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VISION IN LABORATORY RODENTS – TOOLS TO MEASURE IT AND IMPLICATIONS FOR BEHAVIORAL RESEARCH

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HIGHLIGHTS:

- Rodents’ visual pathways are able to carry out complex visual processing
- Vision guides rodent behavior in several laboratory settings
- Rodent vision can be tested using operant and reflex-based behavioral tasks
- Electrophysiological tests of visual function provide an alternative for behavioral tests
- Genetic engineering may have unexpected consequences on vision

ABSTRACT

Mice and rats are nocturnal mammals and their vision is specialized for detection of motion and contrast in dim light conditions. These species possess a large proportion of UV-sensitive cones in their retinas and the majority of their optic nerve axons target superior colliculus rather than visual cortex. Therefore, it was a widely held belief that laboratory rodents hardly utilize vision during day-time behavior. This dogma is being questioned as accumulating evidence suggests that laboratory rodents are able to perform complex visual functions, such as perceiving subjective contours, and that declined vision may affect their performance in many behavioral tasks. For instance, genetic engineering may have unexpected consequences on vision as mouse models of Alzheimer’s and Huntington’s diseases have declined visual function. Rodent vision can be tested in numerous ways using operant training or reflex-based behavioral tasks, or alternatively using electrophysiological recordings. In this article, we will first provide a summary of visual system and explain its characteristics unique to rodents. Then, we present well-established techniques to test rodent vision, with an emphasis on pattern vision: visual water test, optomotor reflex test, pattern electroretinography and pattern
visual evoked potentials. Finally, we highlight the importance of visual phenotyping in rodents. As the number of genetically engineered rodent models and volume of behavioral testing increase simultaneously, the possibility of visual dysfunctions needs to be addressed. Neglect in this matter potentially leads to crude biases in the field of neuroscience and beyond.

Abbreviations: AD, Alzheimer’s disease; CNS, central nervous system; CPD, cycles per degree (of visual angle); CT, contrast threshold; DLGN, dorsal lateral geniculate nucleus; HD, Huntington’s disease; LFP, local field potential; NCL, neuronal ceroid lipofuscinosis; OKR, optokinetic reflex test; OMR, optomotor response test; PD, Parkinson’s disease; PERG, pattern electroretinography; POS, photoreceptor outer segment; PVEP, pattern visual evoked potential; RGC, retinal ganglion cell; RPE, retinal pigmented epithelium; SC, superior colliculus; SCN, suprachiasmatic nucleus; SF, spatial frequency; VA, visual acuity; V1, primary visual cortex.

Keywords: Rodent; Mouse; Vision; Behavior; Visual Evoked Potentials; Electroretinography

INTRODUCTION

Mammals collect information from the world around them with five traditional senses: visual, auditory (hearing), olfactory (smell), gustatory (taste) and somatosensory senses (touch/pain). Evolution modifies and tunes the senses of each species so that they optimally promote survival and reproduction in the species’ natural habitat. Vision in mice and rats follows this principle as demonstrated by two illustrative examples: (1) Their vision is tuned for night-vision (scotopic) rather than for day-vision (photopic) because mice and rats are nocturnal mammals; (2) In the mouse and rat brain, optic nerve axons primarily target limbic areas rather than associative visual areas, likely because rodents are prey animals, decreasing response time to object movement rather than interpreting objects.

Many behavioral scientists may argue that laboratory rodents are practically blind, or at least that they barely use vision when performing behavioral tasks [1]. By today, scientists acknowledge that this is not true. However, fewer may know how sophisticated the rodent visual system actually is. Indeed, recent research shows that there are at least 32 functionally diverse retinal ganglion cell (RGC)
subtypes in the mouse retina, of which a large number convey orientation and/or direction selectivity [2]. Furthermore, the mouse primary visual cortex (V1, striate cortex) has been shown to learn and predict spatiotemporal sequences from visual representations [3]. Also, higher-order visual areas in mice appear surprisingly sophisticated and include similar ”what” (ventral stream) and ”where” (dorsal stream) extrastriate visual pathways as found in primates [4]. The complexity of mouse visual system would not make sense evolutionarily if mice were not using their vision for complex tasks in nature. Indeed, a recent study showed that vision is important for accurate insect prey capture in C57BL/6J mice, supporting the sentiment that mouse vision is not only needed for defensive responses [5]. In addition, recent evidence indicates that mice are even able to perceive subjective contours that form illusory images [6].

Vision clearly guides the rodent behavior in several laboratory settings. Every researcher who has mistakenly tried to train navigation in the Morris swim task to mice carrying the rd1 mutation (a common retinal degenerative gene defect in inbred mice [7]) knows how frustrating it can be. Luckily, the presence of retinal degenerative gene mutations in several inbred rodent strains is well acknowledged and documented by laboratory animal suppliers [7]. However, genetic engineering meant to target other than visual pathways can have unexpected consequences on visual function as well. A dramatic example of this is the R6/2 mouse model of Huntington’s disease [8, 9]. These mice, extensively used in visual-guided behavioral paradigms (e.g. [10-14]), start to lose their retinal responsiveness to bright light flashes already before clear-cut change in their motor function or behavior [8, 9]. Unfortunately, the unexpected effect of genetic engineering on rodent vision is not the only possible biasing factor when it comes to using vision in behavioral task. Albino mice and rats are well known for their poor vision, but they do still respond to visual pattern stimulation [15] and thus likely utilize their vision in a behaviorally meaningful way. However, vision in albino rodents may easily be lost upon acute bright light exposure [16, 17], or chronic low light level exposure even at their standard vivarium [18, 19].
In this article, we will first describe the basics of visual function for general guidance. Second, we will discuss the special characteristics of rodent vision, and third, introduce some well-established and relatively simple techniques to test vision in rodents, with the focus on pattern vision. Finally, we will highlight with appropriate examples the importance of acknowledging the potential problems associated with visual decline in behavioral neuroscience and beyond.

