Is there any difference in human pupillary reaction to acupuncture between light- and dark-adaptive conditions?

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Abstract
Objectives To determine if acupuncture stimulation elicits a pupillary response under light adaptation and whether there is any difference in the pupillary response between light and dark adaptation environments during acupuncture stimulation.

Methods The participants consisted of 55 healthy individuals who had no known eye diseases or pupil abnormalities. Experiment 1 compared pupillary responses between acupuncture stimulation and no-stimulation groups under light adaptation. Experiment 2 compared pupillary responses to acupuncture between two conditions (dark and light adaptation) with a two-period repeated measurement crossover design. For both experiments the pupil diameter was continuously measured for 3 min before stimulation, during stimulation and for 3 min after stimulation. For all acupuncture stimulation interventions an acupuncture needle was inserted superficially at the TE5 acupuncture point followed by gentle tapping stimulation for 90 s.

Results In experiment 1 the pupil diameter was significantly decreased during (p<0.01) and after stimulation (p<0.0001) compared with the pupil diameter before stimulation under light adaptation. No significant difference was noted in the serial changes in pupil diameter in the no-stimulation group. In experiment 2 the pupil diameter was significantly decreased 90 s after stimulation (p<0.05) and 150 s after stimulation (p<0.05) under light adaptation conditions. Furthermore, the pupil diameter was significantly decreased 120 s after stimulation (p<0.05) and 150 s after stimulation (p<0.01) under dark adaptation conditions. No significant difference in the serial changes in pupil diameter was noted between the groups.

Conclusions This study shows that pupil constriction occurs following acupuncture stimulation under light adaptation and this response is no different from that seen under dark adaptation.

INTRODUCTION
Acupuncture stimulation causes various physiological responses through the autonomic nervous system. The influence of acupuncture on autonomic functions has been studied using a variety of assessment methods.1–5 Evaluating pupillary reaction can be useful in acupuncture studies because it can demonstrate, non-invasively, changes in autonomic activities.

Pupil diameter is regulated by the autonomic nervous system as well as by hormones in the blood such as epinephrine and norepinephrine. Constriction of the pupil is caused by the contraction of a sphincter muscle, which is under parasympathetic control. Dilation of the pupil occurs through contraction of the dilator muscle, which is under sympathetic control.

Pupil size is affected by the intensity of the external light stimulus and also by various autonomic influences.6 Pupillary dilation can occur through sympathetic activation due to mental/physical arousal or fear. Conversely, pupillary constriction can be elicited by parasympathetic activation during a relaxed or fatigued state. Thus, sympathetic and parasympathetic nerve functions can be estimated by analysing pupillary responses.

Only a few studies have been published on pupillary reaction in relation to acupuncture in humans. Takeda et al7 reported that pupil constriction was elicited after acupuncture treatment based on observation of a patient with cervical strain. Yamaguchi8 reported that a patient with tension headache demonstrated a reduction in pupil area and an increase in maximum constriction velocity with acupuncture. Ohyama et al9 performed electrical acupuncture treatment for 20 min on subjects in a supine position and observed changes in their pupil diameters. Maximum constriction was seen 15 min after starting electrical acupuncture treatment.

In our previous study10 we examined the influence of gentle superficial acupuncture on pupillary reaction in 12 healthy men. After needle insertion, decreases in pupil diameter, heart rate and pulse wave amplitude were observed. After acupuncture the change in pupil diameter was significantly different compared with controls (no acupuncture). We
suggested that the acupuncture stimulation method used in the study induced a systemic autonomic reaction.

Acupuncture stimulation was administered superficially at the TE5 acupuncture point in our first pupillography study. The points used in clinical acupuncture, however, are distributed over the whole body. We therefore performed another experiment with acupuncture stimulation on the face, abdomen, lower extremity and forearm in order to determine whether different pupillary reactions would be seen at different acupuncture stimulation sites. The results suggested that the pupillary reaction varied with the site at which acupuncture stimulation was administered.

