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Sex and Strain Differences in the Visual Evoked Potentials of Albino and Hooded Rats

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DYER, ROBERT S. AND H. SCOTT SWARTZWELDER. *Sex and strain differences in the visual evoked potentials of albino and hooded rats.* PHARMAC. BIOCHEM. BEHAV. 9(3) 301–306, 1978.—Visually evoked potentials were recorded n male and female rats from albino and hooded strains. Recordings were made at 4 different flash intensities in unanesthetized animals. Clear sex and strain differences were observed. Females had larger amplitude P1-N1 and N1-P2 components and shorter latency N1 peaks than males. Albino rats had larger amplitude P1-N1, N2-Pe and P3-N3 components and longer latency P2 and P3 peaks than hooded rats. Variations in flash intensity produced greater alterations in latencies and N1-P2 amplitudes in hooded rats than in albino rats, but greater alterations in N1, N2 and P3 latencies in albino rats than in hooded rats. Hooded rats are recommended as more valuable for studies of chemically induced change in the visual evoked potential.

Sex differences Male Female Albino rats Hooded rats Strain differences Evoked potentials Visual evoked potentials

RECENT studies in functional neurotoxicity have explored the value of recording sensory evoked potentials as indices of the functional integrity of the central nervous system [12, 13, 14, 16, 26]. Although a considerable literature quantitatively describes the influence of many variables upon the human evoked potential [e.g., 4, 23, 24], only a few studies have quantitatively characterized the consequences of manipulating common laboratory variables upon evoked potential parameters in unanesthetized animals.

Common among many electrophysiological reports of experiments upon animals is either the failure to report what sex was used, or exclusive use of the male. The former approach is probably based upon the never stated assumption that sex makes no difference in electrophysiological matters, and the latter is based upon the rarely stated but widely held supposition that males provide a more stable baseline than females because of the complicating cyclic hormonal variable. Surprisingly, there have been no attempts to directly compare the evoked potentials of male and female rats. Although the hormonal fluctuations of the estrous cycle almost certainly do affect the evoked potential [2], there is at least one good reason to include females in the study of neural function, even in the absence of precise knowledge of the stage of estrous in the animals at the time of recordings. In humans there are sex differences in evoked potentials which are clearly unrelated to gonadal hormones [3], and there is reason to believe that some toxicants selectively affect one sex or the other [13]. A rational approach to neurotoxicity therefore demands the testing of both sexes.

Significant strain differences in visual evoked potential latencies have been reported [5], but amplitude differences have not. In view of the anatomical [7, 17, 20] and functional [25] differences known to exist between albino and hooded rat visual systems, it would be surprising if no differences existed in evoked potential amplitudes.

The present report describes the influence of sex upon the amplitudes and latencies of visual evoked potentials recorded from the cortex of rats from two outbred strains, one of which is albino and one of which is hooded.

METHOD

Long-Evans hooded rats were obtained from Blue Spruce Farms, and Sprague-Dawley albino rats were obtained from the Charles River Breeding Co. The animals used were part of two toxicology experiments reported previously [13,14], and the details of their exposure history as well as surgical and recording procedures can be found in those reports. Briefly the albinos were exposed to either lead or no lead through their dam's milk throughout the suckling period and at maturity (60 days old), 14 female (7 exposed, 7 control) and 18 male (6 exposed, 12 control), derived from 12 exposed and 12 control litters were surgically implanted with recording electrodes over the visual cortex. The hooded rats were either exposed prenatally to low levels of carbon monoxide or not, and at maturity (60 days old), 24 male (9 control and 15 exposed) and 17 female (8 exposed and 9 control), derived from 5 control and 6 exposed litters, were surgically implanted with recording electrodes over the visual cortex. Following a two week recovery period recording sessions were begun. During the recording session the pupils were dilated (Cyclogyl), the animals were habituated to both the recording chamber and the flash, and the averaged response to 100 flashes 10 μsec in duration presented at 0.4 hz was obtained

