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Are the Differential Effects of Chloral Hydrate on Hooded Rats vs. Albino Rats Due to Pigmentation or Strain Differences?

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SISSON, D. F., J. SIEGEL AND I. S. WESTENBERG. *Are the differential effects of chloral hydrate on hooded rats vs. albino rats due to pigmentation or strain differences?* PHARMACOL BIOCHEM BEHAV **39**(3) 665–670, 1991.—Effects of chloral hydrate anesthesia on EEG power spectra and VEP components were examined as a function of both pigmentation and strain differences in rats. Ten albino Westenberg Long Evans rats (WLE A) were compared to ten pigmented Westenberg Long Evans rats (WLE P), and to ten Wistar albino (Wis A) rats. Albino rats required less chloral hydrate to reach a deep level of anesthesia than pigmented rats. Wistar rats remained anesthetized longer than WLE rats. During deep levels of anesthesia, the lowest EEG frequency band contained more power in Wistar rats than in WLE rats. During moderate levels of anesthesia, frequencies less than 4 Hz lost power while frequencies greater than 13 Hz gained across all rats. Wistar rats had more power in the frequencies less than 8 Hz than did WLE rats; pigmented rats had more power in the frequencies greater that 13 Hz than did albinos. VEP component latencies of pigmented rats were shorter than albinos. Component amplitudes were not significantly different between groups.

EEG spectral analysis Visual evoked potential Anesthesia Chloral hydrate Pigmented vs. albino Rat

DIFFERENCES between VEPs in pigmented vs. albino strains of rat have been observed both for flash (6,12) and patterned (4) VEPs. These differences in VEPs are not surprising due to the anatomical differences in the visual pathway (7,15). This paper seeks to differentiate between the effects of strain differences and the effects of pigmentation differences on VEPs. WLE rats were used because of the presence of both pigmented (hooded) and albino individuals in this strain. These rats were contrasted to Wis A rats, which enabled us to separate the specific, single-gene variable of albinism from the nonspecific, multigene variable of strain. Differences due to strain were isolated by comparing Wis A rats to WLE A rats, and differences due to albinism were isolated by comparing WLE P rats to WLE A rats.

Differences in the responses of pigmented and albino strains of rats to pentobarbital anesthesia have been reported (20) with Sprague-Dawley albino rats being more sensitive to pentobarbital than Long-Evans pigmented rats. Westenberg and Bolam (24,25) demonstrated that this difference in susceptibility to pentobarbital was due to general strain differences and not to albinism per se.

We have previously examined VEPs in acute Wis A preparations under chloral hydrate anesthesia (21). To make the appropriate between-strains and within-strain comparison in the current study, we ascertained the doses of chloral hydrate required to induce anesthesia in WLE rats, and whether a moderate level of anesthesia could be produced during which VEPs were minimally different from those recorded in awake, chronic preparations such as we observed in Wis A rats. We also determined whether WLE rats exhibited the EEG power spectrum that was characteristic of moderate anesthesia levels in Wis A rats [low power in the <4 Hz band, a peak in the 4–8 Hz band and some power in the 13–18 and >18 Hz bands, see (21)]. The contrasts between WLE A and either Wis A rats or WLE P would separate strain from pigmentation differences.

METHOD

Male rats (10 Wis A, 10 WLE A and 10 WLE P) weighing between 340 and 620 g were used in this experiment. Weight differences between groups were not significant. The WLE rats were bred at Glendale Community College during the development of a segregated inbred strain. They were from the 22–24th generation of inbreeding (coefficient of inbreeding = .99). Littermate pairs of WLE A and WLE P rats were used for this experiment to reduce nongenetic differences.

The rats were anesthetized with a combination of ketamine HCl (50 $mg \cdot kg^{-1}$ IM), and chloral hydrate (300 $mg \cdot kg^{-1}$ IP). A tracheotomy was performed, and the right jugular vein was catheterized for subsequent maintenance doses of chloral hydrate. Each subject was placed in a stereotaxic head holder.