While our previous studies yielded potentially useful clinical findings, it should be noted that these experiments were conducted in dark adaptation environments. Because clinical acupuncture is presumably conducted in environments with sufficient lighting, we conducted two further experiments to determine (1) if acupuncture stimulation elicits a pupillary response under light adaptation (experiment 1), and (2) whether any difference in pupillary responses exists between light and dark adaptation environments during acupuncture stimulation (experiment 2).

METHODS
Subjects
The participants consisted of 55 healthy individuals (39 men) of mean age 28.6±9.3 years who had no known eye diseases, pupil abnormalities or a diagnosis of diabetes. They were recruited from the student populations of Tsukuba University and Shinjuku Vocational School of Acupuncture and Judo Therapy. In accordance with the ethical principles of the World Medical Association Declaration of Helsinki, we fully explained the purpose and details of this study to the participants and obtained their written consent prior to their participation.

Experiment 1
Protocol
Thirty-five healthy individuals (mean age 32.0±10.2 years) were divided into two groups, an acupuncture stimulation group (n=23) and a no-stimulation group (n=12). For the randomisation, two kinds of envelopes were prepared, identical in external appearance, containing cards labelled ‘acupuncture stimulation group’ and ‘no-stimulation group’. The randomisation procedure was conducted by an individual who was blind to the details of this study.

After entering the room, the participants took a seated position for 10 min, then put on a device to which a CCD camera was affixed to measure pupil diameter. This camera was connected to a digital image processor (IScan, Burlington, Massachusetts, USA). Each subject then underwent light adaptation for 10 min followed by another 10 min of rest before the recordings were made.

Pupil diameter was continuously measured for 3 min before stimulation, 3 min during stimulation and 3 min after stimulation with a sampling rate of 60 frames/s. The mean pupil diameter from the sets of 5 s of data was computed at the following phases for later statistical analysis: the prestimulation phase (25–30 s before stimulation, later referred to as 30 s prestimulation); the during stimulation phase (30–55 s after the onset of stimulation, later referred to as 50 s of stimulation); and the poststimulation phase (30–55, 60–65, 90–95, 120–125 and 150–155 s after the termination of stimulation, later referred to as 30, 60, 90, 120 and 150 s poststimulation, respectively). The dominant side of each subject’s eye was not determined for this study. On every occasion the subject’s right eye, which was located on the same side as the acupuncture administration, was used for measurements. Subjects in the no-stimulation group underwent the same procedures except that they did not receive any acupuncture stimulation during the intervention period. Using an illuminometer (T-10, Konica Minolta, Tokyo, Japan), the luminance conditions (lx) before (Pre30) and at the end of the experimental session (Post150) were obtained. All of the experiments were performed between 09:00 h and 15:00 h.

Experiment 2
Protocol
Twenty healthy individuals (mean age 22.7±1.9 years) participated in a two-period crossover study. They received two kinds of interventions: one with acupuncture stimulation under light adaptation (intervention A) and one with acupuncture stimulation under dark adaptation (intervention B).

The subjects were randomised into two groups using the envelope allocation method. One group of 10 subjects received intervention A in the first experiment session and then received intervention B in the second session; the other 10 subjects completed their sessions in the reverse sequence. Each intervention was administered on a different day with a 1-week interval. As described in experiment 1, the randomisation procedure was conducted by an individual who was blind to the details of this study. The same procedural sequence was used for experiment 2 to maintain the allocation concealment.

Pupil diameter was measured using electronic pupillography (Newopt, Kawasaki, Japan).

For intervention A, subjects took a seated position for 10 min and then wore a goggle-type recording device specifically designed for light-adapted conditions. Each subject underwent light adaptation for 10 min followed by another 10 min of rest before the recordings were made. Exactly the same procedure was followed for intervention B except that subjects wore a goggle-type measuring device specifically designed for dark-adapted conditions and underwent dark adaptation.

The pupil diameter was continuously measured for the same time periods and sequences as those of experiment 1. In addition, as in experiment 1, the right eyes of the subjects were used for measurements, which was the same side as that of acupuncture administration. Using an illuminometer (T-10, Konica Minolta), the luminance con-
ditions (lx) before (Pre30) and at the end of the experimental session (Post150) were obtained.

