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Progressive Effects of Sildenafil on Visual Processing in Rats

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Abstract—Photoreceptors are light-sensitive cells in the retina converting visual stimuli into electrochemical signals. These signals are evaluated and interpreted in the visual pathway, a process referred to as visual processing. Phosphodiesterase type 5 and 6 (PDE5 and 6) are abundant enzymes in retinal vessels and notably photoreceptors where PDE6 is exclusively present. The effects of the PDE inhibitor sildenafil on the visual system, have been studied using electroretinography and a variety of clinical visual tasks. Here we evaluate effects of sildenafil administration by electrophysiological recordings of flash visual evoked potentials (VEPs) and steady-state visual evoked potentials (SSVEPs) from key regions in the rodent visual pathway. Progressive changes were investigated in female Sprague-Dawley rats at 10 timepoints from 30 min to 28 h after peroral administration of sildenafil (50 mg/kg). Sildenafil caused a significant reduction in the amplitude of VEPs in both visual cortex and superior colliculus, and a significant delay of the VEPs as demonstrated by increased latency of several VEP peaks. Also, sildenafil-treatment significantly reduced the signal-to-noise ratio of SSVEPs. The effects of sildenafil were dependent on the wavelength condition in both assays. Our results support the observation that while PDE6 is a key player in phototransduction, near full inhibition of PDE6 is not enough to abolish the complex process of visual processing. Taken together, VEPs and SSVEPs are effective in demonstrating progressive effects of drug-induced changes in visual processing in rats and as the same paradigms may be applied in humans, representing a promising tool for translational research. © 2020 The Author(s). Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key words: sildenafil, PDE6, visual evoked potential, steady-state visual evoked potential, rat.

INTRODUCTION

Photoreceptors are critical for light detection in the retina. The transduction of light into a neural signal occurs in the outer segment of the photoreceptors. During this process, photons excite light-sensitive G-protein coupled receptor proteins called rhodopsins, activating a retina-specific phosphodiesterase type 6 (PDE6) (Wert et al., 2014), which hydrolyzes cyclic guanosine monophosphate (cGMP) causing closure of sodium channels in the outer segment, hyperpolarizing the photoreceptor. This triggers a cascade of cellular processes transforming the light signal into electrochemical signals that are propagated to post-synaptic neurons (Sawinski et al., 2009).

Retinal ganglion cells (RGCs) and especially the intrinsically photosensitive (ipRGCs) are also involved in transduction of light in the rat visual pathways. The ipRGCs are implicated in triggering light-induced reflexes such as pupillary constriction and regulation of the circadian rhythm (Reifler et al., 2015; Jiang et al., 2018). The exact mechanism of photoreception in ipRGCs is not fully elucidated but previous studies propose involvement of the photopigment melanopsin through cGMP-independent and thus PDE-independent mechanisms (Warren et al., 2006; Jiang et al., 2018). The ipRGCs project to cardinal relays of visual cortex in mice and rats, i.e. the dorsolateral geniculate nucleus, but whether they exist in higher order mammals remains to be elucidated (Reifler et al., 2015).

Sildenafil citrate (henceforth, 'sildenafil') is a PDE inhibitor which mainly targets PDE5. Sildenafil also inhibits PDE6, but with a 10-fold lower efficacy (Wallis, 1999). Sildenafil (marketed as Viagra® among others) is generally well tolerated but some patients experience temporary visual impairments such as changes in color discrimination (Laties and Zrenner, 2002;

Martins et al., 2015), blue color tinge to vision, transient blindness and reductions in the amplitude of electroretino-grams (ERGs) (Vobig et al., 1999; Jägle et al., 2004). Due to the localization of PDE6 in the internal membranes of retinal photoreceptors (Cote, 2004) and bipolar cells (Shiells and Falk, 2002; Moschos and Nitoda, 2016), these visual disturbances have been ascribed to inhibition of PDE6. Inhibition of PDEs increases cytoplasmic levels of cGMP (Loughney and Ferguson, 1996) preventing hyperpolarization of the outer segment of the photorecep-tors (Wert et al., 2014). Furthermore, sildenafil is a hypotensive agent causing decreases in systemic blood pressure temporarily increasing the intraocular blood pressure (Gerometta et al., 2011). This effect has been associated with reductions in retinal electrical potentials as measured by ERG (Bui et al., 2005).

Sildenafil has been used to investigate retinal PDE6 function in both humans (reviewed in Laties and Zrenner, 2002), monkeys (Kinoshita et al., 2015), dogs (Wallis, 1999) and rodents (Wallis et al., 1999; Nivison-Smith et al., 2014). But PDE6 inhibitors are not the only source for evaluation of functional effects of deficient PDE6 function. Several severe retinopathies, like achro-matopsia and retinitis pigmentosa, may be caused by mutations in genes encoding PDE6 (Zhang et al., 2005; Heckenlively and Arden, 2006; Gopalakrishna et al., 2017) and diseases impacting retinal function represent some of the most common forms of neurodegenerative disorders (Hartong et al., 2006; He et al., 2014). Conse-quently, these patient populations, and recent murine models (Nivison-Smith et al., 2014), have contributed to the current knowledge on the association between PDE6 and the visual pathway.

The transient or flash visual evoked potential (VEP) is an event-related potential (ERP) representing net changes in post-synaptic electrical potentials as a response to sensory stimulations. The VEP is considered to be a measure of neurological integrity of the visual pathway (Iwamura et al., 2003; You et al., 2011). Steady-state visual evoked potentials (SSVEP) are VEPs evoked by high frequency stimuli presumably causing a summation of the VEPs (Vialatte et al., 2010). SSVEPs are most appropriately analyzed in the frequency domain by a Fourier transform yielding information about the amplitude and the phase of the evoked potential (Regan, 1989; Luck, 2005; Norcia et al., 2015). As opposed to VEPs, it has been proposed that SSVEPs may enable differentiation between different cell types in the retina. These observations are based on recordings of visual evoked potentials in *Drosophila*. Here, photore-ceptors are shown to respond to the frequency of the stim-ulus while second lamina cells (invertebrate RGCs) seem to respond at double rate corresponding to the second har-monic (Afsari et al., 2014). Whether this interpretation is also applicable for rodents remains to be elucidated.

