ABSTRACT

Objectives To determine if there is any difference in pupillary response among different acupuncture stimulation sites.

Methods The subjects were 14 healthy males who had no known eye diseases or abnormality in their pupils. They received five different interventions: no acupuncture stimulation (hereinafter ‘no-stimulation’) and acupuncture stimulation at four sites (TE5, ST7, CV12 and ST36). The Latin square design was used to allocate stimulation order. For all acupuncture stimulation interventions, a disposable acupuncture needle was inserted superficially at the acupuncture point. Gentle repetitive tapping stimulation was applied manually during the subject’s exhalation phase of respiration, for 90 s. The pupil diameter was continuously measured for 2 min before stimulation, during stimulation and for 2 min after stimulation. Statistical analysis was conducted on serial changes in pupil diameter during acupuncture stimulation on each respective site and during non-stimulation session by analysis of variance and Fisher (least significant difference) multiple comparison, with linear analysis using a mixed model.

Results Pupil diameter reduction occurred at 30 s after stimulation on ST7 (p = 0.008) and 60 s after stimulation (p = 0.014) compared with pre-stimulation. The decrease of pupillary diameter occurred 60 s after stimulation on TE5 (p = 0.028) compared with pre-stimulation. On ST36, CV12 and during the non-stimulation intervention, no significant change in the pupil diameter was observed.

Conclusions Pupillary reaction varies depending on the different stimulation sites.

It is known that acupuncture stimulation causes various physiological responses through the autonomic nervous system. The influence of acupuncture on autonomic functions has been studied by using a variety of assessment methods.\(^3\) Evaluating pupillary reaction can be useful in acupuncture study, since it can non-invasively demonstrate the changes in autonomic activities.

Pupil size is affected not only by intensity of external light stimulus but also by various autonomic influences.\(^4\) Constriction of the pupil is caused by contraction of a sphincter muscle, which is under parasympathetic control. Dilation of the pupil is elicited through contraction of the dilator muscle, which is under sympathetic control. Pupillary dilation can occur by sympathetic activation due to mental arousal or fear. Conversely, pupillary constriction can be elicited by parasympathetic activation during a relaxed or fatigued state. Thus, sympathetic and parasympathetic nervous functions can be estimated by analysing the pupillary responses.

Ohsawa et al\(^5\) observed pupillary dilation response following electroacupuncture stimulation to the posterior foot of anaesthetised rats. The pupillary dilation response was not affected by resection of cervical sympathetic nerves innervating the pupil. However, the response diminished by resection of the oculomotor nerves that contain parasympathetic nerves innervating the pupil. This indicates that pupillary dilation is caused by suppression of the oculomotor nerve function. An important role of the parasympathetic nerves in this response is suggested.

There are only a small number of studies on pupillary reaction in relation to acupuncture on humans. Takeda, et al\(^8\) reported that pupil constriction was elicited after acupuncture treatment based on observation of a cervical strain patient. Yamaguchi\(^9\) reported that a tension type headache patient had a pupil area reduction and maximum constriction velocity increase with acupuncture. Ohyama, et al\(^10\) performed 20 min electrical acupuncture treatment on subjects in a supine position and observed changes in their pupil diameter. The maximum constriction was seen at 15 min after starting electrical acupuncture treatment. These studies demonstrate the activation of parasympathetic function following acupuncture.

In our previous study,\(^11\) we examined the influence of acupuncture on pupillary reaction with 12 healthy males. After needle insertion, a decrease in pupil diameter, heart rate and pulse wave amplitude was observed. Post-acupuncture, a change of pupil diameter was significantly different when compared with the control (no acupuncture) condition. We concluded that this stimulation method induced a systemic autonomic reaction. The acupuncture stimulation was administered superficially at the TE5 acupuncture point in the previous study. However, points used in clinical acupuncture are distributed over the whole body. Given this fact, along with the results of our previous study, it would be interesting to observe whether different pupillary reactions are seen at different acupuncture stimulation sites. Therefore, we performed acupuncture stimulation on the face, abdomen, lower extremity and the forearm, and examined whether the pupillary reaction varied by the site where acupuncture stimulation was administered.

METHODS

Subjects

Fifteen healthy males, who had no known eye disease or abnormality in the pupil, were initially
recruited for the study. One subject’s data was excluded from the study, because his pupil diameter was outside of the normal range (1.5–8.0 mm)\textsuperscript{12} at the baseline. Data of 14 subjects (mean age: 20.4 ± 0.6 years) were used for later analysis. Based on 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 written consent prior to their participation.

Interventions
The following acupuncture sites were used (figure 1): TE5 (on the posterior aspect of the forearm, approximately 4 cm proximal to the dorsal wrist crease); ST7 (on the face, in the depression between the midpoint of the inferior border of the zygomatic arch and the mandibular notch; CV12 (on the upper abdomen at the midpoint between the xiphisternal junction and umbilicus; ST36 (on the anterior aspect of the leg, approximately 2 cm lateral to the inferior ridge of the tibial tuberosity). For acupuncture points with a bilateral feature (TE5, ST7 and ST36), the point on the right side of the body was used.