1. PRIMARY VISUAL PATHWAYS IN A NUTSHELL

Vision is the eye’s capability to focus and to detect images of visible light. This is made possible by retinal photoreceptors in the back of the eye. However, before reaching the retina, wavelengths of visible light need to traverse through the anterior parts of the eye: the cornea, the lens and vitreous body. Once the wavefront hits the retina, it still needs to traverse through its entire thickness to reach the photoreceptors, because they are located in the farthest layer of neuronal retina with respect to the vitreous body (see Fig. 1). In anatomical terms, the layer of photoreceptors and their synaptic sites comprise the ”outer retina”, while the ”inner retina” consists of interneurons (bipolar, horizontal and amacrine cells), ganglion cells and their synaptic sites.

The photoreceptors are metabolically very active, having a high demand for blood supply and a mechanism to constantly renew their most active sites, the photoreceptor outer segments (POSs). Oxygen usage by photoreceptors is estimated to be 3-4 times higher than in other central nervous system (CNS) neurons [20]. The blood supply to the photoreceptors is provided by the choroidal vasculature below the retinal pigment epithelium (RPE), which is located adjacent to the POSs. The RPE forms part of the blood-retina-barrier, which is in essence similar to the blood-brain-barrier in the brain [21]. The RPE is also responsible for the removal of shed POSs. Microvilli in the apical side of the RPE intermingle with POSs enabling close and direct contact between these structures. Approximately 10% of the POS tips are phagocytosed and renewed every day by the RPE [22]. As the homeostasis of the retinal photoreceptors is very delicate and its maintenance rather complex, the photoreceptors are highly vulnerable to defects caused by gene mutations as well as environmental
hazards [23]. For example, retinitis pigmentosa, the most common form of inherited retinal degeneration [24], can be caused by a defect in over 200 distinct genes (RetNet: https://sph.uth.edu/retnet/).

Viable photoreceptors cannot generate vision alone, not even at the level of the retina. Photoreceptor activation by light leads to modulation of glutamate release at the synaptic site between photoreceptors and retinal inner neurons, leading to activation of bipolar cells [25]. In the cone-pathway (color and day vision), the bipolar cells relay the signal directly to the end station of the retina, retinal ganglion cells (RGCs), whereas in the rod-pathway (dark and night vision) one more additional relay station at the AII type amacrine cells is needed [26]. The functionality of this vertical retinal pathway would be enough for mere luminosity detection, but is not enough for detection of visual contours or patterns. The pattern vision is made possible by lateral neuronal pathways within the retina, involving lateral inhibitory activity by the horizontal cells, and ON-OFF organization of the RGCs. The retinal signaling utilizes similar neurotransmitter composition as the brain. While glutamate and GABA are the main excitatory and inhibitory neurotransmitters [27], other neurotransmitters such as monoamine neurotransmitters, acetylcholine and neuropeptides have rather a modulatory role [28].

As soon as the visual information is generated in the retina as an appropriate physical stimulus, the electrical signals are conveyed to the brain via RGC axons, which form the optic nerve as they exit the eye. The optic nerves mainly target a few distinct areas in the brain: the dorsal lateral geniculate nucleus (dLGN) in the thalamus, superior colliculus (SC) in the midbrain, suprachiasmatic nucleus (SCN) in the hypothalamus, and pretectum between thalamus and midbrain [29]. In brief, the dLGN is a thalamic relay station where visual signals are conveyed via the optic tracts to the visual cortex. The SC is responsible for coordinating head and eye movements with sensory targets. The SCN is associated with circadian response to light, and the pretectum is mainly responsible for the pupillary light reflex in response to a change in the ambient luminance [30]. In the primary visual pathway,
the visual code is finally interpreted at V1 and further in associative visual areas containing the "what" and "where" visual pathways [4, 31].

2. VISION IN LABORATORY RODENTS

Research by Nobel laureates Hubel and Wiesel in cats and non-human primates laid the foundation to our understanding of the physiology of primary visual pathways [32-35]. Nowadays, half a century later, vision research in cats is rare (Fig. 2). Instead, several thousands of papers on vision research conducted in primates emerge annually, highlighting our great interest to the visual sense. For a long time, the general opinion was that rodent visual system is too distinct from humans [1], hindering the use of rodents in vision research. However, since around year 2000, the paradigm has shifted, and vision-related research in mice has been rising exponentially (Fig. 2). Next, we will briefly discuss some special characteristics of rodent vision that should be taken into an account in meaningful translational vision research.

3.1 Rodent retina is specialized for detection of motion and contrast at night

Mice and rats have a relatively low number of cone photoreceptors (~3 % in mice, ~1 % in rats [36, 37]) as compared to rods, but cone-mediated vision is imperative for their behavior. It should be noted that also human retina is largely rod-dominant, with 5% of photoreceptors being cones [29]. However, in the center of the human retina resides fovea, where cone density increases almost by 200-fold as rod density decreases. The centermost part of the fovea, called foveola, is devoid of rods. By contrast, the peripheral-central cone gradient is negligible in the mouse retina [38]. Compared to humans, the average density of rods and cones is large throughout the mouse retina. Photoreceptor density is ~3-4 times higher in the mouse central retina than in the human macula and therefore the phagocytic load per RPE cell is higher in mice than in humans.

