We have so far compared synaptic properties of β2-TG and WT mice within each
of the slices. We next examined how the medial slice compares to the lateral SC
in β2-TG mice. We tested all synaptic properties previously discussed for
significance between the medial and lateral SC of β2-TG mice. Differences were
statistically significant for the following properties only: (1) as expected from Fig. 2,
at P6-7 AMPA-mini amplitudes were significantly larger in the lateral slice
than the medial slice of β2-TG mice (p=0.0002, two-tailed t-test); (2)
interestingly, AMPA-minis were also significantly larger in the lateral slice than
in the medial slice at P3-4 (p=0.041), suggesting that the dramatic difference
between the two slices seen at P6-7 starts in fact to emerge during the first few
postnatal days; (3) as indicated in Fig. 4, at P6-7 the number of inputs was
significantly larger in the medial than lateral SC (p=0.012), and finally (4) the
NMDA single-fiber response amplitude was larger in the lateral than the medial
SC at P6-7 in β2-TG mice (p=0.015).
Monocular enucleation at birth increases AMPA-mini amplitudes and decreases the number of inputs in the medial SC of β2-TG mice at P6-7

As previously discussed, in the medial SC of β2-TG mice, synapse development was impaired: AMPA-mini amplitudes were smaller than in WT mice, and the number of inputs per SC neuron was abnormally large. We previously proposed (Xu et al., 2011) that binocular competition is impaired in the medial (binocular) SC of β2-TG due to the abnormal propagation of their retinal waves. This model predicts that enucleating the ipsilateral eye at birth will restore normal development of β2-TG mice in the medial SC by eliminating binocular competition. This prediction has been confirmed anatomically (Xu et al. 2011), and we now asked how synaptic properties in β2-TG mice were affected by monocular enucleation at P0. Notably, monocular enucleation at birth “restored” synaptic properties in the medial SC of β2-TG to WT levels (Table 4). Specifically, enucleated β2-TG mice had larger AMPA-mini amplitudes and a smaller number of inputs compared to non-enucleated β2-TG mice, and all synaptic properties were similar between β2-TG and WT mice (see Discussion).

Dendritic branching is enhanced in the lateral SC of β2-TG mice

In some of the recordings, we successfully filled the SC neurons with Alexa Fluor 594 and reconstructed their morphology offline. Importantly, this technique permits us to examine dendritic development specifically in physiologically confirmed retino-recipient neurons (c.f., Krahe et al., 2011). We focused again on P6-7, and asked whether the observed differences in synapse development
between β2-TG and WT mice were correlated with differences in dendritic branching in the postsynaptic neurons.

As reported previously (Furman and Crair, 2012) and confirmed here, at P6-7 dendritic branching of neurons in WT mice is highly elaborate in the medial SC, but not as elaborate in the lateral SC (Fig. 5). Qualitatively, in β2-TG mice, dendrites appeared to be highly and roughly similarly complex in the medial and lateral SC (Fig. 5A, bottom panels). To quantify dendritic morphology and test for differences among the groups, for each neuron we calculated: (1) the number of dendritic nodes; (2) total dendritic length; and (3) the fractal dimension of the dendritic tree, which reflects its space-filling properties as one gradually zooms down to progressively finer scales.

In the medial SC, dendritic branching was similar in β2-TG and WT mice (Fig. 5B-D, Table 1). This suggests that the relatively large number of weak synaptic inputs to medial slice neurons in β2-TG mice has no net effect on dendritic branching. In contrast, neurons form lateral slices in β2-TG mice with increased synaptic strength and single-fiber response amplitudes (Fig. 2 and Fig. 4) also had increased dendritic branching (Fig. 5 and Tables 1, 2).

