Inducible gene targeting in mouse retinal neurons expressing Grm6
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**Purpose:** The purpose of the study is to generate mouse lines for inducible gene targeting in depolarizing bipolar cells (DBCs).
**Methods:** Three engineered transgenic constructs with iCre, mCherry, and ERT2-Cre-ERT2 (ECE) cDNAs downstream of a 10 kb mouse Grm6 promoter fragment were made, respectively. The iCre and mCherry constructs were mixed and injected pronuclearily into embryos of C57BL/6 and Balb/c mixed background and one founder line, MCV11F, was established. The ECE construct was injected alone and one founder line, T1, was established. Cre activity was revealed by commercial Z/EP or Ai9 reporter lines. Cre induction in the T1/Ai9 line was done by intraperitoneal injections of varying doses of tamoxifen (TAM) or by topical application of 4-hydroxytamoxifen (4-HT) loaded nanowafers on the cornea.
**Results:** The MCV11F line has robust mCherry and Cre recombinase expression in all adult DBCs. However, in the Z/EP background, we found GFP expression throughout the entire retina, indicating that Cre was expressed earlier during development. Without induction, the T1/Ai9 line displays Cre activity in ~50 neurons in young adults and ~100 in mice over one year of age. Intraperitoneal delivery of 500 nmol per gram body weight per day of TAM over five days is sufficient to induce Cre activity in >90% of DBCs. By titrating the amount of TAM injected, a single delivery of 10-25 nmol per gram body weight could induce tdTomato expression in ~300 intermittently dispersed neurons per retina. To induce Cre activity in the T1 mice unilaterally, we employed a nanowafer delivery system of 4-HT at the dose of (5 µg/wafer/day). This could also induce reporter expression between 200-1000 DBCs in the treated eye. Under inductive conditions where 100 to 1000 DBCs expressed tdTomato, examination of the axonal stratification levels of ~200 cells in the inner plexiform layer (IPL) showed that rod bipolar cells (RBCs) and all known cone ON bipolar cells except the type-9 (CB9) could be labeled. Interestingly, we encounter a few cells whose axons stratify to the OFF center, suggesting that certain hyperpolarizing bipolar cells may also express GRM6.
**Conclusions:** Despite being a good marker line, the precocious Cre expression during development of MCV11F mice restricts its use. The T1 line is more suitable for inducible gene targeting in DBCs. Its utility may be expanded by further exploration of the type, amount, and routes of reagents used for induction.

**Commercial Relationships:** Yu-Jian Chen, None; Hoon Shim, None; Crystal S. Shin, None; Ghanashyam Acharya, None; Ching-Kang J. Chen, None

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Mapping the purine circuitry in the zebrafish retina
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**Purpose:** ATP is an important signaling molecule in the vertebrate retina and regulated by a variety of retinal driven mechanisms. However, there is a paucity of information regarding the expression of this molecule in the developing retina.

**Methods:** We have utilized transgenic zebrafish lines to express yellow fluorescent protein (YFP) reporter constructs under the control of the regulatory elements of purine biosynthetic enzymes. These reporters include adenylosuccinate synthetase (AdoSyn), purine nucleoside phosphorylase (PNP), and adenosine deaminase (ADA).

**Results:** We have shown that the reporter constructs are expressed in the developing retina and that expression patterns are consistent with the known expression patterns of these enzymes. In addition, we have observed changes in expression patterns during development and in response to light and other stimuli.

**Conclusions:** Our results provide valuable insights into the function of purine signaling in the developing retina and may have implications for the treatment of retinal diseases.
profile of key proteins involved in purinergic signaling in the zebrafish retina. The goal of this study was to map the circuits involved in ATP and adenosine signaling in the zebrafish retina.

**Methods:** Neurochemical circuits were mapped in the zebrafish retina using in situ hybridization techniques and immunohistochemistry. Antibodies targeted to purine receptors (P1 and P2 receptors), components of the purine signaling machinery (vesicular nucleotide transporter (VNUT), 5'-ectonucleotidase, NTDPase-1,-2,-3, and adenosine deaminase) and retinal cell specific markers were used to uncover purine neurochemical and protein signatures in the retina.

