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TITLE:
Pupillary Response as Assessment of Effective Seizure Induction by Electroconvulsive Therapy

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KEYWORDS:
electroconvulsive therapy, automated pupillometer, pupillary response, light reflex, anesthesia, pupillary diameter

SUMMARY:
Pupillary responses (light reflex) were measured for assessment of adequate seizure induction by electroconvulsive therapy using an automated infrared pupillometer immediately after electrical stimulation. Constriction ratio was calculated and compared with seizure quality.

ABSTRACT:
Electroconvulsive therapy (ECT) is reported to be effective for severe neuropsychiatric disorders. In ECT, electrical stimulation is applied to the brain, inducing seizure activity. Adequate seizure induction with ECT is associated with seizure duration, symmetrical high amplitude waveforms during slow-wave activity, postictal suppression, and activation of the sympathetic nervous system. Sympathetic nervous system activation is influenced by anesthetic agents or cardiovascular drugs during ECT. Pupillary responses can reflect sympathetic nervous activity or the degree of brain damage. Pupillary response measurement can be conducted in a simple, precise, and objective way using an automated infrared pupillometer, enabling the measurement of pupil diameter (mm) to two decimal places. The white light used for measuring light reflexes is not overly bright, and patients do not typically report discomfort. Pupillary light reflexes were measured before anesthesia induction and immediately after electrical stimulation using this equipment. Pupil diameter is typically enlarged after brain damage or sympathetic nervous activation. Adequate seizure induction using ECT could induce pupillary enlargement immediately after electrical stimulation. In the current method, the constriction ratio of pupil size

was calculated automatically and compared with seizure quality. Pupillary responses immediately after electrical stimulation may provide a useful assessment of the efficacy of seizure induction with ECT.

INTRODUCTION:

Electroconvulsive therapy (ECT) is considered an effective treatment for severe neuropsychiatric disorders, including refractory psychosis, bipolar disorder, and depression[1]. In ECT, an electrical current is applied to the brain to induce a seizure under general anesthesia[2]. Although the mechanisms underlying ECT remain unclear, its antidepressant effects have been attributed to seizure-induced changes in neurotransmitter levels, improved neuroplasticity, increased functional connectivity, and an increase in the plasmatic production of brain-derived neurotrophic factor[3]. It has also been reported that ECT facilitates serotonin-, norepinephrine- and dopamine-mediated neurotransmission[4]. These findings suggest that ECT could cause activation of the sympathetic nervous system. Previous studies have evaluated adequate seizure induction by ECT using seizure duration, symmetrical seizure amplitude, postictal suppression, and activation of the sympathetic nervous system[4,5]. Among these factors, increased activation of the sympathetic nervous system cannot be measured using electroencephalography. Detection of sympathetic nervous system activation is dependent on increased blood pressure (BP) and heart rate (HR). However, these hemodynamic parameters do not always reflect sympathetic responses because of the administration of antihypertensive drugs to prevent cardiac events during ECT and anesthetic agents, which affect sympathetic nervous function.

Pupillary responses can reflect the degree of brain damage[6]. Thus, pupillary mydriasis is indicated for severe brain damage[6]. Artificial seizures induced by electrical stimulation constitute an abnormal state of brain activity. Thus, evaluating the pupillary response immediately after ECT may be useful for assessing the efficacy of ECT because ECT may also influence pupillary responses[7]. However, measuring pupillary responses in busy clinical situations, as in the current case, is often difficult. To address this issue, a measurement method using an infrared quantitative pupillometer could help to measure pupillary responses easily, accurately, objectively and reproducibly. Quantitative pupillary assessment methods are superior to those obtained manually at the bedside, even by experienced nurses and physicians[8]. The proposed method for measuring pupillary reactivity using an automated infrared pupillometer could be useful for detecting the degree of seizure or sympathetic nervous activation. In a previous study, we reported that the pupillary light reflex was related to the efficacy of seizure by ECT[9]. Specifically, we found that pupillary diameter was not changed after light stimulation, remaining enlarged when adequate seizure was induced. Thus, the aim of the proposed method is to measure the light reflex using an automated infrared pupillometer immediately after electrical stimulation. The proposed method is easy to perform, enabling any clinician, not only psychiatrists, to evaluate the efficacy of seizure induction using ECT.

PROTOCOL:

The study protocol was approved by the institutional clinical research ethics committee of Kyushu University, Fukuoka, Japan (IRB: Clinical Research number #28-77). Although the measurement of pupillary reaction is an essential and standard clinical procedure during anesthesia, informed

consent was obtained for this research. Patients with cataract, glaucoma, intraocular lenses, or insulin-dependent diabetes mellitus were excluded because their pupillary responses may be abnormal.