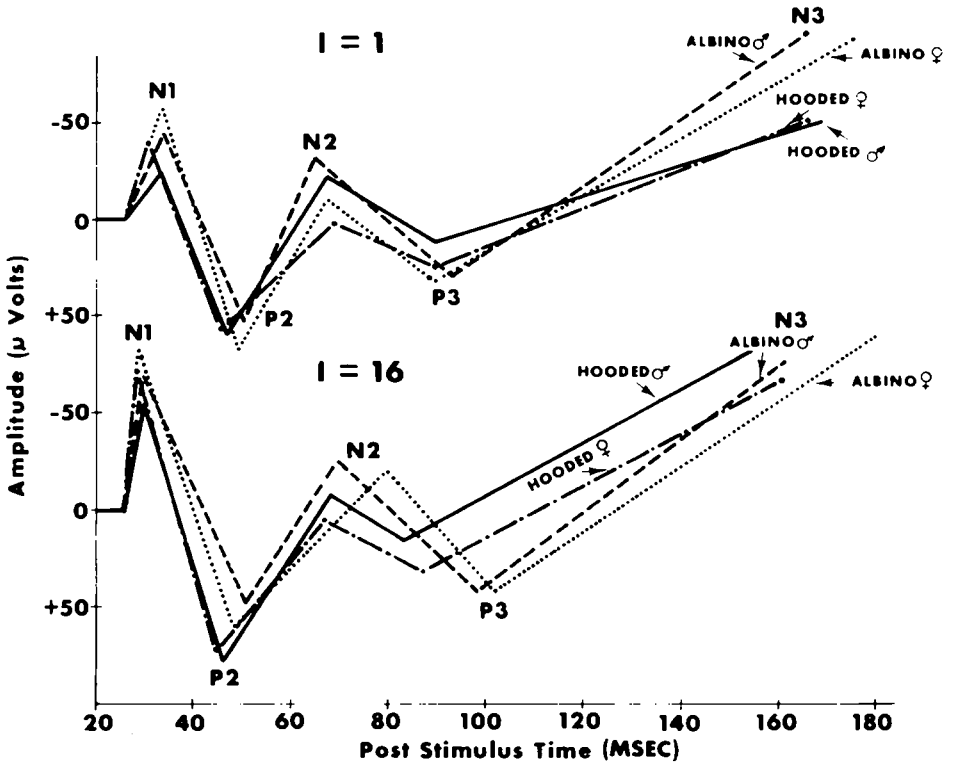

FIG. 1. Composite mean flash evoked responses from visual cortex of male and female albino and hooded rats at the highest (I=16) and lowest (I=1) intensities studied. Negative deflections are upward. Peaks are labeled according to common usage [12], as used in this study.

at each of 4 different flash intensities. The flashes were derived from a Grass strobe unit, and the flash intensities corresponded to settings 1, 2, 4 and 16 or roughly 9.4×10^4, 1.9×10^5, 3.8×10^5 and 1.5×10^6 candlepower. All flashes at a given intensity were completed before the intensity was changed, and the different intensities were presented in counterbalanced order across animals.

Details of the evoked potential measurement and nomenclature used may be found elsewhere [12]. The 5 major positive and negative peak latencies were evaluated as were the 5 major peak-to-peak amplitudes. The analysis performed upon each of these 10 variables was an unweighted means 3 factor (group × sex × intensity) design with repeated measures on the intensity factor. Probabilities less than 0.05 were considered statistically significant. Post hoc comparisons were made using the Scheffe Test.

RESULTS

The effects of the 2 exposures have been reported elsewhere [13,14] and will not be considered here. Figure 1 shows the mean amplitudes and latencies of albino and hooded male and female rats at the highest (I=16) and lowest (I=1) intensities studied. The peaks are labeled according to the designations used in this study.

Figure 2 illustrates the influence of sex upon amplitudes of the visual evoked potentials, averaged across the different light intensities. Females had larger P1-N1, N1-P2 and P3-N3 amplitudes than males, and males had larger P2-N2 and N2-P3 amplitudes than females. The analysis of variance revealed that the differences in P1-N1 amplitude and N1-P2 amplitude were statistically significant. The only significant overall latency difference between males and females was found at the N1 peak, where males had significantly longer latency (32.0 msec ± 0.2 SEM) than females (30.8 msec ± 0.2 SEM).

