THE EFFECTS OF MATERNAL ISOLATION ON THE ONTOGENY OF CIRCADIAN ACTIVITY RHYTHMS AND THE GROWTH OF RAT PUPS

By

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Abstract

The circadian rhythms of young animals can be entrained by prenatal and postnatal maternal cues. The present research examined the effects of early postnatal maternal isolation on the ontogeny of the activity rhythm in rat pups. The studies also examined the effects of different light cycles, as well as other stimuli which may serve as synchronizers of the activity rhythm during the postnatal period. Activity rhythms synchronized to a light-dark (LD) cycle appeared as early as five days after birth in mother-reared pups. This result contrasts with data from other reports which indicate a later onset. Pups reared without their mothers (AR pups) between 3 or 4 to 18 days postnatally on a LD cycle had rhythms which were of lower amplitude and of shorter duration than those in the mother-reared group. AR pups with a LD cycle and a feeding cycle which approximated the normal nursing rhythm showed more synchronization of their activity than pups with only a LD or feeding cycle. The mean period of the activity rhythms of pups raised under constant light deviated the most from 24 hrs. The introduction of a temperature cycle attenuated the AR pups' activity. These results indicate that the nursing rhythm, in conjunction with LD cycles, may serve as synchronizers of rat pups' rhythms. However, nursing and LD cycles represent only a part of the complex postnatal environment which includes temperature, as well as other stimuli not investigated here, such as olfactory cues.

There was also evidence suggesting that rhythmic factors during the early postnatal period may influence growth. AR pups with a cyclic feeding schedule had heavier spleens and lighter livers than animals with a noncyclic schedule. Heavier forebrains were associated with a predominantly diurnal feeding cycle. These growth factors may, in turn, influence the rhythmicity of locomotor activity.

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Most importantly, this dissertation is dedicated to Eric, my best friend, scientific colleague, and partner in life. He endured the hardships and shared the pleasures of my doctoral work.

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Chapter 1

General Introduction

Background

Terminology

Biological rhythms are ubiquitous in both the animal and plant kingdoms and affect such diverse processes as pupal eclosion, predatory hunting, and learning and memory. Biological rhythms may be defined as biological processes which recur or vary in intensity at predictable time intervals (Rusak & Zucker, 1975). The length of the recurring time interval, referred to as the period (Aschoff, 1979), is used to classify rhythms into three general types. Infradian rhythms have periods greater than approximately 28 hrs and encompass phenomena such as female reproductive cycles and seasonal breeding in some mammals. Ultradian rhythms can be represented by sleep-state rhythms and feeding bouts (Bowden, Kripke, & Wyborney, 1978; Daan & Aschoff, 1981) and have periods less than about 20 hrs. Rhythms with periods of approximately 24 hrs (Halberg & Lee, 1974), or circadian rhythms, will be the major focus of this dissertation. Rhythms are also described by other parameters. The peak of a rhythm refers to its maximum value whereas the trough refers to the rhythm's minimum value. A rhythm's amplitude is the difference between the peak value and the trough value.

Circadian rhythms can be entrained by rhythmic stimuli, called zeitgebers or "time giving" stimuli (Borbéley, 1978). When entrained, a circadian rhythm maintains a stable phase relationship with the

zeitgeber (Enright, 1981b). For example, when entrained to a zeitgeber such as the light-dark (LD) cycle, a rat's peak of activity might consistently occur 1 hr after the onset of darkness. The 1-hr lag between the onset of darkness and the activity peak would be the phase relationship. When rhythmic data are fit to a cosine function, the term, acrophase, is used to describe the phase relationship between the zeitgeber and the function's peak (Enright, 1981b). Several zeitgebers have been identified; for example, environmental pressure cycles (Hayden & Lindberg, 1968), social cues (Takahashi & Murakami, 1982), feeding schedules (Bolles & Stokes, 1965; Krieger, 1979; Spiteri, 1982), and most notably, light-dark cycles . One factor determining the entraining effectiveness of a zeitgeber is the circadian rhythm itself. Body temperature rhythms in monkeys are more readily entrained by LD cycles whereas urinary potassium excretion rhythms are more stable with a feeding cycle (Moore-Ede & Sulzman, 1981).

Besides entrainment another property of circadian rhythms is their ability to freerun, or maintain their rhythmicity, in the absence of cyclic input from zeitgebers (Aschoff, 1981). Locomotor activity rhythms in rats (Richter,1971) and cortisol rhythms in humans (Miles, Raynal, & Wilson, 1977) freerun under conditions of constant darkness (DD). Rhythmicity is also maintained under conditions of constant light (LL), although under prolonged LL conditions circadian rhythms may "disappear" and ultradian rhythms may predominate (Albers, Gerall, & Axelson, 1981; Honma & Hiroshige, 1978). The periods of freerunning circadian rhythms deviate slightly from 24 hrs (entrained conditions)

and may depend on the lighting conditions present before placement on LL or DD (Davis & Menaker, 1981).