1. Preparation for electroconvulsive therapy

1.1. Clean the right and left forehead and area behind the ears with an alcohol cotton swab, and then attach electroencephalogram (EEG) monitors at four symmetrical points.

1.2. Clean bilateral temples with normal saline, and then attach adhesive electrical stimulation pads (about 4 cm × 5 cm) to the bilateral temples to prevent interference in the stimulation due to hair. The intensity of stimulation is changed each time, according to the previous degree of inducted convulsion.

1.3. Attach electrocardiogram (ECG) monitors at two points on the chest (base of the heart and cardiac apex) and monitor heart rate.

1.4. Prepare a tourniquet and belt it at the left thigh.

NOTE: The tourniquet is belted until 200 mmHg after the patient is asleep. Convulsion induced by ECT can be observed in the lower leg even after systemic administration of muscular relaxant.

1.5. Attach two electromyogram sensors to the left anterior tibial muscle, with a distance of 5 cm, to measure generalized seizure time in the lower leg.

2. Preparation for anesthesia

NOTE: All patients underwent ECT in the same room, at the same time of day.

2.1. After the forehead is cleaned with an alcohol cotton swab, attach a bispectral index (BIS) measurement device to the patient's forehead. BIS is used to monitor depth of anesthesia.

2.2. Non-invasively measure blood pressure at the arm every 1 min during ECT therapy.

2.3. Continuously monitor saturation of oxygen and pulse rate by a clip attached to the finger.

2.4. Hold a pupillometer (see the **Table of Materials**) over one of the patient's eyes. After the patient opens their eyes, press a button (**Figure 1A**) on the pupillometer, and measurement starts automatically. Maximum (initial) resting pupil size (MAX) and minimum pupil size after light stimulation (MIN) are automatically measured, and the constriction pupil size ratio (%constriction = [MAX–MIN]/MAX expressed as a percentage) is automatically calculated.

NOTE: The duration of light emission is 800 ms, and the required data can be obtained within 1 second. If the patient is unable to keep their eyes open, the examiner assists by holding the eyelid

open. Although anesthetists typically measure the pupillary diameter or light reflex of patients before surgery in the operating room, cooperation with patients is needed before anesthesia induction.

3. Anesthesia

3.1. Supply oxygen (6 L/min) via a mask.

3.2. Administer propofol (sedative: 3 µg/mL, target controlled infusion) via intravenous line until loss of consciousness.

3.3. Tighten the tourniquet belted at the thigh to 200 mmHg.

3.4. Administer succinylcholine (muscle relaxant: 1 mg/kg) via the intravenous line.

3.5. After spontaneous breathing of patients ceases, have the anesthesiologist perform controlled ventilation using a face mask with 100% oxygen without intubation. Perform ventilation from the point at which end-tidal carbon dioxide is 30 mmHg until electrical stimulation[10].

3.6. After BIS value begins to increase, the anesthesiologist stops ventilation and psychiatrists start the ECT procedure.

4. Electroconvulsive therapy procedure

4.1. Conduct ECT via bitemporal electrode stimulation using an ECT instrument. Set the initial electrical stimulus dose (%) at half the value of each individual's age.

NOTE: Maximum stimulus dose is 100%. The electrical stimulation time is about 7-8 s.

4.2. Immediately after electrical stimulation, hold thr automated infrared pupillometer over one of the patient's eyes. At this time, if the patient closes their eyes, keep the patient's eyelid open. Press the device button, as in step 2.4, and measure maximum (initial) resting pupil size (MAX) or minimum pupil size after stimulation (MIN).

4.3. Perform controlled ventilation using a face mask with 100% oxygen until the patient begins breathing spontaneously.

4.4. Using EEG, measure ictal regularity, seizure time and greater postictal suppression (by the psychiatrist [**Figure 2**, **Figure 3**]).

REPRESENTATIVE RESULTS:

Portable pupillometer devices are produced by several companies. These devices are typically conveniently sized and can be operated with one hand (**Figure 1A**), enabling examiners to

accurately detect pupillary responses by pressing a button. Infrared light is used to detect the pupillary edge automatically (**Figure 1B**), accurately representing pupillary data (**Figure 1C**). This device measures maximum (initial) resting pupil size (MAX), minimum pupil size after stimulation (MIN), constriction pupil size ratio (%constriction, [MAX-MIN]/MAX expressed as a percentage), latency duration (LAT, time between initiation of retinal light stimulation and onset of pupillary constriction), constriction velocity (CV, extent of constriction/duration of constriction), maximum constriction velocity (MCV), dilation velocity (DV, extent of pupil size recovery/duration of recovery). The light reflex is acquired using a flash of visible white light of 800 ms duration at the start of each 3.2 s scan[11]. We report the relationship between the pupillary response and efficacy of ECT using this device among 13 patients (**Figure 3**, this figure has been modified from reprinted from Shirozu et al.[9]. As shown in **Figure 3A**, pupillary diameter was increased after electrical stimulation. However, significant differences in constriction by the light reflex were caused immediately after electrical stimulation, reflecting differences in seizure efficacy induced by ECT (**Figure 3B**).

