Human neurophysiological markers of high anxiety level during preparation for visual recognition

Evgeniy A. Cheremushkin1,*, Nadezda E. Petrenko1, Irina A. Yakovenko1, Sergei A. Gordeev2, Nikolay N. Alipov2, Olga V. Sergeeva2

1 Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 Pirogov Russian National Research Medical University, Moscow, Russia

*Correspondence: khton@mail.ru (Evgeniy A. Cheremushkin)
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Abstract

The functional state of subjects with high and low levels of anxiety is studied by electroencephalograph analysis of different temporal periods preceding a cognitive task of visual expression recognition. Several conditions are investigated: background/eyes closed; background/eyes opened; listening to instructions for the cognitive task; operative rest (time lapse between listening to instructions and the beginning of the task); as well as short intervals immediately preceding exposure to target stimuli (preparatory stage), which were paired facial images with identical or different emotional expressions. At all these pre-task stages, high-anxiety subjects exhibit much lower electroencephalograph amplitude values for alpha and theta bands (as compared with low-anxiety subjects). The most prominent differences in electroencephalograph amplitude values revealed during the phases of listening to instructions and operative rest. These datum may provide more precise electrophysiological markers of the level of anxiety during conditions preceding cognitive task performance.

Keywords

Anxiety; electroencephalograph; alpha rhythm; prefrontal cortex; preparatory processes; face recognition

1. Introduction

Today’s world, with its stream of negative information and stressful factors, creates strong prerequisites for the development of neurotic disorders. According to current data, the prevalence of anxiety disorders varies from 13.6 to 28.8% [1], while the prevalence of anxiety in students is reportedly as high as 27% [2]. Such high frequencies of anxiety, notably in the young, drives the search for neurophysiological correlates of anxious states.

The processes underlying cortical resting-state electrical activity changes in anxious people are actually well understood [3–9], so are the neurophysiological mechanisms of facial expression recognition in such subjects [10–12]. It has been shown that both anxiety and depression, influence recognition of facial expression [13, 14]. In studies of eye movement tracking, for example, patients with a generalized anxiety disorder (GAD), unlike healthy subjects, fixed their gaze primarily on stimuli associated with a threat [15].

Current neuroimaging studies clearly show that many cortical and subcortical areas are involved in the recognition of emotions, notably the amygdala, the right fusiform gyrus, and the ventrolateral prefrontal neocortex [16–21]. Functional magnetic resonance imaging (fMRI) studies have revealed significant changes of activation in these areas in subjects with anxious and depressive disorders [16, 20, 22–26].

The organization of the processes of preparation for emotionally significant information recognition in anxious subjects, notably the neurophysiological patterns of brain electrical activity in the phase of listening to the instructions for task performance and the subsequent period of operative rest (waiting for the beginning of the task), is, as yet, quite poorly understood. In healthy subjects, it has been shown analytically that alpha- and theta-rhythms in the state of operative rest provide valuable information about functional state and can be a predictor of visual recognition effectiveness [27–29].

Analysis of the neurophysiological correlates of preparation for visual recognition, apart from single studies of anxiety disorders [30] and obsessive-compulsive disorders [31], has been performed mainly in healthy subjects [32–34]. It is worth noting that preparation for visual recognition is mainly studied for short time periods (no more than 1 second(s) before stimulus presentation). Longer periods, allowing an estimate of trends in electroencephalogram (EEG) rhythms changes, have only been used in single studies of healthy subjects [35].

It was hypothesised that changes in the power characteristics of alpha- and theta- bands, revealed in resting state and the visual recognition of various facial expressions, may be accompanied by changes in these rhythms in preparation for task performance in young adults with signs of anxiety. Such data may provide additional information on brain mechanisms of the onset and development of anxious states, notably in young men with incomplete, especially prefrontal cortex, brain maturation.

The purpose of our investigation is to analyse neurophysiological correlates of the cognitive preparation for a facial recognition task in subjects with high and low levels of anxiety. To do this, we analysed cortical bioelectrical activity in high- and low-anxiety subjects during various periods, or conditions, preceding the performance of a cognitive task: (1) background/eyes closed; (2) background/eyes...
opened; (3) listening to the instructions for the cognitive task; (4) operative rest (time lapse between listening to instruction and the beginning of the task), as well as during short intervals immediately preceding exposure to target stimuli (stage of preparation) – image pairs of faces with same and different emotional expression.

