Title
Task-Relevant Late Positive Component of the Auditory Event-Related Potential in Monkeys Resembles P300 in Humans

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phorylation and dephosphorylation reactions of the 80-, 49-, and 39-kD polypeptides occur in vivo. Moreover, these reactions are readily inducible by exposing the flies to ambient room light. No unusual stimuli are necessary. These observations lead one to conclude that the light-induced phosphorylation-dephosphorylation cycles of these three polypeptides are part of normal physiological reactions occurring in a living system.

Another important point is the tissue specificity of the three polypeptides. By dissecting out the compound eye into its individual components, it was shown that all three polypeptides arise specifically from the photoreceptor layer (*6*). The lamina, which contains the synaptic endings of photoreceptors, in particular, does not make any contribution to these polypeptides in two-dimensional gels stained with Coomassie blue. The tissue specificity makes it highly unlikely that the three polypeptides are involved in general metabolism or housekeeping functions. Nor is it likely that any of these polypeptides are involved in synaptic mechanisms. Rather, the polypeptides are probably part of the molecular machinery underlying photoreceptor mechanisms.

We reported earlier that the light-induced shift in the *p*I (that is, the light-induced phosphorylation) of these polypeptides is blocked by the *norpA* mutation (*6*). Inasmuch as the available evidence strongly suggests that the *norpA* mutation blocks an essential step in phototransduction (*7*), we suggested that the light-induced modification of these polypeptides requires the integrity of the phototransduction process (*6*). These observations, together with our present finding that the phosphorylation of at least the 80- and 49-kD polypeptides is rather rapid, suggest that the 80- and 49-kD polypeptides and their phosphorylation reactions may be involved in some early molecular process underlying the mechanisms of photoreceptor potential generation or modulation (or both).

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8. The dark- or light-adapted ^{32}P-labeled flies were frozen in liquid nitrogen and dehydrated in acetone at −20°C. After dehydration, 100 compound eyes were dissected out from each group of flies and homogenized in 60 μl of a mixture containing 8*M* urea, 2 percent Triton X-100, and 2 percent Bio-Lyte 3/10 (Bio-Rad). The supernatant (about 55 μl), recovered after a brief centrifugation (3000 rev/min for 10 minutes), was subjected to two-dimensional gel electrophoresis by the method of P. H. O'Farrell [*J. Biol. Chem.* **250**, 4007 (1975)] with a slight modification by K. Miyazaki et al. [K. Miyazaki, H. Hagiwara, M. Yokota, T. Kakuno, T. Horio, in *Isoelectric Focusing and Isotachophoresis*, N. Ui and T. Horio, Eds. (Kyoritsu Shuppan, Tokyo, 1978), p. 183 (in Japanese)]. The isoelectric focusing (IEF) gels contained: 1.6 ml of 30 percent acrylamide, 1.5 percent *N,N'*-methylenebis (acrylamide), 1.5 ml of 0.004 percent riboflavin, 0.45 percent *N,N,N',N'*-tetramethylethylenediamine, 0.08 ml of 1.5 percent ammonium persulfate, 0.6 ml of 40 percent Bio-Lyte 3/10, 6.13 g of urea (Ultrapure grade, Schwarz/Mann), 1.2 ml of 20 percent Triton X-100, and 2.5 ml of distilled water. The gels were polymerized by illumination. The sample was applied from the acidic end (0.02*M* H_3PO_4, anode; 1*M* NaOH, cathode) and was focused with constant current mode at 0.5 mA per gel until the voltage reached 300 V and, thereafter, with constant voltage mode at 300 V for 16 hours and finally at 700 V for 2 hours. The second dimension, sodium dodecyl sulfate–10 percent polyacrylamide gel electrophoresis (SDS-PAGE), was carried out on a slab gel (14 by 13 cm) according to L. K. Laemmli [*Nature (London)* **227**, 680 (1970)].
9. Wild-type flies that had been dark-adapted for 24 hours were separated into three test tubes of 60 flies each. The first group was frozen in the dark (dark-adapted; Fig. 2a). The second group was subjected to a white strobe flash of < 1 msec duration (National Panashot PE-200, Matsushita Electric Co., Japan) and frozen as described in the text (flash-illuminated; Fig. 2b). The third group was exposed to room light for 5 minutes and frozen in room light (light-adapted; Fig. 2c). The gel analyses were carried out as described (*8*).
10. To obtain contour profiles, we dried the Coomassie blue–stained gels on filter paper and photographed them. The grain density of the photographic negatives were scanned to 100 μm resolution with Optronix Colorscan System C-4100. The data were stored on a magnetic tape and processed on the Purdue MACE operating system connected to a PDP 11/70 computer with the program developed by M. Laris.
11. The amount of phosphorylation was estimated by integrating each of the two contour peaks in Fig. 3b, representing dephosphorylated and phosphorylated forms of the 49-kD polypeptide.
12. We thank M. Laris for his help with computer analyses of two-dimensional gel electrophoretic profiles. Supported by grant BNS 80-15599 from the National Science Foundation and grant EY 00033 from the National Eye Institute of NIH.

