INVESTIGATION OF ENHANCING VISUAL SPATIAL ACUITY IN DEVELOPMENTALLY COMPROMISED CIRCUITS

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The development of visual acuity requires visual experience, but the neural mechanisms that mediate the experience-dependent maturation of visual acuity remain unknown. After eye opening in mice, visual acuity is very poor but with time and visual experience, matures to an adult-level by 45 days post-birth (p45). Accumulating evidence demonstrates that the development of visual acuity requires the maturation of parvalbumin-expressing (PV) inhibitory neurons. Enhancing the maturation of inhibition accelerates the onset of adult-level visual acuity and the development of PV neurons, while rearing mice without visual experience delays maturation of inhibition and holds acuity at a juvenile state. In this study, we chronically perturbed PV neurons in order to assess their direct role in the development of visual acuity by selectively knocking-out (KO) ErbB4 receptor tyrosine kinase in PV-expressing cells. ErbB4 is a protein important for the stabilization of excitatory input onto PV neurons. When knocked out, excitatory input fails to stabilize onto PV neurons, resulting in a 25% reduced evoked firing rate. This phenotype mimics immature PV neurons. We found that adult PV-ErbB4 KO mice had a behavioral deficit in visual acuity compared to wild type (WT) mice. The difference in visual acuity between WT and PV-ErbB4 KOs is comparable to the difference between a juvenile p35 mouse and an adult p45 mouse with mature acuity. This is a 10-day developmental difference and demonstrates that fully functional, adult-like PV neurons are required to develop mature visual acuity. We also found that the visual acuity of PV-ErbB4 KOs can be rescued by using a behavioral training task. This is significant because we were able to bypass the initial deficit, indicating a role for other circuits in the brain involved in regulating visual acuity in behaviorally relevant environments. We therefore conclude that PV neurons are necessary for establishing the baseline, mature visual acuity, but with training other circuits may be involved in improving and enhancing visual acuity.

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PREFACE

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1.0 INTRODUCTION

1.1 PARVALBUMIN INHIBITORY NEURONS MEDIATE EXPERIENCE DEPENDENT PLASTICITY

Experience dependent plasticity permits external stimuli (eg worldly experiences) to influence both the structure of neural circuits and the function of their neural connections (van Versendaal & Levelt, 2016). During early development, passive exposure to input from the environment is critical for the proper maturation of cortical circuits. One well-studied model of experience-dependent plasticity occurs within the primary visual cortex (V1), the first cortical processing center for visual information. Hubel and Wiesel famously discovered a period of time wherein the connections of neurons within V1 are particularly sensitive to environmental stimulation and perturbation. This developmental window became known as the critical period for ocular dominance plasticity and has since served to inform much of what we know about the mechanisms by which experience-dependent plasticity occur (Wiesel & Hubel, 1963).

The primary visual cortex contains spatially organized clusters of neurons that selectively fire action potentials in response to visual stimulation from a single eye (known as the monocular zone) and to stimulation from both eyes (known as the binocular zone). Most neurons located in the binocular zone are driven by stimuli from one of the two eyes and can be given a score related

to which eye drives the firing rates of neurons the strongest when presented with visual stimulation. This is known as an ocular dominance index.

In cats, primates, and rodents, the developmental window known as the critical period for ocular dominance plasticity (ODP) is traditionally assayed via monocular deprivation during the first few weeks following eye opening (Trachtenberg, 2015). Monocular deprivation entails the obstruction of vision by depriving a single eye of visual experience. This visual obstruction subsequently shifts the responsivity of neurons from the contralateral, occluded, eye to the ipsilateral, open, eye after several days of monocular deprivation (Hubel & Wiesel, 1970). Rodents, which open their eyes about 12 days after birth (~P12), enter the critical period for ocular dominance plasticity around P21-P23, and closes by P32 (Smith & Trachtenberg, 2007). When testing this assay in adults, after the critical period has closed, results in no detectable shift in ocular dominance even after a year of monocular deprivation (Prusky & Douglas, 2003).

The onset and timing of the critical period is intimately connected with the development and maturation of inhibitory cell types and circuitry within the visual cortex. Inhibitory, or GABAergic, neurons are characterized by the production of the neurotransmitter GABA that functions to reduce neuronal excitability in the nervous system. GABAergic neurons make up approximately 10-20% of the neuronal population within the cortex of rodents (van Versendaal & Levelt, 2016). Three major classes of inhibitory neurons account for nearly 100% of GABAergic neurons, namely: parvalbumin-expressing neurons, somatostatin-expressing neurons, and 5HT3a receptor expressing neurons (Rudy et al., 2011).

Ample evidence demonstrates the role of inhibition in mediating the development of the critical period for ocular dominance plasticity. Accelerated maturation of inhibition through overexpression of brain-derived neurotrophic factor (BDNF), a protein important for growth of

GABA-expressing inhibitory neurons, results in a precocious onset of the critical period in mice (Huang et al., 1999; Marty, Berzaghi, & Berninger, 1997). Additionally, administration of benzodiazepines (GABA agonists), which enhance the effects of inhibitory signaling, cause onset of the critical period even in dark-reared mice, which normally fails to occur (Iwai et al., 2003). Conversely, a reduction in inhibition through the loss of GAD65 (a GABA synthesizing isoform) results in a failure to enter the critical period but can be rescued via enhancement of inhibition through application of diazepam (Hensch et al., 1998). Interestingly, GAD65-KO mice can enter the critical period at any age following administration of GABA agonists, which help restore the effects of normal inhibitory signaling, demonstrating the importance of inhibitory maturation and activity for the onset of the critical period (Fagiolini & Hensch, 2000). These studies demonstrate that the maturation state of inhibition is particularly important for the developmental onset and closure of the critical period for ocular dominance plasticity.

Inhibition mediated by parvalbumin-expressing inhibitory neurons is known to play a significant role in the development of the brain, and the onset of the critical period (Fagiolini & Hensch, 2000). PV inhibitory neurons are the largest sub-class of inhibitory neurons, accounting for approximately 40% of GABA-expressing cells within the brain (Rudy et al., 2011). Parvalbumin is a calcium binding-protein and is associated with a fast-spiking firing pattern in which the cells fire at frequencies > 50Hz with brief action potentials (Ascoli et al., 2008; Kawaguchi et al., 1987; Kawaguchi & Kubota, 1998; Rudy et al., 2011). PV inhibitory neurons typically synapses locally and consist of two morphological sub-types. Fast-spiking (FS) basket cells typically synapse on the soma of target neurons, while chandelier cells target the axon initial segments (Ascoli et al., 2008; Kawaguchi & Kubota, 1998). These neurons are known to have several important functions, such as thresholding and scaling the responses of excitatory neurons

in order to prevent runaway excitatory activity (Atallah et al., 2012; Runyan et al., 2010), as well as playing a role in controlling oscillatory activity in the gamma range (50-80 Hz) (Cardin et al., 2009).

Indirect evidence for the role of PV inhibitory neurons in controlling the timing of the critical period was first identified when accelerated development of inhibitory circuitry not only caused an early onset of the critical period, but also caused an early maturation of PV neurons (Huang et al., 1999). This same study also noted a precocious development of adult-level visual acuity, which suggests the role of inhibition in regulation maturation of visual acuity. A study in 2013 specifically identified the role of PV neurons in mediating the timing of the critical period for ocular dominance plasticity (Kuhlman et al., 2013). One day following monocular deprivation, PV inhibitory neurons in V1 have an evoked firing rate reduced by 50% and lose a significant amount of their excitatory input in the hemisphere contralateral to the ocular closure, permitting the other, undisturbed hemisphere to maintain normal responsivity to visual stimulation. When inhibition was artificially maintained, ocular dominance plasticity failed to occur. Therefore, PV inhibitory neurons are key mediators of visual experience for instructing the formation of visual circuits.

Monocular deprivation performed through the closure of the critical period permanently reduces visual acuity through that eye, while visual acuity remains unaffected if performed either before, or after, the critical period (Prusky & Douglas, 2003). Additionally, a review by (Takao K. Hensch, 2005) suggests that enhancement of GABA function not only accelerates onset of critical period plasticity, but also the development of visual acuity, while a reduction in GABA function not only delays the onset of critical period plasticity, but also leads to a delay in the development of, or loss therein, of visual acuity. Indeed, it has been observed that mice with accelerated

inhibitory development not only have accelerated closures of the critical period, but also have accelerated onset of adult-level visual acuity (Huang et al., 1999). While mice that experience monocular deprivation throughout the duration of the critical period experience a permanent loss of visual perception through the closed eye (Prusky & Douglas, 2003).

