DIFFERENT BEHAVIOR OF SOME COMPONENTS OF FLASH-EVOKED POTENTIALS IN DELAYED PERFORMANCE IN CATS

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Summary Cats were trained to press a bar for a food reward in response to a buzzer tone lasting 1 sec (CS). The animals were rewarded when they pressed the bar with a fixed delay after the onset of CS. Flashes were repeated at 1.0 Hz following CS and evoked potentials of the visual cortex to these flashes (FEPs) were recorded and analyzed. When repetitive flashes followed CS, occurrence of bar pressing was timed more accurately than when they did not. It was found in this case that while an initial deflection of FEPs was left unchanged through one trial, the following components were inhibited immediately before bar pressing and facilitated after it.

In the previous experiments (URAMOTO, 1973) cats were conditioned to perform bar pressing with the following procedure: A tone was given for 1 sec as CS, and it was followed by repetitive flashes of light at 1.0 Hz. The cats were rewarded when they pressed a bar after a fixed delay following the onset of CS. With this conditioning procedure (F-procedure) the response latency (time measured from the onset of CS to bar pressing) was found to concentrate around 1-2 sec after the end of the delay with a small variance. In contrast to this, when the same cats were trained by an identical procedure except that CS was not followed by flashes of light (NF-procedure), the response latency was significantly longer and its variance was larger than in the responses established with F-procedure.

From these findings it was suggested that the flashes of light following CS acted on the control mechanism of the internal timing process of the animals so as to trigger bar pressing accurately. Since the accurate timing behavior in this case is obtained by the visual cue, this must be mediated *via* the visual pathways involving the visual cortex.

In the experiment to be reported here, cats were prepared for recording flash-evoked potentials (FEPs) of the visual cortex and were conditioned to per-

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form bar pressings with F-procedure. The purpose of the present paper is to describe how the visual cortex is excited by the repetitive flashes when they are used by the animals as a cue to time the bar pressing accurately.

METHODS

Three cats were used (Cats 52, 61, and 62).

Conditioning. The animals were placed in an observation box ($65 \times 40 \times 45$ cm) and allowed to behave freely. One wall of the box had a window (10×15 cm) which permitted introduction of a flash of light from a conventional photic stimulator. Below this window, a bar and a food tray were provided. When the bar was pressed, a food pellet (UCS) was delivered onto the tray. Near the box a loudspeaker was placed to sound a buzzer lasting 1 sec (CS). The animals were first trained to press the bar for the food reward. Then they were taught that the bar pressing was rewarded only when it was made in response to CS. It took about 2 weeks to complete this preliminary training. After that, the animal's bar pressings were modified so as to follow the conditioning paradigm. Two procedures were employed. One procedure (NF-procedure) was that the delay period of 5 sec was interposed after the onset of CS, and the first bar pressing after the end of the delay was rewarded. Responses occurring more than 15 sec after the onset of CS were not rewarded. The other procedure (F-procedure) was the same as NF-procedure except that the flashes of light were given at 1.0 Hz for 14 sec after the offset of CS.

In each procedure the inter-trial interval was 45–60 sec. The animals received 2–4 training sessions daily, each consisting of 20 trials. The criterion of the establishment of CR was that the CR score remained above 80% in ten successive sessions.

Cats 61 and 62 were first conditioned with NF-procedure and then F-procedure. Cat 52 was conditioned in the order of F-, NF- and F-procedures. The conditioning procedure was changed from one to the other after the previously established response had been extinguished with the criterion that the CR score was below 10% in three successive sessions. The number of sessions required for the establishment was 19–35 and that for the extinction was 5–10.

Recording FEPs. For recording FEPs from the visual cortex the cats were prepared under Nembutal anesthesia (30 mg/kg, i.p.). Screw electrodes were stereotaxically fixed on the skull (A 5, L 3–4) overlying the boundary between areas 17 and 18 or area 18 (OTSUKA and HASSLER, 1962). Other screws were implanted over the frontal sinus and occipital bone as indifferent and ground electrodes, respectively. FEPs were amplified and displayed on two oscilloscopes for photography and monitoring.

Flashes and bar pressings were marked by an ink-writing recorder. The time of the first bar pressing from the onset of CS was taken as a response time

and CR score was calculated in each session.

RESULTS

As described previously (URAMOTO, 1973), the response time mean and its variance with F-procedure were smaller than those with NF-procedure. Table 1

Table 1. Mean (M) and variance (V) of conditioned response times.

