TrkB Activation During a Critical Period Mimics the Protective Effects of Early Visual Experience on the Stability of Receptive Fields in Adult Superior Colliculus

Abbreviated title 50 characters: Early TrkB activity stabilizes receptive fields

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Number of pages: 24
Number of figures: 7
Number of tables: 0
Number of words for Abstract: 248
Number of words for Introduction: 648
Number of words for Discussion: 1268
Conflict of Interest: None

Acknowledgements: Support for this work was provided by a GSU Brains and Behavior Fellowship and a GSU Center for Neuromics student grant awarded to D.B.M., and a GSU Brains and Behavior Seed grant, a National Science Foundation grant (IOS-1656838), and a DARPA grant (HR0011-18-2-0019, TA2), awarded to S.L.P. We would like to thank Dr. Angela Mabb of the GSU Neuroscience department and Hyuk-Kyu Seoh of the GSU Biology department for providing guidance on Western Blot imaging and analysis, members of the Pallas lab for technical support and manuscript review, and the Department of Animal Resources staff at GSU for excellent animal care.

Abstract

During a critical period in postnatal development, spontaneous and evoked retinal activity shape nascent visual pathways in an adaptive fashion. Visual experience increases transcription of the neurotrophin BDNF, activating the BDNF receptor TrkB, which promotes maturation of parvalbumin (PV) positive inhibitory interneurons, a process thought to open a critical period for ocular dominance plasticity in visual cortex. Development of perineuronal nets around PV neurons limits plasticity, ending the critical period and restricting adult plasticity. Another form of critical period plasticity is receptive field (RF) refinement. Spontaneous activity alone is sufficient for spatial refinement of visual receptive fields in superior colliculus (SC) and visual cortex (V1), but visual experience during an early critical period is necessary to maintain inhibitory synapses and stabilize RFs in adulthood (Carrasco et al. 2005, 2011; Carrasco & Pallas 2006; Balmer & Pallas 2015a). We report here that deprivation-induced RF enlargement in adulthood has a behavioral consequence; it impairs fear responses to looming objects in mice and hamsters. The mechanism through which early experience protects RFs from deprivation-induced loss of inhibition in adulthood is unknown. Given that the loss of RF refinement in SC does not occur until adulthood, and that inhibitory PV neurons are rare in SC, we asked whether or not BDNF-TrkB signaling was involved. We find that early TrkB activation is necessary and sufficient to maintain visual RF refinement in adulthood, suggesting a common signaling pathway for maturation of inhibition across neuronal subtypes and locations within the visual pathway.

Significance Statement

Receptive field refinement in superior colliculus (SC) differs from more commonly studied examples of critical period plasticity in visual pathways in that it does not require visual experience to occur; rather spontaneous activity is sufficient. Maintenance of refinement requires brief, early exposure to light to stabilize inhibition beyond puberty. This type of inhibitory plasticity must not depend on parvalbumin (PV)-containing GABAergic interneurons or on the formation of perineuronal nets, because these are very uncommon in SC. Nonetheless, we find that TrkB activation during a critical period can substitute for visual experience in maintaining receptive field refinement into adulthood, and that this maintenance is beneficial to visual survival behaviors. Thus, multiple types of plasticity converge on the same neurotrophin-dependent signaling cascade.

Introduction

As sensory pathways transition from a highly plastic state early in life to a stable state in adulthood, stimulus tuning properties are progressively sharpened through neural activity-dependent plasticity and are then maintained in that state. Much of the investigation into the regulation of critical periods has centered on the development of visual system connectivity, particularly ocular dominance (OD) plasticity, (Wiesel and Hubel, 1963, 1965). The critical period for OD plasticity in visual cortex (V1) opens with the maturation of parvalbumin (PV) containing GABAergic neurons (Fagiolini and Hensch, 2000; Morales et al., 2002; van Versendaal et al., 2012; Kuhlman et al., 2013; Toyoizumi et al., 2013; Gu et al., 2016). Development of inhibition in V1 is controlled by BDNF signaling through its TrkB receptor. Visual deprivation reduces TrkB signaling, resulting in reduced GABAergic inhibition and prolonged OD plasticity in V1 (Huang et al., 1999; Huang and Reichardt, 2003; Jiang et al., 2005; Gao et al., 2014). BDNF over-expression has the opposite effect (Gianfranceschi et al., 2003). Here we address the generalizability of TrkB signaling as a mechanism underlying critical period regulation across different properties and visual regions.

Receptive field (RF) refinement is an essential step in visual system development. Visual experience is required for development of acuity in monkeys (Regal et al., 1976; Teller et al., 1978), cats (Timney et al., 1978; Derrington and Hawken, 1981), and rats (Fagiolini et al., 1994), but not in V1 of mice (Prusky and Douglas, 2003; Kang et al., 2013). Our studies in demonstrated that early visual experience is not necessary for RF refinement in superior colliculus (SC) or V1, but is required to maintain refined adult RFs (Carrasco et al., 2005; Balmer and Pallas, 2015). Chronic dark rearing (DR) beyond postnatal day (P) 60 results in expansion of RFs to juvenile size (Carrasco et al., 2005). Light exposure during a critical period protects RFs against this later loss of refinement (Carrasco and Pallas, 2006; Balmer and Pallas, 2015). Thus, in contrast to OD development and RF refinement in cats and primates (see Shatz, 1996, for review), development of refined RFs in hamster SC and V1 is independent of sensory experience, requiring vision only for maintenance. These results counter the view that vision is required for development but not maintenance of visual receptive field properties (see Shatz, 1996, for review). They caution against over-generalization across features and species, and raise the possibility that RF refinement in hamster SC and V1 may occur through a distinct mechanism. This scenario is reminiscent of developmental disorders such as schizophrenia, autism, or Rett syndrome in which early development appears normal despite a pre-existing defect that reveals itself at later stages Thus, an important goal of this study was to reveal the mechanism through which early developmental insults can set the stage for later failures in maturation.

Because RF expansion in SC of DR adults results from a loss of inhibition (Carrasco et al., 2011; Balmer and Pallas, 2015), we asked whether RF refinement and maintenance might occur through a mechanism other than TrkB directed inhibitory plasticity. This possibility is supported by the fact that PV neurons in SC are largely non-GABAergic (Villalobos et al., 2018), and that PNNs are scarce in the visual layers of SC (Seeger et al., 1994; Bertolotto et al., 1996; Murakami et al., 1996; Grieco et al., 2018). We find, however, that TrkB activation during the critical period for RF refinement is necessary and sufficient to maintain refined RFs in SC and V1 of adults. Thus, BDNF-TrkB activity seems to be a common path through which visual experience influences the development and maturation of inhibition in the visual pathway. These findings raise the possibility that manipulating TrkB activity could reactivate plasticity in adults for therapeutic purposes and could provide insight into the development of schizophrenia, autism, and other disorders that similarly involve the breakdown of mature connectivity stemming from an early developmental error.

Materials and Methods

Subjects

Forty-five adult Syrian hamsters (*Mesocricetus auratus*) of both sexes were bred in-house and used in this study. Hamsters provide a valuable model for studying the developing visual system due to their robust and well-characterized visual responses and short gestation time (Pratt and Lisk, 1989). Hamsters were housed in social groups of up to 5 adults per cage in standard rodent cages, with enrichment items including nestlets and chew toys. All animals were provided ad libitum access to food and water 24 hours per day.

Twenty-four adult mice (C57BL/6) of both sexes were bred in-house and used in the behavioral assay. Mice are a valuable model for perceptual tasks because they are commonly used in behavior experiments and area widely studied animal in visual neuroscience.