Rod photoreceptors in mice and rats are most sensitive for wavelengths at greenish light spectrum at around 500 nm [39, 40]. As most behavioral assays are conducted under mesopic/photopic light levels, and rods bleach and desensitize at relatively low light levels, this article will concentrate on cone-mediated vision. Mouse and rat cone photoreceptors contain two types of opsin pigments: a short wavelength opsin (S-opsin) and a medium/long wavelength opsin (here referred to as M-opsin, but also often called as L-opsin in previous literature). The absorption maxima of these opsins are ~360 nm and 508-510 nm in both rodent species [41,
As conventional visible light spectrum ranges from ~390 nm to 700 nm, the absorption peak of rodent S-pigment is in UV-range. The absorption maximum of the M-opsin (508-510 nm) in laboratory rodents is at relatively low wavelength range compared to human M-opsin at 531 nm (green) [20]. The two other cone opsins in humans have absorption maxima at 420 nm (blue, S-opsin) and 588 nm (red, L-opsin). The lack of long wavelength sensitive opsin in mice and rats renders their vision insensitive under low luminosity to long-wavelength red light (above ~600 nm). This explains why circadian rhythmicity can be maintained in these species in standard vivarium under red light during the dark phase. On the other hand, while mice and rats lack the ability to see the longest wavelengths of visible light, they do see under UV-illumination when we humans are blind. The UV vision in mice and rats highlight the fact that their vision is tuned to work well at night.

Although laboratory rodents have only two cone opsins in their retina, it would be a mistake to say that they possess only two types of cones. A large proportion of cones in mice are neither S- nor M-cones but actually dual-cones, meaning that they are sensitive to both UV and green color range. Although this dual-expression of opsins should be detrimental for color vision, mice can actually learn to discriminate different wavelengths of equiluminous light after extensive training [43]. They do also possess chromatic pathways in the RGC level [44], LGN [45] and V1 [46], which further suggests that mice possess some characteristics of color vision.

The number of RGCs in mice and rats is small due to the small eye size and high convergence of photoreceptors to RGCs, and is estimated to be ~45 000 and 85 000 RGCs per retina, respectively [36, 47]. This leads to average RGC densities of ~3000 cells/mm$^2$ in mice and ~1600 cells/mm$^2$ rats [47, 48], with a substantial variation between strains [49]. In the human central retina, the RGC density goes up to ~35 000 cells/mm$^2$, but the density declines quickly towards the peripheral retina [50]. Human visual acuity is high at the central retina (peak ~60 cycles of degree per visual angle, CPD), but from experience we know that the image is blurry in the peripheral parts of our visual field. Pigmented mouse visual acuity at 0.6 CPD is ~100-fold worse than in humans [51, 52]. However, visual pattern vision is not only determined by visual acuity but also by contrast sensitivity. The peak contrast threshold measured in mice (~2 %) is only 4 times smaller than that reported in humans (~0.5 %) [53, 54]. Finally, there are obviously many anatomical differences between the rodent and primate eyes that lead to large differences in the visual capabilities between these species. Nevertheless, the structure and function of the retina is in principle very similar between rodents and primates,
especially at the retinal periphery [30], a fact that facilitates translational ophthalmic and vision research.

3.2 Rodent optic pathways primarily target limbic midbrain

Once optic nerve axons emanate toward the brain, the majority of them cross at the optic chiasm, i.e. projections from the right eye mainly activate the left side of the brain and vice versa (Fig. 1). The extent of crossing varies between species and is greater in animals with laterally oriented eyes. Around 97% of optic nerve axons radiate to the contralateral side of the brain in the mouse [55], while only 60% do so in humans [29]. Thus, the binocular visual field is small in mice and large in humans. The frontally oriented eyes enable precise visual depth perception that is a useful feature for predatory species. On the other hand, laterally oriented eyes create large visual fields, which may be more important for prey animals. Virtually all optic nerve axons target the dLGN in primates [56], whereas in mice, 70% of optic nerve axons target the SC [57, 58], and in rats virtually all crossing axons [59]. The superficial layers of SC are innervated by optic nerve axons, while neurons in the deeper layers are not generally visually responsive but respond to somatosensory or auditory stimuli [60]. According to current thinking, the superficial part of SC contains a map that represents the visual field, whereas the underlying part of SC contains a vector map of the saccades (a quick reflexive movement of both eyes to fixate in one direction) that shift the direction of gaze [61]. The large number of optic nerves projecting to the SC rather than LGN in rodents highlight the fact that rodent vision is tuned for reflexive behaviors.

3. BEHAVIORAL VISION TESTS

Rodent vision can be tested in numerous of ways. Many of the tests used in published research utilize operant training in mazes [51, 62], and nowadays, modern touch-screen based systems [6, 63]. Laboratory rodents can learn visual tasks even when head-fixed and “exploring” a virtual visible world [64-66]. This allows sophisticated imaging techniques during behavior, such as in vivo two-photon imaging and single-cell electrophysiology without significant movement artifacts [65-67]. While theoretically all aspects of vision could be tested with operant training, practical constraints limit the use of learned tasks in phenotyping or drug studies that often require large number of subjects and repetitions. Training mice to perform operant tasks may require weeks, or even months, and many mice are practically untrainable [68]. As rats learn operant tasks
much more readily than mice [69], rats may be preferred when training-based tasks are a necessity. However, several techniques have been established and well characterized that require little or no training at all. The responses in these tasks rely on instinctive behaviors. These methods include the eye blink response test, visual placing test, visual cliff test, visual water tank, and optomotor response tests.