FIGURE & TABLE LEGENDS:

Figure 1: Automated infrared pupillometer. (**A**) The measurement method. (**B**) Detecting the edge of the pupil. (**C**) Representing the pupillary data. The images are presented with the explicit permission from the commercial supplier.

Figure 2: EEG results during a typical seizure.

Figure 3: Pupillary response changed immediately after electrical stimulation. (**A**) Maximum pupil diameter. (**B**) Pupillary constriction ratio (light reflex). We used two-way ANOVA for comparisons between adequate and inadequate seizure groups at control, and just after stimulation. Adequate seizure status was determined using the criteria described above. Data are presented as mean ± standard deviation. Sidak's multiple-comparison post hoc test was utilized for two-way analysis of variance. $^{***}p < 0.001$, $^{****}p < 0.0001$. This figure has been modified from reprinted from Shirozu et al.[9] with permission.

DISCUSSION:

Automated infrared pupillometer devices have been used to measure pupillary reactions in clinical situation[12]. However, to our knowledge, no previous studies have used this equipment for detecting the efficacy of seizure induction by ECT. Resting pupil diameter size differs between patients, but constriction ratio provides an objective measure. Thus, we selected the constriction ratio change, not the diameter size change. Additionally, small changes of pupillary diameter can only be measured using an automated infrared pupillometer.

Several important factors should be considered in applying this method. Pupillometer measurement should not be used in patients with ophthalmic lesions because pupillary reflexes may be altered as compared with normal patients. Pupillary reactions are influenced by environmental light and time of day, these factors should be kept uniform across patients. Although anesthetic agents and psychiatric drugs have also been found to affect pupillary

reaction,[9,13] these treatments did not affect the results in our previous report[9].

The efficacy of ECT has been evaluated based on electroencephalography and increases in BP or HR. This method is widely used, and has been evaluated in many previous studies. However, an increasing number of patients have adapted to ECT, and rates of cardiovascular disease are increasing. Thus, it is important to prevent abnormal hypertension. The proposed method might be particularly useful for patients who have been administered antihypertensive drugs during ECT. Pupillometry can be used to measure constriction velocity after constriction by light stimulation, and further details regarding the relationship between pupillary reaction and efficacy of ECT should be examined in future studies. These studies may reveal valuable information about the relationship between the efficacy of ECT and pupillary reactions at time points other than immediately after electrical stimulation.

Hemodynamics are substantially changed immediately after electrical stimulation. Then, measurement should be conducted immediately after electrical stimulation because pupillary reactions recover rapidly. Thus, pupillary reaction measurement should be conducted by a person other than the responsible anesthesiologist, and the method requires cooperation with a psychiatrist or nurse.

ACKNOWLEDGMENTS:
none

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1 Saito, S. Anesthesia management for electroconvulsive therapy: hemodynamic and respiratory management. *Journal of Anesthesia.* **19** (2), 142-149, (2005).
2 Ward, H. B., Szabo, S. T. & Rakesh, G. Maintenance ECT in schizophrenia: A systematic review. *Psychiatry Research.* **264** 131-142, (2018).
3 Milev, R. V. et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 4. Neurostimulation Treatments. *Canadian Journal of Psychiatry.* **61** (9), 561-575, (2016).
4 Vutskits, L. General Anesthetics to Treat Major Depressive Disorder: Clinical Relevance and Underlying Mechanisms. *Anesthesia & Analgesia.* **126** (1), 208-216, (2018).
5 Nishikawa, K. & Yamakage, M. Effects of the concurrent use of a reduced dose of propofol with divided supplemental remifentanil and moderate hyperventilation on duration and morphology of electroconvulsive therapy-induced electroencephalographic seizure activity: A randomized controlled trial. *Journal of Clinical Anesthesia.* **37** 63-68, (2017).
6 Ritter, A. M. et al. Brain stem blood flow, pupillary response, and outcome in patients with severe head injuries. *Neurosurgery.* **44** (5), 941-948, (1999).
7 Kobayashi, K. et al. Rapid and lasting enhancement of dopaminergic modulation at the hippocampal mossy fiber synapse by electroconvulsive treatment. *Journal of Neurophysiology.* **117** (1), 284-289, (2017).

