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Some Developmental Observations on the Effects of Prolonged Deprivation of Low-voltage Fast-wave Sleep in the Deermouse, *Peromyscus maniculatus bairdi*

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Abstract

Previous electrophysiological work with adult deermice (*P. m. bairdi*) (Mitler & Levine, 1970) indicated that: three states of arousal could be reliably distinguished: low-voltage, fast-wave sleep (LVF), high-voltage, slow-wave sleep (HVS), and waking (W); low-electromyograph (EMG) activity was concomitant with LVF; and time in LVF was radically reduced in mice perched above a shock-grid on a pedestal too small to permit total loss of muscle tonus. A preliminary aim of the present research was to gather electrophysiological data on juvenile deermice to construct some estimate of developmental sleep changes. Electrocorticograph (ECoG) and (EMG) records, from three 20-day-old mice while motionless with eyes closed, also reflected LVF, HVS, and W, but in proportions different from those reported by Mitler and Levine. Juvenile mice showed 1/3 more LVF than did adults. The main goal of this research was to explore the effects of long-term LVF-deprivation on juvenile and adult mice. Litters were selected at either 20 or 53 days of age. Littermates were assigned to one of four 14-day treatment conditions in an Age $\times$ Treatment design with 3 subjects per cell. The 1st treatment, LVF-deprivation, consisted of perching on a pedestal over a shock-grid. The remaining 3 conditions ran simultaneously with the former. Mice in the yoked control condition had approximately equal free movement volume as those mice restricted to the pedestal, and were 'yoked' to those animals with respect to shock. Mice in the sleep deprivation condition were confined to a treadmill in motion 19 of each 24 hr. Finally, mice in the isolated condition were caged alone. After treatment, animals were placed in activity recording cages for 21 days.

Dependent variables included body weight before treatment, after treatment, and after procedure, brain weight, brain/body weight ratios, activity, and regularity of activity for the 21 days posttreatment. Major findings indicated that animals LVF-deprived from 20 to 34 days of age were more active than controls while animals LVF-deprived from 53 to 67 days of age were less active than controls. There were no interpretable differences among treatments in regularity of activity, body weight, or brain weight. Results were discussed in terms of input requirements for CNS development and/or maintenance. Directions for future research were suggested.

Keywords

REM-sleep; low-voltage, fast-wave sleep; deprivation effects; activity; development; circadian activity; deermice

At least 2 states of sleep are distinguishable in humans and many mammals. Based on electrocorticographic (ECoG) (or electroencephalographic [EEG]) records and

electromyographic (EMG) records of neck muscle activity, observers have distinguished one state characterized by large, slow changes in electrical potential and by little or no muscle movement with moderate levels of neck muscle tonus (high-voltage, slow-wave sleep, [HVS]). The other state, when it appears, follows HVS in most mammals, and is characterized by low-voltage fast-wave ECoG records and by a radical loss of neck muscle tonus (low-voltage, fast-wave sleep, [LVS]), (Meier & Berger, 1965). This latter state has also been termed “REM-sleep” and “paradoxical sleep” (Dement, 1965; Jouvet, 1960). Both states appear in cyclic fashion (Aserinsky & Kleitman, 1953; Meier & Berger, 1965).

Of the 2 states, LVF has received more attention by far. One of the most striking properties of mammalian LVF is its diminution with age. The LVF/total-sleep ratio in humans drops from over 50% in infancy to less than 14% in old age (Roffwarg, Muzio, & Dement, 1966). Research with rhesus monkeys (Meier & Berger, 1965) also indicates a general developmental decline in LVF. Jouvet-Mounier (1968) and Jouvet-Mounier, Astic and Lacote, (1970) reported developmental LVF-declines in the rat, the cat, and the guinea pig. These workers noted an apparent association between the magnitude of LVF decline and the degree of brain immaturity at birth, the rat, most immature at birth, showed largest LVF-decreases, and the guinea pig, least immature at birth, showed smallest LVF-decreases. Such developmental data suggest that the importance of LVF is inversely related to level of maturity.