**DISCUSSION**
We examined the role of retinal wave propagation in the formation of functional synaptic contacts between retinal ganglion cells (RGCs) and their postsynaptic partners in the superior colliculus (SC). To record retinocollicular synaptic currents, we performed patch-clamp recordings in two recently developed brain slice preparations (Furman and Crair, 2012). These slice preparations allow us to examine the difference in retinocollicular functional connectivity in the medial SC, which receives binocular input, versus the lateral SC, which is predominantly monocular. Furthermore, to manipulate retinal wave propagation during the first postnatal week, we employed a transgenic approach: we used β2-TG (“small wave”) mice with conditional expression of β2-nAChRs in the retinal ganglion cell layer only (Xu et al., 2011). In these mice, the spiking activity of individual RGCs is normal, but retinal waves during the first postnatal week do not propagate beyond local “small” domains. We found a surprising synaptic phenotype in β2-TG mice at P6-7. In the medial SC, the number of retinal inputs converging onto postsynaptic cells was abnormally large, but retinocollicular synaptic strength was smaller than normal. In the lateral SC, by contrast, synapses were dramatically stronger in β2-TG, with a similar number of inputs in β2-TG and WT mice. Moreover, the dendritic anatomy of neurons in the lateral SC of β2-TG mice was highly elaborated, resembling that of neurons in the medial SC. These findings suggest that the spatiotemporal properties of retinal waves instruct not only visual map development and the anatomy of retinocollicular connectivity, as previously reported (Xu et al., 2011), but also their morphological and functional synaptic underpinning in the SC.
Patterns of spontaneously generated neural activity have been observed across the developing nervous system (Lippe, 1994, Leinekugel et al., 2002, Blankenship and Feller, 2009, Watt et al., 2009, Warp et al., 2011, Ackman et al., 2012). Clarifying the developmental roles of these activity patterns is of major importance, as environmental or genetic factors (e.g., exposure to nicotine) may interfere with these patterns, and potentially cause abnormal circuit formation (Uusisaari et al., 2002, Graven, 2004). A growing body of evidence supports an instructive role for retinal waves during visual map development, meaning that specific spatiotemporal patterns of retinal waves, and not just normal activity levels at individual RGCs, are necessary for proper wiring of RGCs to their targets in the brain (Stellwagen and Shatz, 2002, Stafford et al., 2009, Xu et al., 2011). In particular, recent work from our lab employed a conditional transgenic approach to manipulate the spatiotemporal properties of retinal waves without affecting the firing properties of individual RGCs. Anatomical analysis demonstrated that “small” retinal waves in these β2-TG mice are sufficient to support spatial retinotopic refinement in the mainly monocular region of the SC, but insufficient to support eye-specific segregation and retinotopic refinement in the binocular (antero-medial) SC. The selective impairment of map development in the antero-medial SC clearly depends on binocular interactions, because
enucleating the ipsilateral eye at birth rescues retinotopic refinement in the antero-medial SC of β2-TG mice.

The finding that manipulating retinal activity results in divergent synaptic phenotypes in the medial vs. lateral SC is significant both conceptually and methodologically. Conceptually, it suggests that the impact of altering the pattern of retinal activity depends on the degree of binocular interactions in a specific sub-region of the visual map. Methodologically, it supports the idea that altered synapse development in β2-TG mice stems from the abnormal retinal waves in these mice, and not simply some global and nonselective impairment in their retina or SC. This is further corroborated by the enucleation experiments (Table 4). In particular, ipsilateral enucleation at birth increased AMPA-mini amplitudes in the medial SC of β2-TG mice, strongly arguing against some general defect in synapse formation mechanisms in these mice. The possibility remains, however, that the altered expression of β2-nAChRs in β2-TG mice, particularly the lack of these receptors in the LGN and SC, contributes to their mapping phenotype, for instance through downstream effects on cell-adhesion processes (cf. Rubin et al., 2011).

**Synaptic and dendritic development in the medial SC of β2-TG mice**

The electrophysiological results from the medial (binocular) SC are consistent with, and complement the anatomical findings of Xu et al. (2011). In particular, the excessive number of inputs to SC neurons fits well with the impaired eye-
segregation and retinotopic refinement of retinal axons in the antero-medial SC: if some of the aberrant retinal axons in the antero-medial SC form synaptic contacts with postsynaptic partners, then one would expect to observe a greater number of inputs in the antero-medial SC. Xu et al. (2011) reported that enucleating the ipsilateral eye at birth restores normal retinotopy in the antero-medial SC of β2-TG mice. Here we show that ipsilateral enucleation also “restored” synaptic properties of β2-TG mice to WT levels (Table 4). It should be noted that AMPA-mini amplitudes in the enucleated β2-TG mice were smaller than in the lateral SC of β2-TG mice, in other words, enucleation increased AMPA-mini amplitudes, but not to the point of the “monocular” part of the map in the same mice. In any case, these findings argue against the possibility that observed differences between the medial and lateral SC stem solely from SC neurons obeying different competition rules for synapse strengthening in the medial vs. lateral SC. Instead, these findings support the idea that between-eye competition interferes with map development in the medial SC of non-enucleated β2-TG mice, both anatomically and physiologically.