EEG-studies of anxious subjects were performed mainly during a state of quiet wakefulness (usually eyes-closed, but also eyes-open). In these cases, the focus was on the state of preparation for visual recognition, i.e. during 1 s EEG fragments immediately before stimulus presentation. In the current study, several extra conditions were analysed, including: listening to instruction, operative rest (the period between listening to instruction and the beginning of task performance), while the preparation for recognition period was increased to 4 s. In studies on healthy subjects conducted in our laboratory, this approach provided new data pertaining to the state of the subject before testing and during the interstimulus intervals of the cognitive task activities [25–36]. The presented methods might be of value for patients not capable, due to some pathological or age restriction, of undergoing or completing standard cognitive testing by EEG recording. In the presence of multiple artefacts during the post-stimulation EEG (i.e. between the presentation of target and trigger stimuli) the pre-stimulation recording could be used for assessment of the status of healthy subjects, and especially patients. Finally, it should be mentioned that in our studies of both restless subjects such as normally developing pre-school and primary school children [37] and deficits in the fronto-thalamic system of selective attention [38], the analysis of long pre-stimulus EEG fragments was highly illuminating.

2. Materials and methods

2.1. Subjects

Following a screening questionnaire testing of 2nd year medical and pediatric faculty students, a prevalence of high anxiety was revealed. Subsequently, an EEG study was undertaken for 1 semester, and 82 respondents, aged from 19 to 21 (mean 19.1 ± 0.7) years, not under neurological or psychiatric supervision, with normal or corrected to normal vision, were investigated. According to the Taylor Manifest Anxiety Scale, 2 groups were selected: low-anxiety (LA: up to 15 points, M = 7.8 ± 0.9) and high-anxiety (HA: 25 points or greater, M = 29.3 ± 1.1). Each group included 8 male and 8 female subjects. All subjects were acquainted with the experimental procedure and gave informed consent. The study was performed according to the ethical principles for medical research involving human subjects of the World Medical Association Declaration of Helsinki.

2.2. Stimuli and stimulation procedure

A model of fixed psychophysiological cognitive set with the recognition of facial expression was used [36]. In the phase of set formation, paired images of a man from the Ekman’s Atlas of Emotions were presented 20 times; the left hand image showed a man with an angry unpleasant expression, the right hand image showed the same man with a neutral expression. In the phase of set testing, the impact of the setting on facial expression recognition, paired pictures of the same man with a neutral expression in both images were presented 40 times. Exposure duration was 0.35 s. After a 2 s delay following the triggering stimulus (a white light spot) the stimulus was presented. The subject determined on which side, right or left, the facial expression was unpleasant (or the same) and reported it orally following the trigger stimulus.

2.3. Data acquisition

The EEG was recorded continuously during the experiment. For EEG recording, amplification, and filtration a Neocortex-Pro (“Neurobotics”, Russia) system was used. Sample frequency was set to 250 Hz, and the frequency bandwidth was 0.5–70 Hz. Twenty Ag/AgCl electrodes (“Micromed”, Hungary) with a resistance < 5 kOhm were applied following the standard 10–20 scheme with additional leads (F3, F4, F7, F8, Fz, FT7, FT8, C3, C4, Cz, FC3, FC4, T3, T4, P3, P4, T5, T6, O1, O2). The EEGs were monopolar; the reference electrode was combined auricular. Stimuli presentation, response recording, and their synchronization with the EEG were controlled by a Neostimul (“Neurobotics”, Russia) system.

2.4. Data analysis

Four EEG fragments (20 s) obtained under the following experimental conditions were recorded and analysed: (1) background/eyes closed; (2) background/eyes opened; (3) listening to the instructions for the cognitive task; (4) operative rest (time lapse between listening to the instructions and the beginning of the task), as well as 4 1-s fragments, immediately preceding exposure to the target stimuli – paired pictures of a face with same and different emotional expressions (preparation stage). To assess the amplitude EEG parameters a wavelet transform was used. Both long (experimental conditions 1–4) and short (pre-stimulus) EEG fragments were analysed. A continuous wavelet transform based on the “maternal” Morlet wavelet complex (Matlab 78.0.1) in the range of 1–35 Hz was performed [39]. Distribution maps of the wavelet transform ratio (WTR) module, representing the amplitude of a potential, were drawn in a bandwidth of 4–13.5 Hz with 0.5 Hz steps and a temporal resolution of 1 ms. The frequency was then averaged across domains of 4–7.5 and 8–13.5 Hz. For each experimental condition (1–4 and preparation stage) a mean value of WTR for both frequency domains was assessed. The statistical significance between LA and HA groups for the calculated data was made by repeated measures analysis of variance (RM ANOVA), where the between-group factor was the “Group” (2 levels – LA and HA), and the within-group factors – the “Condition” (12 levels – conditions 1–4, see above, and 81 s interval immediately before the task performance: 4 for presentation of faces with different expressions, 4 for presentation of faces with identical expression) and the “Lead” (20 levels). Alpha- and theta-activities were studied independently. For analysis of between-group differences for each condition and each lead a one-way ANOVA was used. The number of recognition errors for students with high and low levels of anxiety was determined using Student’s t test. Statistical data analysis was performed with SPSS 13 software.

