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Flash Evoked Potentials from Rat Superior Colliculus[1]

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DYER, R. S. AND Z. ANNAU. *Flash evoked potentials from rat superior colliculus.* PHARMAC. BIOCHEM. BEHAV. 6(4) 453–459, 1977. In view of reports that the superior colliculus evoked potential from rats is uniquely sensitive to toxic gases, the present study characterized normal flash evoked potentials from unanesthetized rats. The waveform was complex, with at least 5 positive and 5 negative peaks. The waveform originated in the SGS layer, and some components were stable over time if conditions of light intensity, stimulus frequency and dark adaptation were held constant. The greater complexity of the waveforms reported here compared to those described by others can be attributed to both an intense flash stimulus and unanesthetized preparation.

Pharmacological methods Evoked potentials Vision Unaesthetized preparation
Superior colliculus

FIELD potentials evoked in the mammalian superior colliculus (SC) by light flash or electrical stimulation of the optic nerve have been studied in both anesthetized and unanesthetized preparations [7, 8, 9, 10]. The complexity of waveforms reported in these studies has varied considerably, ranging from a single biphasic sinusoid to a multipeaked potential with superimposed spikes. Accurate characterization of the normal SC evoked potential is important, since it has been suggested to be extremely sensitive to such toxic agents as carbon monoxide and ozone and on those grounds has been recommended as a procedure useful in assaying CNS responses to environmental agents [12]. Clearly before evaluations of this nature can be confidently made, the evoked potential must be accurately described under normal conditions.

The present paper characterizes the normal SC visual evoked potential from unanesthetized rats. Although more papers have examined the cat than the rat SC, our results suggest similarities between the potentials evoked in the two species. Light flashes were chosen over optic tract stimulation because they more closely approximated natural activation.

METHOD AND MATERIAL

Sixty adult male Long-Evans rats obtained from Blue Spruce Farms were used in the experiments. Animals were anesthetized with Equithesin, following which they had bipolar 0.25 mm twisted nichrome wires, insulated up to the cut tips, lowered into their superior colliculus under stereotaxic guidance. The tips of the electrodes were separated from each other in the vertical plane by about 1 mm, and the electrodes were lowered into the SC at 5.5 posterior to bregma, 1.5 lateral and about 3.8 mm deep. The electrodes were cemented in place with dental acrylic and connected to an Amphenol receptacle. A 0-80 stainless steel screw was screwed into the skull over the frontal sinus and connected by an insulated nichrome wire to the receptacle for purposes of grounding the animal. At least one week recovery was allowed before any recordings were made.

After pupils were dilated (Cyclogyl) and the animals were connected to the recording apparatus via an Amphenol plug and Microdot mininoise shielded cable, they were placed in a chamber 8 cm wide, 20 cm long and 38 cm high, which had mirrors on 3 walls, ceiling and floor. The fourth clear Plexiglas wall had the lamp from a Grass PS-2 photostimulator mounted flush against it. The mirrors were intended to insure that the flash was of roughly equal intensity independent of direction of gaze. Air was blown into the chamber at 6 ℓ/min through a 0.6 cm hole located 5 cm from the floor. Although signs of startle or behavioral reactivity to the photic stimuli were never observed after the first few flashes, each days data collection session was immediately preceded by about 300 flashes at the same intensity and frequency as would occur during the session. This procedure was intended to allow for habituation to the recording chamber, the flash and the air flow. Recordings were made by connecting the animal to conventional preamplifiers with high and low frequency cutoffs set at 10 kHz and 0.2 Hz respectively. Amplified signals were led to an oscilloscope for monitoring, and to a PDP-12 computer for averaging. The post stimulus analysis epoch was 240 msec, each 1 msec representing one bin of a 240 point plot display. Voltage was sampled every 333 μ sec, and the three samples were averaged together to produce the value deposited in a given bin on a given sweep. Unless otherwise noted, 50 responses were averaged for each run. It should be noted that a true average was obtained, thus obviating the need for a calibration pulse on each run.

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A signal from the computer triggered a Grass S44 stimulator, which in turn triggered the photostimulator. Except during experiments which evaluated the effects of varying flash intensity, it was always set at its greatest value (No. 16), which produced a 10 μ sec flash of about 1.5×10^6 candlepower. Except during recovery cycle experiments flashes were presented at 0.5 Hz. Discharge of the strobe unit was accompanied by a week auditory click. This click was largely masked by the noise produced by the stream of air blowing into the chamber, but to insure that no auditory component contributed to the flash evoked potential the strobe face was covered with opaque material and a series of runs was accomplished. No evidence of an auditory component was observed.