We observed no significant difference in dendritic branching between WT and β2-TG mice in the medial SC (Fig. 5). It is possible that the enlarged number of inputs encourages enhanced branching in β2-TG mice, but the weaker synapses in these mice does not support persistent increases in dendritic branching, resulting in no net change compared to WT mice. It is also possible that averaging across a heterogeneous population of neurons in the SC increased the observed variance in
dendritic properties and masked small differences between the genotypes. To address this caveat, it would be worthwhile in future work to record from identified cell types in the SC, e.g. using GFP reporter mice that selectively label particular classes of neurons.

In our previous work (Furman and Crair, 2012), we found that developmental changes in AMPA/NMDA ratios do not follow the same time course as all other synaptic and dendritic properties examined. Specifically, AMPA-minis were similar in the medial and lateral SC at P3-4 but were larger in the medial than in the lateral SC at P6-7. In contrast, AMPA/NMDA ratios were similar in the medial and lateral SC at P3-4 and P6-7, but were larger in the medial than in the lateral SC at P12-13. This suggests that in the retinocollicular system the developmental increase in AMPA/NMDA ratio is delayed compared to AMPA-minis. This same interpretation could explain the AMPA/NMDA profiles of \( \beta_2 \)-TG mice reported here. Specifically, at P6-7 AMPA-minis were dramatically larger in the lateral SC of \( \beta_2 \)-TG compared to WT mice, presumably due to increased competition in the lateral SC of \( \beta_2 \)-TG mice. At this age (P6-7), AMPA/NMDA ratios were similar between \( \beta_2 \)-TG and WT mice (Fig. 3E, F). However, at P12-13, AMPA/NMDA ratios were abnormally large in the lateral SC of \( \beta_2 \)-TG compared to WT mice. In other words, increased competition in the lateral SC of \( \beta_2 \)-TG mice caused an over-maturation of AMPA-minis at P6-7, and, with a delay, atypically large AMPA/NMDA ratios at P12-13. For the moment, it remains unclear whether the developmental changes in AMPA-minis
and AMPA/NMDA ratios are indeed mechanistically linked (with a delay) in the retinocollicular system, or alternatively whether the observed changes in these two measures are only correlational.

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**Synaptic and dendritic development in the lateral SC of β2-TG mice**

In the lateral SC, the electrophysiological results reported here are somewhat unexpected in view of previous anatomical findings. However, these results are consistent with a recently proposed model of retinal axon competition in the SC (Furman and Crair, 2012). In this model, competition through temporal offset in activity enhances synaptic and dendritic growth. This form of competition may operate not only between axons from the two eyes, as previously suggested, but also among RGC axons of the same eye. During normal development, this form of within-eye competition is attenuated in the monocular SC because retinal waves propagate over large distances, generating continuous activation of RGCs along their wave front. In β2-TG mice, in contrast, small patches of retina are activated discontinuously, thus generating “competing” cohorts of RGCs that are activated at a temporal offset on the order of seconds to tens of seconds.

According to the growth-enhancing model of axonal competition, one would predict excessive synaptic strengthening and dendritic branching of neurons in the lateral SC of β2-TG mice, as we observed here.
We previously reported that in WT mice, synaptic strengthening and dendritic branching are enhanced in the medial (binocular) compared to the lateral (monocular) SC (Furman and Crair, 2012). The WT dataset reported here expands and confirms our previous findings by including WT mice backcrossed to β2-TG mice, to control for potential genetic differences due to inbreeding within β2-TG mice. The findings from the lateral SC of β2-TG mice, echo, in a way, the “binocular” WT data: synaptic strength (Fig. 2), number of inputs (Fig. 4E), single-fiber response amplitude (Fig. 4F), and dendritic branching (Fig. 5), are all similar, or even slightly larger in the lateral SC of β2-TG mice compared to the medial SC of WT mice. We interpret these findings as reflecting enhanced competition among retinal axons of the same eye due to small retinal waves in β2-TG mice, similar to the enhanced competition among retinal axons of the two eyes in the binocular SC of WT mice.