**Acupuncture stimulation**

The acupuncture stimulation method used in both experiments 1 and 2 and the rationale for this method are detailed elsewhere. Briefly, a disposable acupuncture needle (40 mm length, 0.16 mm diameter; Seirin, Shizuoka, Japan) was inserted 2–3 mm in depth at the right TE5 acupuncture point using a guide tube. The guide tube was kept in place following needle insertion and gentle repetitive tapping stimulation was applied manually on the head of the tube during the subject’s exhalation phase of respiration. Tapping was performed at a rate of approximately 5 times per second. There was no attempt to induce *de qi* sensation during the stimulation. After 90 s, the stimulation was terminated and the needle was removed.

**Statistics**

SPSS Advanced Model software V.15 was used for the statistical analysis. Serial changes in pupil diameter between groups during the observation periods were analysed by mixed-model two-way ANOVA and linear analysis using Fisher (LDS) multiple-comparison methods. Serial changes in pupil diameter within groups during the observation periods were analysed by mixed-model one-way ANOVA and linear analysis using Fisher (LDS) multiple-comparison methods. A probability of *p*<0.05 was considered significant.

**RESULTS**

**Experiment 1**

For the acupuncture stimulation group there was No difference in the luminance condition between Pre30 and Post150 was seen in the acupuncture stimulation group (376.6±35.0 and 380.8±46.0, respectively, *p=0.984*) or in the no-stimulation group (348.1±28.4 and 347.8±28.3, respectively, *p=0.689*).

Table 1 shows the changes in pupil diameter in subjects with or without acupuncture stimulation under light adaptation conditions. In terms of serial changes in the acupuncture-stimulated group, pupil diameter was significantly decreased during and after acupuncture stimulation compared with pupil diameter before stimulation. No significant difference was noted in the serial changes of pupil diameters in the no-stimulation group. No significant difference was noted between the acupuncture stimulation and no-stimulation groups in pupillary responses.

**Experiment 2**

For light adaptation, there was no difference in the luminance condition between Pre30 (667.3±31.4) and Post150 (670.5±15.6) (*p=0.549*). For dark adaptation, the luminance condition was 0 at Pre30 and Post 150.

Table 2 describes changes in pupil diameter with acupuncture stimulation under light and dark adaptation conditions. With regard to serial changes with acupuncture stimulation under light adaptation conditions, the pupil diameter was significantly decreased 90 s and 150 s after stimulation compared with the pupil diameter before stimulation. With regard to serial changes with acupuncture stimulation under dark adaptation conditions, the pupil diameter was significantly decreased 120 s and 150 s after stimulation compared with the pupil diameter before stimulation.

No significant difference was seen in the serial changes in pupil diameter between the groups receiving acupuncture stimulation administered under light and dark adaptation.

**DISCUSSION**

Our previous studies conducted under dark adaptation conditions, showed that pupillary constriction occurs following gentle acupuncture stimulation. In addition, a decrease in heart rate was observed during acupuncture stimulation. We concluded that the decrease in pupillary diameter with acupuncture was due to the elicitation of parasympathetic nerve activation. Nishijo et al showed that the reduction in heart rate induced by acupuncture involves the excitation of parasympathetic nerves and also inhibition of the sympathetic β receptor function.

Our present study confirmed that the pupillary constriction response occurs following acupuncture stimulation under light adaptation. Furthermore, there was no difference in the pupillary response between light and dark adaptation environments during acupuncture stimulation.

In the previous study, conducted in 2010, we focused particularly on possible differences in pupillary responses among acupuncture points. Acupuncture points on the face (ST7), abdomen (CV12), a lower extremity (ST36) and a forearm (TE5) were stimulated using the same stimulation technique and showed that different pupillary reactions occur when different acupuncture points are stimulated. Our conclusion corresponds with a series of studies on the somatosympathetic reflex performed by Sato et al. In one study they conducted an experiment using spinalised rats under light adaptation in order to clarify whether elicita-