1.2 THE DEVELOPMENT OF VISUAL ACUITY

Visual acuity is a measure of an organism's ability to accurately perceive their surroundings. While necessary for survival, the neural mechanisms by which acuity develops remain unknown. Visual acuity is initially very poor in most mammals and, following eye opening and subsequent visual experience, develops with age to an adult, mature level. This experience-mediated maturation of visual acuity occurs in many different species of mammals, including cats(Freeman & Marg, 1975; Mitchell, Giffin, Wilkinson, Anderson, & Smith, 1976), monkeys (Teller, Regal, Videen, & Pulos, 1978), and rodents (Fagiolini, Pizzorusso, Berardi, Domenici, & Maffei, 1994). For example, a mouse's visual acuity, assessed at 25 days post-birth (p25), has an acuity of ~0.25 cycles/° and gradually improves until about p40-p45 wherein the mouse's visual acuity plateaus. An adult mouse's visual acuity is estimated to be between, depending on the strain of mice and method of behavioral testing ~0.42 – 0.52 cycles/° (**Fig. 1**) (Prusky & Douglas, 2003; Stephany et al., 2014). This period of improvement overlaps with the critical period for ocular dominance plasticity and extends past its closure. Similar to the critical period and the influence of experience on the development of ocular dominance, visual experience is necessary for the development of visual acuity. When reared without visual experience (dark rearing), visual acuity fails to develop past a juvenile stage (Fagiolini et al., 1994; Timney, Mitchell, & Giffin, 1978)

With the maturation of sense organs, the developing brain relies increasingly on sensory experience to guide the establishment of mature cortical circuitry. It is this sensory-dependent activity in the visual cortex that drives numerous changes, including the maturation of inhibition through a cascade of neurotrophic growth factors. The expression and production of neurotrophic factors contribute to the development of inhibitory cell maturation and circuit construction. The neurotrophin family of growth factors are implicated in numerous developmental functions in the brain. BDNF and neurotrophin-4/5 (NT-4/5) regulate synapse formation and, specific to the visual cortex, help mediate differentiation of monocular and binocular-zone spatial organization (Cabelli, Hohn, & Shatz, 1995). Neurotrophin-3 (NT-3) is shown to regulate neuronal proliferation and differentiation (Ghosh & Greenberg, 1995).

Many neurotrophic factors are regulated in an activity dependent manner. High activity promotes expression of BDNF (Isackson et al., 1991) and NT-3 (Patterson et al., 1992). BDNF expression is rapidly regulated by sensory input in the visual cortex, and subsequently neural activity, during development. When rodents are dark reared or excitatory cells are prevented from firing via tetrodotoxin administration, BDNF mRNA levels are dramatically reduced, but when exposed to light (visual stimulation), the levels are rapidly increased (Castren et al., 1992).

Inhibitory neurons may be intimately tied to the development and ultimate maturation of the trajectory of visual acuity. For example, the maturation of visual acuity can be accelerated through the overexpression of brain derived neurotrophic factor (BDNF), a molecule important for the development of inhibitory circuitry. In BDNF-overexpressing transgenic mice, an accelerated development of visual acuity was reported (Huang et al., 1999). The same study also observed an accelerated development of parvalbumin-positive (PV) inhibitory neurons, assessed through a precocious increase in soma sizes and neurite arborizations.

There are numerous methods used to measure the visual acuity of mammals, and particularly mice. At the behavioral level, a common method of testing visual acuity is to utilize a visual water task (Prusky & Douglas, 2003; Prusky, Reidel, & Douglas, 2000; Stephany et al., 2014). This task involves placing the animal into a corridor filled with water and conditioning it to distinguish a low spatial frequency sine-wave grating from an isoluminant gray screen. Sinusoidal gratings are a useful stimulus to assess visual acuity because its parameters are easily controlled, and the higher the cycles per degree become, the more the gratings begin to like a gray screen. Once the animal learns this, progressively higher spatial frequencies are incorporated into the discrimination task in order to probe when the animal fails to accurately discriminate the sinusoidal gratings (and therefore an escape from water) from the gray screen. Traditionally, in the studies cited above, an individual animals' visual acuity is established when the animal fails to accurately discriminate the sinusoidal gratings from the gray screen 70% of the time.

Visual acuity can also be estimated through the use of visually evoked potentials, and this can be performed on both young animals who are too small to be able to perform the behavioral task. Visually evoked potentials (VEPs) are a measure of local cortical activity in the visual cortex measured when a visual stimulus is presented. Acuity is measured by assessing when the cortical activity in V1 is no longer differentiated from the "noise" activity associated when viewing a gray stimulus. This is particularly useful for assessing the effects of visual deprivation and the development of cortical responsiveness, which ultimately underlies the perceptual components of visual acuity. When reared without any visual experience whatsoever, the VEP-assessed visual acuity is not different from an animal that recently opened its eyes, significantly reduced from that of an adult animal that had visual experience (Fagiolini et al., 1994). Additionally, environmental enrichment from birth is able to enhance visual acuity in animals, again giving credence to the

importance of experience in the development of visual acuity likely mediated through PV inhibitory neurons (Prusky, Reidel, et al., 2000).

The underlying physiology of VEP acuity, and behavioral/perceptual acuity, lies within the neural responsive patterns and circuitry that develop. When dark reared, along with the failure to develop visual acuity at the behavioral level, functional response properties of the primary visual cortex (V1) also fail to develop. For example, in rodents, visual experience is necessary for the development of visual responsiveness, binocular visual responses, orientation selectivity, and receptive field size of excitatory neurons in primary visual cortex (Fagiolini et al., 1994).

Understanding the relationship of PV neuron development and the maturation of visual acuity will be useful to understanding a component of the neural mechanism by which acuity develops.

1.3 THE NEURODEVELOPMENTAL ROLE OF ERBB4 RECEPTOR TYROSINE KINASE

The above evidence demonstrates that experience-dependent maturation of the visual cortex is related to the maturation of inhibitory cells and, more specifically, the maturation of parvalbumin-expressing inhibitory neurons. In order for PV neurons fully mature, they must express the protein ErbB4 receptor tyrosine kinase. ErbB4 expression is highly enriched in the postsynaptic density of excitatory (Garcia, Vasudevan, & Buonanno, 2000) and Parvalbumin-expressing neurons (Sun et al., 2016) and it interacts with major scaffolding proteins for the maintenance and control of synaptic function (Ehrlich & Malinow, 2004; El-Husseini et al., 2000)**.** ErbB4 selectively binds

the neurotrophic factor neuregulin-1 (NRG1; Mei & Xiong, 2008). Synaptic activity, such as excitatory firing due to visual stimulation, triggers a release of NRG1 and activation of ErbB4 tyrosine kinase activity, leading to the recruitment and stabilization of AMPA receptors (Li et. al, 2007). Interruption of this signaling causes destabilization of AMPA receptors, loss of spine structure, impairment of plasticity, and the loss of spines and NMDA receptors (Li et al., 2007).

The interaction between NRG1 and ErbB4 is intimately implicated in the formation of synapses between pre-synaptic excitatory neurons and post-synaptic PV neurons. Slice physiology experiments first found that changes in ErbB4 levels altered dendritic spine sizes, synaptic AMPA receptors, and NMDA currents in neonatal slices, demonstrating the importance of ErbB4 in regulating excitatory input onto PV neurons (Li et al., 2007). NRG1 expression and release in excitatory neurons is regulated in an experience-dependent manner and influences the expression of ErbB4 (Sun et al., 2016). Upon binding of NRG1, ErbB4 actively recruits AMPA receptors to the post-synaptic density of PV inhibitory neurons following excitatory neuron action potentials, promoting the formation of long-term synapses through stabilization via PSD-95 (**Fig. 2**.; Ting et al., 2011). This recruitment of AMPA receptors is necessary for the development of excitatory input onto PV inhibitory neurons, and the maturation of PV inhibitory circuitry in the cortex.

Normally, expression of ErbB4 is necessary for the formation of stable excitatory input onto PV neurons (Sun et al., 2016). Visual activity that drives the firing of neurons leads to an increase in the activation of ErbB4 by the binding of its ligand, NRG1, produced both by excitatory neurons and inhibitory neu by phosphorylation of By knocking ErbB4 out, excitatory synaptic input onto PV neurons is dramatically reduced, which has been demonstrated *in vitro* (Sun et al., 2016).

During the critical period for ODP, the expression and localization of ErbB4 receptor tyrosine kinase on PV neurons is particularly sensitive to environmental perturbation. One day of visual deprivation to a single eye dramatically reduces excitatory input onto PV neurons, likely mediated via a reduction of excitatory neuron activity, which results in a reduced evoked firing rate (Kuhlman et al., 2013*)*. Additionally, when dark reared, the evoked firing rates of PV neurons are reduced by ~50% (Kuhlman, Tring, & Trachtenberg, 2011).

These reductions in evoked firing rate through ErbB4 manipulation, related to a failure of excitatory input to develop onto PV neurons, is therefore correlated with the loss of visual acuity that occurs from dark rearing or monocular deprivation through the closure of the critical period. This mutation is a useful tool for preventing the full maturation of PV firing properties, and the Kuhlman lab found that knockout of ErbB4 reduces excitatory drive onto PV neurons by 25%, mimicking a phenotype more similar to a young, visually inexperienced juvenile mouse (Feese, 2017).

1.4 EXPERIMENTAL GOALS

The above studies demonstrate the importance of PV-expressing inhibitory neurons in mediating experience-dependent plasticity. While PV neurons are intimately tied to developmental processes in the visual cortex, such as controlling the opening and closure of the critical period, their role in the development of visual acuity remains unknown. While many of the studies cited above focus on a particular type of neural plasticity and adaptation, namely ocular dominance plasticity, the role of PV neurons in establishing the normal circuitry for visual development must be better understood, and may pave the way to understanding how to improve visual acuity in people who

suffer from amblyopia. In order to bridge this gap, my master's thesis aims to determine the impact of disrupting PV neuron function on cortical processing of high spatial frequency stimuli during normal development and in an improvement scenario.

