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Novel environment as a stress--inducing factor. An event-related potentials study

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Abstract. The effect of stress induced by the novelty of a situation was evaluated by means of event-related potentials (ERPs). Potentials, recorded with Fz, Cz and Pz electrodes, were evoked by flashing red and yellow LED diodes. A standard "odd ball" procedure was used, in which flashes of one color were mentally counted (target stimuli). ERPs evoked by target and non-target stimuli recorded in the first session of the experiment were compared with those recorded at least 40 min later. The early waves and P200 components indicated the increased responsiveness during the initial sessions. Amplitudes of both components were significantly larger. Latencies of the early waves were also significantly shorter. The effects were present in responses to both target and non-target stimuli. In contrast, the latency of P300 wave was significantly elongated during the first recording. Grand-averaged curves indicated also a reduction of P300 amplitude, but when individual waves were analyzed, the effect did not reach the level of statistical significance. It was suggested that the novel situation could be employed as a model of relatively pure stress, useful in the interpretation of other results such as the effects of pain.

Key words: stress, human ERP, P200, P300

INTRODUCTION

The effects of emotional stress on the function of an organism can be dramatic. They range from the mild activation that improves the performance to the strong detrimental effects including the diseases such as hypertension, ulcers, depression or even tumor growths (Riley 1981, Levine 1986). Yet stress remains very difficult to study. One of the main problems is to disentangle its effects from the other aspects of the stimulation. In the case of factors such as the activation or emotion it is not even clear to what extent they can be separated and whether or not they should be incorporated into the definition of stress itself (Levine 1986). One of the useful strategy in such cases is to analyze the large variety of experimental situations in which stress is involved and look for the common effects and differences.

Most of the stress research has been based either on measuring the hormone levels or electrical responses of the organism. Among electrical responses stress can alter the heart rate, blood pressure, skeletal muscle tension (electromyogram; EMG), electroencephalogram (EEG) and galvanic skin response (GSR) (Duffy 1932, 1957, Darrow et al. 1942, Lindsley 1951, Malmo 1959). These alterations co-vary with catecholamine levels, thus, it is generally assumed that they reflect the activity of the autonomic nervous system (Levi 1965).

It seems that the late, event-related components of evoked potentials can be used in stress research. In particular, the P300 component is promising. A number of studies indicate that this wave reflects brain processes more advanced than stimulus perception (Israel et al. 1980a,b, Donchin et al. 1986a,b, Kramer et al. 1987, Polich 1989). The P300 wave could be elicited only when the subject had to react to or mentally count the infrequent target stimuli, presented among the more frequent non-target stimuli. This wave was also relatively insensitive to the physical parameters of the stimulus. Instead, it showed the strong variations with the probability of target stimulus occurrence. Moreover, P300 wave was very sensitive to factors without the direct relation to the main task. Its amplitude declined if any activity, concurrent to the reaction to target stimuli, was introduced. P300 amplitude was also reduced when the subject suffered from chronic pain in the background (Rosenfeld and Kim 1991, Rosenfeld et al. 1993, Michalski 1998). It can be assumed that the concurrent activity (such as performing mental arithmetic while counting the target stimuli) also carried a stress component. Even more obvious is the involvement of stress in the experiments with painful stimulation. Neuronal responses, selective to meaningful stimuli that evoked the P300-like potentials, were found in monkey locus coeruleus, a structure that probably controls the responsiveness of the vast areas of the brain (Aston-Jones et al. 1991, 1996)

One interesting model of stress is based solely on the novelty of the situation. It has been found that in such situations the levels of hormones that reflect the activity of the autonomic nervous system were very effectively altered. These hormones are commonly linked with stress (Mason et al. 1957, Levine and Treiman 1964, Friedman and Adler 1967, Friedman et al. 1967, Basset et al. 1973, Hennessy and Levine 1979).

The aim of the present experiment was to evaluate the effects of stress, produced by the novelty of the situation, on processing of neutral stimuli, using the analysis of P300 event-related potentials. It was expected that the results would facilitate the interpretation of the effects of tonic pain on event-related potentials observed in a similar experiments (Rosenfeld and Kim 1991, Rosenfeld et al. 1993, Michalski 1998).

METHODS

Twenty-seven volunteers of both sexes (7 males and 20 females), aged 27-43 years, participated in the experiment. None of the volunteers had any previous experience as a subject in electrophysiological experiments. Informed consent was obtained from all participants.