Cutaneous innervations of stimulation sites are as follows: TE5 by the C7 spinal nerve; ST7 by the 3rd branch of the trigeminal nerve; CV12 by the T8 spinal nerve; and ST36 by the L5 spinal nerve.\textsuperscript{13}

The acupuncture stimulation methods used in this study and the rationale of these are detailed elsewhere.\textsuperscript{14} Briefly, the method was as follows: a disposable acupuncture needle (40 mm in length, 0.16 mm in diameter, Seirin Co., Shizuoka, Japan) was inserted 2–3 mm in depth at the acupuncture point using a guide tube. The guide tube was kept in place following the 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 at a rate of approximately five times per second. After 90 s, the stimulation was terminated and the needle was removed.

In the rest condition, subjects underwent the same procedures except they did not receive any acupuncture during the intervention period.

Protocol
To record the diameter of the pupil, each subject wore a goggle-type recording device which captured the image of the pupil with a pupillography, infrared CCD camera (Iriscoder C7564, Hamamatsu Photonics, Shizuoka Pref., Japan). The right eye of the subjects was used for measurement, which was the side of acupuncture administration.

The experiment procedures were as follows: After entering into a dark room, the participants took a seated position, used an eye mask for 10 min dark adaptation, then put on the goggle-type recording device and rested for another 10 min before the recordings.

Each participant received five different interventions: no stimulation (A); stimulation on TE5 (B); ST7 (C); ST36 (D); then CV12 (E). By using the Latin square design, subjects were allocated in five different groups depending on different administration orders as follows: ABCDE (n = 3); BCDEA (n = 3); CDEAB (n = 3); DEABC (n = 3); and EABCD (n = 2). Each session was conducted in an identical manner except for the order of interventions.

The pupil diameter was continuously measured for 2 min before stimulation, during stimulation and for 2 min after stimulation; and it was analysed for 5 s with a sampling rate of 60 frames per second. The mean pupil diameter of 5 s data was computed for later statistical analysis at the following phases: pre-stimulation phase (between 25–30 s before stimulation, later referred to as 30 s pre-stimulation); during stimulation phase (between 30–35 s after onset of stimulation, later referred to as 30 s of stimulation); and post-stimulation phases (between 30–35, 60–65, 90–95 and 120–125 s after termination of stimulation, later referred to as 30, 60, 90 and 120 s post-stimulation, respectively). All the experiments were performed during the day in the period from 09.00 to 15.00.\textsuperscript{15}

Statistics
Statistical analysis was conducted on temporal changes in pupil diameter during acupuncture stimulation on each respective site (TE5, ST7, CV12 and ST36), and also during non-stimulation session by analysis of variance and Fisher (least significant difference) multiple comparison, with linear analysis using a mixed model (SPSS V.15). A probability \( p \leq 0.05 \) was defined as significant.

RESULTS
No interaction was detected between the changes in pupil diameters and order of interventions (\( p = 0.825 \)). There was a difference in pupil diameters between TE5 and ST7 (\( p = 0.000 \)), TE5 and ST36 (\( p = 0.000 \)), TE5 and CV12 (\( p = 0.001 \)), TE5 and non-stimulation (\( p = 0.000 \)). The pupil diameter was decreased
30 s after stimulation (Post30, p = 0.009) and 60 s after stimulation (Post60, p = 0.008) when compared with before stimulation (Pre30).

Table 1 indicates the changes in pupil diameters during acupuncture stimulation on each respective site (TE5, ST7, CV12 and ST36), and also during the non-stimulation session. For the pupillary diameter change over time among the stimulation sites, the decrease occurred 60 s after stimulation on TE5 compared with before stimulation (p = 0.028). Pupil diameter reduction occurred at 30 s after stimulation on ST7 and 60 s after stimulation compared with before stimulation (p = 0.008 and p = 0.014, respectively). There was also a trend towards a decrease 90 s after stimulation on ST7 (p = 0.098).

On ST36, pupil diameter showed a decreasing trend during 30 s stimulation compared with before stimulation (p = 0.058). On CV12 and non-stimulation, there was no change in the pupil diameter after stimulation, compared with before stimulation.

**DISCUSSION**

A previous study by Nishijo et al. demonstrated that acupuncture induces a heart rate reduction response. The study clarified, by using autonomic blocking agents, that the heart rate reduction by acupuncture involves inhibition of the sympathetic β receptor and/or excitation of parasympathetic nerves.