**Results:** In situ hybridization revealed that A1 receptors were weakly expressed in the inner nuclear layer and ganglion cell layer. A2A receptors were highly expressed in all cell layers of the zebrafish retina, and A2B receptors were restricted to the horizontal cell layer. Immunohistochemical evidence identified strong P2X receptor expression in the outer retina and ganglion cell layer. NTDPase-1 and -2 were expressed throughout the retina with the strongest expression in the outer and inner retina as described previously. Adenosine deaminase was highly expressed at photoreceptor terminals and faintly in the inner retina. In addition, a likely source of ATP could be derived from VNUT which was localized to both horizontal cells in the outer retina and Müller cells in the inner retina.

**Conclusions:** Our findings indicate that purinergic circuits are regulated by presence of receptors, enzymes and VNUT expression. Potential ATP pools likely originate from Müller cells in the inner retina and horizontal cells in the outer retina. These findings on receptor expression suggest important roles for purines in modulating specific retinal circuits, including the nocturnally driven rod pathway, and inner retinal circuits. The strong expression profile of both adenosine receptors and ecy-enzymes involved in ATP degradation favors an important role for adenosine modulating retinal circuitry and synaptic inputs in both the inner and outer retina. Taken together, these findings highlight the far reaching modulatory implications of purine-mediated signaling on visual processing in the retina.

**Commercial Relationships:** Dillon McDevitt, None; Salvatore L. Stella, None

**Support:** Penn State Start Up Funds

**Program Number:** 2222 **Poster Board Number:** A0398

**Deafferented mouse rod bipolar cells extend their dendrites and synapse with healthy photoreceptors**

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**Purpose:** Previous studies from our group demonstrated constructive retinal restructuring in the rod bipolar cells of the rabbit retina in response to selective ablation of a small patch of photoreceptors. The mouse is a more convenient animal model for investigation of the molecular mechanisms responsible for this restructuring due to availability of the genetically modified lines. We investigate retinal response to photoreceptor ablation in the mouse to test if it can be used as a model for constructive retinal restructuring.

**Methods:** Round lesions of Barely Visible clinical grade were placed in vivo in the mouse retina with a 532-nm laser, using 200µm spot diameter and 20ms pulse duration. Photoreceptor migration and changes in the morphology of the deafferented rod bipolar cells (RBCs) were assessed using confocal microscopy of immunostained tissue. RBCs and their synaptic contacts with photoreceptors were visualized with PKCa, CIBP2, mGlur6 and ELFN1 antibodies. Cone arrestin antibody was used to visualize cone photoreceptors.

**Results:** The photocoagulation procedure resulted in the death of rod but not cone photoreceptors at the lesion site. No damage to the neurons in the inner nuclear layer was observed. We find that over time (3-180 days after photocoagulation), healthy rod photoreceptors partially fill the lesioned area. During this process, RBCs change their dendritic morphology: first they lose thinner processes, leaving one or two dendrites, which then expand, on average, from 10µm to 30µm in length, and from 0.3 to 1µm in thickness. These thickened dendrites extend towards the healthy photoreceptors located around...
Adult mouse retinas from the A/J strain were

3:45 PM–5:30 PM

Retinal neurons of the same type are commonly assumed

These findings indicate that light-evoked release of

The positioning of CBCs in a mosaic is constrained

This work was supported by Burroughs Wellcome Fund

Purpose:

The lesion and make synapses similar to those of the healthy RBCs. The dendrites of the deafferented RBCs also approach the pedicles of the cone photoreceptors that survived the ablation in the middle of the lesion.

Conclusions: Deafferented mouse RBCs restructure their dendritic trees to form new connections with healthy photoreceptors outside the lesion, similar to our earlier observations in the rabbit retina. In the center of the lesion, they approach surviving cone photoreceptors more often than in the healthy retina. The mouse model, with the broad range of its genetic control, can be used to study molecular mechanisms behind the conductive retinal plasticity.

