EFFECT OF STIMULUS UNCERTAINTY ON THE PUPILLARY DILATION RESPONSE AND THE VERTEX EVOKED POTENTIAL

DAVID FRIEDMAN, GAD HAKEREM, SAMUEL SUTTON AND JOSEPH L. FLEISS

Biometrics Research, New York State Department of Mental Hygiene, 722 West 168th Street, New York, N. Y. 10032 (U.S.A.)

(Accepted for publication: October 30, 1972)

In 1965, one of us (SS) reported the existence of a long-latency positive component of the evoked potential which was of large amplitude in response to stimuli about which the subject was uncertain, and was of minimal amplitude or absent when the subject had advance information about the nature of the upcoming stimulus (Sutton et al. 1965). This P₃ component (latency of approximately 300 msec to peak) was subsequently shown by Sutton et al. (1967) to occur at the point in time at which subject's uncertainty about the stimulus was resolved.

Tueting et al. (1971) were able to demonstrate that a monotonic inverse relationship held between P₃ amplitude and the probability of event occurrence in a guessing situation. They found the strongest and clearest relationship between P₃ amplitude and a term which reflected the relative probability of being correct or incorrect when guessing a particular stimulus event. They referred to this as the outcome probability, since it reflected the joint probability of the stimulus and guessing frequencies.

Hakerem and his colleagues (Levine 1969; Levine and Hakerem 1969) had also shown that the averaged peak pupillary dilation (latency of about 1 sec to peak) followed some of the same relationships that held for P₃. We therefore undertook the simultaneous recording of evoked potential and pupillary data in order to determine if peak dilation would also follow P₃ amplitude in its relationship to probability of event occurrence. Since pupillary dilation has often been considered a sign of orienting (Sokolov 1960; Liberman 1965; Shakhnovich 1965; Lynn 1966), a finding that pupillary dilation followed the same systematic relationship with respect to outcome probability as did P₃ would aid in the interpretation of the P₃ component of the evoked potential. The expectation that the pupillary dilation response might be sensitive to probability manipulation is encouraged by the fact that other autonomic measures, the galvanic skin response (Lovibond 1969) and heart rate (Higgins 1971), have been shown to vary as a function of stimulus probability.

In addition, since P₃ and the contingent negative variation (CNV) have often been shown to coexist under certain experimental conditions (Tueting and Sutton, in press), this averaged baseline shift was also recorded in the present experiment.

METHOD

Two double clicks, which differed in the length of their interclick interval, were used as stimuli. One double click had an inter-click interval of 2 msec (S), and was referred to as the “single” click (since it was perceived as a single click), while the other had an inter-click interval of 8 msec (D), and was referred to as the “double” click. These stimuli were presented over a loudspeaker in random order except for the constraint that their relative probabilities were varied under different experimental conditions. The four conditions were: 20%S/80%D, 40%S/60%D, 60%S/40%D, 80%S/20%D. The four probability conditions were presented in sessions which were counterbalanced for order within each subject across 4

1 Now at Albert Einstein College of Medicine.
2 Also at Queens College, City University of New York.
days of running. A different $4 \times 4$ Latin Square with the dimensions being days and order of probabilities was selected at random for each of the 8 subjects. While this design does not control for carry-over effects within individual subjects, the use of different Latin Squares for different subjects controls for carry-over effects in the average across subjects. The subject was always informed of the relative frequencies of the two events at the beginning of that block of trials. Successive stimuli were separated by an interval that varied from 10 to 14 sec; interval lengths were not constant because the subject initiated the trial himself by pressing (and immediately releasing) one of two choice keys (Honeywell microswitches). Exactly 1.78 sec later, the stimulus was delivered. For averaging purposes, sampling time began with the key press and ended 4 sec later.

There were two experimental conditions, an "uncertain" and a "certain" condition. In the "uncertain" condition, the subject guessed whether the next stimulus would be single or double by pressing one of two clearly labeled choice keys.

The presentation of the stimulus confirmed or disconfirmed the subject's guess. In the "certain" condition, instead of guessing, the subject was told before each trial which stimulus would occur, and he simply pressed the appropriate key to start the trial. Prior to each uncertain probability condition, stimuli at that probability were presented under the certain condition. This was done in order to assist the subject in refamiliarizing himself with the probability in that condition. Any bias that may have been introduced is opposite to hypothesis since the prediction was that the evoked potential amplitude in the uncertain condition would be larger, i.e., opposite to habituation effects. Over the 4 days of the experiment, for each probability condition there was a total of 500 uncertain and 200 certain trials.