A number of theories on the biological function of LVF have been offered (Berger, 1969; Dewan, 1968; Ephron & Carrington, 1966; Roffwarg, *et al.*, 1966; Snyder, 1966). The Roffwarg *et al.* (1966) theory addresses the developmental phenomena associated with LVF. These researchers observed that CNS activity during LVF is similar to that resulting from sensory input during waking and they suggest that this endogenous stimulation may promote CNS development. Roffwarg and his associates point out that the characteristically early myelination of the visual tract in humans may be related to corresponding neural activity during LVF. Snyder (1965) points out that LVF involves massive activity in other cortical centers as well, noting that were it not for an efferent inhibitory mechanism, the frenzied activity in motor areas during LVF would result in equally frenzied muscle activity throughout the body. Indeed, some studies with the domestic cat have shown that the *locus coeruleus* of the pons may be a key component in such an efferent inhibitory mechanism (Jouvet & Mounier, 1960).

Roffwarg and his associates argue, essentially, that LVF provides endogenous stimulation needed for the development and/or maintenance of the CNS during periods of low exogenous input. It should be noted that this argument involves two implicit, but plausible assumptions. First, that exogenous input derives from organism-environment interaction, and second, that neonatally immature mammals cannot interact with their environments to the same degree as can mature mammals.

To the author's knowledge, only one attempt has been previously made to assess the significance of LVF for animals of different ages (Berger & Meier, 1966). These workers examined the effects of LVF- and of HVS-deprivation in infant and juvenile rhesus monkeys. They fixed the length of each deprivation period by the time beyond which a tone and shock could no longer arouse infant animals (7 days for LVF, 8 days for HVS). Arousal thresholds were higher for infants throughout deprivation and, across age and deprivation condition, thresholds increased as deprivation progressed, with juveniles showing the greater increases. Postdeprivation analysis continued as long as did the deprivation period. Data indicated no subsequent increases (“attempts to make-up” or “rebound”) in HVS for HVS-deprived monkeys and subsequent LVF-increases only for LVF-deprived juveniles (the older of the 2 groups). No behavior disturbances were observed. These data are clearly not supportive of the Roffwarg *et al.* hypothesis. However, the failure to obtain any LVF-rebound in LVF-deprived infants and the lack of any behavioral disturbances may be explained without dismissing

Roffwarg *et al.* First, it should be noted that the LVF-deprivation period of 7 days is short for a rhesus monkey, representing only a small percentage of prepubescent life. Second, it may have been neurophysiologically impossible for Berger and Meier's LVF-deprived infants to show subsequent LVF-rebound, *i.e.*, at that age LVF was already at a maximum. Finally, Berger and Meier's behavioral testing may not have been sensitive to the kinds of disturbances associated with reduced input early in life.

The present research, stimulated by the Roffwarg *et al.* hypothesis, also attempted to assess the developmental consequences of LVF-deprivation. However, this work was on a longer term basis than that of Berger and Meier, and special attention was given to overall activity, an index assumed to be related to exogenous input. The theoretical framework parallels research on the effects of early sensory deprivation. There has been much work on these and related research topics (*e.g.*, Bennett, Diamond, Krech, & Rosenzweig, 1964; Harlow, 1959; Thompson & Melzack, 1956). The reader is directed to Weinstein, Fisher, Richlin, and Weisinger (1968) for a complete bibliography on sensory deprivation and Mitler (1970) for a theoretical treatment of the relation between LVF-deprivation and sensory deprivation.

Briefly, the reasoning of sensory deprivation research and the present research is that young subjects having undergone some experimental treatment in early life will subsequently behave differently than will appropriately treated control subjects. A critical question for such reasoning is whether or not any observed treatment effects are truly developmental, that is, would or would not an identical treatment during some later period in life produce similar effects? Thus, the present research included adult-juvenile comparisons for preliminary sleep analysis in *P. m. bairdi* and for effects of LVF-deprivation.

The advantages of *P. m. bairdi* as an experimental animal have been amply presented elsewhere (see King, 1968). The usefulness of this species in sleep research has been discussed and demonstrated by Mitler and Levine (1970) who time-sampled sleep of adult *P. m. bairdi* and quantitatively showed that such records could be reliably categorized into 3 arousal states: low-voltage fast-wave sleep (LVF), high-voltage slow-wave sleep (HVS), and waking (W). The mean percent of sleep time judged as LVF was 37.1%. Since low muscle tonus was concomitant with LVF, they further attempted to deprive LVF by confining mice over a shock-grid on a pedestal too small to permit total loss of muscle tonus. In time-sampled ECoG and EMG records from such mice, only HVS and W were observed, but in ratios slightly different from those observed for unrestricted mice. The authors concluded that, by preventing low muscle tonus, the pedestal-over-shock-grid can radically reduce the proportion of LVF in the sleep of *P. m. bairdi*, but that controls for shock and for the disrupted relationship between HVS and W were necessary if such a deprivation technique were used experimentally.

