Original Article

**Influence of mouth guards on autonomic nervous system activities: A quantitative study of pupillary flash responses**

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**Abstract**

Background: Recently, it has been reported that mouth guards (MGs), which reduce the incidence and severity of traumatic oral injuries in contact sports, may actually affect sports performance. We have observed that a majority of subjects showed improved dynamic visual acuity during head rotation when using a MG, but subjects who were unwilling to use a MG showed the opposite effect. Thus, we hypothesized that unpleasant sensations due to MGs may decrease sports performance.

Methods: In this study, we measured autonomic nervous system activity to evaluate unpleasant sensations objectively and quantitatively by measuring the pupillary flash response (PFR) and heart rate variability (HRV) before, during, and after wearing 3- and 5-mm-thick custom-made MGs in 10 healthy subjects.

Results: It was found that the 5-mm MG had a higher incidence of unpleasant sensations (50% of subjects) than did the 3-mm MG (10%). PFR (not HRV) analysis showed that both sympathetic and parasympathetic nervous system activities increased in subjects with unpleasant sensations.

Conclusions: We suggest that the unpleasant sensation induced this unusual autonomic nervous system response, which could not be detected by traditional methods such as HRV analysis. By using PFR analysis, it is possible to make MGs without unpleasant sensations for better sports performance.

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**1. Introduction**

Athletes use mouth guards (MGs) in contact sports, such as football [1], rugby [2], boxing [1,3], ice hockey [4], and basketball [5], because MGs reduce the incidence and severity of traumatic oral injuries, such as teeth fractures and luxation, and oral soft tissue injuries [3,5,6]. Also, to obtain the benefit of MGs, a special educational program is required to promote awareness, knowledge, and motivation [7].

Some studies have also reported that the use of MGs affects sports performance (e.g. muscle strength [8], equilibrium [9], ventilatory gaseous exchange [10,11]). We have also observed that the dynamic visual acuity of some subjects during active head rotation apparently improves when using a MG, but in other subjects it had the opposite effect [12]. As subjects with impaired dynamic visual acuity tended to be unwilling to use a MG, we hypothesized that the unpleasant sensations caused by a MG decrease sports performance, including dynamic visual acuity. To test this, we needed to evaluate these unpleasant sensations objectively and quantitatively.

To date, various parameters of autonomic nervous system activity (ANSA), such as heart rate variability (HRV) [13–15], electrodermal activity [15], and muscle sympathetic nerve activity [16], have been measured to objectively evaluate pleasant/unpleasant sensations. Robin et al. [15] reported that subjects who had fearful dental experiences exhibited stronger ANSA (skin resistance and skin potential responses) compared with non-fearful subjects. Also, using spectral analysis of HRV, bruxism patients showed higher sympathetic nervous system activity than did control subjects, and these responses might be related to stress and occlusal disharmony [14].

We investigated the influence of MGs on ANSA by analysis of pupillary flash responses (PFRs). This method was established by Yamaji et al. [17,18] and has the advantages that it allows independent analysis of sympathetic and parasympathetic nervous system activities, it is non-invasive, and it makes it possible to estimate the instantaneous state of ANSA.
2. Materials and methods

2.1. Participants

Healthy subjects (n = 10; eight males, two females, aged 21–46 years) who were not daily users of a MG participated. All subjects had normal dentition and general medical condition without systemic disease, dental disease, or oral/dental trauma. They provided informed consent according to the World Medical Association’s Declaration of Helsinki.

2.2. Mouth guards

We made custom MGs using ethylene vinyl acetate sheets (Erkodor, Erkodent, Germany) with no taste or scent. The sheet was formed using a thermal-forming pressure machine (Erkopress ES-200E, Erkodent). We prepared two types of MG, 3 and 5 mm thick. The 3-mm MGs were made using 3-mm MG sheet, and the palatal flange was adjusted to the teeth cervical line. The 5-mm MGs were made using 2-mm and 3-mm MG sheets, and the hard palate was covered.

2.3. Subjective unpleasantness scores (SUS)

Subjects were asked to verbally estimate the unpleasant sensation induced by a MG after wearing and removing it. Subjective unpleasantness scores (SUS) was expressed between 100 (maximal unpleasant sensation) and 0 (no unpleasant sensation).

