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Electroretinogram Recording for Infants and Children under Anesthesia to Achieve Optimal Dark Adaptation and International Standards

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TITLE:

Electroretinogram Recording for Infants and Children under Anesthesia to Achieve Optimal Dark Adaptation and International Standards

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KEYWORDS:

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SUMMARY:

Adhering to international standards and maintaining retinal dark adaptation are critical to acquire valid full field electroretinogram responses in the diagnosis and management of inherited retinal diseases. A practical protocol using a portable darkroom is provided to obtain full field electroretinogram for infants and children under sedation or general anesthesia in the operating

room setting.

ABSTRACT:

Electroretinogram (ERG) is the only clinical objective test available to assess retinal function. Full field ERG (ffERG) measures the panretinal rod and cone photoreceptor function as well as inner retinal function and is an important measure in the diagnosis and management of inherited retinal diseases as well as inflammatory, toxic, and nutritional retinopathies. Adhering to international standards and maintaining retinal dark adaptation are critical to acquire valid and reliable dark-adapted (scotopic) and light-adapted (photopic) ffERG responses. Performing ffERG in infants and children is challenging and often requires general anesthesia in the operating room. However, maintaining retinal dark adaptation in the operating room is becoming increasingly difficult given the numerous light sources from anesthesiology monitoring systems and other equipment. A practical and widely applicable method for ffERG testing is described in the operating room that optimizes retinal dark adaptation. The method reduces operating room time by dark-adapting the patient before general anesthesiology is instituted. The operating room is modified for dark adaptation and any remaining light source in the darkened operating room is minimized with the use of a modified portable foldable darkroom that encloses the patient's head and the ERG examiner during ffERG scotopic recordings. The simple method adheres to ffERG international standards and provides valid reliable scotopic and photopic ffERG recordings that are critical to assess objective retinal function in this young age group where subjective assessment of visual function such as visual acuity and visual fields are not possible. Furthermore, the ffERG is the gold standard clinical test in detecting early onset inherited retinal diseases including Leber congenital amaurosis where approved gene therapy has become available. In sedated conditions, very low amplitude ffERG signals can be detected due to minimal orbicularis muscle activity interference, which is particularly relevant in patients after gene therapy to detect improved amplitude responses.

INTRODUCTION:

The electroretinogram (ERG) is the only clinical objective test available to assess retinal function and the full field ERG (ffERG) is the only objective test to assess rod-photoreceptor generated activities[1,2]. The ffERG measures the electrical responses from the entire retina elicited by a full field flash stimulus and is a gold standard test in the diagnosis and management of inherited retinal diseases[2,3]. Thus, the ffERG is an important test in infants and young children to detect early onset inherited retinal diseases such as Leber congenital amaurosis where approved gene therapy and clinical trials are available[4,5].

Adherence to ffERG standards established by the International Society for Clinical Electrophysiology of Vision (ISCEV) are critical to acquire valid and reliable dark-adapted (scotopic) and light-adapted (photopic) ffERG responses[1,3]. Failure to properly maintain adequate retinal dark adaptation during scotopic ffERG recordings results in falsely-impaired recorded responses and patient mismanagement. Performing ffERG in infants and children is challenging given limited cooperation and often requires general anesthesia in the operating room[6]. A recent survey among ISCEV members showed 12-14% of ERG's are performed under sedation or general anesthesia[7]. Maintaining retinal dark adaptation in the operating room is difficult given the

numerous light sources from anesthesiology monitoring systems and other equipment. While anesthetic agents may have an effect in reducing ERG responses, ERG responses under sedation or general anesthesia are reliable in providing accurate diagnosis[6,8,9].

A simple and widely applicable method is described for ffERG testing in the operating room that adheres to the international standards and optimizes retinal dark adaptation. The goal of this practical method is to provide valid reliable scotopic and photopic ffERG recordings to assess objective retinal function in infants and young children, which is particularly relevant in this young age group given subjective assessment of visual function such as visual acuity and visual fields are typically not possible. The operating room is modified to promote retinal dark adaptation, and the procedures reduce operating room time by dark-adapting the patient before sedation or general anesthesiology is instituted. A modified portable foldable darkroom encloses the patient's head and the ERG examiner during ffERG scotopic recordings to minimize any remaining light source including light emission from the ERG system. The portable darkroom allows rapid access to the patient by the anesthesiologist when necessary. After the completion of ffERG, diagnostic retinal imaging including optical coherence tomography (OCT) and fundus imaging as well as venopuncture for genetic testing can easily be performed while the patient remains under anesthesia.

The method is suitable for practitioners and practices that manage pediatric patients with retinopathies. An average sized ocular operating room provides adequate space, and a room with low background electrical noise is desirable to allow quality ffERG recording. While the ERG examiner is inside the foldable darkroom during scotopic ffERG recording, a trained technician is needed to operate the ERG system outside of the foldable darkroom. Conferring with the anesthesiology team is essential in modifying the operating room and to promote the safety of the patient in a darkened environment.

The advantages of the method over alternative techniques include optimizing and maintaining retinal dark adaptation, promoting valid reliable ffERG recordings, improving patient safety, and facilitating additional diagnostic testing such as retinal imaging and venopuncture for genetic testing. Optimal dark adaptation is also critical given ffERG stimulators should be calibrated for complete darkness conditions as recommended by ISCEV[10]. Alternative methods include the use of oral agents such as chloral hydrate with variable sedative responses in infants and children, which affects the quality of ffERG recordings and causes difficulties in monitoring vital signs. While some children can cooperate with ffERG recording in the clinic, the testing session may be prolonged depending on cooperation, and the validity of ffERG recordings may be affected by eye movement and blink artifacts as well as difficulty in maintainng retinal dark adaptation[4]. The current method provides additional dark adaptation and safety measures compared to the previously described deep sedation ffERG method[6].

PROTOCOL:

The protocol follows the operating room guidelines of the Bascom Palmer Eye Institute, University of Miami and is applicable to infants, young children, and uncooperative adults.

Patients who cannot have general anesthesia due to safety issues should not have the procedure.

1. Operating room selection and modification

1.1. Select an operating room with low 60 Hz background electric current noise and proper electrical grounding, to avoid ERG recording interference. Use a room with an isolated electrical circuit without connection to or is near heavy appliances (e.g., refrigerator).

1.1.1. Perform trial ERG recordings in the operating room at the location where the ERG recording will take place. Check the ERG recording baseline as well as the trial recording waveforms to determine the absence of 60 Hz background electric noise.

1.2. Inspect the operating room for light leaks from ceiling, door, and window openings. Perform human observation after full dark adaptation (30 to 45 minutes) given that the normal human eye can detect light as dim as approximately 4 photons, which is better than any man-made light meter except liquid nitrogen-cooled detectors for astronomy.

