Visual performance in mice: physiology meets behaviour

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Neuroscientists frequently need to characterize the sensory abilities of animals under different experimental conditions. For example, it might be of interest to determine how “good” an animal is able to see. In this case, often used benchmarks to determine visual performance are visual acuity and contrast sensitivity, the ability to resolve fine details in the visual scene, and contrast sensitivity, the ability to discriminate between differences in the brightness of visual stimuli. However, behavioral or electrophysiological methods for the determination of visual performance and perception in rodents are often time consuming or require complex and invasive surgeries (1-5). In addition, in most cases it remains elusive whether values of visual acuity and contrast sensitivity determined with behavioral tests are comparable to measurements obtained with physiological techniques at the level of individual animals.

A well-established physiological technique to examine responses of sensory cortices to external stimuli is optical imaging based on intrinsic signals. It enables non-invasive measurements of cortical activity over large brain areas with a high spatial resolution (6). In the primary visual cortex (V1), for example, optical imaging can be performed either episodically (7,8) or with periodically presented stimuli (9-12). Periodic imaging combines continuous-periodic stimulation with continuous data acquisition (9,13). In this way, intrinsic noise can be effectively separated from the stimulus-evoked cortical responses using Fourier analysis, drastically speeding up the imaging procedure (6,9).

Episodic imaging has also been shown to be suitable for determining visual acuity and contrast tuning at the level of V1 (14,15). Although less time consuming, periodic imaging has not yet been adapted to these purposes. In addition, it is unclear whether acuity and contrast sensitivity values obtained by measuring visually driven V1 activation reflect the behaviorally determined thresholds for these visual abilities.

In order to address this concern we developed a novel approach to determine these parameters of vision in mice using periodic intrinsic signal imaging. Since periodic imaging is typically performed in anesthetized animals (9,13,16,17), we first determined the appropriate level of anesthesia that allows to obtain reproducible visuotopic maps of high quality in V1 of mice. We then compared visual acuity and contrast sensitivity thresholds obtained by optical imaging in a group of mice with values from another group of mice where these data were obtained in a behavioral test. For the behavioral experiments we used the visual water task (VWT) which is often used for assessing mouse visual perception (4,11,18,19). Finally, in a last set of experiments, we first trained another group of mice in the VWT and then used the same animals to determine spatial resolution and contrast sensitivity with optical imaging. Remarkably, we could show that our measurements of visual perception obtained with intrinsic imaging were almost identical to measurements determined using the behavioral VWT, even at the level of individual animals. Thus our novel imaging approach provides reliable data of mouse visual perception which can be obtained in a rapid and relatively non-invasive manner.

MATERIALS AND METHODS

Animals and rearing conditions

For the present study we used adult male mice C57BL/6 [SP120, Jackson labs] mice were raised in a group of 2-3 in transparent standard cages [16.5 × 22.5 cm] on a 12 h light/dark cycle, with food and water available ad libitum. Generally, the environment in the cage was minimally enriched with cotton rolls and nest material. In our mouse facility the light intensity was about 150-170 lux. Animal housing in our institution is regularly supervised by veterinaries from the state of Thuringia, Germany. All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive 2010 [2010/63/EU], and were approved by the Thuringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz [Thuringia State Office for Food Safety and Consumer Protection] under the registration number 02650/14 and 02632/16.

Preparation of the animal for optical imaging

As described previously (10-12,20), animals were initially anesthetized with 4% isoflurane in a mixture of 1:1 O2/N2O and placed on a heating blanket [37.5°C] for maintaining body temperature. Subsequently, mice received injections of chlorprothixene [40 µg/mouse, i.m.] and carprofen [4 mg/kg, s.c.]. The inhalation anesthesia was applied through a plastic mask and maintained at 0.5% in control mice during the experiment. The animal was fixed in a stereotaxic frame and we removed the skin of the left hemisphere to expose the visual cortex. The exposed area was covered with 2.5% agarose in saline and sealed with a glass coverslip. Cortical responses were always recorded through the intact skull.

Manipulation and assessment of anesthesia depth

To investigate the influence of anesthesia on optical imaging results, we increased the isoflurane concentration in a stepwise manner to 0.75%, 1% and 1.25%. At each concentration, the animal was allowed to reach a stable state for 10-20 min, before a pair of maps was acquired for 10 min in total using intrinsic signal imaging (12). V1 maps in control mice were recorded with optical imaging. Remarkably, we could show that our measurements of visual performance obtained with intrinsic imaging were almost identical to those determined using the behavioral VWT, even at the level of individual animals. Thus our novel imaging approach provides reliable data on mouse visual perception which can be obtained in a rapid and relatively non-invasive manner.
in the same temporal rhythm, but with stable anesthesia at 0.5% isoflurane.

