

1. Project description — Redefining the visual critical period: natural behavior and neural dynamics

1.1 General Scientific Challenge and Project Overview

At the heart of brain development lies a phenomenon that is both foundational and transformative: the critical period. Critical periods are temporal windows characterized by sensitivity in the nervous system to changes, be it chemical, electrical, or environmental^{1,2}. During embryonic development, these windows describe periods during which genetic programming guides the initial anatomical connectivity and intrinsic neuronal activity primes circuits and synaptic connections for the arrival of sensory information after birth³. However, the environment and physical properties of an individual, into which the brain is born, cannot be genetically encoded². Thus, the arrival of sensory input marks the beginning of a unique critical period, wherein neural circuit maturation is highly malleable and depends strongly on an individual's experience. Deviation or disruption in a normal sensory experience during this time can pose serious consequences, such as, imprecise anatomical connectivity, aberrant wiring of brain circuits, or long-lasting neurodevelopmental disorders⁴. Despite the importance of experience-dependent processes during critical periods, our understanding of this process is severely limited.

According to the classical model, the postnatal critical period of neural development is a feed-forward process: experience shapes neural activity, thereby influencing the structure, gene expression, and function of brain circuits⁵ (**Fig. 1, classic model**). *Missing from this model is the role of a dynamic, evolving experience.* Animals are not a passive sponge during the critical period, but rather, engage with the world through dynamically changing experiences as their natural behaviors emerge. Sensory experience and perception are shaped by active behaviors, which are constrained by peripheral sampling and the computational capacity of neural circuits to process and propagate information. *We propose that the postnatal experience-dependent critical period is a bidirectional or recurrent process, involving feed-forward and feed-back processes (Fig. 1, proposed model).* Sensory experience and behavior drive patterns of neuronal activity, which shape neuron structure and functional properties, which in turn reshape sensitivity to sensory inputs and inform behavioral experience and performance⁶. To study the implications of this new conceptual model, *we plan to construct a novel experimental platform in a highly evolved mammalian species to study the development of natural behaviors and neural network organization and functional activity, utilizing modern technological methods and computational tools.*

Current understanding of the critical period derives primarily from decades of studying the visual system. In essence, there are two milestones used to describe this window. First, there is eye opening, a fundamental *binary* state transition initiating the refinement and maturation of structural (e.g. dendritic morphology and synaptic connections) and functional (e.g. spatial receptive field) properties in visual neurons. Second, there is the window of ocular dominance (OD) plasticity, first discovered by Hubel and Wiesel⁷ in carnivores and primates and measured by artificial sensory deprivation. Even today, OD plasticity via sensory deprivation still serves as the primary method for defining and studying the visual critical period, even though deprivation produces an abnormal experience and underlies amblyopia. Critically missing from this picture are the natural milestones of visual development following eye opening and visually-guided behaviors that concurrently emerge with maturing visual circuits. To address this challenge, *we propose a first-principles approach using the ferret visual system to rigorously characterize the developmental trajectory of natural visually-guided behaviors and map that trajectory to concurrent maturation of synapses and cells in the primary visual cortex (V1).*

The proposed studies will characterize development of visually-guided, ethologically-relevant behaviors in the ferret (**Fig. 2**). *Ferrets are the ideal model system to address this challenge.* Not only are they born immature^{8,9}, allowing for experimental investigation weeks before eye opening, but possess advantages over both rodents and primates. Unlike rodents, their visual system has a columnar organization and is far more similar to primates^{10,11}. Unlike primates, ferrets can be used in a high-throughput manner and are amenable to the application of state-of-the-art neurotechnology¹².

In the first phase (Project 1), we will harness modern technological methods and computational tools to track the natural development of visually-guided behaviors, kinematics, and eye-movements in ferrets. Upon

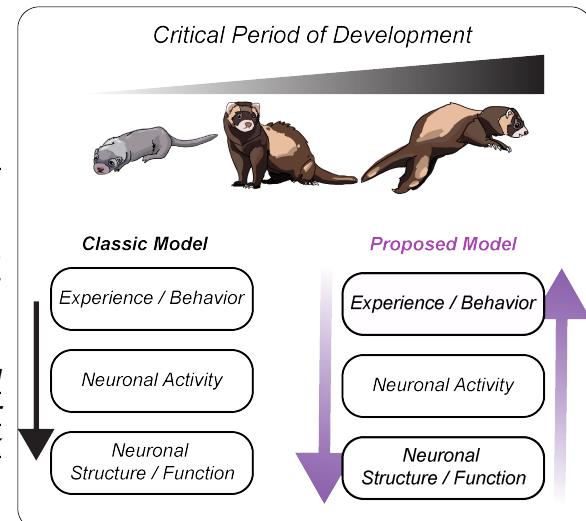


Figure 1: Schematic of conceptual framework

this foundation, we will perform manipulation experiments to test how visually-guided behaviors during the critical period depend on experience and properties of the visual system. In the second phase (Project 2), we will incorporate *in vivo* two-photon (2P) microscopy to map the structural and functional dynamics of neural networks at synaptic and cellular scale. Initially, we will follow established methods, but through a collaborative effort we will employ novel head-mounted miniature 2P microscopes to visualize neural circuits *simultaneously* with the tracking of behavior and eye-movements throughout development. This proposal will produce a novel platform for studying the intersection between dynamic experience, circuit maturation, and the development of natural ethologically-relevant behaviors.

