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The electrophysiological correlates of learning in the developing kitten

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THE ELECTROPHYSIOLOGICAL CORRELATES OF LEARNING
IN
THE DEVELOPING KITTEN

A Thesis
Presented to the
Department of Psychology
and the
Faculty of the Graduate College
University of Nebraska at Omaha

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
A. Derick Dalhouse
September 1971

Accepted for the faculty of The Graduate College of the University of Nebraska at Omaha, in partial fulfillment of the requirements for the degree Master of Arts.

Graduate Committee
Name Department
William A. deGraw Biology

Chairman

2-24-72 Sgd. the author

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Abstract

The objectives of this experiment were (a) to study the development of classical conditioning in the kitten using light as the conditioned stimulus (CS), shock as the unconditioned stimulus (US), and leg flexion as the conditioned response; (b) to investigate physiological correlates of learning during the development of classical conditioning such as visual evoked responses (VER), visual following (VF), electrocardiogram (EKG), and galvanic skin response (GSR); and (c) to ascertain the relationship, during development, between physiological and behavioral changes associated with conditioning.

Four kittens were exposed to two classical conditioning paradigms. Two Ss received a continuous 10 sec. light paired from the 9th to the 10th sec. with shock, and two received 5 light flashes 2 secs. apart, the last being paired with shock. Four control Ss received the same number of photic stimuli and shocks as their experimental counterparts but on no occasion was light and shock paired. GSR, EKG, VERS and leg-flexion responses were recorded from all 8 animals during a 24 day experimental period.

No obvious evidence was found for learning except for suggestive changes in GSR responses. However, it was observed that the development of the short-latency positive and negative N_1 components of the Ss VERs appeared later than normal or not at all. On the other hand, the long-latency N_2 wave demonstrated precociousness showing larger amplitudes and in one of the two paradigms, shorter latencies than normal.

The dominant waveform observed throughout the development of the VER in the kittens used in this study was the long-latency negative N_2 wave followed by a huge positive after-swing. The typical "W"-wave usually observed in the adult cat's VER was over-shadowed by the positive-negative complex and in many cases never appeared.

A comparison of four VERs obtained from the kittens in paradigm II (intermittent light) revealed that they were able to give comparable or nearly comparable VERs at 10 days of age to light flashed 2 seconds apart. This suggests that when shocked, the kittens may develop this ability sometime prior to 10 days postnatally.

The average latencies of the N_2 waves for the kittens used in paradigm I were found to be shorter than that observed in unshocked kittens (Rose, 1971). However, these latencies showed the normal trend of decreasing with age as was observed by Rose.

Table of Contents

List of Figures

List of Tables

Introduction

The Pavlovian procedure for studying learning has been adopted by many researchers and the learning literature is replete with classical conditioning studies. However, it was not until recently that studies on infants of various species, including man, have appeared. At first, these studies seemed contradictory, reporting different ages for the onset of conditioning in a single species, as well as in different species. This apparent confusion was clarified when further investigation revealed that the different sensory systems matured at different rates in a single species, as well as in different species (Bykov, 1960; Eugen, Lipsitt, & Kaye, 1963; Ivanitskii, 1958a, 1958b; Klyavina, Kobakova, & Stelmakh, 1958; Maiorov, 1929; Nikitiana, 1954; Obraztsova, 1955; Panchenkova, 1965; Sadlecek, 1963; Stanley, Cornwell, Paggian, & Trattner, 1963; Tuge, Shima, & Koga, 1957; Veronin, 1948; Volokhov, 1956).

Probably because of Pavlovian tradition, the dog is the most frequently reported on species in the classical conditioning literature. Yet, it was not until 1950 that the first reported attempts were made to obtain a conditioned flexion response in young puppies (Fuller, Easler, & Banks, 1950). These researchers reported that when they paired olfactory, auditory, or visual stimuli (CS) with electric shock to the foreleg (US), they were able to develop conditioned body movements beginning at days 18 to 21 postnatally. Two years later, James and Cannon (1952) reported that when they used a bell as the CS and shock to the foreleg as the US they were not able to get a conditioned orienting response in young puppies before day 21, conditioned body movements before day 22 and a specific foreleg response before day 30. Still later, Volokhov (1956) presented a table showing that he was able to get conditioned food responses to olfactory CS at day 1. In 1961, Cornwell and Fuller reported that when they used a tactile CS on newborn puppies they were able to develop conditioned withdrawal responses prior to day 21, achieving 50% criterion level by day 15 and 90% by day 19. Finally, in 1963, Stanley _et al._ reported developing a conditioned avoidance response to quinine-flavored milk in neonatal puppies, using a 5 sec insertion of a manometer nipple in the _S_'s mouth as CS and a dropper nipple, plus quinine-flavored milk, in the mouth as US.

Though not as voluminous as that on infant puppies, there are a number of conditioning studies on infants of other species. Marquis (1931) reported developing conditioned sucking in human infants at day 1 to an auditory CS. However, Kasatkin and Levikova (1935) reported not being able to develop a conditioned response in human infants to photic stimuli before the second month of life. Veronin (1948) reported developing conditioned responses in neonatal monkeys. Klyavina and Obrastsova (1959) reported obtaining defensive conditioned reflexes in neonatal.goats. Ivanitskii (1958a, 1958b) reported obtaining conditioned food reflexes on day 1 in infant rabbits to olfactory, sound, light, thermal, and tactile stimuli. Panchenkova (1956, 1962) obtained conditioned reflexes in neonatal rats and guinea pigs.

There are no studies relating EEG and behavioral indices of classical conditioning developmentally, but there are studies showing that adult brain oscillations can be modified by classical conditioning.

Pioneer work in the area was undertaken in the U.S.S.R. by Livanov and Kogan in the early 1930's (Morrell, 1961) and in the West by Durup and Fessard (1935). They reported being able to modify brain oscillations with Pavlovian classical conditioning. No more research was reported in the area until 1952 when Artemyev and Bezladnova reported observing remarkable increases in the amplitude and duration of evoked responses to photic and auditory stimulti when they were paired with an aversive or non-aversive USS(electric shock and food). This study apparently revitalized the area as shortly thereafter a number of studies reported on changes in brain electrical activity during conditioning. Morrell and Jasper (1956) reported observing that when intermittent photic stimuli were paired with a tone, the tone was eventually able to induce repetitive responses in the visual cortex similar to that induced by the photic stimuli. A year later, Yoshii, Pruvot, and Gastaut (1957) reported observing that these conditioned repetitive discharges were earlier in onset, higher in amplitude, and more constant in subcortical structures, especially the mesencephalic reticular formation. Several other researchers have reported similar findings (Galambos, 1959; Hernández-Péon, Scherrer, & Jouvet, 1956; Hernández-Péon, Scherrer, & Velasco, 1955; Morrell, 1958, 1959, 1961; Morrell, Barlow, & Brazier, 1960; Rusinov & Rabinovich, 1958; Worden, 1959; and others).

In the adult unanesthetized cat, the visual evoked response (VER) usually observed is a "W"-shaped wave (Marty, 1962; Rose & Ellingson, 1970). This "W"-wave is the end product of a number of VER components which appear at different stages in the VER development and finally combine or disappear leaving the typical adult wave. The first component of the wave is a positive deflection usually labeled P_1. The next is a negative inflection usually labeled N_1. Then there is another positive component P_2 and another negative component N_2. In the ideal animal, all four components of the "W"-shaped wave are easily identified, but in some animals, the "W"-shape may not be as obvious.