In rodents, the superior colliculus is the major retinorecipient nucleus and the visual evoked potential here is comparable to responses obtained by ERG (Sefton et al., 2014). The rodent visual cortex receives visual information relayed from different nuclei and diverse parts of the brain, but no direct signal from the

retina (Sefton et al., 2014). Consequently, we chose to record from these anatomical areas. Some types of RGCs have been shown to be sensitive to light in the blue part of the spectrum (Panda et al., 2005; Baden et al., 2016; Lagman et al., 2016). Thus, stimuli with wavelengths overlapping these spectra were used to probe the contri-bution of different cell types (photoreceptors vs RGCs) to the evoked signal. Surprisingly, no study has yet used electroencephalography (EEG) to characterize visual effects of sildenafil in either clinical or preclinical studies although this technique has a high translational potential. Consequently, we investigated rodent electrophysiologi-cal responses during visual processing by characteriza-tion of flash VEPs and SSVEP recorded from the superior colliculus and the visual cortex. We studied how net electrophysiological activity changed over time following administration of sildenafil and thus PDE6 inhibi-tion, thereby modelling progressive changes in retinal PDE6 function.

EXPERIMENTAL PROCEDURES

All animal experimentation was carried out according to European Communities Council Directive (86/609/EEC) and Danish legislation on care for laboratory animals.

Animals

Eight Sprague Dawley (SD) female rats (Taconic, Denmark) weighing 225 g on arrival, were kept on a reversed 12 h circadian cycle (lights on at 18:00 h). They were single housed in Makrolon type IV cages with wood bedding, food and water ad libitum. The cages were enriched with food enrichment (once a week), a red house, nesting material and wood for gnawing. The temperature in the housing room, was set at $22 \pm 1.5\,°C$ and the humidity at 55–65%.

Surgery

The animals were anesthetized using subcutaneous (SC) injections of Hypnorm® (Lundbeck, Valby, Denmark), midazolam 5 mg/ml (B.Braun, Melsungen, Germany) and saline in a 2:1:1 relation (2.0 ml/kg), yielding 157 μg/kg fentanyl. Norodyl (carprofen 5 mg/kg) (ScanVet, Fredensborg, Denmark) and Noromox prolongatum (amoxicillintrihydrate 150 mg/kg) (ScanVet, Fredensborg, Denmark) were administered during surgery and for five days post-surgery. The animals were mounted in a stereotactic frame and Marcain (2.5 mg/ml bupivacaine, AstraZeneca, Albertslund, Denmark), was administered locally (SC) prior to incision. Coordinates were guided by Paxinos and Watson (1998) and are given in millimeters relative to bregma, AP are the anterior–posterior axis, ML are the medial–lateral axis. The depth is given in DV the dorsal–ventral axis. Holes were drilled bilaterally for implantation of recording electrodes in visual cortex (AP: −6, ML: ±4) and superior colliculus (AP: −6, ML: ±1, DV: −3.5). A reference electrode (AP: +8, ML: −2) and a ground electrode (AP: −2, ML: +4) were also implanted. The electrodes for recording in the superior

colliculus were stranded electrodes E363/3/Spc (PlasticsOne, VA, US). The other four electrodes were E363/20/2.4/S screw electrodes (PlasticsOne). The electrodes were collected in a plastic pedestal MS363 (PlasticsOne) and fixed to the skull with RelyX™ Unicem dental cement (3M, Copenhagen, Denmark) and Fuji plus cement (GC America, US) as a chronic implant. The animals were allowed to recover for 14 days before initiation of experiments.

EEG recording

The recordings were made during the dark phase in awake and behaving animals in a Makrolon type IV cage with wood bedding. The LEDs were positioned in a frame 40 cm above the bedding. First, a 30 min baseline recording was obtained from the rats while the visual stimulation paradigms were presented. Following administration of sildenafil, the same type of recordings was made every 30 min for the first two hours, then after 4, 5, 7, 24 and 28 h, in total ten recordings per animal.

The animals were exposed to whole-field light flashes of 20 lx white LED (~390–700 nm, SMD5050), 20 lx blue (455–460 nm), and 5 lx short wave blue (SWB) (405 nm). Each flash had a duration of 10 ms and 400 flashes were applied at a frequency of 1 Hz to record flash VEPs. Then, the frequency was increased to 14 Hz and the SSVEPs were recorded for 100 s. Light stimulation was controlled by Spike2 software version 7.20 (Cambridge Electronic Design Ltd, Cambridge, UK). Spike2 was also used for recording of the EEG signals. Signals were amplified and filtered using a Brownlee amplifier model 410 (Brownlee Precision, CA, US) at the following settings: low-pass filter; 200 Hz, high-pass filter; 1 Hz, sampling rate; 1000 Hz. EEG recordings were carried out in four animals, while the exposure data included eight rats.

Drug administration, pharmacokinetic characterization and histology

The rats received sildenafil citrate extracted from Viagra® (Pfizer, NY, US) 50 mg/kg in 0.1 M HCl, pH adjusted to 4 with NaOH (Abbott et al., 2004). The solution was administered perorally. The rats were chosen based on visual inspection of the VEP (pilot data, not shown) recorded in the superior colliculus.

The rats were sacrificed 15 min ($n = 4$) or 5 h ($n = 4$) after drug administration (Walker et al., 1999). Trunk blood was sampled, and the cerebellum was harvested and weighed for exposure profiling of sildenafil. The cerebrum was removed and snap frozen on dry ice and used for validating the location of the electrodes. The brains were sliced in a freeze microtome in 20 μm thick coronal slices and placed directly on glass slides for microscopy inspection. Although the electrodes were removed before the cerebrum was snap frozen they left visible traces in the tissue.

Exposure

The cerebellum was thawed and homogenized using a Covaris 220X (Covaris Inc., MA, US). The concentration of sildenafil was quantified in a LC–MS/MS platform (Xevo TQS triple quadrupole) mass spectrometer operated in electrospray MS/MS mode (multiple reaction mode) and coupled to a Waters Acquity UPLC controlled by MassLynx software version 4.1 (Waters, MA, US). Mass spectrometry methods yielded the total concentration of sildenafil in the tissue samples. The free fraction of sildenafil in blood and brain was determined as previously described in (Bundgaard et al., 2012). The free fractions of sildenafil were 7.2% and 3.9% in the blood and brain, respectively. These values were used to calculate the unbound concentrations of sildenafil in the samples.

Data analysis and statistics

The flash VEPs were generated by averaging single sweeps time-locked to the visual stimuli from -0.2 s to $+0.5$ s relative to stimulation offset using Spike2. Grand averages were created and VEP peaks were quantified. Naming of the peaks in the visual cortex was guided by (Creel et al., 1974; Meeren et al., 1998). The first positive deflection was named P1, the first negative deflection is named N1, the second positive deflection is named P2 and so on. For flash VEPs, the amplitude (i.e. from baseline to peak measured in mV) and latency (i.e. from stimulus onset to peak measured in s) of the different peaks were analyzed separately.