Averaged waveforms were displayed on an oscilloscope, and a cursor controlled by the teletype and one analog channel could be moved along the averaged waveform by the experimenter. Upon signal the latency and amplitude of the bin corresponding to the cursor location was printed. In addition, a bin by bin description of the entire evoked potential could be printed by a Centronics printer. Bin values were read to the nearest 1.0 μV. Unless noted otherwise, evoked potentials presented as figures in the paper are either tracings made from polaroid pictures of the oscilloscope display or graphs drawn by plotting every other bin value. Calibration marks appearing on most of the figures are hand drawn and are presented for the reader's convenience. Actual values of amplitudes and latencies were obtained directly as described above.

Recovery cycles were determined for 5 animals according to the method described by Shagass [11]. Each sweep contained 3 flashes; the first, a reference flash, was separated from the second by a fixed 2 sec interval. The assumption was made that on the average, the response to the first two flashes would be the same. This proved a reasonable assumption, since averaged evoked potentials obtained at 2 sec intervals appeared identical to those obtained at longer intervals. The interval between the second and third flashes varied until 100 trials had been run at 2000, 1000, 500, 350, 200, 150, 125, 100, 80, 60, 40, 30, and 20 msec intervals. After each sweep, the 240 bins following the first flash were subtracted from the 240 bins following the second flash, thus leaving the response to the third flash. In the case where interflash intervals were long, this procedure proved unnecessary, but at short interflash intervals it was necessary to separate the second from the third response. In 3 animals the experiment was repeated at least 1 week after the first session.

Effects of varying stimulus intensity upon amplitudes and latency of different components were determined in 6 animals. In these experiments the 5 different relative intensities of the Grass photostimulator were used, and values were expressed as a percentage of the response obtained to the maximum setting. Trials of 100 flashes were run at each setting, and compared to an immediately preceding trial of 100 flashes at the maximum intensity. Order of presentation of the trials was 16, 1, 16, 8, 16, 2, 16, 4. The numbers 1, 2, 4, 8, and 16 correspond roughly to 9.4×10^4, 1.9×10^5, 3.8×10^5, 7.5×10^5 and 1.5×10^6 candlepower.

To verify that all potentials reversed polarity in the stratum griseum superficiale [10] six acute experiments were performed under Equithesin anesthesia. In these experiments a monopolar electrode was lowered through the superior colliculus in 10 μ steps, with evoked potentials

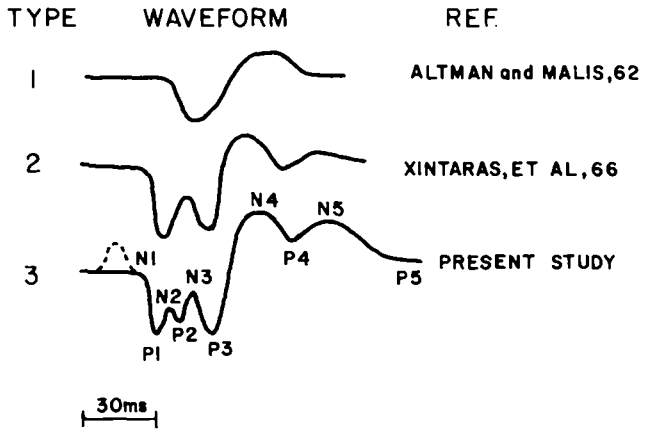

FIG. 1. Diagram of typical waveforms recorded from superior colliculus. See text for further explanation. Time calibration is approximate. Upward deflections are negative.

averaged in blocks of 100 at each step. Most regular recordings were made using the bipolar configuration, but in order to further estimate the origin of different components, each chronic animal had a series of monopolar recordings refering either the deep or shallow pole of the electrode to ground.

Finally, the animals were perfused with normal saline and 10% Formalin, the electrodes were carefully removed, and the brains were frozen, sectioned at 90 μ and stained with cresyl violet to confirm location of the electrodes and lesions.

RESULTS

Waveform

Several different waveforms have been reported as characteristic of the SC. Figure 1 schematizes three basic waveforms described for flash evoked potentials. Active electrodes were below the stratum griseum superficiale (SGS); and negativity is represented by an upward deflection.

The types 2 and 3 waveforms in Fig. 1 were typical of those we have recorded. We have occasionally observed, as have others [5], an early complex, indicated by dotted lines in Fig. 1, however, since its presence, even within animals, was not consistent we have not evaluated it quantitatively. Normative data for 15 animals are presented in Table 1. Nine of these animals were dark adapted for 2 hr, and recordings were made in a dark room. The other six animals were not dark adapted and had recordings taken in a room that was not darkened. Amplitudes are given in μV with the coefficient of variability underneath, and latencies are given in ms with the coefficient of variability underneath [3]. The values used to characterize each animal were the means obtained from 500 flashes.