EEG signals were recorded with disc electrodes glued at Fz, Cz and Pz positions, referenced to linked mastoids and supplemented by vertical and horizontal EOG. Signals were sampled with 2048 Hz frequency, 12 bit resolution, digitally filtered 0.16-30 Hz and reduced to 256 Hz by averaging the adjacent points (Elmiko Paperless EEG system). Data were stored in epochs containing 250 ms before and 1 s after the stimulus onset. Epochs were rejected by the computer program and replaced with the new ones if EOG amplitude exceeded 40 μV.

Event-related potentials were evoked using the standard "odd ball" procedure. Flashes of spatially overlapping arrays of red and yellow LED diodes (2 deg x 2 deg of the visual angle, 10 cd/m^2 luminosity on 1 cd/m^2 background, 100 ms duration) were presented in random order every 2.5 s. Subjects were asked to mentally count the yellow flashes (target stimuli) and ignore the red flashes (non-target stimuli). Target stimuli were presented eight times less frequently than non-target

stimuli. Averaged responses were computed in the sessions that contained at least 30 repetitions of the target stimulus.

Potentials recorded in the first session of the experiment were compared with potentials recorded more than 40 min after the beginning of the recording. Amplitudes and latencies of peaks were measured using cursors on the computer screen and the data were transferred to SYSTAT program for multi-factor analysis of variance (ANOVA). Measurements were done in target and non-target responses and in the curves computed as a difference between them. An alternative way of analysis was based on grand-averaged potentials, computed by averaging responses over all subjects.

RESULTS

Figure 1 shows the potentials recorded with Pz electrode, grand-averaged over all subjects. Pz recordings showed the biggest P300 waves and the most pronounced differences between the responses recorded at the beginning of the experiment and those recorded later. Three sets of curves represent responses to target stimuli (top), non-target stimuli (middle) and the difference between them (bottom). Dotted lines show the waveforms obtained during the first session of the experiment and solid lines show the potentials obtained in later sessions.

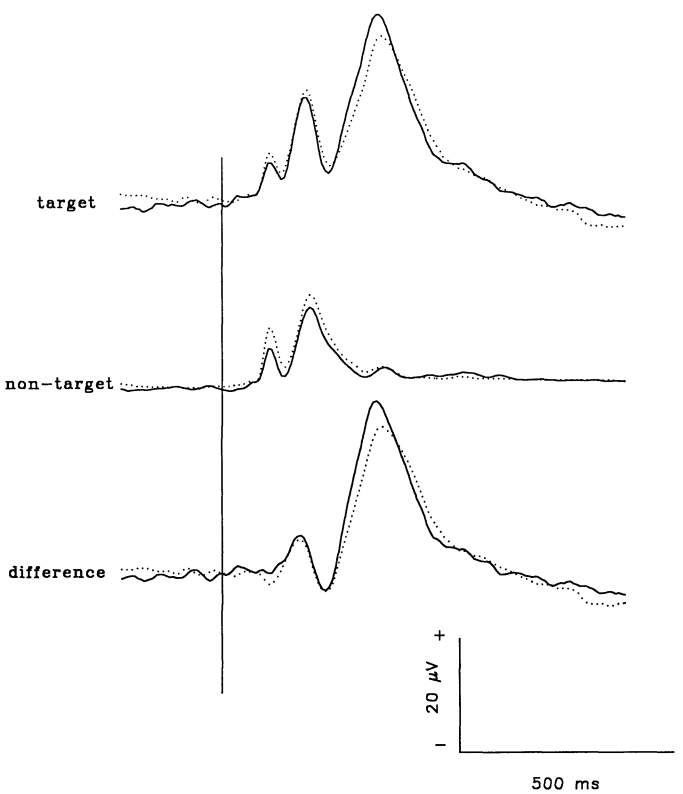

Fig. 1. Grand-averaged responses to target stimuli (top), non-target stimuli (middle) and the curves calculated as a difference between them (bottom). Dotted lines show the waveforms recorded in the first sessions of the experiment. Solid lines represent the recordings obtained at least 40 min later. Potentials were recorded with Pz electrode.

Responses to target stimuli consisted of three clearly distinguishable components: early responses, P200 and P300 peaks. In responses to non-target stimuli P300 peaks were lacking and P200 peaks were reduced. Consequently, the difference curves contained the P300 components and the smaller P200 waves. Early responses were reduced to zero.