8 Meeker, M. et al. Pupil examination: validity and clinical utility of an automated pupillometer. *Journal of Neuroscience Nursing.* **37** (1), 34-40, (2005).
9 Shirozu, K. et al. The relationship between seizure in electroconvulsive therapy and pupillary response using an automated pupilometer. *Journal of Anesthesia.* 10.1007/s00540-018-2566-9, (2018).
10 Sawayama, E. et al. Moderate hyperventilation prolongs electroencephalogram seizure duration of the first electroconvulsive therapy. *JOURNAL OF ECT.* **24** (3), 195-198, (2008).
11 Rollins, M. D., Feiner, J. R., Lee, J. M., Shah, S. & Larson, M. Pupillary effects of high-dose opioid quantified with infrared pupillometry. *Anesthesiology.* **121** (5), 1037-1044, (2014).
12 McNett, M., Moran, C., Janki, C. & Gianakis, A. Correlations Between Hourly Pupillometer Readings and Intracranial Pressure Values. *Journal of Neuroscience Nursing.* **49** (4), 229-234, (2017).
13 Shirozu, K. et al. The effects of anesthetic agents on pupillary function during general anesthesia using the automated infrared quantitative pupillometer. *Journal of Clinical Monitoring and Computing.* **31** (2), 291-296, (2017).

Figure 1

Click here to access/download;Figure;figure 1.tif

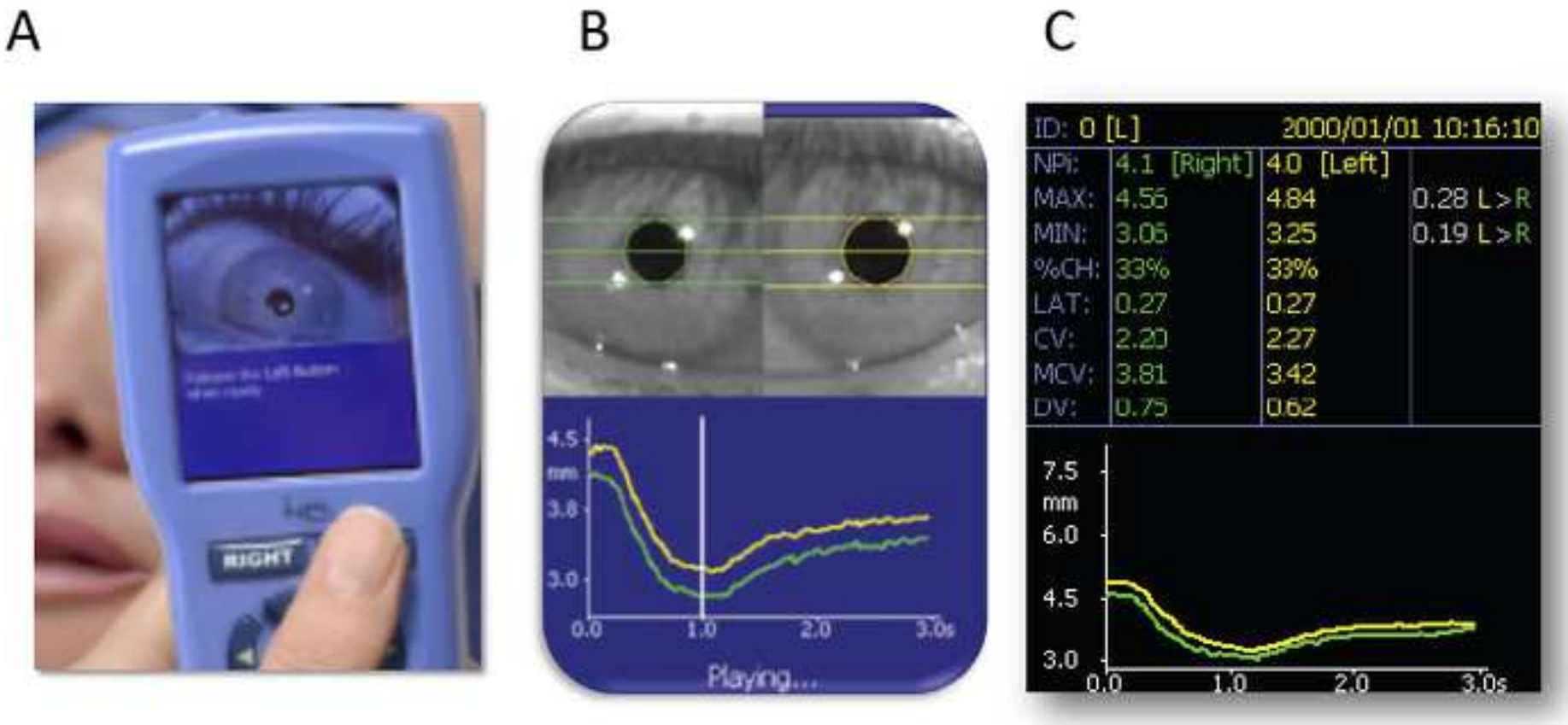
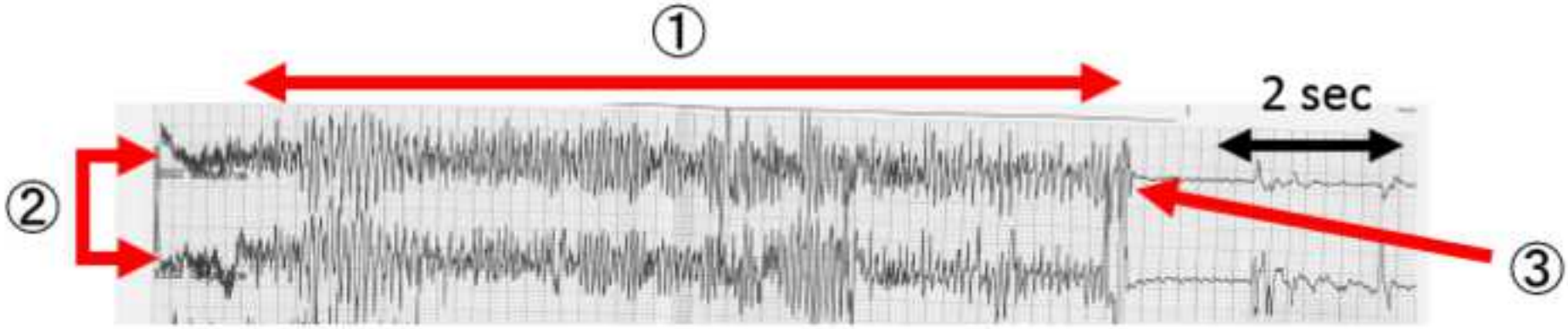