1.3. Install opaque non-reflective black curtains on tracks to cover the operating room door and window openings fully without light leakage (**Figure 1**). Select curtain material that is washable and resistant to staining and bacterial growth. Follow local operating room regulations and procedures for proper interval cleaning. Block light leaks from the ceiling if present.

2. Foldable portable darkroom selection and modification

2.1. Select a portable darkroom that is easy to install and store and large enough to enclose the patient's head, the ERG examiner, and the ffERG stimulus. Use folding portable darkrooms designed for an optic physicist (e.g., www.scientex.co.jp/pdf/pdf-b-lp-eng.pdf, 48"x 48"x81" cross type) which are available commercially and optimizes the maintenance of retinal dark adaptation of the patient during scotopic ffERG recordings (**Figure 2**).

NOTE: The fabric of the portable darkroom mentioned was tested by the eye institute's microbiology department for ease of disinfection and the optical transmission was tested by the eye institute's biomedical department before purchase. This is recommended if a different portable darkroom is used.

2.2. Add a small opening with flaps at the rear of the portable darkroom to allow routing connections and cables (**Figure 3**).

NOTE: During scotopic ffERG testing, the ffERG light stimulus is inside the folding portable darkroom and the ERG recording system is outside of the portable darkroom. The ERG electrode wire connections and the cable connecting the ERG light stimulus to the ERG recording system go through the opening created with a double closure system to guarantee total darkness. We use a small handheld ffERG light stimulus to ease ERG recording inside the folding portable darkroom and record one eye at a time. A larger ffERG light stimulus can record both eyes

simultaneous but will need to be held by a metallic arm requiring a larger opening at the rear of the portable darkroom and will likely require a larger darkroom.

3. Patient preparation and retinal dark adaption

3.1. Confirm medical reason for ffERG and obtain informed consent for examination under anesthesia, ffERG, and other procedures of interest for patient management such as retinal imaging (e.g., fundus imaging, optical coherence tomography, fluorescein angiography) and venopuncture for genetic testing.

NOTE: Most common reasons for ffERG in infants and young children include decreased vision, nystagmus, nyctalopia, visual photosensitivity, abnormal fundus, and medication with risk of retinal toxicity (e.g., vigabatrin). Important to recognize factors that are likely to affect ERG recordings including high myopia and albinism. In general, ffERG responses from infants younger than age 6 months are small and still developing, making interpretation of recorded responses difficult.

3.2. Place ocular anesthetic drop (proparacaine 0.5%) followed by pupillary dilation combo drop (cyclopentolate 1% + phenylnephreine 0.5%) to each eye. Repeat the combo drop to each eye 2 to 3 times with 5 minutes between drops.

NOTE: The proparacaine decreases burning sensation and increases corneal absorption of the dilating drops but may have to be skipped in patients with very poor cooperation.

3.3. Patch both eyes for retinal dark adaptation of at least 30 minutes. With eyelids gently and completely closed, place 2 regular-size self-adhesive eye occlusion patches over each eye without significant pressure on the eye.

3.3.1. Place the first patch conventionally and oriented horizontally with the wider end of the patch temporally. Place the second patch horizontally over the first patch with the wider end nasally and adjust the position typically with a mild tilt clockwise to prevent light leak nasally.

3.4. After placing the eye patches over each eye, place opaque black tape horizontally across to cover both eyes without significant pressure on the eyes. Make a small vertical cut at the inferior edge of the black tape before placement at the location across the bridge of the nose to avoid pressure on the nose.

3.5. Place the black opaque relaxation sleeping mask with head headband over the patched eyes (**Figure 4**).

NOTE: ISCEV international standard for dark adaptation is 20 minutes. Dark adaptation of at least 30 minutes is preferred to facilitate optimal scotopic ffERG recording given the retinal dark adaptation curve reaches a more asymptotic point compared to 20 minutes. Based on our experience, vast majority of infant and young children are tolerant of bilateral patching, and

parental support and encouragement are critical. Explaining the purpose of dark adaptation and the benefit of reducing general anesthesia time helps the parents to understand. Parental tender loving care including cuddling, music from cell phone, and pacifier are very helpful during the dark adaptation period. Of over 120 infants and young children who underwent the method, only 2 patients could not tolerate bilateral patching for dark adaptation. Both patients were dark-adapted after general anesthesia induction instead and the ERG responses were subsequently successfully recorded using the same method.

4. Dark-Adapted full field electroretinogram recording in the operating room

4.1. Prepare the operating room by placing translucent red filter films over monitors and opaque black tape over LEDs and light sources (**Figure 5A-5B**). Set up folding portable darkroom. Close curtains over door and window openings.

4.2. Induce general anesthesia or sedation by anesthesiology team on bilaterally-patched patient followed by continued anesthesiology monitoring. Perform timeout to verify procedures to be performed.

4.3. Place ERG recording electrodes, ffERG light stimulus, a very dim red light mounted on a forehead band, topical 0.5% ophthalmic proparacaine, 2.5% ophthalmic hydroxypropyl methylcellulose (if Burian-Allen electrode is used), and sterile gauze (for wiping excess methylcellulose) close to ERG examiner position before placing the portable darkroom to enclose the head of the patient and ERG examiner (**Figure 6A**). The ERG examiner will be using the mounted red forehead band to perform scotopic ffERG recordings.

NOTE: The red light mounted on a forehead band is modified by placing layers of red light filter films over the LEDs. The red light should be as dim as possible to allow the ERG examiner to perform the procedure so dark adaptation is maintained. Helpful for the examiner to wait a few minutes to have some of his or her own partial dark adaptation before placing the electrodes. Experienced ERG examiners tend to use very dim red light or can do the procedure by feel without any red light if Burian-Allen electrode is used.

4.4. Place the ground ERG electrode with conductive jelly on one ear lobe. Snake the ground ERG electrode connection and ffERG light stimulus cable through the modified flap opening of the portable darkroom and the ERG technician connects them to the ERG system outside of the darkroom.

4.5. Turn off room lights and check and cover any remaining uncovered light sources with black tape.

4.6. Remove the black mask over both eyes. Remove the black tape and patches over the right eye only and place the corneal ERG recording electrode on the right eye to record the scotopic ffERG responses using the hand-held full field light stimulus in accordance to the ISCEV standards (**Figure 6B**).

4.6.1. Snake the ERG recording electrode connection through the modified flap opening of the portable darkroom for the ERG technician to connect it to the ERG system outside of the darkroom. Take care to use the dimmest red light possible, and a brief period of additional dark adaptation, approximately 5 min, is recommended for recovery after lens insertion in accordance to the ISCEV standards.