Methodological considerations

An important limitation of electrically stimulating retinal inputs to the SC is that ipsilateral and contralateral inputs are stimulated non-selectively. This limits the interpretation of the retinal origin of the measured postsynaptic responses. In WT mice from P6-7 on, the measured responses are primarily from contralateral inputs even in the medial SC, because at these ages the SGS layer, where recordings were performed, receives almost exclusively contralateral input. In β2-TG mice the picture is more complicated: eye-segregation is poor in these mice, and the ipsilateral input extends laterally later in development than in WT mice.
Despite these caveats, it is important to note that in the mouse, contralateral input dominates even the binocular SC, with the ipsilateral retinal axons constituting only a small fraction of the total input to the SC. It is possible that ipsilateral inputs contributed to the postsynaptic responses we measured, but given the strong bias toward input from the contralateral eye, we think it is unlikely that the differences in synapse development we observed between β2-TG and WT mice are attributable solely to differences in the relative contribution of ipsilateral vs. contralateral inputs. These issues could be addressed in future work by selectively stimulating ipsilateral vs. contralateral RGC axons, e.g. using optogenetic techniques or an SC version of optic nerve attached LGN preparations (Mooney et al., 1996, Jaubert-Miazza et al., 2005, Ziburkus and Guido, 2006). These techniques may also allow one to examine whether individual SC neurons are dominated by input from one eye, or alternatively receive binocular input at younger ages, as this question is of particular relevance for models of binocular map development.

The graded-stimulation technique for estimating number of inputs (Fig. 4) is prone to a number of methodological limitations. In particular, RGC axons that were severed during slicing downstream of the stimulation location would not contribute to post-synaptic responses. Thus, in practice the calculated number of inputs is likely to underestimate the actual number of RGC inputs. A related point is that two (or more) axons arbors that branch from a single RGC axon at a point upstream of the stimulation location may be counted as separate inputs in the
graded-stimulation protocol (as long as the axon retains those branches and both branches synapse onto the same postsynaptic neuron). In our experiments, the stimulation location is close to the anterior tip of the SC (see Furman and Crair 2012). In WT mice, it is atypical for an axon to branch anterior to this point (Dhande et al., 2011). However, the possibility remains that in some cases RGC axons bifurcate in β2-TG mice anterior to the stimulation point so that our stimulating electrodes recruit, depending on the stimulation strength, one or more axon arbors originating from the same RGC. Such a scenario would compromise the estimated number of inputs in the β2-TG mice. However, the observed difference in the number of inputs between β2-TG and WT mice in the medial slice is quite dramatic, and is therefore likely to reflect, at least in part, an actual difference in the number of RGCs contacting postsynaptic neurons (Fig. 4E).

**Recapitulation: The functional role of long-range retinal wave propagation**

To summarize, our findings suggest that long-range retinal wave propagation plays different and complementary roles in the two sub-regions of the SC (Fig. 6):

1. In the binocular SC, large retinal waves are required for mediating “successful” binocular competition, meaning: proper segregation of eye-specific retinal inputs; pruning of inappropriate retinocollicular synapses; and strengthening of appropriate synapses. When waves are small, binocular segregation is impaired and synapses are weaker than normal; (2) In the lateral (mainly-monocular) SC, large retinal waves attenuate competition among same-eye RGCs: here, small retinal waves cause a transient but dramatic abnormal
increase in synaptic strength. Ultimately, molecular and activity-dependent mechanisms involved in circuit refinement may act in a cell-type specific manner. Based on traditional electrophysiological techniques we obtained a “first-order” analysis of synapse development across the retinocollicular map. It would be beneficial to extend these techniques to allow, for example, recording from identified sub-populations of neurons in the SC, to stimulate specific RGC sub-populations, and to perform refined manipulations of retinal activity in space and time. Recent advances in imaging, stimulation, and genetic techniques (Yazaki-Sugiyama et al., 2009, Cruikshank et al., 2010, Dombeck et al., 2010, Runyan et al., 2010, Koch et al., 2011, Zhang et al., 2011a, Zhang et al., 2011b) are paving the way for examining the mechanistic underpinning of map development in unprecedented detail.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS
Fig 1. Examining synapse development in the medial and lateral SC of WT and β2-TG mice. A. Example spontaneous retinal waves recorded with a multielectrode array in WT (top) and β2-TG (bottom) mice at P4 (from Xu et al., 2011). The size and color of the circles correspond to the activity level recorded from the underlying electrodes, which were spaced 100µm apart, with 500 msec between frames from left to right. Waves in WT mice encompass the entire electrode array, whereas in β2-TG mice, waves are much more circumscribed and encompass a smaller portion of the retina. B. Schematic of mapping phenotype in β2-TG mice. At P7, focal dye injections into the retina (left, different colors) result in dense target zones in corresponding locations of the superior colliculus (SC) of wild-type (WT) mice (middle, dorsal view). In contrast, in β2-TG mice (right), target zones are enlarged for ventro-temporal retinal injections, indicating poor retinotopic refinement in this part of the map, but not in the remaining parts of the map. The ventro-temporal retina and its corresponding antero-medial crescent of the SC (shaded) represent the binocular visual field. To record selectively from the medial (binocular) vs. lateral (mainly-monocular) SC, we designed two brain-slice preparations that differ in their cutting angles. C. Schematic of retino-collicular axons refinement. Panels A-B were adapted with permission from Xu et al. (2011) and panel -C was adapted with permission from Dhande et al. (2011).