Analysis of Rhythmic Data

A variety of methods are available for the analyses of rhythmic data (Enright, 1981a). The two types of analyses used for the rhythm data presented in Chapter 3 will be described here. Day-night differences in a set of data are frequently used to establish rhythmicity. This measure is particularly useful for determining entrainment of a rhythm to a LD cycle when the rhythm has definite peaks or troughs during the light or dark period. Nonsignificant day-night differences do not necessarily imply a lack of rhythmicity or even entrainment. In these cases the rhythm may be freerunning or entrainment may be obscured by the manner in which the data were sampled and/or combined for analysis.

Time series analyses are more sophisticated ways of determining if rhythmicity is present in a set of data. In general, a prerequisite for the use of time series analyses is a long series of data consisting of many daily observations (Enright, 1981a). One of the more simple analyses is autocorrelation analysis. This involves the calculation of a product-moment correlation "between the original data series, and that same series when it is 'lagged' on itself by some fixed number of time units" (Enright, 1981a, p.27). Usually a large number of lags are examined and the correlations are then plotted against the lag. If a stable rhythm is present in the data, the plot of the autocorrelations against the lags will be cyclic and the lags of the peaks will

correspond to the dominant period of the data (Enright, 1981a). Problems in interpreting the results of autocorrelation analyses will be discussed in Chapter 3. Unlike some time series analyses, autocorrelation analysis does not assume a priori the shape of the function which best describes the data.

Physiological Aspects

Physiological studies of circadian rhythms have focussed on the visual system (Rusak & Zucker, 1979). Transections of the optic nerves and bilateral enucleation do not disrupt freerunning circadian rhythmicity in mammals, although entrainment to a LD cycle is lost (Davis & Menaker, 1980; Deguchi, 1975b; Moore, 1975; Richter, 1971). Lesions of the primary optic tract and/or the accessory optic tract do not impede LD entrainment in rats (Moore, 1975) although reentrainment to a shifted LD cycle may be retarded in hamsters (Rusak & Boulos, 1981). Similar results are seen when the lateral geniculate nuclei, a major terminal site for visual fibers, are lesioned (Dark & Asdourian, 1975; Rusak & Boulos, 1981).

The suprachiasmatic nuclei (SCN), two hypothalamic nuclei located dorsally to the optic chiasm, are important to circadian rhythmicity. The SCN receive direct input from the retina via the retinohypothalamic tract (RHT) (Pickard & Silverman, 1980; Sawaki, 1977). Lesions of the SCN in rats and hamsters disrupt a variety of circadian rhythms (Redgate, 1976; Saleh & Winget, 1977; Stephan & Nunez, 1977) although animals may continue to show ultradian rhythms

(Van den Pol & Powley, 1979) and even entrainment to a LD cycle (Rusak, 1979; Rusak & Boulos, 1981) and feeding schedules (Krieger, Hauser & Krey, 1977; Phillips & Mikulka, 1979; Stephan, 1980). There may be species differences in the role of the SCN in the mediation of circadian rhythms. For example, SCN lesions in monkeys abolish circadian rhythms of feeding, drinking, and rest-activity patterns but not body temperature (Albers, Lydic, & Moore-Ede, 1980; Moore-Ede, Lydic, Czeisler, Fuller, & Albers, 1980).

Other structures within or near the central nervous system (CNS) have roles in circadian rhythmicity. For example, lesions in the ventromedial hypothalamus, which receives input from the SCN (Berk & Finkelstein, 1980; Stephan, Berkley, & Moss, 1981), abolish the predominantly nocturnal feeding pattern in rats (Sieck, Nance, & Gorski, 1979). Pinealectomy abolishes freerunning circadian rhythms in some birds (Binkley, 1974) but not mice or rats (Cheung & McCormack, 1982; Quay, 1968,1974). Rats with pinealectomies, however, may reentrain more quickly to a shifted LD cycle (Kincl, Chang, & Zbuzkova, 1970). Hormonal and neurochemical rhythms in the pineal gland (i.e., melatonin) and exogenous melatonin have been postulated to synchronize activity rhythms in blinded rats (Pohl & Gibbs, 1978) and pinealectomized rats (Redman, Armstrong, & Ng, 1983).

Ontogeny of Biological Rhythms

A major goal in many developmental studies is to elucidate the origins of a particular phenomenon and to determine the conditions

which are necessary for the emergence and maintenance of the phenomenon. One issue in the area of circadian rhythm development concerns endogeny versus dependence on external cyclic cues (Petrén & Sollberger, 1967). Endogeny implies that an organism has an "inherited circadian rhythm" (Petrén & Sollberger, 1967) which does not rely on external cyclic cues for its emergence and maintenance. Due to certain maturational processes, this inherited rhythm can then be entrained by zeitgebers. The other view is that external cyclic cues are necessary for the maintenance of circadian rhythmicity. Some investigators (eg., Brown, 1974) claim that it is virtually impossible to produce an environment devoid of time cues since some of those cues might be very subtle. A second issue involves investigating what cues or stimuli are important and effective in entraining rhythms during early development. One or both of these issues underlie most of the studies discussed in the following sections.

Prenatal Rhythms

Evidence on the prenatal aspects of the ontogeny of circadian rhythms is relatively scanty. This is particularly true for mammalian species, largely because of the technical difficulties inherent in obtaining appropriate data from in utero preparations.