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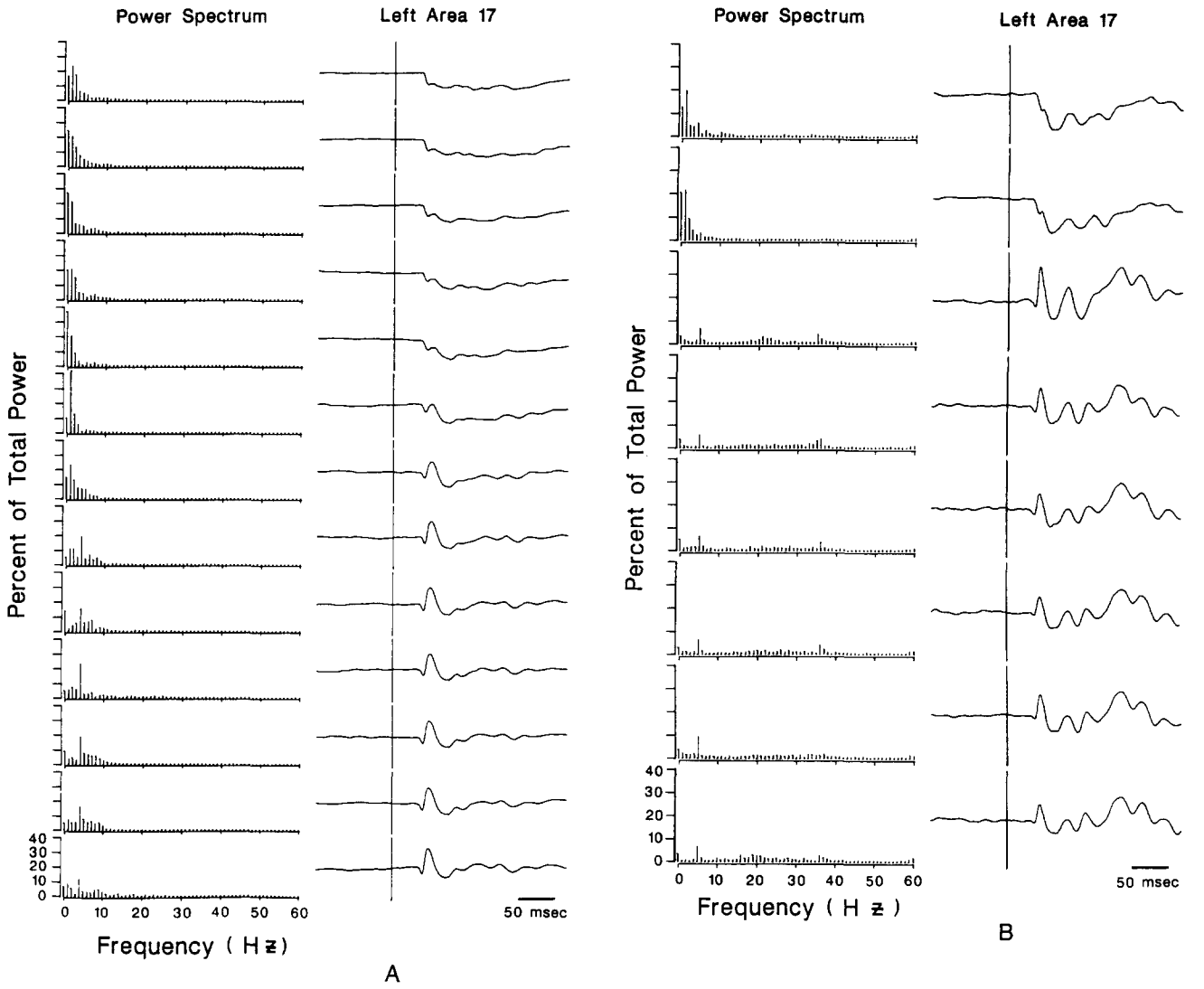