4.6.2. After checking for electrical baseline stability and ERG electrode impedance, proceed with recording of the rod responses (dark-adapted 0.01 $cd \cdot s \cdot m^{-2}$ flash ERG), followed by the combined rod-cone responses (dark-adapted 3.0 $cd \cdot s \cdot m^{-2}$ flash ERG and 10 $cd \cdot s \cdot m^{-2}$ flash ERG) and the dark-adapted 3.0 flash oscillatory potential responses. Be mindful of the recommended time intervals between the light stimulus to maintain dark adaptation.

NOTE: When a handheld ERG light stimulus is used to test one eye at a time, keep the other eye monocular patched to maintain dark adaptation during scotopic recordings of the first eye. Dawson Trick Litzkow (DTL) fiber electrode or bipolar Burian-Allen ERG corneal electrodes are typically used. The DTL electrode is better tolerated by a conscious patient and has lower amplitude-to-noise ratio compared to the Burian-Allen ERG corneal electrode. Given patient tolerance is not an issue during sedation or general anesthesia, Burian-Allen electrode is preferred for sedated ERG recordings given its superior amplitude-to-noise ratio.

4.7. Remove the black tape and patches of the left eye and proceed with scotopic ffERG recording of the left eye following same procedures as for the first eye as in step 4.6 with the hand-held full field light stimulus.

NOTE: Recorded scotopic ffERG amplitudes tend to be mildly lower in the second recorded eye given the retinal dark adaptation of the second eye is typically affected by the ERG stimulus flashes diffusing to the eye through bone and tissue during ERG recording of the first eye.

5. Light-Adapted full field electroretinogram recording in the operating room

5.1. After completion of the scotopic ffERG recordings, turn on all overhead room lights. Disconnect the ERG electrode connections and the ffERG light stimulus cable from the ERG recording system and snake them back to the inside the portable dark room through the modified flap opening. Remove the portable dark room.

5.2. Light adapt both eyes for 10 minutes by using the overhead room lights in accordance to the ISCEV standards (background luminance 30 $cd \cdot s \cdot m^{-2}$). Keep the bipolar Burian-Allen ERG electrodes in place for both eyes given the built-in eyelid speculums of the electrodes will hold the eyes open. If DTL lenses are used, use eyelid speculums to keep both eyes open with instillation of periodic lubricating eye drops to avoid corneal drying.

5.3. Connect the ERG electrode connections and the ffERG light stimulus cable to the ERG system and proceed to record, in accordance to the ISCEV standards, the cone flash responses (light-

adapted 3.0 cd·s·m^{-2} flash ERG) followed by the cone flicker responses (light-adapted 3.0 flicker ERG).

NOTE: This completes the ffERG recording. Other diagnostic retinal imaging including OCT, fundus photos as well as venopuncture for genetic testing can easily follow while the patient remains sedated.

REPRESENTATIVE RESULTS:

Using the method described, valid, reliable, interpretable normal and abnormal ffERG responses are feasibly obtained in the operating room for infants and young children under sedation or general anesthesia. In particular, falsely low scotopic ffERG responses are avoided, and common retinal causes of decreased vision and nystagmus in this age group are readily identified. For instance, the preservation of scotopic ffERG responses is important to differentiate Leber congenital amaurosis from achromatopsia where the cone ffERG responses are diminished in both conditions but the scotopic ffERG responses are preserved in achromatopsia but not in Leber congenital amaurosis (**Figure 7**). Obtaining good quality scotopic ffERG responses is also important to diagnose conditions where distinct scotopic ffERG waveform morphology is present. For example, the presence of a negative b-wave in the scotopic combined rod-cone ffERG response is a key feature of congenital stationary night blindness (**Figure 7**). While anesthetic agents may reduce ERG responses, ERG responses under anesthesia are reliable in providing accurate diagnosis.[6] The lower limit of the normal range of the ERG responses is age dependent and increases with age. For instance, the lower limit of normal for age 12 months to 24 months for the scotopic rod responses with the Burian-Allen electrode is 75 µV. As recommended by ISCEV, individual ERG labs are encouraged to collect own normal values.

The method is used reliably to determine disease progression over time. For instance, the systemic features of Alström syndrome are subtle in very young patients and the initial ffERG responses may be similar to achromatopsia with relative preservation of scotopic ffERG responses and diminished cone responses (**Figure 8**). Over time, the scotopic ffERG responses worsen showing a cone-rod dysfunction pattern that is consistent with conditions including cone-rod dystrophy and secondary syndromic cone-rod degenerations such as Alström's syndrome (**Figure 8**).

FIGURE AND TABLE LEGEND:

Figure 1: Dark proofing of openings of operating room. Opaque non-reflective black curtains cover operating room door and window openings.

Figure 2: Folding portable darkroom. Commercially available foldable portable darkroom (**A**) isolates the patient's head and the ERG examiner (**B**) to optimize the maintenance of retinal dark adaptation during scotopic ffERG recordings (photo taken with lights on before starting case for illustration purposes).

Figure 3: Modification of the rear of the darkroom. Small opening created at the rear of the

darkroom (**A**) covered by double flaps (**B**) allow routing connections and cables to the ERG recording system outside of the darkroom (**C**).

Figure 4: Dark adaptation with bilateral patching. A dark relaxation mask is placed over the patient after each eye is patched by placing a layer of black tape over 2 eye pads over closed eyelids.

Figure 5: Dark proofing of operating room. Translucent red filter films (**A**) are taped over monitors and opaque black tape (**B**) covers LEDs and light sources.

Figure 6: Recoding scotopic ffERG responses inside darkroom. Patient with darkroom in place (**A**). Scotopic ffERG responses are recorded in a very darkened environment inside darkroom (**B**) with a very dim red light mounted on a forehead band used to place corneal ERG recording electrodes in place (photo taken with lights on before starting case for illustration purposes).

Figure 7: Normal and abnormal ffERG examples. Standard ffERG responses obtained with method in infants and young children showing normal responses and valid reliable scotopic and photopic responses that easily differentiate Leber congenital amaurosis (LCA), achromatopsia, and congenital stationary night blindness (CSNB). LCA example is a 6-year-old with RDH12 genotype; achromatopsia example is a 3-year-old with PDE6C genotype; CSNB example is a 3-year-old with TRPM1 genotype.

Figure 8: Recoding scotopic ffERG responses inside darkroom. Standard ffERG responses obtained with method obtained in a 2-year-old patient with follow-up 2 years later. Progression of the scotopic ffERG responses is evident, and patient was found to have Alström syndrome.

DISCUSSION:

The methodology and protocol describe how to effectively perform valid and reliable ffERG in infants and children under sedation or general anesthesia in the operating room. The major concept and aim of the technique are to provide and maintain optimal retinal dark adaptation during scotopic ffERG recordings. This is essential to provide accurate objective assessment of rod photoreceptor function given retinal dark adaptation is rapidly diminished by exposure even to dim light leading to erroneous recorded responses. The critical steps of the method are (i) to choose an operating room with low 60 Hz background electric current noise, proper electrical grounding and to modify and prepare the room meticulously to block light sources (ii) to pharmacologically dilated the pupils fully and to place multi-layer eye patches that completely blocks light to induce retinal dark adaptation (iii) to use a modified portable foldable darkroom that encloses the patient's head and the ERG examiner, and (iv) to use the least amount of dim red light needed inside the darkroom during scotopic ffERG recording.