### Table 1 Pupil diameter responses (mm) with and without acupuncture under light adaptation conditions

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Pre30</th>
<th>Stim30</th>
<th>Post30</th>
<th>Post60</th>
<th>Post90</th>
<th>Post120</th>
<th>Post150</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulation</strong></td>
<td>3.75 (0.69)</td>
<td>3.48 (0.98)*</td>
<td>3.27 (0.67)**</td>
<td>3.30 (0.66)**</td>
<td>3.34 (0.78)**</td>
<td>3.31 (0.64)**</td>
<td>3.36 (0.74)**</td>
</tr>
<tr>
<td><strong>No-stimulation</strong></td>
<td>3.43 (0.86)</td>
<td>3.37 (0.77)</td>
<td>3.36 (0.91)</td>
<td>3.32 (0.85)</td>
<td>3.28 (0.88)</td>
<td>3.42 (0.87)</td>
<td>3.39 (0.86)</td>
</tr>
</tbody>
</table>

Values are means (SD).

Pre30, before stimulation; Stim30, during stimulation; Post30, Post60, Post90, Post120, Post150, 30 s, 60 s, 90 s, 120 s, 150 s after stimulation.

*p<0.01 vs Pre30.

1p<0.0001 vs Pre30.
tion of the sympathetically-mediated pupillary response depends on the stimulated spinal segments. The spinalisation was made at the pupillary parasympathetic preganglionic nucleus in the brain stem (the first cervical level) and the pupillary sympathetic preganglionic nucleus in the spinal cord (first to third thoracic levels). Reflex pupillary dilation occurred when pinching stimulation was applied on the forepaw and chest, which were abolished by bilateral transection of the cervical sympathetic trunk. However, pinching stimulation on the hindpaw did not elicit any reflex pupillary dilation.

It should be noted that a wide variety of acupuncture methods are used in clinical acupuncture. A decrease in the heart rate was observed previously when acupuncture points were stimulated by commonly used acupuncture techniques such as electroacupuncture and manual needling that elicit the de qi sensation. Our study demonstrated pupil constriction following acupuncture stimulation under light adaptation, and this response was no different from that seen under dark adaptation. Although the current study did not systematically evaluate autonomic nerve function, gentle acupuncture stimulation applied superficially on the skin is thought to induce a systemic reaction via the parasympathetic nerves, and the study did confirm the existence of an acupuncture-induced parasympathetic excitation response, as shown by Nishijo et al. Gente superficial acupuncture stimulation, as used in this study, may provide beneficial clinical effects on various conditions related to autonomic dysfunction. It is interesting to note that, while the pupillary constriction response was observed in a majority of participants, some did not exhibit such a response. The exact reason why some individuals did not respond as expected could not be determined, and this is an important area of future research. The major limitation of this study is the possibility of a type II error due to a small sample size. Our findings need to be confirmed with larger samples.

Summary points

- Pupillary testing is usually done in a darkened room
- This study explored testing eyes adapted to the light
- The pupillary response to acupuncture can still be measured

Table 2  Pupil diameter responses (mm) with acupuncture under light and dark adaptation conditions

<table>
<thead>
<tr>
<th></th>
<th>Pre30</th>
<th>Stim30</th>
<th>Post30</th>
<th>Post60</th>
<th>Post90</th>
<th>Post120</th>
<th>Post150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light-A</td>
<td>4.20 (0.76)</td>
<td>4.13 (0.64)</td>
<td>4.23 (0.73)</td>
<td>3.99 (0.66)</td>
<td>3.89 (0.76)*</td>
<td>4.01 (0.77)</td>
<td>3.91 (0.76)*</td>
</tr>
<tr>
<td>Dark-A</td>
<td>7.78 (0.84)</td>
<td>7.76 (0.71)</td>
<td>7.60 (0.77)</td>
<td>7.56 (0.84)</td>
<td>7.63 (0.81)</td>
<td>7.47 (1.14)*</td>
<td>7.34 (1.20)*</td>
</tr>
</tbody>
</table>

Values are means (SD). Light-A, light adaptation condition; Dark-A, dark adaptation condition; Pre30, before stimulation; Stim30, during stimulation; Post30, Post60, Post90, Post120, Post150, 30 s, 60 s, 90 s, 120 s, 150 s after stimulation. *p<0.05 vs Pre30. †p<0.01 vs Pre30.

REFERENCES

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**Notes**