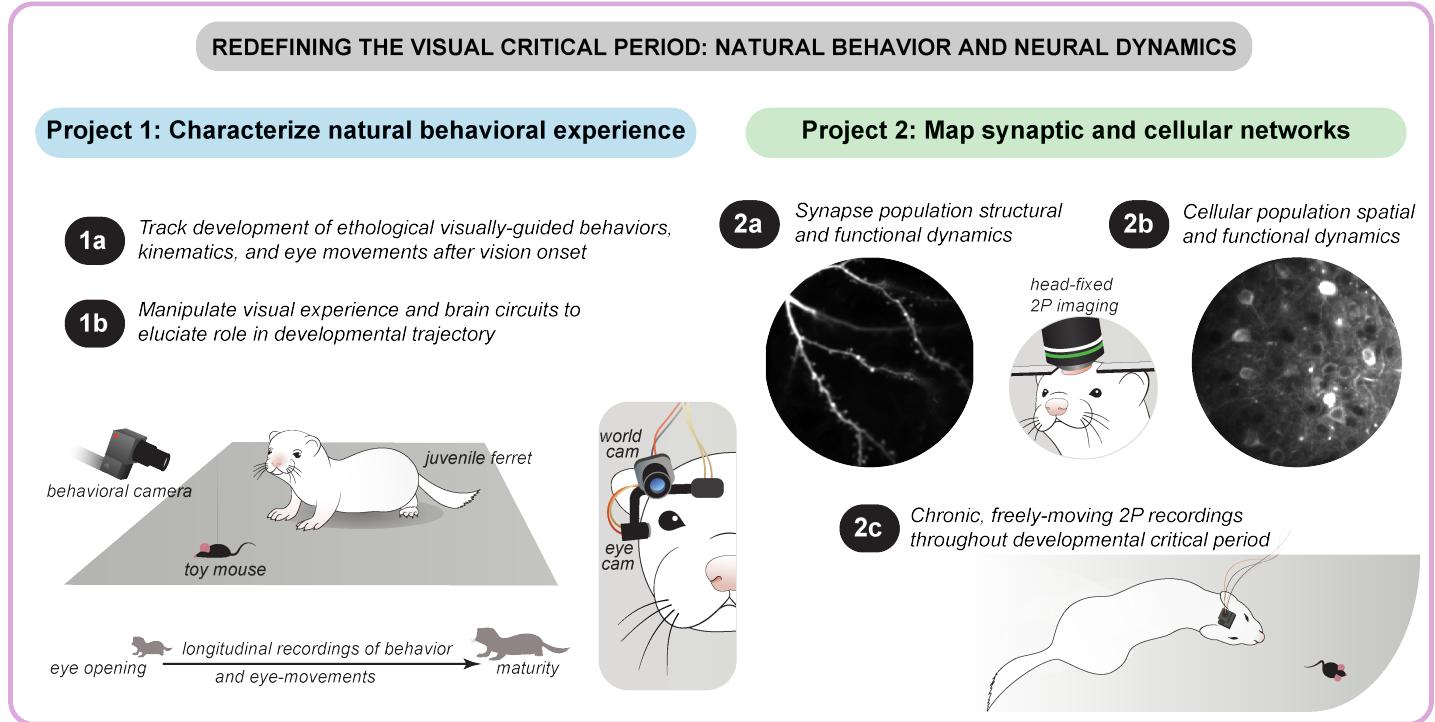


Figure 2: Overview of proposed projects.

1.2 Public Health Relevance

Visual experience during postnatal development is critical for perceptual, cognitive, and social development. Developmental disorders, such as strabismus amblyopia (affecting 2-5% of the human population¹³), remain a significant health problem and few therapeutic interventions have successfully translated from rodent research to clinical promise¹⁴. Other developmental disorders presenting visual deficits, such as autism spectrum disorder and schizophrenia, occur from an otherwise normal environment, however circuit disruption during experience-dependent plasticity may be an underlying cause²⁵. Understanding the normal neurodevelopmental processes during the critical period can aid in the identification of potential targets for therapeutic intervention. By understanding the bidirectional nature of behavioral experience and neural dynamics during development, we can explore potential therapies in a novel framework and identify when they are most impactful. In addition, the ferret model system may offer greater insights to human visual development as compared to rodents; ferrets have forward-facing eyes and possess an early visual system architecture like primates. A deeper understanding of the visual critical period may also open new avenues to adult plasticity, inspiring new strategies for therapy and lifelong learning.

1.3 Project Objective

The goal of this proposal is to redefine the critical period of visual development by the temporal emergence of natural behaviors in ferrets and map concurrent neural dynamics at synaptic and cellular scale. The proposed experiments will shed light on the bidirectional nature of experience-dependent development. We will answer the following major questions:

- How do visually-guided behaviors evolve after eye opening?
- How does the emergence and execution of visually-guided behaviors depend on experience and concomitant changes in visual circuits?
- Do synapse populations and cellular circuits mature in lockstep with behaviors, or do they precede or follow behavioral changes?
- How do visually-guided behaviors and visual experience *directly* drive neural dynamics?

1.4 Approach

As per the Funding Opportunity Announcement (RFA-RM-23-005), a detailed experimental plan and extensive preliminary data are not provided. This section describes our general experimental approaches and some preliminary, unpublished data on which these projects are based.

1.4a Project 1: Characterize natural behavioral experience during development

Scientific challenge and opportunity: The textbook definition of the critical period of visual development is based on the time of eye opening and window of OD plasticity. Neither of these milestones considers the animal's ethological behaviors or natural sensory experience. In addition, neither of these milestones takes into account the recurrent process of development whereby a changing, dynamic sensory experience instructs neural network dynamics, which in turn, constrain perceptual experience and behavior. To address this challenge, we will harness modern techniques and computational tools to track the natural development of visually-guided behaviors, kinematics, and eye-movements in ferrets. This project, already beginning, will serve as a foundation for understanding how the perception of the world changes over time, as well as emergence of ethologically-relevant visual behaviors (e.g. navigation, head/eye orienting, and tracking simulated prey). We will then engage in manipulation experiments to test how the visually-guided behaviors during the critical period depend on experience and specific properties of neural circuits. These manipulations will help redefine the critical period window based on behavioral readouts.

Project 1 details: To describe how visually-guided behaviors evolve after eye opening in the ferret, we will return to a first-principles approach. We will construct an arena (~3x3 m), and provide a number of objects (e.g. balls and toys) for ferrets to interact with. High-resolution cameras to track behavior will be fitted with lenses (Nikon) to optimize pixel resolution over the arena area. This includes a depth camera (Basler Blaze) to capture behaviors in 3D. The walls of the arena will be transparent and computer monitors will be attached to the outside in order to display a range of stimuli (e.g. natural images, gratings, filtered noise)¹⁵. We will engage the visual system using a toy mouse with programmatically controlled movements and speed.