There were 2 min rests after each block of 25 trials and the total session lasted 50 min. Since there were four sessions per day as well as a 30 min rest between sessions, the total testing on each experimental day lasted almost 5 h.

Six males and 2 females (age range 18–24)

---

![Diagram](image)

**Fig. 1.** Averaged pupillary and evoked potential responses for the 80% probability stimulus in the uncertain and certain conditions for subject BR. Time zero on the heavy time line represents the point at which the subject pressed the choice key. Time zero on the inserted time line represents stimulus onset. Calibrations of response amplitudes are shown as vertical distances from the heavy time line.
served as subjects. Beckman biopotential skin electrodes were attached at vertex (C3) and referred to right earlobe. An electrode attached at the neck served as ground. EEG signals were amplified 10,000 times with a specially designed amplifier, with the bandpass set between 0.016 and 50 c/sec (half amplitude points). Pupillary movements were recorded with a Lowenstein-Lowenfeld electronic pupillograph. Data were recorded on 7-channel magnetic tape and electronically labeled to enable the sorting and averaging of responses according to different experimental contingencies. Averaging was accomplished with a computer of average transients (Mnemetron CAT 400A), and averages (not sums) were written out with a Moseley X-Y plotter. Since the data were not found to differ as a function of the stimulus, component amplitudes of the dilation and evoked potential responses to "single" and "double" clicks at the same probability level were combined after averaging.

Evoked potential components were measured in the averaged response from a baseline defined as the level (fitted by eye) for 160 msec prior to stimulus onset to the peak of the component. Measurement from baseline avoids the complication introduced in peak-to-peak measurement where it cannot be specified which of the peaks is altering as a result of some experimental manipulation. However, measurement from baseline does introduce a complication when positive peaks are above baseline or when negative peaks are below baseline (when the convention used is negative up and positive down). For example, when as in these data N2 was almost always below baseline, then a small baseline-to-peak measurement reflects a larger rather than a smaller N2 (Fig. 1). As an additional check, we measured all components peak-to-peak in order to obtain another criterion for the use of the terms larger and smaller.

CNV was measured as the amplitude at the same 160 msec level immediately prior to stimulus onset but referred to the average amplitude for the 160 msec period occurring shortly (180 msec) after the subject's key press which initiated the trial. (The amplifier was disabled during these first 180 msec of the trial to allow time for the calibration pulse.) It should be noted that this is not the classical CNV which begins with a stimulus (S1) but rather is a slow negative shift which in these experiments begins shortly after the key press which initiates the trial.

Unfortunately an additional channel of EEG amplification was not available for monitoring eye movements electrically. This is particularly important in relation to CNV data. However, during all trials the subject's right eye was visually monitored on the pupillometer and all trials showing eye movements or blinks were rejected. While this is not quite as satisfactory as an electrical average response which can be compared to the average CNV, research in an adjacent laboratory (Tueting and Sutton, in press) has consistently shown that under these experimental conditions, averages from eye electrodes to our ear reference are essentially flat.

---

3 Available from Stanley Molner, P.O. Box 667, Allwood Station, Clifton, New Jersey 07012.
TABLE I

Means, standard deviations (in parentheses) and number of trials in the average for evoked potential components, peak pupillary dilation (PD) and CNV across 8 subjects.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stimulus probability</th>
<th>Certain</th>
<th>Uncertain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>P₁</td>
<td>1.9</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>N₁</td>
<td>3.9</td>
<td>(3.9)</td>
<td>2.4</td>
</tr>
<tr>
<td>P₂</td>
<td>12.7</td>
<td>12.1</td>
<td>11.7</td>
</tr>
<tr>
<td>N₂</td>
<td>3.0</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td>P₃</td>
<td>5.9</td>
<td>4.9</td>
<td>4.3</td>
</tr>
<tr>
<td>CNV</td>
<td>0.7</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>PD**</td>
<td>0.014</td>
<td>0.013</td>
<td>0.012</td>
</tr>
<tr>
<td>N</td>
<td>40</td>
<td>80</td>
<td>120</td>
</tr>
</tbody>
</table>

* p < 0.05  
** p < 0.01  
*** p < 0.001

Significance tests (t tests) compare each value in the uncertain condition with the corresponding value in the certain condition. 
¹ The peak of the N₂ component was almost always below baseline, which means that a larger numerical value in the table (i.e., greater distance from the baseline) indicates that the component is actually smaller.