Figure 2



Adequate seizure group meets below three inclusion criteria:

① An induced encephalograph seizure time > 25 s

② Regularity symmetric high amplitude (300–500 μV) spike- and-slow wave complex

③ Postictal suppression > 2, evaluated by a psychiatric specialist

Inadequate seizure group do not satisfy above criteria.

③(PSI: postictal suppression index)

3 : good seizure suppression (very flat), and transition is abrupt

2 : good seizure suppression (very flat), but transition to flat is gradual

1 : seizure termination is clear, but suppression is poor (not flat)

0 : cannot tell where the seizure ends

Figure 3

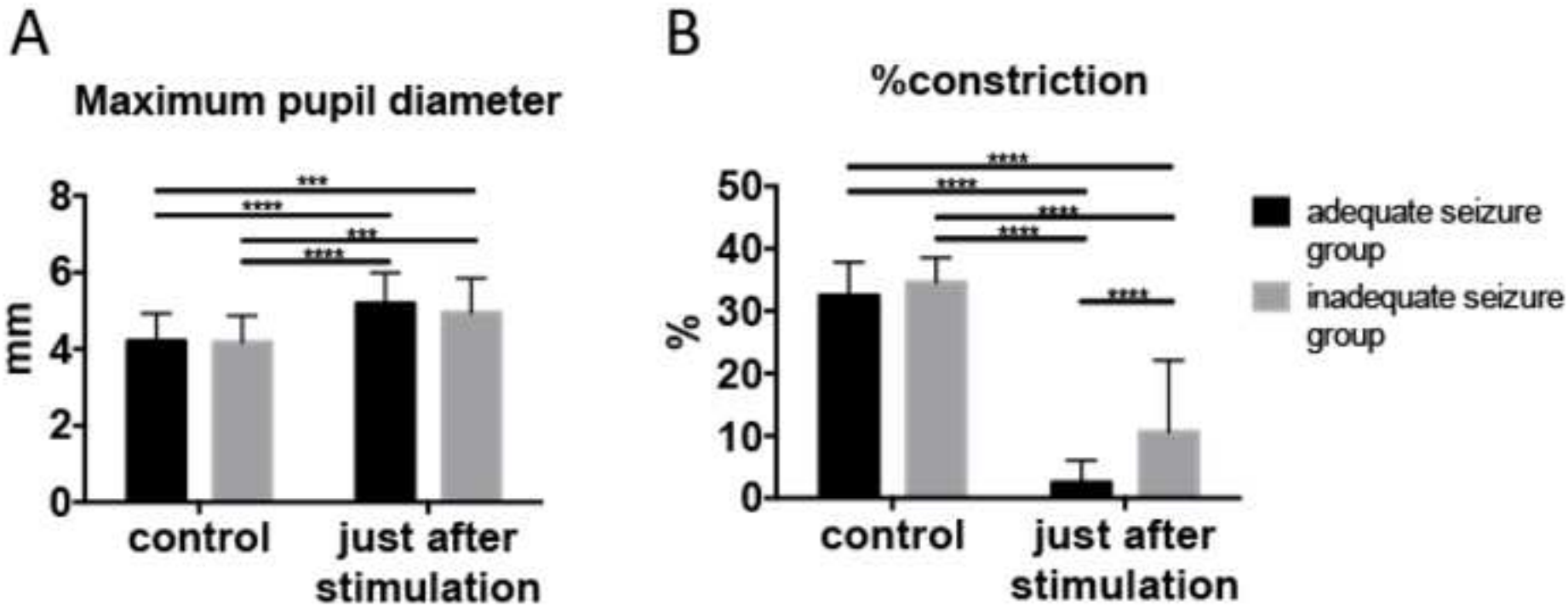


Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
BIS Quatro sensor	medtronic		
ECG	Somatics Inc.	ELDSC-9	
EEG	Somatics Inc.	ECEF-4	
EMG monitoring lead	Somatics Inc.	ELDS-BR	
Finger probe	Nihon Koden	TL-201T	
Non invasive blood pressure cuff	Nihon Koden	YP-713T	
Npi-100/automated infrared pupillometer	NeurOptics		
Thymapads	Somatics Inc.	EPAD-C	
Thymatron IV system	Somatics Inc.		
VBM tourniquet9000	Medizintechnik GmbH		

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Date:	December 4. 2018

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Alisha DSouza, Ph.D.
Senior Review Editor

January 29, 2019

Dear Dr. Alisha DSouza

"Title: Pupillary response as assessment of effective seizure induction by electroconvulsive therapy"

Thank you very much for your consideration to the above-referenced manuscript. We appreciate valuable comments given by the editors and reviewers. The manuscript has been revised in accordance with the their comments. We would like to submit the revised manuscript for publication as Journal of Visualized Experiments. Changes were made in red in manuscript.

Editorial Comments:

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

Answer⇒We asked English editing service.

• **Introduction:**
1) Line 107, 108, 110, : Need references

Answer⇒We added the references.

• **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 add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) 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.

Answer⇒We added the phrase" The study protocol was approved by the institutional clinical research ethics committee of Kyushu University, Fukuoka, Japan (IRB: Clinical Research number #28-77). Although the measurement of pupillary reaction is an essential and standard clinical procedure during anesthesia, informed consent was obtained for this research. ".