3. Results

The number of mistakes in facial expression recognition for low- and high-anxiety groups revealed no difference in performance. On presentation of two pictures of the same subject with different expression, high-anxiety students made 1.00 ± 0.48 mis-interpretations, while low-anxiety students made –0.40 ± 0.23 (t = –1.13, p = 0.27) mistakes. Similarly, no differences in recognition performance were found on presentation of two pictures with...
similar expression: high-anxiety students made $3.87 \pm 1.03$ misinterpretations, low-anxiety students $-4.06 \pm 1.34 \ (t = 0.11, p = 0.913)$.

An analysis of variance of frequency EEG parameters showed significant differences in interactions of factors “Condition × Group” for alpha ($F(9,54) = 6.79, p = 0.001$), and theta ($F(4,109) = 2.91, p = 0.03$) rhythms values (see Table 1) which were higher in the LA group.

Table 1. Results of ANOVA of the amplitudes of the Alpha and Theta band EEG in subjects with high and low levels of anxiety.

|                  | Alpha band | Theta band |
|------------------|------------|------------|
|                  | df | $F$ | $p$ | df | $F$ | $p$ |
| Condition        | 11 | 52 | $18.675$ | 0.000 | 11 | 20 | $6.619$ | 0.000 |
| Condition × Gr   | 19 | 44 | $2.336$ | 0.020 | 4 | 109 | $2.909$ | 0.029 |
| lead             | 19 | 44 | $23.417$ | 0.000 | 12 | 19 | $9.711$ | 0.000 |
| Lead × Gr        | 18 | 44 | $2.511$ | 0.006 | 12 | 19 | $1.146$ | 0.415 |
| Lead × Condition | 1 | 62 | $47.201$ | 0.000 | 1 | 30 | $7.990$ | 0.000 |
| Lead × Condition × Gr | 1 | 62 | $1.270$ | 0.264 | 1 | 30 | $0.806$ | 0.376 |

3.1. Analysis of bioelectrical activity in conditions not directly related to stimuli presentation

A detailed analysis of regional between-group differences revealed their topographical specificity in various experimental conditions and for various frequency ranges.

For the alpha-rhythm (Fig. 1), the least differences between LA and HA groups were noted for the resting conditions: in Condition 1 (background/eyes closed) a significant ($p < 0.05$) difference was revealed only in the right fronto-temporal area (FT8), in Condition 2 (background/eyes opened), additionally, a significant difference ($p < 0.05$) was revealed in the left posterior temporal area (T5). In Condition 3 (listening to the instructions) a less prominent alpha-rhythm amplitude in HA group was detected in the right frontal (F4), bilaterally in fronto-temporal (F7, F8, FT7, FT8), and left fronto-central (FC3) areas. In Condition 4 (operative rest) a significantly less pronounced alpha-rhythm in the HA group was present in all mentioned areas except for FT7.

As for the theta-rhythm (Fig. 2), in the two resting state conditions (1 and 2), there were no significant differences between the two groups. In Condition 3 (listening to instruction), a less prominent alpha-rhythm amplitude in the HA group was detected in the leads F4, F7, F8, FT8, as well as in the anterior temporal (T3, T4) and left posterior temporal area (T5). In Condition 4 (operative rest), between-group differences shift mainly to caudal cortical areas (P3, P4, T5) while persisting in FT8 and T3. Again, in all mentioned cases, the theta-rhythm amplitude was less in the HA group.

3.2. Analysis of bioelectrical activity immediately preceding stimulus presentation

In the preparation period for visual recognition, irrespective of whether the facial expressions were identical of different, the between-group differences in the alpha-rhythm were pronounced mainly in frontal and fronto-temporal areas (Fig. 3). In the phase of set formation, 4 s before stimulus presentation, significant differences were found in F7, FT8 and FC3, 3 s – only in F7 and FT8, 2 s – in F4, F7, F8, FT7, FT8 and FC3. Immediately (1 s) before stimulus presentation significant differences were detected only in fronto-temporal areas, mainly in the right hemisphere. In all cases, the alpha-rhythm amplitude was less in the HA group.