Ferrets are raised in our animal facility. Ferret kits are born and can be acclimated in the behavioral arena multiple days *before* the eyes open. Kits undergo survival surgery for implantation of headposts and/or recording chambers prior to eye opening (see below and Project 2). After acclimation, starting day 1 of eye opening, kits will be placed in the behavioral arena for 10-20 min. We will record their kinematics (i.e. body, head, tail movements) as they explore and navigate around objects placed in the arena. We will also test their ability to visually-track a toy mouse, varying the spatial position, trajectory, and speed. Ferret kits will be recorded in the behavioral arena daily or on alternating days for up to 4 weeks after eye opening, during the classic critical period window defined by ocular dominance plasticity.

DeepLabCut¹⁶ will be used to track the position of different components of each animal (i.e. body, head, tail), training models for each animal and the simulated mouse. Models will be trained across randomly sampled movie frames (total ~1000 per model) to generalize over different behaviors (i.e. volitional exploration vs. directed tracking). Models will be trained on multiple batches of data and several validation steps will be used to quantify model fits. **Pilot data from our lab of DeepLabCut- based tracking over development is shown in Figure 3.** Animal trajectories will be processed and analyzed (Python/Matlab). Simple computational analyses include measuring changes in an animal's speed, acceleration, motor coordination across the head/body, and ability of an animal to track visual objects. In addition, we will deploy novel ethologically inspired unbiased strategies to identify behavioral motifs over development. Such strategies include Time-REsolved Behavioral Embedding (TREBLE)¹⁷ and Motion Sequencing (Moseq)¹⁸, which will uncover continuous behavioral dynamics, variation across individuals, and reveal structure in pose estimation data.

Critical to characterizing visual experience is measuring conjunctive eye-movements. To accompany behavioral recordings, we will mount eye/head tracking hardware to capture, eye-movements, the world view of the animal, and the inertial forces of the animal's head (Rosco Tech) (**Fig. 2**). We will build a custom design for this device following published methods^{15,19,20} and guidance from our collaborator, Dr. Philip Parker (Rutgers). This device will be attached to the animal's head via an implanted titanium D-post and the device's hardware (cameras, IMU sensor) will be attached to a 3D printed holder that slots onto the D-post. Connected wires will be tethered above the arena and through a motorized commutator (AlphaOmega) so the animal can move freely. Eye-movement data will be processed and gaze-corrected using the world view camera. Processing and analysis of eye- and head movements will be conducted with guidance from Drs. Jacob Yates (Berkely) and Philip Parker^{15,21}.

After establishing how visually-guided behaviors and eye-movements develop post eye opening, we will conduct manipulation experiments. These experiments will involve chronic and acute manipulations. Chronic manipulations will test how developmental trajectories depend on visual experience. Acute manipulations will

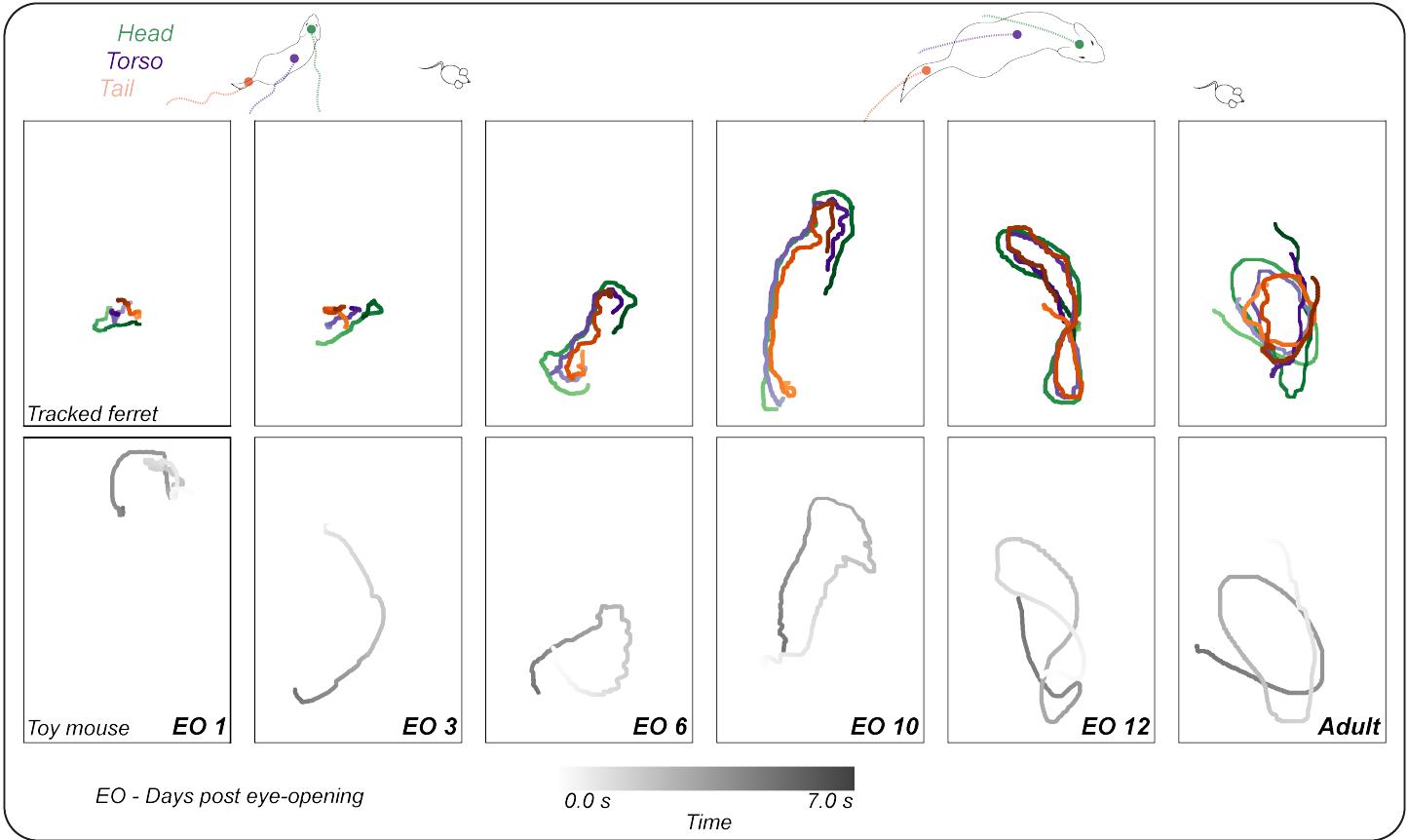


Figure 3: Visually-guided behaviors develop quickly after eye opening. Shown are tracked points on an individual ferret recorded in a behavioral arena for ~2 weeks after EO. Each plot shows movement in time over 7 seconds. In each epoch, a toy mouse is presented and moved for tracking. Immediately after EO, animals do not appear to move or follow visual cues. Two weeks later, animals demonstrate accurate visually-guided tracking and chasing.