Light treatment groups

Normally reared hamsters were housed in a 12/12 hour, reversed light-dark cycle. DR hamsters were housed in a darkroom, within which were several light-tight housing cabinets. Pregnant dams of DR subjects were transferred into DR housing approximately 3 days before parturition. During drug administration and for general husbandry purposes, they were briefly exposed to dim red light at a wavelength not visible to Syrian hamsters (Huhman and Albers, 1994).

To test the effect of TrkB receptor blockade on RF maintenance, strobe light exposure was used rather than a 12/12 light cycle because it was likely that the injected antagonist would not be effective throughout a 12-hour daily light exposure. Strobe-exposed animals were placed in a small enclosure containing a light flashing at approximately 25 Hz for 5 hours a day on each day of the critical period (7 days total). The timing of strobe exposure overlaps the 6 hour effective dose curve of the TrkB antagonist (Cazorla et al., 2011). This method exposed test subjects to a total duration of light exposure similar to the amount sufficient to maintain RF refinement in SC.

Drug treatment groups

In order to test the hypothesis that TrkB activation is sufficient for adult maintenance of RF refinement, all animals were administered a daily injection of either a TrkB agonist (Andero et al., 2011), a TrkB antagonist (Mui et al., 2018), or vehicle during the critical period for RF maturation in SC (P33-40) under dim red light conditions (Carrasco et al., 2005). Specifically, treatment consisted of an intraperitoneal injection of either the TrkB agonist 7,8-Dihydroxyflavone (DHF) (98% Sigma Aldrich CAS#: 38183-03-8) (10mg/kg) made fresh daily, dissolved in diluted Dimethylsulfide (DMSO 60% in DI water), or DMSO alone as a negative control. The antagonist ANA-12 (Sigma Aldrich #SML0209) 0.15g/ml was dissolved in 60% DMSO/40% DI water solution. Animals were weighed prior to each daily injection to ensure that the 1 mg/kg dose of drug or vehicle remained consistent throughout the treatment phase.

Western blotting

Animals were euthanized with sodium pentobarbital (Euthasol >150 mg/kg IP). Brains were immediately extracted and flash frozen in cold 2-methylbutane on dry ice, then stored at -80*C or immediately dissected for lysates. Individual tecta were excised and lysed in RIPA buffer (150mM NaCL, 150mM Tris, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate) containing 2% Halt protease inhibitor (ThermoFisher Scientific). Proteins were visualized using SuperSignal West Pico Chemiluminescent Substrate kits (Life Technologies) or IRdye fluorescent secondaries (Li-Cor), and imaged on an ImageQuant LAS4000 mini

(GE Healthcare Life Sciences), or Odyssey CLx fluorescent imager (Li-Cor). Protein levels were quantified as the optical density of the phosphorylated TrkB proteins relative to the optical density of total TrkB protein using ImageJ. No difference was detected between the two imaging methods using identical membranes, thus data were combined. To assess the effectiveness of the TrkB agonist and antagonist, 33 test animals received the drug doses IP and then either remained in their DR habitat, or were exposed to strobe conditions for 2 hours, followed by euthanasia and tissue harvest. Rabbit anti-pTrkB (Y817) (1:1000, Abcam), and rabbit anti-pan TrkB (80G2) (1:500), Cell Signaling Technologies) were used to confirm that the drugs were having the expected effect on TrkB phosphorylation in SC *in vivo*. The Y817 phosphorylation site was chosen because it is a reliable marker for calcium release (Hubbard and Miller, 2007), as well as an activator of protein kinase C (PKC), which is associated with activity dependent synaptogenesis in visual cortical development (Zhang et al., 2005). Negative controls included lanes with primary antibody but no protein to confirm specificity of the bands identified at the targeted molecular weight. Positive controls for pTrkB included lysates with BDNF added immediately after homogenization.

Assessment of pre- and post-synaptic inhibitory signaling strength

To characterize and compare treatment dependent changes in inhibitory signaling in adulthood we examined expression levels of presynaptic GAD-65 and postsynaptic $GABB_AR$ $\alpha1$ using antibodies (mouse anti-GAD-65 1:10 (Developmental Studies Hybridoma Bank-University of Iowa GAD-6), and rabbit anti-GABAARα1 1:1000 (Abcam ab33299)). Negative controls included lanes without protein and lanes without primary antibody.

Surgery

Electrophysiological recordings were made in sedated animals as described previously (Carrasco et al., 2005). In brief, animals were deeply anesthetized with intraperitoneal injections of urethane (2g/kg, split into 3-4 doses). Surgical levels of anesthesia were confirmed via withdrawal reflexes, respiration rate, and heart rate, with supplemental ¼ doses of urethane given as needed. Preoperative doses of atropine (0.05 mg/kg) were administered after anesthesia onset to stabilize breathing and reduce secretions in the respiratory tract. A single injection of dexamethasone (1mg/kg) was used as an anti-inflammatory. The surgical site was then shaved and cleaned with ethanol, and the head was stabilized with a bite-bar restraint. A midline incision was made in the scalp and the skull sutures were exposed. A 5mm bilateral craniotomy extending from bregma to lambda was then made, and the meninges retracted. The cortex and hippocampus were aspirated unilaterally to expose the underlying SC. Removal of cortex has no observable effect on SC neuron RF properties in hamsters, except for impairments in cortically-mediated direction tuning (Chalupa, 1981).

Electrophysiology

Single unit extracellular recordings were obtained with Teflon coated, glass insulated microelectrodes (2-2.3 MΩ). The electrode was positioned perpendicular to the exposed SC, and lowered into the tissue using a Kopf Model 650 micropositioner. All recordings were obtained within 200 µm of the surface of the SC to ensure they were made from the superficial, retino-recipient layers. Electrical signals were amplified, filtered (10,000x; 0.5-3kHz; Bak Electronics A-1), and digitized at 20 kHz using Spike2 software and CED hardware (Micro 1401-2; Cambridge Electronic Design).

Visual Stimulus Presentation

In order to measure RF size, we used a visual stimulus presented on a CRT monitor (60Hz refresh rate) positioned 40 cm from the left eye. The monitor was maintained at its highest contrast and brightness settings for each experiment. Visual stimulus generation was accomplished using custom MATLAB

(Mathworks) software with the PsychToolBox-3 application. The visual stimulus consisted of a bright white square traveling from dorsal to ventral visual field at 14°/s across a black background. The stimulus size was 1 degree in diameter and each vertical traverse shifted 2° along the x axis of the monitor after each presentation, with a 3 second inter-stimulus interval, as in (2015).

Analysis of RFs

Spike2 software (Cambridge Electronic Design) was used for offline spike sorting of single units (approximately four unique visually responsive neurons were isolated per recording site). Analysis of RF size was completed by a researcher blind to treatment group. Receptive field diameter along the azimuthal axis was measured by plotting the visual field location from which spiking responses were produced as the stimulus traversed the monitor from nasal to temporal visual field. A uniform fraction of the peak response (20%) was defined as the minimum stimulus-evoked response threshold, as in a previous study (Balmer and Pallas, 2015). Responses were normalized by setting the peak response of each single unit to 1.0 to account for differences in response strength between individual units. RF size data was compared between treatment groups to quantify the effect of TrkB activation vs. vehicle treatment.