During set testing, for 4 s before stimulus presentation, significant differences in alpha-rhythm amplitude (less in the HA group) were found in F7, F8, FT8 and FC3, 3 s and 2 s – in F4, F7, F8, FT8 and FC3. 1 s before the presentation significant differences were detected bilaterally in fronto-temporal areas (F7, F8, FT7; FT8).

In the case of the theta-rhythm amplitude, significant between group differences (greater in the LA group) were found mainly in fronto-temporal areas (Fig. 4). During set formation (presentation of a pair of faces with different expressions), 4 and 3 s before stimulus exposure, significant differences were detected in areas F7 and FT8, then (2 and 1 s before exposure) also in F8. In the phase of set testing (presentation of a pair of faces with an identical expression), 4 s before stimulus presentation, significant differences were found in F7, FT8 and FC3, 3 and 2 s – also in F8. Immediately (1 s) before stimulus presentation significant differences were detected in areas F7, F8 and FT8.

4. Discussion

4.1. Analysis of bioelectrical activity in conditions not directly related to stimuli presentation

EEG recording and analysis in resting (background) conditions, without stimulus presentation, is a widely used method in neurological practice [3–6, 9, 40]. In clinical studies aimed to find characteristic EEG signs of depression and anxiety disorders, significant changes in alpha range with higher activation pattern in the right fronto-temporal area have been found in patients as compared to healthy subjects [4, 5, 7]. Current neuroimaging studies also demonstrate a correlation between high anxiety and increased blood flow in the ventral medial prefrontal cortex of the right hemisphere [41]. The increase of activation in the right prefrontal cortex is associated with the enhancement of hypothalamic–pituitary–adrenal activity, regulating the level of cortisol – a hormone involved in human and primate response to conditions of high anxiety and fear [42, 43]. The decrease in amplitude of the alpha-rhythm in the right fronto-temporal area in the resting condition, suggests increased activation in high-anxiety subjects and well fits the existing data.

It is worth noting that more pronounced intergroup differences appear in the phases of instruction listening and operative rest, manifesting in high-anxiety students as a decrease of amplitude parameters in their alpha- and theta-bands. In addition to differences in fronto-temporal areas, the right dorsolateral prefrontal, anterior and posterior temporal, central frontal areas also became involved. Previously, it has been shown that the verbal instruction subjects listen to before task performance generates some internal condition that persists in operative memory and largely determines the spatial organization of bioelectrical brain activity specific for a performed task [27–29]. In the present study, the higher values of the alpha-rhythm in LA subjects indicates that they are closer to a state of physiological rest than the HA subjects, in which a more pronounced activation of anterior central cortical areas is typical. The theta-rhythm plays an important role in the processing of information coding in episodic memory [44–46]. This occurs while listening to instructions and during their retention in operative memory, the latter being a key feature of operative rest. These results suggest that high-anxiety subjects suffer from a deficiency of processes essential for working memory.
Fig. 1. Amplitude values of the EEG alpha-waves, calculated by means of wavelet-transform, in different leads in subjects with high and low anxiety levels. On the vertical axis – the mean values of the wavelet transformation coefficient (WTC), standard units (s.u.), on the horizontal axis – experimental conditions: 1 – background/eyes closed; 2 – background/eyes opened; 3 – listening to instruction; 4 – operative rest. Dashed boxes – high-anxiety group, open boxes – low-anxiety group. The asterisk (*) corresponds to significant ($p < 0.05$) between-group differences, as calculated by one-way ANOVA. Standard errors are shown.

Table 2. Mean values of the oscillation power of alpha band (EEG leads FT7, FT8) in groups with low (LA) and high (HA) levels of anxiety in different conditions of the experiment. Conditions: (1) background/eyes closed; (2) background/eyes opened; (3) listening to instructions for the cognitive task; (4) operative rest; 5–12: 1 s intervals immediately before the task performance; 4 for presentation of faces with different expressions, 4 for presentation of faces with identical expression. $F$, $p$ – one-way ANOVA results. Error of mean is shown.