The initial positive deflection, P1, was probably analogous to the initial deflection described by others. Under constant conditions it had a relatively constant within animal latency to peak, but zero crossing of the wave at N1 varied in latency from trial to trial.

The P1 wave was followed by a complex and variable waveform which was best characterized as a negative wave which frequently had 1 or 2 positive going spikes riding upon it. In about 50% of animals the positive spikes were not observable, while in others they were as large as P1. The

TABLE 1

AMPLITUDES (μV) AND LATENCIES (MS) OF SC EVOKED POTENTIAL COMPONENTS IN 15 ANIMALS COEFFICIENT OF VARIABILITY ($\frac{SD}{\bar{X}}$ 100) GIVEN IN PARENTHESIS

Amplitudes	N1-P1	P1-N3	N3-P3	P3-N4	N4-P4	P4-N5	N5-P5
Dark Adapted	400	122	134	690	189	142	252
	(16.8)	(26.0)	(25.4)	(15.9)	(31.2)	(47.9)	(23.0)
Non	277	145	132	522	160	109	197
Dark Adapted	(31.0)	(44.8)	(51.5)	(29.5)	(33.8)	(56.9)	(32.5)
Latencies	P1	N3	P3	N4	P4	N5	P5
Dark Adapted	27.9	34.6	40.1	50.5	62.6	70.4	166.4
	(2.5)	(2.3)	(1.8)	(1.6)	(4.5)	(4.6)	(5.1)
Non	28.3	34.0	40.2	51.7	61.2	69.2	173.2
Dark Adapted	(3.2)	(1.5)	(1.2)	(1.6)	(3.8)	(3.2)	(4.7)

most common configuration (about 95% of all animals showing the positive spikes) had only 1 of the positive spikes, and for this reason we refer to this waveform as the P1-N2-P2-N3-P3 complex. Even within animals this waveform varied considerably from trial to trial. In describing this waveform, amplitude measurements were taken from P1 to N3, unless N2 was clearly larger and more distinct, in which case amplitudes were measured from P1 to N2. None of the procedures tested selectively affected just one of these two negative peaks. Variability was so great that this complex is not likely to be useful in evaluating the effects of any agent upon CNS function unless the effect of the agent is to reduce variability.

Assessing amplitudes of P3 and N4 was occasionally difficult if one attempted to measure from baseline, since there were some trials on which P3 was only positive with respect to N3, and not with respect to baseline. In most animals trials of this sort were unusual, but their presence could not be ignored. It seemed possible, therefore, that amplitudes measured from P3-N4 might provide a more stable trial to trial description of the waveform. To test this possibility it was necessary to obtain an estimate of the variability of amplitudes obtained using each of the different measurement techniques. This was done by recording seven consecutive trials of 50 flashes each for 7 different animals. For each trial the amplitude of P3 from baseline, N4 from baseline and P3-N4 was obtained, and the coefficient of variability was then calculated for each animal, and for each measure. Paired t-tests showed that the P3-N4 variability was significantly less than P3 from baseline ($p<0.05$) and less but not significantly so than N4 from baseline ($0.05<p<0.10$).

The N4-P4-N5 complex rode on a long (about 115 msec) negative wave which returned to baseline at P5. Occasionally after discharge waves with a period similar to N5 could be seen following N5 for the entire 240 msec epoch.

Variability

For these experiments to be useful it is necessary to know how stable the different components of the evoked potential are. Reference to the coefficient of variability values in Table 1 makes it clear that there is more amplitude variability from one animal to the next than latency variability, and a considerable range of variability depending upon which peak is examined. It will be shown