Study I: Sleep Analysis in Young *P. m. bairdi*

The purpose of this preliminary study was to analyze the sleep of young *P. m. bairdi* with a procedure comparable to that used by Mitler and Levine (1970) in their study of adult mice.

Method

Three *P. m. bairdi* (2 males), selected from the Peromyscus colony at Michigan State University's Biology Research Center, were weaned at 17 days of age. For the next 2 days, procedure followed that of Mitler and Levine (see Mitler and Levine, 1970 for more detail), surgery taking place at 18 days and recordings at 20 days of age. Animals were examined individually, each examination being regarded as a separate replication.

Implantation, performed under ether anesthetic, consisted of fixing 3 steel screws with connected nichrome wire, 2 over the left parietal cortex and 1 over the right parietal cortex, to

the skull so that the screw tips met the dura. In addition, 2 nichrome wires were placed in the flesh over the neck muscles. Leads from the screws and the neck wires were connected to female components of 1 three-socket and 1 two-socket amphenol plug. The plugs were then built into a dental acrylic head platform.

Two channels of a Grass Model III electroencephalograph were used. The 2 electrodes over the left parietal area gave push-pull ECoG records. The 2nd channel recorded EMG superimposed on a record of heart activity from the neck wires. The remaining screw grounded the animal.

After surgery, while under anesthetic, each animal was connected with male-component plugs and placed in a recording cage. A commercial mouse food and tap water were provided on an *ad libitum* basis. During 24 hr of recovery and 24 hr acclimation to the recording apparatus, sample records were taken and gain controls were adjusted to the most convenient gain setting for each preparation. Then, 5 one-minute parallel ECoG and EMG records were taken throughout the next 24 hr, sampling from periods when the animal appeared motionless with eyes closed. (See Mitler & Levine, (1970) for a justification of time-sampling approaches).

Results and Discussion

Since the quantification methods of Mitler and Levine upheld the accuracy of the qualitative categorization procedure, only qualitative analysis was performed on these data. Each of the 60 five-second intervals was judged as LVF, HVS, or W on the bases of ECoG wave amplitude and EMG activity. For animals 1-3 the percents of total sleep time judged as LVF were 34.0%, 54.2%, and 60.0%, respectively, yielding an average of 49.4% for 20-day-old animals as compared with 37.1% for Mitler and Levine's 53-day-old adults.

These data mesh well with the much more extensive findings for the rat, cat, and guinea pig reported by Jouvet-Mounier (1968) and Jouvet-Mounier et al. (1970). The present findings suggest that in *P. m. bairdi*, as in rats and cats, LVF comprises more total sleep time in young animals than in adults. As limited as these data are, they indicate an average LVF decrease of 25% between 20 and 53 days of age, and, together with the findings of Mitler and Levine, comprise sufficient ground work for an exploratory study of the developmental significance of LVF.

Study II: Developmental LVF-Deprivation

The aim of this study was to explore developmentally some of the relations among LVF, CNS development, circadian activity patterns, and regulative function in *P. m. bairdi*. The experimental design evolved from several methodological considerations.

First, several confounding factors operate during LVF-deprivation. Such factors stem from the fact that LVF occurs only during certain phases of circadian activity. Thus, any LVF-contingent arousal system also has the effect of disrupting these phases. The type and frequency of arousal may also have effects distinct from those of LVF-deprivation. The pedestal-over-shock method of LVF-deprivation employed here has an important advantage over the pedestal-above-water method often used in LVF-deprivation studies on adult cats and rats (e.g., Dement, 1965). The former allows a grid cage to be wired in parallel to the deprivation cage and activated by a weight-operated pedestal switch. Such a yoked control cage was employed here. This small cage also controlled for the effects of restricted movement experienced by the animal confined to the pedestal.

Second, if any reciprocal relation between endogenous and exogenous input does operate, then LVF-deprived animals, in order to compensate for losses in that endogenous source of input,

may increase exogenous input at the expense of some HVS. To control for this possible sleep sacrifice, an unselective sleep deprivation condition was included.

Third, since all three of the above conditions involved isolation, an isolated but unmanipulated control condition was added.

And last, to insure that the data would be interpretable developmentally, both juvenile and adult mice were studied.