Commercial Relationships: Anahit Hovhannisyan; Corinne Beier, None; Lee Daeyoung, None; Philip Huie, None; Sydney Weiser, None; Daniel V. Palanker, None; Alexander Sher, None

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Program Number: 2224 Poster Board Number: A0400

Presentation Time: 3:45 PM–5:30 PM

Variation in mosaic patterning in the mouse retina: from regular to random

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Purpose: Retinal neurons of the same type are commonly assumed to be distributed as regular arrays known as mosaics, but relatively few different types have been thoroughly assessed. To determine the generality of mosaic regularity in the retina, the present study has analyzed the spatial organization of six different cell types, including the horizontal cells (HCs), two types of amacrine cells (ACs), and three types of cone bipolar cells (CBCs).

Methods: Adult mouse retinas from the A/J strain were immunolabeled to identify the populations of HCs, VGluT3+ (VG3) ACs, cholinergic (ChAT) ACs, and Types 2, 3b and 4 CBCs. Eight fields from each wholemount retina were sampled to identify the X-Y coordinates of each cell body, from which the Delauney tessellation and the spatial autocorrelation of each field were computed. Various spatial properties of the mosaics were then calculated, and compared to those from simulations of random mosaics matched in density and constrained by soma size.

Results: The VG3 AC mosaics, as well as the mosaics from the three CBC types, were found to be conspicuously less regular and less efficiently packed than the mosaics of the HCs or ChAT ACs. Despite their lesser order, the VG3 ACs were still discriminable from random distributions, while the three CBC mosaics, however, had spatial properties that were comparable to such distributions. Critically, when their spatial properties were considered as a function of the variation in cellular density across fields, all types of CBCs behaved similar to random simulations, whereas the HCs and ChAT ACs spaced themselves apart more uniformly, evidenced by a strong significant negative correlation between the effective radius and cell density. VG3 ACs, while having comparable densities to the HCs in the A/J mouse retina, do not space themselves apart uniformly, thereby exhibiting reduced regularity and packing.

Conclusions: The positioning of CBCs in a mosaic is constrained only by the physical size of their somata, rendering their mosaics irregular to the point of being random. VG3 ACs, by contrast, fail to come into contact with one another as frequently as a random simulation would predict, yet they do not modulate their intercellular spacing uniformly with all of their homotypic neighbors. HCs and ChAT ACs exhibit the critical hallmark of regular retinal mosaics, minimizing proximity to all of their near neighbors.

Commercial Relationships: Patrick W. Keeley, None; Jason J. Kim, None; Benjamin E. Reese, None

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Program Number: 2225 Poster Board Number: A0401

Presentation Time: 3:45 PM–5:30 PM

Spiking dopaminergic amacrine cells strongly modulate ON-cone bipolar cell surrounds and direct signaling from horizontal cells to ON-cone bipolar cells

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Purpose: Surround light responses are strongest following bright illumination. ON-cone bipolar cells (ON-cBCs) produce surround responses that are 1) mediated by their dendritic GABA_{R}s and 2) modulated by the ambient light level, via activation of their dendritic dopamine D_{Rs}, which regulate GABA_{R} expression and activity (Chaffiol et al., submitted). TTX (blocks sodium spiking) greatly reduces ganglion cell surround responses (Cook, McReynolds, 1998; Taylor, 1999), suggesting that spiking neurons, which are only in the inner retina, play a significant role in surround responses. Because dopaminergic amacrine cells (DACs) in the inner retina, the only retinal neurons that release dopamine (Witkovsky, 2004), spike in response to light stimulation (Zhang et al. 2007), we studied whether spiking DACs, acting via D_{Rs}, modulate the strength of ON-cBC surrounds.