allow us to identify which specific components of the visual system are being used during behaviors (i.e. cone- vs. rod-based)²². Chronic manipulations will be conducted by rearing animals in artificial environments. Chronic manipulations may also be initiated at difference timepoints before eye opening and during the classically-defined window of OD plasticity. Acute manipulations will be conducted by changing the illumination and visual statistics of the arena. Table 1 shows proposed manipulations, duration, and specific outcomes expected.

TABLE 1

Manipulation	Duration	Expected Outcome
Dark rearing	Chronic	Visually-guided behavioral trajectory strongly delayed until vision onset
Precocious eye opening	Chronic	Behavioral maturation begins earlier
Monocular deprivation	Chronic	Complex behavioral repertoires require proper binocular vision
Spatial acuity: low (M-pathway) vs. high (P-pathway)	Acute Chronic	High spatial acuity-based behaviors develop at slower rate than low spatial acuity Natural spatial frequencies (1/f) are required for emergence of natural behaviors
Luminance levels: scotopic (M-pathway) vs. photopic (P-pathway)	Acute Chronic	Rod-photoreceptor based behaviors develop at slower rate than cone based Eye-movement statistics and behavior emergence depend on visual pathway driven
Color wavelength	Acute	L-cone (558nm), S-cone (430nm), and rod (505nm) based behaviors develop at different rates
Reverse motion training	Chronic	Signals from locomotion-driven optic flow shape emergence of visually-guided behaviors

Project 1 expected results: Pilot data collected in our lab shows innate and directed behaviors are quickly emerge in ferrets after eye opening (Figs. 3 & 4). Around 4 days post eye opening, animal locomotion speed and acceleration are increase. Surprisingly, animals reach adult-levels after ~2 weeks of vision. As expected, we observed coordination in motor movements: the angles of head and body velocity vectors became more similar as animal's make coordinated head-body movements. We observed enhanced

visual tracking of the simulated prey mouse (**Fig. 3**), evident by animals beginning to track and chase the toy after ~1 week and increased complexity in trajectories. Quantitative analysis (**Fig. 4, bottom right**) demonstrated animals progressively getting better at tracking. Overall, we expect (and observed) motor and visual behaviors to emerge progressively. While we have not recorded eye-movements, we expect eye-movement statistics and head movements to progressively change after eye opening as brain circuits mature (like humans and primates)⁶. Specifically, we hypothesize that saccade amplitude will increase, saccade frequency will increase as acuity is enhanced, saccade latency will decrease, any head-eye fixation will become more stabilized, and the oculomotor system will be refined. As we propose to conduct many manipulations, the type and expected outcome are outlined in Table 1. We expect each of these to produce exciting and novel behavioral readouts. For example, monocular deprivation has never been studied dynamically or behaviorally, but is expected to produce profound hinderances in complex visual behaviors.

Project 1 limitations and alternative approaches: High-risk, explorative experiments accompany a number of unforeseen variables. (1) We cannot predict the temporal resolution (hourly, daily, weekly) required to capture the emergence of visually-guided behaviors. We may need to collect data for several weeks after eye opening or make multiple measurements daily around particularly sensitive period. (2) It is not clear how much developmental variation there will be between animals, but will be able to use computational tools^{17,18} to quantify this variation, novel and interesting in itself. (3) Eye-movement statistics may be difficult to measure and the exact designs described previously^{15,19} may not work for ferrets. Thus, we plan to construct several different designs and create a ‘dummy’ apparatus to acclimate animals to the device. Notably, as ferret kits are 10x larger than a mouse (~200-400g) and have particularly strong neck muscles, we believe there will be greater flexibility in design choice. (4) Simulated prey (i.e. toy mouse) shows promise but may not drive sufficient naturalistic behaviors or be able to be moved in a reliable, naturalistic manner. If this is the case, we can use a real prey (i.e. crickets)²³ and look for inspiration from wild ferret species. (5) A redefinition of the visual critical period in terms of behaviorally-driven experience may not be a straightforward endeavor. Likely, this is a complicated process involving multiple developmental windows and recurrent interactions. While this poses a gap between our proposed conceptual framework and project, we hope these initial experiments will provide a foundation and can bring collaborators to help apply effective computational analysis to unravel the dynamics. (6) Another conceptual limitation is a lack of consideration of underlying molecular changes which are proposed to gate OD plasticity in rodents^{24,25}. It is possible that visually-guided behaviors mature following the release of ‘molecular breaks’ or inhibitory interneuron maturation. While some proposed manipulation experiments may test this possibility (Table 1), more direct tests will require optogenetic or chemogenetic manipulation experiments.