Method

Juvenile Group—This group consisted of 3 animals in each of the 4 treatment conditions. The conditions were filled simultaneously by randomly assigning each of 4 littermates, 1 to a condition, with the restriction that LVF-deprived animals and their yoked controls were of the same sex. Both sexes were represented in each treatment condition. Each litter was weaned at 18 days of age and treatment began on Day 20 with the weighing of each animal. Throughout the following 14-day treatment period, a 12 hr light:12 hr dark schedule prevailed, the light onset being at 0700 hours local time. A commercial mouse food and tap water were provided on an *ad libitum* basis.

Animals in the experimental of LVF-deprivation (E) condition spent all 14 days in a $10 \times 4 \times 12$ in ($25.4 \times 10.2 \times 30.5$ cm) cage with an electrically gridded floor. The animal was trained to perch on successively smaller pedestals mounted on a column in the center of the cage. The smallest pedestal was just large enough to sit on and sleep on but too small to permit total loss of muscle tonus. When such a loss in tonus occurred, the animal would fall from the pedestal. Its weight leaving the column triggered a switch which activated the grid floor. The animal could successfully avoid shock only by remaining on the pedestal. Animals remained in the pedestal cage continuously except for approximately 5 min daily while the apparatus was cleaned.

Animals in the yoked control (Y) condition were housed in a small cage with approximately the same free movement volume as that for the animal restricted to the pedestal. The animal was removed from this cage approximately 5 min per day for cage cleaning. The cage's electrically gridded floor was connected in parallel with the grid of the LVF-deprivation cage, so that a shock was delivered to each cage when no weight was on the pedestal.

Animals in the sleep deprivation (S) condition were housed in a $6 \times 5 \times 7$ in. ($15.2 \times 12.7 \times 17.8$ cm) Plexiglas cage suspended over a clock-triggered, motor-driven disk 4 ft (1.22 m) in diameter. The cage had no floor and was situated 1/8 in. (.32 cm) over the outer portion of the disk with the 7-in. (17.8 cm) side parallel to the direction of rotation. This arrangement forced the animal to live on a treadmill floor moving 5 ft (1.52 m) per minute (measured from cage center) for 19 of each 24 hr. Pilot work revealed that young *P. m. bairdi* could endure no more than 19 hr per day of floor movement. The five hr interval without floor movement was timed so that the onset of darkness exactly split the interval.

Animals in the isolated (I) condition spent their 14 days undisturbed in laboratory Plexiglas cages.

After the respective treatments, each animal was again weighed and transferred for the following 21 days to an activity recording cage. Each cage was equipped with a jiggle-switch which sent records of gross motor movement with lateral force equal to or greater than 0.35 g to a Rustrak event recorder. Thus a continuous record of activity was obtained for each animal.

In order to gather data for “nominal” lighting, for free-running activity, and for reentrainment of activity into a light:dark cycle, the first 7 days had the same 12 hr light:12 hr dark lighting schedule as during the treatment phase. During the second 7 days, free running activity was recorded in continuous darkness. For the last 7 days, the same light schedule was reinstituted.

Following the circadian rhythm measurements, each animal was weighed, killed with chloroform, and its brain, rostral to the pyramidal decussation, was removed and weighed fresh to the nearest milligram.

Adult Group—This group consisted of 3 animals per condition. The entire procedure for these animals was exactly the same as for the juvenile group, except that after weaning, the litter was separated by sex and left undisturbed until 53 days of age. It was not possible to match adult litters to juvenile litters with respect to sex; however, the frequency distribution of adult male and female mice over the 4 treatments was identical to that for the juvenile group.

Results and Discussion

Dependent Variables—Body weight was measured 3 times throughout the 35 day procedure: on Day 1, on Day 14 (posttreatment), and on Day 35 (postprocedure). Brain weight was taken immediately after the last body weighing, and the brain to body weight ratio was computed using the last body weight.

The activity records were scored by a naive laboratory technician and analyzed by computer. Five successive days from each 7-day interval were selected for analysis. For each 5 days, the mean number of movements per half-hour period was computed. In addition, a Fourier analysis was performed by computer on the activity scores. With this analysis, major cycles of activity were marked by extracting from the data those period lengths with a signal-to-noise ratio greater than 1.0. The output was examined by hand and periods with signal-to-noise ratios greater than 2.54 ($p < .01$ against the null hypothesis of no periodic activity) were selected for intergroup comparisons. Finally, a crude rhythmicity index was devised which could be used to measure the predictability of each animal's daily activity pattern. For each 24 hr in each 5-day interval, the total activity score for hours 0700 hours to 1900 hours was subtracted from the total activity score for hours 1900 hours to 0700 hours. This process yielded 5 difference scores for each 5-day period. The rhythmicity index was obtained by extracting the 4th¹ root of the variance of those 5 difference scores for each 5-day period. With this index, the smaller the value, the more regular was the activity pattern. This procedure produced 3 variables for each 5 day interval: mean activity, number of significant periods, and rhythmicity index.