In the present study, changes in the charactertistics of the VER (CS) in developing kittens were the major physiological dependent variables. Rose and Ellingson (1970), Rose and Lindsley (1962, 1965, 1966, 1968), Marty (1962), and others have provided a wealth of information on the development of VERs in the unanesthetized and anesthetized cat. In the chronically implanted unanesthetized kitten, there is a single long-latency negative VER which appears between 2 and 4 days postnatally. By days 5 to 8, a short-latency negative component appears, yielding a double negative VER complex. A positive wave of still shorter latency appears between days 9 to 14, giving a positive-negative-negative complex; and by day 30, a positive-negative-positive-negative "W"-shaped wave, typical of the unanesthetized adult, is observable (see Fig. 3, p. 15). Rose and Lindsley (1966, 1968) and Marty (1962) reported that the early appearing long-latency negative VER can be recorded in the primary visual areas, as well as non-visual areas such as the ectosylvian gyrus and the posterior sigmoid gyrus; whereas, the shorter latency positive-negative complex is confined to the suprasylvian gyri encompassing visual area I and a part of visual area II. They subsequently labeled the long-latency negative response an "indirect visual response" (Rose &

Lindsley, 1965, 1968) and the shorter latency positive-negative component a "visual specific response" (Rose & Lindsley, 1968). Rose and Lindsley (1965, 1968) performed studies involving subcortical lesions which led them to postulate that the long-latency negative (N_2) component is dependent on indirect pathways which probably includes the brachium of the superior colliculus and, possibly, the reticular formation via the tectal and pretectal pathways. The short-latency positive-negative complex, on the other hand, follows the more direct visual pathways via the lateral geniculate.

If the Rose-Lindsley hypotheses are correct, it is conceivable that an attempt to condition the VER in infant kittens may result in a change in the development of the N_2 component as it is implicated as the attentional or arousal component of the VER. Such a change may be in the latency or the amplitude of the N_2 component. On the other hand, one would not expect classical conditioning per se to have much effect on the latency or amplitude of the short-latency VER components which are believed to use the more direct visual pathways.

There are only few reports in the literature on visual following and none of these deal with the development of classically conditioned visual following. From the works of John (1961), John and Killam (1959, 1960), Grastyan, Lissak, Madarasz, and Donhoffer (1959), Majkowski (1958), Morrell et al. (1960), Verzilova (1958), Yoshii et al. (1957), and Zukerman (1959) on the conditioning of frequency-specific potentials in adult animals, it is known that adults of many species can be conditioned to give 4 cps and 10 cps VERs when electric shock of similar frequencies is used as US. There are no reports on what to expect from infant animals.

Under non-reinforced conditions, Hunt and Goldring (1951) observed that as many as 3 to 5 minutes were required between flashes in order to obtain comparable VERs over numerous stimulus presentations in 7- to 10-day-old rabbits. Rose and Ellingson (1970) reported that at least 20 sec intervals were required between photic stimuli in order to produce comparable evoked responses in 0 to 4-day-old kittens. The present study was designed to investigate whether or not the pairing of repetitive light flashes with electric shock would affect the infant kitten's ability to produce comparable VERs.

Specific Aims

The specific aims of this study were the following: first, to investigate the conditionability of infant kittens using photic stimuli as CS, shock as US, and leg flexion as UR; second, to investigate relative amplitude, latency, and waveform changes of VERs in the primary visual areas; third, to investigate changes in EKG frequency to the CS onset, fourth, to investigate whether GSR changes become time-locked to the CS onset or the US onset; and fifth, to investigate what relationship exists between physiological and behavioral changes during the conditioning procedure.

Methods

Subjects

Ss were eight kittens obtained from mother cats reared and bred in the Laboratories of Developmental Psychobiology, Nebraska Psychiatric Institute, Omaha, Nebraska.

Procedure

The eight kittens were implanted with cortical electrodes between 3 and 4 days postnatally. The implantation of electrodes and attachment of the connector sockets to the skull were executed under Nembutal anesthesia, as previously described by Rose (1966). Small stainless steel jewelers's screws were placed over the visual cortex 8 mm anterior to lambda and 5 mm lateral and to the right of the midline. Another was placed over the association area 13 mm anterior to lambda and 4 mm lateral to the midline. Reference electrodes were embedded in the neck muscles. These measurements were worked out in preliminary studies. EKG and GSR were not recorded via chronic electrodes but via leads attached to the forelimbs with collodion (described below) prior to the beginning of recording sessions and removed thereafter with acetone.

Ss were given 2 or 3 days recovery and the first habituation run began on day 6 for all animals. All Ss received three days habituation (days 6-9) to the restraints (which will be described), the number, duration and type of photic stimuli to be used in their respective paradigms, and the experimental chamber. No shock was administered during the habituation sessions.

During the recording sessions, Ss were restrained gently by wrapping them in a towel. Slits in the underside of the towel permitted the exposure of both front legs and the left rear leg to which EKG, GSR, and shocking electrodes were attached. They were then placed in a hammock suspended from the ceiling of a lightproof, electrically shielded box and oriented toward the light source projected through an optical system housed in an adjacent box. The stroboscopic light source (a Grass PS-1 Photostimulator) was placed 1.2 meters from the Ss, a distance from which the click of the gas discharge tube could not be discerned by the human ear, nor could electrical responses be detected over the auditory or other regions when the stimulator was activated and the light was blocked. The light beam was collimated so as to completely flood both eyes with light. Flash intensity was kept constant at setting #8, the next to the highest setting, and all Ss were dark adapted for twenty minutes before the commencement of daily sessions.

On day 6, prior to the first habituation run, Ss were assigned randomly to Paradigm I which consisted of a 10 sec continuous light, paired from the 9th to the 10th sec with an electric shock to the left rear leg, or to Paradigm II which consisted of five light flashes 2 sec apart, the 5th being paired with a shock to the left rear leg. All Ss received 22 trials daily with randomly presented inter-trial intervals of 4, 5½ and 7 sec.

On day 10, prior to the first experimental session, Ss were tested for their threshold to electric shock and the current adjusted above threshold so as to give a clear response. They were then randomly assigned to experimental and control groups. Each pair of experimental and control animals were implanted on the same day. The experimental

Ss received the treatment specified for their respective groups above, and the controls received the same number of light flashes and shocks as their experimental counterparts, but the light was never paired with the shock. Daily experimental sessions were conducted from day 10 to day 34 postnatally.

Materials

The shock was dispensed by a Beede Model 250 constant current generator with Ag-AgCl electrodes fixed to the S's left rear leg with collodion and filled with EKG Sol. The constant current generator was triggered by the Grass S8 Stimulator which controlled the Grass PS-1 Photo Stimulator. Leg flexion was recorded via a Grass Force Displacement Transducer FT 03C plugged into a Grass D-C Amplifier. The evoked responses were recorded via leads plugged into the chronic surface electrodes sitting on the S's head (Rose, 1966) and feeding into a Grass Model 78 Polygraph by way of a Grass High Impedance Electrode Board, Model Thiebn 24. EKG and GSR were recorded via Ag-AgCl electrodes fixed to the S's front foot pads, as described above, and feeding into the polygraph via the high impedance board. All data were recorded and stored on magnetic tape by a Precision Instrument Model 6204 tape recorder to be subsequently analyzed by a Digital Equipment Corporation Model PDP 12A computer.

Hypotheses

Two hypotheses were formulated for this study. The first predicted that the short-latency VER components would follow the normal developmental sequence but that the long-latency N_2 component would change if it was at all influenced by the reticular formation. This change may be in the latency and/or the amplitude of the N_2 component and would result from the conditioning procedure demanding greater attention or being a source of arousal for the Ss. The second predicted that the pairing of shock with the intermittent photic stimuli, two seconds apart, would lead to 10-day-old kittens giving comparable VERs. It was further hoped that an examination could be made of the relationship between the gross behavioral and physiological indices of development during conditioning.