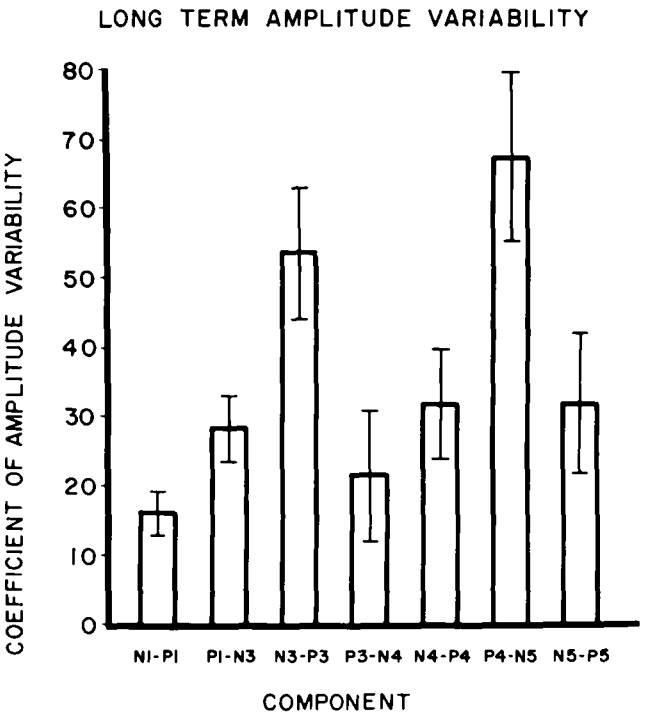

FIG. 2. Coefficient of within animal variability in amplitude of 7 different peaks derived from 5 recording sessions in 6 different animals spread over 11 days. The figure demonstrates that N1-P1, P3-N4 and N4-P4 vary less than other peaks. Variance bars represent standard error of the mean.

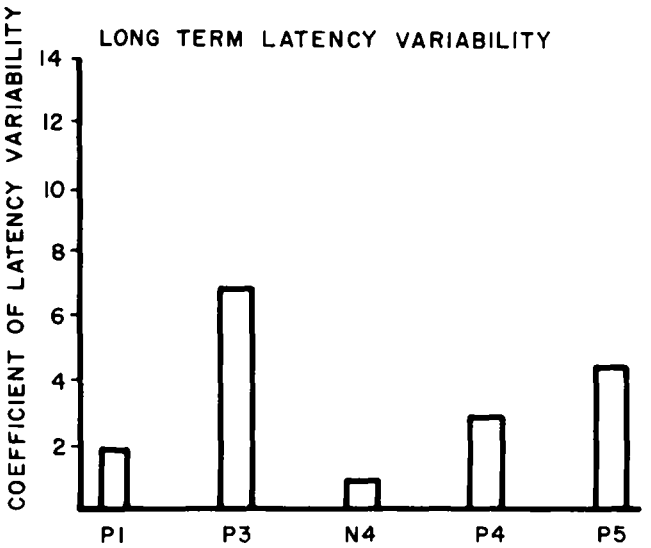

FIG. 3. Coefficient of within animal variability in latency of 5 different peaks derived from 5 recording sessions in 6 different animals. The figure demonstrates the greater stability of early peaks. See text for further explanation.

that within animals some peak amplitudes were stable while others were not.

To answer the question of long-term stability, a group of 6 animals had 500 evoked potentials averaged 5, 7, 8 and 11 days after an initial session. The coefficients of variability were calculated for all peaks. The results for amplitudes are shown in Fig. 2, and for latency in Fig. 3.

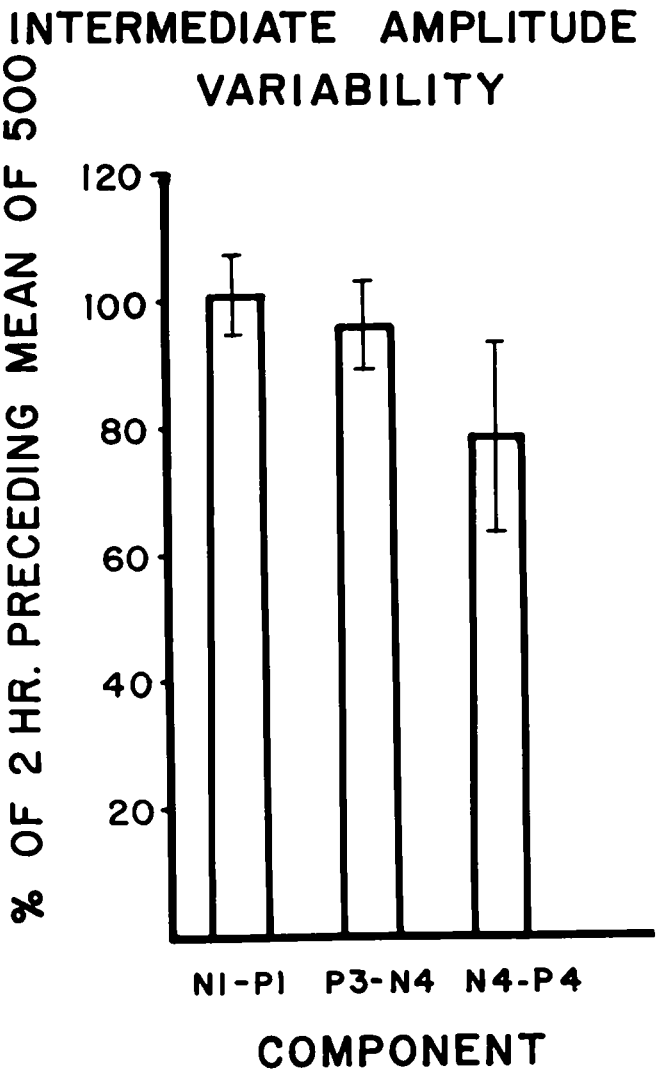

FIG. 4. Estimate of within animal variability in amplitude of 3 peaks derived from 2 recording sessions in 7 different animals. The two sessions were 500 flashes long, and were separated by 2 hr. The figure demonstrates stable recordings for the N1-P1 and P3-N4 peaks. Variance bars represent standard error of the mean.