Based on the seminal work described above, we have come to appreciate that the ability to see requires visual experience. The broad goal of my thesis work is to identify the role of a specific class of inhibitory neurons (PV-expressing) in the development of visual acuity. Our first primary goal was to identify if perturbation of PV inhibitory neurons through KO of ErbB4 disrupted the development of visual acuity. We hypothesized that PV-ErbB4 KOs would display a deficit in visual acuity. By selectively knocking out ErbB4 in PV-expressing cells, I assessed the behavioral impact of a molecular perturbation to PV neurons on visual acuity. A second goal was identifying if we could rescue the KOs and even improve their visual acuity in a behavioral task. We hypothesized that both the WTs and the PV-ErbB4 KOs would improve their visual acuity. Lastly, we wanted to assess if the mice would generalize their improved performance to stimuli at different orientations than what they were trained on. We hypothesized that the mice would be able to generalize. In addition, preliminary data suggests that we can train our mice to improve their visual acuity higher than that of a rat. By providing insight into the relationship between the development of PV inhibitory neurons and the development of visual acuity, we will be one step closer to understanding the complex phenomenon that is our ability to perceive the world around us.

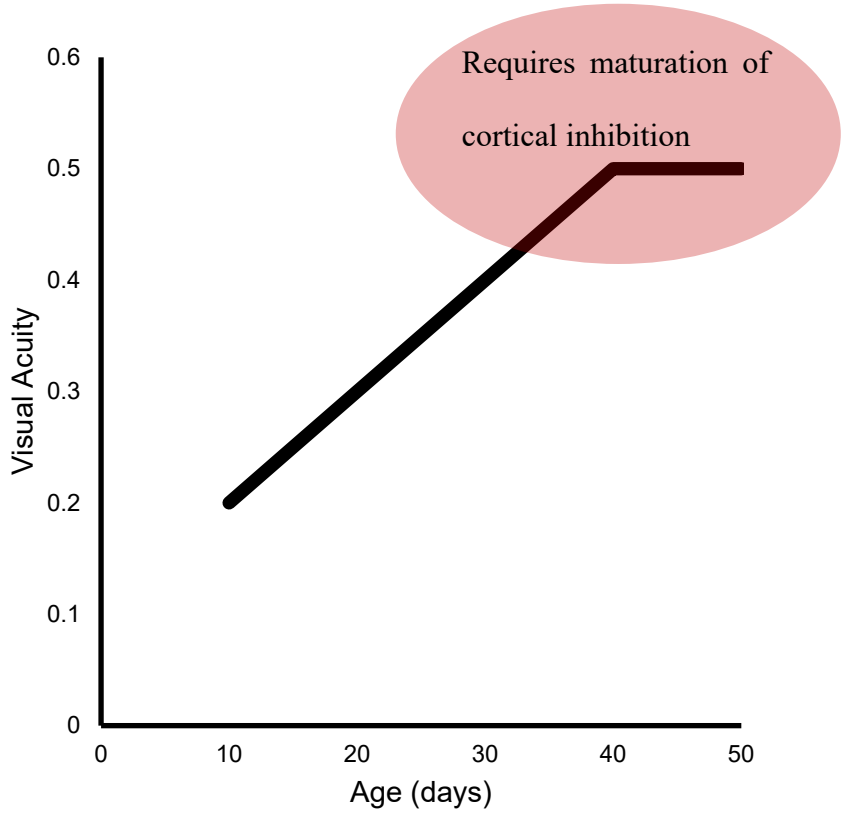

Figure 1. Schematic of maturation of visual acuity in mice. Initially, mammals are born with very poor visual acuity. However, with time and visual experience, our acuity improves to an adult, mature level. Accumulating evidence suggests that the maturation of visual acuity, and subsequently the ability to discriminate fine details, requires the maturation of cortical inhibition.

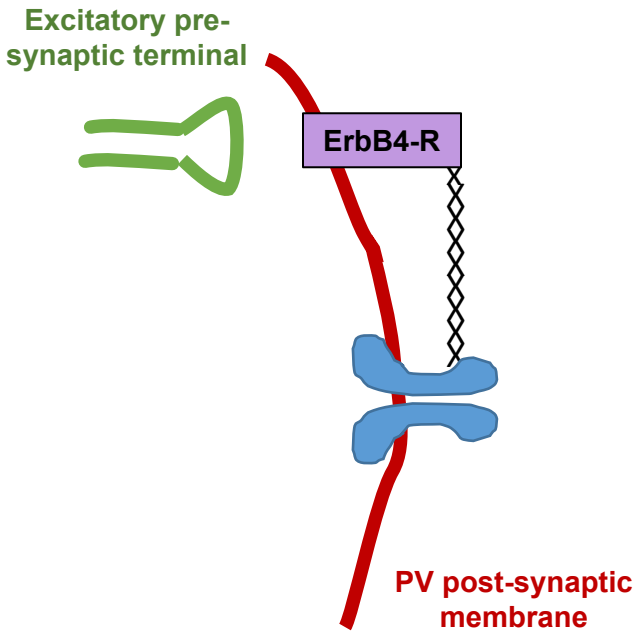

Figure 2. Schematic of ErbB4 Receptor Tyrosine Kinase interaction between excitatory and PV-inhibitory neurons. This receptor is necessary for excitatory input to stabilize on PV neurons, contributing to a mature phenotype. By selectively knocking out ErbB4 in PV neurons, we chronically hold these cells in a developmentally immature state.

2.0 MATERIALS AND METHODS

Experimental procedures were compliant with the guidelines established by the Institutional Animal Care and Use Committee of Carnegie Mellon University and the National Institutes of Health

2.1 ANIMALS

22 mice were used for our experiments, consisting of a total of 11 wild-type (WT) mice and 11 knockout (KO) mice. Knockout mice had the gene ErbB4 selectively knocked out in cells expressing parvalbumin through expression of cre-recombinase under the control of the parvalbumin promotor. Generation of our experimental mice requires two generations of breeding. First, we breed homozygous ErbB4-floxed mice (MMRC, stock number 010439-UCD) with homozygous Ai14 mice (Jackson Laboratories, stock number 007914) to produce a line we call ErbB4Ai14 mice, which are hemizygous for the ErbB4 floxed allele and the Ai14 allele. Additionally, we utilize is a homozygous parvalbumin-cre mouse (Jackson Laboratories, stock number 005628) bred with a homozygous ErbB4 floxed/hemizygous PV-cre mouse to generate a strain of mice ErbB4PV_hets, which are hemizygous for both the ErbB4 floxed allele, and are

subsequently genotyped to select mice that are homozygous positive for PV-Cre (50% of offspring). We then breed the ErbB4Ai14 mice with ErbB4PV_het mice to generate our experimental line. Of the experimental pups, 25% are wildtype (that is, their ErbB4 gene is not floxed), 25% are selective ErbB4 knockouts (that is, both alleles of ErbB4 gene are floxed), and 50% are heterozygous (only one of two ErbB4 alleles are floxed) which we do not use in our experiments. All experimental pups are heterozygous for expression of cre-recombinase under the control of the parvalbumin promotor.

The 22 experimental mice used in the studies were split into two cohorts. Cohort 1 was used to establish the baseline acuity of WT and KO mice, and consisted of 7 WT (2 female, 5 male; ages postnatal day 53-101) and 7 KO (3 females, 4 male; ages p68-92) mice. Cohort 2 was used to test for an improvement of visual acuity in an extended training paradigm, and consisted of 4 WT (1 female, 3 male; ages p58-75) and 4 KO (4 males; ages p71-97) mice. The ages of mice are defined as the age in which the mice began the first session of the acuity task after completing their shaping.

Experimental mice were housed with at least one cage mate, and maximally with four other cage mates. The mice experienced a reverse light cycle, which was dark from 11:00 am to 11:00 pm. All training and behavioral testing occurred during the dark phase.

2.1.1 Head-post surgery

In order to perform behavioral testing, mice of at least 28 days (postnatal) of age were anesthetized with isoflurance (3% induction, 1-2% maintenance) and had a stainless-steel bar glued to the right side of their skull and secured with dental cement. This surgery, on average, took between 25-45

minutes. The steel bar was used to immobilize their head during behavioral tasks. Carprofen was administered during, and 24-hours after, the surgery to reduce inflammation and provide pain relief for the mice. 48-hours after the initial surgery, the water bottle was removed from the cage and the mice began water deprivation. During this period, the mice were given 750uL once each day for 8 to 10 days until their weights stabilized. Once stabilized, mice began acclimation and behavioral training. The weight and health of all water-deprived mice were monitored and daily. Food was provided *ad libitum*.

2.2 BEHAVIORAL TASK

2.2.1 Acclimation task

For acclimation, shaping, and acuity tasks, the mice were briefly anesthetized (3% induction, 1.5% maintenance) with isoflurance and attached to a steel bar within less than 5 minutes. Mice were given at least 5 minutes to recover from isoflurance prior to beginning any of the tasks.

Prior to shaping, mice were acclimated to the behavioral rig and lickport, which provided their water. Mice were placed on the behavioral rig and head-attached for 1 to 2 acclimation sessions prior to shaping. During this task, the mice were given approximately 10-15 minutes to get used to their new position and then were run on a task acclimating them to both the behavioral rig, as well as to the lickport which provides a small amount of water upon the mouse sticking out its tongue and licking. The task consisted of a manually controlled stimulus presentation identical to the shaping task, followed by a black screen. During the stimulus presentation, if the mouse licked at any point it would receive a small amount of water. Upon a manual keyboard press, the

screen would switch to a black screen, which would not release water upon a mouse's licking. The screen would then be manually switched back to a stimulus presentation for the mouse to obtain water. Within one to two acclimation sessions, the mice became familiar with the behavioral rig as well as learning that they were able to obtain water via licking while head-attached. The mouse received ~5-6 uL for each lick recorded on the lickport when the stimulus (0.1 cycles/°) was on the screen. The acclimation task took approximately 20-30 minutes to complete each session. By the end of their acclimation training, mice typically understood that water released from the lickport upon licking and were prepared to begin the shaping task.