There are some data from various species which indicate that ultradian and circadian rhythms are present prenatally. Petrén and Sollberger (1967) observed ultradian rhythms with approximately 8-hr periods in liver glycogen concentrations in prehatchling chicks. Fetal

lambs also exhibit ultradian rhythmicity in the occurrence of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep states (Ruckebusch, 1972; Ruckebusch, Gaujoux, & Eghbali, 1977). There was a change in the period of the REM-NREM cycle, starting at 35 to 40 min around 3 weeks before birth and increasing to approximately 60 min 28 hrs before birth (Ruckebusch et al., 1977). A similar trend towards an increase in period with age was observed by Sterman (1967) and Dierker, Rosen, Pillay, & Sorokin (1982) in human fetal movements during the last 4 calendar months of pregnancy. It should be mentioned that the quiet and active cycles of the fetus (Dierker et al., 1982) may not necessarily correspond to the different behavioral states (i.e., quiet sleep, paradoxical sleep, etc.) usually seen in neonates and older infants (Nijhuis, Prechtl, Martin, & Bots, 1982).

Circadian rhythms are also observed during the prenatal period. Fetal lambs have a higher incidence of rapid breathing movements (Boddy, Dawes, & Robinson, 1973), more respiratory periods (Ruckebusch et al., 1977), and peak tracheal blood flow (Boddy et al., 1973) during the dark portion of 24 hrs. These animals also show fetal circadian heart rate patterns (Lawler & Brace, 1982) although the daily change in amplitude is not very great (2% variation over the mean heart rate value). The heart rate of human fetuses at 38 to 40 weeks of gestation is also rhythmic with a circadian period (Patrick, Campbell, Carmichael, Natale, & Richardson, 1981; Patrick, Campbell, Carmichael, & Probert, 1982). Barr (1973) found greater fetal rat weight gain

during the dark portion of a 9:15 LD cycle. Similarly, Challis, Socol, Murata, Manning, and Martin (1980) observed higher dihydroepiandrosterone sulfate (a by-product of the fetal adrenal glands) and progesterone levels during the evening hours in fetal rhesus monkeys.

Postnatal Rhythms

Postnatal aspects have been extensively studied because they are relatively easy to investigate and many circadian rhythms first appear during the early postnatal period. The attainment of adult-like rhythms may involve changes in several parameters, for example, the amplitude, period, phase relationship, and the shape of the rhythm. This section is divided into two general parts; a discussion of the "normal" development of some biological rhythms and a survey of studies involving manipulations of the postnatal environment and the subsequent effects on rhythm development.

There is large variation in the postnatal appearance of biological rhythms. For example, greater day than nighttime values of 2-deoxyglucose uptake in the SCN of the rat are present at birth (Fuchs & Moore, 1980) whereas plasma corticosterone rhythms are not evident until 3 to 4 weeks postnatally (Levin, Fitzpatrick, & Levine, 1976). Other circadian rhythms, such as intestinal maltase and lactase (Saito, Suda, & Matzuda, 1978) and sucrase activity (Henning & Guerin, 1983), may not appear until an adult pattern of nocturnal food intake is attained around 3 to 4 weeks of age (Bernstein, 1976; Redman & Sweney,

1976). Several rhythms exhibit ultradian periods during early development which later are predominated by circadian periods. This appears to be the case for activity rhythms in rats (Richter, 1927; Teicher & Flaum, 1979), sleep-wake behavior in human infants (Hellbrügge, 1960, 1974a; Jacklin, Snow, Gahart, & Maccoby, 1980; Meier-Koll, 1979), and levels of plasma corticosterone in rats (Ramaley, 1978a; Takahashi, Hanada, Kobayashi, Hayafuji, Otani, & Takahashi, 1979). Some researchers (e.g., Hellbrügge, 1974b) have suggested that the circadian period evolves from the ultradian periods. However, the observations that ultradian rhythms persist into adulthood (Aschoff, 1981) and are still evident in the presence of circadian rhythms in infants (Morath, 1974) makes this hypothesis somewhat tenuous (Minors & Waterhouse, 1981).

Amplitudes of rhythms also undergo ontogenetic changes. Female rats at puberty and children exhibit increases in the amplitude of plasma corticosterone (Itoh, Hirota, & Katsuura, 1980; Ramaley, 1978a) and serum cortisol levels (Onishi et al., 1983). Increases with age in peak values are also observed in rat whole brain serotonin (Okada, 1971) and pineal N-acetyltransferase (NAT) levels (Ellison, Weller, & Klein, 1972) and hamster pineal melatonin levels (Rollag & Stetson, 1981; Tamarkin, Reppert, Orloff, Klein, Yellon, & Goldman, 1980). Both increases in peak values and decreases in trough values account for amplitude changes in rat liver tyrosine transaminase levels (Honova, Miller, Ehrenkranz, & Wov, 1968) while the converse appears to be true

for ontogenetic changes in the human body temperature rhythm (Abe, Sasaki, Takebayashi, Fukui, & Nambu, 1978).

Often concurrent with changes in the amplitude of rhythms are shifts in the phase relationship, or the time of occurrence of the circadian rhythm's peak. For example, the peak levels of thyroid stimulating hormone levels in rat serum advance with age (Kimura, Okano, & Kawakami, 1981). In contrast, the peaks in rat serum follicle stimulating hormone levels (Kimura et al., 1981), hamster pineal melatonin levels (Rollag & Stetson, 1981), and human body temperature (Abe et al., 1978) delay with age.