For these figures it is clear that the amplitude of N1-P1 and P3-N4 components were most stable, and that latencies of all early peaks were quite stable. As might be expected, when variability was expressed as a function of day 1 values increasing variability from the initial reading was found with the increases in time.

Amplitude variability was also studied under 2 conditions with shorter time scales than above. In the first condition, the averaged response of 7 animals to 500 flashes was compared to a second average of 500 flashes which began 2 hr after the end of the first set. This seemed a likely paradigm for evaluating the effects of short exposure to carbon monoxide, since it takes about 2 hr for rat COHb levels to reach equilibrium at a constant concentration of inspired CO. The results of this experiment are shown in Fig. 4. The amplitudes of the 3 peaks recorded during the second set of flashes are expressed as a percentage of the amplitudes recorded in the first set. From this figure it is

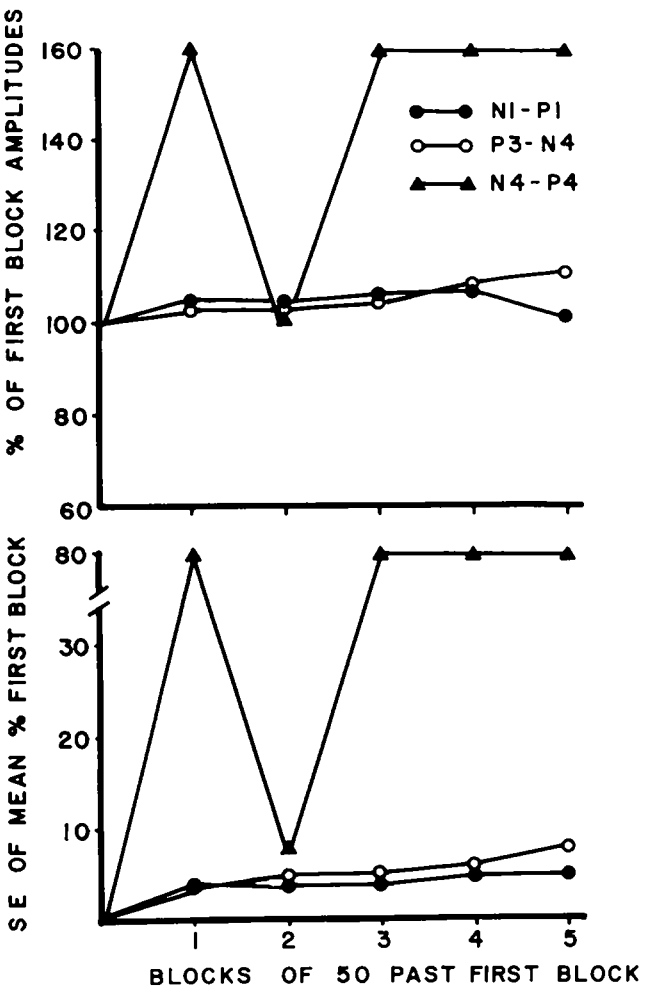

FIG. 5. Estimate of within animal variability in amplitude of 3 peaks derived from 6 consecutive recording sessions in 7 different animals. Each session consisted of 50 flashes and followed the preceding session by about 4 min. The top graph shows changes in amplitude expressed as a percentage of the session (block) 1 amplitude. The lower graph plots the standard errors of the means in the upper graph. See text for further explanation.

clear that using such a procedure one could expect only slight variability in the N1-P1 and P3-N4 amplitudes.

In the second condition, the averaged response of 7 different animals to 6 consecutive blocks of 50 flashes was compared. These results are shown in Fig. 5. Each animal's average amplitude obtained on a given block of flashes was taken as a percentage of that animal's mean amplitude for the first of the 6 blocks. Next the mean and standard error across animals of these percentage of first block within animal means was calculated for each block. Figure 5 shows the block means and block standard error plotted separately for three components. Again, N1-P1 and P3-N4 appeared quite stable, although both showed a trend towards gradually increasing variability with time from the first block. Peak N4-P4 appeared to have a high amplitude and a low amplitude mode.