2.2.2 Shaping task

The shaping task stimuli consisted of a 4-second go-stimulus (0.1 cycles/°) or no-go stimulus (a gray screen), and a two second black screen which signaled the start of a new trial. The sequence for each trial was chosen randomly, subject to the constraint that a go stimulus was unable to be followed by another go stimulus in the next trial. The first day of shaping consisted of the go stimuli presented ~45-48% of the time, and the no-go stimulus being presented for ~55-58% of the time. Upon licking on a go-stimulus, the mice were rewarded with 5-6 uL of water. As the shaping days progressed, a timeout was implemented to facilitate an improvement of performance. The timeout consisted of an extension of the two-second black screen, and would only occur if the mouse licked on a no-go stimulus. Initially the timeouts were one to two seconds. As shaping sessions progressed, the frequency of go trials decreased while the no-go increased until the mice were being tested at ~25% go, and ~75% no-go with an eight second time out. When the mice reached 3 sessions of 90% total accuracy, the mice then performed the acuity task.

2.2.3 Acuity Task (Cohort 1)

The acuity task consisted of a set of six different go stimuli shown 23 times, randomly, throughout a given session. The go stimuli were presented at a frequency of 28 to 31%. Two different tasks were used to measure acuity of mice. Initially, a task consisting of the spatial frequencies [0.1, 0.2, 0.4, 0.5, 0.6, 0.8 cycles/°] was used with 3 WT and 4 KOs. Additionally, a task consisting of [0.1, 0.2, 0.4, 0.45, 0.5, 0.6 cycles/°] was used with 4 WT and 3 KOs. An acuity measurement was established using data from the stimuli [0.1, 0.3, 0.4, 0.5, 0.6 cycles/°], which all 7 WT and 7 KOs were shown. A mouse's given performance was assessed for each spatial frequency with the following equation:

Performance = (#hits + #correct rejects*w)/(#total go stimuli + (#total no-go stimuli * w))

Where # hits are the number of licks a mouse performed on a given spatial frequency, the # of correct rejects are the number of time the mouse did not lick on a no-go stimulus. The total # of go stimuli is the total number of stimuli for a given spatial frequency (23, in our case) while the total # of no-go stimuli varied from ~350 to 400 no-go stimuli. The term w refers to a weighting factor defined as (23/total no-go stimuli), which was used to weight the number of no-go stimuli more closely to the go stimuli. After three consecutive days of acuity assessment, the performance was averaged for an individual mouse and fit with a linear regression to estimate the spatial frequency at 70% performance. This was allowed us to relate our behavioral findings to the literature.

2.2.4 Extended Acuity Training (Cohort 2)

In a separate cohort of mice, we extended the acuity training of both WT and KOs in order to assess if they improve acuity over time. The range of spatial frequencies we used was initially [0.1, 0.22, 0.34, 0.46, 0.58, 0.70 cycles/°]. In the case of three wild-types, their performance at high spatial frequencies [0.58, 0.7 cycles/°] became high enough that we extended their acuity assessment to [0.1, 0.34, 0.46, 0.58, 0.70, 0.82 cycles/°]. Three of four KOs experienced 29 sessions acuity testing 1 to 7 with 0° oriented stimuli, followed by 3 days of testing for generalization with stimuli shifted to a 45° orientation. A fourth KO experienced 3 days of acuity testing at 0°, followed by 2 days of generalization at 45°, and an extended acuity testing of 17 days. The WT cohort was slightly more varied, with two of the WTs experiencing 2 and 3 days of 45° orientation first, followed by extended acuity testing. One additional WT mouse experienced 3 days of acuity 0° orientation, 2 days of 45° orientation for generalization baseline, and an additional 10 days of the original acuity task ranging from 0.1 to 0.7 cycles/°. A fourth wild-type had 3 days of acuity testing at 0°, 2 days at 45°, 12 days at 0.1 to 0.7 cycles/°, and an additional five days at 0.1 to 0.82 cycles/°. In all cases, the first 3 days of acuity at 0° were used to determine the mouse's baseline performance at a given spatial frequency.

2.3 STIMULI CREATION AND STATISTICS

All stimuli were generated in real time using Psychophysics Toolbox (http://psychtoolbox.org) in Matlab (Mathworks, Boston, MA). Stimuli were presented on a computer screen position 25 cm away from the right eye of the mouse, angled at 50° to the midline of the animal. The stimuli

presented to the mice were at 60°, with the edges blurred into a gray background by a Gaussian function.

In cohort 1, a two-factor mixed ANOVA was used to establish if there was a significant difference between WT and KO performances across spatial frequencies [0.1, 0.2, 0.4, 0.5, 0.6 cycles/°]. In cohort 2, a linear regression was used to establish if there was a significant improvement in performance across sessions while a binomial test was used to establish if a mouse's licks were significantly above chance ($p \leq 0.05$).

3.0 RESULTS

Our first goal was to define the role of parvalbumin-expressing inhibitory neurons in the development of visual acuity. To assess the visual acuity of mice, we developed a go/no-go behavioral paradigm in which mice report the presence of a stimulus for a water reward. Prior to their acuity assessment, the mice are water restricted for 8-10 days, acclimated to the experimental set-up, and shaped to learn a simplified behavioral task.

The shaping task consists of the mice learning to respond accurately to a stimulus (go trial) by licking when it is displayed on screen, and to withhold licks on a gray screen (no-go trial). The go-stimulus consists of a circle with vertically oriented (0°) static, sinusoidal gratings displayed at 60° to the mouse's location. The go-stimulus is displayed at 0.1 cycles/°. The experimental paradigm consists of two options on a go trial and two options on a no-go trial. The mouse can lick on a go trial (a hit) or not lick (a miss), while on a no-go trial the mouse can withhold licks (a reject) or lick (a false alarm) **(Fig. 1A)**. When first beginning, the go-stimuli display at a frequency of ~50% go, 50% no-go but reduce to ~25% go, ~75% no-go as the mouse learns the shaping task. When a mouse achieves a performance of 90% for three individual sessions, it then begins the acuity assessment task.

The design and progression of the acuity task is similar to the shaping task. However, the singular 0.1 cycles/° go-stimulus is replaced by six go-stimuli ranging from 0.1 cycles/° to 0.6

cycles/°. Each stimulus is presented 23 times randomly, leading to 138 go stimuli shown in a given acuity assessment session. This design allows us to test if the number of hits in a given session were significantly above chance using a binomial test, ensuring that each session is interpretable. A performance score for each of the spatial frequencies is obtained at the end of a session, and averaged over three consecutive sessions leading to a total of 69 presentations of a given stimulus spatial frequency.

A total of seven WT and seven PV-ErbB4 KO mice were tested in the visual acuity assessment paradigm. Importantly, both cohorts performed highly at both 0.1 cycles/°, which they were shaped on, and on 0.2 cycles/°, which they had no prior visual experience of, demonstrating that the mice understood the structure of the acuity task despite having new stimuli that they had never seen. At spatial frequencies 0.4 cycles/° and above, the WTs consistently performed higher than their PV-ErbB4 KO counterparts. The WTs differed significantly in their performance compared to PV-ErbB4 KOs **(Fig. 1B).** When using a linear regression to estimate the spatial frequency that both the WT and PV-ErBb4 KO cohort achieve 70% performance, the difference is 0.066 cycles/°.