Fig 2. Development of AMPA-mini currents in β2-TG and WT mice. A,B. Examples of evoked AMPA miniature currents ("AMPA-minis") recorded from
medial (A) and lateral (B) slices of β2-TG and WT mice at different ages. In these experiments, synchronous evoked AMPA responses were first recorded under a Ca²⁺-ACSF bath (gray, average trace over 10-20 sweeps). The extracellular Ca²⁺ was then replaced by Sr²⁺ to desynchronize synaptic release and evoke “miniature” AMPA currents (black). C,D. Quantification of AMPA-mini amplitudes in the medial (C) and lateral (D) slice. Number of cells per group is indicated in the figure. WT data include recordings from Furman and Crair, 2012 (see Methods; 9/10, 11/14, and 8/10 cells for P3-4, P6-7 and P12-13 respectively in panel C; and 7/9, 7/11, 5/7 in D). Also, traces WT-P7 (panel A) and WT-P4 and WT-P12 (panel B) were reproduced from Furman and Crair, 2012. In all recordings only one cell per slice and one slice per animal were used. Effect of age was tested using one-way ANOVA with Tukey’s post-hoc test; differences between WT and TG mice were tested using two-tailed Student’s t-test with false discovery rate procedure for multiple-comparisons.

Fig 3. Development of AMPA/NMDA ratios and NMDA decay time-constant.

A,B. Examples of AMPA- and NMDA-mediated currents recorded from medial (A) and lateral (B) slices of β2-TG and WT mice at different ages. Each trace represents average response of 10-20 sweeps. C-F. Quantification of NMDA decay time-constant, τ_{NMDA} (C,D) and AMPA/NMDA ratios (E,F; one-way ANOVA with Tukey’s post-hoc test for the effect of age; Student’s t-test with false discovery rate procedure for differences between WT and β2-TG mice at each age). WT data includes recordings from Furman and Crair, 2012, as follows
(see Methods): 7/8, 14/15, and 7/9 cells for P3-4, P6-7 and P12-13 respectively in panels C,E; and 5/6, 11/13, 5/8 cells in panels D,F.

**Fig 4. Number-of-inputs analysis. A-D.** Examples of graded-stimulation experiments at P6-7 in medial and lateral slices of β2-TG and WT mice. In these experiments, we measured NMDA-mediated responses at different stimulation strengths. The lower stimulation strength was adjusted to obtain a mixture of no-response trials (“failures”) and responses (“successes”). The mean peak amplitude of the success responses at the minimal stimulation strength is an estimate of the single-fiber response. Stimulation intensity was then gradually increased until saturation response amplitude was obtained. The saturation response divided by the single-fiber response is an estimate of the number of retinal inputs to the neuron. **E-G.** Quantification of the number-of-inputs (E), single-fiber response (F), and saturation response (G), for the four experimental groups, color coded as indicated in the schematic. Differences were tested using one-way ANOVA with Tukey’s post-hoc test. The WT traces in panels A and B were reproduced from Furman and Crair, 2012. Also, part of the WT data in E-G (medial slice: 10/12; lateral slice: 8/10 cells) is from Furman and Crair, 2012.

**Fig 5. Morphological analysis of SC neurons. A.** In some of the experiments, we labeled neurons during recording and reconstructed their morphology. Shown are examples of reconstructed neurons from medial and lateral slices of β2-TG and WT mice at P6-7. All cells are oriented so that the pial surface is facing upward; scale bar: 100µm in all panels. **B-D.** Dendritic complexity of
reconstructed neurons was quantified using: (B) the number of dendritic nodes; (C) total dendritic length; and (D) fractal dimension of the dendritic tree. Differences were tested using one-way ANOVA with Tukey’s post-hoc test. One of the reconstructed cells in A (WT P6-7, lateral slice, bottom) and part of the WT data in B-D (medial slice: 10/12, lateral slice: 9/11 cells) is from Furman and Crair, 2012.