Flash Intensity

Changing the flash intensity produced dramatic changes in the evoked potential. Figure 6 shows quantitatively the

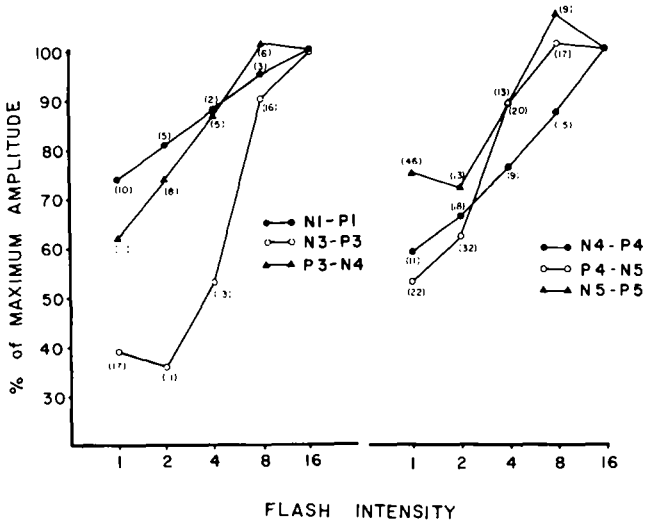

FIG. 6. Average effect of varying flash intensity upon amplitude of different components. The ordinate is percentage of the average response to the highest intensity flash tested. Curves are based on 6 animals, each having an average of 100 flashes taken at each intensity. Standard errors are in parentheses.

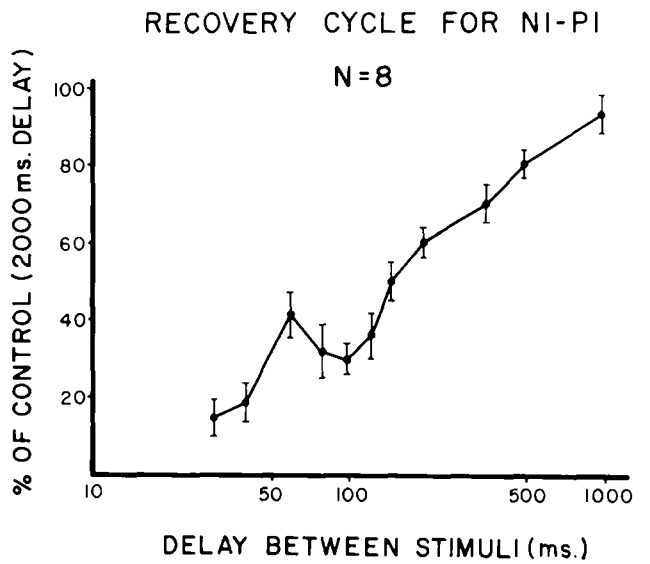

FIG. 7. N1-P1 recovery cycle plotted for 5 animals. Three of the animals were used twice. Variance bars represent standard error of the mean.

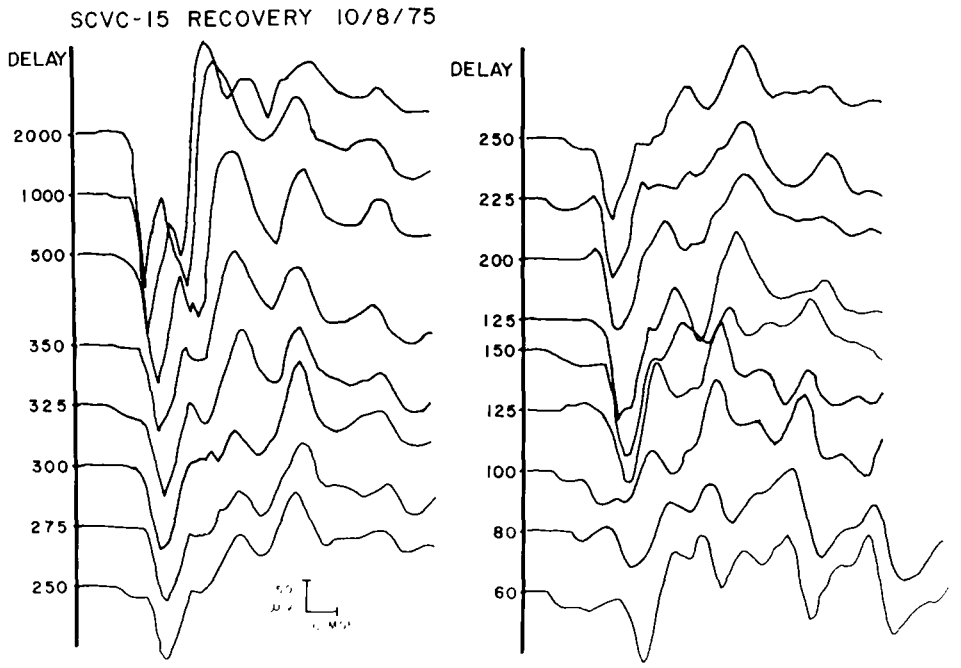

FIG. 8. Averaged waveforms of the evoked potential at different interstimulus intervals. Each potential represents the mean response to 100 flashes at the indicated delay. Negativity is up.