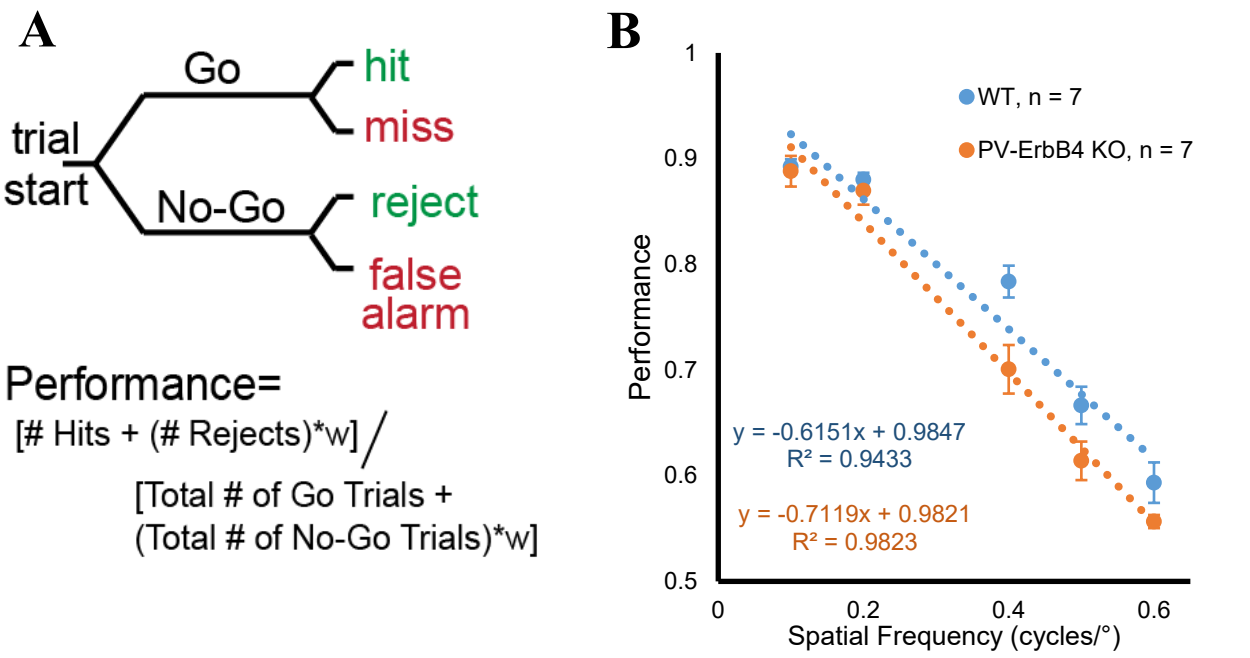

Figure 3 PV-ErbB4 KOs have a deficit in visual acuity. (A) Schematic of behavioral paradigm used to assess visual acuity. The task is a go/no-go task in which animals are trained to lick for a water reward when presented with static grating stimuli (vertically oriented bars of varying spatial frequencies (cycles/°). The equation is used to calculate a mouse's performance of a given spatial frequency. W is a weight factor used that controls for the unequal presentation of no-go gray screen stimuli (~75%) to go grating stimuli (~25%). (B) Performance of WT and PV-ErbB4 KOs differed significantly (Two Factor Mixed ANOVA, $p = 0.02$). Linear regression estimated the 70% performance of PV-ErbB4 KOs at 0.3961 cycles/° and the WTs at 0.469 cycles/°. The estimated difference between WT and KO at the estimated 70% acuity threshold is 0.066 cycles/°. Six go-stimuli spatial frequencies are displayed during each acuity task session, however plotted above are data from only five. This is because a subset of the total 14 mice were assessed at 0.45 (four WT, three PV-ErbB4 KOs) and 0.80 cycles/° (three WT, four PV-ErbB4 KOs), data not shown. Ages range from p60 to p100. Error bars are standard error of the mean.

Our second goal was to use our behavioral paradigm to improve the visual acuity of WTs and particularly PV-ErbB4 KOs despite their initial deficit in visual acuity. Hosang et al. (2018) demonstrated that extended training in a behavioral discrimination task improved the visual acuity of mice. Using our go/no-go paradigm, we tested mice for at least 20 sessions. For this task, we displayed go stimuli at spatial frequencies of 0.1, 0.22, 0.34, 0.46, 0.58, and 0.70 cycles/°. This range of spatial frequencies permitted both WT and PV-ErbB4 KOs to show improvement on at least two frequencies above their 70% threshold established in our first set of experiments **(Fig. 1)**.

We tested eight mice (four WTs, four PV-ErbB4 KOs) in the extended acuity paradigm for improvements in their performance across multiple spatial frequencies. Regression analysis was used to establish if the performance across multiple sessions improved via the slope being significantly different from zero. Three of the four PV-ErbB4 KOs demonstrated a significant improvement in their performance at 0.46 cycles/° **(Fig. 2A)**. Across all spatial frequencies, one of the four mice improved at 0.1 and 0.22 cycles/°, while three of the four KOs improved at 0.34, 0.46, 0.58, and 0.7 cycles/° **(Fig. 2B)**. One of the four PV-ErbB4 KOs (7819_NC) failed to improve at any of the tested spatial frequencies. At 0.58 cycles/°, four of the four WTs demonstrated a significant improvement **(Fig. 3A)**. Across all spatial frequencies, four of the four mice improved at 0.46, 0.58, and 0.70 cycles/°. Two mice significantly improved at 0.34 cycles/°. One mouse significantly improved at 0.10 and 0.22 cycles/° **(Fig. 3b)**.

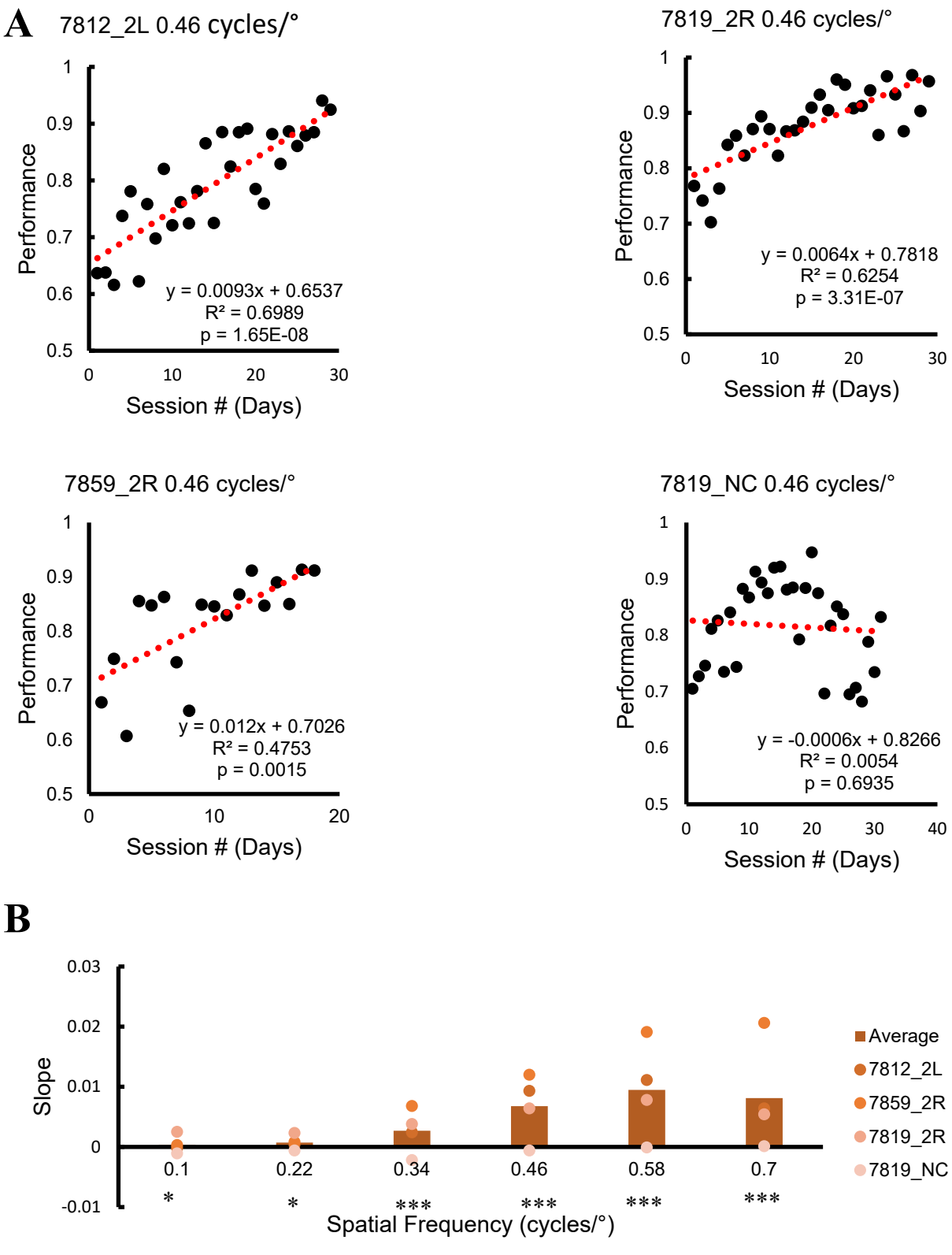
A
7812_2L 0.46 cycles/°
Performance
Session # (Days)
y = 0.0093x + 0.6537
R² = 0.6989
p = 1.65E-08
7819_2R 0.46 cycles/°
y = 0.0064x + 0.7818
R² = 0.6254
p = 3.31E-07
7859_2R 0.46 cycles/°
y = 0.012x + 0.7026
R² = 0.4753
p = 0.0015
7819_NC 0.46 cycles/°
y = -0.0006x + 0.8266
R² = 0.0054
p = 0.6935
B
Slope
Spatial Frequency (cycles/°)
0.1
0.22
0.34
0.46
0.58
0.7
*
*
***
***
***
***
Average
7812_2L
7859_2R
7819_2R
7819_NC

Figure 4. Three of four PV-Erbb4 KOs improve visual acuity at 0.46 cycles/° following extended acuity training. (A) Plots of the performance at 0.46 cycles/° of four separate PV-ErbB4 KO mice across multiple training sessions. Three of the mice improved their performance significantly, as denoted by their significant p-values from regression analysis, demonstrating that the slope is significantly different from zero across the multiple session days. One PV-ErbB4 KO mouse did not improve significantly across the entirety of its training sessions at any spatial frequency. (B) Summary plot of the slopes of four PV-ErbB4 KO mice across the range of tested spatial frequencies (cycles/°). * indicates the number of mice that had slopes significantly ($p < 0.05$) above zero at a given spatial frequency.