Thus the overall procedure involved 14 dependent variables per animal: 3 body weights, 1 brain weight, 1 brain to body weight ratio, 3 mean activity scores, 3 counts of significant periods, and 3 rhythmicity indices.

Data Analysis—With 14 dependent variables and the high likelihood of nonindependence among them, a multivariate analysis of variance (Morrison, 1967) was performed by computer on the entire data matrix. The program also provided univariate F -statistics corrected for nonindependence and for the inflated probability of alpha-error associated with repeated univariate analyses. Each variable was regarded as a separate dependent variable for the purposes of this analysis.

¹Since the variances were typically very large, and since only relative magnitude was of interest, the 4th root of the variance (square root of the standard deviation) was employed to facilitate tabulation and analysis.

Analysis Design—Since the treatment conditions were run simultaneously with littermates, a matched sample design seemed appropriate. Such a design involved analyzing treatments as repeated measures in a 2×4 age group by treatment factorial design with 3 replications per cell. While males and females were equally distributed throughout the design, the sex effect could not be formally analyzed with this small number of subjects. The design of the analysis produced 1 contrast test for the age-at-treatment effect (hereafter referred to as the “age effect”), 3 pair-wise contrasts for treatment and, 3 pair-wise contrasts for Age $\times$ Treatment. These contrasts successively compared: S vs. I, Y vs. I + S, and E vs. I + S + Y. Such a priori tests permitted precise examination of all differences between conditions.

Missing Data—Five data points were missing due to the death of a juvenile animal in the E condition. The animal apparently died of starvation in the morning of the 28th day of the procedure. The animal’s record indicated it was highly active throughout its last 14 days. During the 24 hr dark condition it was provided as much food as were its counterparts in other conditions. Yet, on the morning of the 28, after 1st light onset for the final 7-day period, all had surplus food but the E animal who had apparently eaten all its food. The animal seemed extremely weak and died during examination. Its missing weights were estimated with linear projections based on the body and brain weights observed for the other juvenile animals in the E condition. The first 5 days of the second 7-day period were used for analysis so as to minimize the contamination of activity records by effects of the animal’s food shortage. This procedure seemed appropriate since *P. m. bairdi* starve to death in 24–36 hr after removal of food and 72 hr were allowed prior to the animal’s death. With respect to activity score, period count, and rhythmicity index for the third 5-day interval, those measures on the dead animal for the second 5-day interval were used. This decision seemed fair, if not conservative, since in all conditions there was a tendency for activity to increase, number of periods to remain constant, and rhythmicity index values to increase over the three intervals.

Weight Measures—Table 1 presents mean body weights for each of the 3 weighings as a function of condition, age group, and age at weighing. There were no significant treatment or age by treatment effects for any of the weighings (all $F < 3.96$; all $p > .12$). Juveniles, however, showed reduced weight gain from the 1st to the 2nd weighing in the E and Y conditions relative to the S and I conditions. The age effects for the 1st and 2nd weighings were significant ($F = 51.68$ and 30.84 ; $df = 1/4$; $p < .002$ and $.006$, respectively). These age differences were trivial and, since the juveniles grew, the weight differences disappeared by the time of the final weighing ($F = 3.15$; $df = 1/4$; $p > .15$).

Aside from the reduced weight gain for juveniles in the E and Y conditions, the nonsignificant treatment and Age $\times$ Treatment effects suggest that growth and body maintenance were not appreciably affected by any treatment.

With respect to brain weight, neither treatment nor Age $\times$ Treatment effects were significant (all $F < 1.40$; all $df = 1/4$; all $p > .28$). The age effect, however, was noteworthy. Mean brain weights were 538.25 mg for juveniles and 502.00 mg for adults ($F = 5.80$; $df = 1/4$; $p < .03$).

