# Two different analyzing methods for inhibitory reflexes: Do they yield comparable outcomes?

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## HIGHLIGHTS

- For the analysis of inhibitory jaw reflexes the cumulative sum (CUSUM) error box and the *t*-test method can be used.
- This study aimed to assess the interexaminer reliability and test whether both methods yield similar results.
- Inhibitory jaw reflexes were recorded from the right masseter muscle of 11 participants.
- The interexaminer reliability was fair-to-good to excellent. The comparability of the two analyzing methods was fair-to-good.
- When analyzing the inhibitory jaw reflex data, both the CUSUM error box and the *t*-test method can be used.

## ARTICLE INFO

## ABSTRACT

*Background:* For the analysis of inhibitory reflexes, no consensus exists regarding the methodology that should be used. The most commonly used methods are the cumulative sum (CUSUM) error box and the *t*-test. The aim of this study was to assess the interexaminer reliability of those two analyzing methods and to test whether both methods: yield similar results.
*Methods:* Inhibitory jaw reflexes were recorded from the right masseter muscle of 11 participants (6 males, 5 females). Electrical stimuli were applied at the hairy skin of the upper lip on the right side. In total, 16 stimuli were applied while the participants maintained their clenching level at 10% of their maximum voluntary EMG activity. Two different examiners analyzed the reflex data with two different methods: the CUSUM error box and the *t*-test. The outcome variables were the number of reflex parts, the reflex area size, and the reflex onset. Comparability between examiners and between the two analyzing methods: was assessed with the use of the intraclass correlation coefficient (ICC).
*Results:* The interexaminer reliability was fair-to-good to excellent for both the CUSUM error box and the *t*-test analyses and for all the variables tested. The comparability of the two analyzing methods: was fair-to-good.
*Comparison with existing methods/conclusion:* When analyzing the inhibitory reflex data, both the CUSUM error box and the *t*-test can be used.

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## 1. Introduction

The reflex control of the mandible is of vital importance for the normal masticatory function of humans. Excitatory jaw reflexes are responsible for the rapid reaction to external stimuli to the masticatory muscles, while inhibitory jaw reflexes protect the system when sudden loads are applied to the muscles. The fine coordination of the mandibular function is the result of the balanced activation of these reflexes together with the activity of the masticatory muscles, the temporomandibular joints and the associated tissues (Lobbezoo

et al., 2006; Miles, 2007). The inhibitory jaw reflex can be evoked experimentally by the electrical stimulation of the upper lip. It is shown as a decreased activity on the jaw closing muscle's EMG activity following the electrical stimulus (Lund et al., 1983).

For the analysis of the inhibitory reflex data, two different methods are most commonly used, viz., the cumulative sum (CUSUM) error box method (Türker et al., 1997) and the *t*-test method (van der Glas et al., 1995; van der Kaaij et al., 2009). Since it is not known yet which method better describes the reflex, the aim of the present study was to test whether these two techniques:

1. Have an acceptable interexaminer reliability; and
2. Yield comparable outcomes, so that the outcomes of studies using either one of those methods can be readily compared.

## 2. Materials & methods

### 2.1. Participants

After receiving detailed information about the experiment, 11 volunteers (6 males, 5 females; mean age $\pm$ SD [range] = $30.9 \pm 11.4$ [20–58] years) gave their written informed consent and agreed to participate in the study. All participants were healthy, did not use any medication, and had no recent history of dental pathology or orofacial pain (de Leeuw and Klasser, 2013). Individuals who reported oral parafunctions (i.e., tooth grinding or clenching, adverse behaviors such as nail biting, etc.) were excluded. The experiments were conducted according to the Helsinki declaration, and the protocol was approved by the Clinical Research Ethics Committee of the Medical Faculty at Ege University (September 7th, 2009; No: 09-9/48).

### 2.2. Protocol overview

The reflex data were recorded and subsequently analyzed by two different examiners with the use of two different analyzing methods: the cumulative sum (CUSUM) error box method and the *t*-test method.