2.4. Pupillary flash response (PFR)

Pupillary flash response (PFR) was monitored by goggles-type video pupillography (Newopto, Kawasaki, Japan). The device was equipped with an LED light that was controlled by a PowerLab system (ADInstruments, Bella Vista, NSW, Australia), for producing flash stimulation to the left eye and an infrared camera for recording video images (30 frames/s) of the right eye. After recording, video images were converted into picture images (bitmap) to detect pupil diameter. According to the method established by Yamaji et al. [17,18], we estimated a difference between the initial pupil diameter and that at 2.4 s after flash stimulation (RA: recovery amount) as sympathetic nervous system activity and the maximum velocity of pupil constriction (Vcmax: max contraction velocity) as parasympathetic nervous system activity. This method was proposed by taking into account different dynamics of the iris sphincter and dilator that are innervated by parasympathetic and sympathetic nervous system, respectively. The different dynamics of the iris muscles were predicted by simulations of a homeomorphic pupillary muscle plant model [19] that demonstrated that the initial phase of a PFR is mediated by active constriction of the sphincter whereas the late re-dilatation phase is mainly due to the dilator relaxation. The validity of the method to independently estimate the sympathetic and parasympathetic nervous activities has been confirmed by pharmacological experiments in human subjects [17] and applied successfully in various experiments including those under prolonged micro-gravity [18], hyper-gravity [20], and alternate gravity [21]. Fig. 1 shows an example data set for PFR with RA and Vcmax. As the response dynamics of PFR vary non-linearly depending on the initial pupil diameter, RA and Vcmax should be compared with the control measurements in the same initial pupil diameter range to eliminate any effect of range non-linearity (RLN) [22]. For this reason, we collected over 300 data points with varying brightness in the room and made an RNL map for each subject before the experiment as proposed by Yamaji et al. [17,18]. Based on these PFR properties, percentage sympathetic nervous system activity estimated by PFR (%SNSA_PFR) and percentage parasympathetic nervous system activity estimated by PFR (%PNSA_PFR) were evaluated using the following equations:

\[
\%\text{SNSA}_{-}\text{PFR} = 100 - 100 \times \left(\frac{\text{RA}_d}{\text{RA}_c - 1}\right)
\]

\[
\%\text{PNSA}_{-}\text{PFR} = 100 + 100 \times \left(\frac{\text{Vcmax}_d}{\text{Vcmax}_c - 1}\right)
\]

where RA_d and Vcmax_d are RA and Vcmax during the experiments, and RA_c and Vcmax_c are control values obtained from the RNL map (see Yamaji et al. [17,18] for details). Thus, %SNSA_PFR (%PNSA_PFR) above 100 indicates increased sympathetic (parasympathetic) nervous system activity, and %SNSA_PFR (%PNSA_PFR) below 100 shows decreased sympathetic (parasympathetic) nervous system activity. Fig. 2 summarizes the possible conditions of ANSA according to %SNSA and %PNSA for the PFR analysis.

2.5. Heart rate variability (HRV)

Electrocardiographic (ECG) data were collected continuously using surface electrodes, an amplifier (VC-11, Nihon Koden, Tokyo, Japan), and a data-acquisition system (PowerLab, ADInstruments) during experiments and also for control measurements for the RNL map at a 1-kHz sampling rate. RR intervals recorded over a 3-min period were selected from ECG data for the HRV analysis, which

![Fig. 1. Example of pupillary flash responses. The upper figure represents change in pupil diameter, and the lower figure represents change in pupil contraction velocity. RA, recovery amount; Vcmax, maximum contraction velocity.](http://example.com/fig1.png)