Methods: ON-cBC surround light responses and the effects of artificially polarizing horizontal cells (HCs) on simultaneously-recorded nearby ON-cBCs in rabbit retinal slices were studied in the day in the presence and absence of gabazine (GBZ, GABA_{R} antagonist), SCH23390 (SCH, D_{R} antagonist), APB (blocks cone input to ON-cBCs), and TTX (HCs: sharp pipettes; ON-cBCs: graninidic toperated patch pipettes).

Results: Following maintained (30 min) bright illumination, double labeling of rabbit retinal sections with bd-17 (labels GABA_{R}s-β/3 subunit) and Goa (labels ON-cBCs) revealed that ON-cBC dendritic GABA_{R} expression was significantly greater under control conditions and in the presence of both TTX and dopamine, compared to TTX alone. ON-cBCs exhibited surround light responses and polarization of HCs that had a sign-conserving effect on nearby ON-cBCs when the superfusate contained dopamine, TTX, and APB, but not when the superfusate also contained GBZ or SCH. These data show that TTX acts upstream of dopamine and GBZ.

Conclusions: These findings indicate that light-evoked release of dopamine from spiking DACs, by activating D_{Rs} on ON-cBC dendrites, strongly modulates ON-cBC surround strength. GABA_{R}-mediated ON-cBC surround responses and direct sign-conserving GABA_{R}-mediated signaling from HCs to ON-cBCs are strongest following maintained bright illumination when spiking-evoked dopamine release and D_{R} activation are high, but minimal when spiking is blocked with TTX or when D_{Rs} are blocked.

Commercial Relationships: Stuart C. Mangel, None

Support: Plum Foundation Grant

Program Number: 2226 Poster Board Number: A0402

Presentation Time: 3:45 PM–5:30 PM

Developmental endocrine disruption alters adult visually-guided behaviors in the Zebrafish

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Purpose: To determine the short- and long-term effects of two anti-estrogenic endocrine disruptors, formestane and PCB, on larval visual system anatomy and adult visually-guided behaviors.

Methods: Zebrafish larvae were transiently exposed to environmentally-relevant concentrations of each chemical at 24 hpf (hours postfertilization), 72 hpf, 7d (days) pf, and 21 dpf and the acute effects on overall anatomy were determined. At 3 months of age, adult optomotor responses were assessed. To determine the mechanism of action of the endocrine disruptors, aromatase activity was measured using adult zebrafish brain tissue.

Results: There were no significant anatomical differences nor an effect on aromatase activity (One Way ANOVA, p=0.86) as a result of acute exposure to PCB. In contrast, acute development exposure to formestane potently inhibited aromatase activity (One way ANOVA, p<0.01) and significantly diminished adult visually guided behaviors, as there was a main effect of treatment between the control stimulus ON and treatment stimulus ON groups (2x2x2 ANOVA, p=0.01). Interestingly, however, there were no significant gross morphological differences as a result of formestane exposure.

Conclusions: Inhibition of aromatase activity by formestane during specific developmental time points results in diminished visually-guided responses in adults suggesting long-term consequences.

Commercial Relationships: Cassie J. Gould; Colin J. Saldanha, None; Victoria P. Connaughton, None

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Program Number: 2227 Poster Board Number: A0403

Determining the localization of Tubulin Polymerization Promoting Protein TPPP/p25 in the mice and human retina: effect of zinc supplementation

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Purpose: Tubulin Polymerization Promoting Protein (TPPP/p25) plays a major role in stabilizing microtubule structures and is able to bind zinc through a zinc finger motif that stabilizes the protein. Therefore, in zinc rich tissues like the retina, if it is present, TPPP/p25 might play an important role in tubulin polymerization and visualization of lipid raft fractions. TPPP/p25 might play a role in the organization and reorganization of the synaptic architecture and visual integration. The increased immunoreactivity suggests that changes in zinc levels can directly affect level of TPPP/p25 in the IPL and as such affects the synaptic connectivity mainly on the central retina.