The overall effect of sildenafil was assessed by two-way ANOVA, testing the effect of time and wavelength condition using R via the open source software Rstudio. The three wavelength conditions were analyzed separately, and analysis was carried out on four timepoints as the temporal resolution of the effect of sildenafil is quite low (i.e., baseline, 0.5, 5 and 28 h). These four timepoints were chosen from visual inspection and PK/PD from a previous study suggesting that the maximum plasma concentration of sildenafil in female rats is $T_{max} = 15$ min and a half-life of 1 h (Walker et al., 1999). Separate ANOVAs were computed for the latency and amplitude of each peak, yielding 72 one-way ANOVAs testing the variance over time (DF = 3). These were followed by Tukey post-hoc tests, when the level of significance was $p < 0.05$. The p-values were adjusted for multiple comparisons using the false discovery rate (FDR) method (Benjamini and Yekutieli, 2001).

The SSVEP were analyzed using a custom-made script programmed in Matlab 2016a (Mathworks, MA, USA) fitting sinusoids to determine amplitude by applying a fast Fourier transform. To test for an effect of sildenafil, the signal-to-noise ratios (SNRs) of the amplitudes were quantified at the same timepoints as for VEP: 30 min, 5 and 28 h and compared to the baseline condition using one-way ANOVA. P-values were adjusted using FDR.

RESULTS

Pharmacokinetic profile of sildenafil in rats

Before starting the electrophysiological experiments, we assessed the pharmacokinetic profile of sildenafil in

naïve rats to estimate the temporal dynamics of PDE6 inhibition (Fig. 1). Fig. 1A shows the structure of sildenafil citrate. The total concentrations of sildenafil in blood and cerebellum were measured at 15 min (T_{max}) and at 5 h after peroral administration of 50 mg/kg sildenafil, (Fig. 1B). The free unbound brain concentration of sildenafil at 15 min and 5 h were 817 ± 298 nM and 380 ± 159 nM (mean ± STD; $n = 4$), respectively. Previously reported half-maximal inhibitory concentrations (IC_{50}) of PDE6 in cones and rods are 34 nM and 38 nM, respectively (Wallis, 1999). This suggests that PDE6 activity was more than 90% inhibited up to 5 h after sildenafil

The effect of sildenafil is visualized as the grand average of VEPs for visual cortex and superior colliculus, respectively, during the white light condition (Fig. 1C, D). Seven discernible VEP peaks were detected in the visual cortex, four positive peaks denoted P1–4 and three negative peaks denoted N1–3. Five discernible VEP peaks were detected in the superior colliculus, 3 positive peaks denoted P1–3 and two negative peaks denoted N1 and N2. The peak designation is illustrated in (Fig. S1). Corroborated by the exposure results showing high concentration of sildenafil in plasma and brain during EEG recordings (Fig. 1B), the phototransduction was compromised following sildenafil administration. Generally, sildenafil administration increased the peak latency (Figs. 3 and 5) and reduced the amplitudes (Figs. 2 and 4) of the

majority of VEP peaks recorded from the superior colliculus and visual cortex. The statistical evaluation of the overall effects of sildenafil are summarized in Tables S1 and S2 in Supplementary. The statistical evaluation shows temporal effects following administration of sildenafil as well as general effects dependent on the different wavelength conditions. Furthermore, interactions between the wavelength condition and time points were observed. In the sections below, the data is analyzed separately for the three wavelength conditions using the one-way ANOVA (all ANOVAs are summarized in Tables S3 and S4 in Supplementary), the difference of mean is reported if both the ANOVA and the Tukey post-hoc showed $p < 0.05$.

Sildenafil-induced changes in VEPs in the visual cortex

Fig. 2 depicts the amplitudes of the individual VEP peaks from recordings performed in the visual cortex. Thirty minutes after administration of sildenafil, there were statistically significant differences in the amplitudes of P2, N2 and N3 during stimulation with white light. The changes in mean amplitude were −0.057, −0.079 and 0.072 mV, respectively for the peaks. At timepoint 5 h, there were significant differences from the baseline condition on P2, N2 and N3 (−0.067, −0.057 and 0.066 mV, respectively). 28 h after sildenafil administration there was no detectable changes in VEPs. In the blue light condition, the amplitude of the P4 peak was increased with 0.020 mV after 30 min. After 5 h the P3 was changed by −0.032 mV, the N3 had was changed by −0.039 mV in amplitude while the P4 was increased by 0.186 mV. After 28 h, only the N2 was affected (amplitude changed by −0.032 mV). In the SWB condition there was a statistically significant change in amplitude of the N3 peak after 30 min (−0.0742 mV) and this effect was the same after 5 h. The P4 was modulated throughout all timepoints (decreased after 30 min; 0.011 mV, decreased after 5 h; 0.013 mV and increased after 28 h; 0.017 mV). Furthermore, the amplitude of N1 was increased by 0.040 mV while the N2 amplitude was changed by −0.054 mV. However, these effects were only apparent at timepoint 5 h.

The latencies of the waveforms for the three wavelengths are depicted in Fig. 3. Thirty minutes after administration, sildenafil significantly increased the latency of all peaks (P1; 0.013 s, N1; 0.023 s, P2; 0.025 s, N2; 0.039 s,
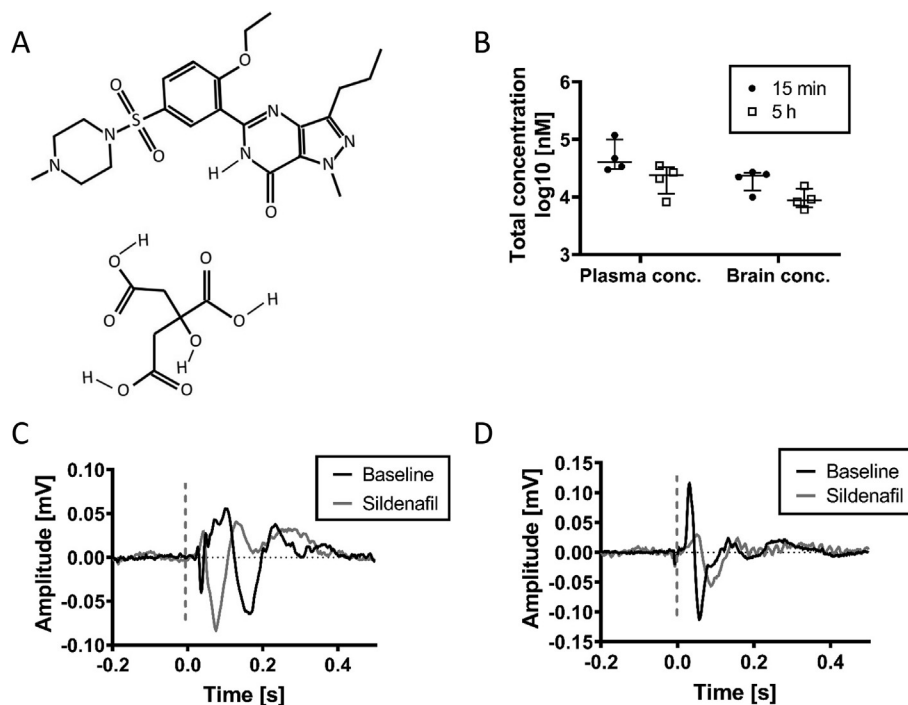