Results

The 24-day experimental session was divided into 6 successive sets of 4 days and the GSR, leg flexion, and EKG responses for each S were summed over these periods. Four separate 2 (groups) x 2 (paradigms) x 6 (trial blocks) repeated measures analysis of variance were completed on each of the dependent variables.

In analyzing VERs, the computer averaged sixteen 500 msec epochs of the 22 VERs obtained daily from each S. This was accomplished by instructing the computer to begin each 500 msec spoch at the onset of the CS. Thus, 24 averaged VERs for each S were obtained, all time-locked to the onset of the CS (there were 24 experimental days). Some editing of the data had to be done to avoid averaging gross head and body movements.

GSR Findings

The GSR responses were of two kinds: those occurring 1.5 to 3 sec after the CS onset and those occurring 0 to 5 sec before the US onset. The former was labeled GSR_1 and the latter, GSR_2.

The GSR_1 data yielded statistically significant main effects for trial blocks F = 2.282, df = 5/20, $p < .05$ and statistically significant paradigm x trial blocks interaction F = 2.930, df = 5/20, $p < .05$. Main effects for groups and groups x trial blocks interaction were not significant, however (F = 5.527, df = 1/4, $p > .05$; F = 0.850, df = 5/20, $p > .05$ respectively).

The GSR_2 data also yielded statistically significant main effects for trial blocks F = 8.843, df = 5/20, $p < .01$. However, main effects for groups, groups x trial blocks and paradigm x trial blocks interaction yielded no statistical significance ($F = 4.770$, $df = 1/4$, $p > .05$; $F = 1.727$, $df = 5/20$, $p > .05$ and $F = 0.523$, $df = 5/20$, $p > .05$ respectively).

Figs. 1 and 2 show that in GSR_1 and GSR_2 the experimental and control animals did respond differentially as a function of trial blocks. *Post hoc* Wilcoxon Rank Sum Tests revealed that in Fig. 1 (GSR_1) the experimental animals were indeed responding at the .01 level higher than the control animals at trial blocks 5 and 6. Similarly in Fig. 2 (GSR_2), the experimental animals were also responding at the .05 level higher than the control animals at trial blocks 2 and 3 and at the .025 level by trial block 5.

EKG Findings

The EKG frequencies were measured prior to and during the CS presentation. Like the GSR_2 data, the EKG data yielded statistically significant main effects for trial blocks $F = 2.198$, $df = 5/20$, $p < .05$ but no statistically significant F-values for groups main effects, groups x trial blocks, or paradigms x trial blocks interaction ($F = 0.144$, $df = 1/4$, $p > .05$; $F = 0.789$, $df = 5/20$, $p > .05$; $F = 1.410$, $df = 5/20$, $p > .05$ respectively).

Behavioral Findings

Only leg flexion responses occurring between the onset of the CS and the onset of the US were counted. The leg flexion data yielded no statistically significant F-values (main effects for groups $F = 1.02$, $df = 1/4$, $p > .05$; main effects for trial blocks $F = 1.361$, $df = 5/20$, $p > .05$, paradigm x trial blocks interaction $F = 0.995$, $df = 5/20$, $p > .05$).

From day 2 to day 24 of the experimental session, increasing crying and struggling was observed in all *S*s when they entered the experimental

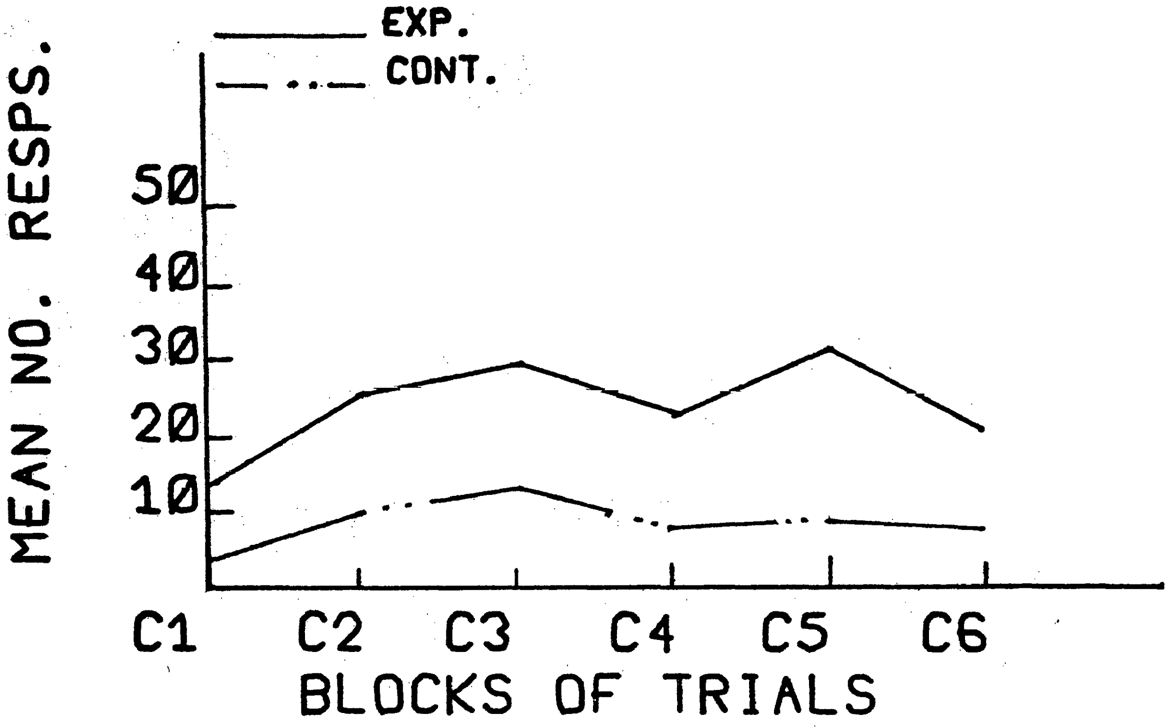

Figure 1. GSR responses occurring 1.5 to 3 seconds after the onset of the CS (GSR_1)

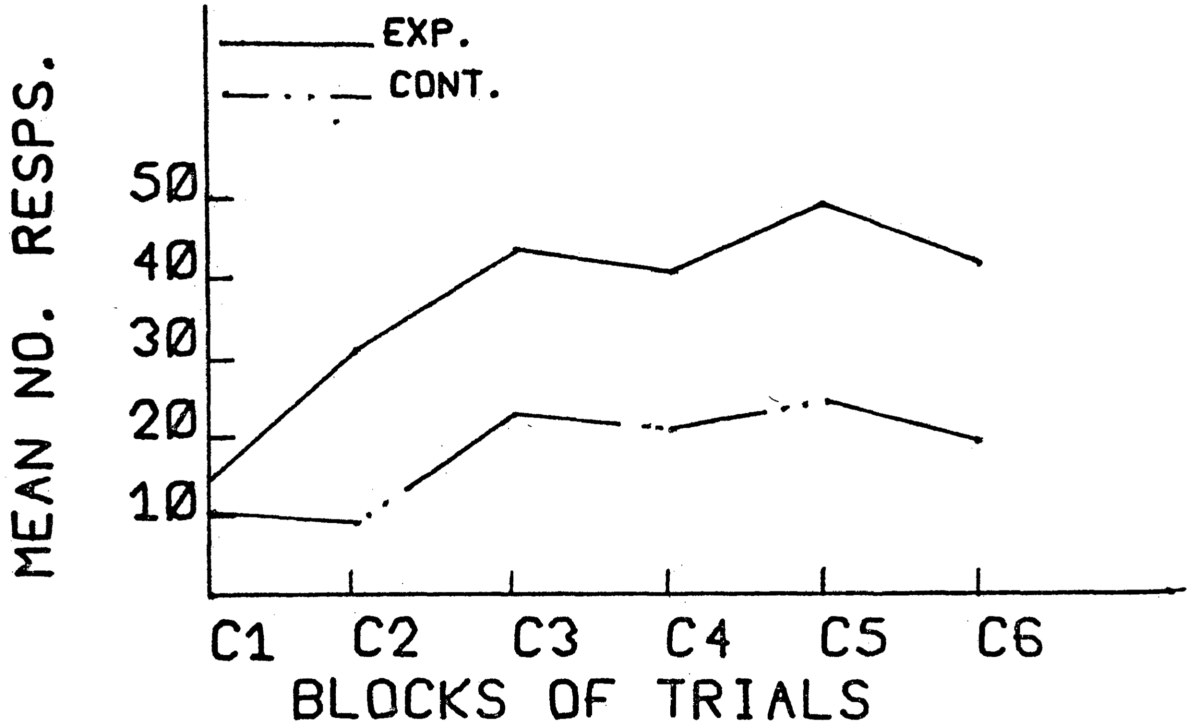

Figure 2. GSR responses occurring 0 to 5 seconds prior to the onset of the US (GSR_2)