Figure 3 indicates the variation in evoked potential amplitudes as a function of strain. In all cases the albinos had larger amplitude evoked potentials than the hooded rats, but only in the case of the P1-N1, N2-P3 and P3-N3 components was this difference statistically significant. The differences in peak latencies between the two strains are shown in Fig. 4. The albino rats tended to have longer peak latencies than the hooded rats, but these differences were only significant at Peaks P2 and P3.

Increasing flash intensity produced variations in most evoked potential amplitudes. Only in the case of the P2-N2 amplitudes were these differences not significant. The P1-N1, N1-P2 and P3-N3 amplitudes increased with increasing flash intensity, while the N2-P3 amplitudes dropped slightly at I=4, but increased at I=16.

The variations in peak latency with increasing flash intensity are presented in Table 1. Only the changes observed in the N1, N2 and P3 peaks were significant. The N1 latencies decreased with increasing stimulus intensity, the N2 latencies increased at the highest intensity and the P3 latencies decreased at I=4, but increased at I=16.

The changes which occurred in evoked potential parameters with increasing flash intensity were parallel in the males and females tested. Thus there were no significant sex

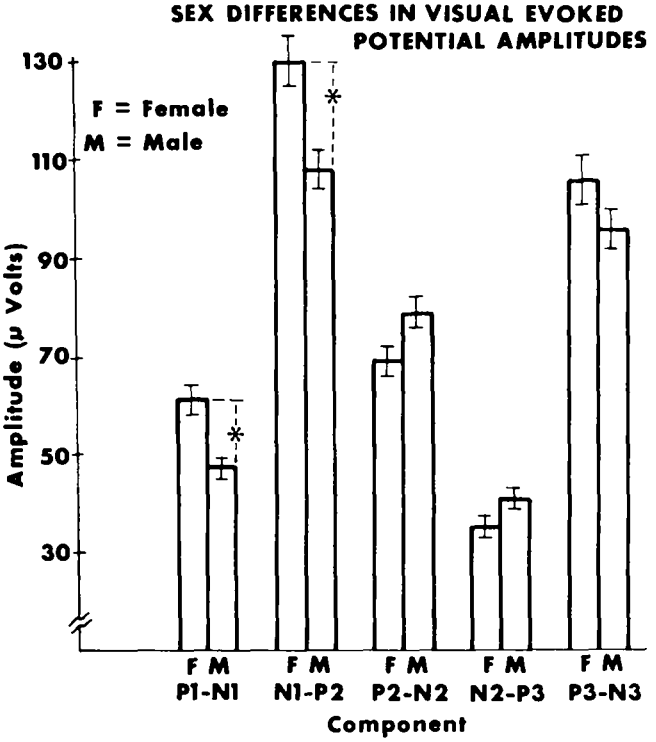

FIG. 2. Mean peak-to-peak flash evoked potential amplitudes, ±SEM, of male and female rats. *$p<0.05$. The figure illustrates the main effect of sex, and is averaged across intensity and strain.