Looming Response Task

An open-top box with dimension of 58.7cm x 42.9cm x 32.4cm was used to test the fear reflex to an expanding spot approaching from above. The test subjects were light and dark reared hamsters and C57BL/6J mice, aged at least P60, housed either in 12h:12h light:dark cycle or in 24h dark. Five trials were conducted under white light, in their subjective daytime between the hours of 1900-2200. Both groups were exposed to white light for less than 90 minutes per trial. Alcohol (70%) was used to clean the apparatus of odors before and between each trial. A plastic cup was spray painted black and used as a hiding chamber. Each subject was placed in the center of the apparatus with the white monitor screen above and given at least 10 minutes for acclimation. The visual stimulus was programmed and displayed using the Psychtoolbox module for MATLAB. A spherical, black looming stimulus was initiated on a white background once the subject was out of the hiding chamber and in the center of the arena. The stimulus expanded from 3.5 degrees of visual angle to 56.5 degrees in 2.35 seconds, remained at that size for 250ms, and then restarted the sequence with a 250ms delay. The fear response was considered positive if the subject either froze or fled into the cup within 5 seconds of the stimulus initiation. The fear response was considered negative if the subject did not demonstrate any freezing or fleeing behavior.

Statistical Analysis

A Student's *t*-test or a One-Way Analysis of Variance (ANOVA), followed by post-hoc Bonferroni tests were used to compare parametric data with equal variance between groups and a normally distributed data set. Descriptive statistics for these analyses are provided as mean ± standard error of the mean (SEM). For data not meeting these criteria, a Mann-Whitney rank sum test or a Kruskal Wallis One-way ANOVA on ranks was used, followed by a Dunn's post hoc test, with data presented as median ± interquartile range (IQR).

Results

Stability of receptive field properties in adulthood maintains the activity driven changes that occur during development. Early visual experience is required for refined RFs to stabilize (Carrasco et al.,

2005), but it remains unclear what molecular changes are responsible. BDNF protects against degradation of visual acuity in visual cortex of dark reared rats (Gianfranceschi et al., 2003), thus we examine here whether BDNF-TrkB signaling might also be protective of acuity in SC of dark reared hamsters, in which case it may be a general mechanism through which sensory experience has its maturational effects. To investigate whether TrkB activation can substitute for visual experience during the critical period for RF plasticity, we pharmacologically manipulated TrkB activation, and measured superior colliculus RF sizes in adulthood (>P90).

7,8 DHF and ANA-12 are both effective modulators of TrkB receptors throughout the visual midbrain. Genetically increasing BDNF expression throughout life rescues RF size and visual acuity in dark reared visual cortex of mice (Gianfranceschi et al., 2003). We asked a more time-limited question- whether increasing BDNF receptor signaling specifically during an early critical period would maintain RF refinement in adult SC. If so, it would suggest that a common mechanism for stabilizing inhibitory synapses across different visual brain areas despite the differences in timing. In order to avoid the difficulties in interpreting results obtained from gene knockout, we tested whether a pharmacological approach would provide sufficient activation of BDNF receptors to address the question.

Previous studies have used the isoflavone 7,8 DHF as a TrkB receptor agonist (Andero et al., 2011; Liu et al., 2014), and ANA-12 as an antagonist (Lawson et al., 2014; Ren et al., 2015). In order to test that the pharmacological manipulations intended to affect TrkB receptors were working as intended, test subjects from each group (7,8 DHF n=9, Strobe + ANA-12 n=5 Strobe n=7, Vehicle n=11) were euthanized following the initial injection. We then measured the amount of activated (pTrkB) relative to total TrkB from V1, SC and hippocampus (as a non-visual region control) for visual experience driven TrkB activity with Western blotting. Immunoblotting with antibodies against phosphorylated (activated) and total TrkB receptors revealed strong treatment induced changes in TrkB activation throughout the brain (**Figure 2)**. In all three areas 7,8 DHF had a robust effect on increasing TrkB phosphorylation well beyond that of vehicle; SC ($F(3,24) = 12.503$ $p < 0.001$, ANOVA) V1 ($F(3,12) = 24.757$ $p <0.001$, ANOVA), hippocampus ($F(3,12) = 6.070$ $p = 0.009$, ANOVA). Visual experience (strobe) also induced increases in pTrkB in both visual brain areas compared to vehicle: SC (0.523 ± 0.0916 $p = 0.046$ n=5), V1 (0.529 ± 0.0812 $p = 0.004$ n=4), but not in hippocampus. ANA-12 treatment reduced pTrkB in both SC (0.132±0.243 $p = 0.034$ n=3) and V1 (0.098±0.0265 $p<0.001$ n=4). These findings support the use of pharmacological TrkB agonists, antagonists and short stroboscopic light treatments in modulating TrkB activity in hamster SC.

Elevating TrkB receptor phosphorylation levels during the critical period maintains SC receptive field refinement into adulthood.

To test the sufficiency of TrkB activity in stabilizing RF refinement, we dark reared hamsters from <P0 to >P90 and provided daily treatment with the TrkB agonist 7,8-DHF throughout the critical period (P33-P40). Hamsters that were treated with the agonist maintained a significantly smaller RF size (12° ± 6°, n = 92) compared to vehicle injected controls (18° ± 4°, $p<0.05$, n = 84) **(Figure 3)**. Importantly, these are measurements of single unit RF sizes, and not due to a change in overlap of adjacent RFs that might be measured from multiunit extracellular recordings. These results support the hypothesis that RF refinement can be maintained in adulthood by TrkB activation during the critical period in SC.

Decreasing TrkB phosphorylation levels during the critical period prevents maintenance of SC receptive field refinement into adulthood

To test the necessity of TrkB activation for the maintenance of RF refinement in SC, we used a TrkB antagonist (ANA-12) to block the light-induced phosphorylation of TrkB receptors that occurs during

visual experience. ANA-12 was administered during a stroboscopic presentation of light for 5 hr/ day throughout the critical period (P33-P40), followed by return to the dark until adulthood (>P90). This stroboscopic light treatment was sufficient to maintain RF refinement in SC into adulthood (supplementary Figure 1). Single unit recordings from SC neurons in animals (n=6) receiving the antagonist revealed significantly larger RFs (20° ± 4° (n=82) than in neurons from normally reared animals 8.580° ± 3.78° (n=71); $p<0.05$) **(Figure 4)** and interestingly, larger RFs than either vehicle injected (18° ± 4° n = 84) or DR controls (14.48° ± 6.4° n=66; (H(3) = 153.507, p = < 0.001, Kruskal Wallis One-Way ANOVA on Ranks). This finding further supports the hypothesis that TrkB activity is responsible for maintenance of RF refinement, because blocking TrkB activity during the critical period resulted in enlarged RFs in adulthood, despite visual experience.

TrkB activity during the critical period preserves RF refinement by maintaining adult levels of inhibition

We sought a potential mediator of the delayed effect of early TrkB activity on RF size using post hoc examinations of presynaptic GABA precursor enzyme (GAD65) and postsynaptic $GABA_AR$ receptor expression. We measured adult (>P90) levels of GAD65 and receptor using Western blotting. 7,8 DHF treated animals had higher relative exogenous GAD65 expression (0.216 ± 0.006 n = 7) compared to vehicle treated (0.104 ± 0.0121 n = 5), and antagonist (ANA-12+strobe) treated hamsters (0.085 ± 0.0092 n=4) (F(4,28) = 102.747 p < 0.001, One-Way ANOVA). Normally reared and DR control groups performed as expected, with normally reared hamsters having much higher GAD65 expression (normal: 0.242 ± 0.0057 n = 6; DR: 0.0737 ± 0.0048 n=4; One-Way ANOVA p<0.001) **(Figure 5A)**. In contrast, we observed no differences in $GABA_AR\alpha1$ expression between our treatment groups (p = 0.97) **(Figure 5B)**.