### 2.3. Electromyography

Surface EMG was recorded at a sample frequency of 2000 Hz from the right masseter muscle with the use of two custom-made bipolar electrodes of silver amalgam (4 mm diameter; 15 mm center-to-center inter-electrode distance; orientation parallel to the direction of the masseter muscle fibres) placed in the area of the greatest lateral distension upon clenching. A common ground electrode was attached to the right arm. EMG signals were amplified (10,000 times) and bandwidth filtered (5–1000 Hz).

### 2.4. Jaw-reflex responses

The jaw-reflex responses of the right masseter muscle to electrical stimulation were studied. Stimuli were applied using two custom-made surface stimulating electrodes (silver amalgam, diameter 4 mm) that were placed 15 mm apart over the hairy skin of the upper lip on the right side. The stimuli were generated by an isolated constant-current device (DS7A, Digimeter, Herthordshire, UK) and consisted of single 1 ms rectangular wave pulses. Stimulation delivery was under the control of a custom-made data acquisition computer program that was built with LabVIEW (National Instruments, Austin, TX). In order to establish the reflex threshold, the intensity of the stimulus was gradually increased until the first reflex response was visible on an oscilloscope. The upper lip was then stimulated using an intensity of six times the reflex threshold with an average of $6.36 \pm 1.48$ mA and 0.1 ms

width. The inter-stimulus interval randomly varied between 1 and 3 s. A total of 16 stimuli were delivered while the participant maintained his/her clenching level at 10% of the maximum voluntary contraction (MVC). MVC was recorded while participants were asked to bite as hard as they could on a custom-made force transducer, which allowed contact of all teeth and was covered with a tough dental impression material (Provil® Novo, Heraeus Kulzer GmbH, Hanau, Germany) in order to protect the dentition. In total, three recordings were performed, lasting five seconds each with 30 s rest time in between. The maximum value of these records was considered the maximum voluntary bite force (MVBF). The EMG activity corresponding to the MVBF was considered the MVC and was used in the remainder of the experiment.

#### 2.4.1. Reflex data analysis

As to enable the analysis of the reflex data, the surface EMG signal of the right masseter was full-wave rectified and averaged around the time of the stimulation (150 ms prestimulus and 300 ms poststimulus).

For analyzing the reflex responses, two different, commonly applied methods were used: the CUSUM error box method (Türker et al., 1997) and the *t*-test method (van der Glas et al., 1995; van der Kaaij et al., 2009) by two experienced examiners (Examiner 1 and Examiner 2).

The CUSUM error box method is based on the analysis method put forward by Ellaway (Ellaway, 1978), and is typically used for monitoring subtle but persistent change detection. In the CUSUM error box method, we determined the maximal prestimulus deflection, either upwards or downwards, and took its symmetry to form an error box. Any poststimulus CUSUM deflection that is larger than the error box and occurring before the conscious reaction to that stimulus was accepted as a genuine reflex response (Türker et al., 1997). We then determined the size of the reflex response using the method put forward by Brinkworth and Türker (Brinkworth and Türker, 2003). Briefly, the size of the significant CUSUM error box deflection 'R' was normalized against 100% of the reflex. 100% of the reflex was determined by multiplying the measured reflex size from the CUSUM error box deflection (k′) by 100 and dividing this value by the multiplication of the prestimulus mean bin value (k) and the duration of the reflex in bins (n). Therefore, 'R' is defined as a percentage of the reflex response:

$$R = k' \times 100/(k \times n)$$

The CUSUM error box method is illustrated in Fig. 1.

The *t*-test method uses rectified and averaged surface EMG records. It then calculates the mean and standard deviation of each of the data points to generate a *t*-test line on the graph. Any *t*-test line that crosses the significance level (t-value for n-1, where n represents the number of stimuli applied) is then indicated as the start points and endpoints of significant reflex responses. The *t*-test method is illustrated in Fig. 2.

#### 2.4.2. Reflex data reliability assessments

Each reflex data series was analyzed four times: two experienced examiners analyzed each series using both analyzing methods (CUSUM error box and *t*-test). As outcome variables, the number of reflex parts, the size of the reflex area, and the onset time of the reflex were calculated.

### 2.5. Statistical analysis

First, the inter-examiner reliability of both examiners was determined, using the Intraclass Correlation Coefficient (ICC) test with absolute agreement. To that end, both examiners analyzed each series of the data using both analyzing methods (CUSUM error box