Project 1 future directions: After completing initial experiments, there are many directions we are considering to enhance behavioral readouts and manipulation of visual experience. We plan to incorporate time-of-flight cameras to capture behaviors in 3D (Basler Blaze; **Fig. 5**) and bolster data for extracting motifs. We would like to incorporate an overhead projector illumination system to create visual stimuli or illusory objects/prey in real-time. Once we collect robust visual-tracking data, we plan to extract quantitative temporal, behavior kernels as used for human psychophysics²⁶. Finally, as

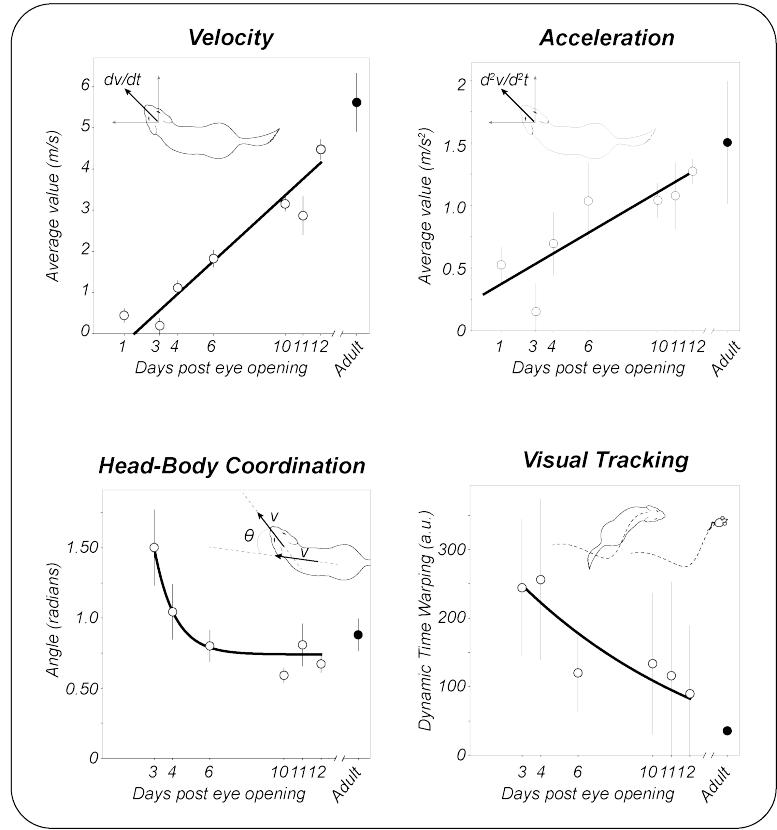


Figure 4: Ferret locomotion, motor coordination, and visually-guided behaviors mature quickly after eye opening.

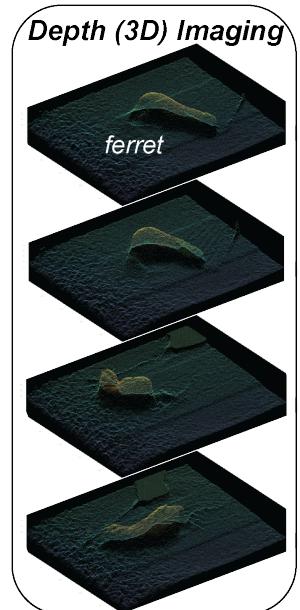


Figure 5: Ferret movements in 3D

mentioned above, there are several possible manipulations to conduct. We would like to test the role of ON/OFF pathways with APB retinal injections to suppress the ON pathway, the functional role of the lateral geniculate nucleus and primary visual cortex with acute optogenetic or chemogenetic inactivation, and eventually incorporate the ability to disrupt the expression of signaling molecules or postsynaptic receptors important for neural plasticity (e.g. CaMKII and NMDA receptors).

1.4b Project 2: Map concurrent maturation of synaptic and cellular networks

Scientific challenge and opportunity: To understand the bidirectional, recurrent process during the critical period of development it is important to assess the dynamic visual experience and underlying maturing neural circuits. While decades of research have characterized how visual cortical circuits mature after eye opening²⁷, few have related those circuits to natural visual experience^{15,21} or have attempted to characterize how each develops concurrently²⁸. Thus, in this second phase, we will incorporate *in vivo* two-photon (2P) microscopy to map the structural and functional dynamics of neural networks at synaptic and cellular scale. Genetically-encoded activity-indicators will be imaged through a chronically-implanted window *in the same animals* undergoing longitudinal tracking of visual behavior development (building on Project 1). Initially, this project will rely on established methods for imaging (i.e. head-fixed), but through a collaborative effort, we plan to employ head-mounted miniature 2P microscopes for capturing neural circuit activity simultaneously during active visual behaviors in developing animals.

Project 2 details: To record neural dynamics at synaptic and cellular scale we will used *in vivo* 2P imaging of the newest GCaMP variants (8s & 8f)³⁴ expressed with adeno-associated viruses (AAV1/2) in the primary visual cortex—routine procedure in our lab. Ferrets born on site will be injected with AAVs around 2 weeks of age in a sterile surgical suite. To record from synapses, we will sparsely express GCaMP8 in excitatory neurons by injecting a diluted virus (1:50,000) expressing Cre recombinase under the hSyn promoter and injecting a second, Cre-dependent virus expressing GCaMP8s under a CAG promoter^{10,29}. In this way, we will visualize calcium signals in dendritic spines^{10,29}, the exclusive site of excitatory synaptic inputs on layer 2/3 pyramidal neurons in ferret visual cortex. To record from excitatory neuron populations, we will inject a single virus to express hSyn-GCaMP8f. Following AAV injection and a few days before eye opening (Postnatal day 28-30), we will implant both a cranial window over the injection site and the mounting apparatus for recording eye/head movements (**Fig. 2**). The window will be sealed and protected with a custom 3D printed protective cap.

In the first phase of project 2 we aim to characterize the maturation of (1) dendritic spine structure and function²⁹ and (2) the organization and functional response properties of excitatory neuron populations¹². In this first phase we will use head-fixed 2P imaging procedures, routine in the lab. We propose to record behavior and neural dynamics on alternating days during the critical period from the same animal (**Fig. 6**). Behavioral recordings will be conducted as optimized in Project 1. For 2P imaging, we place the window under a state-of-the-art 2P microscope (Bruker 2P Plus, Coherent Ultra-2 laser). On imaging days, we will identify suitable field of views for recording and present a large battery of visual stimuli (PsychoPy) with a high-resolution 120Hz monitor. Visual stimuli will include drifting gratings (varying in spatial frequency, temporal frequency, and direction of motion), noise stimuli (white noise and wavelets) for mapping spatial receptive fields, and natural movie sequences. For dendritic spine imaging, we will collect data in a 50x50 μm area ($\sim 0.1 \mu\text{m}/\text{px}$) for optimal synaptic resolution. For cellular imaging, we will collect across a 1x1 mm area ($\sim 0.5 \mu\text{m}/\text{px}$). Data collected will be stored on a 100TB server onsite and processed locally. Processing involves nonrigid registration (NoRMCorre) and state-of-the-art denoising methods using deep neural network learning and inference from the Allen Brain Institute. (DeepInterpolation)³³. Subsequent analysis will be conducted using custom software code (Python/Matlab). We will analyze the classic stimulus-dependent receptive field properties (e.g. feature selectivity, spatial frequency sensitivity), activity correlations between neighboring and