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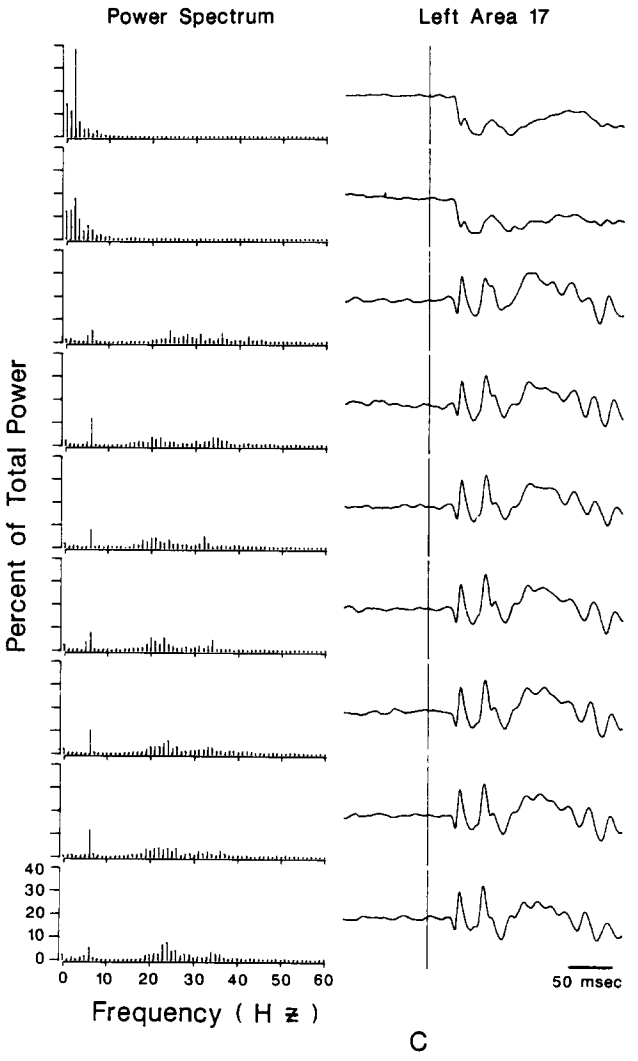
FIG. 1. The effects of anesthetic depth on EEG power spectrum and VEPs in Wis A, WLE A and WLE P rats. In column 1 of these figures are power spectra derived from 4-s epochs of EEG activity. The Y-axes are plotted in units of percent of total power; the X-axes are plotted in units of frequency from 0–60 Hz; each vertical bar represents the relative power in a 1 Hz band. In column 2 are flash VEPs recorded over left OC1. Data in each row were collected at 5-minute intervals starting when the animal was at a deep level of anesthesia and ending when an animal showed signs of requiring more anesthesia. Representative data from a Wis A (A), a WLE A (B) and a WLE P (C) rat are shown (see Fig. 4 for component labels).

Screws were threaded into the skull over both visual cortices (2.0 mm anterior to lambda and 4.0 mm lateral to the sagittal suture) to record VEPs, and over the cerebellum to ground the preparation. A stainless-steel insect pin was embedded in dorsal neck muscle as an indifferent electrode.

Pupils were dilated with a 0.5% solution of tropicamide and cornea were kept moist with a wetting solution. Body temperature was maintained between 37.8 and 38.0°C. Heart rate, EEG and expired carbon dioxide were monitored as indications of the animal's state. Except for the initial ketamine HCl prior to surgery, only chloral hydrate at a concentration of 100 $mg \cdot ml^{-1}$ was administered via the jugular catheter to maintain adequate levels of anesthesia.

EEG activity was recorded from the cortical screw over left visual cortex (OC1). Immediately preceding each block of 50 flashes, 4096 samples of cortical activity were taken at a 1 kHz sampling rate. The power spectrum of this 4 s epoch of EEG activity was divided into 6 bands: <4 Hz, 4–8 Hz, 8–10 Hz, 10–13 Hz, 13–18 Hz, and >18 Hz.

VEPs were evoked using a Grass photostimulator at intensity 4 with the flash tube in a sound-attenuating box positioned 25 cm in front of both eyes of the subject. This arrangement provided binocular stimulation with a luminance of 2.36×10^5 lux. EEG was filtered to pass frequencies between 0.1 and 300 Hz. Evoked activity was digitized at a sampling rate of 1 kHz for 100 ms prior and 250 ms following each flash and averaged over 50 successive light flashes at a stimulus rate of one flash per second using a computer (IBM AT) with 12-bit analog-to-



digital converters (Data Translation).