2) Mention inclusion and exclusion criteria to select subjects, and also mention if consent was obtained.

Answer⇒We added the phrase "Patients with cataract, glaucoma, intraocular lenses, or insulin-dependent diabetes mellitus were excluded because their pupillary responses may be abnormal.".

3) 1.1: Attach where? On the patient? Which parts of the body? EEG requires clean surfaces and is done at specific points. Please mention all details to replicate the step.

Answer⇒We added the phrase "After the right and left forehead and area behind the ears are cleaned with an alcohol cotton swab, electroencephalogram (EEG) monitors are attached at four symmetrical points.".

4) 1.2: What kind of pad? What is the intensity of stimulation?

Answer⇒We added the phrase "After bilateral temples are cleaned with normal saline, gummous electrical stimulation pads (about 4 × 5 cm) are attached to the bilateral temples to prevent interference in the stimulation due to hair. The intensity of stimulation is changed each time, according to the previous degree of inducted convulsion.".

5) 1.3: Which 3 points? How are the contact points prepped?

Answer⇒We added the phrase "Electrocardiogram (ECG) monitors are attached at two points (base of the heart and cardiac apex) and heart rate is monitored.".

6) 1.4: Attach how? How tight? Where exactly?

Answer⇒We added the phrase "A tourniquet is prepared and belted at the left thigh. A tourniquet is belted until 200 mmHg after the patient is asleep. Convulsion induced by ECT can be observed in the lower leg even after systemic administration of muscular relaxant.".

7) 1.5: Where exactly? Please provide more specific details.

Answer⇒We added the phrase "Two electromyogram sensors are attached to the left anterior tibial muscle, with a distance of 5 cm, to measure generalized seizure time in the lower leg.".

8) 2.1: How is the site prepped? What is the device measuring?

Answer⇒We added the phrase "After the forehead is cleaned with an alcohol cotton swab, a bispectral index (BIS) measurement device is attached at the patient's forehead. BIS is used to monitor depth of anesthesia.".

9) 2.2: How? Through a standard monitor?

Answer⇒We added the phrase "Non-invasive blood pressure is measured at the arm every 1 minute during ECT therapy.".

10) 2.3: how? Using a clip on monitor?

Answer⇒We added the phrase "Saturation of oxygen is continuously monitored by a clip attached to the finger, and the pulse rate is monitored.".

11) 2.4: how is this done? Please elaborate.