Fig. 1. Effect of sildenafil on visual evoked potentials (VEPs) in rats. (A) Structure of sildenafil citrate. (B) Total concentration of sildenafil in the plasma and cerebellum. The concentration of unbound sildenafil is depicted with median and interquartile range. (C, D) Grand average VEP waveforms from rat visual cortex and superior colliculus, respectively. Waveforms evoked by a 10-ms white-light flash stimulus are shown at baseline before sildenafil administration (black) and 15 min after (grey) per oral administration of sildenafil. The broken gray line marks the stimulus flash. More information about peak designation and the waveforms of steady-state responses can be found in the supplementary material.
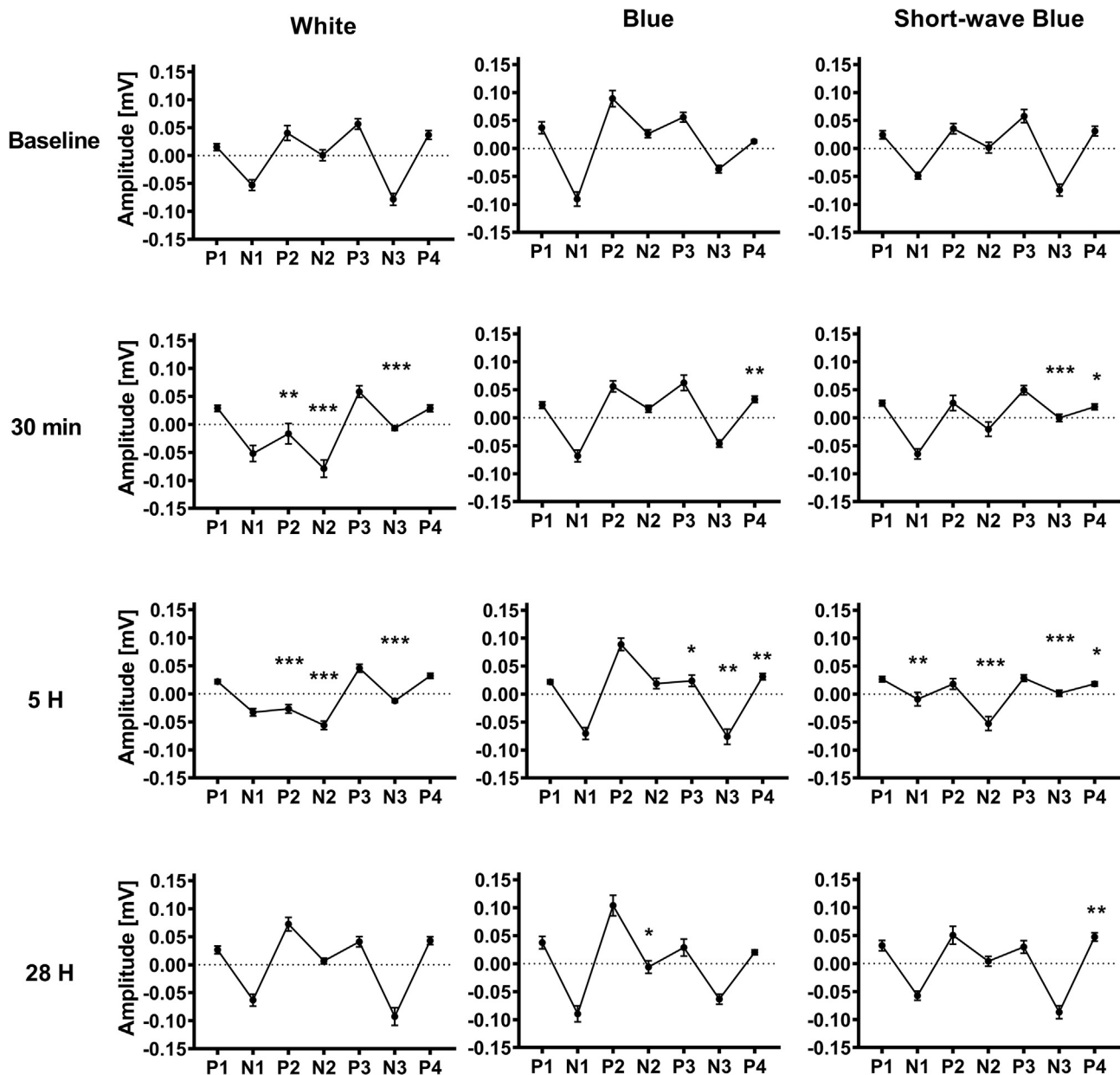
Fig. 2. Sildenafil-induced changes in amplitude of visual evoked potentials from rat visual cortex. Amplitudes are plotted as the mean with standard error of mean (SEM). The columns depict data obtained from stimulation with different visual stimuli: white, blue and short-wave blue light. The rows represent time related to dosing: baseline (before dosing), 30 min post, 300 min post, and 28 h after administration of sildenafil. The asterisks refer to significant differences after sildenafil administration compared to baseline with significance levels of $p < 0.05 = *$, $p < 0.01 = **$, and $p < 0.001 = ***$.

P3; 0.083 s, N3; 0.096 s and P4; 0.110 s) in the white light condition. 5 h after drug administration, the latency of the P1, N1, P3, N3 and P4 components were still increased (0.014, 0.023, 0.043, 0.056 and 0.058 s, respectively). In the blue condition the latency of P1, N1, P2, N3 was increased after 30 min (0.006, 0.0082, 0.006, 0.013 s, respectively). Effects on P1, N1, P2 and N2 persisted for 5 h (0.0042, 0.0065, 0.005 and 0.012 s). For the SWB condition, the latency of the VEP components was increased for all peaks 30 min after sildenafil administration (0.016, 0.038, 0.072, 0.093, 0.138, 0.124 and 0.103 s). After 5 h, the differences were still

significant (0.017, 0.028, 0.026, 0.048, 0.097, 0.081 and 0.079 s). No changes from baseline VEPs were detectable 28 h after sildenafil administration in any of the wavelength conditions.