The method is significantly superior to existing alternative methods. While performing ffERG without sedation in infants less than 1 year old may be possible by using a feeding bottle and some children can cooperate with ffERG recording, cooperation is poor in most infants and young children. Performing ffERG with oral sedation in a conventional ERG recording darkroom lacks

the ability to monitor the patient's cardiopulmonary function safely and the spectrum of responses to the oral sedation is wide and unpredictable. Performing ffERG in a darkened operating room without a portable darkroom is typically not dark enough to adequately achieve and maintain dark adaptation given the growing number of new anesthetic and operating room equipment.

The method works well with a small handheld ffERG light stimulus given the limited space inside the portable darkroom, and ffERG recording is done one eye at a time. A larger full-size full field ERG stimulus dome will allow simultaneous ERG recording of both eyes and shorten examination time. A full-size ERG stimulus dome would require a metallic arm to hold it securely over the supine patient and a larger portable darkroom with a much larger flap opening would be required to accommodate the metallic arm. Such modification is possible with care to create a large flap opening that is not prone to light leak.

Limitations of the method are few and include the effect of sedative and general anesthetic agents in reducing ERG responses, which is not substantial enough to influence accurate clinical diagnosis and follow-up testing to assess progression. The method requires the cooperation of the pediatric anesthesiology team to monitor patient in a dimly lit operating room for 15 minutes during scotopic ffERG recording. If anesthetic emergency arises, the procedure can be aborted immediately and the portable darkroom can be moved rapidly to allow full patient access.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