Answer⇒We added the phrase "The examiner holds a pupillometer over one of the patient's eyes. After the patient opens their eyes, the examiner presses a button (Figure 1A) on the pupillometer, and measurement starts automatically. Maximum (initial) resting pupil size (MAX) and minimum pupil size after light stimulation (MIN) are automatically measured, and the constriction pupil size ratio (%constriction, [MAX-MIN]/MAX expressed as a percentage) is automatically calculated. The duration of light emission is 800 ms, and the required data can be obtained within 1 second. If the patient is unable to keep their eyes open, the examiner assists by holding the eyelid open. Although anesthetists typically measure the pupillary diameter or light reflex of patients before surgery in the operating room, cooperation with patients is needed before anesthesia induction.".

12) 3.3: Which tourniquet?

Answer⇒We added the phrase "A tourniquet belted at the thigh is tightened to 200 mmHg.".

13) 3.2,3.4: Mention administration routes.

Answer⇒We added the phrase "via intravenous line".

14) 4.1: What are the dosage units? What is the max and min range? How long is the stimulation applied for?

Answer⇒We added the phrase "Conduct ECT via bitemporal electrode stimulation using an ECT instrument. The initial electrical stimulus dose (%) was set at half the value of each individual's age. Maximum stimulus dose is 100 %. The electrical stimulation time is about 7-8 seconds.".

15) 4.2: Please add more details to describe how this is done.

Answer⇒We added the phrase "Immediately after electrical stimulation, the examiner holds a pupillometer over one of the patient's eyes. At this time, if the patient closes their eyes, the examiner keeps the patient's eyelid open. The examiner presses the button, as in step 2.4, and maximum (initial) resting pupil size (MAX) or minimum pupil size after stimulation (MIN) is measured using an automated infrared pupillometer.".

16) 4.4? how? Needs details.

Answer⇒We added the phrase "Measurement of ictal regularity, seizure time and greater postictal suppression using EEG by the psychiatrist (Figure 3)".

• **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.

Answer⇒We appreciated your suggestion. We rephrased.

• **Figures:**
1) Do you have explicit permission from NeuroOptics inc to use fig 1?

Answer⇒Yes. The images are presented with the explicit permission of NeurOptics Inc.

2) Fig 2: define error bars, sample sizes, ****,***

Answer⇒We added the phrase "Sidak's multiple-comparison *post hoc* test was utilized for two-way analysis of variance. $^{***}p < 0.001$, $^{****}p < 0.0001$.".

• **References:**Please spell out journal names.

Answer⇒We wrote. Could you tell me how?

• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Thymatron IV system (Somatics),

Answer⇒We rewrote.

1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

Answer⇒We added the phrase.

• **Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as EEG, electric stimulator, pulse ox, etc.

Answer⇒We added the products in xls.

• Please define all abbreviations at first use.

• 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]."

Answer⇒We added the supplement file.

Comments from Peer-Reviewers:

Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please revise the manuscript to thoroughly address these concerns. Additionally, please describe the changes that have been made or provide explanations if the comment is not addressed in a rebuttal letter. We may send the revised manuscript and the rebuttal letter back to peer review.

Reviewer #1:

Manuscript Summary:
The authors present an interesting method to measure pupillary response and pupillary mydriasis as an indicated of brain damage.

Major Concerns:
JOVE manuscripts tend to be very detailed, methodology-driven, manuscripts. This paper lacks these details. For instance more details were given about ECT, than measurement of pupil size

Answer⇒We added the phrase as pointed by editor and reviewers.

Minor Concerns:
A video would help

Reviewer #2:

Manuscript Summary:
Authors employed the pupillary responses for assessment of adequate seizure induction, and then calculated the constriction ratio. It was shown that pupillary responses immediately after electrical stimulation provide a precise assessment of the efficacy of seizure induction with electroconvulsive therapy. Research topic is interesting and meaningful. However, some major issues should be considered by authors.

Major Concerns:
1. How many patients were evaluated in this test? Authors should clarify this issue.

Answer⇒We added the phrase "We reported the relationship between the pupillary response and efficacy of ECT using this device among 13 patients (Figure 3, this figure has been modified from reprinted from Shirozu, K. *et al.* The relationship between seizure in ECT and pupillary response using an automated pupilometer. *J Anesth.* 10.1007/s00540-018-2566-9, [2018]).[9]".

2. The description of ECT procedure is quite briefness, authors should describe more details.

Answer⇒We added the phrase as pointed by editor and reviewers.

3. I don't understand the definition of "constriction ratio", which is a key factor of this paper.

Answer⇒We added the phrase “Maximum (initial) resting pupil size (MAX) and minimum pupil size after light stimulation (MIN) are automatically measured, and the constriction pupil size ratio (%constriction, [MAX-MIN]/MAX expressed as a percentage) is automatically calculated.”.