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Program Number: 2228 Poster Board Number: A0404

Interactions of zebrafish Olfactomedin 1 with the AMPA receptor and SNARE complexes

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Purpose: Olfactomedin 1 (Ofm1) is a component of the AMPA receptor complex in the vertebrate brain and retina. Ofm1 and ofm1b double knockout zebrafish (ofm1/null) demonstrated changes in the retinal structure and visual motor function (ARVO 2014, Abstract #1918670). Changes in the composition of the AMPA receptor complex in the ofm1 null fish were investigated to elucidate the molecular mechanisms of Ofm1 action in the brain and retina.

Methods: Brains of adult fish were used to isolate synaptosomal and lipid raft fractions. Ofm1, GluR2 and SNARE complex proteins were immunoprecipitated with specific antibodies and analyzed by Western blotting. GluR2 internalization was investigated in retinal sections of adult fish.

Results: In the adult wild-type (wt) brain, Ofm1 was preferentially localized to the synaptosomal membrane fraction together with GluR2, PSD95, Syntaxin and SNAP25. In this fraction, Ofm1 interacted with Syntaxin, SNAP25, Synaptophysin, VAMP2 and GluR2, as judged by co-immunoprecipitation indicating participation of Ofm1 in both pre- and post-synaptic events. The palmitoylation of GluR2 was greatly reduced in the synaptosomal fraction of ofm1 null fish compared with wt. Ofm1 co-precipitated with GluR2 was also palmitoylated. Changes in palmitoylation may cause reduced internalization of GluR2, as observed in retinal explant cultures from ofm1a/b null larvae compared with wt. Analysis of the composition of lipid raft prepared from adult brain demonstrated the presence of Ofm1. The levels of several proteins (GluR2, SNAP25, Flotillin1,
Expression of Kv11.1 in retinal ON-bipolar cells

Catherine W. Morgans\textsuperscript{1}, Gaoying Ren\textsuperscript{1}, Tammie Haley\textsuperscript{1}, Weihong Xiong\textsuperscript{1}, Maria Borisovska\textsuperscript{2}, Cyrus McHugh\textsuperscript{1}, Margaret Veruki\textsuperscript{1}, Robert M. Duvoisin\textsuperscript{1}.

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**Purpose:** This study aims to determine if ON-bipolar cell responses to light are influenced by dendritic potassium channels. We focused on the Kv11.1 (erg1, hERG) channel as it has been shown to be expressed in bipolar cells.

**Methods:** Kv11.1 antibodies were validated by immunofluorescence and Western blotting using Kv11.1-transfected HEK293 cells. Kv11.1 expression in mouse retina was confirmed by Western blotting, and its distribution in the retina determined by immunofluorescence confocal microscopy. The physiological role of Kv11.1 in bipolar cells was assessed by measuring the effect of the Kv11.1 blocker, E-4031, on electroretinogram (ERG) recordings and patch-clamp recordings of bipolar cells.

**Results:** Two validated antibodies against different epitopes of Kv11.1 revealed immunofluorescent labeling of the inner and outer plexiform layers (IPL and OPL) of the mouse retina. Western blots of retinal proteins revealed a band of approximately 150 kDa with both antibodies, consistent with expression of Kv11.1 isoform a (ERG1a). In the IPL, Kv11.1 co-localized with TRPM1 and GPR179 in the dendritic tips of ON-bipolar cells, and occasionally with TRPM1 in ON-bipolar cell bodies. Kv11.1 immunofluorescence was unchanged in TRPM1 knockout and PKCa knockout retina. Patch-clamp recordings from rod bipolar cells in retinal slices revealed an E-4031 sensitive potassium current. The putative Kv11.1 current was present in both intact cells and cells where the axon terminal was severed, indicating that the current is likely to arise from the dendrites.

**Conclusions:** Kv11.1 is expressed in retinal ON-bipolar cells, where it is localized to the dendrites. ERG recordings in the presence and absence of E-4031 indicated a role for Kv11.1 in shaping the light response of ON-bipolar cells. The lack of effect of E-4031 on ERGs from PKCa knockout mice suggests that Kv11.1 activity may be regulated by phosphorylation by PKCa.