effects of changing intensity on the amplitude of 6 components; each point is the average of 7 animals and each animal's point is the average of 100 responses. Values are expressed on the semi-log plot as a percentage of the response to a flash of maximal intensity (No. 16 on the Grass Strobe), and thus are not contaminated by inter-animal variability in raw amplitude. The N3-P3 component was most sensitive, dropping off quickly at lower intensities. The P3-N4, N4-P4, and P4-N5 components reached maximum amplitude at ½ the maximum flash intensity (1 = 8). The flash intensity scale represents the 5 settings on the Grass Strobe unit, corresponding roughly to 9.4×10^4, 1.9×10^5, 3.8×10^5, and 1.5×10^6 candlepower.

Recovery cycle

Recovery cycles were plotted for five animals. The amplitude of N1-P1 fell to 50% of control when the

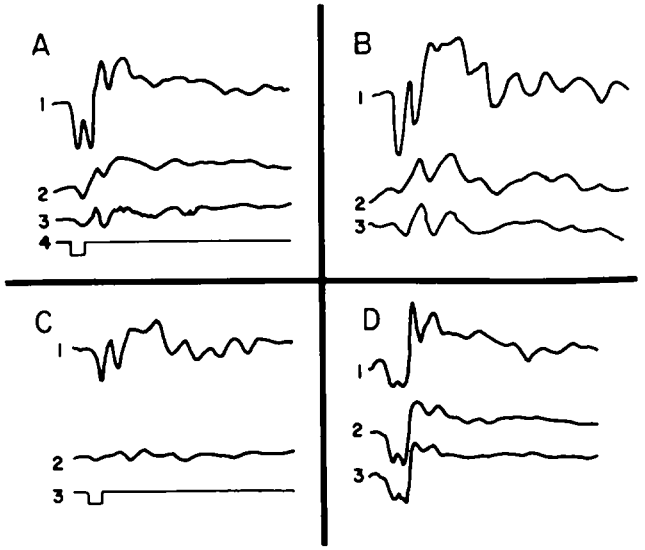

FIG. 9. Photographs taken from the oscilloscope display of the averaged SC EP. Records in A, B and C were averages of 50 EP's obtained sequentially from the same animal and illustrate the effects of varying flash intensity and arousal level. Total time from A1 to C2 is about 45 min. In A the animal was unanesthetized and in the recording chamber. A1 flash intensity = 1.5×10^6 cp; A2 flash intensity = 9.4×10^4 cp; A3 flash intensity = 9.4×10^4, but distance between bulb and chamber is now 9 cm instead of flush; A4 calibration pulse 100 μV, 10 ms, total trace duration 240 ms. Calibration pulse applies to both A and B. Immediately after A3 animal was anesthetized with 160 mg/kg Equithesin, and series B was begun 15 min later. B1, B2, and B3 are the same as A1, A2, and A3, except animal was anesthetized. Immediately after B3 the animal was removed from the recording chamber. C1 and C2 repeat B1 and B2 outside the chamber. C3 calibration pulse 100 μV, 10 ms, total trace duration 240 ms, applies to C and D. D1 same animal shown in A, B, and C, mean of 500 responses recorded under conditions identical to A1 except 60 days previously. Note the similar P3-N4 amplitudes. D2 and D3, different animals recorded under the same conditions as A1. Note the type 3 waveform. Negativity is represented by upward deflections.

interstimulus interval was decreased to 150 msec. Subsequent peaks became increasingly difficult to identify at intervals less than 150 ms, probably because latencies of the different peaks tended to vary somewhat independently of one another. Thus, the latency of N3 may have increased with decreasing interstimulus intervals more rapidly than N4, resulting in an apparent decrease of its amplitude to 0, when it overlapped N4 in time. For this reason only the N1-P1 amplitudes were evaluated quantitatively. Figure 7 shows the average recovery cycle plotted for P1. This figure is based on 8 experiments, since 3 animals were tested twice. Figure 8 shows the changes in waveforms observed in one animal at different interstimulus intervals. Each potential in Fig. 8 represents the mean of 100 trials at the indicated delay.