The striking finding that juveniles had significantly heavier brains than did adults may be consistent with findings of increased brain weights in young animals reared in enriched environments (Bennett et al., 1964). This interpretation, however, does not satisfactorily explain the fact that the isolated juveniles, presumably those experiencing the least varied environment, had the 2nd heaviest brains. In view of this difficulty in interpretation and the small number of animals, it seems wiser to reserve judgment. The possible age effect must be further investigated with more precise measuring techniques involving a variety of neuroanatomical structures such as those studied by Bennett and his associates (1964).

For brain weight divided by final body weight, again, the treatment and Age $\times$ Treatment effects were not significant (all $F < 1.00$; all $df = 1/4$; all $p > .68$). The age effect was significant. The mean ratios were 0.0357 for juveniles and 0.03105 for adults ($F = 6.63$; $df = 1/4$; $p < .02$). This result, however, is clearly related to the age differences in brain weight and the lack of age differences in final body weight. Therefore, these ratios add little additional information, but further underscore the necessity for more elaborate future research.

Circadian Activity Measures—Figure 1 illustrates mean activity per half-hour for juvenile and adult animals as a function of condition. Activity means, expressed in frequency of movement, were computed for each 5-day interval. Contrasts disclosed no treatment effect (all $F < 1.00$; all $df = 1/4$; all $p > .25$). With respect to Age $\times$ Treatment interactions, the I vs. S and the Y vs. I + S contrasts revealed no significant interactions (all $F < 1.00$; all $df = 1/4$; all $p > .25$). However, the E vs. I + S + Y Age $\times$ Treatment contrasts, disclosed significant differences. Table 2 presents mean activity for the E and I + S + Y groups as a function of age and 5-day interval ($F = 11.07$; 6.71, and 11.92; all $df = 1/4$; $p < .03$, .062, and .03 for the three 5-day intervals, respectively). The age effect was significant for the first two 5-day intervals ($F_s = 9.78$ and 10.98; $df = 1/4$; and $p < .035$ and .030, respectively). The age differences appeared to diminish by the third 5-day interval ($F = 3.53$; $df = 1/4$; $p > .133$).

There seemed to be no systematic differences among conditions or age groups in the time of activity onset. All animals began activity shortly after light offset during the first and third 5-day intervals. During continuous darkness (second 5-day interval), all animals tended to begin activity successively earlier by 10 to 15 min increments across successive 24 hr intervals. This finding is consistent with other circadian activity research on dark-active animals (Marler & Hamilton, 1967). In this tendency to initiate activity successively earlier, treatment or age differences were not apparent. A further age difference was apparent during examination of data for individual subjects. For the 11 juveniles who survived the entire procedure, 5 were most active during continuous darkness the remaining 6 were most active during the third 5-day interval. For adults, 9 of 12 were most active during continuous darkness and 3 most active during the third 5-day interval. There seemed to be no relation between interval of greatest activity and treatment condition. These age differences may be due to a true Age $\times$ Light schedule interaction. On the other hand, they may be artifacts of either diminishing differences in body weights or of the juveniles reaching sexual maturity between the second and third 5-day intervals. Changing differences in body weight may have differentially influenced the likelihood of movements being recorded between the second and third 5-day intervals. The onset of sexual maturity, and hence estrous, in juvenile females may have directly increased female activity and indirectly increased male activity. In view of the high sensitivity of the activity recording devices, the weight-gain interpretation seems least likely. The sexual maturity interpretation is plausible, yet Layne (1968) concluded from a review of the ontogenetic literature on *Peromyscus* that *P. m. bairdi* reach sexual maturity between 33 and 40 days of age. This would place the onset of sexual maturity in juveniles well before activity recording even began. Nevertheless, more data are certainly necessary before any conclusions may be drawn on the Age $\times$ Light schedule interaction implied by this individual subject analysis.

Table 3 summarizes the data on number of significant periods computed from a Fourier analysis of movement frequency over each 5-day interval. Such significant period lengths ranged from a maximum of 294.95 hr (projected) to a minimum of 5.05 hr. There seemed to be no systematic pattern among conditions, age groups, or lighting schedules either in period length or likelihood of a particular length to emerge repeatedly over successive 5-day intervals. This was true with only one exception; virtually, each animal showed a significant period in the range of 23.50 to 24.50 hr in length. Such periods emerged even during constant darkness. Such a finding

suggests that in the present study, neither age nor treatment factors disrupted the tendency for at least one activity period to be 'circadian' in nature.