Procedure		52	Cat 61	62
without	M	7.7	7.5	7.1
flashes	V	9.17	8.84	5.98
with	M	6.4	6.7	6.8
flashes	V	2.87	3.40	2.61

(In Cat 52 the data for with flashes are those obtained from the last conditioning with flashes.)

shows response time mean (M) and variance (V) obtained with F- and NF- procedures for the three cats. They are the data from the last five sessions (100 trials). It was found that Vs and Ms were consistently smaller in the conditioning with flashes (F-procedure) than without flashes (NF-procedure). A statistical analysis was made on Vs and Ms by F- and Welch-tests, respectively. The difference in Vs was highly significant ($P < 0.001$) in all the animals. The values of M in Cats 52 and 61 were significantly smaller with F-procedure than with NF-procedure ($P < 0.005$ and $P < 0.05$, respectively) while the difference in Cat 62 failed to reach the significant level of 5%. Figure 1 shows an example of distributions of response times. The distribution with flashes (top) is unimodal with a small variance as compared with that without flashes (below). Thus, it seems that the cats used in the present experiment learned to utilize the repetitive flashes as a cue to time the bar pressing accurately.

Modification of FEPs associated with learning of bar pressing and its performance

When animals were well conditioned with flashes, they were usually standing still near the food tray with attention toward the small window through which the repetitive flashes were introduced. (Note that FEPs were obtained from the conditioning with F-procedure after the cats had been previously conditioned with NF-procedure.) On hearing the click noise of a microswitch which was produced by a bar pressing, the cats seemed to alter their attention level but they remained standing still as they had been. In Cat 62, however, there was a slight behavioral difference from other cats; when the buzzer tone was introduced, this cat turned aside for 2-3 sec and watched the window again.

The major components of FEPs were two positive-negative biphasic waves. They will be called "a-wave" and "b-complex" (STERIADE, 1968). The peaks of the

Fig. 1. An example of distribution of response times. Data were obtained from Cat 61 in the final five successive sessions (100 trials) of the conditioning with (top) and without (bottom) flashes. Ordinates, number of responses. Abscissas, response time in sec.

first positivity, the first negativity, the second positivity, and the second negativity were at 17–22, 24–28, 33–40 and 45–55 msec, respectively (Figs. 2 and 3).

It was reported in cats trained to press a bar for a food reward that a large enhancement of both “a-wave” and “b-complex” was found in a trace conditioning paradigm when photic stimuli were given contingently upon correct responses (URAMOTO, 1972). An increase in amplitudes of FEPs was also found along with the present conditioning.

FEPs were superimposed for the three cats in Fig. 2. Nine to thirteen FEPs were sampled from one trial out of the first session (left row) and the last session (right row) of the conditioning for each cat. As the conditioning progressed to a certain extent FEPs were found to increase gradually as compared with those in the very initial stage. This was true for both “a-wave” and “b-complex.” After the cats had been conditioned to the extent to which the CR score was 40% or more, the amplitude of the “a-wave” was increased about 1.2–1.3 times and that of the “b-complex” about 1.3–1.5 times as compared with their amplitudes in the first session of the conditioning. Although with further progress of the conditioning this enhancement of FEPs was lessened, it was still observed (Fig. 2, FEPs in right row as compared with those in left row).

There was one more notable finding in FEPs. The difference in the ampli-

Fig. 2. Changes in amplitudes of FEPs along with the conditioning with flashes. For each cat, FEPs were superimposed in one trial out of the first session (left row) and the last session (right row) of the conditioning. In each trace, N denotes a number of sampled FEPs. In this and the following figures, positivity is indicated downward and stimulus onset by an arrow. Voltage and time calibrations, 50 μ V and 20 msec.

tude of "b-complex" between FEPs sampled immediately before and after bar pressing became manifest as the conditioning continued while such was not the case in the amplitude of "a-wave". The "b-complex" of FEPs was inhibited just before the bar pressing and facilitated after it, but the "a-wave" was not changed significantly. This is most evident in the final stage of the conditioning in which the CR score was as high as 80% or more. This is exemplified in the records of Fig. 3. These records were obtained from the three cats in the last 20 trials. Out of 20 series of FEPs, those with the same temporal relations to CS, bar pressing and reward were sampled and superimposed. The sampled FEPs were the first ones after CS (A), the ones just preceding the bar pressing (B), the ones immediately after the bar pressing (C), and the ones just before the reward (D). Taking the first FEP elicited after CS (A) as control, one can see that the "b-complex" decreased before the bar pressing (B) and increased after it (C, D). It was also noted that the "b-complex" was slightly reduced in the peak latency immediately before the bar pressing.