According to some investigators, the changes which occur throughout the prenatal and postnatal periods may be indicative of the maturation of various processes which underly circadian rhythmicity (Minors & Waterhouse, 1981). Although some of the physiological mechanisms presumed to underly circadian rhythmicity have been studied developmentally their links with the observed changes in rhythmic parameters are largely correlative. One example is the physiological control of several rhythms in the rodent pineal gland (Klein, Namboodiri, & Auerbach, 1981). Functional innervation of the pineal gland by superior cervical ganglion fibers is associated with the normal expression of circadian rhythmicity in rat pineal NAT activity (Deguchi, 1982; Illnerová & Skopková, 1976) and possibly for circadian changes in "synaptic ribbon" numbers in the pinealocytes (King & Dougherty, 1980).

The SCN have also been studied developmentally. The presence of an intact connection between the retina and SCN appears to be necessary for the normal entrainment of rhythms to a LD cycle (Mosko & Moore, 1979a; Stephan & Nunez, 1978). Despite the observation that retinal fibers are present in the SCN around 3 to 4 days after birth (Mason, Sparrow, & Lincoln, 1977) maturation of other components are probably necessary before entrainment to a LD cycle occurs. For example, the plasma corticosterone rhythm (Takahashi et al., 1979), is not entrained by a LD cycle until 2 to 4 weeks postnatally. Based on electrophysiological recordings from hypothalamic slices, Shibata, Liou, and Ueki (1983) and Shibata, Oomura, Liou, and Ueki (1984) suggested that the appearance of some circadian rhythms may correlate with increases in SCN neuronal activity and the appearance of circadian rhythms in SCN firing rates. Intact SCN from birth seem to be necessary for the expression of freerunning rhythmicity, at least in activity, drinking, and estrous behavior, although the types of deficits following SCN lesions depend partially on the extent and the location of destruction (Mosko & Moore, 1979 a, b).

Information on the developmental effects of other lesions is scanty. Subcutaneous injections of monosodium glutamate (MSG) in neonatal rats (Miyabo, Ooya, Yamamura, & Hayashi, 1982) and hamsters (Pickard, Turek, Lamperti, & Silverman; 1982) destroy a large portion of the inner retinal cell layer. The retinal input to the SCN, however, remains relatively intact and animals with MSG lesions do not

differ from intact animals in the entrainment and pattern of the circadian plasma corticosterone rhythm (Miyabo et al., 1982) or activity rhythms (Pickard et al., 1982). These data suggest that neonatal destructon of retinal projections other than those going to the SCN may not seriously impair certain aspects of circadian rhythm entrainment.

The preceding discussion indicates that postnatal changes in several parameters which define rhythmicity, including period, amplitude, and phase relationship, occur for a variety of biological rhythms. In addition, there are differences among rhythms in the kinds of changes which occur as well as in the onset of overt rhythmicity. Ontogenetic changes in the SCN and pineal gland seem to be associated with some of the postnatal changes although other, as yet unidentified, components are also probably involved.

Manipulations of the Postnatal Environment

The most intensively studied manipulations are those involving the LD cycle. These include raising animals under conditions of LL or DD or under non-circadian (other than 24 hrs) LD cycles.

There are differential effects of LL and DD on the ontogeny of circadian rhythms as well as differences between rhythms in their responses to LL or DD. One problem with interpreting data from animals kept on LL or DD is that group data may obscure the presence of freerunning rhythms in individual animals. This may also occur when few time points are sampled. Data from individual rats that were blinded at birth and kept with sighted dams until 3 to 6 weeks of age

indicate freerunning circadian rhythms in plasma corticosterone levels (Hiroshige, Honma, & Watanabe, 1982a; Itoh et al., 1980; Takahashi et al., 1979). In contrast, based on group data Krieger (1973) found no circadian rhythms at 80 days of age in plasma corticosterone in animals enucleated at birth, although at 30 days of age a circadian rhythm of low amplitude was evident. Data from individual rats at 80 days old, however, revealed that some of the animals may have had freerunning circadian rhythms. It is possible that prolonged exposure to DD may depress the circadian rhythm in plasma corticosterone. Data on the pineal NAT rhythm indicate that rat pups kept with their dams on DD from either conception or birth showed freerunning circadian pineal NAT rhythms at 23 days of age (Deguchi, 1975a); these rhythms, however, were considerably reduced in amplitude at 7 weeks of age. Similar amplitude reductions in circadian rhythms of urinary constituent levels (chloride, potassium, etc.) are observed in blind humans (Hollwich & Dieckhues, 1974).