Optical imaging

Responses of the mouse visual cortex were recorded as originally described by Kalatsky and Stryker (9). Briefly, the method uses a periodic stimulus that is presented to the animal for some time and cortical responses are extracted by Fourier analysis. We used different periodic stimuli in our experiments. For the assessment of map amplitude and map quality in relation to anesthesia, the eyes were stimulated with a vertically drifting horizontal bar of 2° width, moving at a temporal frequency of 0.125 Hz, with 80° distance between bars.

The stimulus was created by custom software [Kalatsky & Stryker (9)]. Both maps for upward and downward drifting bars were acquired and averaged for the removal of the hemodynamic delay. For the measurement of visual acuity and contrast tuning, we created horizontal sine-wave patterns which reversed every eight seconds, using the program Psychopy (11,12). All stimuli were presented on a high refresh rate monitor [HiRacchi Accuview HM 4921D] placed 25 cm in front of the animal. Visual stimulation was adjusted such that it only appeared in the binocular visual field of the recorded hemisphere [-5° to +15° azimuth, -17° to +60° elevation]. The stimulus was presented alternately to each eye for 5 min [for the moving bar stimulus].

All images were all acquired directly through the skull. A Dalsa 1M 30 CCD camera [Dalsa, Waterloo, Canada] with a 135 mm × 50 mm tandem lens [Nikon, Inc., Melville, NY] first recorded images of the surface vascular pattern via illumination with green light [550 nm] and, after focusing 600 µm below the cortical surface, the intrinsic signals were obtained via illumination with red [610 nm] light.

Frames were acquired at a rate of 30 Hz and temporally averaged to 7.5 Hz. The 1024 × 1024 pixel images were spatially binned to a 512 × 512 resolution. For determination of contrast sensitivity we used maximum responses of the amplitude maps to each contrast. Data points were fitted using a Naka-Ruston equation (15,22,23) to obtain the contrast-tuning-curves. For each animal we determined the C50 as the contrast at the half maximum response. This value was defined as cortical contrast sensitivity (15). All data were analyzed using MATLAB.

Visual water task

To assess behavioral contrast sensitivity and visual acuity, we used the VWT, a visual cortex dependent visual discrimination task based on reinforcement learning (1,14,24,25). Initially, animals were trained to distinguish vertical sine wave grating with a low spatial frequency [0.1 cyc/deg] and 100% contrast from a grey with the same luminance. Subsequently, their ability to perceive varying contrasts at a spatial frequency of 0.2 cpl was tested. In another group of mice we determined visual acuity at 90% contrast. The apparatus is a water filled trapezoidal-shaped pool, with two monitors placed side by side on the wider end. A midline divider between the two monitors sets the choice point between both visual stimuli. Below the monitor showing the sine wave grating a submerged platform is placed invisible to the animals. The position of the grating and the platform was changed in a pseudorandom manner during training and testing. Animals were trained and tested 10 times in one session, and two sessions separated by 3 h were run in a single day. The task was performed with off-switched room lights. After achieving 90% accuracy, we determined the contrast or spatial frequency thresholds by reducing the contrast or spatial frequency of the sine wave gratings until the level of correct attempts dropped below 70%.

After achieving a stable level of visible contrasts or spatial frequencies for 10 days, all runs within this time were used to calculate frequency-of-seeing curves for each animal, i.e., percentage of correct responses was plotted against contrast or spatial frequency, respectively. The values at which the curve dropped below 70% were calculated by interpolation.

Statistical analysis

Visual cortex response amplitudes and the scatter of the obtained maps under constant and varying isoflurane concentrations were compared by a paired of unpaired t-test. Optical imaging and VWT data of individual animals were compared by a Wilcoxon signed-rank test. The level of significance was set as *p<0,05; **p<0,01; ***p<0,001. Data are presented with mean and standard error of the mean [سع.]

RESULTS

Anesthesia levels affect the strength and the scatter of the visually evoked V1 maps

As a first step, we investigated whether alterations in the anesthetic level affect cortical maps of the primary visual cortex [V1] obtained by Fourier based periodic imaging. For this, we gradually increased the concentration of isoflurane from 0.5% to 1.25% isoflurane during one optical imaging session. At each step animals were stimulated visually with a moving light bar displayed on a black monitor screen and we recorded the responsiveness of V1 of the left hemisphere. Figure 1A depicts representative color coded phase and greyscale magnitude V1 maps evoked by visual stimulation of the contralateral eye at constant [left] and increasing [right] isoflurane concentrations.