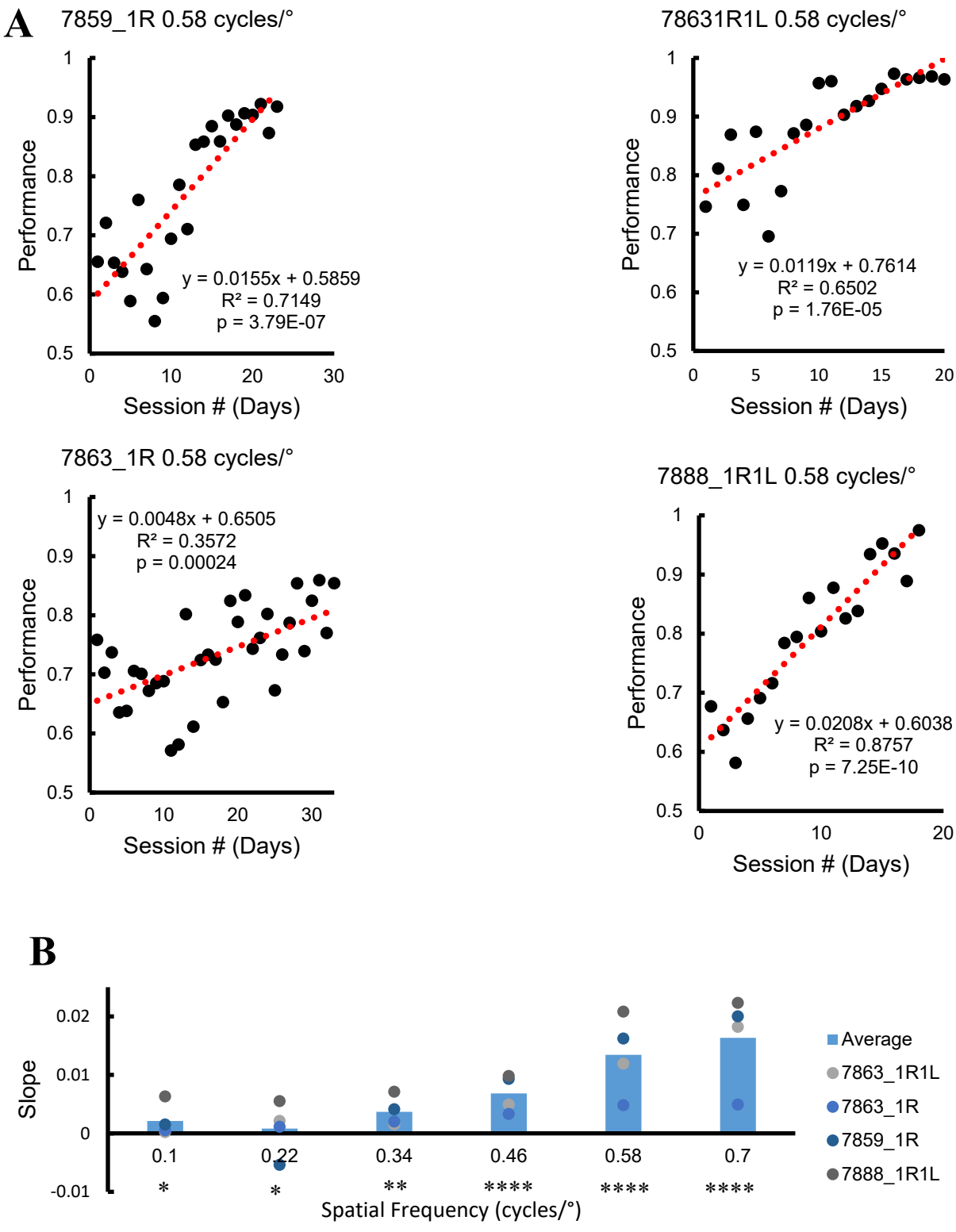
A
7859_1R 0.58 cycles/°
Performance
Session # (Days)
y = 0.0155x + 0.5859
R² = 0.7149
p = 3.79E-07
78631R1L 0.58 cycles/°
y = 0.0119x + 0.7614
R² = 0.6502
p = 1.76E-05
7863_1R 0.58 cycles/°
y = 0.0048x + 0.6505
R² = 0.3572
p = 0.00024
7888_1R1L 0.58 cycles/°
y = 0.0208x + 0.6038
R² = 0.8757
p = 7.25E-10
B
Slope
Spatial Frequency (cycles/°)
0.1
0.22
0.34
0.46
0.58
0.7
*
*
**
****
****
****
Average
7863_1R1L
7863_1R
7859_1R
7888_1R1L

Figure 5. Four WTs improve visual acuity at 0.58 cycles/° following extended visual acuity training. (A) Plots of the performance at 0.58 cycles/° of four WT mice across multiple training sessions. Four of four mice improved their performance significantly, as denoted by significant p-values demonstrating that the slope of the regressions are significantly different from 0 across training days. (B) Summary plot of the slopes of 4 WT mice across the range of tested spatial frequencies (cycles/°). * indicates the number of mice that had slopes significantly ($p < 0.05$) above zero at a given spatial frequency.

Our third and final goal of this study was to assess whether the improvement of visual acuity at 0° orientation stimuli transferred to stimuli at an orientation the mice were not trained on. Therefore, following improvements in stimuli at 0° orientations, the mice were assessed over two days at 45°. Both PV-ErbB4 KOs **(Fig. 4A)** and WTs **(Fig. 5A)** improved their performance at 0.46 and 0.58 cycles/° respectively. Across all tested spatial frequencies, the PV-ErbB4 KOs and WTs **(Figs. 4B and 5B, respectively)** maintained high performance when tested at 45°.

Lastly, we tested one WT mouse at progressively higher spatial frequencies. The goal of this set of experiments was to see if it were possible to push mice to an even higher level of performance. We re-tested the mouse at spatial frequency ranges 0.10, 0.22, 0.34, 0.46, 0.58 and 0.70 cycles/°. If the mouse achieved three consecutive days of a false alarm rate below 30%, and a number of hits significantly above chance (minimally 16 of 23 hits, binomial test $p<0.05$) at the highest tested spatial frequency (in this case, 0.70 cycles/°), the mouse was progressed up by 0.12 cycles/° and the spatial frequency immediately following 0.10 cycles/° was removed. We maintained 6 spatial frequencies, shown 23 times each, throughout all tested sessions. The mouse was subsequently tested up to ranges 0.10, 0.58, 0.70, 0.82, 0.94, 1.06 cycles/°. The mouse was then performing well enough that we jumped the highest stimulus spatial frequency to 1.30 cycles/°. After achieving three days of high performance, we jumped the highest spatial frequency

to 1.54 cycles/°. The mouse performed significantly above chance for three days in a row (binomial test, $p<0.05$) at 1.54 cycles/° **(Fig. 6)**.

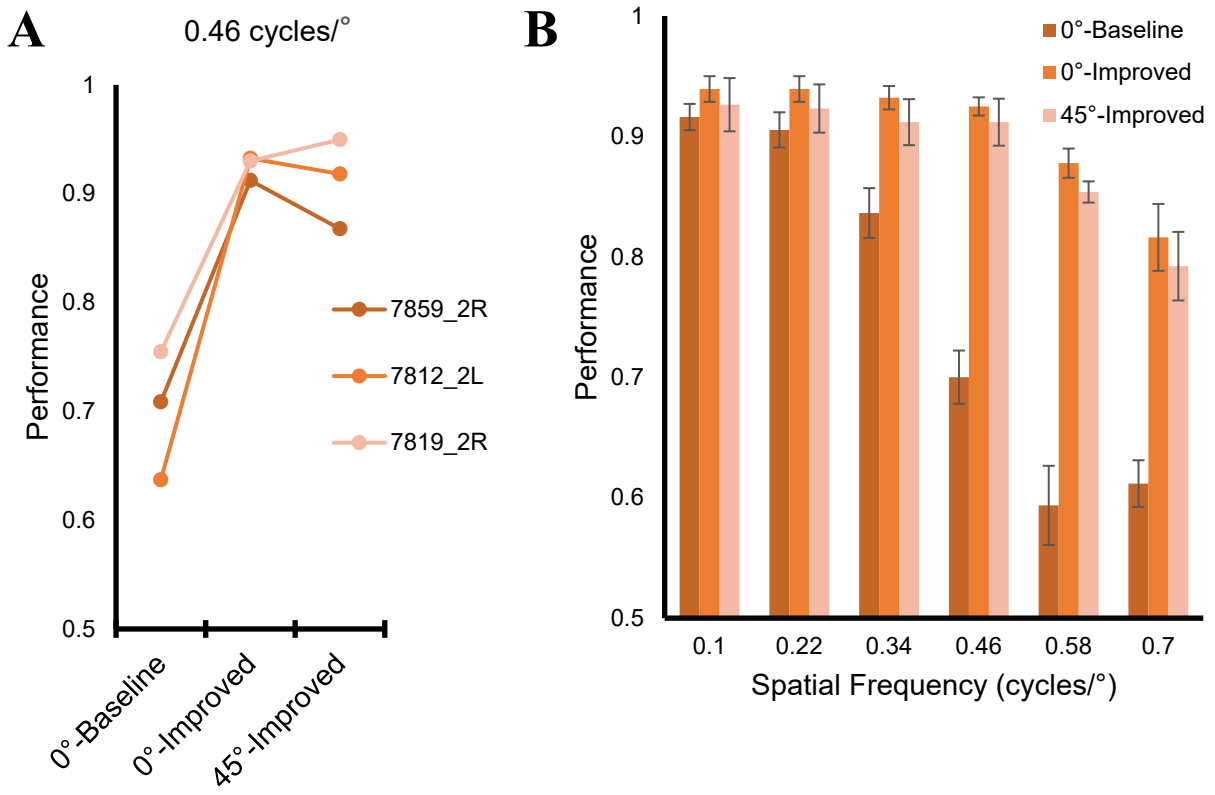

Figure 6. Qualitative plots of PV-ErbB4 KOs demonstrating high performances at 45° oriented stimuli. (A) Plot of the three PV-ErbB4 KO mice that improved their visual acuity at 0.46 cycles/°. The mice initially perform poorly (0°-baseline) but then improve their performance (0°-improved) and maintain a high performance when the stimulus is switched to 45° (45°-improved). Each point is an average of two days of testing. Either the first two days of acuity testing at 0° (baseline), the last two days of testing at 0°, followed by the first two days of testing at 45° after the improvement training at 0° concluded. (B) Summary plot of the three PV-ErbB4 KO mice average performances across all shown spatial frequencies. Each bar is an average of six performance measurements, two from each mouse of the first and second days of testing. Error bars are standard error of mean.