Conditions of LL can also influence the development of circadian rhythms. Group (Krieger, 1973; Ramaley, 1975) and individual data (Krieger, 1973) suggest that the adrenal corticosterone circadian rhythm is not evident in animals kept under conditions of LL from a few days before or soon after birth. Some of Krieger's data, however, show what may be ultradian rhythms in plasma corticosterone levels. This agrees with the observations that when placed in LL for 3 months in adulthood, rats show ultradian rhythms in locomotor activity (Honma

& Hiroshige, 1978). Unlike plasma corticosterone levels, activity rhythms of chicks (Broom, 1980) and pineal NAT levels in rats (Deguchi, 1975a) exhibit circadian rhythmicity following LL conditions from hatching or birth, respectively. The latter finding is somewhat surprising since light has a very strong suppressive effect on pineal NAT levels in animals raised on a LD cycle (Klein & Weller, 1972; Minneman, Lynch, & Wurtman, 1974). Deguchi (1975a) hypothesized that "in the rats born and raised in continuous light a mechanism that partially prevents the suppressive effect of light develops" (p. 2818). This remains to be tested although similar effects are seen in the circadian rhythms of the sleep-wake behavior in rats (Hagino, Nakamoto, Saito, & King, 1979) and of sleep-states in the potoroo (rat kangaroo), a marsupial (Astic, Royet, & Saucier, 1976). Even though animals kept on LL or DD conditions from birth do not experience cyclic changes in ambient light they retain the ability to entrain their rhythms to a subsequent 24-hr LD cycle. This occurs for rhythms in plasma corticosterone (Krieger, 1973; Ramaley, 1978a, b) and pineal NAT levels (Deguchi, 1975a). Therefore, it appears that at least for these rhythms the capacity for entrainment to LD cycles may mature in the absence of cyclic light input.

In addition to LL or DD, non-24-hr LD cycles may modify the ontogeny of rhythms. Rat pups conceived and raised by dams kept on a 9:9 LD cycle show rhythms in corticosterone and plasma thyroid stimulating hormone which seem to be synchronized to the LD cycle

(Ooka-Souda, 1981). Similarly, rats reared from 2 to 3 days before birth on 6:6 LD cycles can entrain their pineal NAT rhythms to that cycle (Deguchi, 1977) and mice reared on 20 or 28-hr LD cycles also entrain to the non-24-hr cycles (Davis & Menaker, 1981). Entrainment to the non-24-hr LD cycles temporarily influences the period of the freerunning rhythm when the animals are subsequently placed on DD (Davis & Menaker, 1981). Despite obvious entrainment to the exotic light cycles, a circadian rhythm may still be present (Deguchi, 1977). Also, being reared under and entrained to 20 or 28-hr LD cycles does not prevent later entrainment of locomotor activity to a 24-hr LD cycle (Davis & Menaker, 1981).

Several other manipulations modify the development of circadian rhythms. For example, female rats that ingest ethanol in a liquid diet during the first postnatal week have offspring that are retarded in showing a day-night difference in blood corticosterone levels (Taylor, Branch, Cooley-Matthews, & Poland, 1982). This retardation, however, may be confounded by some other nutritional factor or the times of the data sampling. Ader (1969) and Ader and Deitchman (1970) have shown that the appearance of day-night differences in plasma corticosterone levels can be accelerated by shocking or handling rat pups daily or handling the dams during gestation. Acceleration of the maturational processes or changes in the interactions with the dam may account for these results (Ader, 1969; Ader & Deitchman, 1970). The development of the corticosterone rhythm can also be delayed or attenuated in rats

when they are injected with the corticosteroids, dexamethasone or cortisone acetate, during early prenatal life (Krieger, 1972; Poland, Weichsel & Rubin, 1981). The drug's influence depends partly on the sex (Miyabo & Hisada, 1975) and age of the animals (Poland, Rubin, & Weichsel, 1981).

Various manipulations during the early postnatal period can retard (corticosteroid injections, alcohol or other nutritional factors) or accelerate (shocking or handling) the development of some circadian rhythms. The long term consequences of these manipulations should be investigated. Prolonged exposure to LL or DD conditions from birth attenuates or disrupts some circadian rhythms, although subsequent entrainment to LD cycles appears normal. The persistence of circadian rhythms after extended DD or dim LL, or exposure to non-24 hr LD cycles from birth has been taken as evidence for the maturation of an endogenous clock (Minors & Waterhouse, 1981; Richter, 1971).

Maternal Synchronization and Entrainment

Prenatal and Postnatal Aspects

During the prenatal and preweaning period the mother exhibits rhythmicity in several processes which may potentially entrain or influence the biological rhythms of her offspring. For example, during the prenatal period mothers exhibit hormonal (Klein, 1972; Patrick, Challis, Campbell, Carmichael, Natale, & Richardson, 1980) and feeding (Barr, 1973; Strubbe & Gorissen, 1980) circadian rhythms. Similarly, during the preweaning period in rats circadian rhythms are apparent in

feeding (Strubbe & Gorissen, 1980; Tachi, Tomogane, & Yokoyama, 1981), nursing bouts (Ader & Grota, 1970; Croskerry, 1976; Grota & Ader, 1969; Hughes, Harlan, & Plaut, 1978; Leon, Adels, Coopersmith, & Woodside, 1984), and possibly in the transport of melatonin in the milk (Reppert & Klein, 1978). Since some of these maternal rhythms persist even under LL (Ader & Grota, 1970), the assumption that LL or DD are noncyclic environments for the offspring is incorrect.