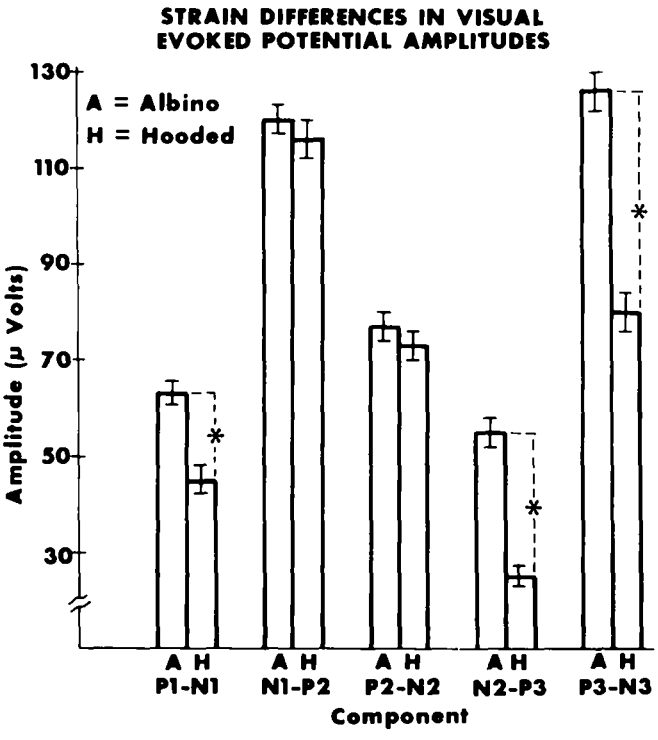

FIG. 3. Mean peak-to-peak flash evoked potential amplitudes, ±SEM, of albino and hooded rats. *$p<0.05$. The figure illustrates the main effect of strain, and is averaged across intensity and sex.

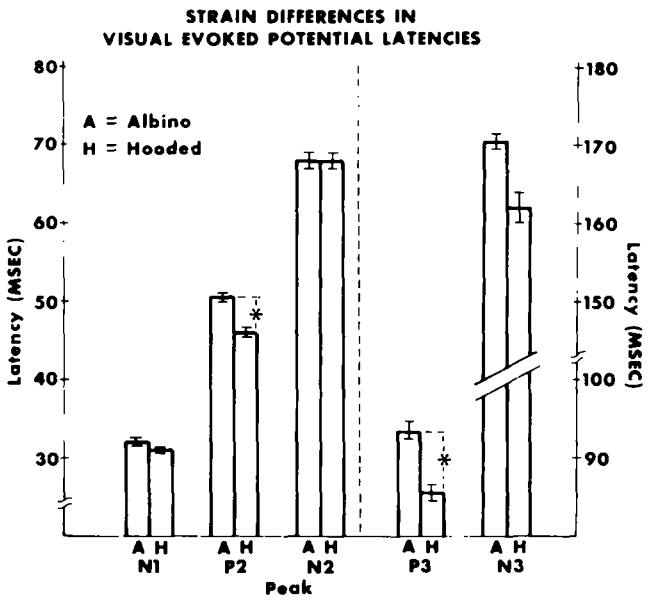

FIG. 4. Mean peak flash evoked potential latencies, ±SEM, of albino and hooded rats. ±$p<0.05$. The figure illustrates the main effect of strain, and is averaged across intensity and sex.

TABLE 1

EFFECTS OF FLASH INTENSITY UPON EVOKED POTENTIAL PEAK LATENCIES*

	Intensity 1	2	4	16
N1	32.8 ± 0.3	32.0 ± 0.3	31.4 ± 0.3	29.7 ± 0.2
P2	48.3 ± 0.4	48.4 ± 0.5	48.3 ± 0.5	47.7 ± 0.5
N2	67.1 ± 1.2	67.6 ± 1.2	66.3 ± 0.9	70.6 ± 1.7
P3	90.3 ± 1.7	88.8 ± 1.6	86.3 ± 1.6	91.2 ± 1.7
N3	169.1 ± 2.1	165.6 ± 3.1	162.4 ± 2.6	165.3 ± 2.2

*All values are expressed as msec ± SEM, and are the means collapsed across the sex and strain variables.

by intensity interactions observed in any of the 10 analyses of variance. There were no significant interactions between sex and strain with any of the evoked potential parameters measured.

Variations in the evoked potential parameters produced by increasing flash intensity were strain dependent. Figure 5 depicts the influence of strain upon the changes in amplitude of the P1-N1, N1-P2, N2-P3 and P3-N3 amplitudes produced by increasing flash intensity. There was a significant interaction in the case of the N1-P2 amplitudes. Hooded rats increased at a greater rate than did the albinos at higher intensities.