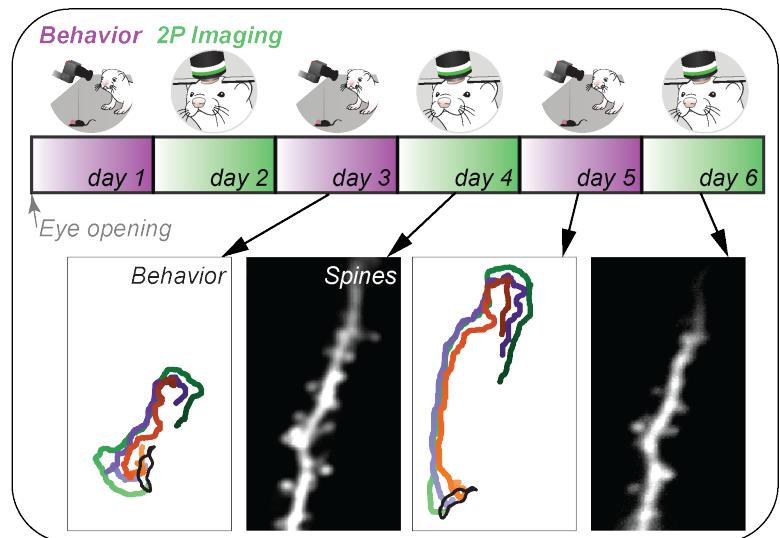


Figure 6: Experimental paradigm for recording developing behaviors and neural dynamics.

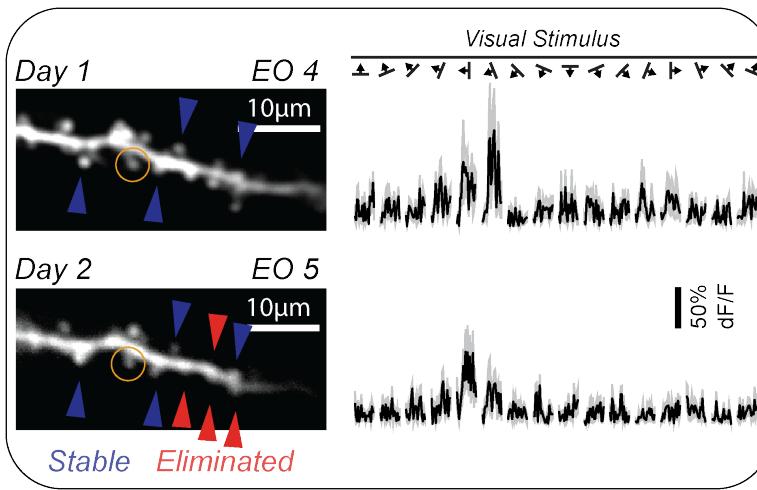


Figure 7: Synaptic pruning and functional dynamics captured with 2P longitudinal imaging.

which include not visual tuning, but also other properties such as calcium event amplitude, reliability, and capture the emergence of local dendritic organization (i.e. clustering, dendritic nonlinearities).

Phase 2 of this project presents a novel and exciting direction for our lab: simultaneous 2P imaging in freely-moving, behaving ferrets during development. In this phase, we will implant a cranial window with a custom headplate to attach a head-mounted miniature 2P microscope designed by Dr. Emily Gibson (UC Denver), a major collaborator (**Fig. 8**). Instead of imaging and recording behavior on alternate days, we will image excitatory cellular circuits in the primary visual cortex using the 2P miniscope while ferrets are exploring and interacting with toy mouse in the arena. We will illuminate the walls of the arena with visual stimuli (e.g. gratings, noise) to drive visually-evoked activity. Coupled fibers for transmitting excitation laser pulses and collecting emitted light will be tethered above the arena. Data collected will be stored, processed, and analyzed similar to head-fixed 2P imaging data. It will be critical to gaze-correct the world view camera for accurate estimates of visual stimuli driving neural activity, assisted by Dr. Jacob Yates. With these data we will be able to analyze neural activity driven by optic-flow, saccadic eye-movements, pursuit during tracking, and locomotion.

Project 2 expected results: As previously reported, we expect a number of functional response properties to change over development. Receptive fields will become smaller, coinciding with tuning to higher spatial frequencies, and the selectivity to specific visual stimuli will become more pronounced. In addition, reliability in responses will increase. With respect to behavior, we posed a simple but profound question: do changes in the visual cortical neurons precede, lag, or occur in lockstep with an animal's behavioral development? We might expect any outcome, but it is most likely that neural dynamics will occur in concert. For example, the spatial acuity of an animal will depend on neural circuit processing of high spatial frequencies and inform the specific eye-movements used to sample the world (i.e. larger saccades occur for animals with lower acuity). For this reason, we propose the high-risk experiments of capturing the visual experience of the animal simultaneously with neural activity, putting us in the best possible position to align trajectories of behavioral development with neural circuit maturation.

