Effects of shock-induced arousal on the elicitation and waveform elaboration of photically evoked afterdischarges

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The effects of an activating stimulus (unsigned tailshock) on the elicitation and waveform elaboration of photically evoked afterdischarges (PhADS) were examined. Tailshocks administered 1 sec prior to each photic stimulus presentation resulted initially in significant suppression of PhAD activity. With time PhADs could be elicited, but at attenuated levels. Hippocampal EEG rhythms simultaneously recorded during shock treatment initially exhibited a predominance of rhythmical slow-wave activity (RSA). With time, hippocampal EEG was characterized by a mixture of RSA and irregular large-amplitude slow waves. The results are discussed in terms of a proposed heuristic model relating PhAD activity to "arousal" level.

The rat visually evoked response (VER, see Figure 1), cortically recorded, is characterized by a primary positive-negative (P1-N1) wave complex followed by secondary components (P2-P3) and afterdischarge activity (P3 on). The examination of photically evoked afterdischarge (PhAD) bursting in the rat has revealed a number of important neurobiological events. For example, systematic changes in PhAD activity correlate well with different behavioral processes (Klingberg, 1970; Klingberg & Pickenhain, 1967; Pickenhain & Klingberg, 1965; Pond & Schwartzbaum, 1972; Schwartzbaum, Kreinick, & Gustafson, 1971); the effects of x-ray irradiation can be quantified in terms of changes in PhAD bursting patterns (Klingberg, 1967); alterations in PhAD elicitation and waveform correlate well with neural maturation (Klingberg & Schwartz, 1966) as well as with developmentally induced sensory changes (Heron & Anchel, 1964; Rhodes & Fleming, 1970); PhAD activity has been shown to be functionally dependent upon visual stimulation parameters that have long been known to affect visual

Figure 1. A typical averaged VER from rat occipital cortex recorded over 25 trials with five early waves labeled P₁ - P₃ followed by rhythmic afterdischarge activity. Stimulus onset was delayed 250 msec. Negativity is indicated by an upward deflection (Calibrations: 30 microV; 100 msec).

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perception and functioning (Gelbrich, Klingberg, & Rebentisch, 1972; Kohler & Klingberg, 1969; Klingberg & Pickenhain, 1966; Theil & Klingberg, 1971; Yellin & Jerison, 1973); cortical PhAD activity directly relates to dorsal lateral geniculate activity (Bigler, 1975; Kohler & Klingberg, 1969; Sumitomo & Klingberg, 1972a) as well as to other diencephalic, mesencephalic, and limbic system activity (Fleming & Bigler, 1974; Kimura, 1962; Schwartzbaum, 1975; Sumitomo & Klingberg, 1972b,c; Theil & Klingberg, 1971); the occurrence of PhAD activity has been utilized in the assessment of visual system morphology

(Creel, Dustman, & Beck, 1970, 1973; Kohler & Klingberg, 1969, 1971; Sumitomo & Klingberg, 1972a,b,c); pharmacological modulation of PhAD responding provides an excellent paradigm for the evaluation of central drug action (Bigler, 1975; Bigler & Fleming, in press; Bigler, Fleming, & Shearer, 1974; Fleming, Rhodes, Wilson, & Shearer, 1972, 1973) as well as a neuropharmacological model for experimental epilepsy (Bigler, 1975; Shearer, Fleming, Bigler, & Wilson, 1974).