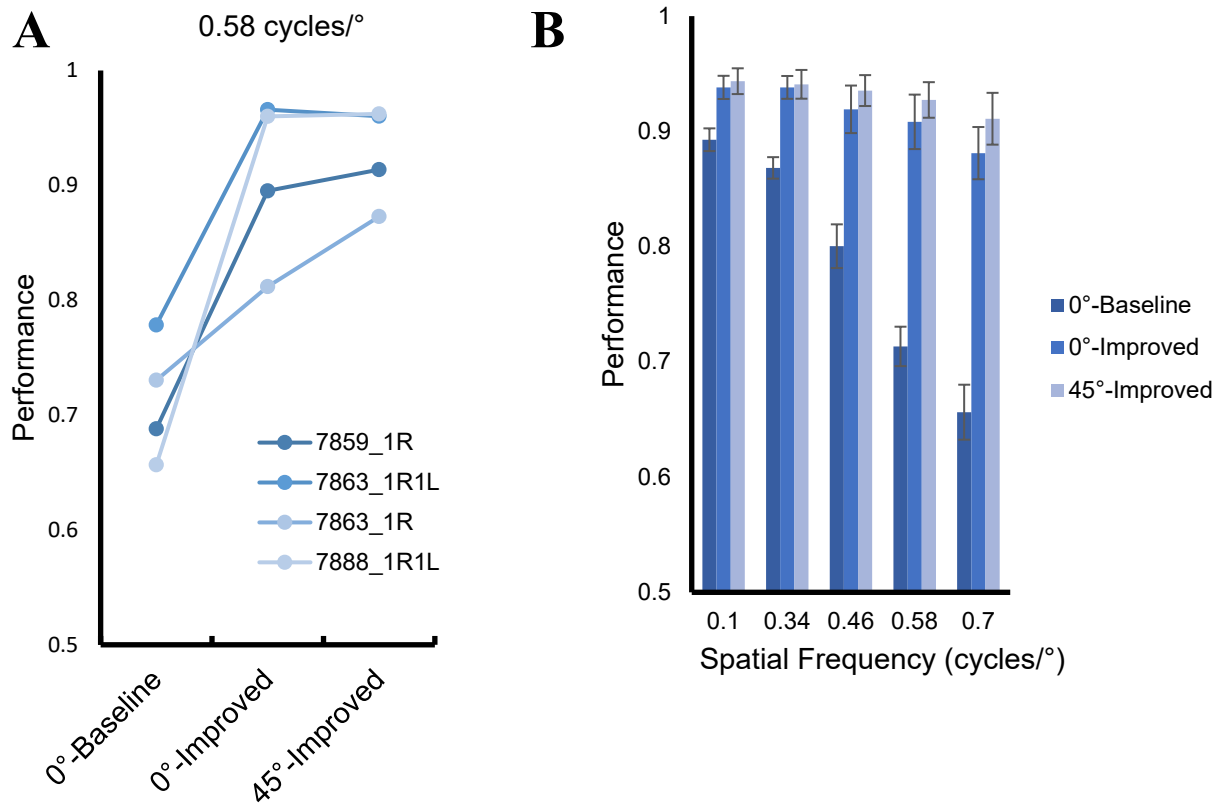

Figure 7. Qualitative plots of WTs demonstrating high performances at 45° oriented stimuli. (A) Plot of the three WT mice that improved their visual acuity at 0.58 cycles/°. The mice initially perform poorly (0°-baseline) but then improve their performance (0°-improved) and maintain a high performance when the stimulus is switched to 45° (45°-improved). Each point is an average of two days of testing. Either the first two days of acuity testing at 0° (baseline), the last two days of testing at 0°, followed by the first two days of testing at 45° after the improvement training at 0° concluded. (B) Summary plot of the three WT's average performances across spatial frequencies excluding 0.22 cycles/ °. This was excluded because three of the four WTs were shown a stimulus set extended to 0.82, and excluding 0.22, cycles/° during the extent of their training. Each bar is an average of six performance measurements, two from each mouse of the first and second days of testing. Error bars are standard error of mean.

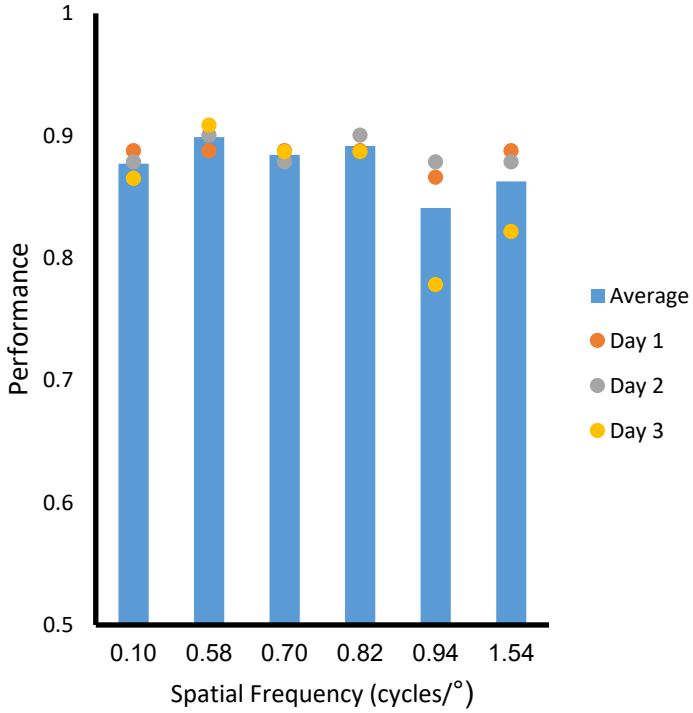

Figure 8. One WT mouse demonstrated high performance at 1.54 cycles/°. Following multiple sessions of testing the mouse's performance at progressively higher spatial frequencies, this mouse performed very well across three testing sessions at 1.54 cycles/°. On days one, two, and three the number of hits the mouse achieved at all 6 spatial frequencies was significantly above chance (binomial test, $p<0.05$). Bars are an average of the performance values from days one through three. Mouse ID: 7859_1R.

4.0 DISCUSSION

This study provides a systematic characterization of the direct perturbation of parvalbumin-expressing inhibitory neurons on the development of visual acuity assessed in a novel go/no-go behavioral paradigm. Using a transgenic mouse line in which ErbB4 receptor tyrosine kinase was selectively knocked out in parvalbumin-expressing neurons we chronically perturbed a key-component of the development of PV-expressing inhibitory neurons. Normally, expression of ErbB4 is necessary for the formation of stable excitatory input onto PV neurons (Sun, Ikrar, Davis, Holmes, et al., 2016). By knocking ErbB4 out, excitatory synaptic input on PV neurons is dramatically reduced, which has been demonstrated *in vitro*. (Sun, Ikrar, Davis, Holmes, et al., 2016).

We specifically assessed the visual acuity of 7 WT and 7 PV-ErbB4 KO mice in a novel behavioral paradigm. This study revealed that adult PV-ErbB4 KO mice have a developmental deficit in visual acuity. It also revealed that adult PV-ErbB4 KOs can be rescued and even improve their visual acuity following extended testing in our novel behavioral paradigm despite an initial deficit in acuity. It revealed that when mice (both WTs and KOs) are tested on one particular orientation, they are able to generalize their performance at high spatial frequencies to stimuli at orientations they were not tested on (45°). Lastly, we have preliminary evidence that for at least

one WT mouse, they can perceive high spatial frequency stimuli extending up to 1.54 cycles/° - higher than what a rat can accurately discriminate at 1.36 cycles/° (Hosang et al., 2018).

These findings reveal that direct perturbation of parvalbumin-expressing inhibitory neurons results in a developmental deficit of visual acuity than can be subsequently rescued with behavioral training. Specifically, we found that (1) PV-ErbB4 KOs have a deficit in visual acuity, (2) that PV-ErbB4 KOs are able to improve their visual acuity in a rewarded behavioral task, and (3) that PV-ErbB4 KOs are able to generalize their high-performance to stimuli trained at 0° oriented stimuli to 45° oriented stimuli. These findings lead to questions regarding the neural mechanisms by which the development of visual acuity is regulated, and also augmented in behaviorally relevant situations.

4.1 PV-ERBB4 KO MICE DISPLAY A DEVELOPMENTAL DEFICIT IN VISUAL ACUITY

Indirect evidence demonstrated that overexpression of BDNF accelerates the development of parvalbumin-expressing inhibitory neurons and also accelerates the maturation of adult-level visual acuity in mice (Huang et al., 1999). Along with that, PV neurons are known to mediate the timing of the critical period for ocular dominance plasticity (Kuhlman et al., 2013). Based on these data, we hypothesized that perturbing PV neurons through the knockout of ErbB4 would result in a deficit in visual acuity. We used a novel go/no-go behavioral paradigm on head-fixed, adult mice to assess their visual acuity **(Fig. 1A)**. In agreement with our hypothesis, the PV-ErbB4 KO mice displayed a developmental deficit in visual acuity compared to their WT counterparts **(Fig. 1B)**.