Peak latencies of flash evoked potentials recorded from albino and hooded rats were also differentially affected by changes in flash intensity. Figure 6A shows the statistically significant N1 and N2 strain by intensity interactions. The N1 latencies of both albino and hooded rats decreased with increasing flash intensity, but the albino latencies decreased at a more rapid rate. The albino N2 latencies remained relatively unchanged at low stimulus intensities, but increased at

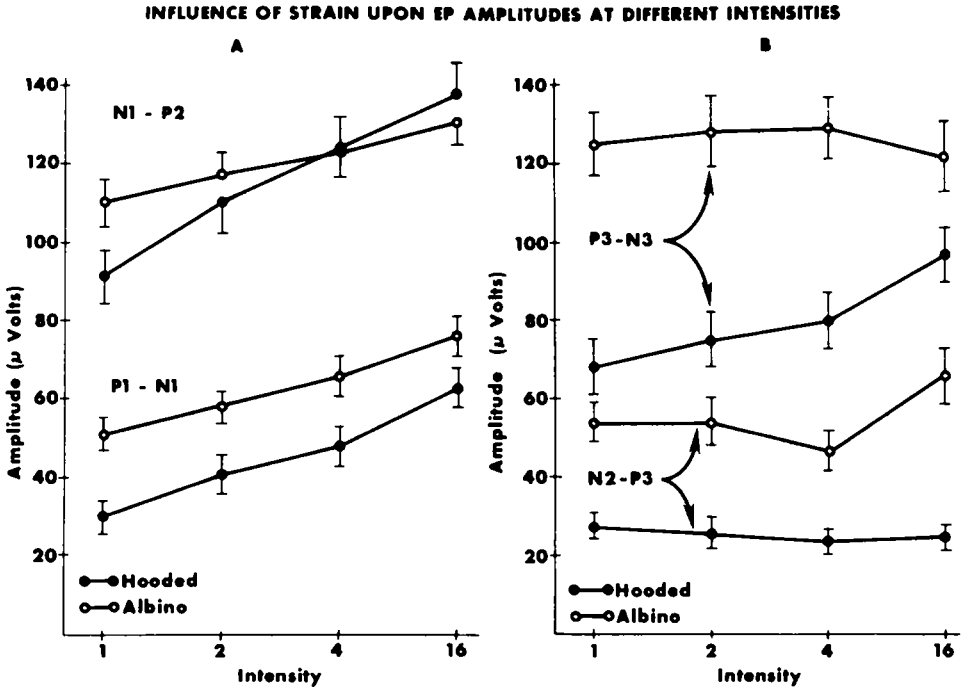

FIG. 5. Mean peak-to-peak flash evoked potential amplitudes, ±SEM, of albino and hooded rats at different flash intensities. (A) P1-N1 and N1-P2 amplitudes. (B) N2-P3 and P3-N3 amplitudes. The N1-P2 strain × intensity interaction was statistically significant. Flash intensity $1=9.4\times10^4$ candlepower (cp), $2=1.9\times10^5$ cp, $4=3.8\times10^5$ cp and $16=1.5\times10^6$ cp.