Dark rearing impairs fear response to visual looming stimuli

RF refinement is critical for the development of visual acuity and environmental awareness. DR animals have a number of visual deficits in cortex such as poorer visual acuity, orientation tuning, and direction tuning (Fagiolini et al., 1994), but deficits in SC have rarely been characterized. We tested the effect of visual deprivation on object detection by examining differences in fear responses (escape or freezing behavior) to visual looming stimuli **(Figure 6A)**, an SC dependent behavior (Zhao et al., 2014; Shang et al., 2018).

We found that DR animals with larger RFs are less likely to respond to overhead looming stimuli (0.25 ± 0.25 n=9) than normally reared animals (0.75± 0.38 n=9) **(Figure 6C,E)** (Mann-Whitney Rank Sum test $p<0.05$). Dark rearing had a particularly large effect on the escape ("flight" to shelter) behavior, with almost no occurrences of it throughout all trials **(Figure 6C)**. These data suggest that the failure to maintain RF refinement in SC has a negative impact on instinctual fear responses to a looming visual object.

We also demonstrated the effect of DR on looming response in mice with a similar outcome. DR mice were less likely to respond to overhead looming stimuli (0.20 ± 0.20 n=12) than normally reared mice (0.80 ± 0.35 n=12) (Mann-Whitney Rank Sum Test $p<0.001$). Surprisingly the significant decrease in fear responses in DR mice was even more substantial than the decrease in DR hamsters **(Figure 6D,F)**, something we would not have predicted in a nocturnal species like mice. These data suggest that both hamsters and mice are susceptible to DR induced disruptions of visual function.

Together, these results demonstrate that TrkB activation can substitute for visual experience during the critical period of RF plasticity, and they support the hypothesis that TrkB activation is both necessary and sufficient for the maintenance of RF refinement in SC. In addition, RF refinement in SC is important for visual behavior, because larger receptive fields result in impaired responsivity to looming visual

stimuli. Our results also provide evidence that this early increase in TrkB expression is reducing presynaptic GAD65 expression in adult SC.

Discussion

Our previous study in dark reared hamsters showed that spontaneous activity alone is sufficient for the receptive fields of visual neurons in superior colliculus and visual cortex to refine during postnatal development. However, unless the animal experienced a normal light cycle for several days during an early critical period, that refinement is not maintained into adulthood, and RFs unrefine (Carrasco et al., 2005). These results were surprising because vision is thought to be necessary for development of visual function, but not for adult maintenance of function. A further surprising result was the observation that early deprivation did not have any detectable effect until adulthood (Carrasco et al., 2005). Visual deprivation eliminates visually driven activity without affecting spontaneous activity, and can permanently impair the normal development of some visual tuning properties, yet have little effect on others. For example, orientation selectivity in visual cortex will begin to develop in DR ferrets (Chapman and Stryker, 1993; Chapman et al., 1996; Chalupa and Snider, 1998; Issa et al., 1999; White et al., 2001) but fails to sharpen by adulthood (Huberman et al., 2008) and direction selectivity in V1 fails to develop at all (Li et al., 2006). Additionally, ocular dominance columns in V1 will form in DR animals (Wiesel and Hubel, 1974; Horton and Hocking, 1996), but ocular dominance plasticity is inappropriately extended into adulthood as a result (Mower et al., 1981; Cynader, 1983). In contrast, as seen with RF refinement, some other RF properties (orientation selectivity) develop without visual experience (Huberman et al., 2008), but continued deprivation results in a degradation of tuning in adulthood (Hensch, 2005). This suggests that one role of early experience may be to fine tune previously established connections and stabilize them in a mature state. We report that increasing TrkB phosphorylation during the critical period in dark reared animals can substitute for visual experience and forestall the negative effects of visual deprivation on RF refinement, whereas blocking TrkB activity during critical period light exposure prevented RF refinement from being maintained into adulthood. These findings clarify the requisite role of experience-driven TrkB activity during an early critical period in stabilizing adult RFs and preventing deleterious adult plasticity. They suggest that TrkB receptor signaling is the convergence point through which visual activity drives the maturation of inhibition in the superior colliculus, as has previously been reported for the visual cortex (Gianfranceschi et al., 2003).

TrkB signaling mediates activity-dependent maturation of visual processing circuits in V1 and SC

The development of ocular dominance is perhaps the most commonly studied example of receptive field maturation in the visual system. In carnivores and primates, eye-specific columns begin to form before birth and they progressively sharpen with visual experience (Crowley and Katz, 2000; 2002). Although rodents do not have visible columns, individual cortical neurons do develop an ocular dominance preference (Niell and Stryker, 2008; Huberman and Niell, 2011). Ocular dominance development is subject to a critical period during which monocular deprivation can result in a shift in representation of cortical cells away from the closed eye (Wiesel and Hubel, 1963, 1965). Dark rearing causes a prolongation of the critical period for ocular dominance plasticity, perhaps as a result of prolonged immaturity of NMDA receptors (Carmignoto and Vicini, 1992) and GABAergic neurons (Jiang et al., 2005). In contrast, RF refinement in SC and V1 is unaffected by deprivation, and early experience is necessary only for stabilizing RFs in adulthood, suggesting a different mechanism may regulate its timing.

Visual experience is thought to have its effects on ocular dominance development through a BDNF-mediated signaling pathway that promotes maturation of inhibitory synapses and regulation of critical

period plasticity. Visual input drives NMDA receptor activity, which is required for light to increase BDNF levels and thus trigger maturation of inhibition (Castren et al., 1992; Greenberg et al., 2009; Park and Poo, 2013). Transcription factors such as Npas4 regulate transcription of genes associated with plasticity, including BDNF (Lin et al., 2008; Bloodgood et al., 2013). BDNF in turn binds to and activates TrkB receptors (Pollock et al., 2001; Viegi et al., 2002), and TrkB activity promotes the expression of GAD, GABA, and $GABA_A$ receptor expression throughout the brain (Rutherford et al., 1997; Huang et al., 1999; Jovanovic et al., 2004; Porcher et al., 2011; Sanchez-Huertas and Rico, 2011). Critical period closure and the ensuing restriction of visual cortical plasticity are associated with visual experience-induced changes in NMDA and GABA receptor composition (Carmignoto and Vicini, 1992; Fox et al., 1992; Stocca and Vicini, 1998; Chen et al., 2001; Li et al., 2017), increases in GABA expression (Jiang et al., 2005), and perineuronal net development (Sur et al., 1988; Bavelier et al., 2010; Beurdeley et al., 2012; Ye and Miao, 2013; Wen et al., 2018). Spontaneous activity is not sufficient to drive these changes in the context of ocular dominance plasticity (Sur et al., 1988; Huberman et al., 2008; Chalupa, 2009).

The similar effects of TrkB signaling on RF refinement in both SC and V1 are surprising for a number of reasons. V1 requires several more days of early (P33-P40) visual experience to stabilize adult RF refinement (>P90) compared to SC (P37-P40) (Balmer and Pallas, 2015). V1 also has different classes of GABAergic cells than SC, which contains very few GABAergic parvalbumin positive interneurons (Mize, 1992; Choi et al., 2009; Villalobos et al., 2018), which are essential in V1 ocular dominance plasticity (Hensch, 2005). It seems likely that there are some as yet undiscovered differences in the TrkB signaling pathway downstream of TrkB receptor activation.

How does early TrkB signaling maintain RF refinement?