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Task-Relevant Late Positive Component of the Auditory Event–Related Potential in Monkeys Resembles P300 in Humans

Abstract. *A long-latency (300-millisecond), vertex-positive component of the event-related potential recorded from monkeys was present only when the eliciting stimulus was relevant to the task. The amplitude of this component varied inversely with stimulus probability and was dissociable from motor responses.*

The event-related brain potential (ERP) in humans, which is evoked by a task-relevant, rare stimulus, contains a prominent positive wave with a latency of about 300 msec (*1*). This P300 component can be elicited by the detection of infrequent and unpredictable target signals, such as pitch changes, that occur in a train of repetitive tone stimuli. A number of investigators have shown the P300 to be relatively independent of physical stimulus parameters and significantly related to cognitive processing of task-relevant stimulus information (*2*). These findings have led to attempted clinical application of the P300 component of the auditory ERP in the evaluation of cognitive functioning associated with aging, dementia, and alcoholism (*3*). Although the neural processes responsible for the

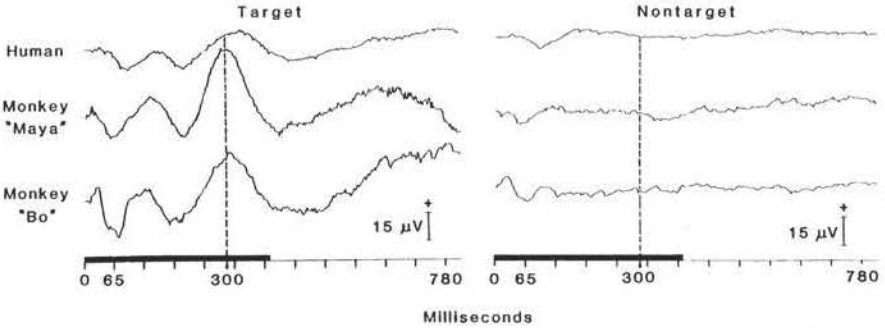

Fig. 1. Event-related potentials recorded from a human and two monkeys engaged in a similar discrimination task. The human subject was instructed to operate a lever and to respond to the rare event by releasing the lever in the same fashion as the monkeys. Note the strong similarity in ERP wave form morphology between species and the presence of a P300 component to the target stimuli that does not appear to the nontarget stimuli. The thickened line along the abscissa represents the period of tonal stimulation.

P300 are unknown, research on its origins would be stimulated by an appropriate animal model that would allow application of precise depth recording and lesion experiments. Endogenous components in the rat, cat, and monkey have recently been reported (*4–6*), but in none of these studies were the animals performing a differential instrumental response to the target stimulus, which is frequently required in human P300 paradigms. We report a positive component, occurring at about 300 msec in behaving monkeys (*Macaca nemestrina*), that has features comparable to the human P300: it occurs when the stimulus is task-relevant, varies inversely in amplitude with stimulus probability, and is dissociable from motor responses.