In the acute experiments the reversal of potential at the level of the stratum griseum superficiale (SGS) was confirmed.

GENERAL DISCUSSION

Few authors have described flash evoked potentials in SC as complex as type 3 in Fig. 1, but variations may probably be accounted for by variations in stimulus intensity and level of arousal. Bishop and O'Leary [2] described an equally complex wave recorded from decorticate rabbits lightly anesthetized with Nembutal. In their case a long duration (23 ms) flash was used. In a preparation apparently similar to the one used in the present study, Xintaras *et al.* [12], described only a type 2 wave. Type 1 waveforms were reported with short duration, low intensity flashes [1]. Figure 9 illustrates the effects of variation in stimulus intensity and arousal level with a series of 8 successive averages of 50 evoked potentials obtained from the same animal. In A1-A4 the animal is unanesthetized and in the recording chamber surrounded by mirrors. A1 shows the averaged response to maximal intensity flashes (No. 16 on the photostimulator). A2 shows the response to a flash 0.0625 times that of A1, and A3 shows the response to the same stimulus as A2, except the flash bulb is now 10 cm from the recording chamber instead of flush against it. A4 is a calibration pulse 10 ms long and 100 μV positive. Part B of Fig. 9 repeats the same series as Part A, except 15 min before B1 the animal was lightly anesthetized with an injection of Equithesin (160 mg/kg). Part C shows the response of the anesthetized animal after it has been removed from the mirror chamber. Part D1 shows the average of 500 responses of the same animal under conditions similar to A1, recorded 60 days previously.

It appears possible to account for the variety of waveforms reported in the following way. In the anesthetized animal a dim flash produces an evoked potential characterized by a deep positive wave, followed by a deep negative wave [1], which we have called the type 1 response. A slightly brighter flash produces a small negative wave at the peak of the initial positive wave, called here type 2. Type 2 responses can be observed following flashes in animals anesthetized with chloralose [6] or Nembutal, but in the Nembutal anesthetized animal the flash must be bright. Doty and Kimura [4] described type 2 responses to flashes in the decorticate midpontine pretrigeminal preparation. In the unanesthetized or lightly anesthetized animal, the present results show that the negative wave which appears on the positive wave may become more complex, developing one or occasionally two positive spikes (the type 3 response) if the stimulus is a very bright flash. Observations made while animals in the present experiment were anesthetized during implantation of the electrodes confirm this analysis.

It is appropriate to compare the present characterization of the flash evoked potential from SC with the electric shock evoked potential described by Pickering and Freeman [9]. According to that analysis, SC EP is well characterized as a damped sine wave, the frequency of which depends upon the arousal level of the animal. The analysis is intriguing, because it implies that some of the great variability observed in later components of the present study might be accounted for in terms of varying frequencies and amplitudes resulting from different arousal levels. However, if such is the case, it seems likely that analysis of these later components will not be useful for studies of toxicants, since a method for controlling and monitoring arousal would be necessary. On the other hand, the relative stability of the amplitudes and latencies of early components suggests that a more complex analysis may be unnecessary. Finally, it is of interest to point out that on a number of occasions the averaged evoked potentials from the present study showed oscillations with the same period as N5 extending throughout the 240 msec epoch. This

finding suggests that under some conditions the sine wave may not be damped at all.

The present study was undertaken to examine the normal superior colliculus visual evoked potential as a preliminary step to examining its feasibility as an index of CNS sensitivity to toxic agents. The enormous size of the structure guarantees accessibility, and the large amplitude and temporal stability of potentials recorded from it under constant conditions recommend it as a potentially useful tool. Investigators are cautioned, however, that the stability of potentials recorded decreases markedly when the light intensity and interstimulus interval are not held constant from one recording session to another, and when amplitude comparisons are made between instead of within animals. It seems quite likely that the wide interanimal variability in amplitudes represents small variation in electrode placement, and that careful monitoring of amplitudes during electrode implantations might reduce it. When experiments using within subjects designs are undesirable such an approach might be attempted. Further, not all components are equally stable. The P1, P3 and N4 peaks have been the most stable from trial to trial and day to day. Finally, it remains to be demonstrated that under carefully controlled conditions SC EP is in fact sensitive to toxic agents.

It perhaps should be noted that if the SC EP is found to be a sensitive index of the effects of toxic agents upon the CNS, it does not necessarily follow that the SC itself is sensitive. Changes could represent changes at retinal or tract locations or might be characteristic of all locations within the brain, but where the changes occur is initially less important than whether or not they do occur.

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