Visual experience-regulated TrkB activity may function in both a permissive and an instructive role in development of the visual system. In the permissive role scenario, experience driven activity increases overall BDNF production and subsequent TrkB activity. The TrkB activity would then allow the maturation of the GABAergic synapses necessary to develop or to stabilize RF properties. In the instructive role scenario, visual experience would be driving TrkB activity to form specific ensembles of visual neurons designed to respond to different types of visual input. Our finding that early TrkB activity maintains RF refinement in adult SC suggests that the BDNF-TrkB signaling pathway plays more of a permissive than an instructive role. This view is supported by our previous result that RF refinement will occur without any visual experience(Carrasco et al., 2005), and that the loss of refinement in DR adults coincides with reductions in GABA expression and postsynaptic GABA receptor function (Carrasco et al., 2011). Thus RF refinement does not require instructive signaling from the BDNF-TrkB signaling pathway to occur, but rather requires permissive signaling to maintain its stability long term.

Which inhibitory forces close the critical period irreversibly?

Our experimental activation of TrkB receptor during an early critical period led to adult RF plasticity several weeks after the typical critical period in SC. The argument for maturation of an inhibitory mechanism as the causal factor is strong. GAD65 expression in SC was higher in our TrkB agonist treated animals relative to vehicle, consistent with previous studies showing decreased GABA expression in SC following dark rearing (Carrasco et al., 2011). We also note that TrkB antagonist treatment resulted in even larger RFs than dark rearing alone, suggesting that spontaneous TrkB activity during the critical period may provide a buffer against further RF enlargement in DR subjects. Even though these results point to a presynaptic mechanism involving changes in neurotransmitter levels, we do not rule out changes in postsynaptic receptor function as a possible factor in shaping inhibitory circuit development

especially given our previous results showing decreased GABAA receptor function after chronic DR (Carrasco et al., 2011).

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Figure Legends

Figure 1. Graphical description of experimental procedure for measuring visual RF sizes with *in vivo*, extracellular, single unit recordings of stimulus provoked action potentials in SC.

Figure 2. Drug treatments were effective at modulating TrkB activity in SC, V1, and Hippocampus. 7,8 DHF administration at P33-40 increased TrkB receptor activation in the all measured brain areas, and ANA-12 treatment blocked TrkB activation. **(A-C)** Example blots of treatment groups generated using 20 µg of protein per lane. All presented lanes are from the same gels, with nonadjacent lanes revealed by vertical lines between them. **(D-F)** Densitometric analyses of Western blots generated from SC **(D)** V1 **(E)** or Hippocampus **(F)** lysates prepared from juvenile hamsters (~P33) receiving the TrkB receptor agonist (7,8 DHF), visual experience (strobe light for 1 hour), strobe light + the TrkB antagonist ANA-12, or vehicle injection revealed differences in activation levels of TrkB receptors between groups. Agonist and strobe light exposure greatly increased levels of phosphorylated TrkB in SC compared to vehicle. Analysis of ANA-12 treatment on TrkB phosphorylation during one hour of strobe light exposure revealed that the antagonist is effective in preventing visual experience evoked TrkB activation. The density of the anti-phosphorylated TrkB (Y817) band is normalized to the anti-total TrkB (80G2) protein band to measure differences in TrkB activity. Data presented as mean ± SEM. * $p<0.05$ ** $p<0.01$

Figure 3. TrkB activation during the critical period maintains RF refinement into late (>P90) adulthood. (A) Experimental design and summary of findings. A): Experimental timeline of treatments and resulting RF changes throughout development. **(B** RF sizes for each experimental group measured in visual degrees and plotted as individual data points. Open pair of eyes across the top of graph indicates the group was given visual experience. Closed eyes indicate group was dark reared throughout development. Data presented as median ± IQR. * $p<0.05$

Figure 4. **TrkB blockade with ANA-12 during the critical period blocks the protective effects of visual experience for SC RF refinement in adulthood (>P90).** DR animals were given sufficient stroboscopic visual experience during the critical period to maintain RF refinement in SC, but TrkB blockade during that time period blocked the protective effects of light. **(A)** Experimental timeline for treatment and resulting RF changes during development. **(B)** RF sizes for each experimental group measured in visual degrees and plotted as individual data points. Symbols as in Figure 3. Data presented as median ± IQR. * $p < 0.05$.

Figure 5. **Early TrkB expression is both necessary and sufficient to maintain increased presynaptic inhibition in adult SC. (A)** Adult GAD65 expression levels were maintained in TrkB agonist injected animals but decreased in DR, vehicle, and ANA-12 injected animals. **(B)** $GABA_AR$ $\alpha1$ expression remained constant across all treatment groups and rearing conditions. Data presented as mean relative optical density ± SEM. * $p<0.001$.

Figure 6. Dark rearing and subsequent enlargement of RF size in SC reduces fear responses to looming stimuli in hamsters and mice. (A) Schematic of apparatus. A box with a monitor (M) suspended above it projecting the looming stimulus, and a shelter (S) placed at the far end. Animals responded to looming stimuli either by fleeing (FL) into the shelter or freezing (FR) in place. Unresponsive animals continued exploratory behavior (EB). **(B)** Expansion of the looming stimulus from the start of a cycle to the end (approximately 2s). **(C/D)** Occurrences of each response type to looming stimuli per animal in each experiment group for 5s after stimulus presentation in hamsters (C) and mice (D). Responses were determined on an ascending scale from **exploratory behavior (EB)** < **freezing** < **flight**, with only the last? observed behavior reported. **(E/F)** The frequency of an escape response (freezing/flight) to looming stimuli compared between normally reared and DR groups in hamsters (E) and mice (F). Data presented as mean fear response ± SEM *$p<0.05$.

Figure 7. A summary of findings regarding the dependence of RF size maintenance in adult SC on early visual experience, and the role that TrkB activation plays within that process.