Responses to target stimuli indicated that the effects of first recording on P300 waves and on earlier components were opposite: P300 waves were reduced and delayed in the first recordings whereas earlier components were enlarged. The earliest peaks were more enlarged in response to non-target stimuli than in response to target stimuli. Consequently, the dotted grand-averaged difference curve shows a downward deflection within this region. The enlargement of P200 component was similar in target and non-target responses thus, solid and dotted difference curves overlap in this region. Since the non-target stimuli did not produce the P300 waves, this wave appears in the difference curve as it was in the grand-averaged target response.

Figure 2 shows the difference curves computed from Fz (top), Cz (middle) and Pz (bottom) recordings. As in Fig. 1, dotted lines show the waveforms obtained during the first session of the experiment and solid lines show the data obtained in the later sessions. Downward deflections within the latency region of the earliest components are seen in all recordings. Thus, the enlargement of the earliest component during the first session was always bigger in the non-target than in target responses. The amplitudes of P200 components overlap in all recordings, thus, the enlargement of this component was similar in target and non-target responses. In contrast, the alterations of P300 waves seem to depend on the recording site. The reduction of the amplitude and the elongation of latency is clearly seen in Pz recordings. The effect is smaller in Cz recordings, and in Fz recordings P300 waves are practically identical.

P300 peak was identified as the most positive value between 250 ms and 400 ms after the stimulus onset. P200 peak was identified as the most positive value be-

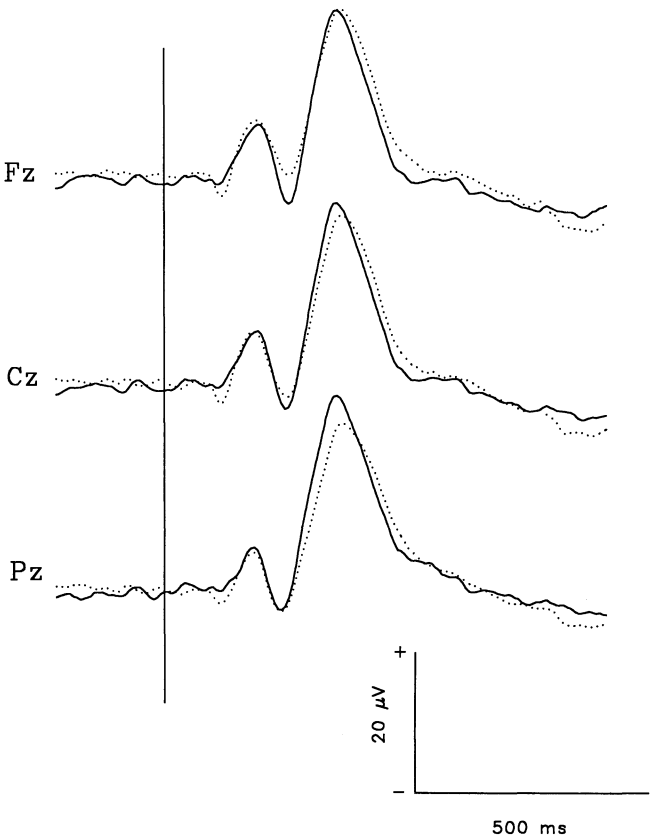

Fig. 2. Grand-averaged difference curves recorded with Fz (top), Cz (middle) and Pz (bottom) electrodes. Waveforms were computed as a difference between target and non-target responses averaged for all subjects. Dotted lines show the curves computed from the first recordings and solid lines show the curves computed from potentials recorded at least 40 min later.

tween 150 ms and 290 ms. Finally, the early peak was identified as the most positive value between 90 ms and 140 ms after the stimulus onset. Amplitudes of these peaks were measured in individual ERPs relative to 250 ms pre-stimulus baseline.

Amplitudes and latencies of early components were analyzed using a three-way ANOVA (electrode x target x recording). The target variable indexed the target and non-target responses and recording variable indexed the first and later recordings. The amplitudes of early components were significantly higher in the first recording of the experiment than in the later recordings ($F_{1,287}$ = 7.369, P<0.007). The mean amplitude in the first recordings was 8.9 μV (SD = 5.9). In the later recordings it was 7.096 μV (SD = 5.2). The amplitudes of the early component were also significantly higher in response to non-target stimuli than in response to target stimuli ($F_{1,287}$ = 11.316, P<0.001). Mean amplitude of the early components was 9.5 μV (SD = 5.4) in non-target responses and 6.9 μV (SD = 5.8) in target responses. There were no significant interactions.