Perhaps the simplest and fastest way to test if an animal sees altogether is to approach the eyes of the animal with an object, for instance a cotton swap. If the animal is able to see the approaching object, it will blink before the object touches the cornea. A closely similar test is called visual placing test. Here, the animal is lifted from its tail to a height of ~ 15 cm and lowered within 0.5 - 1 s and decelerating as the base, a grid or a tabletop, is approached. A simple scoring for the test can be applied based on the distance of the snout from the base before the animal extends its forepaws toward it [70]. A bit more complex test is the visual cliff test, which was developed by psychologists Gibson and Walk to study depth perception in human infants and several animal species [71]. In this test, the subject is placed in an arena with a continuous glass pane floor. On one side of the arena, a sheet (cloth, paper etc.) with high-contrast checkerboard pattern is attached to the glass pane, while on the other side, the checkboard pattern is placed on a lower level, yielding an impression that there is a cliff separating the two sides. The scoring in the test can be based, for example, on the percentage of time walking over the elevated, ‘safe’ side vs. the lowered, ‘danger’ region. Another option is to test the animal’s choice when it is lowered by the experimenter onto the glass pane in the border between the elevated and lowered regions. This test can distinguish differences between mouse strains [72]. While the above-mentioned tests are fast and easy to perform, they are basically limited to test whether the animal sees at all, but tell little about what an animal can see.

4.1 Visual Water Test

There are a few well-characterized behavioral tests that can effectively assess what rodents can see; i.e. they test pattern vision. Prusky and Douglas established the visual water task that exploits rodents’ instinctive urge to escape from water as soon as possible [73]. The test arena is essentially a Y-maze where the upper two arms are separated with a central divider (see Fig. 3). The animal starts swimming from the start compartment at the maze stem when a gate is opened. The ends of both arms are covered by monitor screens, providing visual cues on which side the submerged escape platform is situated. The positive visual cue can be a horizontal or
vertical sinusoidal grating with adjustable spatial frequency (SF) or contrast. The screen in the other arm without an escape platform shows only a uniform grey pattern. According to Prusky and Douglas, the animals do not need to be explicitly reinforced in this task [68]. Instead, the animals seem to spontaneously compare the screens before entering either of the target arms at the “choice line”. The SF of the stimulus is set by the length of the central divider in the arena (which determines the distance from the choice line to the screens) and the width of visual cycle in the pattern stimulus. Other advantages of the water tank are that water does not easily generate odor trails, and in water the mice tend to focus on the monitors whereas on land the multiple sensory inputs may dampen task performance.

The visual water task has been used to assess many aspects of vision, such as contrast sensitivity, visual acuity, visual discrimination (e.g. distinguishing vertical from horizontal gratings), motion coherence detection, and visual system development in laboratory rodents [73-79]. Importantly, the visual water task yields similar visual acuity measures as acuity determination by pattern VEP (PVEP) studies or learned visual tasks at around 0.5-0.6 CPD in C57BL mice [51, 73, 80]. The test performance clearly depends on visual cortical function, as lesioning a part of V1 dramatically drops visual acuity measures [74]. However, a drawback in the visual water task is that it might still be too labor-intensive for very large-scale studies. According to Prusky and Douglas, the training phase alone takes two to four sessions of 10-15 trials in C57BL mice and Long-Evans rats [73]. The relative time-demanding nature of the visual water task might explain why it has not gained as much popularity as the optomotor response test. In addition, a test that requires learning and motor skills is prone to performance effects that are not dependent on vision.

4.2 Optomotor response test

The optomotor response test (OMR) relies on animal’s reflexive head movements when a grating stimulus is moving in the animal’s visual field [81]. The visual system is not only a sensory but also a sensorimotor system in which proprioceptive and vestibular stimuli drive the direction of gaze. In the OMR test, the animal is placed on an elevated platform that is surrounded by a mechanical drum or by monitor screens from all directions (see Fig. 3). The inner walls of the drum present similar vertical gratings as used in other pattern vision tests. As the drum starts to move, the vestibular input dissociates from the visual input, and in response, the animal reflexively moves its head or the whole body to compensate for the sensory mismatch. The head or body
movements are detected by a video camera overlying the platform. As the SF in the grating increases or the contrast decreases, the animal’s reflexive movements become more and more indistinguishable, and finally cease. Thus, the mechanical OMR can be used to test visual acuity and contrast threshold of laboratory animals in scotopic, mesopic and photopic conditions [82]. A more modern version of the drum-based OMR, where different stimuli needed to be mechanically changed, was first represented by Prusky et al. [83]. Here, monitor screens surround the animal (see Fig. 3) and allow quick alterations in the stimulus characteristics. Fundamentally, the mechanical and computerized versions of OMR are the same.

The computerized OMR has gained relatively broad popularity in mouse vision research perhaps partly due to commercialization of the platform. Recently, sophisticated techniques have been developed where the head movements are automatically detected and quantified [84]. Automated analysis decreases experimenter-bias compared to subjective assessment of head/body movements and should lead to better reproducibility of results. One major advantage of OMR compared to maze-based behavioral vision tests is that it allows assessment of monocular vision without mechanical occlusion of either of the eyes [85]. This is achieved by tracking the horizontal orientation of the animal’s head automatically during testing and projecting the stimulus restrictively only at the monocular visual field. The OMR has been extensively used to assess visual acuity, contrast threshold and motion sensitivity of laboratory animals [81, 83, 86-88]. Numerous research projects have utilized the OMR to show visual restoration in rodent models of retinal degeneration and dysfunction by drug and gene therapies, as well as by insertion of light-activated channels and photoreceptor transplants into the retina [89-93].