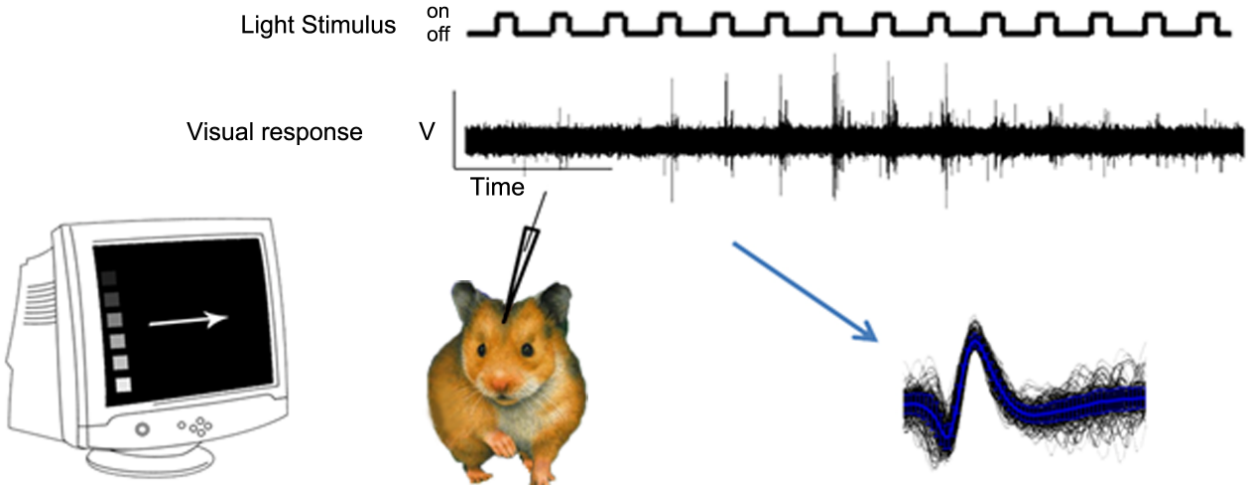

Figure 1

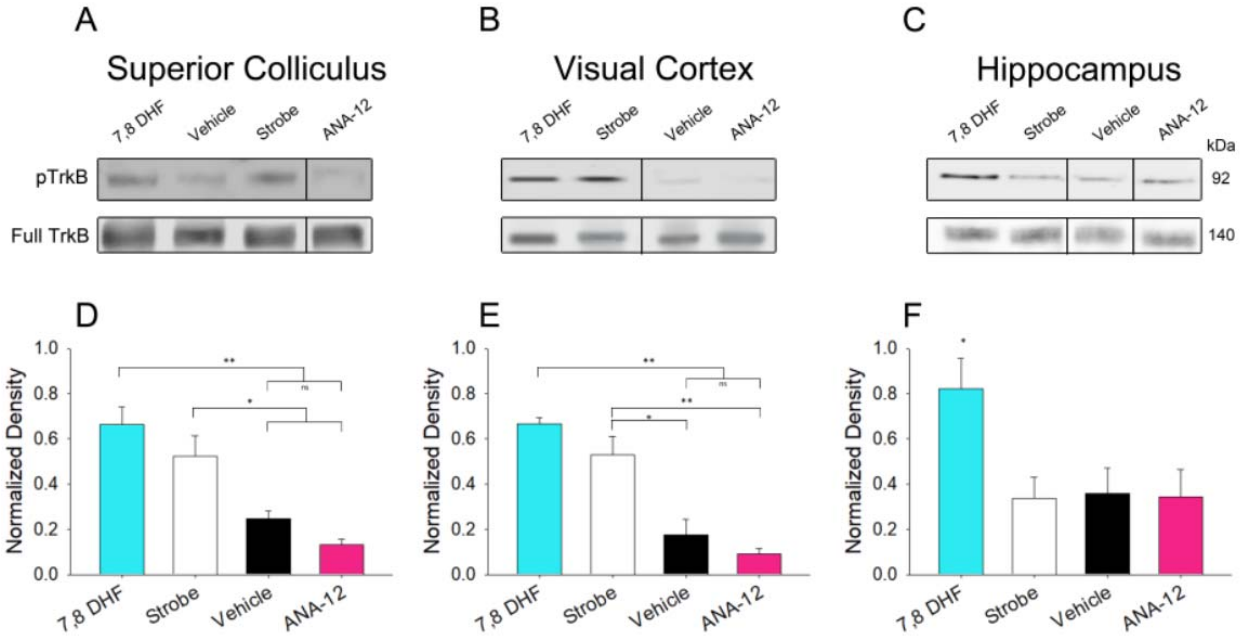
A
Superior Colliculus
7,8 DHF
Vehicle
Strobe
ANA-12
pTrkB
Full TrkB
B
Visual Cortex
7,8 DHF
Strobe
Vehicle
ANA-12
C
Hippocampus
7,8 DHF
Strobe
Vehicle
ANA-12
kDa
92
140
D
E
F
Normalized Density
1.0
0.8
0.6
0.4
0.2
0.0
7,8 DHF
Strobe
Vehicle
ANA-12

Figure 2

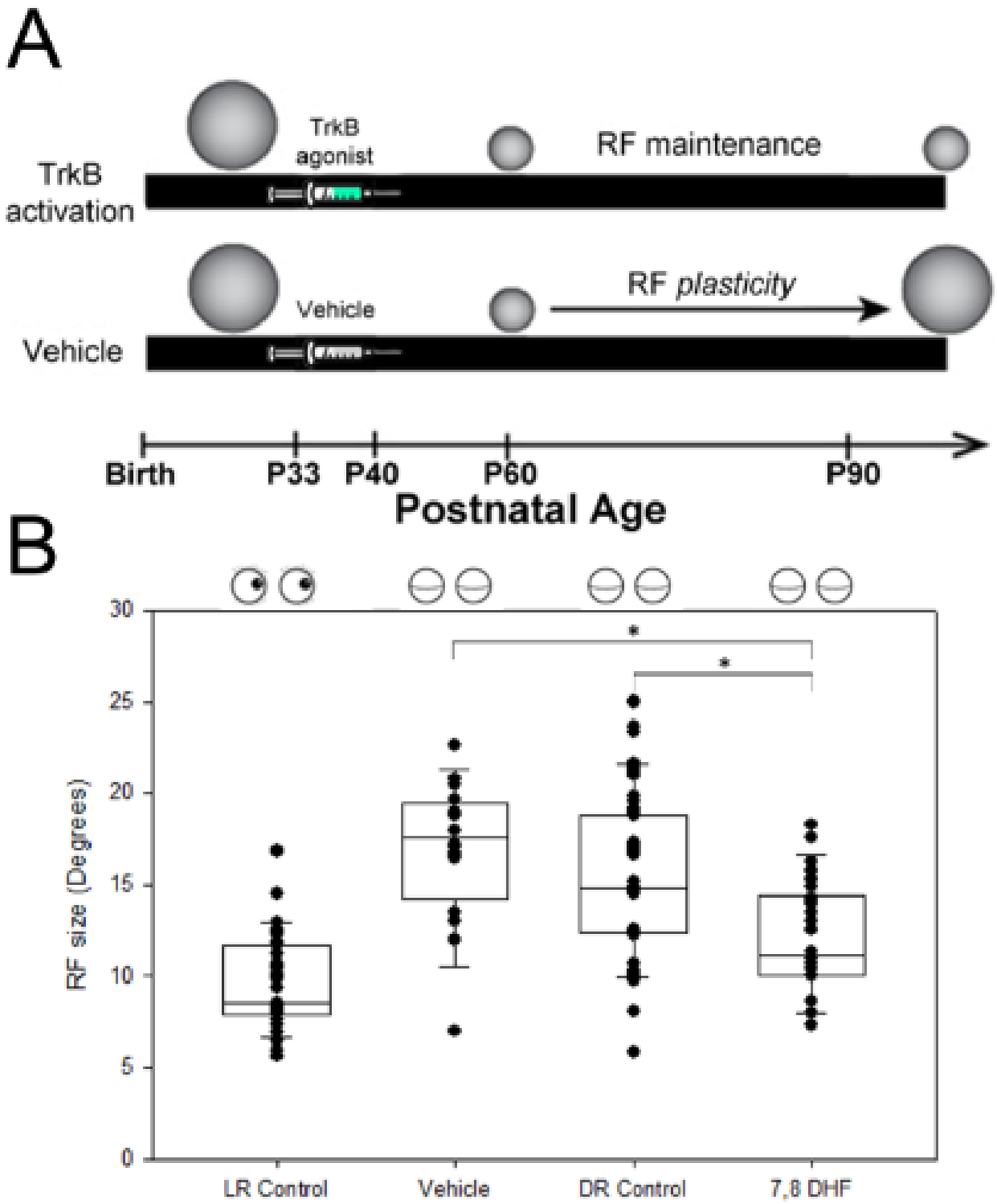
A
TrkB agonist
RF maintenance
TrkB activation
Vehicle
RF plasticity
Vehicle
Birth
P33
P40
P60
P90
Postnatal Age
B
RF size (Degrees)
30
25
20
15
10
5
0
LR Control
Vehicle
DR Control
7,8 DHF