Following surgery, each animal was allowed to reach an anesthetic level at which a booster dose of anesthetic was required. The onset of spontaneous whisker movements were the criterion for this anesthetic level (21). Chloral hydrate was then administered IV in 0.1 ml (10 mg) doses until the subject exhibited an EEG with most of its power in the <4 Hz band which is indicative of a deep anesthetic state (21). Starting at this point, EEG and VEPs were recorded at five minute intervals until the animal again required more anesthesia per the whisker-twitch criterion. A cluster analysis (26) of power in the spectral bands of EEG epochs collected during the recovery from chloral hydrate administration divided the recovery time into deep and moderate states of anesthesia. A repeated-measures ANOVA (26) was used to examine the effects of strain, pigmentation and state on anesthetic dose required to induce a deep level of anesthesia and on EEG spectral power and the effects of strain and pigmentation on VEP component amplitudes and latencies. Differences due to strain were isolated by comparing the Wis A and WLE A rats; differences due to albinism were isolated by comparing WLE P and WLE A rats.

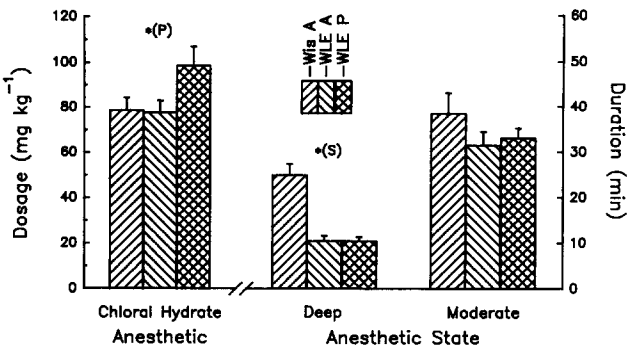


FIG. 2. The mean minimum dose of chloral hydrate necessary to induce a deep level of anesthesia in the three groups of rats (left Y-axis) and the duration of the subsequent deep and moderate anesthetized states (right Y-axis). WLE A rats require significantly less chloral hydrate ($p<0.05$) then did the WLE P rats. There is no difference in the dosage required by Wis A and WLE rats, yet Wis A rats remained in a deep state significantly longer ($p<0.01$). In this and all subsequent figures, an asterisk (*) denotes a significant difference among the three groups of rats. When the asterisk is followed by a "P," the difference is due to pigmentation (WLE P vs. WLE A); when the asterisk is followed by an "S," the difference is due to strain (Wis A vs. WLE A).

RESULTS

Chloral Hydrate Anesthesia: Strain and Pigmentation Differences

Figure 1 shows the time course of chloral hydrate anesthesia in Wis A (Fig. 1A), WLE A (Fig. 1B) and WLE P rats (Fig. 1C). Just after chloral hydrate administration, most EEG activity (60–70% of total power) was in the low frequency (<4 Hz) band. At this deep level of anesthesia, the amplitudes of VEP positive components were greatly enhanced while the negative components were greatly reduced compared to VEPs recorded in the later (moderate) state of anesthesia (14). With time, EEG activity in the <4 Hz band decreased, and peak power moved to the 4–8 Hz band. VEPs measured at this moderate level of anesthesia exhibit components very similar to those observed in awake, behaving animals (6,12).

The dose of chloral hydrate required to induce deep anesthesia and the mean duration of the deep and moderate levels of anesthesia for the three groups of rats are shown in Fig. 2. The amount of chloral hydrate required to induce deep anesthesia averaged 78.6 $mg \cdot kg^{-1}$ for Wis A, 77.6 $mg \cdot kg^{-1}$ for WLE A and 98.5 $mg \cdot kg^{-1}$ for WLE P. The difference in dosage between Wis A and WLE A rats is not significant; pigmented rats required more chloral hydrate than albino rats, $F(1,27)=4.99$, $p<0.05$. In contrast, there is a difference in the duration of anesthesia between Wis A and WLE A rats, but not between WLE A and WLE P rats. The difference between strains stems from the significant difference in the duration of the deep state [25.0 min for Wis A rats versus 10.5 min for WLE A rats, $F(1,27)=38.10$, $p<0.01$]; differences in the duration of the moderate state are not significant either for strain or pigmentation. The interaction between state and EEG power spectrum is highly significant, $F(5,135)=82.71$, $p<0.001$, as expected. As can be seen in Fig. 3, the <4 Hz band lost power between deep and moderate states, $F(2,27)=3.79$, $p<0.05$, and the 13–18 and >18 Hz bands gained power, $F(2,27)=7.95$, $p<0.01$; $F(2,27)=6.00$, $p<0.01$, respectively. In the deep state, Wis A rats had signifi-