Investigations on prenatal maternal synchronization of fetal rhythms in non-rodent species suffer from a variety of problems. Aside from technological and ethical considerations, some investigations lack the appropriate statistical analyses (Ruckebusch et al., 1977; Sterman, 1967) or the investigators draw tenuous conclusions from insufficient data (Evans, Carter, Brooke, & Smith, 1979; Ullner, 1974). Some studies are better controlled. For example, Patrick et al. (1981, 1982) observed human fetal heart rhythms which were positively correlated with the maternal heart rate rhythm. Recently, Taylor, Martin, and Nathanielsz (1983) demonstrated that the circadian rhythmicity of myometrial activity in pregnant monkeys is partly determined by the fetal hormonal system. The occurrence of human infants' REM sleep does not correspond to the occurrence of maternal REM sleep within five days after birth (Anders & Roffwarg, 1973), although the infants' REM sleep may be associated with maternal NREM sleep (Bertini, Antonioli, & Gamlei, 1978). Using time series analysis, Lawler and Brace (1982) observed fetal lamb circadian heart rate rhythms which were 180 degrees out of

phase with the ewe's heart rate rhythm. These authors suggested that the rhythms were independent of each other, although an alternative explanation is that they were synchronized in a negatively correlated manner. One problem in interpreting correlated data is determining whether the two rhythms are synchronized, or independent of each other but with similar periods.

Evidence for maternal entrainment of offspring rhythms in rodents is accumulating. For example, the acrophases of the corticosterone rhythms of blind pups born to and raised by dams entrained to a reversed cycle (DL) have peak levels that are about 12 hrs out of phase with the acrophases of pups born to and raised by dams on a LD cycle (Hiroshige et al., 1982a; Takahashi & Deguchi, 1983). In addition, the corticosterone rhythms of blind pups fostered to blind dams on a reversed light cycle were synchronized to the dam's rhythm, suggesting that the pups were not using extraretinal cues from the light cycle (Hiroshige et al., 1982b; Takahashi & Murakami, 1982).

There is some debate whether the initial entrainment observed in these studies occurs during the prenatal or postnatal period. In order to distinguish between these two possibilities a fostering procedure was adopted in most of the relevant studies. At birth, the pups are fostered to a lactating mother entrained to a light cycle reversed to that of the natural mother. The pups are either blinded at birth or kept with the foster mother on DD or dim LL to eliminate the effects of a light cycle on the pups' rhythms. The acrophases and

periods of the pups' rhythms are determined at several ages and then compared with the natural and foster mothers' rhythms or the rhythms of non-fostered pups.

Using the fostering procedure, both prenatal and postnatal influences are observed. Deguchi (1975a) found that at 3 weeks of age the pineal NAT rhythms of rat pups were most similar to their natural mothers' rhythms, suggesting a prenatal influence. Similar conclusions were made regarding the plasma corticosterone rhythms of blinded rat pups (Hiroshige et al., 1982c; Hiroshige, Honma, & Honma, 1983) and the locomotor activity rhythms of hamster pups (Davis & Gorski, 1982). Also, examination of fetal rat brains near parturition revealed prenatal maternal entrainment of deoxyglucose uptake in the SCN (Reppert & Schwartz, 1983). In an attempt to attenuate possible prenatal rhythmic influences, Honma, Honma, Shirakawa, and Hiroshige (1984a,b) lesioned the SCN of female rats 10 days after conception. At birth, the offspring were blinded and fostered to an LD-entrained dam. The offspring of SCN-lesioned dams had circadian corticosterone rhythms which were phase-shifted relative to the rhythms of pups from sham-operated dams, suggesting a prenatal effect. Davis and Gorski (1983) also reported desynchronized acrophases of locomotor activity rhythms within litters of hamster pups born to mothers whose SCN were lesioned at 7 days gestation. In addition, the acrophases of plasma corticosterone rhythms in pups fostered to dams on a different postnatal LD cycle tend to be more variable than those of pups reared

on the same light cycle from conception (Hiroshige et al., 1982b; Takahashi et al., 1982).

Predominantly postnatal effects are reported in other studies. Deguchi (1979) reported that 2 weeks after fostering, pups fostered at 10 days after birth had pineal NAT rhythms that resembled the foster mother's rhythm (Deguchi, 1979). Also, after fostering at birth, the pineal NAT acrophases of blinded pups resembled their foster dams most at 4, 6, and 8 weeks of age (Takahashi & Deguchi, 1983). Similar results were reported on the circadian plasma corticosterone (Takahashi, Hayafuji, & Murakami, 1982; Takahashi & Murakami, 1982; Yamazaki & Takahashi, 1983) and locomotor activity rhythms (Takahashi, Murakami, Hayafuji, & Sasaki, 1984) of rat pups.

Given the preceding results it is probably safe to propose that, at least in rodents, both prenatal and postnatal maternal entrainment can occur (Takahashi & Deguchi, 1983). The use of different statistical methods to determine rhythmicity and acrophases, data sampling problems (particularly in the hormonal studies), and the fostering technique (Ackerman, Hofer, & Weiner, 1977; although see Reppert, Coleman, Heath, & Swedlow, 1984) may also influence the results. It is also possible that some rhythms are entrained more effectively prenatally, and others postnatally. Furthermore, the studies involving SCN lesions of the pregnant dams (i.e., Honma et al., 1984a, b) present complications because it is not clear whether the offspring of the SCN-lesioned animals differ because of changes in the maternal rhythms

or some other lesion-induced effects. For example, Honma et al. (1984a) mentioned that 100% of the dams that had a major portion of their SCN lesioned before 10 days of gestation had spontaneous abortions. Also, some pups from SCN-lesioned mothers had poorly defined rhythms (see Figure 4 in Honma et al., 1984a).