RESULTS

Certain–uncertain effects

Profound amplitude differences in all components measured were obtained between certain and uncertain conditions. Fig. 1 shows sample data for one subject for the 80% probability condition and demonstrates certain–uncertain differences. Summary data averaged across all 8 subjects at each stimulus probability value are shown in Fig. 2 and the means and standard deviations are presented in Table I. As can be seen at all probability values, P₃ was larger in the uncertain than in the certain condition. Peak pupillary dilation showed the same relationship at all probability values. The CNV was more negative in the uncertain condition than in the certain condition at all probability values. For the other evoked potential components, N₂ was smaller (see Method Section above for explanation of this term) in the uncertain condition at all probability values, but for other components effects of stimulus probability were less consistent across probabilities. Component P₂, like P₃, tended to be larger in the uncertain condition (statistically significant in 3 out of 4 probabilities), while N₁ and P₁ tended to be smaller in the uncertain condition (N₁ statistically significant in 2 out of 4 probabilities, P₁ statistically significant in 1 out of 4 probabilities).
TABLE II

Average slopes (b) and standard errors (in parentheses) for the regression of amplitude on probability.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stimulus probability</th>
<th>Outcome probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certain</td>
<td>Uncertain</td>
</tr>
<tr>
<td>P₁</td>
<td>-0.6</td>
<td>-1.4*</td>
</tr>
<tr>
<td></td>
<td>(0.6)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>N₁</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>P₂</td>
<td>-0.9</td>
<td>-6.2***</td>
</tr>
<tr>
<td></td>
<td>(0.8)</td>
<td>(1.6)</td>
</tr>
<tr>
<td>N₂</td>
<td>-0.6</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>(0.9)</td>
<td>(2.9)</td>
</tr>
<tr>
<td>P₃</td>
<td>-3.2*</td>
<td>-18.5***</td>
</tr>
<tr>
<td></td>
<td>(1.4)</td>
<td>(1.3)</td>
</tr>
<tr>
<td>CNV</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>(0.9)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Peak dilation</td>
<td>-1.8</td>
<td>-22.2***</td>
</tr>
<tr>
<td></td>
<td>(1.5)</td>
<td>(4.8)</td>
</tr>
</tbody>
</table>

* p < 0.05.
** p < 0.01.
*** p < 0.001.
† Not calculated since the CNV occurs prior to the event and cannot differ as a function of correctness or incorrectness of the guess.

Stimulus probability effects

As also can be seen in Fig. 2, in the uncertain condition both P₃ amplitude and pupillary dilation are largest at the lowest probability and smallest at the highest probability. For each response variable, the significance of the mean curve was assessed by estimating the slope of the regression of amplitude on stimulus probability for each subject, averaging across subjects and testing the mean slope (b) for significant departure from zero using a t ratio with 7 (n - 1) df. These values are presented in Table II (left side).

Significant negative slopes were found in the uncertain condition for both P₃ amplitude and for pupillary dilation but not for the CNV. Thus both P₃ amplitude and pupillary dilation are inverse monotonic functions of stimulus probability. In the certain condition, only P₃ shows a significant relationship to stimulus probability. In the uncertain condition, however, P₁ and P₂ also have significant negative slopes in relation to stimulus probability.

Outcome probability effects

In order to consider the effects of right and wrong guesses separately, we calculated “outcome probabilities”. The term, outcome probability, has been fully explained elsewhere (Tueting et al. 1971). Briefly it is a term which reflects the interaction of the a priori stimulus probability, which was set by the experimenter, and the subject’s guessing rate. Thus in the experimental condition in which the “single” click is presented in 100 out of 500 trials, a correct guess in 25 of those trials yields a hit probability for the “single” click of 25/500 = 0.05. The corresponding miss probability for the “single” click is 75/500 = 0.15. Tueting et al. (1971) have given two arguments for analyzing data of guessing experiments in terms of outcome probabilities.


On logical grounds, they provide separate quantitative estimates for hits and misses. On empirical grounds, only with outcome probabilities were the same functions obtained for different methods of manipulating the probability of two events.

The outcome probability data for all evoked potential components and peak pupillary dilation are shown in Table III and amplitudes for the P3 component and pupillary dilation are plotted for each of the 8 subjects in Fig. 3. It can be seen that for the hits almost all subjects show an inverse relationship between each measure and outcome probability. The data for the misses are a great deal more variable (Table III) and more attenuated in range (Fig. 3), but as can be seen in the average data across the 8 subjects (bottom row of Fig. 3), the general trend is in the same direction as for the hits.

Slope analyses were also performed for the regressions of amplitude on outcome probability (Table II, right side). For both the hits and misses, significant negative slopes were found for both P3 amplitude and pupillary dilation.