**Commercial Relationships:** Catherine W. Morgans, None; Gaoying Ren, None; Tammie Haley, None; Weihong Xiong, None; Maria Borisovska, None; Cyrus McHugh, None; Margaret Veruki, None; Robert M. Duvoisin, None

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**Orexin-B modulates GABAAergic synaptic transmission on rat retinal rod bipolar cells**

Yong-Mei Zhong, Gong Zhang, Guo-Zhong Xu, Shi-Jun Weng, Xiong-Li Yang. Institutes of Brain Science, Fudan University, Shanghai, China.

**Purpose:** Orexin-A and orexin-B have been implicated in arousal and feeding behaviors by activating two G-protein-coupled receptors: OX,R and OX,R. While the expression of orexins and orexin receptors is immunohistochemically revealed in retinal neurons (Liu et al., 2011), the function of these peptides in the retina is largely unknown. Here we investigated whether and how orexin-B modulates GABAAergic synaptic transmission on rod-dominant ON type bipolar cells (RBCs) in the rat retina.

**Methods:** In vivo scotopic flash electroretinogram (ERG) recordings were conducted to monitor the effect of orexin-B on ERG b-wave that mainly represents RBC depolarization. Whole cell patch-clamp recording techniques in both retinal slices and freshly dissociated RBCs were used to investigate the effect of orexin-B on GABAAergic synaptic transmission on RBCs.

**Results:** Intravitreal injection of orexin-B produced a marked increase in the amplitudes of scotopic ERG b-waves, suggesting orexin-B modulates RBC activity in vivo. We recorded GABA receptor (GABAR)-mediated inhibitory postsynaptic currents (IPSCs) from RBCs in rat retinal slices to test whether orexin-B modulates GABAAergic feedback from amacrine cells (ACs) to RBCs in the inner plexiform layer (IPL). We found that orexin-B significantly suppressed GABAR-mediated IPSCs of RBCs. We further investigated the effect of orexin-B on GABAR-mediated currents (GABA currents) in rat isolated RBCs. Orexin-B suppressed GABA-R\(_\text{R}_,\) but not GABA-R\(_\text{R}-\text{mediated currents of RBCs and the effect was partially eliminated by an OX,R antagonist or an OX,R antagonist.}

**Conclusions:** Orexin-B suppressed GABAA currents of RBCs and GABAAergic inhibitory feedback from ACs onto RBCs, and the suppression may cause increased activity of RBCs, as evidenced by the fact that orexin-B potentiated the scotopic ERG b-wave.

**Commercial Relationships:** Yong-Mei Zhong, None; Gong Zhang, None; Guo-Zhong Xu, None; Shi-Jun Weng, None; Xiong-Li Yang, None

**Support:** The National Natural Science Foundation of China: 31171055, 31571075

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**Accurately assessing natural and artificial visual function in behaving animals**

Tamar Arens-Arad, Nairouz Farah, Yossi Mandel. Faculty of life Sciences, Optometry Track and Bar-Ilan Institute for nanotechnology and Advanced Materials (BINA), Bar Ilan University, Ramat Gan, Israel.

**Purpose:** Accurately assessing natural and artificial visual function performance in awake and behaving animals is of great importance. Performance in awake and behaving animals is of great importance. Instrumentation for measuring the performance of the visual system in behaving animals is subject to systematic drifts due to movements of the head or eyes. To overcome these problems, we used a Head-Mounted Projection (HMP) system for visual stimulation and cortical recordings as a novel method for studying natural and artificial vision in behaving animals.

**Program Number:** 2230 Poster Board Number: A0406
**Presentation Time:** 3:45 PM – 5:30 PM
**Support:** NEI/NIH Intramural Research Program

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for studying various retinal diseases and treatments. Here we present the development of a novel customized head-mounted projection system integrated with electrodes for recording visual evoked potentials (VEP) in response to natural and artificial stimuli for assessing visual functions in awake and behaving animals.