The data of FEPs obtained from all three cats in the final 20 trials of bar pressing were treated quantitatively by measuring amplitudes of "a-wave" and "b-complex". For "a-wave" the amplitude was measured from the first positive peak to the first negative one. For "b-complex" the amplitude from the first negative to the second positive peak was measured. The amplitudes so measured

Fig. 3. Dynamic changes in FEPs when CRs were well established. FEPs were taken from the last session of the conditioning. A, B, C, and D denote the specified time points (see text). FEP samples were collected every period and 10 FEPs were superimposed in each period except D for Cat 52 (only 7 FEPs were available). In the left side a sequence of events was indicated. For Cat 62, FEPs for A were discarded, because this cat turned aside and did not watch the flashes for 2-3 sec on hearing the buzzer tone.

were expressed as percent of the average value taken over all FEPs obtained in the last 20 trials and shown in columns in Fig. 4 for FEPs sampled from the specified time points of one trial. It is clearly seen that "b-complex" suffered a strong reduction before the bar pressing and enhancement after it. In addition, the amplitude from the second positive to the second negative peak was greatly increased. Although, as described above, "b-complex" was modified dynamically in one performance of the well-conditioned bar pressing, such was not seen with "a-wave." In this regard "a-wave" seems to be distinguished from "b-complex" in the mechanism of potential generation.

DISCUSSION

The present experiment has provided further support for the previous finding that bar pressings were more accurately timed when repetitive flashes at 1.0 Hz followed CS than when they did not (URAMOTO, 1973).

Fig. 4. Quantitative analyses of amplitudes of FEPs for all three cats in the stage of well-conditioned bar pressings. For details, see text. A statistical analysis was made by *t*-test. It was manifested that "a-wave" (open columns) was not significantly different between the four specified time points (A, B, C, and D) ($P > 0.05$). In "b-complex" shown by filled columns, differences were significant between the two time points (B and C, immediately before and after bar pressings, respectively); $P < 0.05$ for Cat 52, $P < 0.005$ for cat 61, and $P < 0.05$ for Cat 62.

It has been found in acute experiments that there were different behaviors between "a-wave" and "b-complex" when steady retinal illumination was employed to enhance the visual cortical excitability; "b-complex" was strikingly potentiated, while the amplitude of "a-wave" showed diminution or no significant change (STERIADE, 1967, 1968). It was demonstrated in the present conditioning paradigm that "b-complex" was inhibited before bar pressing and facilitated after it with a conspicuous enhancement before food-delivery, while "a-wave" was left unchanged through one trial.

Visual input is modulated by pupillary mechanism (FERNÁNDEZ-GUARDIOLA *et al.*, 1964; NAQUET *et al.*, 1960). However, amplitudes of FEPs are less dependent upon it at the level of the visual cortex than at the level of the optic tract or lateral geniculate body (NAQUET *et al.*, 1960). It was found in various sites of cat visual cortex that amplitudes of primary components of FEPs were dependent on the intensity of light flashes (KUHN, 1967). Since the cats showed no marked behavioral changes while flashes were introduced and "a-wave" is supposed to reflect the cortical arrival of visual input (STERIADE, 1968), the fact that "a-wave" was kept approximately constant in one trial may suggest that the visual input does not fluctuate much. Therefore, the variability of "b-complex" in one trial in the stage of well-conditioned bar pressing can reasonably be ascribed not to peripheral but to some intrinsic mechanism.

JASPER (1963) reported that FEPs were consistently depressed prior to the

performance of conditioned avoidance responses when a monkey was well conditioned to intermittent brief flashes of light. It was also evident that the latency of FEPs was reduced when distinct periods of arousal were produced by external stimuli (STEINBERG, 1965). Taking into account JASPER's (1963) notion, one may say that the remarkable depression of "b-complex" of FEPs before bar pressing was induced by a distinct arousal of the animals.

On the other hand, it was found in monkeys (RICCI *et al.*, 1967) and cats (KOBAYASHI and SUZUKI, 1972) that conditioned motor responses followed a large enhancement of the visual cortical evoked potentials to shocks delivered to the lateral geniculate body. However, this discrepancy may be due to the differences between the conditioning procedures and/or properties of the stimuli used.

It is of interest to note a marked augmentation of "b-complex" of FEPs after as compared with before bar pressing when the cats were well conditioned. In cats trained to press a bar for milk reinforcement, it was found that a high voltage slow burst of EEG synchronization occurred after bar pressing and during consumption of a milk reward (BUCHWALD *et al.*, 1964; CLEMENTE *et al.*, 1964). A number of investigators have focused attention on the mechanisms underlying these phenomena and assumed the participation of internal inhibitory process (BUCHWALD *et al.*, 1964; CLEMENTE *et al.*, 1964; MARCZYNSKI *et al.*, 1971; MARCZYNSKI and SHERRY, 1972; ROTH *et al.*, 1967). On the other hand, HACKETT and MARCZYNSKI (1969, 1971) made a comparison of FEPs during these EEG synchronizations with those obtained in various behavioral states such as during nonrewarded bar pressing, emotional excitement, and so on. A conspicuous enhancement was found in "b-complex" during bursts of EEG synchronization. Thus, it is probable that a marked augmentation of "b-complex" of FEPs has resulted from inhibitory mechanisms which were triggered by bar pressing in this situation.

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