|      | FT7          |      | FT8          |
|------|--------------|------|--------------|
|      | LA           | HA   | $F/p$        | LA           | HA   | $F/p$        |
| 1    | 148.52 ± 19.50 | 137.63 ± 16.43 | 0.42/0.67   | 128.39 ± 5.96 | 108.19 ± 8.63 | 1.92/0.063 |
| 2    | 104.19 ± 7.28  | 94.59 ± 7.57  | 0.91/0.364  | 95.84 ± 5.43  | 78.36 ± 4.32  | 2.51/0.014  |
| 3    | 130.82 ± 11.37 | 101.07 ± 9.02 | 2.04/0.044  | 107.73 ± 5.52 | 79.31 ± 4.71  | 3.91/0.000  |
| 4    | 118.87 ± 9.21  | 97.23 ± 9.76  | 1.61/0.112  | 110.49 ± 5.15 | 86.70 ± 4.94  | 3.33/0.001  |
| 5    | 110.16 ± 7.32  | 98.42 ± 5.52  | 1.28/0.205  | 98.26 ± 3.54  | 82.01 ± 4.44  | 2.85/0.005  |
| 6    | 110.51 ± 6.60  | 96.96 ± 5.35  | 1.59/0.116  | 100.12 ± 3.68 | 84.19 ± 4.87  | 2.60/0.011  |
| 7    | 112.14 ± 7.16  | 94.18 ± 5.28  | 2.01/0.048  | 100.46 ± 3.75 | 82.91 ± 4.74  | 2.90/0.005  |
| 8    | 112.42 ± 7.23  | 95.36 ± 5.09  | 1.93/0.058  | 100.17 ± 3.86 | 83.22 ± 4.26  | 2.94/0.004  |
| 9    | 111.24 ± 6.15  | 99.75 ± 6.92  | 1.24/0.219  | 98.07 ± 3.28  | 84.26 ± 4.27  | 2.55/0.012  |
| 10   | 112.01 ± 6.40  | 98.52 ± 7.42  | 1.37/0.173  | 97.62 ± 3.45  | 81.67 ± 4.26  | 2.90/0.005  |
| 11   | 111.53 ± 6.10  | 98.25 ± 7.43  | 1.38/0.172  | 96.44 ± 3.49  | 82.73 ± 4.24  | 2.49/0.015  |
| 12   | 109.00 ± 5.88  | 98.49 ± 7.05  | 1.14/0.257  | 95.87 ± 3.75  | 81.48 ± 4.21  | 2.55/0.013  |

4.2. Analysis of bioelectrical activity immediately preceding stimulus presentation

The differences in the spatio-temporal pattern of brain activity between LA and HA groups at the stage of preparing for visual expression recognition is in many ways similar to that of instruction listening and operative rest. Namely, HA subjects have lower spectral values in alpha and theta ranges for all 4 s preceding exposure to the pairs of faces either with different or identical expression. The mechanisms of preparation for task performance have been intensively studied [32, 47–50]. The available data suggest that in healthy subjects a decrease in alpha-rhythm immediately before the target
Fig. 2. Amplitude values of the EEG theta-waves, in different leads in subjects with high and low anxiety levels. Other indications same as in Fig. 1.

stimulus presentation indicates a state of mental readiness, indispensable for the detection and analysis of objects [50–52]. In HA subjects lower values of alpha-rhythm parameters are detected during the 4 s preceding stimulus presentation, without the decrease immediately before the exposure. It has previously been shown that low values of alpha-rhythm power during preparation for task performance is typi-
Fig. 3. Schematic maps of differences in alpha-rhythm power (8–13.5 Hz) between HA and LA groups in the condition preceding the presentation of facial stimuli. 4 EEG 1-s fragments were recorded; times in seconds before stimulus presentation are given below corresponding maps. I – pairs of faces with different expression; II – pairs of faces with identical expression. Gray-filled circles represent leads with significantly ($p < 0.05$) higher values in the LA group, as calculated by one-way ANOVA. Top – a schematic map of EEG leads.

Fig. 4. Schematic maps of differences in theta-rhythm power (4–7.5 Hz) between HA and LA groups in the condition preceding the presentation of facial stimuli. Other indications are the same as for Fig. 3.

Pronounced differences between the HA and LA groups are also a characteristic of the theta-rhythm. It was shown that the theta-activity in frontal areas increases when task performance required involvement of attention or operative memory [55]. The rise of theta-rhythm in intervals preceding stimulus presentation in the LA group, according to the available data, can be a predictor of effective memorizing [56–58]. Wang and coll. [59] suggest that theta-activity in
the anterior cingulate cortex might reflect a process of active inhibition. Alternatively, decreased theta-rhythm in high-anxiety subjects might be linked directly to the anxious state [60]. Accordingly, the lateral prefrontal cortex where in high-anxiety subjects the alpha- and theta- rhythm power is relatively low (Table 2 and Table 3), is associated with explicit emotion regulation and top-down cognitive control [35, 61, 62], and is disturbed in anxious subjects [23, 63].