**Methods:** We devised and customized a Digital Mirror Device (DMD) based head-mounted system, to project high quality images at visible and near IR light onto the rat retina and performed computer simulations to characterize and optimize the optical properties of the system. The design included a periscope like system to relay the DMD projected image onto the rat retina, and fitted onto the rat skull using a customized head plate and adapter. VEPs were recorded using electrodes implanted into the visual cortex and embedded into the mounting head plate. VEPs induced by flashes with varying pulse durations (ranging from 0.25 msec to 8 msec), varying frequency (ranging from 1Hz to 32Hz) and varying contrast levels projected by the head mounted projector were investigated in both anesthetized and awake animals.

**Results:** The system enabled the projection of images with MTF values higher than 0.85, with optimal image quality obtained at a 1mm pupil diameter, with a retinal image diameter of 3 mm corresponding to 45 degrees visual field in the rat. Robust VEP signals were recorded in response to images projected at various contrast and light intensity. The VEP amplitude decreased as a function of temporal frequency reaching the noise limit for frequencies higher than 32 Hz and increased as a function of stimuli duration, reaching a plateau at pulses longer than 10 ms. Similarly, a decrease in VEP amplitude for decreasing contrast was also observed, reaching the noise level at 6% contrast.

**Conclusions:** Our results demonstrate the feasibility of investigating visual function performance in rats using a novel head-mounted projection system. This system may prove to be a vital tool in studying natural and artificial vision in awake and behaving animals, and for the evaluation of various treatments or other interventions, such as training for the studying of visual cortex plasticity.

**Commercial Relationships:** Tamar Arenas-Arad, None; Nairouz Farah, None; Yossi Mandel, None

**Support:** Israeli Ministry of Health and Lirot association

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**A high-threshold rod input drives retinal dopamine release in response to light**

**Poster Board Number:** A0409

**Presentation Time:** 3:45 PM–5:30 PM

**Purpose:** The origin of light input to dopaminergic amacrine cells of the retina has received significant attention in recent years. However, measurement of dopamine release in the retina in response to light does not correlate with electrophysiological and/or immunohistochemical reports. The purpose of this study was to describe the photoreceptive origin of light-induced dopamine release using transgenic mouse models.

**Methods:** We used UHPLC-MS/MS analysis to quantify DOPAC:DA ratio in the mouse retina in response to 4 light intensities (max 1.6 log W/m²; ~10,000 lux). Wild-type, Gnat2⁺/⁺ (cone-functionless), and rd/rd (lacking rods and cones) animals were light pulsed and the gap junction blocker meclofenamic acid (MFA) applied intravitreally.

**Results:** We found that light caused a significant increase in dopamine turnover (DOPAC:DA ratio) in the retinas of both wild-type and Gnat2⁻/⁻ mice (p<0.001; n=3) but only at very bright light intensities (> 0.65 log W/m²; ~1000 lux), with no significant difference between the two groups. In agreement with previous studies, no significant increase was obtained in response to light in rd/rd mice at any light intensity. The gap junction blocker MFA (1 mM) caused a significant reduction in light induced dopamine release in both wild-type (p<0.01; n=3) and Gnat2⁻/⁻ mice (p<0.05; n=3) animals, but did not completely abolish the light-induced increase in comparison to dark conditions.

**Conclusions:** We find a surprising role for rods as the primary photoreceptor contributing to light-induced dopamine release in the mouse retina. We have previously shown that light-induced dopamine release in rod-inactivated Gnat²⁻/⁻ mice was significantly reduced, indicating little cone input to this response. Our data with the Gnat²⁻/⁻ mice supports this finding, but given that this input has a very high threshold, at least 6 orders of magnitude above the rod threshold, the mechanism of this light gating remains unknown. It
is possible that ipRGCs are playing a role in this input, but require a functional rod input to convey this message.

Commercial Relationships: Victor Perez Fernandez, None; Morley W. John, None; Cameron Morven, None

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