![Fig. 2. Relationship between change in autonomic nervous system activity and pupillary flash responses. SNSA, sympathetic nervous system activity; PSNA, parasympathetic nervous system activity.](http://example.com/fig2.png)
was conducted using AcqKnowledge Software (BIOPAC Systems, Goleta, CA, USA) to quantify the power spectra of RR intervals into two frequency bands: LF band (low frequency: 0.04–0.15 Hz) and HF band (high frequency: 0.15–0.4 Hz). The LF/HF ratio was used to reflect sympathetic nervous system activity and the HF power was used to reflect parasympathetic nervous system activity [23,24]; %SNSA_HRV and %PSNSA_HRV were estimated using the following equations, as in (1) and (2):

\[
\%\text{SNSA}_{\text{HRV}} = 100 + 100 \times \left( \frac{\text{LF/HF}_d}{\text{LF/HF}_c} - 1 \right),
\]

\[
\%\text{PSNSA}_{\text{HRV}} = 100 + 100 \times \left( \frac{\text{HF}_d}{\text{HF}_c} - 1 \right),
\]

where LF/HF_d and HF_d are LF/HF and HF during the experiments, and LF/HF_c and HF_c are LF/HF and HF during control measurements.

2.6. Procedures

Each subject was seated in a chair with a natural head position in dim light for 20 min for dark adaptation and relaxation. Then, we conducted a series of nine PFR measurements at a rate of one measurement every 20 s (total 3 min), followed by a 2-min rest. Thus, one series took 5 min. Then, the subject used a MG for 25 min, and five series of PFR measurements were performed. Finally, two series of PFR measurements were performed after the subject removed the MG. In total, eight series of PFR measurements were performed (Fig. 3). SUS was estimated immediately after each PFR measurement. The order of experiments using the 3- and 5-mm MGs was pseudorandomized.

2.7. Statistical analyses

Student’s t-tests were used to estimate ANSA parameter differences.

![Diagram of the experiment](image)

**Fig. 3.** Measurement process of autonomic nervous system activity in this experiment. MGs, mouthguards; PFR, pupillary flash response; SUS subjective unpleasantness score.

![Time course of changes in autonomic nervous system activity and subjective unpleasantness score by pupillary flash responses using 3-mm and 5-mm MGs. Student’s t-test for paired data: * p < 0.05. MGs, mouthguards; SUS subjective unpleasantness score; PFR, pupillary flash response; SNSA, sympathetic nervous system activity; PSNA, parasympathetic nervous system activity.](image)
3. Results

The unpleasantness of the sensations induced in subjects by the MG were divided based on low and high SUS values. If the maximum SUS during an experiment was above 60, we considered the subject to be a high-SUS subject; otherwise, the subject was considered a low-SUS subject. Among 5-mm MG users, five subjects were deemed high-SUS subjects (Fig. 4c) and the other five were deemed low-SUS subjects (Fig. 4f). Among 3-mm MG users, only one subject was deemed a high-SUS subject (Fig. 4i) while nine were deemed low-SUS subjects (Fig. 4l).

3.1. Time course of %SNSA and %PNSA

The mean of nine flash stimulations in one series is plotted in Fig. 4. %SNSA_PFR in high-SUS subjects reached a maximum (120.0%) after 10–13 min wearing a 5-mm MG, showing a significant increase compared with the original level (p < 0.05) (Fig. 4a). In low-SUS subjects wearing a 5-mm MG, %SNSA_PFR tended to decrease and showed a minimum (91.7%) after 10–13 min, with a significant difference compared to the original level (p < 0.05) (Fig. 4d). The SUS increased immediately after inserting the MG and returned to baseline levels immediately after its removal under all conditions (Fig. 4c, f, i, l).

Changes in %SNSA_HRV and %PNSA_HRV in both high- and low-SUS subjects in both experiments using 5- and 3-mm MGs were very small throughout the experiment relative to those in SNSA_PFR and %PNSA_PFR (data not shown).

3.2. Comparison between PFR and HRV analyses

Cumulative changes in %SNSA_PFR, %PNSA_PFR, %SNSA_HRV, and %PNSA_HRV from the original levels while wearing a MG (5–23 min data: 4 series), excluding data for 0–3 min, which was regarded as the accommodation time, were calculated and are summarized in Fig. 5. Positive values indicate that these activities were increased by MG wearing, and negative values indicate decreases. Results of the PFR analysis in high-SUS subjects using a 5-mm MG showed a positive averaged cumulative %SNSA_PFR (33.8%), and the averaged cumulative %PNSA_PFR was also positive (15.4%; Fig. 5a).