room while they were being prepared for their daily experimental run. When the E went to their cages, both experimental and control animals came to the door of their cages and purred if stroked or picked up. However, as soon as they entered the experimental room, they began crying. This crying was more obvious in the control animals than in the experimental group. While the GSR and EKG electrodes were being attached to the Ss' foot pads, increasing perspiration was observed from day to day. This was again more evident in the control animals. On day 20, one control animal, M 267 Br/W, defecated while being placed into the experimental apparatus.

During the training sessions, the experimental animals were observed to cry while being placed in the restraints and after receiving shock. Thereafter, all crying ceased as soon as the light appeared. Some of the control animals cried throughout the training sessions on some days while others cried for a while and then stopped. In no case, however, did the onset of the light result in a cessation of crying as with the experimental animals. At the end of the experimental session, both experimental and control animals demonstrated anxiety to get out of the restraints and in many cases purred when they did.

Visual Evoked Response (VER) Findings

Fig. 3 is a reproduction of the developmental sequence of VERs in kittens (Rose, 1971). Fig. 3A shows the VERs obtained from unanesthetized kittens with implanted cortical electrodes (similar to the present study). Fig. 3B shows the VERs obtained from unanesthetized but Flaxedilized kittens. This latter figure is believed to give a truer

Figure 3

A. Computer-averaged visually evoked potentials from awake unanesthetized kittens with implanted cortical electrodes. Stimulation (binocular) occurs at onset of trace. Monopolar recordings; neck reference. Numbers indicate age in days; letters indicate recordings from same animal (longitudinal series). Traces are algebraic summations of 25 successive responses to single flashes.

B. Computer-averaged visually evoked potentials from contralateral cortex of unanesthetized (Flaxedilized) kittens at ages indicated. Focused monocular stimulation occurs at onset of trace. Monopolar recordings; neck reference (with ear reference check). Traces are algebraic summations of 25 responses.

(Taken from chapter written by Rose in Brain Development and Behavior, 1971).

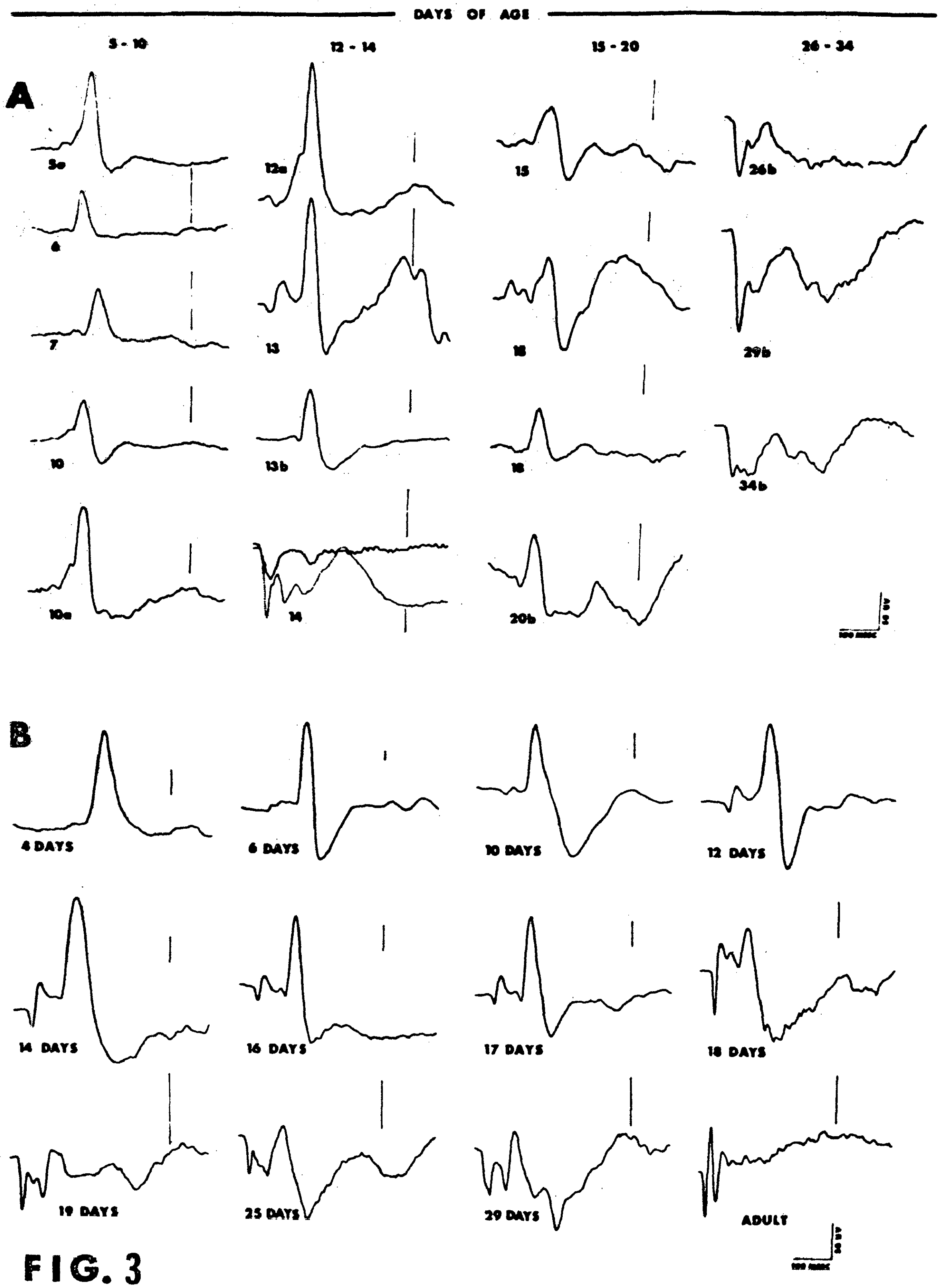
DAYS OF AGE
5 - 10
12 - 14
15 - 20
26 - 34
A
5a
12a
15
26b
6
7
13
18
29b
10
13b
18
34b
10a
14
20b
50 uV
100 msec
B
4 DAYS
6 DAYS
10 DAYS
12 DAYS
14 DAYS
16 DAYS
17 DAYS
18 DAYS
19 DAYS
25 DAYS
29 DAYS
ADULT
50 uV
100 msec

FIG. 3

picture of the VER development as light focusing could be maximized and the situation remained more constant. Figs. 4,5,6, and 7 are the VERs obtained from the kittens used in this study.

A comparison of the figures reveals that the long-latency negative (N_2) wave dominated the VERs obtained from all the animals used in this study. In addition, the N_2 wave is followed by a very pronounced positive deflection forming a negative-positive complex which dominated the waveform up to and during the period that the typical "W"-wave observed in the adult normally appears.