Click here to access/download;Figure;Figure 1 OR black Curtain.png

Figure 2A

Click here to access/download;Figure;Figure 2A Portable Darkroom.png

Figure 2B

Click here to access/download;Figure;Figure 2B Darkroom closed.png

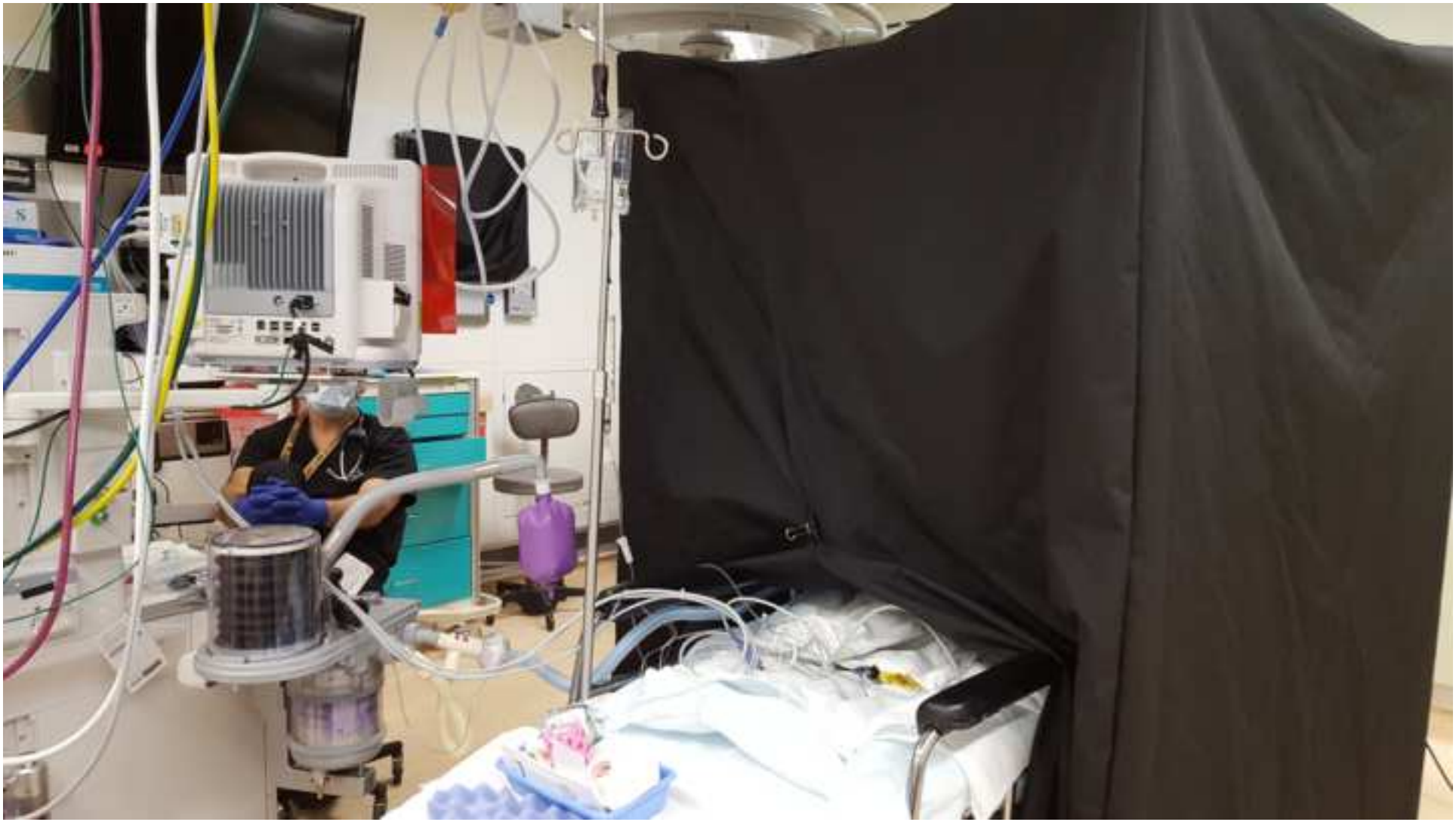

Figure 3A

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Figure 3B

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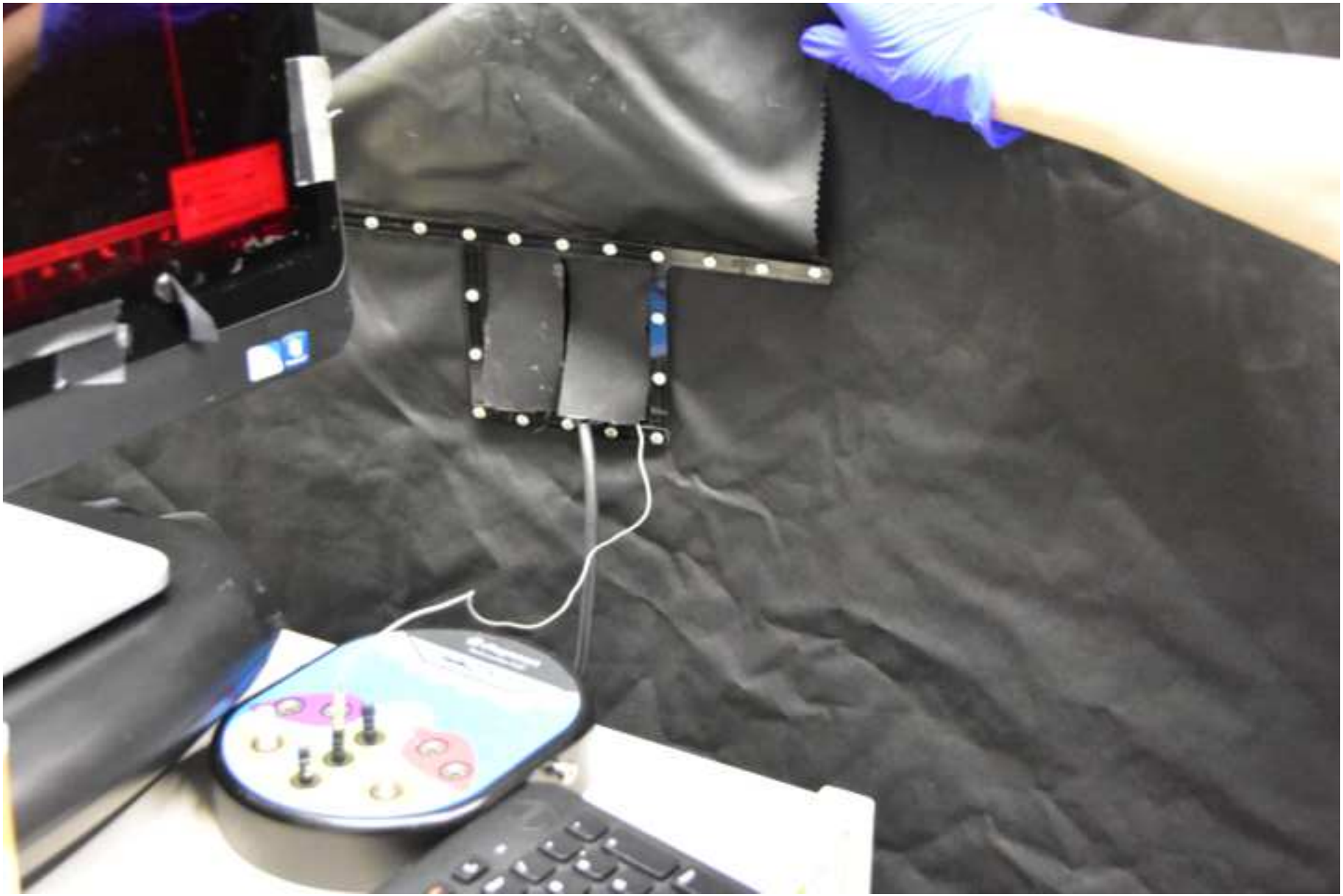

Figure 3C

Click here to access/download;Figure;Figure 3C ERG Tech Outside of Darkroom.png

Figure 4

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Figure 5A

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Figure 5B

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Figure 6A

Click here to access/download;Figure;Figure 6A Patient ready in darkroom.png

Figure 6B

Click here to access/download;Figure;Figure 6B inside darkroom before starting.png