The parameters affecting the elicitation of the primary and secondary components of the VER have received extensive documentation (see Beck, 1975), while those affecting PhAD elicitation have not been as extensively documented even though afterdischarge activity is a very common feature in VERs of many species including man (see Creel, Dustman, & Beck, 1973). Current research has demonstrated that PhADs are readily suppressed or not elicited during conditions that apparently alter organismic arousal levels. Thus suppression or elimination of PhAD activity occurs during the acquisition phase of instrumental avoidance responding (Pickenhain & Klingberg, 1965), behavioral reactivity (Schwartzbaum, Kreinick, & Mello, 1972), brainstem electrical stimulation (Kimura, 1962), general ambulation (Fleming & Bigler, 1974), genetically determined reactivity (Fleming, Wilson, & Shearer, 1973), habituation (Bigler & Fleming, 1974; Fleming et al., 1972; Rhodes & Fleming, 1970), pharmacologically induced arousal (Bigler & Fleming, in press; Bigler et al., 1974; Fleming et al., 1972), and sensory restriction (Rhodes & Fleming, 1970). One unexplored parameter of arousal level on PhAD suppression has been the effect of delivering an activating stimulus to the animal immediately preceding the photic pulse. Using unsignaled tailshock as such a stimulus, the present investigation examined its effects upon the elicitation and waveform elaboration of PhAD activity. Dorsal hippocampal EEG was monitored as an ancillary measure because of the existing relationships between PhAD occurrence and certain hippocampal rhythms (see Bigler & Fleming, in press; Fleming & Bigler, 1974).

METHOD

Subjects and Surgery

Five Holtzman albino rats served as subjects. All subjects were at least 100 days old prior to surgery. Each animal was anesthetized with pentobarbital sodium (40 mg/kg) and surgically prepared with chronic indwelling silver-ball electrodes placed on the dura over the right and left visual cortices (7.0 mm posterior to the bregma, 3.0 mm lateral to the midline). A stainless steel screw electrode was placed in the frontal sinus for grounding. A nichrome wire attached to the perimeter of the skull provided a reference attachment. In three subjects, a bipolar electrode consisting of two stainless steel Size 00 insect pins insulated with epoxylite except for the etched tips was stereotactically positioned in the right dorsal hippocampus (3.0 mm posterior to bregma, 2.0 mm lateral to midline, 3.5 mm

ventral from cortical surface). All electrodes were attached to female amphenol receptacles and imbedded and affixed to the skull surface with dental acrylic plastic and the wound closed. A minimum of 7 days recovery was allowed after which each subject was given at least 4 days acclimation training to the experimental environment. The location of hippocampal electrodes was histologically verified to be within the pyramidal cell layer.

Apparatus

A Grass PS22 photostimulator was positioned 42.5 cm behind and slightly above the midpoint of a flat white reflecting hemicylinder. The subject was placed in a restraining hammock which was positioned such that the subject's head region was encompassed 180 deg by the reflecting hemicylinder. This insured equal presentation of the photic stimulus irrespective of momentary changes in subject's head position. The photostimulator was programmed to deliver 10 microsec pulses every 7 sec to the reflecting surface. Photostimulator intensity was 4 on a scale of 1-16, with the illuminance of the reflecting surface being approximately 5 ftC. The entire apparatus was located within a shielded enclosure in a darkened room with background white noise. All procedures were carried out in unanesthetized animals with mydriatic pupils (1% atropine sulfate ophthalmic solution). Brain responses were amplified with Grass 5P5 preamplifiers and Model 5 polygraph driver amplifiers (bandwidth, .3 - 60 Hz), and ink-written records were obtained.

Procedure

Each subject was placed in the hammock and held under light restraint. A bipolar electrode connected to a Grass Model S9 stimulator was affixed to the tail which had been treated with electrode paste to insure electrical conductance. The subject was then given a 15-min period to dark adapt and acclimate to the restraining apparatus. Following this acclimation period, iterative photic stimulation was initiated for a total of 75 photic stimulus presentations. Commencing with the 26th photic presentation (the first 25 photic stimulus presentations constituted a preshock control period) a tailshock (1.5 mA for 100 msec) was delivered 1 sec prior to each photic pulse for the next 25 flashes (Trials 26-50). Shock was discontinued following the 50th flash with iterative photic stimulation continuing for 25 more presentation trials (these last 25 trials constituted a postshock control). Thus, a total of 75 photic stimuli were presented in the following manner: preshock control (Trials 1-25); shock treatments administered 1 sec prior to each photic flash (Trials 26-50); postshock control (Trials 51-75). The entire procedure took approximately 30 min to complete.