Project 2 limitations and alternative approaches: Imaging GCaMP signals with 2P microscopy presents a number of known limitations. Recording depth is limited to the superficial cortex in high-scattering tissue like that of the ferret. Temporal resolution is limited (~30 Hz), mostly due to the decay constant of calcium signals (~0.5-1 sec), and could cause difficulties in relating neural activity to dynamic behavior evolving over a few seconds (**Fig. 3**). Signal-to-noise can be an issue for low expression or immune-responses due to AAVs or surgical implantation, although this is somewhat abated by application of denoising procedures³³. As an alternative approach we will use implanted extracellular electrodes developed by our collaborator DR. Chong

correlation structure of cells/synapses, and apply computational approaches to assess the fidelity of stimulus representations. We will track the same field of view over multiple imaging sessions to assess changes in the properties of cells and synapses. For cellular populations, we will measure micro- (columnar) and macro- (map) circuit organization of stimulus-driven activity. For synaptic populations we will be able to measure structural and functional dynamics, as shown for the **pilot data shown in Figure 7**. Structural dynamics include the maintenance (stabilization), elimination, and growth of new dendritic spines. This is important as the critical period of development involves enormous increase of synaptic density and concurrent pruning as circuit matures. Alongside structural dynamics, we will assess the functional properties of synapses

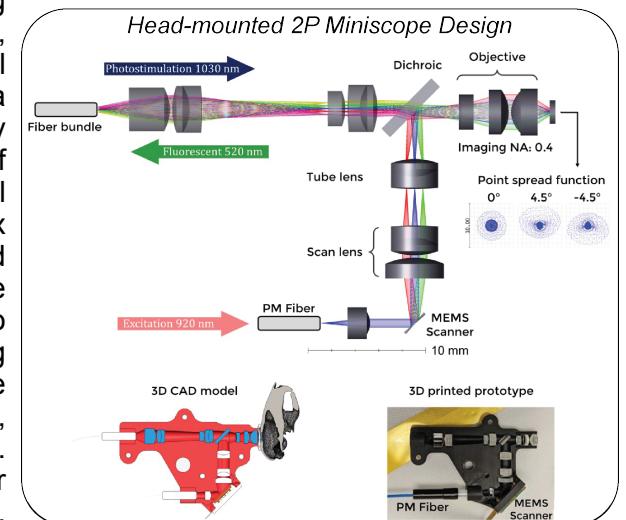


Figure 8: Schematic of light path and head-mounted miniature 2P microscope design (provided by Dr. Emily Gibson).

Xie (Rice). Dr. Xie's lab has created flexible silicon electrodes³¹ (NeuroThreads) that can be implanted early in development, integrate with the neural tissue, and move with the brain as it grows during development. Spiking activity will provide much higher temporal resolution of neural activity and we will not be limited to superficial cortical layers, however it will be more difficult to follow the same cells day-to-day. Separately, we may encounter a conceptual limitation: developmental trajectories of visually-guided behaviors and dynamic vision may be too complicated to directly relate to changes in the receptive field properties of cells and synapses in primary visual cortex. While we may not be able to achieve our overarching goal, the experimental data collected will still be great value to the neuroscience community. In this case, however, we will attempt to design simple visually-guided behavioral tasks that could be more directly related to visual cortical processing (e.g. processing of motion information). Relatedly, it is possible that plasticity in primary visual cortex poorly reflects developing behaviors, as other brains (e.g. motor cortex, superior colliculus) may be more critically involved. We hope to use the foundation developed in Project 1 to push forward towards future experiments exploring neural dynamics of these other brain areas.

Project 2 future directions: We are particularly excited about two different directions these experimental paradigms will allow us to explore. First, we will expand our investigation to inhibitory interneurons. Tools expressing GCaMP in specific interneuron types in nonmurine species are rapidly advancing. This will provide us the opportunity to examine inhibitory network maturation and visually-guided behaviors during critical period plasticity. Second, we will expand our investigations beyond primary visual cortex, moving both closer to the retinal projections that transmit information to the visual pathway, farther up the hierarchical to higher visual areas, and into other sensory areas that are undoubtedly involved with shaping visual experience. Third, we will engage in manipulation experiments (Table 1) using this platform providing *both* behavioral and neural readouts.

1.5 Rigor and Reproducibility

Our lab maintains high scientific rigor, includes male and female subjects in all experiments, blinds experimenters for analyses when necessary, and uses appropriate, robust statistical analyses. A key tenet of the lab is reproducibility: we strive for reproducibility in experiments or data analyses both within the lab and with collaborating labs. Power analyses will be used to determine sample sizes for each experiment, but we expect effect sizes to differ across experiments. We will continue to record from both males and females, testing for any potential sex differences in behavioral development. Rigorous controls for all experiments will be performed as previously described^{12,15,21,29,32}. Statistical analyses will be performed in Matlab and Python using available packages and custom code. The type of statistical analyses chosen will depend on properties of the data (e.g. normally distributed, circular variables) and based on the specific hypothesis being tested (i.e. linear regression vs. principal components analysis). Typically, data we collect requires nonparametric analyses. To ensure proper statistical analyses we will consult with our expert collaborators (Drs. Jacob Yates and Philip Parker) and members of the Computational Neuroscience Initiative at University of Pennsylvania.

2. Innovations

- **Innovation 1 (conceptual):** We will redefine the critical period of visual development as a recurrent process. Venturing into new territory, we will consider how the visual experience changes after eye opening and describe the critical period in terms of naturally emerging behaviors. This fresh perspective can lead to the identification of novel developmental milestones for linking to synaptic and cellular plasticity.
- **Innovation 2 (experimental):** Project 1 proposed experiments are unique and will produce first-of-a-kind datasets on the developing behaviors and visual experience (i.e. ferret head and eye-movements). These data will be invaluable for the field of neuroscience. Moreover, these datasets will not only contain observations, but also perturbation experiments manipulating sensory experience and the early visual system. This will provide novel behavioral readouts of sensory and circuit manipulations.
- **Innovation 3 (analytical):** We will apply state-of-the art computational methods (i.e. DeepLabCut, TREBLE, MoSeq) to analyze behavioral data from individual developing ferrets. Not only will this be the first time these methods are applied to a mammalian developmental model, but they will reveal rich behavioral repertoires.
- **Innovation 4 (experimental):** Project 2's proposed experiments are unique and will produce first-of-a-kind datasets of 2P recordings of synaptic populations or cellular circuits collected from individual developing ferrets as visually-guided behaviors emerge. In addition, 2P data will be processed using state-of-the-art methods for registration and processing (i.e. DeepInterpolation).
- **Innovation 5 (experimental):** Experiments in project 2 will lead to data collected from cortical circuits *simultaneously* while capturing emerging visually-guided behaviors. This will be the first-time head-mounted miniature 2P imaging will be used in ferrets or a developing mammalian species.