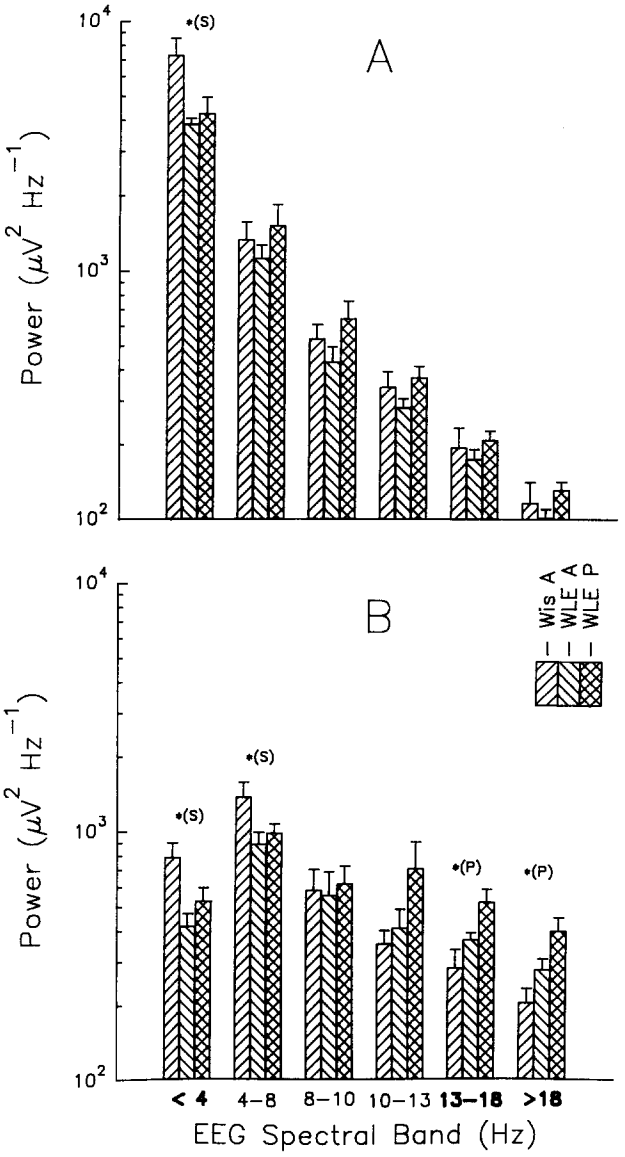


FIG. 3. The distribution of power across the different EEG spectral bands for the three different groups of rats in deep (A) and moderate (B) levels of anesthesia. There is a significant effect of state on the power in these bands ($p<0.001$); specifically a change in power of the <4 Hz frequency band and the frequency bands above 13 Hz as indicated by the boldface labels.

cantly more power in the <4 Hz band than did WLE A rats, $F(1,27)=8.03$, $p<0.01$. Significant between-strains effects are found in the moderate state; the <4 and 4–8 Hz bands contained more power for Wis A rats than WLE A rats, $F(1,27)=9.89$, $p<0.01$, and $F(1,27)=5.84$, $p<0.05$, respectively. Differences between pigmentation genotypes in the moderate state are significant in the 13–18 and >18 Hz bands with WLE P rats having more power in these bands than WLE A rats, $F(1,27)=4.51$, $p<0.05$ and $F(1,27)=4.61$, $p<0.05$, respectively.