Under a variant of the "oddball" paradigm, two monkeys, Maya and Bo, were trained to discriminate infrequent target tones (1 kHz, 400-msec duration) from frequent, nontarget tones (2.5 kHz, 400-msec duration). The monkeys were required to press and hold a lever that initiated a random sequence of target and nontarget tones (one per second) and turned on a small "ready" light. The animals were trained to selectively release the lever within 600 msec of target tone offset for a juice reward, then immediately press and hold the lever again to continue the stimulus sequence. Reinforcement for release at target tone offset was employed to minimize muscle and movement-related potentials that might confound evoked potential averaging initiated at tone onset. The self-paced trials terminated when the animal erred by releasing the lever during or following a nontarget tone, or failed to release the lever in the allotted response time. Errors were followed by a variable "time-out" period that was signaled by extinction of the ready light. This procedure promoted attentiveness to both tones, while the target tone alone was task-relevant.

After training to criterion (*7*), the monkeys were implanted with a head holder and 15 stainless steel screw electrodes placed in coronal and parasagittal arrays according to a modified 10-20 system (*8*). Bipolar electrooculogram (EOG) electrodes were implanted over the supraocular ridge and to the side of the orbit; a noncephalic reference electrode was implanted 1 cm below the inion. After the animals recovered from surgery the electroencephalogram (EEG) was recorded from up to eight channels while the animals performed the discrimination task (*9*). The EOG channel was also recorded to define eye movements in the latency range of ERP components.

Each monkey was tested on at least four separate occasions at each of three target stimuli probabilities: 0.10, 0.30, and 0.50. Twenty-five target stimuli made up each averaged ERP, with a minimum of two replications of each condition obtained during each recording session (*10*). At least two averaged ERP's were obtained at each probability level for the nontarget tones as well, but the number of stimuli comprising each average varied with the probability levels.

The ERP results obtained from monkeys were strikingly similar to those found in human subjects performing in the same paradigm (Fig. 1). The late positive component elicited by rare target stimuli in the monkey ERP is broadly distributed, being maximal over sensorimotor and parietal cortical areas. We found the occurrence of the P300 to be negligible or absent over frontal and temporal cortical regions (*11*).

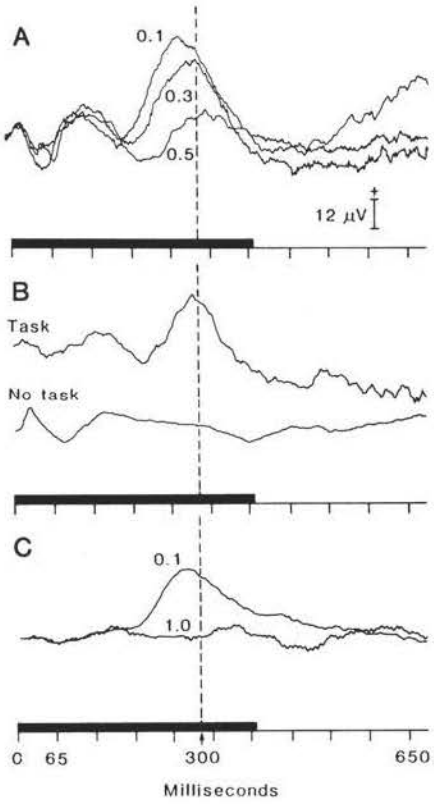

Fig. 2. Effect of target stimulus probability, task relevance, and motor response on the amplitude of a late positive component of the ERP recorded in two monkeys. (A) Grand averages (Cz) for the monkeys, demonstrating that the amplitude of the late positive component elicited by target signals varies inversely with stimulus probability. (B) ERP's elicited by rare stimuli ($P = 0.30$) exhibit a late positive component only while the animal is performing the discrimination task. To improve signal-to-noise ratios, grand averages for one animal were made across midline electrode locations Fz, Cz, and Pz. (C) ERP's averaged when 100 percent of the tones are targets do not elicit the P300 component (as they do when the target occurs with a 10 percent probability), even though there were equal numbers of trials. ERP's were averaged across midline electrode locations for one monkey.