Higher amplitudes of the early components were accompanied by shorter latencies. Latencies recorded in the first session of the experiment were significantly shorter than latencies recorded later ($F_{1,232}$ = 4.826, P<0.029). Mean latency was 119.1 ms (SD = 7.3) in the first session and 122 ms (SD = 7.6) in the later recordings. Latencies of early components in non-target responses were significantly shorter than those measured in target responses ($F_{1,232}$ = 5.648, P<0.018). Mean latency was 119 ms (SD = 6.7) in non-target responses and 121 ms (SD = 8.2) in target responses. There were no significant interactions.

A similar three-way ANOVA (electrode x target x recording) was used to analyze the P200 components. Like the early component, the P200 wave was significantly larger in the first session of the experiment than in the later sessions ($F_{1,288}$ = 15.836, P<0.001). Mean amplitude was 18 μV (SD = 6.4) in the initial recordings and 15 μV (SD = 6) in the later sessions. Unlike the early response, P200 component was significantly bigger in target responses ($F_{1,288}$ = 65.925, P<0.001). Mean amplitude was 19.6 μV (SD = 6.6) in target responses and 14.3 μV (SD = 4.8) in non-target responses. There were significant differences in P200 amplitudes recorded with different electrodes ($F_{2,288}$ = 7.845, P<0.001). Post-hoc Bonferroni test indicated that amplitudes recorded with Pz electrodes were significantly lower than amplitudes recorded with Cz (P<0.007) and Fz electrodes (P<0.001). Mean P200 amplitudes were 18.1 μV (SD = 6.9) in Fz, 17.6 μV (SD = 6.6) in Cz and 15 μV (SD = 5.2) in Pz recordings. All the interactions were insignificant.

The only significant effect in P200 latencies (including the interactions) was the difference between responses to target and non-target stimuli. Again the higher amplitudes in response to target stimuli were accompanied by shorter latencies ($F_{1,286}$ = 27.86, P<0.001). Mean latency of P200 component was 208 ms (SD = 15.3) in target responses and 222 ms (SD = 20.4) in non-target responses.

Since the P300 component was recorded only in response to target stimuli, it was analyzed with a two-way ANOVA (electrode x recording). Surprisingly, the differences in P300 amplitudes that looked apparent in Fig.1 did not reach the level of statistical significance.

On the other hand, the elongation of P300 latency during the first session of the experiment was significant ($F_{1,141}$ = 4.694, P<0.032). Mean latency of P300 component recorded in the first session of the experiment was 396 ms (SD = 26.5). In the later sessions it was reduced to 387 ms (SD = 22). All the other effects and interactions were insignificant.

Components specific to potentials evoked by target stimuli and absent in non-target responses were represented by difference curves. These components could be affected by stress in an unique way. To test this possibility, peaks were measured and analyzed also in difference curves. The earliest peaks were reduced almost to zero and could not be measured (they were identical in target and non-target responses). A two-way ANOVA (electrode x recording) was used for both the amplitudes and latencies of the remaining P200 and P300 peaks. The results, however, did not differ from those obtained with target responses. Again, the amplitude reduction of P300 component did not reach the level of statistical significance but the elongation of latency of this component in the first session of the experiment was even more clearly significant ($F_{1,147}$ = 8.056, P<0.005). Mean amplitude of P300 wave recorded in the first session was 25.16 μV (SD = 8.5) and 25.9 μV (SD = 7.5) in the later sessions. Mean latency was 406 ms (SD = 29) in the first session and 391 ms (SD = 33) in the later sessions. The amplitude of P200 component was significantly higher in the first recording ($F_{1,138}$ = 5.012, P<0.0027). Mean P200 amplitude was 9.3 μV (SD = 4.2) in the first recording and 7.6 μV (SD = 4.8) in the later recordings. The amplitude of P200 wave was also significantly higher in Fz recordings than in Pz recordings ($F_{2,138}$ = 3.692, P<0.03 and Bonferroni test). All the other effects on amplitudes and latencies of both P300 and P200 components and all the interactions were insignificant. Thus, the only discrepancy between the results obtained in target responses and the difference curves was that the latency of P200 component measured in the latter did not differ significantly in the first and later sessions of the experiment.

DISCUSSION

The present results show that ERPs recorded at the beginning of the experiment differed significantly from potentials recorded later. But is there sufficient justification for interpreting the results in terms of stress? It is reasonable to assume that in the first session of the experiment the stimuli were better attended and that the fatigue or stimulus habituation induced by the long session affected the results. It should be remembered, however, that 40 min of the experiment actually consisted of a recording sessions that lasted around 6 min with 10 min long breaks between them. These breaks should reduce the subject fatigue.