The OMR can be supplemented with an optokinetic reflex test (OKR; also called as optokinetic nystagmus) that assesses reflexive eye movements rather than head/body movements in head-fixed animals [81]. The OKR has gained popularity in studies of neuronal circuits [81, 94-96]. In addition, preclinical CNS drug research could greatly benefit from the OKR. Cahill et al. published a comprehensive survey about OKR’s utility in assessment drug actions using dozens of compounds, such as psychostimulants, antipsychotics, antidepressants and opioids [97]. They were able to demonstrate dose-response relationships and provide insight of drug pharmacokinetics, blood-brain barrier penetration and drug-receptor interactions. However, while the OMR and OKR are relatively efficient tests and may allow the fastest quantification of rodents’ pattern vision, a
major downside is that these reflex-based tasks are not dependent on the functionality of the visual cortex but instead are subcortically driven [87]. Furthermore, OMR and OKR share the same challenge as practically all behavioral tests in rodents: the behavioral state affects the test performance, and sometimes the animals may simply “ignore” the test.

4. ELECTROPHYSIOLOGICAL VISION TESTS

Electrophysiological vision tests, i.e. recording electrical activity upon visual stimulation, are objective and not so easily constrained by factors other than vision. Basically, every aspect of vision and every location of primarily visual pathways (at single-cell and network level) can be separately tested using electrophysiology. Electoretinography (ERG) and visual evoked potentials (VEP) are relatively noninvasive and optimal for translational research, since similar methodology can be used in laboratory animals and humans. Guidance for ERG and VEP recordings in laboratory rodent can be found in published literature [98, 99].

5.1 Electroretinography (ERG)

The ERG is recorded from the corneal surface, with electrodes placed either on the bulbar conjunctiva or the lower eyelids in humans [100]. In laboratory animals, the recording is generally performed in anesthetized animals, although a non-anesthetized procedure has also been published [101]. The conventional ERG response is based on changes in ion currents within the retina upon a flash of light. The currents formed in radially oriented retinal cells (i.e. photoreceptors, bipolar cells, Müller glia cells and RPE cells) preferentially flow in the same direction and generate a summed potential, readily be recorded by the distal, corneal electrode. Instead, currents formed in horizontally oriented retinal cell classes (amacrine cells, horizontal cells and RGCs) have negligible contribution to the flash ERG, because the laterally oriented currents cancel each other out and are not recordable at the corneal electrode [102]. The flash ERG can be obtained using a variety of stimulation and background light parameters, and numerous distinct ERG components can be analyzed, each indicating functionality of distinct retinal cell classes [103]. A dark-adapted state is used when one is interested in rod-
mediated vision. Light-adaptation effectively saturates the rods, and thus light-adaptation can be used to extract cone-driven responses. In a general ERG experiment, only the amplitudes and latencies of a- and b-waves are analyzed (see Figs. 5 & 6). The a-wave is a primarily driven by photoreceptor activation [104], whereas the b-wave is mainly generated by activation of glutamatergic ON bipolar cells [25].

As mentioned, the laterally oriented retinal cells have minor contribution to the conventional flash ERG; instead they form the pattern electoretinogram (PERG) response. The PERG is a special technique used to record inner retinal responses [105]. Here, luminance is kept unaltered and the eye is stimulated by contrast reversing patterns on a computer screen [99]. During PERG recording, the radial currents are not seen on the corneal electrode because the luminance in pattern stimulus does not change. Although radial currents are necessary for generating the PERG response, they remain undetected because ON and OFF pathways are stimulated exactly to the same extent (see [106] for a detailed description). Instead, horizontally evoked nonlinear responses will constitute a small local field potential (LFP) in the corneal surface to be recorded, i.e. the PERG.

PERG can be used to test retinal visual acuity, contrast threshold and temporal resolution. Porciatti et al. found that retinal acuity in C57BL mice corresponds to cortical visual acuity as measured by VEPs or behavioral visual tests [51, 52, 74, 102, 107]. Importantly, in disease states, such as in human glaucoma [108] or mouse optic neuropathy [109], the PERG impairment has been shown to be greater than expected from retinal nerve fiber layer thickness and RGC count. It is likely that some RGCs that are viable are not functional [108, 110]. Thus, the PERG holds promise to be able to detect RGC pathologies prior to cell death when the RGCs may be still curable. By current knowledge, PERG may be the most sensitive method to test RGC function [109], exceeding the sensitivity of two other ERG responses that can be used to measure RGC function; scotopic threshold response [111] and photopic negative response [112].

5.2 Visually evoked potentials (VEP)
The VEP is collected from electroencephalogram (EEG) signal and thus its cellular generators are in the brain [99]. The spontaneous EEG activity can be disturbed by sensory stimuli that generate evoked potentials (EPs). Although a single EP is negligible in amplitude compared to the background EEG, averaging tens of responses phase-locked to the visual stimulus will fade out the random EEG fluctuation and bring up the VEP.