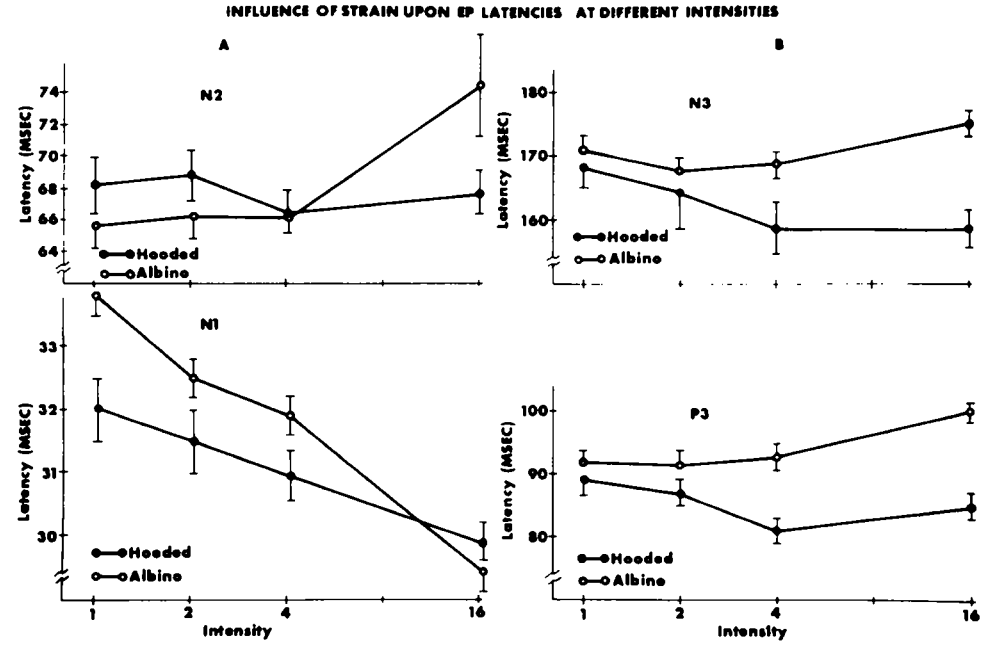

FIG. 6. Mean peak flash evoked potential latencies, ±SEM, of albino and hooded rats at different flash intensities. (A) N1 and N2 latencies. (B) N3 and P3 latencies. The N1, N2 and P3 strain × intensity interactions were statistically significant. Intensities are as indicated in Fig. 5.

the highest intensity. The N2 latencies of hooded rats decreased slightly at high stimulus intensities. There were no significant P2 interactions between strain and intensity. Figure 6B demonstrates the significant P3 strain by intensity interaction. The albino rat latencies increased at high stimulus intensities, while the hooded rat latencies decreased at high stimulus intensities.

DISCUSSION

Clear sex differences were found in the amplitudes of flash evoked potentials in this study. Although this is perhaps not surprising, there are several points which should be made about the finding. First, it is of particular interest that the increased amplitude of early components in females compared to males is the same finding that has been reported in humans [3]. In recordings taken from humans there is some concern that the finding may be related to skull thickness (but see [3]), while in the present experiment this cannot be the case since in all animals the stainless steel screw electrodes penetrated the skull. Indeed, if the findings were related to thinner skulls in females than males, then all components of the evoked potential should be increased, not just the early ones.

The most likely explanation for differences in evoked potentials between males and females is that they are secondary to hormonal influences. There is considerable support for the suggestion that gonadal hormones may influence evoked potential parameters. Estrogen has been shown to increase the amplitude of auditory evoked potentials in the cat [11] and visual evoked potentials in the frog [22]. Neonatal administration of estradiol to rats increases the amplitude of their transcortical evoked responses [9]. However, there is another possibility which may account for the sex differences. There is good evidence that in humans the larger visual evoked potentials observed in females are not mediated by gonadal hormones. First, the differences exist before puberty, and secondly they have been observed in 45 XO karyotype patients with gonadal dysgenesis who were removed from estrogen replacement therapy 30 days prior to testing [3]. Although it is not possible to determine from the present experiment which of these explanations is the most likely in the case of rats, some of the data do indirectly bear upon the issue. As the female hormonal cycle was not monitored in this study, the evoked potential measurements must have occurred during different phases of the cycle in different rats. If the differences between male and female rats evoked potentials were related to the presence of one of the cyclic gonadal female hormones, the results obtained from the females should be more variable than those from the males. To test this possibility the coefficients of variation (SEM/mean$\times$100) were calculated for the females at each of the 4 different flash intensities at each of the two amplitudes which differed significantly from the males. The resulting 8 coefficients were then compared to corresponding coefficients derived from the males by using the Wilcoxon matched pairs signed rank test. The resulting T value of 3 indicated, that for the eight pairs, the probability that the females were not more variable than the males was <0.02. This increased variability in the P1-N1 and N1-P2 amplitudes may be taken as indirect support for the notion that the differences in amplitude between the two sexes are related to gonadal hormones. It was not the case that all amplitudes and latencies were more variable in females than males. A separate Wilcoxon test was performed using as pairs the coefficients of variation of each sex determined for each amplitude and each latency but collapsed across intensity, and under these conditions no significant difference was observed between males and females. The results thus indicate that there are clear differences between male and female early component amplitudes, and that these differences are correlated with an increased variability in the responses of females. The results do not suggest any sex differences in amplitude or variability of late components, nor do they suggest any sex differences in latency variability.

