Postnatal Development of Flash Visual Evoked Potentials in the Jaundiced Gunn Rat¹

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ABSTRACT. In this study, the postnatal development of flash visual evoked potential (VEP) has been monitored in the jaundiced (jj) Gunn rat model of neonatal hyperbilirubinemia to determine whether this evoked response is affected by bilirubin-induced neurotoxicity. VEP could first be recorded at 16 d of age. The jj rats exhibited prolonged wave latencies and lower wave amplitudes during the 3rd wk of postnatal life, when compared with their nonjaundiced littermates. There was no correlation at 21 d of age between VEP parameters and either bilirubin levels or body weight. About one third of the jj animals died between 21 and 28 d of age. The average VEP wave latencies at 21 d of age of the rats who were to die was prolonged compared with those of rats who survived till at least 28 d of age. Thus, the latency of VEP waves at the age of 21 d appears to be related to the further outcome of jj Gunn rats. Although wave amplitudes were lower in jj as compared with nonjaundiced 21-d-old animals, there were no amplitude differences between the jj rats who would die and those who would survive during the 4th wk of life. These findings may contribute to the understanding of the pathogenesis of bilirubin encephalopathy in the neonatal period. (*Pediatr Res* 30: 469–472, 1991)

Abbreviations

VEP, visual evoked potential
ABR, auditory nerve and brain-stem response
jj, jaundiced
Jj, nonjaundiced

Bilirubin, a neurotoxic degradation product of Hb, enters the brain after excessive elevation of its levels in blood, and, in connection with complications such as hypoxia and acidosis, it may cause various degrees of brain damage. The resulting condition, bilirubin encephalopathy, leads to the impairment of motor, sensory, and behavioral functions. The pathogenesis of bilirubin encephalopathy has not been so far fully elucidated. The severe nature of the neurologic sequelae of neonatal hyperbilirubinemia has stimulated the search for reliable and accurate means of following the kinetics of bilirubin entry into the brain, predicting the nature and extent of the subsequent damage caused by this neurotoxin, and preventing this neurotoxicity by

appropriate therapeutic intervention. The availability of a functional marker capable of detecting early and, hopefully, reversible shifts of bilirubin from blood to brain would be of great value in preventing even the minimal manifestations of neonatal brain damage, e.g. learning disabilities and behavioral deficits (1–3).

The ABR has been studied in human neonates with hyperbilirubinemia because hearing loss is one of its sequelae (4–7). Reversible as well as permanent changes in ABR patterns have been observed in conjunction with neonatal jaundice (8–12). Much of the experimental research in this field has been conducted on the homozygous recessive jj Gunn rat model (13–16). This mutant of the Wistar rat develops severe congenital hyperbilirubinemia immediately after birth as a result of its inability to conjugate bilirubin with glucuronic acid, which is the normal pathway for excretion of this neurotoxin in the Jj animal. Some jj Gunn rats die before reaching adulthood, and the survivors exhibit clear signs of bilirubin encephalopathy.

Shapiro and Hecox (17) have recently studied the development of ABR in Gunn rats. The ABR abnormalities observed in jj Gunn rats by these authors were minimal. Because these and similar ABR studies conducted in other animal models of neonatal hyperbilirubinemia (18) have not yielded uniform and conclusive results, we have attempted to assess an additional developing neural pathway, the visual pathway.

The present report describes the postnatal development of the flash VEP in jj and Jj Gunn rats. The VEP, which is generated in the visual cortex, has been extensively used for detecting both short- and long-term effects of the perinatal exposure to neurotoxic chemicals (19–21). Our results suggest that VEP may be of value in assessing the severity of bilirubin-induced neurotoxicity.

MATERIALS AND METHODS

Animals. jj and Jj Gunn rats were obtained from our colony at the Hebrew University-Hadassah Medical School. Female Jj rats were mated with jj males, and the resulting litters contained about the same numbers of jj and Jj pups. The offspring were kept with the mother for 4 wk at a constant room temperature of 23–25°C and a 12 h light-12 h dark cycle and were fed rat food containing 11% fat (Weizmann Institute, Rehovot, Israel) and water *ad libitum*.