During the last decade a theory has developed that declares the lateral prefrontal cortex during the phases of listening to different emotional expressions. The greatest differences were detected in lateral prefrontal cortex during the phases of listening to and waiting for a cognitive task. High-anxiety subjects exhibited significantly lower amplitude values in alpha and theta ranges (as compared with low-anxiety subjects). The analysis of alpha- and theta- rhythms in the prefrontal cortex in conditions of instruction listening and of waiting for a cognitive task may provide EEG anxiety markers in preclinical settings.

5. Conclusion
Amplitude parameters of alpha- and theta- rhythm were studied in high- and low- anxiety subjects in various stages of preparation for a visual recognition (background/eyes closed; background/eyes opened; listening to instructions for the cognitive task; waiting for task initiation – operative rest), including 41 s intervals immediately preceding exposure to target stimuli – image pairs of faces showing different emotional expressions. The greatest differences were detected in lateral prefrontal cortex during the phases of listening to instructions and waiting for the cognitive task. High-anxiety subjects exhibited significantly lower amplitude values in alpha and theta ranges (as compared with low-anxiety subjects). The analysis of alpha- and theta- rhythms in the prefrontal cortex in conditions of instruction listening and of waiting for a cognitive task may provide EEG anxiety markers in preclinical settings.

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Conflict of Interest
All authors declare no conflicts of interest.

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Table 3. Mean values of the oscillation power of theta band (EEG leads FT7, FT8) in groups with low (LA) and high (HA) levels of anxiety under the different experimental conditions. Designations as in Table 2.

| LA       | HA       | F/p  |
|----------|----------|------|
| FT7      |          |      |
| 1        | 148.52 ± 19.50 | 137.63 ± 16.43 | 0.42/0.67 |
| 2        | 139.16 ± 15.89 | 122.63 ± 16.36 | 0.72/0.47 |
| 3        | 192.62 ± 20.82 | 142.75 ± 16.50 | 1.87/0.07 |
| 4        | 202.7 ± 25.14  | 147.16 ± 15.16 | 1.89/0.06 |
| 5        | 145.88 ± 14.46 | 126.47 ± 10.56 | 1.08/0.28 |
| 6        | 149.72 ± 13.35 | 126.93 ± 11.21 | 1.30/0.20 |
| 7        | 145.07 ± 14.83 | 121.34 ± 8.90  | 1.37/0.18 |
| 8        | 147.64 ± 14.06 | 119.36 ± 9.19  | 1.68/0.10 |
| 9        | 145.86 ± 11.95 | 126.36 ± 11.78 | 1.16/0.25 |
| 10       | 141.96 ± 11.30 | 122.37 ± 12.23 | 1.17/0.24 |
| 11       | 141.78 ± 10.37 | 123.06 ± 12.73 | 1.13/0.26 |
| 12       | 136.57 ± 10.33 | 121.42 ± 12.02 | 0.95/0.34 |

| FT8      |          |      |
|----------|----------|------|
| 1        | 128.39 ± 5.96  | 108.19 ± 8.63  | 1.92/0.63 |
| 2        | 123.66 ± 8.52  | 102.15 ± 8.83  | 1.75/0.08 |
| 3        | 168.07 ± 13.27 | 118.12 ± 11.14 | 2.88/0.07 |
| 4        | 166.01 ± 11.39 | 131.44 ± 10.49 | 2.23/0.33 |
| 5        | 132.44 ± 5.53  | 112.07 ± 8.79  | 1.96/0.05 |
| 6        | 133.14 ± 5.98  | 112.73 ± 7.81  | 2.07/0.04 |
| 7        | 135.41 ± 7.97  | 105.58 ± 7.80  | 2.67/0.02 |
| 8        | 135.10 ± 8.08  | 106.12 ± 7.86  | 2.57/0.15 |
| 9        | 134.55 ± 7.13  | 112.81 ± 7.94  | 2.04/0.04 |
| 10       | 129.35 ± 7.20  | 106.92 ± 7.38  | 2.17/0.03 |
| 11       | 130.37 ± 6.80  | 107.51 ± 8.01  | 2.17/0.03 |
| 12       | 129.08 ± 7.20  | 103.22 ± 7.15  | 2.54/0.01 |
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