4. Discussion

Based on participants’ estimation of subjective unpleasantness (SUS), the 5-mm MG induced unpleasant sensations in 50% (5/10), whereas the 3-mm MG caused unpleasant sensations in only 10% (1/10) of subjects. These results suggest that the thickness of the MG is an important factor in unpleasant sensations. Additionally, because the 5-mm MG covered palatal tissue more widely than did the 3-mm MG, the contact area of the MG may also be an important factor in unpleasant sensations. A previous study reported that foreign bodies in the oral cavity, such as palatal plates, cause unpleasant sensations as measured in higher centers in the brain using electroencephalographic examination [25]. We suggest that the trigeminal somatosensory inputs elicited by a MG induce unpleasant sensations, although the detailed mechanism remains unknown. We focused on ANSA in high-SUS subjects using a 5-mm MG as a model of unpleasant sensations.
The analysis of ANSA in high-SUS subjects using a 5-mm MG showed a large discrepancy between PFR and HRV. Both cumulative %NSA_PFR (33.8%) and %PNSA_PFR (15.4%) were positive (Fig. 5a), but cumulative %NSA_HRV (13.6) and %PNSA_HRV (~12.7) were opposite each other in sign (Fig. 5e). In the HRV analysis, sympathetic and parasympathetic nervous system activities were interdependent, because HF power reflects both sympathetic (LF/HF) and parasympathetic (HF) nervous system activity. For example, increased HF power leads to decreased %NSA_HRV and increased %PNSA_HRV. Thus, from HRV analysis alone, it is hard to detect the condition in which both sympathetic and parasympathetic nervous system activities increased independently. On the other hand, from the PFR analysis, it is possible to measure sympathetic and parasympathetic nervous system activities independently, as shown in the results of pharmacological experiments performed by Yamaji et al. [18]. Although it is commonly assumed that sympathetic and parasympathetic nervous system activities act antagonistically, some studies have reported that both sympathetic and parasympathetic nervous system activities increase under specific conditions, such as motion sickness [26] and hypergravity [20]. Consequently, we consider that the PFR analysis (but not the HRV analysis) showed unpleasant sensation, which increased both sympathetic and parasympathetic nervous system activity in high-SUS subjects who used a 5-mm MG.

In the present study, SUS changed immediately after inserting the MG; following removal of the MG, SUS returned to baseline (Fig. 4c and f). However, in high-SUS subjects using a 5-mm MG, the increase in %NSA-PFR and %PNSA-PFR seemed to appear not immediately after inserting the MG, but after 10–13 min had elapsed, and then continued until the end of the experiment (Fig. 4a and b). Thus, ANSA responses evaluated by PFR were delayed compared with changes in SUS. Previous studies also showed that sympathetic nervous system activity increased significantly within 5 min after smoking [13], significant pupillary dilatation was induced 30 s after the cold-pressor test, and blood pressure was significantly increased 2 min after the cold-pressor test [27]. Our results suggest that an increase in SUS precedes ANSA responses. It is likely that the ANSA response is induced by an unpleasant sensation of incompatible MGs.

Zadik et al. said that unpleasantness and interference with sport performance are the most common reasons for sportsmen to not use MGs, and education and motivation are important [28]. Also, MG use has not spread to many athletes in Japan [29]. One important reason may be that some athletes associate wearing a MG with unpleasantness [30]. However, the influence of MGs on sports performance remains controversial, because of the variety of sports.

The present study showed that both sympathetic and parasympathetic nervous system activities were increased by PFR analysis in subjects with strong unpleasant feelings when wearing a MG. This result leads us to believe that measures of ANSA responses are available for objectively estimating unpleasant sensations induced by MGs. Additionally, this means that making MGs conform better with the anatomy of the oral cavity will reduce athletes’ unpleasant sensations. We expect that producing better MGs will lead to increased use of MGs, and athletes will play their sports under stable physiological conditions, with their concentration undisturbed by any discomfort due to their MGs. Studies are in progress to assess the relationship between sports performance and unpleasant sensations induced by wearing MGs.

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References