VEP. Animals were weighed and anesthetized by ether inhalation. Body temperature was maintained at 36–37°C with a heating pad and monitored with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). The VEP was recorded with a specially adapted Micro-Shev C-ERA 100 evoked response system (Micro-Shev, Efrat, Israel). The VEP was elicited by high intensity ($\times 16$) flashes delivered by a Grass PS-2 photostimulator (Grass Instruments, Quincy, MA) triggered by the evoked response system at a rate of 0.5/s. Flash duration was 10 μ s. The flash lamp was placed at a distance of 15–20 cm from the animal's head. The electrical activity was recorded by means of subdermal needle electrodes (Grass Instruments) placed at the occipital region and chin, with a ground electrode placed at the

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hind limb, filtered (band pass 0.8–30 Hz), amplified, and averaged ($n = 64$). Duplicate VEP were recorded in response to flash stimuli and compared with a control recording obtained by shielding the lamp. Typical VEP recordings showing waves P_1 , N_1 , and P_2 are presented in Figure 1. The average latencies and amplitudes of each of these waves were calculated.

Plasma bilirubin. Plasma bilirubin was determined by the method of Michaelsson (22).

Results are presented as mean $\pm$ SD, and statistical evaluation was performed using t test and repeated measures analysis of variance.

RESULTS

Experiment 1: development of VEP. A group of 12 jj and 12 Jj pups, originating from four litters, were examined at 16, 18, 21, and 28 d of age and compared with 12 jj and 14 Jj adult (3–4 mo old) Gunn rats. Eight jj and 10 Jj rats were alive at the age of 28 d. At this age, the jj rats were significantly smaller (50.6 ± 3.6 g, mean $\pm$ SD) than their Jj littermates (59.5 ± 2.7 g; $p < 0.001$).

Typical VEP recordings obtained between 16 and 28 d of age are shown in Figure 1. VEP could first be recorded on d 16 in nine out of the 12 jj and in nine out of the 12 Jj animals. However, wave P_1 was not present at this time in an additional three jj and two Jj rats and was inconsistently recorded during further development. The latencies of all three VEP waves markedly shortened after d 16 (Fig. 2). In the Jj rats, waves P_1 and N_1 reached their adult latency values by d 21, whereas the latency of wave P_2 continued to decrease beyond this age. In the jj rats, there were significantly longer latencies for waves N_1 and P_2 at 18 d of age and for all three VEP waves on d 21, compared with the Jj rats (Fig. 2). No differences in latency between jj and Jj rats were observed in either 28-d-old or adult animals.

The amplitudes of waves N_1 and P_2 initially increased and later decreased toward the adult values, which were similar to those obtained on d 16 (Fig. 3). Jaundiced rats exhibited lower amplitudes for wave N_1 at 21 d of age and for wave P_2 at 16 and 18 d of age, compared with the Jj rats (Fig. 3).

Experiment 2: prognostic significance of VEP in jj Gunn rats. Retrospective analysis of the data obtained in the developmental

Fig. 1. Flash VEP patterns during postnatal development of a Jj Gunn rat. The VEP was recorded as described in Materials and Methods.

Fig. 2. VEP wave latencies during postnatal development of jj (●) and Jj (○) Gunn rats. Differences between means are statistically significant at: * $p < 0.05$; ** $p < 0.025$; and *** $p < 0.005$.

Fig. 3. VEP wave amplitudes during postnatal development of jj (●) and Jj (○) Gunn rats. Differences between means are statistically significant at: * $p < 0.025$; and ** $p < 0.01$.

study (experiment 1) revealed that three out of the four nonsurviving jj animals died between 21 and 28 d of age. These three animals showed a trend toward longer latency values and lower amplitudes when compared at 21 d of age with the eight jj animals that remained alive for at least another 7 d. We therefore extended the study to a much larger population totaling 34 jj and 32 Jj rats, which were examined at 21 and 28 d of age. Eleven out of the 34 jj rats died during this period. In these 11 rats, the average latencies of waves N_1 and P_2 on d 21 were significantly longer ($p < 0.005$ and $p < 0.01$, respectively) than those observed for the 23 jj rats that were still alive on d 28 (Fig. 4). The latter exhibited the same average latency for N_1 , and a slightly longer latency for P_2 ($p < 0.05$), when compared with the Jj heterozygotes. On d 28, there were no differences in latencies between the Jj and the surviving jj rats (Fig. 4), confirming the earlier results obtained in the developmental study (Fig. 2). The amplitudes of waves N_1 and P_2 were significantly smaller for 21-d-old jj rats as compared with their Jj littermates ($p < 0.01$ and $p < 0.025$, respectively), but there were no differences in amplitude values at this age between the jj rats who would die and those who would survive during the 21–28 d age period (Fig. 5). The 11 jj rats who died between 21 and 28 d of age weighed less on d 21 (23.4 ± 4.0 g) than the 23 jj survivors (32.5 ± 8.4 g; $p < 0.025$), whereas the latter did not differ from the Jj rats (33.4 ± 5.7 g).