Sildenafil-induced changes in VEPs in the superior colliculus

Grand average VEP waveforms recorded from the superior colliculus are displayed in Fig. 4. Five discernible VEP peaks were detected, three positive peaks denoted P1–3 and two negative peaks denoted
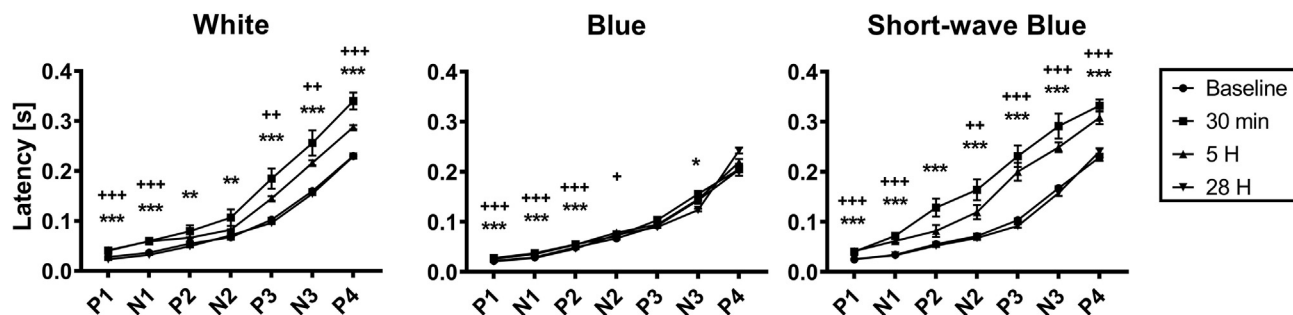
Fig. 3. Sildenafil-induced changes in latency of visual evoked potentials from rat visual cortex. Effects are depicted with mean ± SEM. Latency of peaks for each time point, for each color: white, blue and short-wave blue light. Asterisks refer to significant differences between baseline and 30 min. Plus signs depicts differences between baseline and 300 min. Significance levels: $p < 0.05 = +$ and *, $p < 0.01 = + +$ and **, $p < 0.001 = + + +$ and ***.
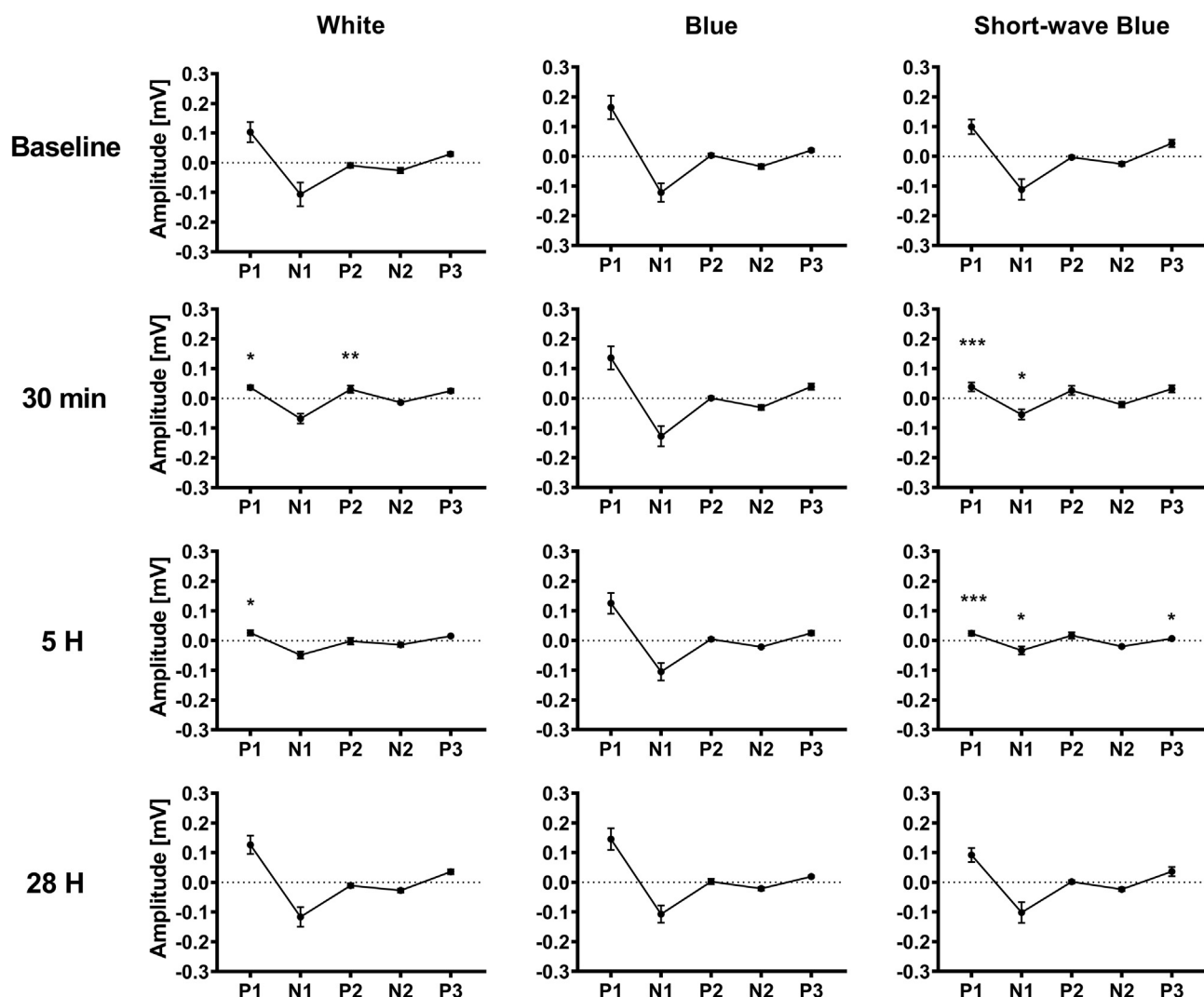

Fig. 4. Sildenafil-induced changes in amplitude of visual evoked potentials from rat superior colliculus. The graphs show the mean ± SEM of the amplitude of peaks under three different color conditions at four timepoints. The columns depict data obtained from stimulation with different visual stimuli: white, blue and short-wave blue light. The rows represent the four time points: baseline (before dosing), 30 min, 5 h, and 28 h after administration of sildenafil. The asterisks refer to significant differences after sildenafil administration compared to baseline with significance levels of $p < 0.05 = $ *, $p < 0.01 = $ **, and $p < 0.001 = $ ***.