VEPs Under Chloral Hydrate: Strain and Pigmentation Differences

The effects of chloral hydrate on amplitudes and latencies of

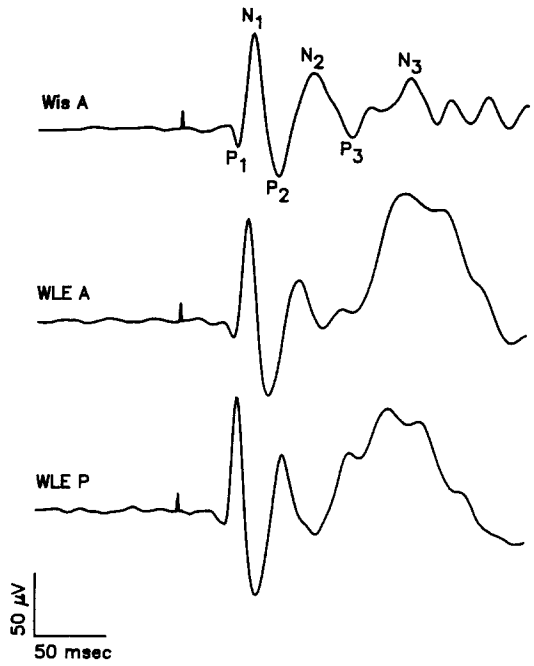


FIG. 4. Group average VEPs for the three types of rat used in this study. Each trace has a 100 ms baseline interval taken just prior to a light flash (shown by the artifact, the first upward deflection in each trace) and 250 ms of evoked activity. To calculate these group averages all VEPs taken from a rat during moderate anesthesia were averaged to produce a grand average VEP for each rat; these VEPs were then averaged across the rats of each type.

the major VEP components are analyzed for differences due to strain and pigmentation. Fig. 4 shows these components for the three types of rats. The components after N_2 were not analyzed, because these late components are quite variable and Dyer (10) has shown that past visual experience is a major factor influencing these components. The overall differences between the component amplitudes for rats (Fig. 5A), whether grouped by strain or pigmentation, are not significant. There are significant differences in the stimulus-to-peak latencies of VEP components (Fig. 5B); pigmented rats have significantly shorter latencies for all components than albinos, $F(1,27)=8.63$, $p<0.01$ for P_1; $F(1,27)=14.66$, $p<0.01$ for N_1; $F(1,27)=9.64$, $p<0.01$ for P_2; $F(1,27)=30.13$, $p<0.001$ for N_2. Strain has a significant effect on the latency of P_2, $F(1,27)=7.34$, $p<0.05$, with Wistar rats having the longest latency.

DISCUSSION

Chloral Hydrate Anesthesia: Strain and Pigmentation Differences

The above results demonstrate that Wistar and Long Evans rats respond differently to chloral hydrate anesthesia. Some of these differences can be attributed to strain while others are due to pigmentation. Albino rats required less anesthesia to reach a deep state from a common light level of anesthesia than did pigmented rats. One possible reason for this difference could be that some drugs bind to melanin in pigment granules (5, 8, 27). If chloral hydrate were such a drug, the results reported here sug-

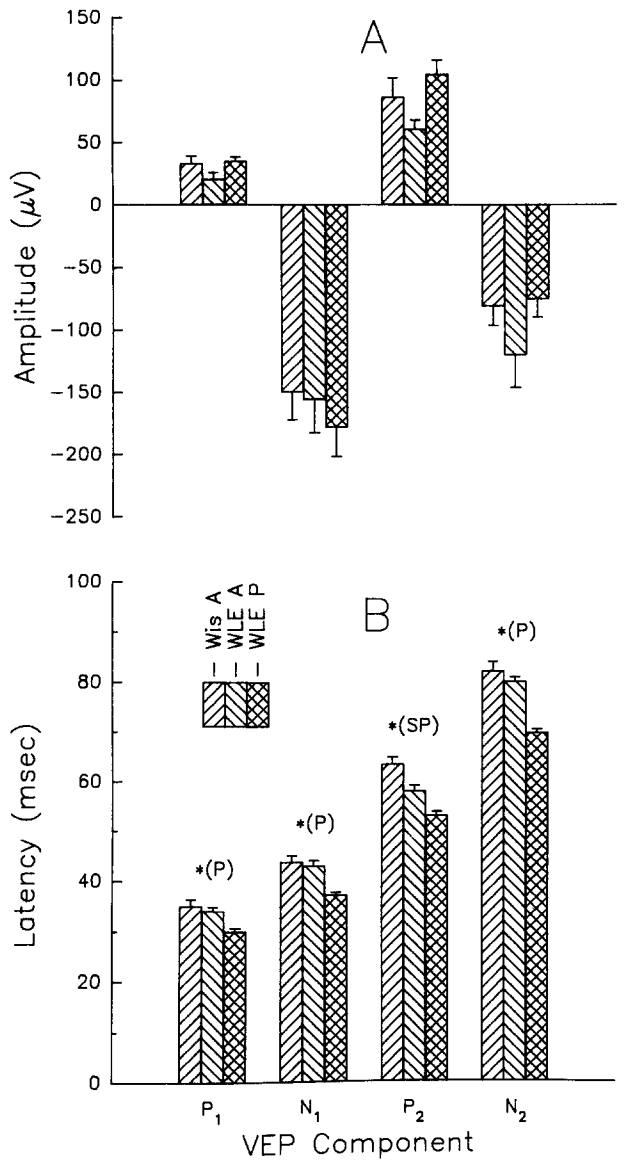


FIG. 5. VEP component amplitudes (A) and latencies (B) for the three groups of rats. Components with positive peaks are shown with amplitudes above the zero voltage line; negative peaks are shown below zero voltage. Latencies of each component are measured from the flash artifact.