In rodents, the VEPs can be recorded from the skull surface overlying the V1 (subcutaneous electrodes), from the cortical surface (screw electrodes) or inside the V1 (LFP electrodes) [99]. Generally, visual stimulation in VEP consists of pattern stimulation rather than flashes of light. The flash-VEP is strongly variable between human subjects [113, 114] and in rats it has a lower predictability from the retinal output than PVEP [99]. The PVEP waveform depends on the recording location as well as on spatial and temporal properties of the stimulus. Porciatti and coworkers performed a laminar analysis at the binocular site of V1 noting a polarity change in PVEP waveforms at ~ 200 µm from the cortical surface [52]. Similar laminar analysis was performed later in non-anesthetized mice by others [115]. The PVEP reached its peak amplitude and fastest latency at ~ 400 µm depth, corresponding to cortical layer IV [52, 115]. In the previous PVEP experiments, visual acuity of the C57BL/6J mouse was determined at 0.6 CPD (see Fig. 4 for an example of visual acuity estimation by PVEP), which is in accordance with behaviorally acquired acuities [51, 74]. Since the PVEP can be recorded in conscious head-fixed mice using chronic electrode assemblies [99], the activation of V1 may be studied in conjunction with behavioral responses. One such behavioral response of a mouse is fidgeting its front paws when visual stimuli are delivered [116]. Cooke and colleagues quantified the amplitude of fidget with a piezo electric sensor to be used in meaningful way, and coined the visually evoked fidget as a “vidget” [116]. In their study, the vidget amplitude corresponded relatively well to the PVEP amplitude. Furthermore, the PVEP can be also combined with simultaneous PERG recording in anesthetized animals [117], providing a comprehensive functional assessment pattern vision channels throughout the primary visual pathways.
Despite its effectiveness and objectivity, the PVEP recording has been little used in vision research conducted in laboratory rodents so far, perhaps due to relative difficulty of the technique. For instance, the setting up and calibration of pattern stimuli is not a trivial task, and an optimal PVEP recording in rodents practically requires stereotaxic surgery. Instead, rodent PVEPs have been used pretentiously in cortical plasticity research. A pioneering study demonstrating ocular dominance plasticity in the adult visual cortex was performed with intracortical PVEPs in behaving mice [115]. A few years later, Shuler and Bear reported that rat V1 neurons learned reward timing in a behavioral task [118]. Recently, mouse V1 was shown to be able to learn and predict spatiotemporal sequences [119]. Collectively, the rodent V1 can be considered as a dynamic cortical area that could be used to study “higher cortical functions” at an easily accessible level [3].

5. DANGER ZONE – VISION PROBLEMS IN RODENTS USED IN BEHAVIORAL ASSAYS

The mouse visual system differs from the human in many ways. Yet, gene defects that cause ocular disease in humans often translate well into genetically engineered mice. In addition to the induction of these purposeful visual defects when studying ophthalmological conditions, genetic engineering may sometimes have unexpected consequences on mouse vision either by confined knowledge of disease manifestations (for instance Alzheimer’s disease can cause visual problems that many are not well aware of) or by lack of knowledge of target protein functions. Indeed, accumulating evidence suggests that most, if not all, neurodegenerative diseases deleteriously manifest in the retina [99, 120]. The most common neurodegenerative diseases, Alzheimer’s disease (AD) and Parkinson’s disease (PD) manifest retinopathy and optic neuropathy where retinal nerve fiber layer degenerates faster than in normal aging [121, 122]. Visual problems reported in AD patients are multifaceted, including problems in depth perception, color recognition, motion detection, spatial contrast sensitivity, and reading and finding objects [123-128]. In PD, the characteristic motor symptoms are wellknown to be caused by neurotransmitter dopamine deficiency, but this may also affect vision in PD patients.
In particular, decreased spatial contrast sensitivity was a common clinical research finding already a few decades ago in PD patients [131-133]. Indeed, dopamine has a central role in retinal light adaptation and contrast sensitivity [134, 135]. Besides human patients, retinal pathology has been observed in several mouse models of AD (see [136] for a review). In PD mouse models, retinopathy and optic neuropathy are less studied although abnormal α-synuclein deposition, a major pathological characteristic of PD, has been found in the retinas of mice modeling PD [137], as well as in retinas of PD patients [138, 139]. While the manifestation of retinopathy in mouse models of AD and PD provides a great opportunity for translational research with respect to vision, it is intimidating from the perspective of behavioral research conducted in these models. Significantly declined photopic vision will certainly cause performance defects in, for instance, Morris water maze test [140], which is a primary research tool in assessment of hippocampal-dependent spatial learning [141].

Pattern vision deficits have been reported in one of the most commonly used AD models, the APP (amyloid precursor protein) and presenilin-1 double-transgenic mice (APP/PS1) [142, 143]. In contrast to previous findings, we did not find pattern vision deficiencies for stationary PVEP stimuli in 13-month-old male APP/PS1 mice [144]. Instead, Grienberger and coworkers found impaired orientation and direction tuning, and hyper and hypoactive neurons in the VI of APP/PS1 mice, as well as decreased performance in visual discrimination task [145]. These findings were likely independent of retinal changes and coincided with progressive amyloid-β pathology in V1. However, none of the above-mentioned studies has directly assessed visual contrast sensitivity function with disease progression in APP/PS1 mice. This should be addressed in future research in conjunction with spatial learning tests.

Several mouse models of rarer neurodegenerative diseases, such as Huntington’s disease (HD) and Neuronal ceroid lipofuscinoses (NCL) manifest retinal pathology [8, 146]. We and others have found that R6/2 mice modeling HD develop impaired photopic vision already before the manifestation of
overt motor symptoms [8, 9]. We recorded delayed photopic ERG responses in these mice already at 4 weeks of age, and by 8 weeks of age the photopic vision was dramatically affected (see Fig. 5). Despite impaired retinal function, the morphology of the retina stayed relatively intact at light-microscopy level. More recently, Karam et al. found evidence that the reduced expression of normal huntingtin protein in R6/2 mouse retinas cause retinal ciliopathy and lead to photoreceptor degeneration [147], providing one explanation for the dramatic visual phenotype in these mice. In several publications, the R6/2 mice have been used in behavioral assays [10-14] and tests of circadian rhythmicity [148, 149]. Results and conclusions derived from behavioral assays that are visually-guided may require re-formatting. Furthermore, a recent report strongly suggests that impairments in the photoreception in HD mice indeed contribute to the progressive dysregulation of circadian rhythmicity and entrainment [150].