Similar analyses were undertaken for the other evoked potential components. For the hits, P2 showed a similar relationship to outcome probability as did P3, whereas N1 and N2 showed an opposite relationship to outcome probability—in other words they decreased as outcome probability decreased. For the misses, only P1 showed a significant relationship to outcome probability in the same direction as the relationship between P3 and outcome probability.
Fig. 3. O–P₃ and peak dilation amplitudes for each subject plotted as a function of outcome probability when subject was right (hits), and when subject was wrong (misses). Set of curves at bottom (labeled X) is an average for the 8 subjects.

DISCUSSION

The evoked potential data clearly replicate earlier findings (Sutton et al. 1965) that P₃ amplitude is greater in the uncertain condition than in the certain condition. In the present study, it was also shown that both the pupillary dilation to these stimuli as well as the CNV preceding these stimuli are larger in amplitude in the uncertain condition. In addition, N₂ which has previously been reported to be smaller for higher states of vigilance (Wilkinson et al. 1966) was found to be smaller in the uncertain condition than in the certain condition (p < 0.01). All of these findings taken together are consistent with Tueting's (1968) suggestion that the uncertain condition is characterized by a higher arousal level associated with greater task involvement on the part of the subject.

The major new finding is that pupillary dilation, like P₃ amplitude, is inversely and monotonically related to outcome probability in the uncertain condition. (This present study also confirms Tueting et al. (1971) in showing that there is a similar relationship for P₃ in the certain condition.) Unlike the uncertain vs. certain differences for both pupil and P₃ which can be attributed to an overall difference in state between the two tasks, the outcome probability effects on the pupil and P₃ within the uncertain condition cannot reflect a difference in prior state, since before the occurrence of the stimulus the subject does not know whether a high or a low probability stimulus will occur. Yet both P₃ and pupillary dilation am-
amplitude in response to the stimulus clearly reflect the effect of outcome probability. In consonance with the lack of differential pre-stimulus set for different probabilities, no relationship is found between the CNV and stimulus probability. This is in contrast to the uncertain vs. certain differences in the CNV which go along with, and may even determine, those $P_3$ and pupillary dilation differences.

The finding of greater $P_3$ and pupillary dilation amplitudes in the uncertain condition, and within the uncertain condition greater amplitude for less probable outcomes, lends some support to an interpretation of $P_3$ as a cerebral correlate of the orienting response (Ritter et al. 1968). But it should be noted that $P_2$ is also inversely and monotonically related to outcome probability (although only in the hits and not as strongly as $P_3$). Yet, $P_2$ has in general not been interpreted as a cerebral correlate of the orienting response.

Consideration of the large variety of experimental situations which reliably influence $P_3$ amplitude (Tueting and Sutton, in press) suggests that at best the orienting response concept with its emphasis on novelty is too narrow a rubric to subsume all the findings—unless the concept is broadened to include informational properties as has been proposed by Pribram (1967). Alternatively, it might be more parsimonious at the present time only to attempt to group the situations which result in an increase in $P_3$ under some broad term whose precise definition and physiological implications will require further research. Jenness (1970) has suggested that the term “salience” is consistent with most of the available data on $P_3$; i.e., any operation which increases the salience of the stimulus to the subject increases the amplitude of $P_3$. In the current experiment, it is easy to see that a stimulus which confirms or disconfirms a subject’s guess is more salient than a stimulus whose identity is known in advance. Similarly, when the subject has guessed that an infrequent stimulus will be presented, it is clear that a stimulus which confirms this guess will have high salience since this is a relatively improbable occurrence. On the other hand, when the subject has guessed that a frequent stimulus will be presented, a disconfirmation of this guess is a relatively improbable occurrence and will have high salience (Tueting et al. 1971).

**Summary**

Scalp recordings of slow baseline shifts (CNV) preceding auditory stimuli, as well as pupillary dilations and evoked potentials to these stimuli, were recorded and averaged in awake adult humans. The experimental situation manipulated the degree of advance knowledge by the subject as to which of two auditory stimuli would be presented next. In the certain condition the subject was told prior to each stimulus which of the two stimuli would be presented next; in the uncertain condition the subject made a guess prior to each stimulus and the occurrence of the stimulus confirmed or disconfirmed the subject’s guess. In both the certain and uncertain conditions, the relative probability of occurrence of the two stimuli was varied in different blocks of trials so that each of the two stimuli occurred at 20, 40, 60 and 80% probabilities.