There are currently no data to suggest that the sex differences in latency are due to anatomical factors. For example, it is not likely that the observed difference in N1 latency between males and females is based on a longer conduction distance in the males. The fiber diameters of the rat optic nerve have been estimated at between 0.6 and 8.0 μ [15,21]. Extrapolating from the data of Hursh [18] and Bement and Olson [1], a multiplication factor of about 4.7 is appropriate for 4.0 μ diameter fibers to give the conduction velocity, which would thus be in the range of 15–20 M/sec for fibers of median size in the rat optic nerve. With these conduction velocities it would be necessary to have a conduction distance difference between males and females of 15–20 mm in order to have a 1 msec shorter latency in the females. A difference of such magnitude is possible but unlikely. In the absence of the necessary anatomical differences, it seems most likely that the sex differences in N1 latencies are secondary to altered excitability induced by humoral factors. Females have a higher resting level of corticosterone secretion than males [8], and cortisol levels may significantly affect neuronal excitability [10]. Thus, the shortened latency of the females in this study may be a result of their increased secretion of corticosteroid. In spite of the reservations mentioned above with regard to the cyclic gonadal hormones, it is also possible that the N1 latency difference is due to estrogens. Estrogens have been shown to increase excitability in a number of neuronal systems [19], and they may do so in the rat visual system.

The strain differences in latencies of the visual evoked potential peaks were similar to those described by Creel *et al.* [5]. In that study, statistical analyses were performed only upon the latency data at one stimulus intensity, but it was found that albinos had longer latency P1, N1, P2, N2 and P3 peaks than did hooded rats. In the present experiment the albinos also had longer latencies than did the hooded rats, but the overall difference was only significant for the P2 and P3 peaks. Significant strain $\times$ intensity interactions revealed that the albinos also had longer N1 and N2 peak latencies, but that these differences were only manifested at high flash intensities.

The Creel *et al.* [5] study did not find any strain differences in evoked potential amplitudes. Amplitudes are more variable between animals than are latencies, and for this reason a larger number of animals per group is required to demonstrate statistically significant group differences. The small numbers of animals used by Creel *et al.* [5] may have precluded demonstration of amplitude differences. In a later study by the same group, in which amplitudes of evoked potentials recorded from both albino and hooded rats were reported in tabular form [6], differences, although not statistically evaluated, were in the same direction as those reported here.

In general terms the strain differences may be characterized as follows. Albino rats have higher amplitude and longer latency responses than hooded rats. As flash intensity

is increased, the rate of change in the evoked potential amplitudes of albino rats is less rapid than in hooded rats. With the exception of N1, albino rat peak latencies generally increase with increasing stimulus intensity, while hooded rat peak latencies generally decrease with increasing stimulus intensity. No simple explanation is available to account for these differences between albino and hooded rats.

The most important conclusions of this study may be summarized as follows. There are clear sex differences in the evoked potentials of rats which parallel those seen in humans, except the rat sex differences may be due to gonadal hormones, while the human differences probably are not. There are also clear strain differences in both amplitude and latency of the flash evoked potential. Albino and hooded rats do not respond in parallel fashion to changes in flash intensity. These findings have important implications for studies using evoked potentials as either a pharmacologic or toxicologic tool. The generally steeper flash intensity-effect functions of hooded rats make them a more attractive candidate than albinos for pharmacologic manipulation. Since the albinos are less sensitive to change induced by altered flash intensity, the only reasonable inference is that they are also likely to be less sensitive to change induced by other variables.

Based on this study it is not possible to recommend a particular sex as preferable for studies of CNS function using the visual evoked potential technique. There were no significant sex by intensity interactions, thus indicating that both sexes responded in a similar way to altered flash intensity. It is not sufficient to rule out studies involving females because of the increased variability associated with the estrous cycle. In spite of this increased variability, females have been shown to be more sensitive to change induced by some toxicants than males [13]. Nor is it possible to argue against using males, since exposure to other toxicants has indicated that they may be more sensitive than females [14]. Thus, the most sensible tactic is to use both males and females of the hooded rat strain for studies of drug or toxicant induced change in the visual evoked potential.

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