gest that such binding requires intact melanin.

Wistar rats remained in the deep state longer than did WLE rats; Wistar rats also remained in the moderate state longer, although the increased duration was not statistically significant. These differences in state duration are probably indicative of differences in the rate of chloral hydrate catabolism between the two strains. If the increase in dosage required by WLE P rats stems from sequestration of chloral hydrate by melanin-containing pigment granules, then the lack of difference in state durations between WLE A and WLE P rats indicates that sequestered chloral hydrate is not returned in an active form.

Because similar findings exist for pentobarbital (20, 24, 25), dosage of any anesthetic should be determined not simply in terms of $mg \cdot kg^{-1}$ but rather in terms of some measure responsive to anesthetic depth such as EEG power spectrum analysis. In studies that use identical doses of anesthesia with different strains, the animals could be at different levels of anesthesia confounding any between-strains comparisons.

The similarity of the between-strains effects of chloral hydrate and pentobarbital (20) suggests that studies of genetically different subjects (i.e., rats of different strains) might elucidate the mechanism(s) of operations of these drugs. The similarities between WLE A and WLE P rats in terms of anesthetic states induced by chloral hydrate indicate that the mutant gene at the c locus that produces albinism is not, by itself, responsible for the between-strains difference.

VEPs Under Chloral Hydrate: Strain and Pigmentation Differences

Significant differences were found between the stimulus-to-peak latencies of VEP components of the three types of rats used in this experiment. In agreement with Dyer and Swartzwelder (12) and Creel, Dustman and Beck (6), VEP components of albino rats had longer latencies than those of pigmented rats. When our data are reanalyzed using peak-to-peak latency, the latency between P_1 and N_1 remains significantly longer in albino rats while the latencies between the later components were no longer affected by pigmentation. We, also, found significant between-strains differences between P_2 and N_2 latencies. The differences between stimulus-to-peak and peak-to-peak latency indicate that the long latency to N_1 in albinos depends on factors that are independent of those that affect the latency of P_1. The longer latency to P_2 and N_2 in albinos, on the other hand, is a direct result of the latency shift exhibited by the early components.

These results suggest that comparisons between VEP component amplitudes and latencies of Wis A, WLE A and WLE P rats are complex with differences in strain and differences in pigmentation both affecting VEPs. When the number of synapses and the length of the transmission pathway are constant, differences in latency are commonly attributed to differences in fiber diameter and myelination. While the range of fiber diameters in the optic nerve of albino rats is the same as that in the optic nerve of pigmented rats, the mean fiber diameter is smaller in albinos. Optic nerve fibers of albino rats are also less myelinated than optic nerve fibers of pigmented rats (13,22). Both of these differences would increase transmission time from retina to cortex and, therefore, explain at least part of the increased latency of the early VEP components of WLE A rats relative to WLE P rats.

Biochemical differences can also affect latency. A possibility exists that a difference in retinal dopamine between albino and pigmented rats can influence P_1 latency. Dopamine depletion, whether by systemic injection of drugs that block synthesis (11,18) or in patients with Parkinson's disease (2,3), slows retinal transmission. The mechanism of dopamine's effect stems from the role dopamine plays in the light-adaptation of the retina (9, 16, 19); namely, dopamine appears to drive most of the retina's light-adaptive responses. Without dopamine the retina remains in a partially dark-adapted state with its relatively slow responses to light (1).

The density of dopaminergic cells is reduced in retinas of human albinos (23). In rats and mice, however, differences in retinal densities of dopaminergic cells between strains mask any differences that might be due to albinism (17,23). WLE A and

P rats would be a natural choice for use in experiments to separate differences in retinal dopamine due to pigmentation from those due to strain.

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