Infrared pupilography used in the present study is considered to be useful in an acupuncture study, since it is one of the very few available methods to evaluate parasympathetic function non-invasively. In our previous study, a significant pupillary reaction was observed following gentle acupuncture stimulation. In addition, in the previous study, a statistically significant change in the subject’s heart rate was seen during acupuncture stimulation. We concluded that the decrease of pupillary diameter by acupuncture was due to elicitation of parasympathetic nerve activation. It was considered that the difference in the reaction time of pupil diameter and heart rate may have been caused by the difference in the reflex centres (i.e., mid brain, medulla) but a detailed mechanism remains unclear.

In the present study, we particularly focused on possible differences in pupillary responses among acupuncture points. Acupuncture points on the face (ST7), the abdomen (CV12), a lower extremity (ST36), and a forearm (TE5) were stimulated using the same stimulation technique and examined to see if there was a difference in reaction by stimulation site. Among the stimulation sites, there was a pupillary constriction response following ST7 and TE5 points. With ST36 point stimulation, a decreasing pupillary trend was observed, but the response was not statistically significant.

To date, there have been only a few acupuncture studies using pupillary reaction as the index. The studies used different stimulation sites and stimulating techniques. Both Takeda et al. and Yamaguchi used an in situ needle technique on the neck for 15 min. Takeda et al. reported a pupillary constriction response following acupuncture in patients with cervical injury. Yamaguchi reported a decreased pupil area and an increased maximum velocity of pupillary constriction in patients with tension headache, suggesting acupuncture induced activation of parasympathetic nerves. Ohyama et al. used low frequency electrical acupuncture stimulation on LI4 for 20 min. Maximum pupillary constriction occurred 15 min after the onset of electroacupuncture stimulation, and the pupil size returned to that of the pre-stimulation state at 10 min post-stimulation. In addition, a transient tendency of pupillary dilation was observed.
observed immediately after the onset of electrical stimulation. It is known that pupillary dilation is induced by psychoemotional factors such as fear. The transient pupil dilation response observed in the study by Ohyama et al. could be elicited by sympathetic arousal due to pain or anxiety associated with needling, although this remains unclear since the study did not employ any control groups.

In order to elucidate neural mechanisms on pupillary response by somatosensory stimulation, basic experimental studies using anaesthetised animals to eliminate emotional factors are needed. A series of studies by Sato et al. suggest that somatosensory stimulation to the skin, muscle and joints elicits reflex responses in various visceral functions via autonomic efferent nerves in animals. Somato-autonomic reflexes involve both segmental spinal reflexes and non-segmental supraspinal reflexes.

For pupillary responses in particular, Ohsawa et al. demonstrated that the reflex dilation of the pupil of anaesthetised rats upon electroacupuncture stimulation to their hind paws was caused by inhibition of pupillary parasympathetic efferent activity. Shimura et al. conducted a study using specialised rats upon light adaptation, in order to clarify if elicitation of 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 (the first to third thoracic level). Reflex pupillary dilation occurred when pinching stimulation was applied on the fore paw and chest, which were abolished by bilateral transection of the cervical sympathetic trunk. However, pinching stimulation on the hind paw did not elicit any reflex pupillary dilation. Their results suggest that sympathetically mediated pupillary dilation occurs in accordance with spinal segmentations, which corresponds with the somato-sympathetic reflex suggested by Sato et al. When sensory stimulus was applied to the face however, the sensory signals reached the brain stem via the afferent trigeminal nerve without being affected by the transaction of spinal cord or sympathetic nerve. This suggested that pupillary response upon facial stimulation involved only parasympathetic inhibition.

Although the current study did not evaluate autonomic nerve functions such as heart rate, gentle acupuncture stimulation, as it was performed in our previous study, is thought to induce a systemic reaction via the parasympathetic nerves. The reaction difference arising from varied stimulation sites may have been caused by the difference in exciting various afferent fibres and ultimately the stimulus receptors that act upon pupillary reaction. The response on ST7 was most particularly remarkable. Because this point is located on the area of skin innervated by the trigeminal nerve, the nearby oculomotor nerve might have been influenced via its afferent pathway. The detailed mechanism is not clear and should be elucidated further in future study.

There are a wide variety of acupuncture methods adopted in clinical acupuncture. Acupuncture response is known to be affected by many factors, including site of needling. In clinical practice, needling points have been selected primarily based on empirical theoretical rationale. This study was conducted as a basic neurophysiological study. Whether or not, the use of a particular point based on the present data would lead to a greater clinical efficacy is out of the scope of the present study. We consider however, that elucidation of acupuncture point characteristics could lead to providing a scientific rationale for using specific acupuncture points and validating the importance of point selection.

**CONCLUSION**

This study demonstrated that there is a difference in pupillary reactions when different acupuncture points are stimulated. Most pronounced responses were observed after acupuncture on ST7.

**Competing interests** None.

**Ethics approval** This study has been approved by the Research Ethics Committee of National University Corporation, Tsukuba University of Technology.

**Patient consent** Obtained.

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**REFERENCES**


Is there any difference in human pupillary reaction when different acupuncture points are stimulated?

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