NCLs are a group of inherited neurodegenerative diseases known to cause early vision loss and blindness [151]. Many groups have shown that mice modeling different forms of childhood NCLs manifest progressive vision loss [89, 146]. The good news is that the retinopathy has been taken into account in the study design when visually-guided behavior has been studied in NCL mice (e.g. [152-154]). Hopefully, the same trend will continue in studies of mouse models of other neurodegenerative diseases as knowledge of visual abnormalities in genetically engineered mice spreads.

Genetic predisposition is not the only risk factor for retinopathy. Ironically, as the radiation of the visible light spectrum is a prerequisite for vision, it is also detrimental for retinal health if exposed in excess. Light exposure, especially in the blue light spectrum, can lead to photochemical damage if retinal protective mechanisms have been overcome [155]. Wild-type pigmented laboratory rodents are resistant to light damage whereas albinos are highly sensitive. We found that albino Balb/C mice experience drastic retinal dysfunction when maintained in cyclic 250 lux light exposure for one month (12h/12h light-cycle; see Fig. 6). Otherwise the same protocol, but at 2500 lux, led to complete abolishment of the ERG signal. For reference, 250 lux is less than recommended for office working
conditions by a European Union regulation [156] and 2500 lux could be measured at midday during an overcast day [157]. In fact, albino Balb/C mice and Sprague-Dawley rats have been reported to develop retinal degeneration even under vivarium conditions [18, 19]. As albino strains are extremely sensitive for light damage, we strongly suggest housing them in the dimmest part of the laboratory animal facility (for instance, lowest rows of cage racks) whenever possible. We are not aware of other controllable unintended environmental risk factors that would have caused significant visual impairment in laboratory conditions, but some special diets depleted of vitamin A or carotenoids, could lead to such impairment. Some mice and rats develop visible ocular problems (e.g. cataract, atrophy of the eye) by various reasons such as fighting. Needless to say, animals with obvious ocular problems should not be used in visually-guided behavioral tasks.

6. CONCLUSIVE REMARKS

In a few decades, the opinion of laboratory mice being practically useless in vision research has evolved to a great epiphany; we may be able to learn more about mammalian visual processing using laboratory mice than any other species, although research requiring complicated visual tasks or foveal vision still needs to be accomplished in primates. Mice can learn to perform visual tasks in virtual environments while head-restrained, which enables simultaneous optical and electrophysiological imaging at a single-cell and network level [65-67]. This combined to the vast library of genetically engineered mice, and the ease to develop new ones, will lead to better understanding of how the vision, sensory systems and the whole CNS works. Studies on the visual system have been integral to understanding how the sensory systems function [32-35]. Furthermore, a lot what we know about nerve degeneration and regeneration, neuronal plasticity, protection, development and outgrowth has been achieved through the study of the visual system [3, 158-168]. Today, the field of vision and eye research seems to be going through another renaissance. Rapidly developing optical imaging methods of the eye, which may enable non-invasive retinal visualization of single blood cell flow and “brain-neuron-like” RGCs in real-time [169, 170] are opening us an indirect window to the brain and hope for earlier diagnosis of brain diseases [99, 120].
A few well-established behavioral and electrophysiological techniques to test rodent vision were presented in this article. We recommend a bottom-up sequence in visual phenotyping of laboratory rodents. First, one should test whether animals can see at all. This could be done with simple eye-blink response or visual placing tests. If some visually-derived behavior is detected, the next step would determine what the animals can see, \textit{i.e.} to test their pattern vision. The choice whether to choose behavioral or electrophysiological approach is multifaceted. The OMR test may be the fastest way to test pattern vision in laboratory rodents, but the caveat is that the response does not draw on the functionality of the visual cortex. The visual water task is sensitive for dysfunctions in the primary visual pathways but training animals to perform adequately in the task may take a long time and human resources. Further, compromised motor or cognitive functions may affect behaviorally derived pattern vision parameters. Electrophysiological recording of PVEPs is an effective and objective way to test pattern vision in photopic conditions. However, obtaining reliable and meaningful results derived from the EEG signal, such as the VEP, requires expertise of electrophysiology, and setting up the system needs to be undertaken carefully to avoid artifacts. To our knowledge, few, if any satisfactory commercial platforms are available for rodent PVEPs. Fortunately, several user-friendly and proper ERG systems are commercially available helping the initial steps of visual testing in several laboratories. Most visual problems are caused by defects at the photoreceptor level and can be readily detected by the conventional ERG. Finally, regardless of the rather complex nature of vision testing in rodents we certainly hope that it becomes a more common practice. Genetic engineering and environmental factors may have unexpected consequences on vision that may lead to crude biases in the field of behavioral neuroscience and beyond.

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DECLARATION OF INTEREST

The authors declare no relevant commercial interest on the topic.
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**FIGURE LEGENDS**

Fig. 1. Illustration of rodent primary visual pathways. A: A schematic illustration of the rodent eye showing major refractive media. Note the remarkably large lens of the rodent eye. Retinal ganglion cell (RGC) axons exit the eye at the optic nerve head and form the optic nerve. B: Majority of the optic nerves cross at the optic chiasm. Thus, RGC axons from the left (L) eye primarily innervate lateral geniculate nucleus (LGN) and superior colliculus (SC) in the right brain hemisphere. In rodents, a larger proportion of optic nerves target the SC than the LGN. The SC is responsible of coordinating head and eye movements in relation to sensory targets, whereas the LGN is a thalamic relay station between the eye and the primary visual cortex (V1). C: Histological cross-section of a mouse eye showing the layered anatomy of the retina. RGCL (retinal ganglion cell layer) is the closest layer in relation to the vitreous. IPL, inner plexiform layer (site of interneuron-RGC synapses); INL, inner nuclear layer (interneuron nuclei); OPL, outer plexiform layer (site of photoreceptor-interneuron synapses); ONL, outer nuclear layer (photoreceptor nuclei); PIS, photoreceptor inner segments; POS, photoreceptor outer segments; RPE, retinal pigment epithelium.