It is clearly visible that at constant isoflurane concentrations the phase maps remained smoothly retinotopically organized and the activity patches of the amplitude maps were equally strong during the time tested [4-5 h]. However, increasing the isoflurane concentration led to an impairment of the phase map quality, predominantly at 1.25% isoflurane, and the activity spots of the amplitude maps got gradually weaker.

Our quantification revealed that in control animals [with constant 0.5% isoflurane anesthesia], V1 responsiveness to both contralateral and ipsilateral eye stimulation remained stable during the imaging session (Figures 1B and 1C) (Table 1), n=4, V1 activation elicited by contralateral eye input: all p-values >0,05, paired t-test; V1 activation elicited by ipsilateral eye input: all p-values >0,05, paired t-test). In contrast, V1 activity evoked by visual stimulation of the contralateral or ipsilateral eye stimulation gradually decreased with increasing isoflurane concentrations (Figures 1B and 1C) (Table 1), n=4, V1 activation elicited by contralateral eye input, 0.5% vs. 0.75% [isoflurane]: p=0.35, 0.5% vs. 1%: p=0.03; 0.5% vs. 1.25%: p=0.02; 0.75% vs. 1%: p=0.02; 0.75% vs. 1.25%: p=0.02, paired t-tests; V1 activation elicited by ipsilateral eye input, 0.5% vs. 0.75%: p=0.009, 0.5% vs. 1%: p=0.003; 0.5% vs. 1.25%: p=0.003; 0.75% vs. 1%: p=0.003; 1% vs. 1.25%: p=0.04, paired t-tests). These changes of V1 responsiveness were significantly different from control values at 1% and 1.25% isoflurane concentration for
both, V1 contralateral and ipsilateral eye input (Figures 1B and 1C) (Table 1). Contralateral eye input, 0.5% vs. 1%: p=0.03; 0.5% vs. 1.25%: p=0.009, unpaired t-tests; ipsilateral eye input, 0.5% vs. 1%: p=0.0005; 0.5% vs. 1.25%: p=0.0002, unpaired t-tests).

In summary, our data indicate that the concentration of isoflurane and thus, the depth of anesthesia markedly affect the strength of V1 responses evoked by visual stimuli.

| TABLE 1 |

V1 responses evoked by contra or ipsilateral eye stimulation under constant [Control group] or decreasing [Experimental [Exp. group]] isoflurane concentrations

<table>
<thead>
<tr>
<th>Control group [n=4], % isoflurane</th>
<th>Contra [×10^5]</th>
<th>Ipsi [×10^5]</th>
<th>Exp. group, % isoflurane</th>
<th>Contra [×10^5]</th>
<th>Ipsi [×10^5]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2.34 ± 0.42</td>
<td>1.76 ± 0.11</td>
<td>0.5</td>
<td>2.18 ± 0.23</td>
<td>1.55 ± 0.11</td>
</tr>
<tr>
<td>0.23 ± 0.23</td>
<td>1.44 ± 0.02</td>
<td>0.75</td>
<td>1.9 ± 1.19</td>
<td>1.29 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>0.21 ± 0.24</td>
<td>1.4 ± 0.04</td>
<td>1</td>
<td>1.4 ± 0.12</td>
<td>0.82 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2.2 ± 0.23</td>
<td>1.33 ± 0.1</td>
<td>1.5</td>
<td>1.0 ± 0.21</td>
<td>0.45 ± 0.01</td>
</tr>
</tbody>
</table>

Often, there is a positive correlation between the strength of the intrinsic signals and the quality of the map which can be quantified by the so called map scatter (21). However, in some cases stronger responses can also weaken the quality of the corresponding maps (26). In order to examine the effects of the anesthesia level on the quality of the retinotopic polar maps, we calculated the map scatter in each recording session.

Our quantification revealed that the scatter of the V1 maps evoked by visual stimulation of the contral and ipsilateral eye remained constant within 4.5 h under stable isoflurane concentration of 0.5% (Figures 1D and 1E) (Table 2) n=4. Scatter of the V1 maps evoked by contralateral eye input: all p-values >0.05, paired t-tests; Scatter of V1 maps evoked by ipsilateral eye input: all p-values >0.05, paired t-tests.