The short-latency negative (N_1) wave, which usually appears around 5 to 8 days postnatally, is not really obvious until around day 17 in most of these animals. Similarly, the short-latency positive wave which is normally observed around 9 to 14 days postnatally is either very immature or does not appear at all. In general, the increasing complexity in the early components of the waveform accompanying increasing age that was observed in Rose's animals is absent in these animals.

Table I compares the average latencies and standard deviations (S.D.) for the N_2 wave in kittens at different ages (Rose, 1971) with that obtained from the Ss in this study receiving paradigm I (continuous light) and paradigm II (intermittent light). Here the average latencies obtained from the animals receiving paradigm I are shorter than those obtained by Rose and the S.D. are larger. For those Ss receiving paradigm II, the average latencies are longer and the S.D. are smaller than Rose's group with the single exception of those obtained from the first age group. A one-way analysis of variance was completed on

Figures 4, 5, 6, and 7

Computer-averaged visually evoked potentials from awake unanesthetized kittens with implanted cortical electrodes. Stimulation (binocular) occurs at onset of trace. Monopolar recordings; neck reference. Numbers indicate age in days. Traces are algebraic summations of 16 successive responses to single flashes. Paradigm I, M 271 Br 1 (Exp), M 271 G 1 (Cont), M 268 Blac (Exp), M 267 Br/W (Cont). Paradigm II, M 266 Br 1 (Exp), M 264 B/W 1 (Contd), M 264 B/W 3 (Exp), M 266 Blac/B 1 (Cont).

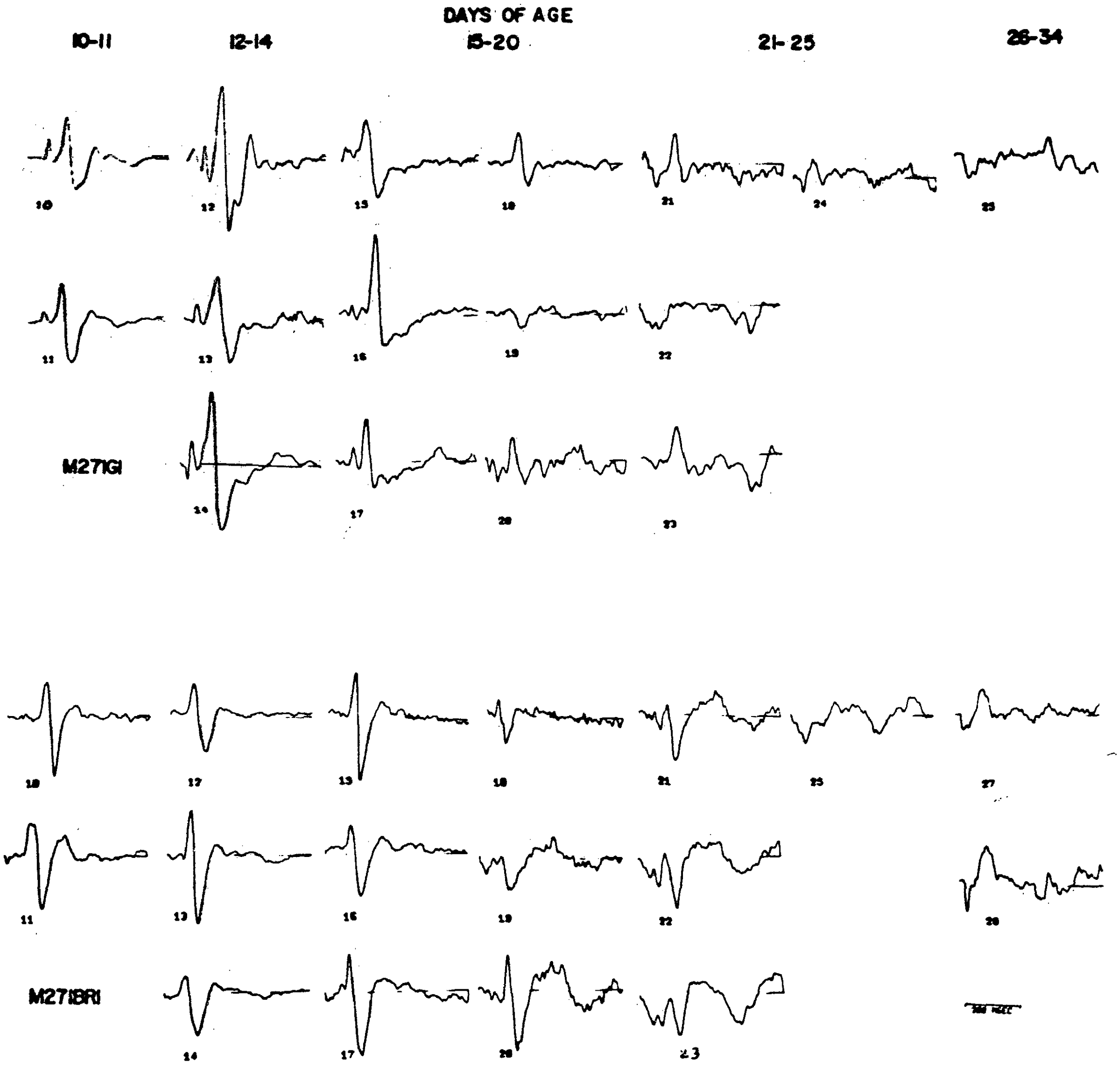
DAYS OF AGE
10-11
12-14
15-20
21-25
26-34
10
12
15
18
21
24
26
11
13
16
19
22
M271GI
14
17
20
23
10
12
15
18
21
25
27
11
13
16
19
22
28
M271BRI
14
17
20
23

FIG. 4

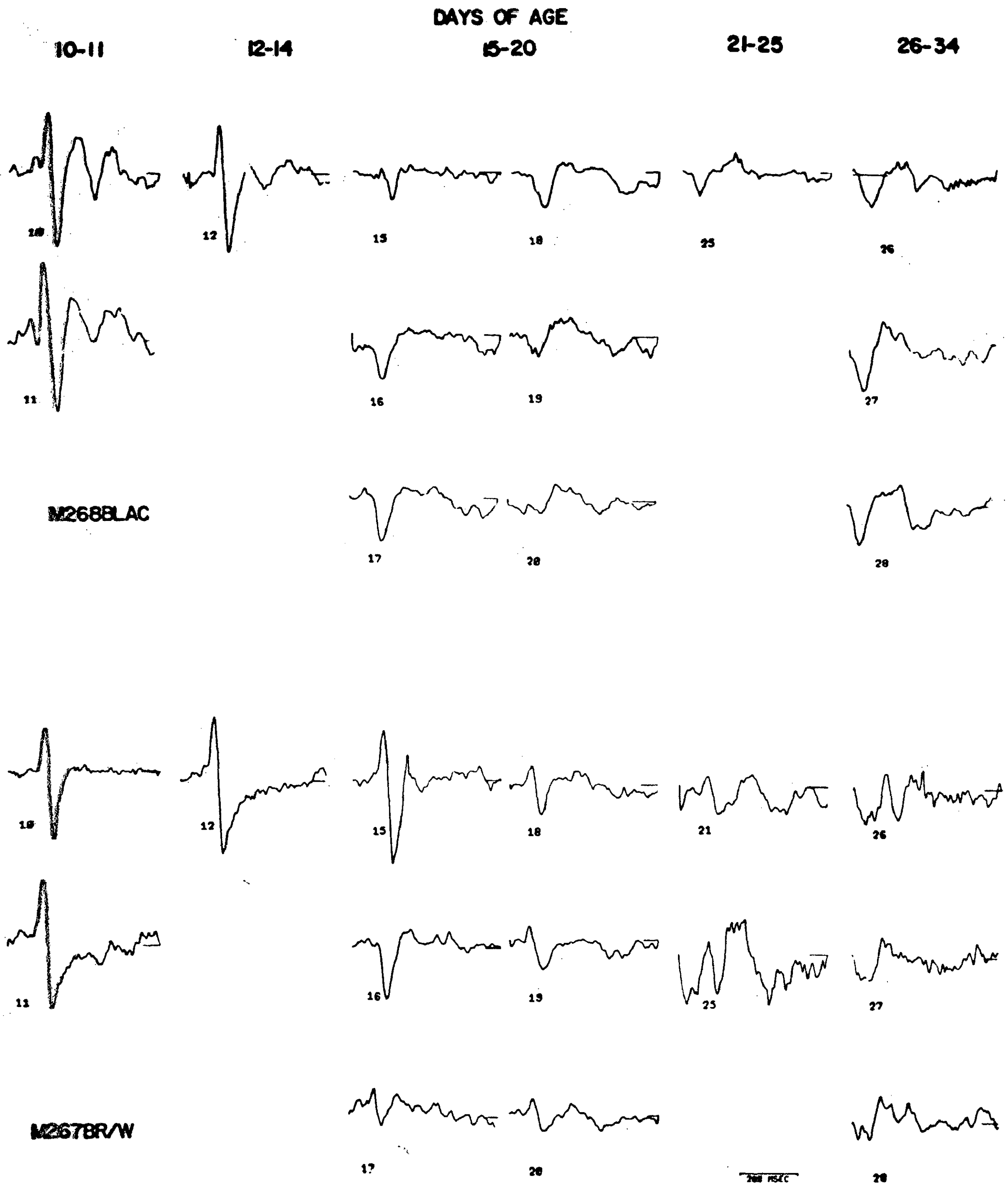
DAYS OF AGE
10-11
12-14
15-20
21-25
26-34
10
12
15
18
25
26
11
16
19
27
M268BLAC
17
20
28
10
12
15
18
21
26
11
16
19
25
27
M267BR/W
17
20
200 MSEC
28