To determine whether VEP parameters are correlated with serum bilirubin levels or body weight, a separate group of 48 jj animals was studied on d 21. No such correlations were found. Moreover, in 12 of these rats who died during the 4th wk of life, plasma bilirubin levels (13.46 ± 3.42 mg/dL; 230.17 ± 58.48

Fig. 4. VEP wave latencies as related to the survival of Gunn rats during the 4th wk of life. jj rats that were still alive on d 28 (▨) were compared, on d 21, with jj rats that died between 21 and 28 d of age (▨) and with Jj rats (□). Statistically significant differences between the means of any two of these three groups are indicated on top of the bars. Average latencies on d 28 for groups of animals that survived are statistically different from their latencies at 21 d of age: * $p < 0.01$; and ** $p < 0.001$.

Fig. 5. VEP wave amplitudes as related to the survival of Gunn rats during the 4th wk of life. See Figure 4 for details of animal groups. Average amplitudes are statistically different between 28 and 21 d of age at * $p < 0.025$.

$\mu\text{mol/L}$) did not differ from those of the 36 survivors (13.04 ± 3.13 mg/dL; 222.98 ± 53.52 $\mu\text{mol/L}$).

DISCUSSION

The present study of flash VEP in Gunn rats was designed to assess the effects of neonatal hyperbilirubinemia on the postnatal development of the visual pathway. jj Gunn rats exhibited significantly prolonged average VEP wave latencies and reduced amplitudes when compared with their Jj littermates during the 3rd wk of postnatal life. Moreover, the significance of this finding of VEP disturbances at the time at which plasma bilirubin reaches peak values was further strengthened after analyzing the outcome of jaundiced animals at the age of 28 d. Most of the jj Gunn rats who showed prolonged latencies of VEP waves at 21 d of age died during the 4th wk of life, whereas most of those who survived till at least 28 d of age exhibited on d 21 significantly shorter wave latencies. Thus, the prolonged latencies of VEP waves at the age of 21 d could be considered a sign of neurologic deterioration in rats exposed to neurotoxic serum levels of bilirubin. In contrast, although VEP wave amplitudes were significantly lower in jj compared with Jj 21-d-old Gunn rats, there were no amplitude differences between the jj rats who died and those who survived during the 4th wk of life.

Our developmental VEP study started at the postnatal age of 16 d, when clear VEP recordings could first be obtained in Gunn rats. The patterns displayed by the VEP waves (N_1 , P_1 , and P_2) in our ether-anesthetized Gunn rats were similar to those reported for awake and unsedated Long-Evans rats (23). The developmental changes in the VEP of Gunn rats were similar to those previously reported for rodents and other species (23–26), i.e. as animals matured, the VEP wave components became clearer, their latencies decreased, and their amplitudes initially increased and thereafter decreased. Further research is required to 1) localize the lesion(s) in the visual pathway that is responsible

for the VEP abnormalities observed in this study with young jj Gunn rats; 2) determine whether these VEP abnormalities are indicative of impending death; and 3) elucidate the mechanism by which bilirubin affects the visual and other neural transmission pathways.

A single study of VEP has been conducted so far in two jaundiced human neonates, before and after exchange transfusion for treatment of the hyperbilirubinemia (8). In both cases, the latency of the negative wave component of the VEP shortened after treatment. This finding suggests that at least some of the VEP disturbances observed in either jaundiced human neonates or newborn jj Gunn rats may be reversible and could disappear as soon as bilirubin is removed from blood and cleared from the brain, as is the case after exchange transfusion. In this respect, it is of interest that the wave components of both VEP (this study) and ABR (13, 15) of adult jj Gunn rats are not very different from those of their Jj controls. It is possible that young jj rats with abnormal evoked response patterns die at the earlier stages of development. The survivors, however, may exhibit other signs of bilirubin encephalopathy, such as ataxia, and the VEP or ABR may not reflect them.

It should be pointed out that difficulties with visual perception and gaze (2, 3, 27) have been reported in infants who developed jaundice in the neonatal period. Thus, the present findings of VEP abnormalities in jj Gunn rats may ultimately be of clinical relevance in the management of neonatal jaundice. Additional studies of VEP and other cortical evoked responses (e.g. somatosensory) may shed further light on the pathogenesis of bilirubin encephalopathy and, thus, contribute to improve the early diagnosis and prevention of impending bilirubin-induced brain damage in the neonatal period.

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