However, increasing the isoflurane concentration led to a slightly increased scatter of the evoked V1 maps, which then markedly increased at the highest isoflurane concentration [1.25%] (Figures 1D and 1E) (Table 2) n=4. Scatter of the V1 maps evoked by contralateral eye input, 0.5% vs. 0.75% vs. 1%: all p-values >0.05; 0.5% vs. 1.25%: p=0.03; 1% vs. 1.25%: p=0.04, paired t-tests; Scatter of the V1 maps evoked by ipsilateral eye input, 0.5% vs. 0.75% vs. 1%: all p-values >0.05, 0.5% vs. 1.25%: p=0.0002; 1% vs. 1.25%: p=0.02. Taken together, our data suggest that increasing the isoflurane concentration and thus altering the anesthetic state of the animal dramatically affects the strength and quality of visually evoked V1 maps. Particularly, V1 maps with the strongest amplitudes and the best quality were always obtained at the lowest concentration of isoflurane [0.5%].

| Table 2 |

Scatter of V1 maps elicited by contra or ipsilateral eye stimulation under constant [Control group] or decreasing [Exp. Group] isoflurane concentrations

<table>
<thead>
<tr>
<th>Control group [n=4], % isoflurane</th>
<th>Contra</th>
<th>Ipsi</th>
<th>Exp. Group, % isoflurane</th>
<th>Contra</th>
<th>Ipsi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2.36 ± 0.49</td>
<td>4.8 ± 1.45</td>
<td>0.5</td>
<td>4.27 ± 1.16</td>
<td>4.04 ± 0.38</td>
</tr>
<tr>
<td>0.5</td>
<td>2.8 ± 0.62</td>
<td>6.55 ± 2.0</td>
<td>0.75</td>
<td>8.34 ± 0.68</td>
<td>4.2 ± 0.39</td>
</tr>
<tr>
<td>0.5</td>
<td>4.37 ± 1.77</td>
<td>7.28 ± 2.55</td>
<td>1</td>
<td>7.1 ± 1.74</td>
<td>12.7 ± 2.56</td>
</tr>
<tr>
<td>0.5</td>
<td>3.87 ± 1.31</td>
<td>6.58 ± 1.82</td>
<td>1.5</td>
<td>19.6 ± 3.44</td>
<td>23.89 ± 1.22</td>
</tr>
</tbody>
</table>

Determination of contrast sensitivity and visual acuity using Fourier based periodic intrinsic optical imaging

Knowing the anesthetic conditions to obtain high quality visually evoked V1 maps [0.5% isoflurane], we further asked whether periodic optical imaging [OI] under these conditions is also appropriate for assessing the visual perception of mice. To determine contrast sensitivity and visual acuity, we adapted the periodic OI method originally developed by Kalatsky and Stryker (9). Here, the account of our initial failures is interesting and instructive in its own right.

Our first approach was to measure V1 responses after binocular stimulation with the standard drifting white-on-black square bar. Surprisingly, visual cortical retinotopic maps did not deteriorate with decreasing bar width, but remained strong and well organized even when the resolution limits of the screen were reached (data not shown). We therefore next tried a drifting horizontal sine wave [consisting of one black and one white bar on grey background] of different spatial frequencies at a temporal frequency of 0.125 Hz. This, too, failed to provide reliable values of visual acuity. Although there was a clear negative correlation between map amplitude and spatial frequency, the resulting visual acuity values were widely scattered and always far beyond the behaviorally confirmed visual capabilities (data not shown).

Therefore, instead of a drifting visual stimulus, we next presented a stable horizontal sine wave grating of varying contrasts or spatial frequencies in the binocular visual field for 2.5 min, reversing after different time periods [2s, 4s, 8s, 12s and 16s].

Pattern reversal after 2s and 4s provided only weak maps with poor quality. Using pattern reversal after 12s and 16s evoked strong maps, which were, however, of low quality [data not shown]. However, V1 maps with both high amplitudes and high quality were always obtained by reversing the pattern after 8s (Figure 2A). Hence, we used this visual stimulus timing to determine contrast sensitivity and visual acuity. In order to quantify contrasts sensitivity, we measured the V1 response amplitudes evoked by visual stimulation with 5 different contrasts [90%, 50%, 20%, 10% and 5%] at 0.2 cycles/degrees per degree, which has been described as the spatial frequency at which the behavioral contrast sensitivity of mice peaks (27).

Data points were fitted using a Naka-Rushton equation (15,22,23) to obtain contrast-tuning-curves. For each animal we determined the C50 as the contrast at the half maximum response and defined this value as contrast sensitivity.