4. In this paper, I didn't find the conclusion about how ECT therapy affects seizure induction by reflecting on pupillary responses.

Answer⇒We appreciated your suggestion. We wrote the phrase “we found that pupillary diameter was not changed after light stimulation, remaining enlarged when adequate seizure was induced.” In introduction and “We reported the relationship between the pupillary response and efficacy of ECT using this device among 13 patients (Figure 3, this figure has been modified from reprinted from Shirozu, K. *et al.* The relationship between seizure in ECT and pupillary response using an automated pupilometer. *J Anesth.* 10.1007/s00540-018-2566-9, [2018]).[9] As shown in Figure 3A, pupillary diameter is enlarged after electrical stimulation. However, significant differences in constriction by the light reflex were caused immediately after electrical stimulation, reflecting differences in seizure efficacy induced by ECT (Figure 3B).” in results.”.

Reviewer #3:

Manuscript Summary:
Pupillary responses (light reflex) were measured for assessment of adequate seizure induction by electroconvulsive therapy using an automated infrared pupillometer immediately after electrical stimulation. Constriction ratio was calculated and compared with seizure quality.

Minor Concerns:

1. In protocol, pulse rate or heart rate should be monitored and mentioned

Answer⇒We added the phrase.

2. Line 148, 176, 177 says" either maximum or minimum pupilary diameter is measured.".but I think both are measured to calculate constriction ratio

Answer⇒We appreciated your suggestion. We added the phrase “2.4. The examiner holds a pupillometer over one of the patient’s eyes. After the patient opens their eyes, the examiner presses a button (Figure 1A) on the pupillometer, and measurement starts automatically. Maximum (initial) resting pupil size (MAX) and minimum pupil size after light stimulation (MIN) are automatically measured, and the constriction pupil size ratio (%constriction, [MAX-MIN]/MAX expressed as a percentage) is automatically calculated.”.

3. Lines 165, 166 & 167 regarding IPPV are not very clear. Please elaborate

Answer⇒We added the phrase "After spontaneous breathing of patients ceases, the anesthesiologist performs controlled ventilation using a face mask with 100% oxygen without intubation. Perform ventilation from the point at which end-tidal carbon dioxide is 30 mmHg until electrical stimulation.[10] After BIS value begins to increase, the anesthesiologist stops ventilation and psychiatrists start the ECT procedure.".

4. Lines 213, 214 says criteria for adequate Adequate seizure status was determined using the criteria described above. But there are no criterias anywhere

Answer⇒We appreciated your suggestion. We wrote the phrase "we found that pupillary diameter was not changed after light stimulation, remaining enlarged when adequate seizure was induced." In introduction and "We reported the relationship between the pupillary response and efficacy of ECT using this device among 13 patients (Figure 3, this figure has been modified from reprinted from Shirozu, K. *et al.* The relationship between seizure in ECT and pupillary response using an automated pupilometer. *J Anesth.* 10.1007/s00540-018-2566-9, [2018]).[9] As shown in Figure 3A, pupillary diameter is enlarged after electrical stimulation. However, significant differences in constriction by the light reflex were caused immediately after electrical stimulation, reflecting differences in seizure efficacy induced by ECT (Figure 3B)." in results.

Sincerely,

Kazuhiro Shirozu, M.D., Ph.D.

****

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Dear Dr. Kazuhiro Shirozu,

Thank you for your email.
This is Okagawa of JSA Office.

Do you want to get permission about reprinting the JA article of own in other journals, is that right ?

Regarding this case, as a result of the examination, it became "free license" as academic purpose, so I will notify you here.

If you have any questions about this, please contact us.

Best regards,

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Dear the Japanese Society of Anesthesiologists and the Society

Recently, my accepted journal ” The relationship between seizure in electroconvulsive therapy and pupillary response using an automated pupillometer • DOI: 10.1007/s00540-018-2566-9” was published in “Journal of anesthesia”.

After publication, we received mail from “journal of visualized experiments”.
They requested me to submit manuscript about the method of above journal.
Can I used the figure published in JOA in journal of visualized experiments?
If can, I included statement “This figure has been modified from [citation].” in JOVE.

I attached the mail from JOVE.
“I recently came across your paper, "The relationship between seizure in electroconvulsive therapy and pupillary response using an automated pupilometer". As a Science Editor with *JoVE* I am interested in speaking with you about the possibility of publishing your methods as a peer-reviewed video article.

JoVE is the leading peer-reviewed video methods journal. Authors submit a traditional text manuscript, and we take care of the entire process of filming and producing your video. The JoVE video article below by the Payne lab at University of Notre Dame is an example of the

high quality video articles we produce.
”

Sincerely,
Kazuhiro Shirozu
Department of anesthesiology and critical care medicine, Kyushu University Hospital