Maternal Zeitgebers

There have been relatively few investigations examining what maternal rhythms or behaviors are capable of entraining the offspring's rhythms. More research has been done on postnatal, as opposed to prenatal, synchronization.

The most studied of potential maternal synchronizers of rat pups' rhythms are the maternal feeding and nursing rhythms. Although both these rhythms change during the preweaning period and are affected by variables such as the weight of the dam (Croskerry, 1976) and the size of the litter (Tachi et al., 1981), maternal feeding occurs mainly during the dark period (Levin & Stern, 1975; Stern & Levin, 1976) and nursing occurs mainly during the light period (Croskerry, 1976; Hughes et al., 1978). The changes in the rhythms have been attributed to the energy (Tachi et al., 1981) and thermal (Croskerry, 1976) demands of the offspring. The offspring of dams kept on ad lib food and water show greater diurnal than nocturnal weight gain up to about 3 weeks postnatally (Levin & Stern, 1975), indicating an association between offspring weight gain and the dam's feeding or nursing rhythms. Similar, but somewhat attenuated, nocturnal-diurnal differences in

weight gain are seen in blind offspring reared by sighted dams. In both these groups, nocturnal weight gain appears when the rat pups initiate nocturnal food intake, around 19 to 20 days postnatally (Levin & Stern, 1975). In contrast, sighted or blind pups raised by blind dams do not show day-night differences in weight gain up through about 19 days of age. It is possible that a rhythm in weight gain was present but was obscured since only two time periods were sampled in this experiment (Levin & Stern, 1975). The sighted pups raised by blind dams do show greater nocturnal weight gain starting around 21 days of age.

One way to manipulate nursing and feeding rhythms is to restrict the dam's food availability to either the light or dark portion of a 24-hr period. Day-restricted feeding shifts the nursing rat's plasma corticosterone peak such that it is opposite to that seen in ad lib fed animals (Tachi et al., 1981). Other investigators have observed synchronization of plasma corticosterone (Krieger, 1979) and activity rhythms (Spiteri, 1982) of adult rats to restricted feeding schedules. Not surprisingly, female rats restricted to diurnal or nocturnal feeding have litters that gain most of their weight during the night or day, respectively (Stern & Levin, 1976). Both of these groups gain less total daily weight than offspring of ad lib fed dams. Restricting the female rat's feeding to a certain period during the day not only changes the nursing rhythm, but also affects other types of contact with the litter. For example, Stern and Levin (1976) found that dams

with feeding restricted to the diurnal period spent more time nursing, licking, or sniffing their litter during the nocturnal period. In contrast, rats receiving food ad lib or only during the night spent more time with their pups during the day, although the ad lib dams spent signficantly more time with their pups at night than did the food-restricted animals (Stern & Levin, 1976).

The nursing rhythm can also be modified by separating the litter from the mother at different times during the day. For example, separating rat pups from the mother during the day is, in some respects, similar to restricting nursing to the nighttime. Deguchi (1977) observed that when rat pups were separated from the dam daily for 3 weeks during the light period (nursing occurs during the night) their pineal NAT rhythms were reversed relative to the dam's rhythm. In other words, the offspring's peaks occurred during the light period. However, when pups were separated during the dark period (nursing occurs during the day which is more similar to the natural situation), their rhythms were similar to the dam's rhythms; that is, peaks occurred during the dark period. Comparable results were seen in the plasma corticosterone rhythms of blind and sighted pups kept on day or night-restricted nursing (Hiroshige et al., 1982c; Miyabo, Yanagisawa, Ooya, Hisada, & Kishida, 1980). Besides changes in the acrophases of the corticosterone rhythms, some animals restricted to nocturnal nursing exhibited bimodal peaks at 4 weeks of age (Hiroshige et al., 1982c; Miyabo et al., 1980). This bimodal pattern is usually found

mainly in immature, 3-week-old rats (Ramaley, 1978a; Takahashi et al., 1979).

To summarize, maternal feeding and nursing rhythms appear to be involved in synchronizing circadian rhythms in offspring weight gains, pineal NAT levels, and plasma corticosterone levels. The offspring's rhythms can be shifted by shifting the mother's feeding (food-restriction) or nursing (separation) rhythms. It is also interesting that immature circadian plasma corticosterone rhythms were observed under the more unnatural, nocturnal nursing schedule.

One possible confounding factor in the separation and restricted feeding studies is that the pups on the restricted schedules tend to weigh less than rats on ad lib conditions (Hiroshige et al., 1982c; Stern & Levin, 1976). In fact, Hiroshige et al. (1982c) noted that a substantial proportion of their animals from both restricted nursing regimens failed to survive (25%) or failed to show rhythms at 4 weeks of age (29%). Postnatal undernutrition, produced by large litters (Macho, Fickovâ, Strbâk, & Földes, 1982) or a deficient maternal diet (Austin, Bronzino, Kelly, Cordova, Siok, Resnick, & Morgan, 1982) may retard the onset of the circadian plasma and adrenal corticosterone rhythms and change the pattern of REM sleep, respectively.