Figure 2B depicts the presented visual stimuli of different contrasts and the related magnitude maps of V1 obtained after binocular visual stimulation of one representative animal. It is clearly visible that the activity spots got weaker along with decreasing the contrast, while a grey blank stimulus did not evoke activity in V1.
In order to determine visual acuity, we used the same visual stimulus pattern as for contrast measurements (Figure 3A). Here, we measured the V1 response to binocular visual stimulation with reversing sine wave gratings at 5 different spatial frequencies [0.1 cpd (cycles per degree), 0.2 cpd, 0.3 cpd, 0.4 cpd, 0.5 cpd] at 90% contrast. For determining the background signal of V1, we presented a grey blank with the same luminance as the sine gratings. The obtained amplitude values for each condition were fitted by a linear regression and the spatial frequency at the null response was used as a proxy for visual acuity.

Figure 3A illustrates the visual stimuli and the related amplitude maps of V1 evoked after binocular stimulation with decreasing spatial frequencies of one individual animal. Activity spots got weaker from the lowest to the highest spatial frequency and the blank stimulus evoked no activity patch within the V1 area. Our quantification showed that in all 7 animals tested the amplitudes of the V1 response gradually decreased with increasing spatial frequencies.

Fitting the amplitude values of the single animals with a linear regression revealed a high correlation with the spatial frequency [n=7, mean R square =0.9539 ± 0.037] (Figure 4B). Calculating the mean of the intersections of the linear regressions with the y axis revealed an average visual acuity of 0.53 ± 0.03 cyc/deg (Figures 4B and 4C). Taken together our results show that Fourier based periodic imaging is useful method to determine V1 contrast and spatial frequency tuning.

In summary, these data show that determination of mouse visual perception with periodic OI provides reliable data almost identical to those obtained by conventional methods like the VWT. Strikingly, these results reveal a close match of visually evoked V1 amplitudes and V1 dependent behavior.

**DISCUSSION**

In the present study we described a novel physiological technique which allows the determination of contrast sensitivity and visual acuity in mice in a fast and simple manner. Notably, we found a close match of visual acuity and contrast sensitivity values obtained by optical imaging and by a commonly used behavioral task [VWT], even at the level of individual animals. Hence, our novel method provides highly reliable values of mouse visual abilities.

**Quantification of visual abilities by optical imaging**
In experimental animals, visual acuity is commonly operationalized as the threshold for behavioral discrimination of sine wave gratings from equi-luminant grey (1,27).

Unlike measures depending on subcortical reflexes [e.g. the threshold for the optokinetic reflex (24)], discrimination tasks depend on the visual cortex. It has therefore become experimental standard to target the visual cortex directly for measuring visual acuity. Recordings of visually evoked potentials [VEP] are routinely used to this end (2,5,28) delivering visual acuity values of approximately 0.5-0.6 cpd in mice. Similar visual acuities have been directly for measuring visual acuity. Recordings of visually evoked potentials [VEP] are routinely used to this end (2,5,28) delivering visual acuity values of approximately 0.5-0.6 cpd in mice. Similar visual acuities have been measured by episodic optical imaging (14) and now, in the present study, by periodic optical imaging (Figure 2). The values are in the same range as those obtained by behavioral testing (1,4,25,29) and, remarkably, show a close correspondence with behaviorally obtained values in individual animals, confirming the validity of our method (Figure 4).

In order to generate valid data, we had to stimulate the animals with a reversing sine-wave grating, rather than with the usual moving single bar. If a moving bar of decreasing width was used, the elicited visual cortical activities would either-for a sine bar-scatter widely around too high values, or even-for a square, white-on-black bar remain at constant high levels all through to the limits of screen resolution. These observations illustrate the important difference between detection and discrimination: Whereas single moving visual events merely need to be detected, pattern-reversing sine wave gratings stimuli must be discriminated from uniform grey. The latter kind of stimulus is therefore used by researchers measuring visual acuity with VEPs or episodic optical imaging (2,3,14), and needed to be employed in order to adapt periodic imaging to this end (Figures 2A and 3A). Unlike visual acuity, contrast sensitivity has rarely been measured in mice. Using the visual water task, Prusky and colleagues (24) have established a contrast tuning curve for mice which peaks at 0.2 cyc/deg, where mice are able to distinguish a contrast of approximately 17%. This value is precisely confirmed by VEP recordings (2,3,5) and now by the present study by intrinsic signal imaging (Figure 3). Again, there was a close correspondence between these physiological data and the data obtained by behavioral tasks. We therefore believe that the method developed here will become useful for researchers studying longitudinal changes in the visual capabilities of experimental animals.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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