Fig. 2. Evolution of vision-related research in common laboratory animals and humans. Vision-related research utilizing mice has been in steep increase during the past few years. If this linear trend continues, a few thousand vision-related articles using mice will be indexed to PubMed annually by 2036. However, the number of research articles on vision in primates is over 10-fold greater than research articles on vision in mice currently.

Figure 3. Schematic drawings of the visual water tank (left panel) and optomotor response (OMR, right panel) apparati. The visual water task always starts with a pre-training phase where the animals are taught that there is an underwater escape platform in front of a monitor screen displaying a pattern stimulus [73]. In the next
training phase, the animals start swimming from the start compartment once the release gate is opened. The correct choice, and the hidden platform, are cued with a high-contrast, low spatial frequency (SF) stimulus. Once the animals reach near-perfect (> 90% correct choices in at least 10 trials) performance, testing can be initiated. In the testing phase, the stimulus characteristics can be modulated by altering SF or contrast of the gratings, allowing determination of the animal’s visual acuity (VA) and/or contrast threshold (CT). The OMR test does not require any other training but a brief acclimatization to the apparatus. The animal is placed onto an elevated platform surrounded by a 360 deg grating stimulus. When the gratings start to move, the animal reflexively follows the movement direction with its head and body. By changing the SF or contrast, VA and CT can be determined. The figures are re-drawn and adapted from [73, 83].

Figure 4. Measurement of visual acuity by pattern visual evoked potentials (PVEPs) in mice. PVEPs are recorded in response to grating reversal stimuli (here vertical sinusoidal “white and black” bars). The left graph shows averaged PVEP responses at 5 different spatial frequencies (SF) of 5 mice. As the spatial frequency increases (bars get narrower) it becomes more and more difficult for the mouse to see the stimulus. Consequently, the VEP amplitude decreases and the latency of the main component increases. Note also the flat response to 0.914 cycles per degree (CPD), indicating that the mouse does not see the stimulus. When the VEPs are recorded for 3 or more SFs, the visual acuity can be extrapolated as shown in the right graph. The data were collected in awake head-fixed mice (n=5, 13-month-old, C57BL background) that were implanted with a chronic intracortical electrode assembly. The data are adapted from [144].

Figure 5. Illustrative figures of retinopathy in mouse models of Huntington’s disease (HD) and CLN5 form of neuronal ceroid lipofuscinosis. HD mice (R6/2 model) represents an example where retinopathy is not expected given the lack of prominent visual abnormalities in HD patients. In contrast, childhood forms of NCLs are clinically characterized by strong retinal degeneration. A: Group-averaged photopic ERG traces (band-filtered between 70-150 Hz) in R6/2 (grey) and wild-type (WT, black) control mice show progressive and robust decline in photopic retinal function in R6/2 mice. B: Especially, the latency of photopic ERG components were significantly delayed in R6/2 mice. C: A representative example of WT retina at 12 weeks
of age (scale bar 50 μm). D: Apart from the wavy appearance of the outer retina and thinning of the OPL in R6/2 mice, the retinal morphology remained relatively normal in these mice at the light-microscopy level. E: Group-averaged scotopic ERG traces (band-filtered between 70-150 Hz) in CLN5 mice and their WT controls illustrate declined a-wave amplitude at 2 months and strongly impaired retinal function at 6 months of age in CLN5 mice. F: The scotopic ERG a- and b-wave amplitudes correlate (a-wave: $R^2=0.80$, p<0.001; b-wave: $R^2=0.75$, p<0.01; Pearson rho) with the average thickness of ONL in a disease state. G: A representative example of WT retina at 6 months of age. H: The retinopathy in CLN5 mice is characterized by loss of ONL nuclei and degeneration of photoreceptor outer and inner segments, as expected from the ERG anomalies. RGCL, retinal ganglion cells layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PIS, photoreceptor inner segments; POS, photoreceptor outer segments; RPE, retinal pigmented epithelium. The data are adapted from [8, 146].

Figure 6. Light-damage in albino Balb/C mice as shown by decrease in scotopic ERG responses. Mice were kept under fluorescent tubes at cyclic (12 h lights on – 12 h lights off) 250 klux and 2500 lux illumination for 1 month. Note that 250 klux is less illumination than in general office conditions [156]. A: Scotopic ERG (thin traces are individual responses and the thick line is averaged response from those) at baseline in 6-week-old Balb/C. B: Similar recording in the same mice after a month of cyclic 250 lux light exposure. The ERGs were recorded for at least 14 h after the last light cycle in fully dark-adapted mice. C: Three mice were kept at 2500 lux cyclic light and did not yield detectable ERG response after 1-month exposure. D: Statistical analysis of b-wave amplitudes in mice that were exposed to 250 lux cyclic light (the reference flash, log 0, yielded approximately 4500 photoisomerization per rod). The data were analyzed by ANOVA for repeated measurements followed by Bonferroni’s posthoc test (***, p<0.001).
Figure 2
Fig 4
Fig. 5
Fig. 6