FIG. 5

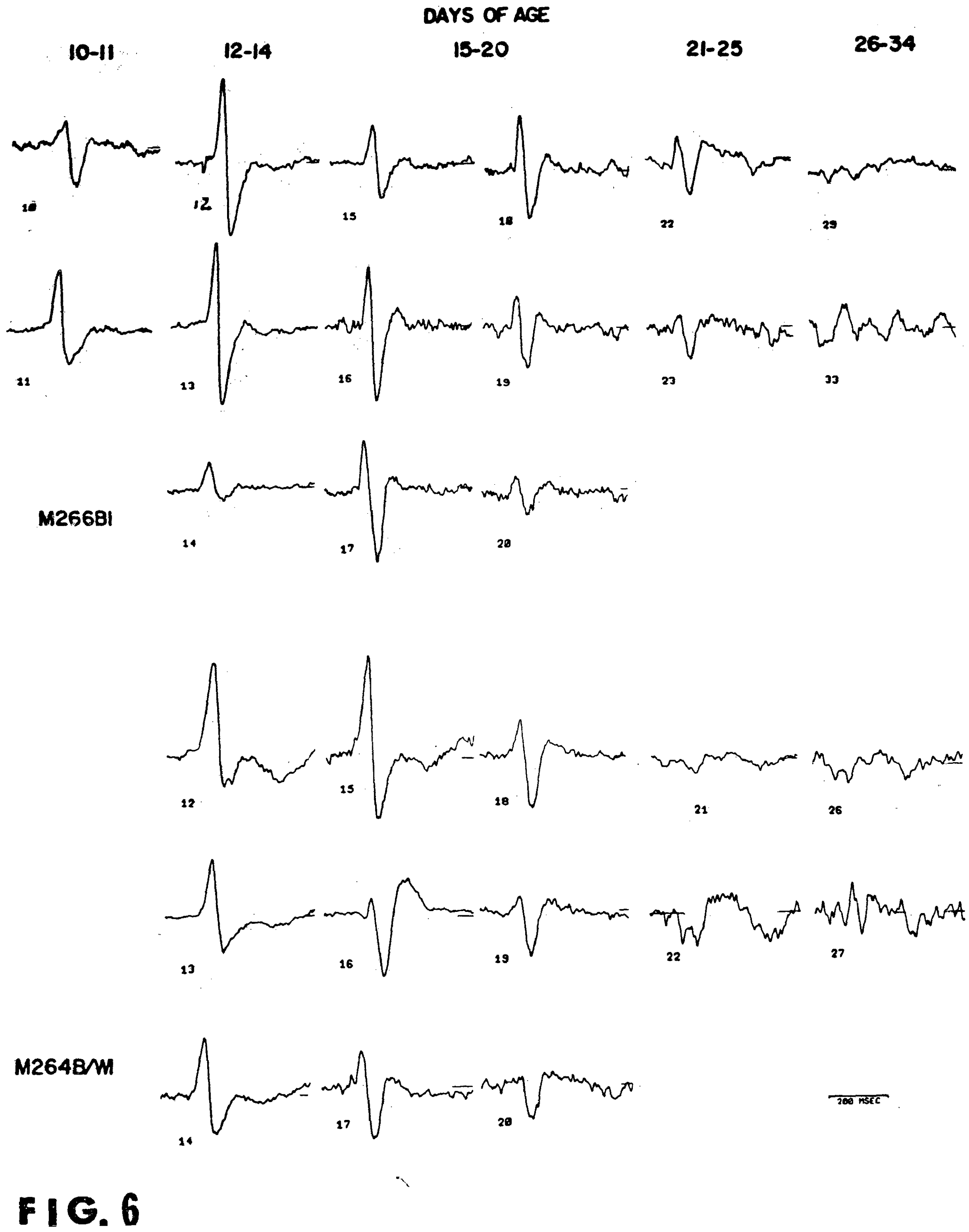
DAYS OF AGE
10-11
12-14
15-20
21-25
26-34
10
12
15
18
22
29
11
13
16
19
23
33
14
17
20
M26681
12
15
18
21
26
13
16
19
22
27
M2648/WI
14
17
20
200 MSEC