**Fig 6. Results summary and conceptual model.** Top row: Retinocollicular synapse development was examined using brain slice preparations that cut through the medial (binocular) or lateral (mainly monocular) SC (*left/right respectively*). Middle/bottom rows: Schematic of synaptic and anatomical phenotype at P6-7 in WT and β2-TG mice, respectively. In WT mice, retinal waves propagate over relatively long distances. Thus, within each eye, RGCs at different locations are activated consecutively (when participating in the same wave). These within-eye correlations support segregation of retinal axon branches into eye-specific layers in the binocularly innervated, medial SC. Large green circles represent formation of strong synaptic contacts to the postsynaptic neuron. Both in the medial and lateral SC, WT waves support retinotopic refinement of the map, so that RGC-axons branch in the corresponding retinotopic location of the SC. SC neurons are shown as receiving monocular input, though in reality the possibility exists of binocular input in the medial SC. In β2-TG mice, retinal wave propagation is truncated resulting in “small waves” that are insufficient for
supporting eye-specific segregation and retinotopic refinement in the binocular
SC. Physiologically, SC neurons receive a larger number of retinal inputs. Some
of these inputs could potentially arise from the ipsilateral eye (as shown), or
alternatively from the contralateral eye only. In the lateral SC, where binocular
interactions are less prominent, the local spatial information contained in small
waves is sufficient for retinotopic refinement of the map. Furthermore, synaptic
strength is enhanced in the lateral SC of β2-TG mice due to large temporal offsets
in the bursting activity of nearby regions in the retina. These temporal offsets are
nearly absent during normal development, and according to a recently proposed
model may promote synaptic strengthening through enhanced axonal competition.

Table 1. Medial SC Neuron Properties at P6-7. Data are presented as means ±
SE. SC, superior colliculus. Percent difference between β2-TG and WT mice
defined as $100 \cdot (x^{\text{TG}} - x^{\text{WT}})/x^{\text{WT}}$, where $x^{\text{TG}}$ and $x^{\text{WT}}$ are the corresponding values in
β2-TG and WT mice, respectively. Part of the WT data is from Furman and Crair,
2012, as follows (see Methods): 11/14, 14/15, 14/15, 10/12, 10/12, 10/12, 28/33,
10/12, 10/12 and 10/12 cells for each property at the order listed, respectively.
Differences in means tested with a 2-tailed Student’s $t$-test: *$P<0.05$, **$P<0.01$.
Synaptic/morphological properties that differed significantly between the 2 mice
strains are in boldface.

Table 2. Lateral SC Neuron Properties at P6-7. Differences in means tested
with a 2-tailed Student’s $t$-test: *$P<0.05$, **$P<0.01$, ***$P<0.001$. 
Synaptic/morphological properties that differed significantly between the 2 mice strains are in boldface. See Table 1 for further details. Part of the WT data is from Furman and Crair, 2012, as follows (see Methods): 7/11, 11/13, 11/13, 8/10, 8/10, 8/10, 18/26, 9/11, 9/11 and 9/11 cells for each property at the order listed, respectively.

Table 3. Development of input resistance of SC neurons. Input resistance of recorded neurons in the medial (A) and lateral (B) slices at indicated ages. Effect of age was tested using one-way ANOVA with Tukey’s post-hoc test. No statistical differences were detected between β2-TG and WT mice in any given age group (Student’s t-test with false discovery rate procedure). Part of the WT data is from Furman and Crair, 2012, as follows (see Methods): 16/17, 28/33, and 15/19 cells in the medial slice for P3-4, P6-7 and P12-13 respectively; and 12/15, 18/26, 10/15 in the lateral slice.

Table 4. Medial SC neuron properties at P6-7 of β2-TG mice following ipsi-lateral enucleation at P0. Differences in means tested with a 2-tailed Student’s t-test: *P<0.05, **P<0.01. Synaptic/morphological properties that differed significantly between mice strains are in boldface. See Table 1 for further details.