Table 4 summarizes rhythmicity index data as a function of condition, age group, and 5-day interval. No contrast disclosed significant treatment or Age $\times$ Treatment effects (all $F < 2.02$; all $df = 1/4$; all $p > .23$). For the age effect, analyses indicated that across treatments juveniles had smaller rhythmicity indices. Tests for the first and third 5-day intervals disclosed significant age differences in rhythmicity ($F = 8.55, 1.96, 39.40$; all $df = 1/4$; $p < .043, .24, .003$ for the three 5-day intervals, respectively). These data suggest that neither treatment nor the Age $\times$ Treatment interaction affected regularity of activity patterns, and that animals in the juvenile group appeared to be more regular than their adult counterparts. Activity data indicated juveniles were also less active. Thus, this age effect may be simply an artifact of a tendency for the index itself to co-vary with mean activity. To check this possible association, a Pearson product-moment correlation between scores for activity and rhythmicity for all animals was performed and disclosed a figure of .70. This result strongly suggests that the age effect in rhythmicity is artifactual.

With respect to overall development, the body weight data suggest that prolonged LVF-deprivation had no significant effect. However, in view of the activity differences that such deprivation seemed to induce subsequently in *P. m. bairdi*, it seems unlikely that anatomical differences will not be found with more exacting techniques.

Brain weight also seems too crude a measure to reveal treatment or Age $\times$ Treatment effects. The age effect observed in brain weights is only suggestive of possible developmental differences in brain plasticity such as those noted by Bennett and his associates (1964). For further research at the CNS level, such techniques as brain section weighings, histological examination of cell-size and density, neurochemical analysis, and single cell activity recordings may be very useful. For example, it would be interesting to compare cell activity during various stages of arousal, brain weights, histological results, and acetylcholinesterase content in an Age $\times$ Treatment factorial design similar to that of the present research. Considering the observed differences in activity, areas in the brain stem reticular formation (the reticular activating system [Hernandez-Peon, 1961]) should surely be included among the portions of brain selected for such analyses. In view of the great activity in the visual cortex (Evarts, 1962), motor cortex (Snyder, 1965), and oculomotor system (Berger, 1969) during LVF, these areas might also be included for such extended analysis.

The data on circadian activity generally indicate that with measures employed here, age and Age $\times$ Treatment interactions can be detected in quantity of daily activity. Differences in the regularity of such activity were not convincingly disclosed excepting the age effect on the first and third rhythmicity indices. But even these differences are suspect because of the high correlation noted between activity scores and the index itself. Thus, there seems to be a clear need for a measure of rhythmicity which is less dependent on quantity of activity.

The significant Age $\times$ Treatment interaction observed in activity does not mesh well with any theory of LVF function. This difficulty arises because the data actually indicate a double, or mirror-image interaction. When treatment began on the 20th day of life, LVF-deprived animals were subsequently more active than animals in the three control groups, but when treatment began on the 53rd day, LVF-deprived animals were less active than controls. The Roffwarg *et al.* hypothesis can explain the effect on juvenile animals in terms of CNS input regulation acting to counteract losses in endogenous input by increasing exogenous input. However, the mirror image effect observed for adults is not so simply handled. According to the supplemental stimulation hypothesis of Roffwarg and his associates, LVF is a less important source of input for adults than for juveniles. Therefore, such a hypothesis would predict activity differences

in the same direction as those of the juvenile group, but decidedly less pronounced. This was clearly not the case.

An alternative interpretation combines the hypothesis of Roffwarg and associates with findings of large increases in LVF proportions after LVF-deprivation (LVF-rebound). With such a combination, the Age $\times$ Treatment interaction could be explained solely in terms of differences in input regulation 'strategies'. The LVF-deprived juveniles should have lost more input than LVF-deprived adults, (since juveniles have more LVF). In counteracting such losses, LVF-rebound was insufficient and required supplemental input via increases in exogenous activity. For LVF-deprived adults, LVF-rebound was sufficient provided sleep time was adequate to allow required LVF. Adults could then get by with just slight increases in their sleep time. Such increases could account for the reduced activity observed in LVF-deprived adults.

Nevertheless, both the former and latter interpretations fail to deal with the fact that 21 days post-treatment seems long enough for any input deficit correction to take place. Yet the data do not show any merging trends in activity among treatments or among age groups. On the contrary, activity differences seem to increase over the 21 days. Such results suggest that Age $\times$ Treatment effects are nonstatic and long-term (if not permanent). Perhaps some of these questions could be answered with continuous polygraphic monitoring of arousal states in posttreatment animals.