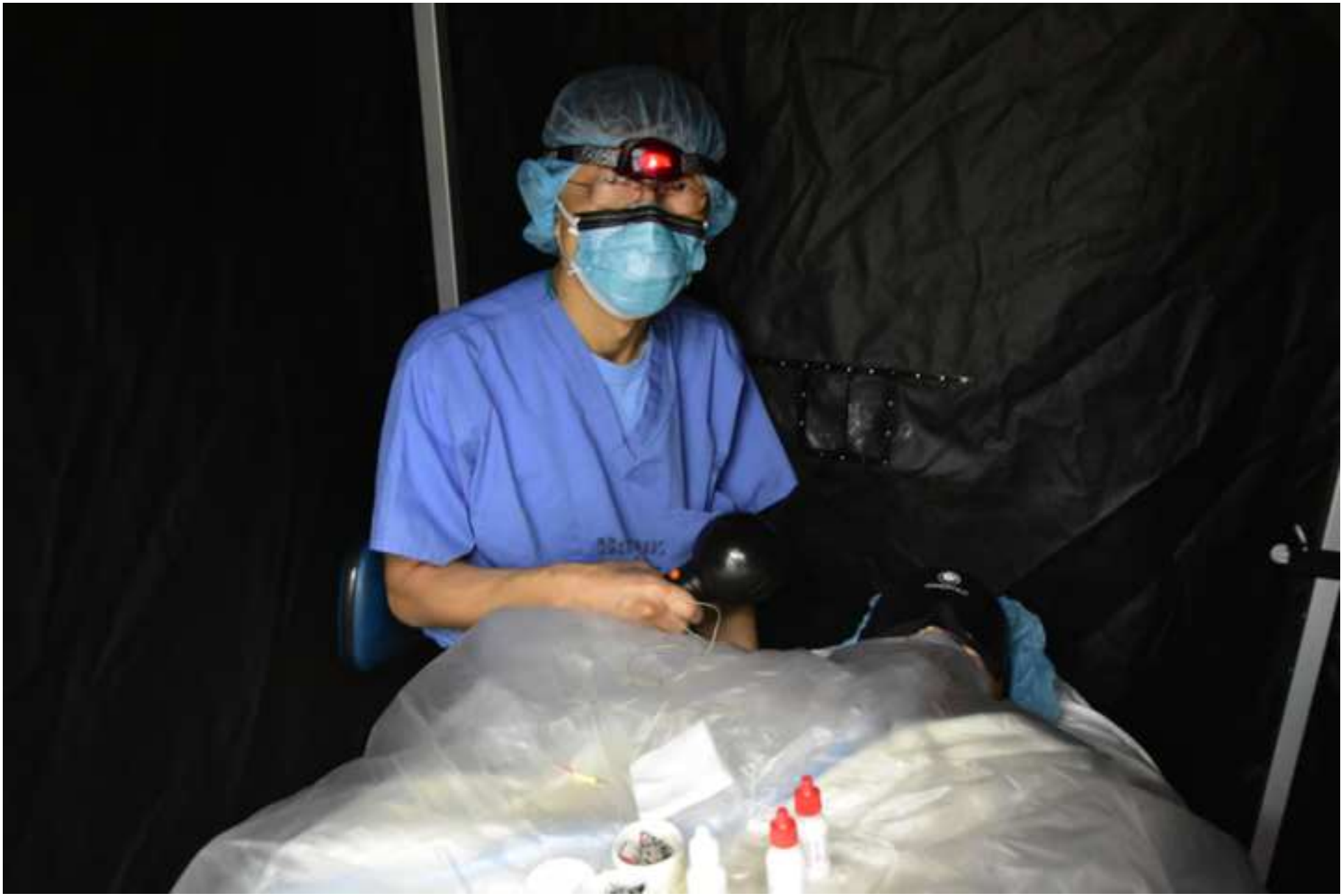

Figure 7

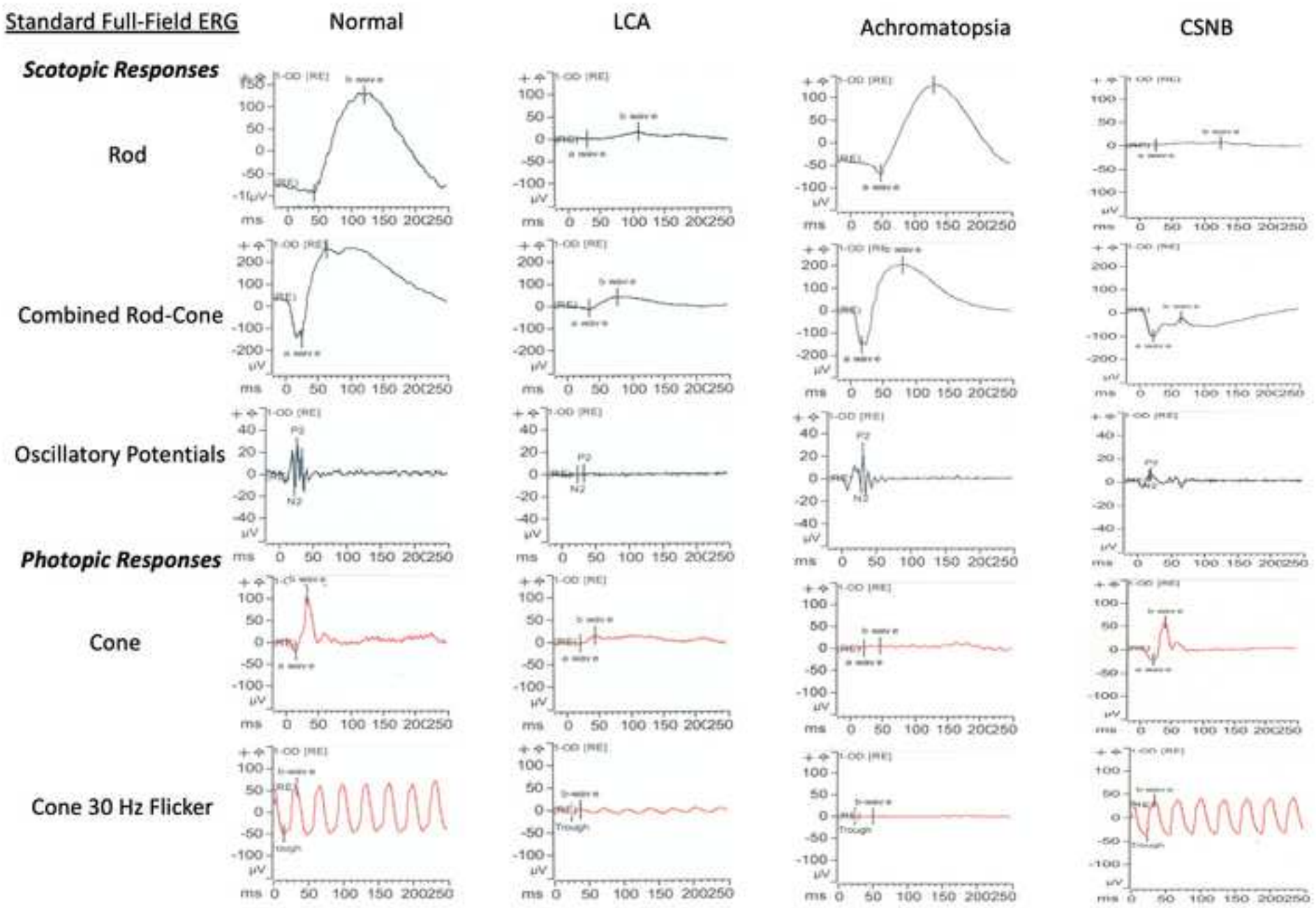

Figure 8

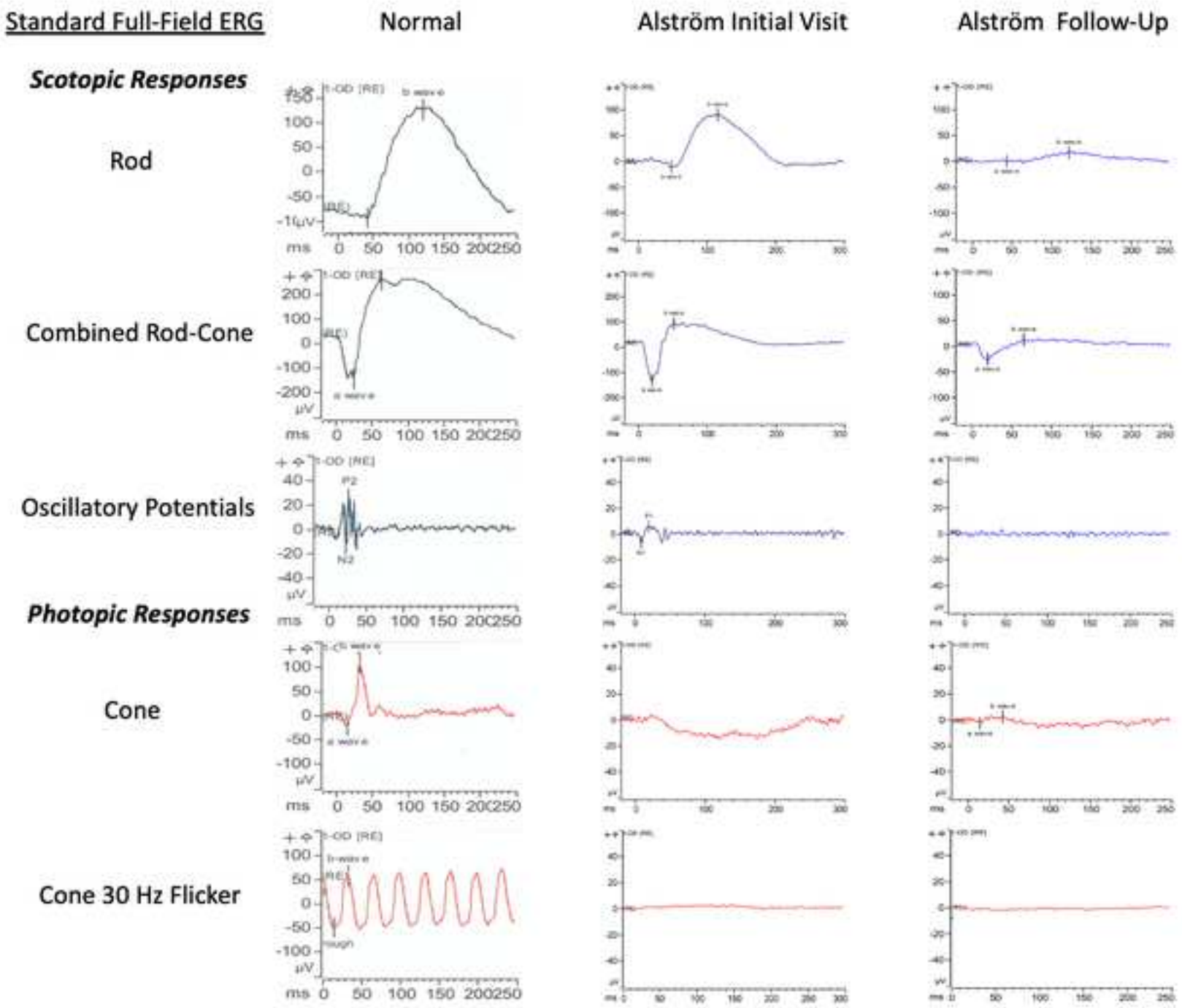

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Black tape	3M Industrial Adhesives and Tapes Division, St Paul, MN 55144-1000 USA	3M ID 70016070396	
Conduction and abrasive paste	Redux Paste (Electrolyte Paste) Hewlett Packard company, USA. Nuprep (Sking Prep Gel) and Ten20 (Conductive Neurodiagnostic Electrode Paste) Weaver and Company, CO, USA	67-05	
Darkroom - Portable foldable	Scientex	B-LP1/B-LP1-X	Available in different sizes
Dark adaptation mask (relaxation sleeping mask)	Mindfold Inc, Durango, CO, USA	6576493	Flexible black plastic face plate backed with a high-density soft foam padding that allows total darkness.
Ear clips for electric grounding	Grass	F-E34DG-72	Grass 10mm Gold Cup EEG Ear Clip with touchproof connector 72" wire - Set of 2

Electrodes ERG recording (Burian-Allen, DTL)	Burian-Allen, Hansen Ophthalmic Develoment Lab, Iowa, USA; DTL, Diagnosys, Lowell, MA 01854, USA.	303-20LA, 303-20A, 303-20P, 303-20I, 303-20SI	Available in different sizes, requires modification as described in Protocol.
ERG systems including handheld full-field stimulus	Any system meeting the standards established by the International Society for Clinical Electrophysiology of Vision (ISCEV).		Authors use Diagnosys and Roland systems; other ISCEV standard systems available.
Eye drops, propracaine, metilcellulose, phenilephrine, ciclopentolate,	Tropicamide 1% Phenylephrine 2.5% Cyclopentolate 1% Proparacaine 0.5% Akorn, Inc. Forest, IL 60045 GONIOTAIRE (Hypromellose 2.5%) Altaire Pharmaceuticals, Inc. NY, NY, USA 11931		
Eye Patch	BSN Medical Inc, Rutherford College, NC	46430-00	Coverlet eye occlusor for treatment of lazy eye
Head band with light	REMIX PRO. Princeton Tec, Trenton, NJ 08650	RMX300PRO-RD-BK	Requires placing layers of red filters over LED as described in protocol

July 19, 2020

Vineeta Bajaj, Ph.D.
Review Editor
JoVE

Re: JoVE61734 "Electroretinogram Recording for Infants and Children under Anesthesia: Simple Ways to Achieve Optimal Dark Adaptation and International Standards,"

Dear Dr. Bajaj:

Please see the responses below to the editorial and reviewer comments. We thank the editor and the reviewers for their constructive comments which have improved our paper.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
Thorough proofreading has been performed to ensure no spelling or grammatical errors.

• Avoid punctuating the title and make it concise.
The title is shortened with punctuation removed. The title now reads: "Electroretinogram Recording for Infants and Children under Anesthesia to Achieve Optimal Dark Adaptation and International Standards"

• **Introduction:** Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.
The following paragraph is added to the end of Introduction:
"The advantages of the method over alternative techniques include optimizing and maintaining retinal dark adaptation, promoting valid reliable ffERG recordings, improving patient safety, and facilitating additional diagnostic testing such as retinal imaging and venopuncture for genetic testing. Optimal dark adaptation is also important to achieve best possible ffERG recordings given the ISCEV guidelines for calibration of stimulus and scotopic recording parameters are most applicable in complete darkness conditions [10]. Alternative methods include the use of oral agents such as chloral hydrate with variable sedative responses in infants and children, which affects the quality of ffERG recordings and causes difficulties in

monitoring vital signs. While some children can cooperate with ffERG recording in the clinic, the testing session may be prolonged depending on cooperation, and the validity of ffERG recordings may be affected by eye movement and blink artifacts as well as difficulty in maintainng retinal dark adaptation[4]. The current method provides additional dark adaptation and safety measures compared to the previously described deep sedation ffERG method.[6]"

• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Some examples NOT in the imperative: 3.3,4.2,
2) Split up long steps (e.g., 3.3)
Imperatives are now used and long steps are split throughout the Protocol.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
1) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.
The following is added immediately after the Protocol heading "The protocol follows the operating room guidelines of the Bascom Palmer Eye Institute, University of Miami …" The method is clinical and not research and requires no IRB approval.
2) Mention patient age, sex, and any other inclusion/exclusion criteria.
The following is added immediately after the Protocol heading "…is applicable to infants, young children, and uncooperative adults. Patients who cannot have general anesthesia due to safety issues should not have the procedure."
3) 4.3: Mention recommended drugs if any.
The dilation eye drops are mentioned in 4.3.

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform

the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
The protocol highlight is revised based on these instructions.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
The protocol highlight is revised based on these instructions.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
The protocol highlight is revised based on these instructions.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.
The Discussion has been reviewed and covers the above in detail.

• Please provide each figure (if multiple panels are present per figure, keep them within 1 file) as an individual SVG, EPS, AI, TIFF, or PNG file.
The figures are changed from JPEG to PNG.

• **References:**
1) A minimum of 10 references is required.
There are now 10 references.
2) Please spell out journal names.
The journal names are spelled out in the clean Word file of the paper included in the submission of this revision. We used Endnote JOVE format which apparently doesn't spell out journal names.