N1–2. In the white light condition, there was a statistically significant change in the amplitude of P1 (-0.067 mV) and an increase in the amplitude of P2 (0.040 mV) after 30 min. After 5 h, the amplitude of P1 was still changed (-0.078 mV). Sildenafil did not affect the amplitude of the VEP in the blue light condition. For the SWB light
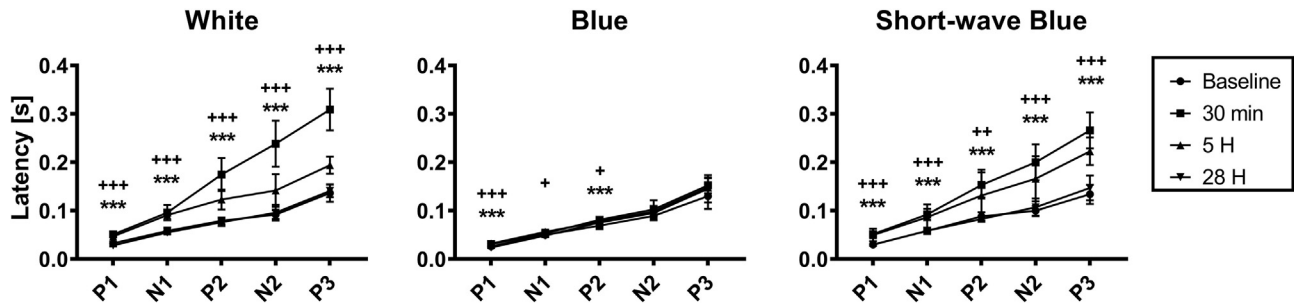
Fig. 5. Sildenafil-induced changes in latency of visual evoked potentials from rat superior colliculus across three color conditions and four time points. Effects are depicted with mean ± SEM. Asterisks represent differences between baseline and 30 min. Plus signs delineate differences between control and 300 min. Significance levels: $p < 0.05 = +$, $p < 0.01 = ++$, and $p < 0.001 = +++$ and ***.

condition, there were statistically significant changes in the VEP amplitude of P1 and N1 after 30 min (−0.061 and 0.057 mV) and 5 h (−0.075 and 0.078 mV, respectively). Furthermore, the P3 was reduced with 0.038 mV after 5 h. After 28 h, there was no detectable differences in the amplitudes of the VEPs in any light condition.

Fig. 5 shows the latencies of the VEPs recorded from the superior colliculus. In the white light condition, the latency of all peaks was increased 30 min after sildenafil administration (0.018, 0.038, 0.096, 0.146 and 0.173 s). These differences were also present after 5 h (0.014, 0.033, 0.044, 0.050 and 0.058 s). In the blue light condition, sildenafil increased the latency of the early positive deflections; P1 and P2. The difference of mean was 0.005 s and 0.012 s, respectively. After 5 h, the latencies of both peaks were still increased with a change of 0.006 s and 0.008 s, and the latency of N1

was increased with 0.006 s. In the SWB condition, the latency of all VEP components were significantly increased 30 min after sildenafil administration (0.021, 0.034, 0.065, 0.1 and 0.132 s). This effect was also apparent after 5 h (0.020, 0.028, 0.043, 0.066 and 0.089 s). After 28 h, there was no effect of sildenafil on the latency of the VEPs in any light condition.

Sildenafil-induced changes in SSVEPs

Representative waveforms of SSVEP are depicted in Fig. S 2. Both from baseline and 30 min after administration, from the two sites of recording.

Fig. 6 shows the impact of sildenafil on the SSVEPs recorded in the visual cortex. In the white light condition, there were no changes compared to the control condition in either harmonic. In the blue condition (*F*
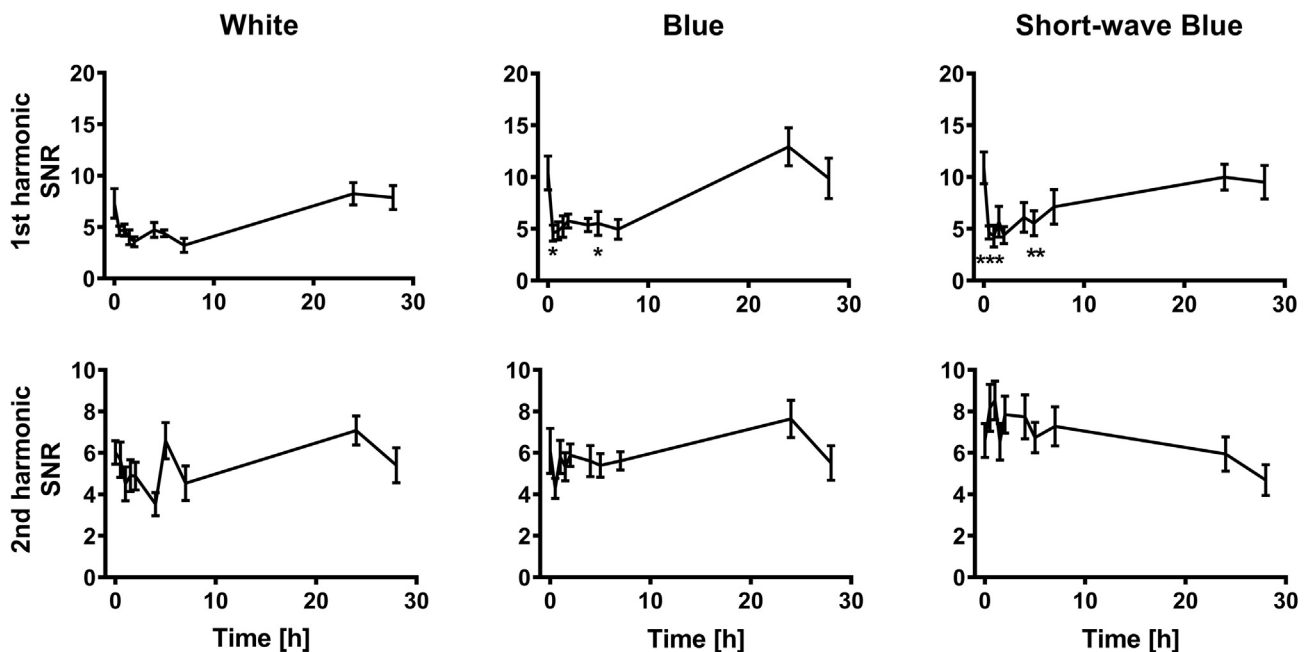

Fig. 6. Signal-to-noise ratio of the 1st and 2nd harmonic of steady-state visual evoked potentials from visual cortex is affected by sildenafil. The SNR (mean and ± SEM) of the SSVEP for 1st and 2nd harmonic of the visual cortex during exposure to white, blue and short-wave blue light. The line plots show time points: 0 = baseline; 30 min after administration of sildenafil, 1, 1.5, 2, 4, 5, 7, 24 and 28 h. The asterisks refer to significant differences after sildenafil administration compared to baseline with significance levels of $p < 0.05 = *$, $p < 0.01 = **$, and $p < 0.001 = ***$. There are significant changes for the 1st harmonic of the blue and the SWB conditions.