Difficulties also arise in trying to integrate the present data with other findings on the effects of LVF-deprivation. Other than the present findings for juveniles, there seems to be no long-term, LVF-deprivation data for young animals. For adult subjects various measures have been used. Dement (1965) has noted, in LVF-deprived cats, increased sexual activity and a general inability to attend to cues in a Y-maze learning task. Dewson and associates (Dewson, Dement, Wagener, & Nabel, 1965) have observed threshold decreases in excitability of the auditory system and in electroconvulsive seizures for LVF-deprived rats. These findings would seem in direct opposition to the present findings of decreased activity in LVF-deprived adult *P. m. bairdi*.

One possible explanation of this apparent discrepancy is that LVF-deprived adult animals may be both inactive and hyperresponsive. Another observation by Dement (1965) lends some support to such an interpretation. He noted that some LVF-deprived cats, presumably some of the same animals who could not learn his Y-maze, tended to seek dark corners when they were undisturbed. It may be that, had circadian activity been measured in Dement's LVF-deprived cats, less than normal activity would have been found. In qualitative observations in the present research, it was also noted that the posttreatment E animals tended to be most violent in avoiding capture for cage transfer and most responsive when someone entered the laboratory. However, these speculations must be tested with more extensive research.

The entire body of Study II data should be regarded as exploratory. The findings raise more questions than they answer. The use of locomotor activity as a dependent variable clearly indicated that LVF-deprivation during early life affects *P. m. bairdi* differently than does such deprivation during adulthood. Furthermore, such differences appear to increase over time. Substantially more work will be necessary to uncover this phenomenon's biological underpinnings.

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Fig. 1.

Mean activity based on the total number of movements per half-hour for juvenile and adult animals as a function of condition. Activity is expressed in terms of movement frequency. Means were computed over each of three 5-day intervals. Vertical lines represent ± 1 standard error of the mean. The "E," "Y," "S," and "I" represent experimental, yoked control sleep deprived, and isolated groups, respectively.

Table 1
Mean Weights in Grams for Juvenile and Adult Animals as a Function of Condition, Age Group, and Weighing

Weighing	Experimental		Yoked		Sleep Deprived		Isolated	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
1								
Age (days)	20	53	20	53	20	53	20	53
X	8.80	12.68	8.68	14.47	8.82	13.76	8.64	14.75
2								
Age (days)	34	67	34	67	34	67	34	67
X	10.34	12.71	10.26	13.76	11.73	13.77	13.42	16.03
3								
Age (days)	55	88	55	88	55	88	55	88
X	14.89	16.23	14.35	16.49	15.01	16.45	16.73	15.76

TABLE 2
Activity Means Expressed in Frequency of Movement Per Half-hour for the E vs. I + S + Y Contrasts, as a Function of Age Group and 5-Day Interval

5 Day Interval	E	I+S+Y	F Age × Treatment	df	p
1	Juveniles Adults	1.02 16.81	11.07	1/4	.03
2	Juveniles Adults	11.03 22.78	6.71	1/4	.06
3	Juveniles Adults	17.51 21.36	11.92	1/4	.03

Table 3
Mean Number of Significant Periods as a Function of Condition, Age Group, and 5-Day Interval

5-Day Interval	Experimental		Yoked		Sleep Deprived		Isolated	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
1 Age (days) X	41 9.33	74 9.00	41 9.33	74 9.67	41 7.67	74 9.33	41 9.33	74 11.67
2 Age (days) X	48 11.67	81 11.67	48 10.67	81 11.00	48 15.00	81 10.00	48 9.00	81 9.67
3 Age (days) X	55 9.67	88 11.00	55 10.00	88 8.33	55 10.33	88 8.67	55 8.00	88 10.67

Table 4
Mean Rhythmicity Index as a Function of Condition, Age Group, and Age at Computation

5-Day Interval	Experimental		Yoked		Sleep Deprived		Isolated	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
1 Age (days)	41	74	41	74	41	74	41	74
X	7.26	9.36	2.14	11.86	3.06	12.65	5.71	10.34
2 Age (days)	48	81	48	81	48	81	48	81
X	10.46	11.92	2.61	10.14	3.71	13.80	5.38	11.74
3 Age (days)	55	88	55	88	55	88	55	88
X	11.92	8.09	8.08	7.18	3.55	8.93	8.68	10.22