• **Table of Materials:** Please sort in alphabetical order.
The Table of Materials is sorted in alphabetical order.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Our figures and tables are original and not published previously

Comments from Peer-Reviewers:

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
In this paper, Lam et al describe in detail a method for obtaining electroretinograms from infants in an OR setting with sedation of the infant. This test provides important information on the status of the retina in infants suspected of vision loss or of having an inherited retinal disease. The authors are experienced in this procedure and a video presentation would be quite informative to other pediatric ophthalmologists who would like to provide a similar service.

Major Concerns:
1. When recording the video, it would be useful to show other procedures, such as OCT, which would commonly be performed under sedation in this patient population in addition to the ERG.
Thanks for the comment, the protocol highlight for the video now includes the other diagnostic retinal imaging including the OCT that follows the ffERG recording.
2. The potential portable ERG equipment shown in the Table at the end of the document does not include the portable RETeval system (LKC Technologies, Gaithersburg, MD). I believe that this system also meets requirements and should be listed. If the authors do not believe that it is suitable for this purpose, the reason should be stated.
The method can be used with any portable ERG equipment that meets ISCEV standards. To list all available systems is beyond the scope of this paper. We have modified the Table of Material to state "Any system meeting the standards established by the International Society for Clinical Electrophysiology of Vision (ISCEV)"
Minor Concerns:
3. I do not understand the use of yellow highlight in the manuscript
The yellow highlight is for protocol highlight per authors' instructions to include what needs to be included in the video.
4. Page numbering is off (Page 9 of 6).
This is corrected.

Reviewer #2:
Manuscript Summary:

Good depiction of recording dark-adapted ERGs in operating room.

Major Concerns:
None

Minor Concerns:
Authors might consider mentioning abbreviated O.R. procedure where full dark adaptation they recommend is not feasible.
Typically, when full dark adaptation in the O.R. is not feasible is because of poor communications with the anesthesiology team to discuss the importance of dark adaption. As stated in the Introduction, Paragraph 4, "Conferring with the anesthesiology team is essential in modifying the operating room and to promote the safety of the patient in a darkened environment."

Reviewer #3:
Manuscript Summary:
This is a well written and very useful description of the novel technique the authors utilize to obtain high quality dark adapted ERGs in the operating room in patients unable to cooperate with testing while awake. The authors describe a portable dark room setup which can be brought to the OR as well as their pre-anesthesia induction dilation and patching regimen which shortens the OR time.

Major Concerns:
The introduction is good but could more clearly state the exact ISCEV standards, especially for dark adaptation.
The ISCEV standards is mentioned in Introduction, paragraph 2 and referenced as Reference #1. The importance of compliance to ISCEV standards is emphasized throughout the paper. The light stimulus levels recommended by ISCEV is added to the protocol steps. The ISCEV standard for dark adaptation of 20 minutes is stated in step 3.5 note. Step 4.6 is revised to add more info from the ISCEV standards. Given the length of the ISCEV standards, a 10-page document, inclusion of further details of the ISCEV standards in the Introduction is beyond the scope of this paper.

Under "Operating Room Selection and Modification" it should be stated how to determine the 60 Hz background noise in an OR; it may differ in different parts of the OR.
Step 1.1 is revised to include the following:
"Perform trial ERG recordings in the operating room at the location where the ERG recording will take place. Check the ERG recording baseline as well as the trial recording to determine the absence of 60 Hz background electric noise."

Under "Inspect Operating room for light leaks" please state how light leaks are corrected if found.
Step 1.3 states how light leaks are blocked if found.

Please state how the curtains are affixed to the OR room door. The authors note that the fabrics used are washable; it would be helpful to know where and how often the fabrics are laundered, i.e. can they go in the hospital laundry?
Step 1.3 is revised to include tracks used to install the curtains as demonstrated in Figure 1. The method and frequency of washing curtains varies by local operating room regulations and procedures.
"Install opaque non-reflective black curtains on tracks to cover the operating room door and window openings fully without light leakage (Figure 1). Select curtain material that is washable and resistant to staining and bacterial growth. Follow local operating room regulations and procedures for proper interval cleaning. …"

There could be a section on: Equipment/materials required. This should include what types of materials are needed to cover lights, where to get the red filters, signage such as shown in the figures, etc. There is an excellent table at the very end and this should be placed here for better understanding of what follows.
We will follow the JoVE publication format with respect to where the table of material is placed.

Under Portable Darkroom, when I go to the website for the portable darkroom it says "*This darkroom can't use in clean room." This implies that the material cannot be cleaned? The authors must clarify this.
Step 2.1 Note is revised to "Note: The fabric of the portable darkroom mentioned was tested by our eye institute's microbiology department for ease of disinfection and the optical transmission was tested by our eye institute's biomedical department before purchase. This is recommended if a different portable darkroom is used."

There could be a section headed, "Results" showing the beautiful waveforms obtained. The waveforms are extremely clean, and the Alstrom example is excellent. It would be useful to provide the genotype of the patient with LCA whose waveforms are shown; LCA patients usually have a nonrecordable ERG and this patient has a very low but recordable SCR, cone isolated and 30 Hz flicker so knowing the genotype would be helpful. In addition, because anesthesia is known to reduce ERG amplitude by up to 50%, it would be helpful for the authors to state what the lower limit of normal is with their system.
We follow the JoVE publication format and the section is headed as "REPRESENTATIVE RESULTS"

The legend for Figure 7 is revised to include genotypes of the ERG examples: "…LCA example is a 6-year-old with RDH12 genotype; achromatopsia example is a 3-year-old with PDE6C genotype; CSNB example is a 3-year-old with TRPM1 genotype."
REPRESENTATIVE RESULTS, end of paragraph 1 is revised to "While anesthetic agents may reduce ERG responses, ERG responses under anesthesia are reliable in providing accurate diagnosis. The lower limit of the normal range of the ERG responses is age dependent and increases with age. For instance, our lower limit of normal for age 12 months to 24 months for the scotopic rod responses with the Burian-Allen electrode is 75 uV. As recommended by ISCEV, individual ERG labs are encouraged to collect own normal values."

Please include an estimate of how many patients have undergone this type of testing and what percent cannot tolerate the dark adaptation pre-induction; the authors say it is a small percent but do you have numbers to back this up? This would be very helpful for physicians trying this for the first time.
Step 3.5, end of Note is revised to "Note:…Of over 120 infants and young children who underwent the method, only 2 patients could not tolerate bilateral patching for dark adaptation. Both patients were dark-adapted after general anesthesia induction instead and the ERG responses were subsequently successfully recorded using the same method.

Minor Concerns:
It should be mentioned in the discussion that this technique is for those cases where a patient cannot cooperate in the clinic, because some children can cooperate in clinic.
This is now added to Discussion, paragraph 2, "… some children can cooperate with ffERG recording…"

Thank you for your time and consideration.

Sincerely,

Byron L. Lam, M.D.