REFERENCES


Fig 2
FIG 3

Medial slice

Lateral slice

WT, P3  β2-TG, P4

WT, P6  β2-TG, P6

WT, P12  β2-TG, P12

AMP A response (~70mV)
NMDA response (+40mV, NBQX)

40pA
50ms

WT, P3  β2-TG, P4

WT, P6  β2-TG, P7

WT, P12  β2-TG, P13

NMDA decay time (msec)

WT

β2-TG

n=6
n=8

13
12

15
17

AMP A/NMDA ratio

WT

β2-TG

n=6
n=8

15
17

17
18

n=6
n=8

15
17

17
18

FIG 3
Fig 4
Fig 5

A

WT, P6-7 medial slice

WT, P6-7 lateral slice

β2-TG, P6-7 medial slice

β2-TG, P6-7 lateral slice

100μm

B

Number of nodes

C

Dendritic length (μm)

D

Fractal dimension

- Medial slice, WT
- Medial slice, β2-TG
- Lateral slice, WT
- Lateral slice, β2-TG

n=12 n=13 n=11 n=8

n=12 n=13 n=11 n=8

n=12 n=13 n=11 n=8

n=12 n=13 n=11 n=8

** * *
<table>
<thead>
<tr>
<th>Retinal activity</th>
<th>Superior colliculus (SC)</th>
<th>Retinal axons</th>
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<tr>
<td><strong>WT</strong></td>
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<tr>
<td>Contralateral retina</td>
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<td></td>
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<tr>
<td>Ipsilateral retina</td>
<td></td>
<td></td>
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<tr>
<td><strong>β2-TG</strong></td>
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**Strong synapses**

**Weak synapses**

*Fig 6*
<table>
<thead>
<tr>
<th>Property</th>
<th>WT</th>
<th>β2-TG</th>
<th>Relative difference (β2-TG vs. WT)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantal AMPA current amplitude, pA</td>
<td>21.62 ± 0.78 (n=14)</td>
<td>18.66 ± 0.98 (n=15)</td>
<td>-14%</td>
<td>0.028 *</td>
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<tr>
<td>NMDA decay time constant, ms</td>
<td>188 ± 25 (n=15)</td>
<td>228 ± 30 (n=17)</td>
<td>+21%</td>
<td>0.32</td>
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<td>AMPA/NMDA current ratio</td>
<td>1.85 ± 0.17 (n=15)</td>
<td>1.74 ± 0.12 (n=16)</td>
<td>-6%</td>
<td>0.62</td>
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<tr>
<td>NMDA Single-fiber response amplitude, pA</td>
<td>15.17 ± 0.82 (n=12)</td>
<td>13.52 ± 1.11 (n=9)</td>
<td>-11%</td>
<td>0.24</td>
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<tr>
<td>NMDA saturation response amplitude, pA</td>
<td>61.84 ± 6.62 (n=12)</td>
<td>97.07 ± 15.08 (n=9)</td>
<td>+57%</td>
<td>0.03 *</td>
</tr>
<tr>
<td>Number of retinal inputs</td>
<td>4.22 ± 0.54 (n=12)</td>
<td>7.15 ± 0.71 (n=9)</td>
<td>+69%</td>
<td>0.003 **</td>
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<tr>
<td>Input resistance, Ω</td>
<td>1141 ± 147 (n=33)</td>
<td>1036 ± 128 (n=31)</td>
<td>-9%</td>
<td>0.59</td>
</tr>
<tr>
<td>Number of dendritic nodes</td>
<td>62.2 ± 10.9 (n=12)</td>
<td>50.5 ± 9.7 (n=13)</td>
<td>-19%</td>
<td>0.43</td>
</tr>
<tr>
<td>Dendritic length (μm)</td>
<td>2202 ± 302 (n=12)</td>
<td>1920 ± 218 (n=12)</td>
<td>-13%</td>
<td>0.45</td>
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<tr>
<td>Fractal dimensions of dendritic tree</td>
<td>1.089 ± 0.014 (n=12)</td>
<td>1.090 ± 0.014 (n=13)</td>
<td>+0%</td>
<td>0.99</td>
</tr>
<tr>
<td>Property</td>
<td>WT</td>
<td>β2-TG</td>
<td>Relative difference (β2-TG vs. WT)</td>
<td>P value</td>
</tr>
<tr>
<td>---------------------------------------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>-----------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Quantal AMPA current amplitude, pA</td>
<td>18.48 ± 0.88 (n=11)</td>
<td>26.50 ± 1.61 (n=12)</td>
<td>+43%</td>
<td>0.0004 ***</td>
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<tr>
<td>NMDA decay time constant, ms</td>
<td>309 ± 27 (n=13)</td>
<td>240 ± 27 (n=12)</td>
<td>-22%</td>
<td>0.082</td>
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<tr>
<td>AMPA/NMDA current ratio</td>
<td>1.53 ± 0.13 (n=13)</td>
<td>1.52 ± 0.15 (n=12)</td>
<td>-1%</td>
<td>0.97</td>
</tr>
<tr>
<td>NMDA Single-fiber response amplitude, pA</td>
<td>10.33 ± 0.59 (n=10)</td>
<td>20.44 ± 2.30 (n=9)</td>
<td>+98%</td>
<td>0.0003 ***</td>
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<td>NMDA saturation response amplitude, pA</td>
<td>42.93 ± 6.22 (n=10)</td>
<td>94.80 ± 19.35 (n=9)</td>
<td>+121%</td>
<td>0.016 *</td>
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<tr>
<td>Number of retinal inputs</td>
<td>4.13 ± 0.54 (n=10)</td>
<td>4.37 ± 0.48 (n=9)</td>
<td>+6%</td>
<td>0.75</td>
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<tr>
<td>Input resistance, Ω</td>
<td>1077 ± 110 (n=26)</td>
<td>1002 ± 102 (n=25)</td>
<td>-7%</td>
<td>0.62</td>
</tr>
<tr>
<td>Number of dendritic nodes</td>
<td>26.7 ± 2.2 (n=11)</td>
<td>49.0 ± 7.2 (n=8)</td>
<td>+83%</td>
<td>0.04 *</td>
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<td>Dendritic length (μm)</td>
<td>1417 ± 173 (n=11)</td>
<td>2067 ± 191 (n=8)</td>
<td>+46%</td>
<td>0.023 *</td>
</tr>
<tr>
<td>Fractal dimensions of dendritic tree</td>
<td>1.060 ± 0.006 (n=11)</td>
<td>1.072 ± 0.014 (n=8)</td>
<td>+1%</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Table 3. Input resistance (Ω) in the medial and lateral SC of β2-TG and WT mice