$(3,25) = 4.57$, $p = 0.011$), the post-hoc test showed that sildenafil induced a reduction in SNR of the 1st harmonic 30 min after administration (56% decrease relative to baseline level). After 5 h, the SSVEP SNR was reduced 47% relative to baseline level. There were no changes in the 2nd harmonic. In the SWB condition $(F(3,25) = 6.85$, $p = 0.0032)$, there was a similar trend with a statistically significant depression 30 min and 5 h after administration (57% and 49% reduction, respectively). As for the blue light condition the SSVEP was normal after 28 h and there was no effect on the 2nd harmonic.

The SNR of the SSVEP recorded from the superior colliculus is shown in Fig. 7. Both harmonics were affected in the white light condition: The 1st harmonic was significantly affected by sildenafil $(F(3,25) = 3.06$, $p = 0.047)$. Here, the SNR was reduced by 36% after 30 min. This effect was not detectable at later timepoints. Sildenafil also affected the 2nd harmonic $(F(3,25) = 5.76$, $p = 0.0078)$. The SNR was decreased by 31% after 30 min. This effect persisted for 5 h but the SSVEP was normal after 28 h. In the blue light condition, there were no changes in the first harmonic. In the 2nd harmonic there was a statistically significant $(F(3,25) = 6.04$, $p = 0.0062)$ depression of the SNR after 30 min (35%) and after 5 h (22%). The SSVEP was normalized after 28 h. In the SWB condition, there was no significant difference in the SNR of the 1st harmonic between baseline and recordings obtained from sildenafil-treated rats. For the 2nd harmonic, there was an increase of the SNR at both 30 min (40%) and 5 h (42%) $(F(3,25) = 4.5$, $p = 0.012)$. Again, the SNR was normalized after 28 h.

DISCUSSION

The purpose of the study was to investigate the progressive effect of PDE6 inhibition on visual processing by two different electrophysiological assays following administration of a high-dose sildenafil. The visual cortex and superior colliculus were both severely affected by the administration of sildenafil generally causing reductions in amplitude and increases in latency of the majority of the VEP peaks. Sildenafil affected both the superior colliculus and the visual cortex. This suggests that the compound is primarily impacting early parts of the visual pathway such as rod and cone function, as the superior colliculus and the visual cortex are not part of the same downstream functional pathway (Sefton et al., 2014). The observed change in the flash VEPs corresponds well to the observed reductions in amplitude of a- and b-waves commonly reported from ERG measurements (Vobig et al., 1999; Nivison-Smith et al., 2014). Additionally, previous ERG studies evaluating flicker-ERG responses found prolonged implicit times for both a- and b-waves (Jägle et al., 2004) which is also in line with the results of the present study. Studies on cGMP-specific PDE inhibitors suggest that these effects may be ascribed to elevated cGMP levels (Estrade et al., 1998). We did not observe a complete reversal of the electrophysiological changes after 28 h although fewer VEP peaks were generally affected at this timepoint. Although sildenafil-induced retinal side effects in humans are commonly referred to as acute and transient, our results are supported by sildenafil-induced effects on ERGs in mice that persisted for at least two days (Nivison-Smith et al., 2014).
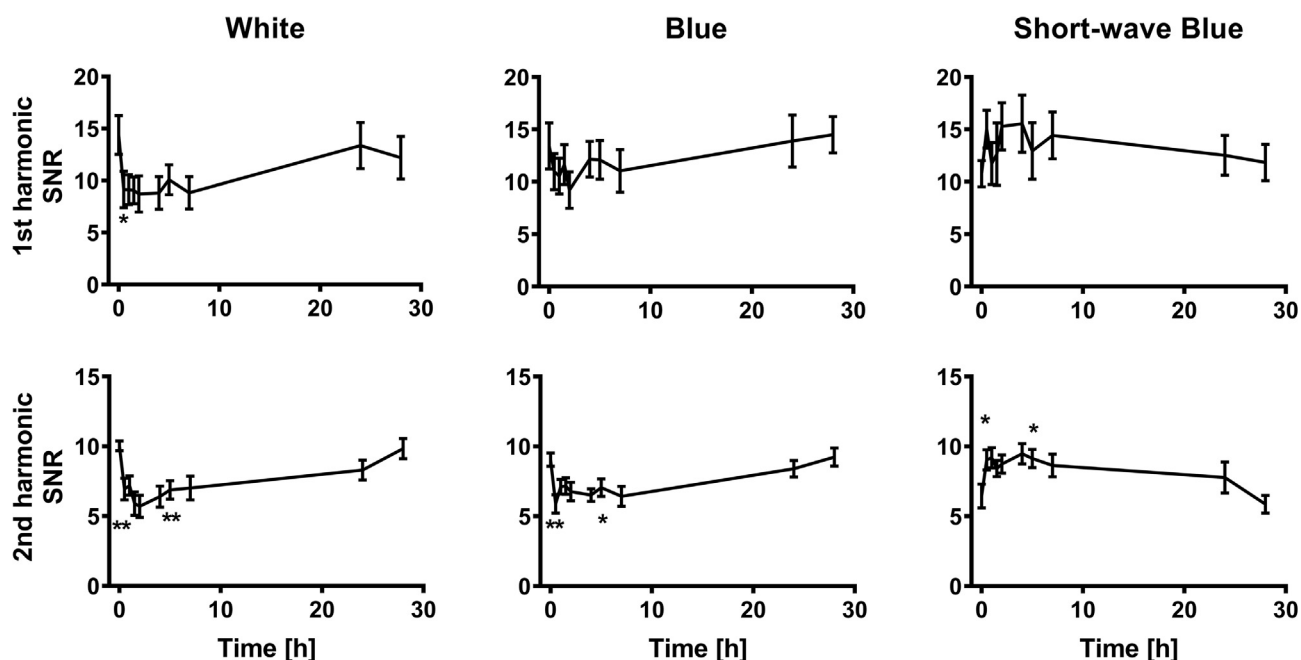

Fig. 7. Signal-to-noise ratio of the 1st and 2nd harmonic of steady-state visual evoked potentials from superior colliculus during exposure to white, blue and short-wave blue light. The line plots show the mean and ± SEM from each time point: baseline at time = 0 and the last recording at 28 h. The asterisks refer to significant differences after sildenafil administration compared to baseline with significance levels of $p < 0.05 = *$, $p < 0.01 = **$, and $p < 0.001 = ***$.