Numerous studies estimate the behavioral visual acuity of mice at the point in which a stimulus' spatial frequency elicits a correct response 70% of the time (Prusky & Douglas, 2003; Stephany et al., 2014). The behavioral tasks in the cited studies, and our own go/no-go behavioral task, were designed differently in that the cited tasks assess acuity using a water-maze discrimination task. This requires that mice swim through a pool and must choose between an isoluminant gray screen, and a screen with sinusoidal gratings. Choosing the grating screens is a "correct" response, and will reward the mouse with removal from the water. However, we estimated the 70% acuity threshold using the slopes obtained from a linear regression of the performance data **(Fig 1B)**. This method estimated the WT 70% visual acuity threshold at 0.468 cycles/°. This is similar to findings in the water task, in which adult mice have an acuity of ~0.5 cycles/° (Prusky & Douglas, 2003). The average acuity for the PV-ErbB4 KOs was 0.396 cycles/°. The difference in estimated visual acuity between the WTs and PV-ErbB4 KOs was 0.066 cycles/°.

This difference of 0.066 cycles/° is relevant both developmentally, as well as in relation to amblyopia. The difference in acuity between a p35 mouse, considered 'juvenile' because its visual acuity is still developing, and a p40-45 mouse, considered 'mature' because their acuity has finished developing, is estimated to be ~0.06 cycles/° (Stephany et al., 2014). This suggests that the chronic perturbation of PV inhibitory neurons through knockout of ErbB4 receptor tyrosine kinase impacts the development of visual acuity. Additionally, our estimated difference approaches the difference found between normal mice and mice with amblyopia, a condition in which visual acuity is degraded in an eye. The difference between normal and amblyopic mice was found to be 0.09 cycles/° (Stephany et al., 2014). Again, this suggests that the deficit in acuity found by disrupting PV neurons approaches a medical condition in which visual perception is permanently degraded.

These data therefore suggest that our mice have a deficit in visual acuity that is both relevant developmentally, and related to visual degradation observed from amblyopia. The circuit-basis for the role of PV neurons in the development and maintenance of visual acuity remains unknown. Going forward, we aim to elucidate how the neural response properties change in the visual cortex of PV-ErbB4 KO mice. Perhaps the loss of ErbB4 function leads to lower evoked firing rates, and subsequently raises the level of noise in the primary visual cortex, leading to reduced perception. It is also known that area PM of V2 (higher visual cortex) receives input of from neurons in V1 that respond to high spatial frequencies (Glickfeld et al., 2013). Perhaps aberrant development of PV inhibitory neurons results in a failure for these neurons to functionally connect to area PM, leading to reduced visual perception at high spatial frequencies.

4.2 PV-ERBB4 KO DEFICIT CAN BE RESCUED WITH A BEHAVIORAL TASK & IMPROVED

Despite an initial deficit in visual acuity, both WT **(Fig. 5)** and PV-ErbB4 KOs **(Fig. 6)** were able to improve their performance to high spatial frequency stimuli following numerous behavioral training sessions. This finding is supported, at least for WTs, by another study which found that in normal mice, their visual acuity can be improved with training in a behavioral water task (Hosang et al., 2018). These data are in agreement with our hypothesis that both the WT and PV-ErbB4 KOs would demonstrate improvement, and that the initial deficit in visual acuity could be rescued with training.

We hypothesized this for several reasons. First and foremost, it is likely that standard cage rearing of mice presents an artificial environment that is visually impoverished. That is, the mice

do not experience visual stimuli that prove to be useful for their continued survival, and thus, their visual acuity fails to develop to its highest capacity. Furthermore, when mice are reared in enriched cages (a larger cage, more social interactions, and tools for playing and exercise), mice develop a higher acuity. Indeed, mice reared in enriched cages, when tested in the behavioral water task, had a visual acuity 18% higher than their standard-reared counterparts (enriched: 0.56 cycles/°, standard: 0.46 cycles/°). Therefore, it is likely that in standard lab conditions, the acuity of mice are maturing and plateauing, but that their acuity can improve somewhat plasticly in a behaviorally relevant context. In the case of Hosang (2018), the mice were able to get out of the water by discriminating high spatial frequency stimuli. In our task, the water-restricted mice were rewarded with water by discriminating high spatial frequency stimuli. In both cases, reward likely played an important role in facilitating the improvement of visual acuity.

In the future, we aim to elucidate the mechanism by which the mice are improving their visual acuity. It is well established that another circuit involving VIP and SST inhibitory neurons influence response properties in visual cortex (Batista-Brito et al., 2017). When mice are in motion, the gain of response of excitatory neurons in V1 is significantly higher than stationary, and loss of ErbB4 in VIP neurons impairs this gain of response from occurring. It may be possible that the development of visual acuity to some standard, mature level is dependent on PV inhibitory neurons, but that improvement of visual acuity in behaviorally relevant and demanding situations for both WT and PV-ErbB4 KOs involves this VIP-SST inhibitory circuit.

4.3 BOTH WT AND PV-ERBB4 KO MICE CAN GENERALIZE THEIR IMPROVEMENTS TO DIFFERENT ORIENTATIONS

Recently, data suggest that the processing of orientation information is independent of spatial frequency (Jeon et al., 2018) and therefore hypothesized that if the mice improved at one orientation, then they would also generalize their improvement to different orientations. The mice were initially tested, and trained for improvement, on static grating visual stimuli that were vertically (0°) oriented. Interestingly, in addition to performing at high spatial frequencies following training, the mice are also able to generalize their high spatial performance to stimuli at an orientation that they were not trained on initially **(Figs 6 & 7)**. This therefore is in agreement with our hypothesis that high spatial frequency performance at 0° would be generalized to 45°.

4.4 CONCLUSION & CAVEATS

Lastly, we demonstrated that one WT mouse was able to perform highly accurately at 1.54 cycles/° **(Fig. 8)**. We have reason to believe that this is the highest visual performance that a mouse has been able to accurately perceive. In 2000, the visual acuity of a mouse was estimated to be 0.51 cycles/° and a rat to be 0.92 cycles/° (Prusky, West, & Douglas, 2000). An individual mouse has been reported to have improved its visual acuity to 1.49 cycles/°, much higher than that of a rat's acuity, and likely the highest-ever reported visual acuity observed in mice (Hosang et al., 2018).

The largest potential caveat to the interpretation of the results of this study is that we are knocking out ErbB4 receptor tyrosine kinase in all parvalbumin-expressing inhibitory neurons. These neurons are found throughout the brain, and as such it is possible that the location of the

deficit is not found within the visual cortex, but may arise prior to the visual information arriving at the cortex (namely in the lateral geniculate nucleus) or in higher-order visual areas such as the superior colliculus. We have identified six possible brain areas that may influence behavior and cortical processing of spatial information. Most broadly, the cortex, along with the reticular nucleus of the thalamus, lateral geniculate nucleus of the thalamus, lateral posterior nucleus of the thalamus, intergeniculate leaflet of the thalamus, and the superior colliculus. Using *in-situ* hybridization data from the Allen Brain Institute, we identified that co-expression of parvalbuminin and ErbB4 occurs most strongly in the cortex, and in the reticular nucleus of the thalamus, as well as the superior colliculus. Preliminary studies demonstrate that both the SC and primary visual cortex project to area LP, suggesting a convergence there (Bennett et al., 2018). It is not thought that SC processes spatial information, however, and we will therefore restrict an important control set of experiments to the reticular nucleus of the thalamus as well as the cortex **(Fig. 9)**.

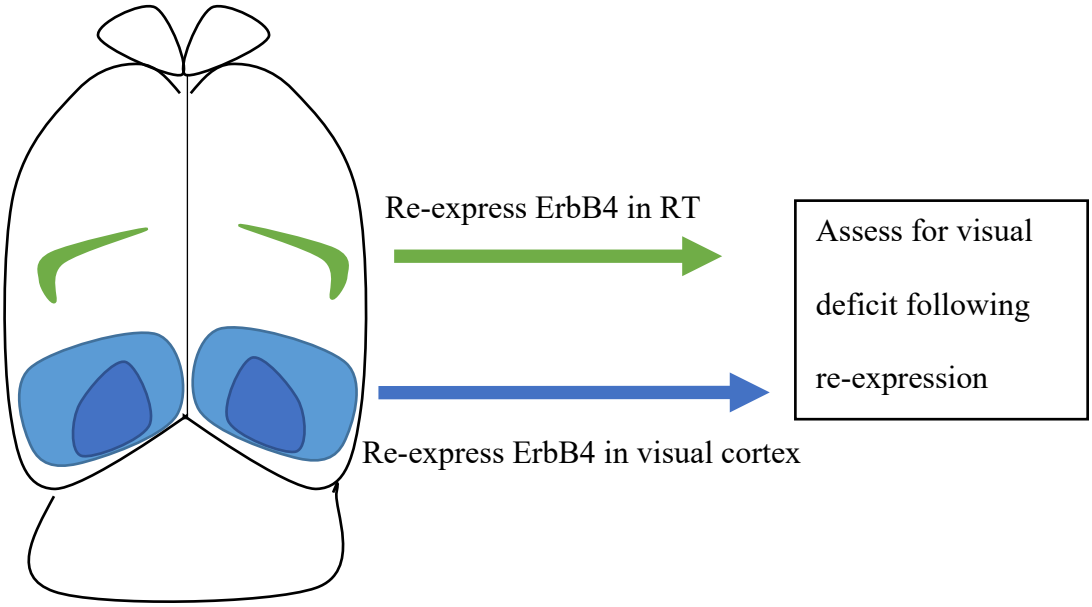

Figure 9. ErbB4 re-expression experimental design as a key control for future experiments. Using an AAV5-CAG-FLEX-GFP-T2A-ErbB4rc virus, we will inject ErbB4 back into either just the visual cortex, or the reticulate thalamic nucleus and re-assess a cohort of mice to see if ErbB4 expression in a localized area restores visual acuity function.

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