As mentioned in the introduction it is probably impossible to find an experimental model of the pure stress, isolated from the other factors. In an attempt to evaluate the role of stress in the present results the discussion will follow two lines: the effects of different experimental paradigms will be compared and the different behavior of early and late ERP components in the present experiment will be analyzed.

Different experimental paradigms introduced the different "contaminating factors" but they all attempted to produce stress. Therefore the effects common for the different paradigms are most likely correlated with stress. If two different measures, such as hormone levels and electrical responses, correlate, the interpretation becomes still less ambiguous. Thus, the simple comparison of the results of different experiments can reduce the ambiguity of interpretation.

It was shown that in animals the initial exposure to the experimental setting evoked the large elevations in plasma cortisol (Mason et al. 1957, Hennessy and Levine 1979). In human experiments, the introduction to the experimental situation for the first time was often more effective in increasing steroid level than anything the experimenter could devise, including electric shock (Levine and Treiman 1964, Friedman and Adler 1967, Friedman et al. 1967, Basset et al. 1973).

The effects of a novel situation on event-related potentials has not been studied before, thus, the discussion has to be limited to the indirect comparisons. The effect of tonic pain on responses to neutral stimuli was analyzed using an experimental paradigm almost identical to that presently used. (Rosenfeld and Kim 1991, Rosenfeld et al. 1993, Michalski 1998). In these studies pain strongly reduced the amplitudes of P300 waves, whereas earlier components were unaffected. It is reasonable to assume that sustained painful stimulation was accompanied by a tonic stress component. In the present experiment the reduction of P300 amplitude was insignificant but the grand-averaged curves showed the tendency for such a reduction, clearly different from the significant enlargement of earlier components. On the other hand the latencies of P300 component were significantly elongated in the present experiment. Similar

elongation was produced by tonic pain in the experiment of Rosenfeld and co-authors (1993) but not in the remaining two experiments (Rosenfeld and Kim 1991, Michalski 1998). The elongation of P300 latency was found in patients suffering from chronic pain that was not experimentally induced (Tandom and Kumar 1993) and in patients suffering the migraine attacks (Mazzotta et al. 1995). These data, however, should be treated with caution because they were obtained from ill patients in which a number of brain functions could be affected.

Introducing a second, parallel task (e.g., performing mental arithmetic while counting the target stimuli) should also increase the stress level. The reduction of P300 amplitudes by such a parallel task was reported in a number of papers (Israel et al. 1980 a,b, Kramer et al. 1987).

An interesting clue is provided by the opposite behavior of the P300 and the earlier ERP components, recorded in the present experiment. Earlier components were significantly enlarged and their latencies shortened in the initial recordings. Could these be the effects of better attention? And why did P300 waves behave clearly differently? In a number of experiments P300 waves were used in the studies of emotions in which the same stimuli that evoked the responses also carried the emotional component. In the majority of these experiments both pleasant and unpleasant pictures produced higher P300 amplitudes than neutral images. (Radilová et al. 1983, 1984, Johnston et al. 1986, 1987, Radilová 1989, Carretié et al. 1991, 1995, Johnston and Wang 1991, Laurian et al. 1991, Palomba et al. 1993, 1997, but see Vanderploeg et al. 1987). These experiments were obviously designed to detect the differences between the effects of positive and negative emotions. Stronger responses to both types of emotional images were attributed to higher attention levels. But if a higher attention level increases the P300 amplitude (the same way as the earlier components) why was this component reduced and delayed in the initial sessions of the present experiment? And why did the earlier components show the opposite effect? If the behavior of earlier components reflects the alterations in the attention level or stimulus habituation, these components could provide a measure of these effects and enable the separation of the other process - the inhibition of P300 waves during initial trials. Unfortunately the full separation of these processes is not possible because it is not known to what extent they overlap in time. If they do, the reduction of P300 amplitude during the first trials could be even stronger but it may be masked by the excitatory process.

It can be concluded that the experimental procedure based solely on the novelty of the situation produced the significant differences in the event related potentials that were complementary to the earlier recorded alterations in hormone levels. The effects observed in the short and medium latency ERP components can be easily explained by better attention level, habituation or general fatigue. The effects on P300 components were opposite. It is tempting to interpret them as the effects of stress.

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