Figure 2A shows that the amplitude of the P300 component in monkeys varied inversely with stimulus probability: it was largest at the 0.10 probability level, smaller at 0.30, and smallest at 0.50 [$F(2, 20) = 3.78$, $P < 0.05$] (one-way repeated-measures analysis of variance) (*12*). This effect was specific to the P300 component. Changes in probability did not significantly affect the first negative component (N1) elicited by the target ($F < 1$) or the nontarget ($F < 1$) stimuli. A separate analysis of P300 latency showed no significant differences between the 0.10 and 0.30 conditions; however, P300 latency in the 0.50 condition was significantly delayed (*13*).

The P300 component was present only when the stimulus was relevant to the task. Figure 2B compares results from a monkey performing the discrimination task with a "no-task" condition in which the lever was removed from the animal's control, reinforcement was withheld, and the random stimulus sequence was presented to the animal. With this manipulation, the P300 disappeared or was attenuated [$F(1, 8) = 8.98$, $P < 0.05$]. In previous studies a late positive component was recorded from monkeys in passive, no-task conditions (*5, 6*). However, those monkeys were not trained in a behavioral task and were therefore naïve with respect to the stimulus sequence. In the present study ERP's were recorded only from trained animals that had extensive prior experience listening and responding to the stimulus train. This fundamental difference between the two procedures may account for the different results.

To examine the possibility that the task-relevant condition elicits a motor-related potential corresponding in latency to the P300, animals were also tested when all tones were targets (that is, when there were no nontarget tones). The monkeys had to release the lever after each tone offset for reinforcement and then immediately press and hold the lever again to obtain another stimulus. Thus, the motor components of this task were identical to the discrimination paradigm, but since the stimulus sequence was predictable ($P = 1.0$), the conditions for elicitation of the P300 were not met. Figure 2C compares the results from the 100 percent condition with results from the same animal when the target occurred only 10 percent of the time. The occurrence of a P300 only in

the latter condition demonstrates a dissociation of the P300 from a motor response [$F(1, 19) = 9.38$, $P < 0.01$].

The stimulus and behavioral requirements of this monkey study were similar to those used in human experiments measuring P300. Wave form morphology elicited in monkeys paralleled that of the human ERP. In the monkey the P300 was markedly attenuated if elicited by rare stimuli that were not relevant to the task; its amplitude varied in a systematic fashion with stimulus probability, and it was independent of motor responses. Thus the late positive peak recorded from monkeys in this study behaved essentially like the P300 component recorded from humans in similar experimental situations.

As far as we know, this is the first report of a nonhuman primate P300 obtained from animals engaged in a stimulus discrimination task (*14*). The component was robust and was reliably present in both animals tested. The demonstration in monkeys of a late positivity in the ERP that appears comparable to the human P300 component provides opportunities to further explore the neurophysiological and anatomical bases of its underlying neural processes.

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7. Criterion was achieved when monkeys successfully discriminated rare target tones from frequent nontargets better than 85 percent (number of correct responses divided by total number of responses and errors times 100) of the time of five successive training sessions.
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9. EEG (bandpass, 0.1 to 100 Hz; gain, 1000) was recorded on magnetic tape, digitized and averaged off-line, and stored on disks only when the behavior was at least 75 percent correct.
10. All three probability levels were not always tested in a given recording session.
11. The ERP's recorded from Maya were more than twice as large as those recorded from Bo at the same locations. For this reason, we normalized the data for each monkey by setting the base-to-peak amplitude of the P300 for the precentral region (Cz) at 100 percent. The amplitude of each of the other potentials was then recalculated as a percentage of P300's amplitude at Cz. The results are as follows: Cz, 100 percent (number of averages = 32); parieto-occipital region (Pz), 73 percent ($N = 37$); medial parietal region (C3), 80 percent ($N = 18$); medial parietal region (C4), 79 percent ($N = 24$); occipital region (P3), 70 percent ($N = 14$); and occipital region (P4), 61 percent ($N = 14$). The underlying cortical regions for these locations were derived from W. D. Winters, R. T. Kado, and W. R. Adey [*A Stereotaxic Brain Atlas for Macaca nemestrina* (Univ. of California Press, Berkeley, 1969)].
12. The data were amplitude-normalized for each monkey. The 0.10 and 0.50 conditions were significantly different (Duncan's multiple-range test).
13. The F ratio was 5.70 [$F(2, 19) = 5.70$, $P < 0.05$)]. The mean latencies of the 0.10 (286 msec) and the 0.30 (296 msec) conditions were significantly different from that of the 0.50 condition (323 msec) (Duncan's test).
14. E. Donchin, D. Otto, L. K. Gerbrandt, K. H. Pribram [*Electroencephalogr. Clin. Neurophysiol.* **31**, 115 (1971)] showed a P300-like wave recorded from the postcentral electrode in a nonhuman primate.
15. Supported by PHS grant MH14599-06 and NIH grant NS11876-08. We thank J. K. Manago for technical and surgical assistance and H. Michalewski, T. O'Connor, and J. Polich for comments on the manuscript.