Data Analysis

PhAD activity was analyzed in only the right visual cortex although both cortices were simultaneously monitored. Each block of 25 responses was broken down into blocks of five consecutive responses for data analysis. Three measures of PhAD activity were taken for each trial. The first was a measure (in millimeters) of the linear excursion of PhAD bursting. This was achieved by tracing the EEG inscription of the PhAD with a map-reading wheel. The second was to measure the duration (in milliseconds) of PhAD bursting. The third was to measure the number of spindle-like waves per PhAD burst. Each measurement initiated with the N_3 wave and continued until PhAD activity could no longer be differentiated from background EEG. For all three measures of PhAD waveform activity means were calculated for each block of five responses, and analysis of variance techniques were used for statistical examination of the data.

RESULTS AND DISCUSSION

The results are summarized in Table 1 and Figure 2. The effect of introducing an activating stimulus (tailshock) prior to the presentation of a photic pulse was to significantly suppress all PhAD

Table 1
Mean ($\pm$ Standard Error of the Mean) Treatment
Effects on PhAD Parameters

Trial Block	Linear Excursion (in mm)	Duration (in msec)	Spindle-Like Waves
Control			
1-5	104.96 $\pm$ 28.95	387.96 $\pm$ 65.66	2.92 $\pm$.51
6-10	110.25 $\pm$ 16.47	477.29 $\pm$ 50.00	3.36 $\pm$.43
11-15	161.53 $\pm$ 13.10	703.93 $\pm$ 81.86	4.72 $\pm$.63
16-20	153.09 $\pm$ 20.85	578.61 $\pm$ 99.99	4.40 $\pm$.94
21-25	132.17 $\pm$ 16.51	529.28 $\pm$ 49.33	3.96 $\pm$.28
Shock			
26-30	75.35 $\pm$ 32.22	290.64 $\pm$ 114.66	2.24 $\pm$.94
31-35	63.25 $\pm$ 22.74	245.31 $\pm$ 78.99	1.76 $\pm$.65
36-40	91.35 $\pm$ 18.65	377.30 $\pm$ 59.66	2.60 $\pm$.46
41-45	72.70 $\pm$ 24.10	274.64 $\pm$ 75.99	2.24 $\pm$.49
46-50	110.12 $\pm$ 27.72	449.29 $\pm$ 122.32	3.52 $\pm$.94
Control			
51-55	173.00 $\pm$ 32.67	629.27 $\pm$ 85.66	4.88 $\pm$.74
56-60	128.65 $\pm$ 19.88	494.62 $\pm$ 45.00	3.48 $\pm$.48
61-65	134.69 $\pm$ 26.93	510.62 $\pm$ 113.66	3.52 $\pm$ 1.40
66-70	135.58 $\pm$ 13.45	551.94 $\pm$ 59.66	4.00 $\pm$.32
71-75	143.64 $\pm$ 13.45	609.27 $\pm$ 85.66	4.28 $\pm$.67

parameters measured (PhAD linear excursion, $F = 2.71$, $df = 14/56$, $p < .005$; PhAD burst duration, $F = 3.13$, $df = 14/56$, $p < .001$ waves per PhAD burst, $F = 2.24$, $df = 14/56$, $p < .025$). Based on these significant findings, Newman-Keuls tests examining all pairs of means comparisons (Winer, 1962) revealed that the most suppressive effects on all PhAD parameters occurred during shock trials 26 - 30, 31 - 35, and 41 - 45 as compared to control blocks 11 - 15, 16 - 20, 51 - 55, and 71 - 75.

PhAD parameters have been shown to be differentially affected during pharmacological arousal (Bigler & Fleming, in press) or following habituation procedures (Bigler & Fleming, 1974). However, following the induced arousal procedures of the present experiment, suppression of PhAD parameters could not be interdifferentiated; they were all equally suppressed.