FIG. 6

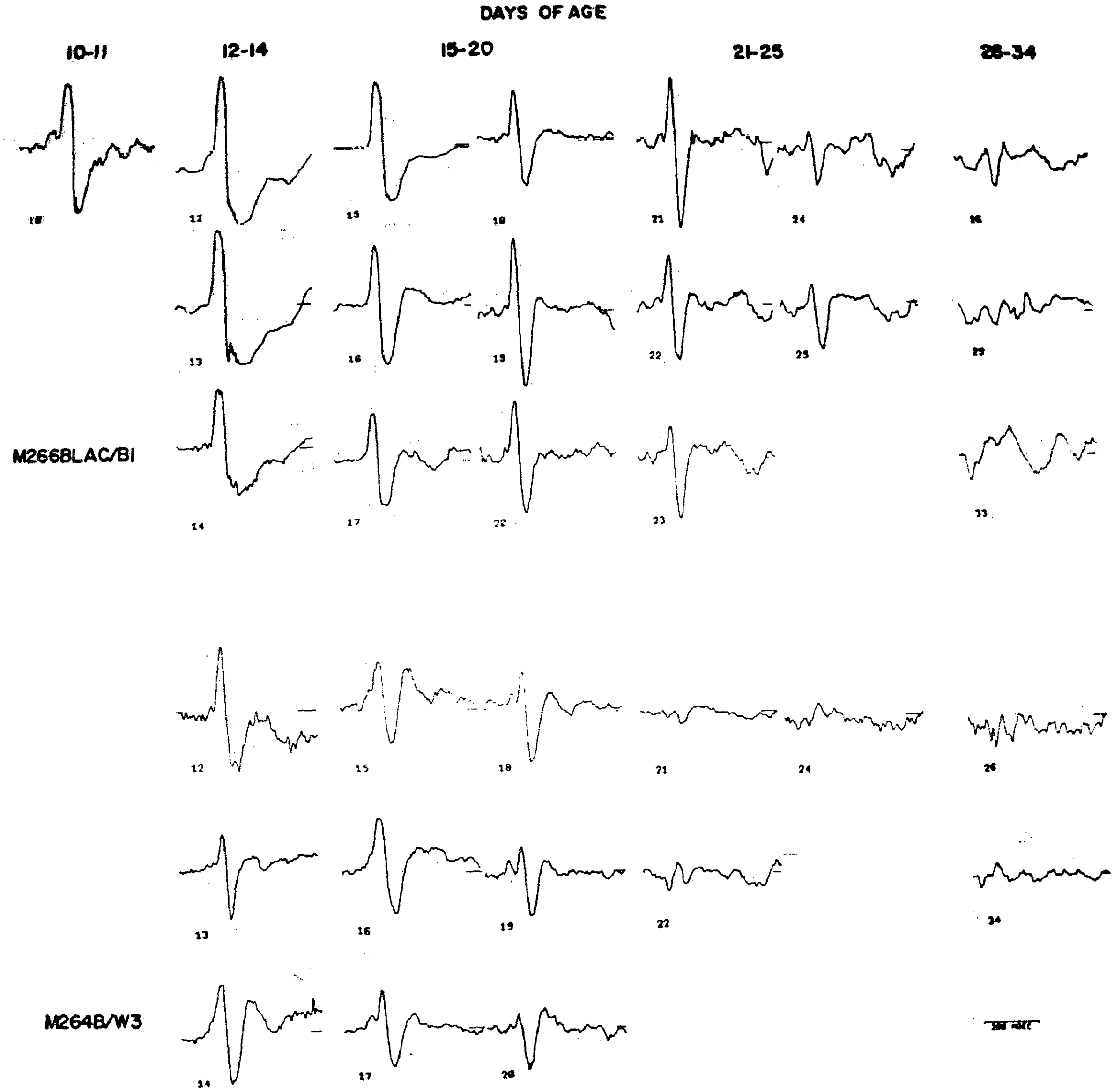
DAYS OF AGE
10-11
12-14
15-20
21-25
28-34
10
12
15
18
21
24
28
13
16
19
22
25
29
M266BLAC/BI
14
17
20
23
33
12
15
18
21
24
28
13
16
19
22
34
M264B/W3
14
17
20

FIG. 7

Table I

Comparison of Peak Delays (msec) of Long-Latency Negative (N_2) VERs Found by Dalhouse With Those Found by Rose (1971)

Rose

Age Range in days	Group N	$\bar{X}$	S.D.
9-12	6	127	7.4
13-15	6	132	13.6
18-21	6	118	10.6
24-30	4	110	16.3

Paradigm I

Age Range in days	Group N	$\bar{X}$	S.D.
10-12	4	114	16.7
13-15	4	93	12.7
18-21	4	84	14.4
24-30	4	89	18.0

Paradigm II

Age Range in days	Group N	$\bar{X}$	S.D.
10-12	4	164	10.2
13-15	4	145	7.7
18-21	4	123	6.7
24-30	4	116	14.3

the latency data obtained from Ss receiving paradigm I and the data obtained from Ss recieving paradigm II. This yielded statistical significance $F = 43.57$, $df = 1/51$, $p < .01$, thus indicating that paradigm I produced significantly shorter VER latencies than paradigm II. No statistical comparison could be made between the latencies obtained by Rose and those obtained in this study as the figures for Rose's study were not available.

Visual Following Findings in Paradigm II

In analyzing visual following, the computer averaged sixteen 500 msec epochs of the 22 daily VERs obtained from the first light flash on each trial. This procedure was then repeated for the three subsequent light flashes. The fifth light flash was presented simultaneously with the shock and could not be averaged.

Fig. 8 illustrates that it was possible to obtain in all Ss except M 266 Blac/Bl comparable or nearly comparable VERs to the four light flashes on day 10 postnatally, the first day of the experiment, as well as on subsequent days. On the average, the initial responses were only slightly larger than the others. Hunt and Goldring (1951) reported that 3 to 5 minutes were required between flashes to produce comparable VERs in 7- to 10-day-old rabbits. Rose and Ellingson (1970) reported that at least 20 seconds were required in 0 to 4-day-old kittens. The present findings seem to imply that at 10 days of age or sometime prior to 10 days the kittens used in this study were capable of giving comparable or nearly comparable VERs to light flashes as close as 2 sec apart.

The latencies of the N_2 wave for each of the VERs obtained from Ss used in paradigm II on days 10, 11, 14, and 19 postnatally are

Figure 8

Computer-averaged visually evoked potentials from awake unanesthetized kittens with implanted cortical electrodes. Stimulation (binocular) occurs at onset of trace. Monopolar recordings; neck reference. Bold print numbers indicate age in days. Small numbers indicate averages obtained for the first, second, third and fourth VER. Traces are algebraic summations. All Ss received paradigm II (intermittent light). M 266 Blac/B 1 and M 264 B/W are control animals. M 264 B/W 3 and M 266 B 1 are experimental animals.

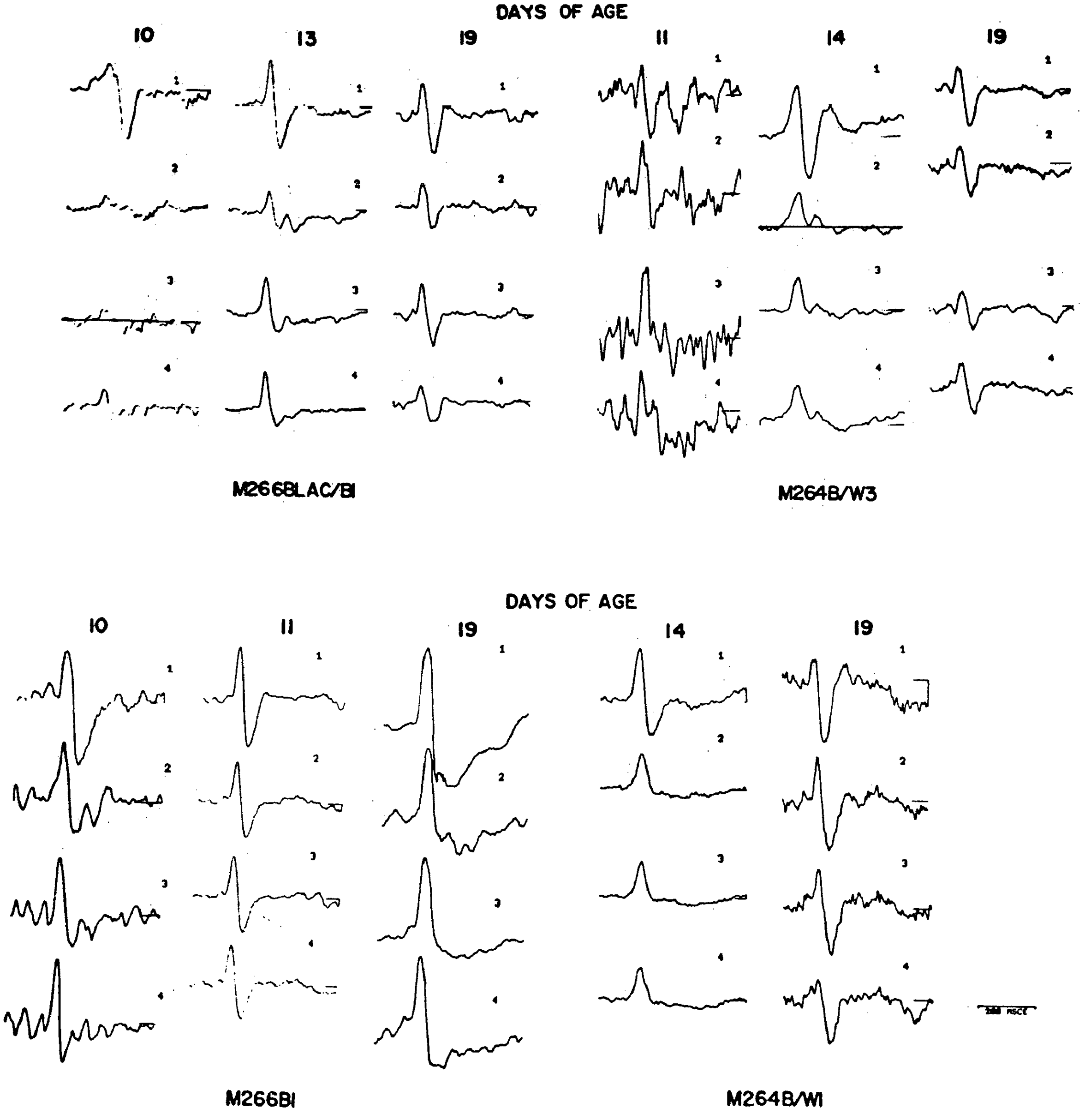
DAYS OF AGE
10
13
19
11
14
19
1
2
3
4
M266BLAC/BI
M264B/W3
DAYS OF AGE
10
11
19
14
19
1
2
3
4
M266BI
M264B/WI