The superior colliculus receives direct input from the retina and may thus represent a more 'clean' visual response than EEG recordings obtained from the visual cortex. This structure does not receive any direct projections from the retina but integrates information relayed via other nuclei primarily the dorsolateral geniculate nucleus (Sefton et al., 2014). Thus, the only relay that is shared between the two paths is the retina. Furthermore, EEG was obtained from the visual cortex using a cortical screw electrode. Consequently, the cortical flash VEPs from this structure may be influenced by volume conduction from other anatomical areas.

There was a general trend that the profiles of change, observed in the white light and the SWB conditions, were similar whereas the blue wavelength condition was affected to a smaller degree. Interestingly, there was no detectable change in the amplitude of the VEPs from the superior colliculus in the blue wavelength condition, but the latency was significantly increased for the early peaks, suggesting a partial compensation of the sildenafil-induced changes.

The exposure after dosing of 50 mg/kg sildenafil suggests near full inhibition of PDE6 in both rods and cones up to 5 h after administration of sildenafil. Our estimate was based on the free unbound brain concentration, since the physical properties of the blood–brain barrier and the blood–retinal barrier are comparable (Toda et al., 2011). The present data are suggestive of a half-life close to 3 h rather than 1 h in female rats as suggested by Walker et al. (1999). The dose described in Walker et al. was 1 mg/kg, which is only 2% of the dose used in the current study. This discrepancy could either result from reaching the threshold of liver enzymes responsible for metabolizing sildenafil or it could be ascribed to slower absorption.

The inhibitory action of sildenafil on PDE6 is believed to involve changes in rod and cone outer segment function along with changes in inner retinal function (Wallis et al., 1999). Even though PDE6 theoretically was considered fully inhibited in the present study, the visual processing was not fully abolished. In common with other fast-acting enzymes, the function of PDE6 is resistant to complete inhibition and it is challenging to quantify the level of inhibition necessary to produce visual deficits (Laties and Zrenner 2002). The catalytic dimer of PDE6 has different forms in rods and cones as it is a heterodimer with two subunits PDE6A and PDE6B in rods, and a homodimer of PDE6C subunits in cones (Lagman et al., 2016). Also, different IC50 values are reported for rods and cones (Wallis, 1999). Thus, it is likely that these isoforms are not equally inhibited by sildenafil.

In some cases of retinitis pigmentosa, the disease is caused by mutations in the PDE6 gene (Zhang et al., 2005). In this disease, the rod cells degenerate first leading to night blindness at the early stages of the disease (Hartong et al., 2006). The signal recorded in the visual cortex after the administration of sildenafil differs from EEG recordings in rats during dim light conditions (unpublished data), suggesting that the inhibition of PDE6 is not directly comparable to low-light conditions. Though sildenafil inhibits PDE6 in both photoreceptor types, Nivison-Smith et al. reported that a heterozygous mouse model of retinitis pigmentosa only showed a limited photoreceptor response to the administration of sildenafil compared to wildtype mice (Nivison-Smith et al., 2014). This supports that sildenafil and retinitis pigmentosa work by similar mechanisms, if the effect of sildenafil is smaller when PDE6 is already compromised.

Studies have shown that the inhibition of PDE5 may also contribute to the effect detected. So far, tests to determine whether sildenafil causes visual adverse events in humans have been largely restricted to methods unrelated to electrophysiological assessment. One rodent study reported ERG effects in mice in line with the present study, i.e. reduced amplitudes of visual responses (Nivison-Smith et al., 2014). In humans, PDE5 inhibition causes increased flow in the ophthalmic artery (Foresta et al., 2008; Gerometta et al., 2011) and a similar change in rodents ultimately affecting visual processing is not unlikely. Consequently, part of the sildenafil-induced changes in the visual processing may be due to increases in intraocular pressure, however, assuming that changes in pressure affects all photoreceptors equally. Then this change does not explain why the EEG response depends on the wavelength of the stimuli. This rather confirms the photoreceptors of the retina are more sensitive to specific wavelengths in the blue part of the visual spectrum.

In vertebrates, the cascade of linear and non-linear processing stages in the visual system will convert the signal frequency associated with periodic full-field illumination changes into a set of odd- and even-harmonic response frequencies in the EEG (Regan, 1989). Neurons that respond to overall illumination changes or neurons with very low spatial frequency pattern sensitivity will contribute to odd harmonics with the largest response at the input frequency. Neurons that respond to stimulus changes will generate responses at both phases of the input, contributing to even harmonics, with a dominant response at two times the input frequency. It has been suggested that low-frequency SSVEPs originate early in the visual pathway prior to cortex (Krolak-salmon et al., 2003), and must derive from the earliest retinal processing stages. As the ipRGCs are light sensitive, without expressing PDE6 (Hatori and Panda, 2010), this may explain why the second harmonic in SSVEPs recorded in the visual cortex is not affected in any of the wavelength conditions; as signal from these neurons could compensate for the loss of signal from the photoreceptors. Because of these projections, we expected the two harmonics from the superior colliculus to be affected in a comparable manner. In the white light condition, sildenafil depressed both the first and second harmonic, as expected. Intriguingly, the ipRGCs have a peak sensitivity in blue part of the visual spectrum (480 nm) (Panda et al., 2005). This correlates well with our results demonstrating a smaller change of VEP and SSVEP-responses in the blue wavelength (455–460 nm) condition relative to the others.

Here we demonstrate that translational EEG assays may be used to study pharmacological-induced changes in visual processing in rats. In the present study, both

VEPs and the SSVEPs was negatively modulated by sildenafil which supports the existing literature on ocular side effects of PDE6 inhibition. The profile of change was dependent on the wavelength condition, given that the response to blue light was less affected by sildenafil than the responses to white or SWB light. VEPs successfully probed the temporal changes, in this case elucidating a slowed and weakened visual response, while the SSVEPs effectively demonstrated effects on the SNR.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2020.06.033.