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Opioid Peptides Mediate the Suppressive Effect of Stress on Natural Killer Cell Cytotoxicity

Abstract. *The cytotoxic activity of natural killer cells was investigated in rats subjected to one of two inescapable footshock stress paradigms, both of which induce analgesia, but only one via activation of opioid mechanisms. Splenic natural killer cell activity was suppressed by the opioid, but not the nonopioid, form of stress. This suppression was blocked by the opioid antagonist naltrexone. Similar suppression of natural killer activity was induced by high doses of morphine. These results suggest that endogenous opioid peptides mediate the suppressive effect of certain forms of stress on natural killer cell cytotoxicity.*

Exposure to stress can suppress the immune system, and it is widely held that this process renders organisms more vulnerable to certain diseases, including neoplasia (*1*). For example, stress reduces the level of circulating antibodies (*2*), delays skin allograft rejection (*3*), and suppresses the reactivity of lymphocytes to mitogenic (*4*) and antigenic (*5*) stimulation. Natural killer (NK) cells are a subpopulation of lymphocytes that spontaneously recognize and selectively kill certain tumor cells and hence seem to be particularly involved in immune surveillance against neoplastic disease (*6*). Thus, it is especially noteworthy in this context that activity of NK cells is markedly reduced in animals by stressors such as surgery, starvation, and transportation (*7*). Moreover, NK activity is suppressed in college students who cope poorly with life-change stress (*8*).

Exposure to stress can also release opioid peptides from central and peripheral sites (*9*), and opioids have recently been implicated in immune regulation. For example, in vitro studies show that morphine and opioid peptides alter the percentage of T cells forming active rosettes (*10*), the reactivity of T cells to mitogenic stimulation (*11*), and the cytotoxic activity of NK cells (*12*). Additionally, opioid receptors have been identified on various components of the immune system, for example, granulocytes, monocytes, lymphocytes, and terminal complexes of complements (*10, 13*). Thus, it may be that opioid peptides released by stress mediate some of the effects of stress on the immune system.

To test this hypothesis, we investigated the effects on NK cell cytotoxicity of two types of inescapable footshock stress: (i) applied intermittently, causes analgesia that, by several criteria, appears to be mediated by opioid peptides ("opioid stress") and (ii) applied continuously, induces equally potent analgesia not involving opioids ("nonopioid stress") (*14*). We find that the opioid, but not the nonopioid, form of stress suppresses the cytotoxic activity of NK cells and that this suppression is blocked by the opioid antagonist, naltrexone. Furthermore, this suppression is mimicked by morphine administration (*15*).

Fischer 344 (F344) female rats, 50 to 60 days old, were maintained on a 12-hour light cycle with free access to food and water. Animals were subjected to one of two footshock paradigms identical in shock intensity and total "shock on" time but differing in the temporal parameters of their application. The intermittent footshock (2.0 mA, 60-Hz sine waves, on 1 of every 5 seconds for 10 minutes) caused an opioid-mediated analgesia; the continuous footshock (2.0 mA, 60-Hz sine waves, on continuously for 2 minutes) caused an equally potent