FIG. 8

presented in Table II. In keeping with the developmental data (Rose, 1971), the latencies for each of the N_2 waves decreased with age. However, with the exception of day 10, when one moves from the first VER to the fourth VER on each day, one finds consistently longer latencies for the N_2 component in the two middle VERs than for the two end ones and this trend was observed throughout the experimental session.

Table II

Peak Delays (msec) of Long-Latency Negative

(N_2) VERs to 4 Light Flashes at 2 sec Intervals

Subjects		N_2 Waves from Marginal Gyrus							
		1		2		3		4	
Age Range in Days	Group N	$\bar{\bar{X}}$	S.D.	$\bar{\bar{X}}$	S.D.	$\bar{\bar{X}}$	S.D.	$\bar{\bar{X}}$	S.D.
10	3	172.80	3.84	170.88	2.72	175.33	7.68	177.91	8.58
11	2	163.20	8.58	167.04	2.72	172.80	10.86	161.28	5.43
14	3	142.08	3.84	148.47	6.65	147.19	6.65	144.63	7.68
19	4	122.08	7.68	133.44	13.30	134.40	14.68	127.68	8.58

Discussion

Failure to obtain statistical evidence of learning from the apriori statistical analyses may be due to one of two reasons. First, it is generally believed that the establishment of leg flexion responses in cats is achieved only with difficulty, as such responses are not native to the species (Bolles, 1970). That response was chosen because it was the most convenient and it was hypothesized that the plasticity of infants in general would serve to overcome the rigidity observed in the adult cat. This hypothesis was not upheld.

Second, by presenting shock simultaneously with the fifth light flash in paradigm II, this procedure probably approximated a simultaneous conditioning paradigm which is known to achieve conditioning only with difficulty. In paradigm I (continuous light) although learning has been shown with long delay intervals, 9 sec may not have been ideal. In addition, the duration of the CS was 9 sec, while the interval between the offset of the US in one trial and the onset of the CS for the subsequent trial varied from 4 to 7 sec. This time relationship between the CS duration and the US offset- CS presentation may have resulted in the CS loosing its cue value. Future studies with varied CS intervals may resolve these problems.

On the other hand, the behavioral observations reported in the Result section imply that the control and experimental animals in both paradigms were responding differentially to the experimental setting and treatments. The fact that the experimental animals were observed to cease crying at the onset of the CS seems to imply that they learned

The CS-US relationship and prepared themselves for the shock as soon as the light appeared (probably by tensing their limbs). On the other hand, the control animals had no such control over the situation as they never received paired presentations of light and shock, thus they cried without regard to the light onset.

In the Result section on GSR, it was pointed out that the Wilcoxon tests did reveal some differential conditioning of the experimental and control animals' GSR. However, the variance within and between Ss was large. This seems to imply that failure to obtain overall significance on this indice may have been due to the small sample size.

It is difficult to explain why statistically significant changes in EKG were not obtained having found evidence for increased emotionality. One may argue that there was simply no EKG learning, but the erratic nature of the EKG obtained within as well as across animals may also lead to the conclusion that that was the cause of statistical non-significance. If so, small sample size and large variance may have also been responsible for failure to obtain overall statistical significance in EKG.

In the background literature, reference was made to the Rose-Lindsley hypothesis concerning the possible neural pathways of the N_2 wave in relation to arousal. They postulated that the N_2 component of the VER is dependent on indirect pathways which include the brachium of the superior colliculus and possibly the reticular formation via the tectal and pretectal pathways. There is no control for shock in this study but the findings that the N_2 component dominated the VER throughout development and well into the period when the typical adult "W"-wave is observed seem to support this hypothesis. When the information on the role of the RAS in arousal (Hernández-Peón et al., 1955; Hernández-Peón & Scherrer, 1955a, 1955b) is added to the findings reported here, they seem to suggest further that the N_2 component may be the arousal component of the VER.

In comparing the relative amplitudes of the VERs obtained from Ss in this study with those obtained by Rose, it was found that in general the relative amplitudes of the N_2 waves were larger for the animals used in this study and this was true for Ss receiving both paradigms. Hall (1971) reported finding that increased emotionality in adult cats led to an increase in the amplitude of their evoked responses. Thus, our findings seem to be consistent with Hall's. It appears that the shock per se may have had an arousing effect on the Ss.

By looking at the overall development of the VER in both paradigms, it was found that the short-latency negative (N_1) wave and the short-latency positive wave were generally either later in appearing or did not appear at all. This may imply immaturity or retardation in their development. On the other hand, the long-latency N_2 wave for the Ss receiving paradigm I displayed shorter latencies than those reported by Rose. This may imply hastened maturity of the N_2 wave as it is expected to get shorter in latency with age. Thus the shocking of Ss in paradigm I may have had a paradoxical effect on their VER development. The Ss reflected early maturity in the shorter latencies of the N_2 component and at the same time immaturity in the late development or absence of the N_1 and short-latency positive components. Garcia-Austt and Pattetta-Queirolo (1961) and Paulsen (1965) reported that chickens and ducklings reared in bright light demonstrate prococious development in their VERs. The findings reported here may be suggesting that a combination of the 9 sec light bombardment plus shock throughout the VER development of Ss receiving paradigm I may have resulted in the selective maturation of the N_2 component.

In Paradigm II, it was found that the latencies were longer than those reported by Rose and that the S.D., with the exception of that for the first age group, were all smaller. These Ss may be reflecting immaturity in the slow development or absence of their short-latency N_1 and short-latency positive components as well as in the longer latencies of the N_2 VER components. These animals did not receive the constant light bombardment as in Paradigm I and the reversed latency of the N_2 may be due to the interaction of the intermittent nature of the stimulus (with concurrent on-off effects) and the shock.

The very pronounced positive after-swing that followed the N_2 component throughout the VER development in the Ss used in this study but not in Rose's animals may be also providing further evidence for emotional arousal in the Ss (Hall, 1971). Possibly, shocking per se facilitated or enlarged the positive after-swing and kept it in the VER complex up to and during the period that the adult VER is usually observed. The significance of the large positive after-swing is very complicated, however, and may best be resolved with proper controls for shock as well as extra- and intra-cortical microelectrode recordings from the relevant areas.

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