Figure 3

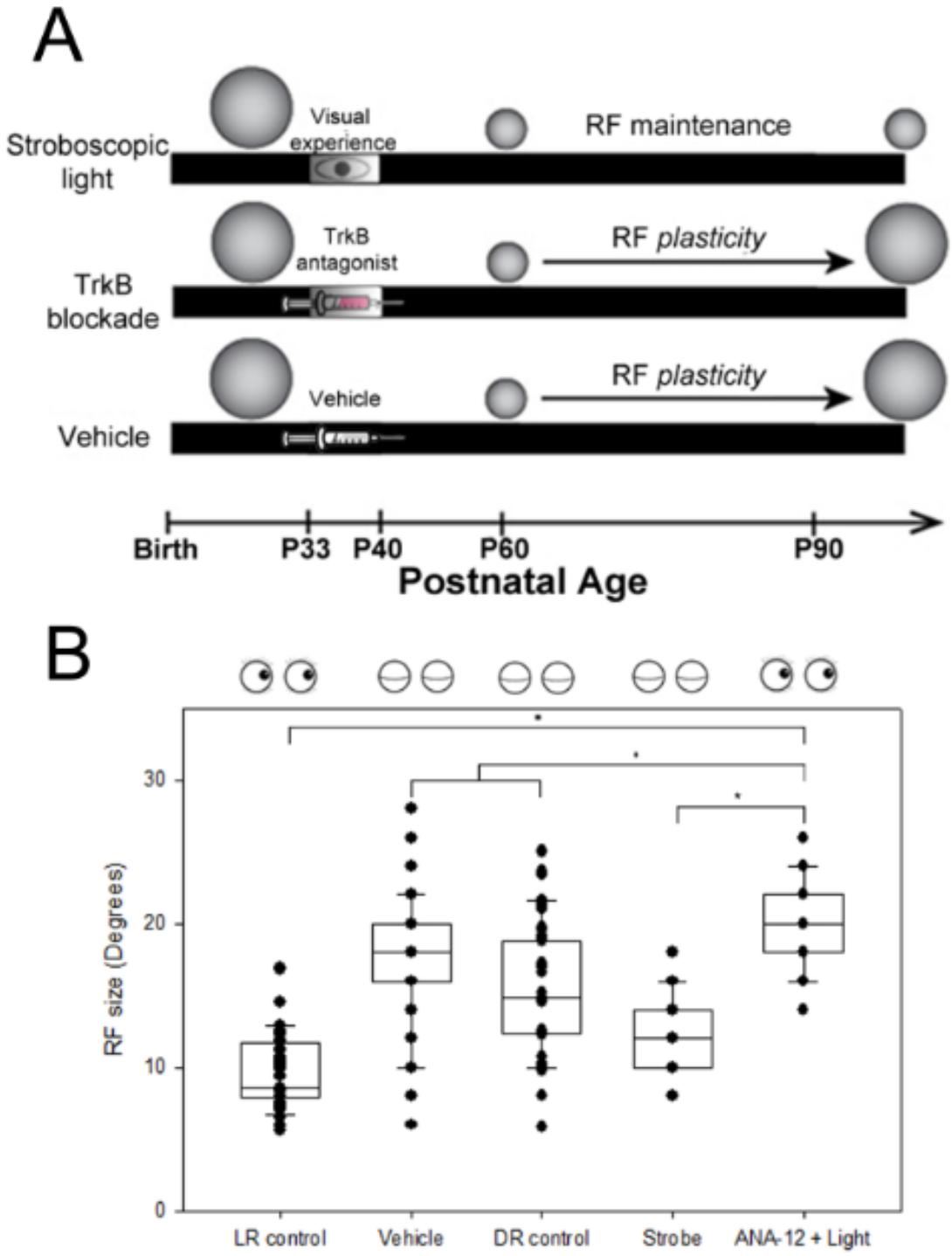
A
Stroboscopic light
Visual experience
RF maintenance
TrkB blockade
TrkB antagonist
RF plasticity
Vehicle
Vehicle
RF plasticity
Birth
P33
P40
P60
P90
Postnatal Age
B
RF size (Degrees)
0
10
20
30
LR control
Vehicle
DR control
Strobe
ANA-12 + Light

Figure 4

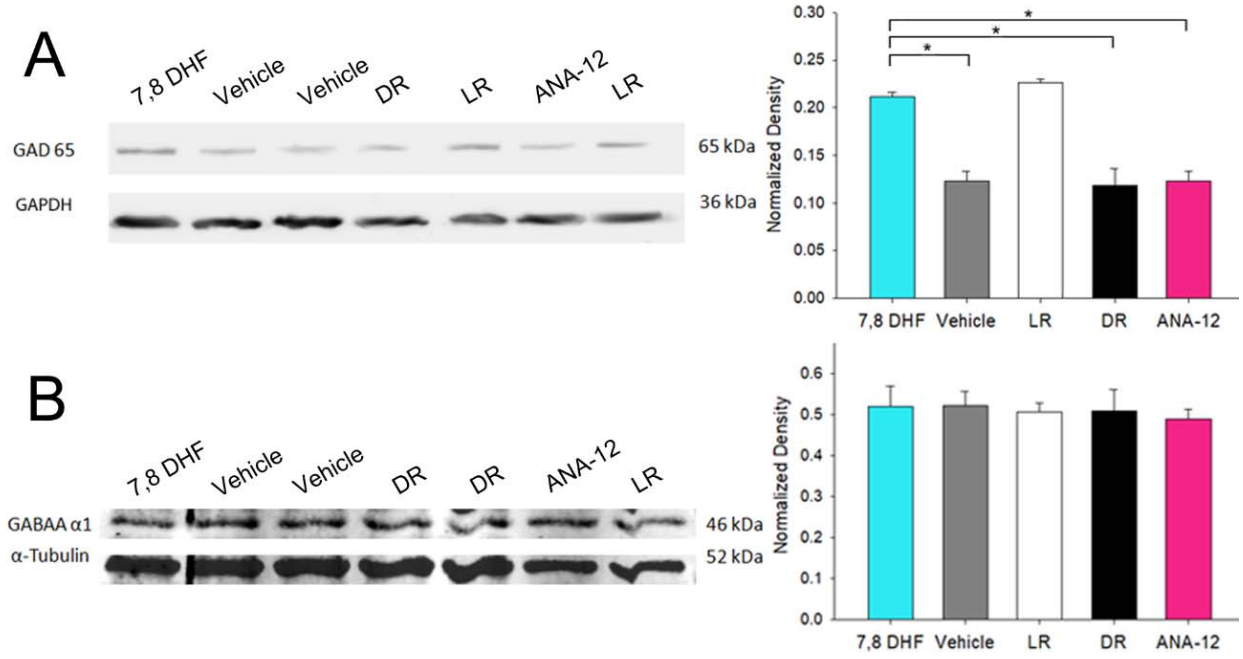
A
7,8 DHF
Vehicle
Vehicle
DR
LR
ANA-12
LR
GAD 65
GAPDH
65 kDa
36 kDa
Normalized Density
0.30
0.25
0.20
0.15
0.10
0.05
0.00
7,8 DHF
Vehicle
LR
DR
ANA-12
B
7,8 DHF
Vehicle
Vehicle
DR
DR
ANA-12
LR
GABAA α1
α-Tubulin
46 kDa
52 kDa
Normalized Density
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7,8 DHF
Vehicle
LR
DR
ANA-12