Figure 3 depicts the initial effects of shock treatment on PhAD activity and hippocampal EEG rhythms. In terms of hippocampal EEG, the period prior to the first shock stimulus exhibited a relative predominance of large-amplitude irregular slow waves (LIA). However, with the initiation of the first shock stimulus, hippocampal EEG shifted to a relative predominance of rhythmical slow-wave activity (RSA or "theta") with PhAD bursting being noticeably absent. These findings complement and extend the previous work by Fleming and Bigler (1974) on the relationship between PhAD elicitation and spontaneous hippocampal RSA by demonstrating that a similar relationship exists during periods of induced or elicited hippocampal RSA and PhAD suppression.

In terms of shock modulation of PhAD waveform, inspection of Table 1 reveals that some degree of adaptation to the shock occurred. During shock treatments 46 - 50, all PhAD parameters were above

the initial control blocks (1 - 5 and most of 6 - 10 preshock control samples). Also, the differences between PhAD parameters during 46 - 50 shock treatments and 51 - 55 postshock control trials were not reliable even though PhAD parameter values reached a maximum during trials 51 - 55. Thus PhAD waveform during the latter shock treatments was stabilizing, albeit at a somewhat suppressed level. Concomitant with this stabilization of PhAD waveform during the latter shock treatments, hippocampal EEG shifted from the early predominance of RSA back to a mixture of LIA and RSA.

Bigler and Fleming (1974) have argued that if arousal can be considered on a continuum, then an optimal arousal level may exist for PhAD elicitation and waveform maximization. Conversely, changes in arousal level above (e.g., those induced by pharmacological arousal, sensory restriction, etc.) or below (e.g., those following habituation) this optimal level would be associated with PhAD suppression. That PhAD activity occurs only within an optimal

Figure 2. Effects of tailshock on PhAD activity in one subject. Each grouping is composed of five consecutive EEG inscriptions which are superimposed. The number range in the lower right-hand corner for each grouping designates which five consecutive responses were superimposed. The EEG tracing at the base of the illustration represents a typical "raw-data" evoked potential followed by PhAD bursting during control procedures. In all cases, negativity is an upward deflection.

Figure 3. A continuous sequence of left visual cortical (LVC), right visual cortical (RVC), and dorsal hippocampal (HIPP) EEG during control photic stimulation (24th and 25th photic stimulus trials) and tailshocks administered 1 sec prior to each photic stimulus (26th-28th photic stimulus trials). Negativity is an upward deflection.

arousal interval appears to be a reasonable heuristic paradigm in explaining the present results. Thus, the initial effects of the shock were to heighten arousal to a level incompatible with PhAD elaboration. With time, arousal level gradually diminished from the heightened state to a point where PhADs were elicited, although at suppressed levels. If arousal level is playing the key role in PhAD elicitation, then it may be possible that a titration type of methodology could be applied. Accordingly, this could be quantified by systematically increasing and decreasing arousal level while concomitantly recording PhAD elicitation/nonelicitation.

Although the objectives of this investigation were not directed towards testing the effects of classical conditioning on PhAD activity, the experimental design employed was similar to a form of classical conditioning (i.e., backward conditioning, Hilgard & Bower, 1966, p. 54). Since the results of this investigation indicate that PhADs undergo systematic changes during behavioral arousal per se, it seems likely that PhADs will exhibit orderly relationships with conditioning processes. Presently, PhADs have only been investigated during the development of an instrumentally conditioned avoidance response (Pickenhain & Klingberg, 1965) and during different behavioral sequences of fixed-ratio operant responding (Pond & Schwartzbaum, 1972; Schwartzbaum et al., 1972). Since PhADs are intimately related to the VER secondary components (Creel, Dustman, &

Beck, 1974; Fleming, Shearer, Creel, 1974; Schwartzbaum et al., 1971) and VER secondary components are systematically related to a variety of neural and behavioral processes (Begleiter & Platz, 1969; John, Bartlett, Shimokochi, & Kleinman, 1973; Rosenfeld & Owen, 1972) the study of the effects of a variety of classical and operant-conditioning paradigms on PhAD parameters could yield more profitable results.

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