Although this combination of innovative experiments are high-risk, we will take great care to ensure that high-quality data is collected from any or all experiments proposed, regardless of the results. To improve our chances in achieving positive results, we have partnered with expert scientists familiar with the suggested methods and analyses (Dr. Philip Parker, Dr. Jacob Yates, Dr. Emily Gibson, and Dr. Chong Xie). That said, our work has the capacity to revolutionize how we study early visual development and models of plasticity which inform therapeutic approaches to neurodevelopmental visual disorders.

3. Investigator qualifications

Since my time as an undergraduate at the University of Oregon, I have shown a sustained track record of scientific creativity, perseverance, and a willingness to take on challenges in innovative ways. In total, **my current scientific contributions have led to 32 publications, including 21 first-author papers and 3 (recent) senior-author papers.**

As a physics undergrad with Dr. Mike Wehr, I learned highly-technical *in vivo* electrophysiological techniques (whole-cell patch-clamp), generating critical data for several projects and the publication of 3 first-author papers. As a doctoral student with Dr. Nicholas Priebe at UT Austin, I expanded my experimental repertoire (*in vivo* microscopy) and became committed to the study of early vision processing and visual development. Supported by an NIH/NEI Training Grant and the broad vision community at UT Austin, my work with Nicholas resulted in 12 publications, including 10 first-author papers. During my postdoctoral work at the Max Planck Florida Institute for Neuroscience with Dr. David Fitzpatrick, I expanded my expertise in vision and experimental techniques, receiving training on state-of-the-art neuroscience techniques such dendritic spine calcium and glutamate imaging (2P microscopy), manipulation of cell-type specific neural activity (optogenetics), and volumetric reconstruction of ultrastructure (electron microscopy)— all applied to a nonmurine model system (the ferret). As a postdoc at MPFI, I had the opportunity to pursue independent projects that did not stem from an established research program and form the necessary collaborations to facilitate those projects. These projects, largely spearheaded by my own ideas and hypotheses, **led to 5 first-author papers in the journals *Neuron* and *Nature* and helped me obtain independent funding through a NIH K99/R00 Pathway to Independence Award.** These projects also modernized and advanced the ferret model system, providing an example to the field of neuroscience how novel techniques could be applied, and even developed for the first time, in a nonmurine mammal.

I started my lab at the University of Pennsylvania in the Department of Neuroscience in July 2021. My research environment is perfectly suited for the completion of this project. I have close collaborations with several visual neuroscientists at Penn (Drs. Diego Contreras, Johannes Burge, and Mike Acaro) and discuss ongoing work regularly. I am a member of the Vision Resource Center, providing direct access to high-precision machining and electronics shops that will facilitate development of the tools proposed to be used. **I have maintained a consistent publication track-record, continuing to develop new ideas and projects, which have already led to 3 senior-author papers.** As an Alfred P. Sloan Research Fellow and Brain Research Foundation awardee, I am connecting with exceptional junior faculty colleagues that will become the next generation of neuroscientists.

I have already assembled a diverse and competent research team comprising two technicians (Joe Barreto and Greg Bond), a senior technician with 2 decades of research experience (Tammi Coleman), and an undergraduate (Amelia Demopoulos), who I have already trained to perform some of the techniques and approaches proposed here. We are expecting at least one postdoc to join the lab in 2024 after their informal commitment. In fact, Barreto, Coleman, and Bond were instrumental in collecting and analyzing preliminary data presented. I have already begun advancing the ferret model system, receiving an NIH/NEI grant to establish CRISPR/Cas9 editing for molecular perturbations. In addition, **critical collaborations have been established with:** 1) Dr. Philip Parker (Rutgers) who is an expert at head-mounted eye-tracking hardware and software, 2) Dr. Jacob Yates (Berkeley) who is an expert visual neuroscience and developing data-driven models incorporating eye-movements of the early visual system, 3) Dr. Emily Gibson (UC Denver) who is an expert in head-mounted miniature 2P microscopy, and 4) Dr. Chong Xie (Rice) whose lab develops flexible chronically-implantable electrodes. As I am not (yet) an expert in these technologies, their assistance and advice is vital.

Overall, I have the scientific foundation, technical expertise, experimental creativity, scientific personnel, and unique team of collaborators to successfully complete this work. As a young investigator embarking on my independent research career, I am determined to carve my own research niche that uniquely leverages my experience and environment. This high-risk, high-reward New Innovator Award would provide an unprecedented opportunity to jumpstart my research program and grant me the intellectual freedom to develop a novel conceptual and methodological framework for studying the critical period of visual development.

4. Suitability for the New Innovator Award program

Discoveries require risk. Compared to a typical R01 application, this award provides an opportunity to studying early visual development with new approaches and a fresh perspective. The NIH Director's New Innovator Award Program affords a unique opportunity to blend technological, experimental, and conceptual risks to redefine the visual critical period of development in terms of natural behaviors and simultaneous recordings of neural dynamics. My proposal is suitable for the following reasons:

(a) This interdisciplinary project allows me to venture into a new field. My previous training and current research program have focused on synaptic and cellular mechanisms of visual processing in mature, visually-experienced animals. This project will allow me to take advantage of the ferret model system and study visual development. In addition, my previous work has not involved natural behaviors. This project will provide an entry point into studying vision in the behaving ferret. As mentioned, a traditional R01 mechanism would not support this proposal given the lack of extensive experience and abundant preliminary data on these subjects.

(b) The proposed experiments are high-risk and innovative. This application does not directly follow any of my previous publications. Instead, it leverages my technical expertise, creative thinking, collaborative disposition, and broad scientific interests. The high-risk experiments serve multiple purposes: they have the potential to be more impactful to the field than incremental follow-up projects and provide the opportunity to test my innovative ideas as I carve a research niche as an independent investigator.

(c) Dependence of project 2 on project 1 will yield valuable insights and transformative data. While the experiments proposed in each project could be conducted separately, the neural recordings proposed will be most impactful if collected alongside or simultaneously with developing, active visual behaviors. In particular, the ability to track an animal's body and head movements, accurately capture eye-movements, and collect high-quality neural data is a holy grail of modern neuroscience research. This interdependence among projects is discouraged in R01 applications but will be highly effective if funded through this mechanism.

Statement of research effort commitment: If selected for this award, I will commit at least 25% of my research effort to this project.

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