Figure 5

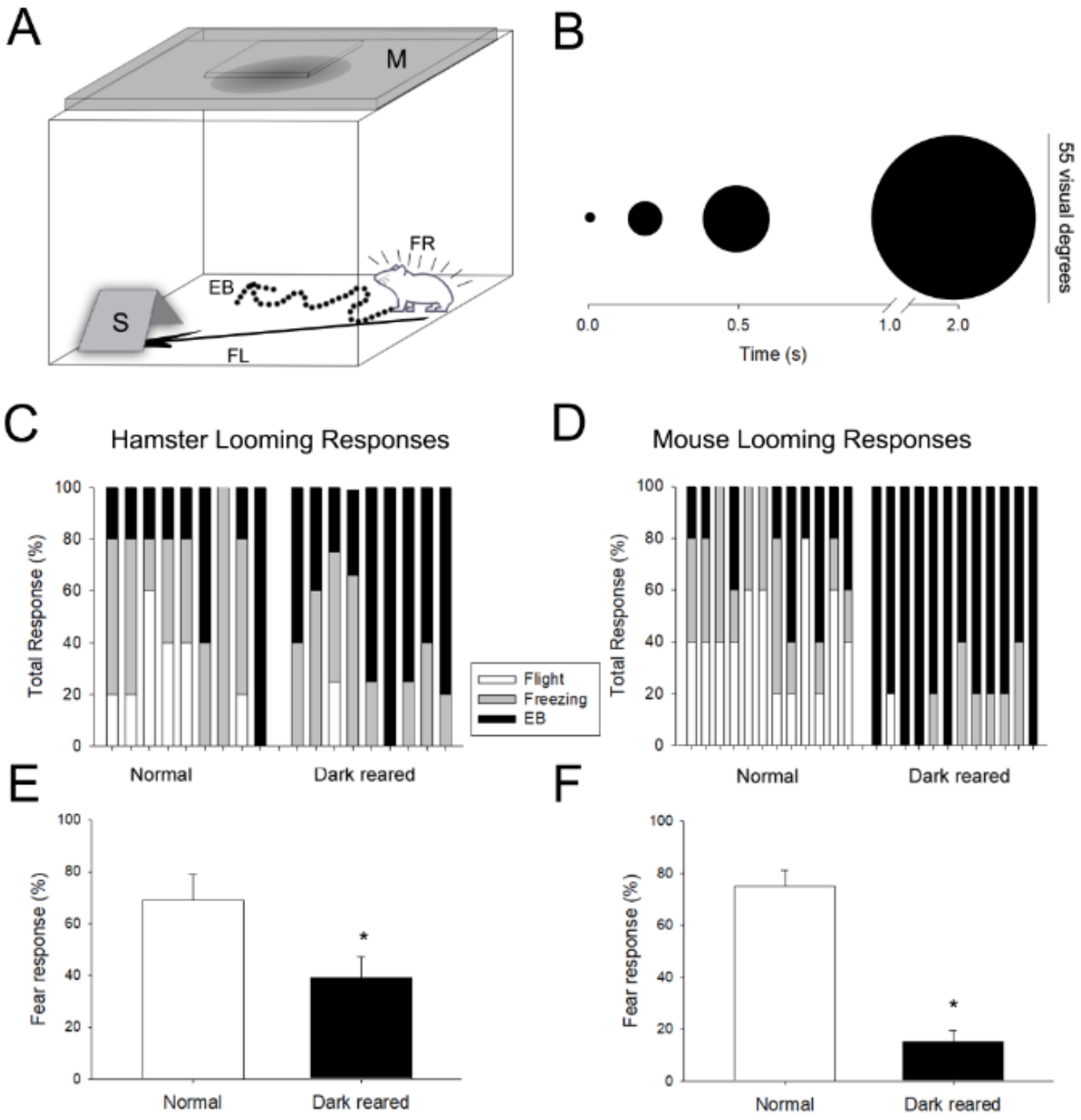
A
M
FR
EB
S
FL
B
55 visual degrees
0.0
0.5
1.0
2.0
Time (s)
C
Hamster Looming Responses
Total Response (%)
Normal
Dark reared
Flight
Freezing
EB
D
Mouse Looming Responses
Total Response (%)
Normal
Dark reared
E
Fear response (%)
Normal
Dark reared
F
Fear response (%)
Normal
Dark reared

Figure 6

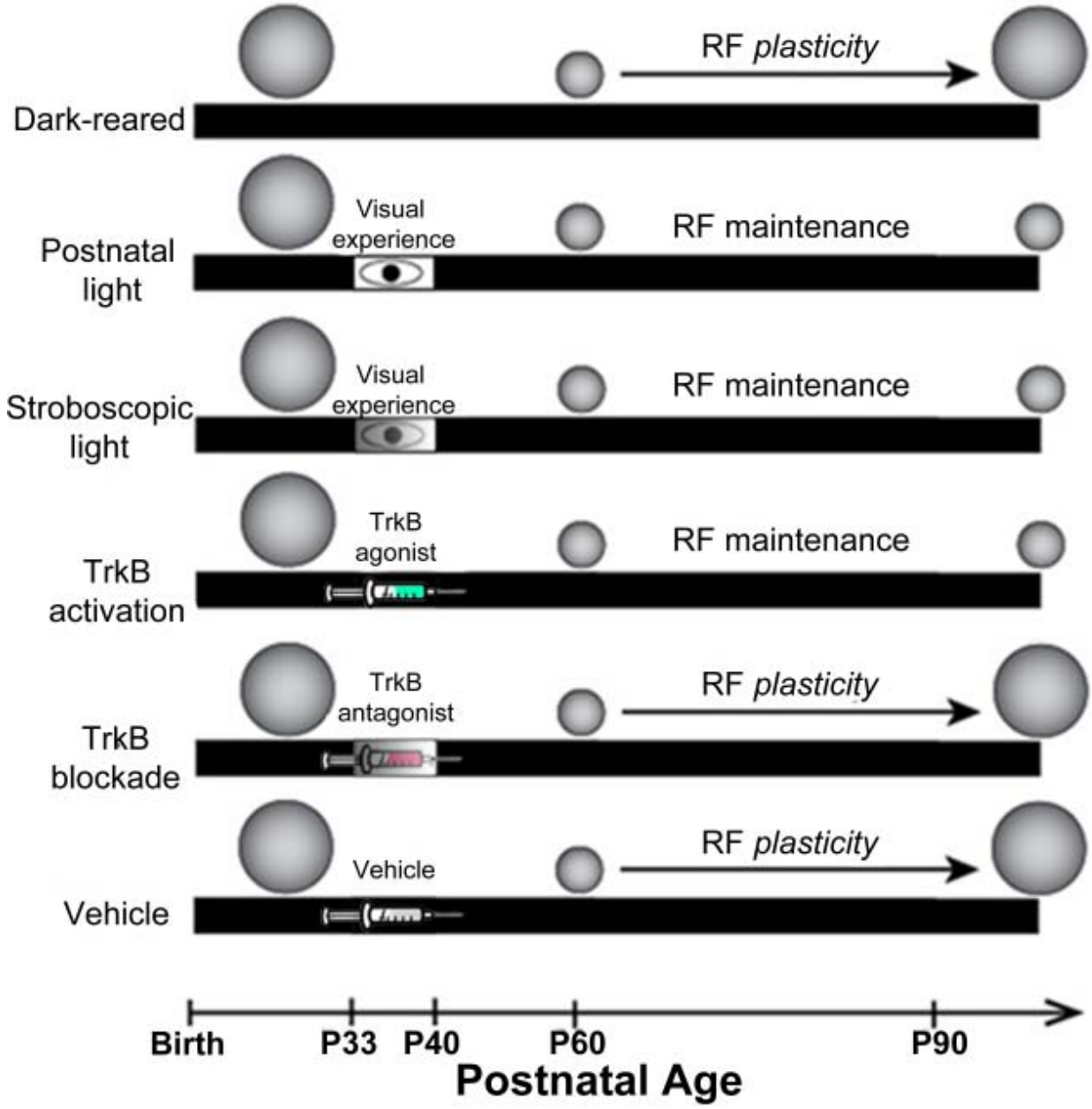
RF plasticity
Dark-reared
Visual experience
RF maintenance
Postnatal light
Visual experience
RF maintenance
Stroboscopic light
TrkB agonist
RF maintenance
TrkB activation
TrkB antagonist
RF plasticity
TrkB blockade
Vehicle
RF plasticity
Vehicle
Birth
P33
P40
P60
P90
Postnatal Age

Figure 7