<table>
<thead>
<tr>
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<th>Medial slice</th>
<th>Lateral slice</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>β2-TG</td>
</tr>
<tr>
<td>P3-4</td>
<td>1205 ± 128 (n=17)</td>
<td>1373 ± 166 (n=15)</td>
</tr>
<tr>
<td>P6-7</td>
<td>1141 ± 147 (n=33)</td>
<td>1036 ± 128 (n=31)</td>
</tr>
<tr>
<td>P12-13</td>
<td>647 ± 69 (n=19)</td>
<td>736 ± 143 (n=12)</td>
</tr>
</tbody>
</table>
Table 4. Summary of electrophysiological and morphological properties of β2-TG mice at P6-7 following ipsi-lateral monocular enucleation at P0

<table>
<thead>
<tr>
<th>Property</th>
<th>β2-TG enucleated, medial SC</th>
<th>Compared to β2-TG, medial SC</th>
<th>Compared to WT, medial SC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative difference</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Quantal AMPA current amplitude, pA</td>
<td>22.61 ± 0.87 ($n=5$)</td>
<td>+21%</td>
<td>0.041 *</td>
</tr>
<tr>
<td>NMDA decay time constant, ms</td>
<td>221 ± 44 ($n=5$)</td>
<td>-3%</td>
<td>0.90</td>
</tr>
<tr>
<td>AMPA/NMDA current ratio</td>
<td>2.27 ± 0.25 ($n=5$)</td>
<td>+28%</td>
<td>0.075</td>
</tr>
<tr>
<td>NMDA Single-fiber response amplitude, pA</td>
<td>12.36 ± 1.56 ($n=5$)</td>
<td>-9%</td>
<td>0.55</td>
</tr>
<tr>
<td>NMDA saturation response amplitude, pA</td>
<td>44.12 ± 9.02 ($n=5$)</td>
<td>-55%</td>
<td>0.030 *</td>
</tr>
<tr>
<td>Number of retinal inputs</td>
<td>3.43 ± 0.38 ($n=5$)</td>
<td>-52%</td>
<td>0.0031 **</td>
</tr>
<tr>
<td>Input resistance, Ω</td>
<td>1110 ± 159 ($n=10$)</td>
<td>+7%</td>
<td>0.75</td>
</tr>
<tr>
<td>Number of dendritic nodes</td>
<td>78.6 ± 18.5 ($n=7$)</td>
<td>+56%</td>
<td>0.15</td>
</tr>
<tr>
<td>Dendritic length (μm)</td>
<td>2253 ± 342 ($n=7$)</td>
<td>+17%</td>
<td>0.40</td>
</tr>
<tr>
<td>Fractal dimension of dendritic tree</td>
<td>1.118 ± 0.021 ($n=7$)</td>
<td>+3%</td>
<td>0.27</td>
</tr>
</tbody>
</table>