Pupillary dilation was found to be larger and the CNV was found to be more negative in the uncertain condition than in the certain condition. Of the evoked potential components, $P_3$ was larger and $N_2$ was smaller in the uncertain condition. Somewhat less consistently, $P_2$ was larger and $P_1$ and $N_1$ were smaller in the uncertain condition.

$P_3$ amplitude and peak dilation amplitude decreased monotonically as a function of the rareness of event occurrence (as measured by either stimulus or outcome probability). This relationship held for $P_3$ in both certain and uncertain conditions, but only in the uncertain condition for pupillary dilation. The $P_2$ component also showed similar relationships to probability, but less strongly than did the $P_3$ component. CNV did not vary as a function of stimulus probability in either condition.

The results are consistent with the interpretation that the uncertain condition is characterized by a higher arousal level associated with greater task involvement on the part of the subject. The similarity between the pupillary dilation relationship to probability and the $P_3$ relationship to probability is consistent with an interpretation of $P_3$ as a “cerebral correlate of the orienting response”. But this would require a broadening and redefinition of the orienting response concept in “informational” terms and in any case would fit
all the available data on $P_3$ only with difficulty. A less ambitious formulation at this time would simply suggest that any operation which increases the “salience” of the stimulus to the subject tends to increase the amplitude of $P_3$.

**RESUME**

**EFFET DE L’INCERTITUDE DE STIMULATION SUR LA REPONSE DE DILATATION PUPILLAIRE ET LE POTENTIEL EVOQUE AU VERTEX**

Les enregistrements sur le scalp des déflexions lentes de la ligne de base (VCN) précédant des stimuli auditifs, de même que des dilatations pupillaires et potentiels évoqués à ces stimuli, ont été enregistrés et moyennés chez des sujets adultes éveillés. La situation expérimentale manipule le degré de connaissance anticipée du sujet concernant celui de deux stimuli auditifs qui va être présenté. Dans la condition de certitude, le sujet a été informé avant chaque stimulus, de celui des deux qui lui serait présenté; dans la condition d’incertitude, le sujet doit faire un pari avant chaque stimulus, que la survenue du stimulus confirme ou infirme. Dans les deux conditions de certitude ou d’incertitude, la probabilité relative de survenue des deux stimuli est rendue variable d’une séquence expérimentale à l’autre, de telle sorte que chacun des deux stimuli survient dans des probabilités de 20, 40, 60 et 80%.

La dilatation pupillaire s’est révélée être plus grande et la VCN être plus négative dans la condition d’incertitude que de la condition de certitude. En ce qui concerne les composantes des potentiels évoqués, $P_3$ est plus grand et $N_2$ plus petit dans la condition d’incertitude. D’une manière un peu moins constante, $P_2$ est plus grand et $P_1$ et $N_1$ plus petits dans la condition d’incertitude.

L’amplitude de $P_3$ et l’amplitude maximale de la dilatation décroissent de façon monotone en fonction de la raréfaction de la survenue d’événements (mesurée soit par la probabilité des stimuli, soit par la probabilité du résultat). Cette relation vaut pour $P_3$ dans les deux conditions de certitude et d’incertitude, mais seulement dans la condition d’incertitude pour la dilatation pupillaire. La composante $P_2$ montre également des relations similaires avec la probabilité, mais moins fortes que ne le fait la composante $P_3$. La VCN ne varie pas en fonction de la probabilité de stimulus dans les deux conditions.

Ces résultats sont conformes à l’interprétation suivant laquelle la condition d’incertitude est caractérisée par un niveau plus élevé d’éveil ainsi que par une participation plus grande des sujets à la tâche. La similarité des rapports entre la dilatation pupillaire et la probabilité et entre $P_3$ et la probabilité s’accordent avec une interprétation de $P_3$ comme “corrélats cérébraux de la réponse d’orientation”. Mais ceci nécessiterait un élargissement et une redéfinition du concept de réponse d’orientation en termes “informationnels” et en tout cas ne serait que difficilement conforme avec toutes les données connues sur $P_3$. Une formulation actuelle moins ambitieuse suggérerait simplement que toute opération qui augmente la “prégnance” du stimulus pour le sujet doit augmenter l’amplitude de $P_3$.

The authors would like to thank Ray Simon for technical assistance, and Marion Hartung for her help in the production of figures. Drs. Lloyd Gilden, Walter Ritter and Patricia Tueting read the manuscript critically and provided many helpful suggestions. This research was supported in part by a grant from General Motors and by Grant MH 14580 from the National Institute of Mental Health, U.S. Public Health Service.

**REFERENCES**