Outline of Experiments

The major purpose of the following experiments was to investigate the contributions of the mother-offspring environment to the development of the offspring's rhythms in more detail. A detailed discussion of the artificial rearing procedure and an examination of

the effects of artificial rearing on physical growth are presented in Chapter 2. This was deemed necessary since pilot studies indicated differences between artificially reared animals and mother-reared animals on several growth parameters. Other manipulations which influence physical growth (eg., undernutrition) have been shown to influence circadian rhythm development (Macho et al., 1982). This examination also provided an opportunity for studying the influence of different light cycles and feeding schedules on physical growth.

Chapter 3 presents the experiments on the development of the locomotor activity rhythm in rat pups. The experiments revolved around three general topics:

1. Unlike other circadian rhythms, such as plasma corticosterone, descriptions of the ontogeny of locomotor activity rhythms are either scant or incomplete. One of the initial studies involved recording the activity of mother-reared pups (MRLD group) between days 5 and 18 postnatally (PN).
2. Previous studies involving maternal separation dealt mainly with ultradian rhythms, such as sleep-wake states. In addition, the separation was usually for less than 24 hrs at a time. The artificial rearing paradigm allowed for very early and prolonged maternal separation, as well as for longer-term recordings needed for circadian rhythm detection.

3. What are the maternal and other environmental cues which may serve as synchronizers for the offspring's rhythms? Using the artificial rearing procedure, aspects of the complex mother-offspring environment which may influence rhythm development could be studied in isolation or in combination with other factors. Light cycles (ARLD and ARDL groups), constant conditions (ARLL and ARDD groups), and feeding cycles (ARLL-LF and ARLL-DF groups) were investigated. In addition, studies looking at the interactions between the light cycles and feeding schedules (ARLD-LF and ARLD-DF groups), as well as an investigation on environmental temperature (ARLD-Temp group), were included.

A synthesis of Chapters 2 and 3 as well as a general discussion of issues in developmental rhythm research are presented in Chapter 4.

Chapter 2

Growth of Artificially Reared Rats

Efforts to investigate particular aspects of mother-offspring interactions have led to the development of several methods of rearing offspring in the absence of the mother and/or littermates. Many investigations involve an acute separation (less than 24 hrs) of infant rats from their mother. Results from these studies indicate that heart rate (Hofer, 1973a,b; Koch & Arnold, 1976) and even survival rate (Holloway, Dollinger & Denenberg, 1979) are influenced by relatively brief periods of maternal separation. Questions involving the effects of long-term or chronic maternal separation require a rearing environment which will guarantee survival of the young animal. Ackerman and Shindledecker (1977) have successfully reared rat pups without their mother beginning at 2 weeks of age but their technique is not applicable to younger pups.

Initial attempts at rearing rat pups soon after birth under chronic maternal separation involved hand-feeding (eg., Dymsza, Czajka & Miller, 1964; Thoman & Arnold, 1968a). Every 2 to 4 hrs the pups were hand-fed by passing soft tubing down the esophagus and injecting a fluid diet through the tubing. In 1969, Messer, Thoman, Terrasa, and Dallman developed a method for implanting a gastric cannula in pups within 24 hrs after birth. A similar method was reported by Juvancz (1981). Delivery of an evaporated milk-based diet was automatically controlled by an infusion pump, eliminating the frequent handling of

the pups necessary in the hand-feeding procedure. Body weight gain was slightly less in the artificially reared (AR) pups than in mother-fed controls. At 20 days PN, AR animals had heavier cecums, livers, and kidneys than control animals. Other organ weights, such as heart, spleen, lungs, and brain, did not differ significantly. Hall (1975) modified Messer et al.'s (1969) artificial rearing technique. He reported a survival rate of 80% when animals were cannulated between 12 and 48 hrs after birth, although at weaning (18 days PN) the body weight of the AR animals was less than normally reared rats. Femur length was not significantly different between the two groups. Thompson (1983) also reported that normal innervation of the soleus muscle occurs in AR pups.

One problem with the Messer et al. (1969) and Hall (1975) technique is the continuous infusion of the diet. Messer et al. reported overdistension of the stomach, especially at younger ages. Also, survival rates and body weight gains are not always optimal (Goldenring, Shaywitz, Wool, Batter, Anderson & Cohen, 1982; Juvancz, 1981). Earlier groups of pups run in our laboratory with continuous feeding also showed similar problems. We adopted an intermittent feeding schedule with rats being fed 2 to 3 hrs four times daily. This decreased the incidence of abdominal bloating and the pups gained more weight. Similar results were reported by Anderson, Raffety, Birkhofer, and Fomon (1980) who found that AR pups with more frequent feedings had higher survival rates and greater body weight gains than AR pups

receiving the same amount of food but in fewer feedings. The more frequently fed animals also had smaller livers, stomachs, and small intestines and larger spleens. Since in the normal rearing situation the mother rat exhibits frequent nursing bouts (Croskerry, 1976; Hofer & Grable, 1971) it is not surprising that intermittent feeding is more beneficial to the pups than continuous feeding.

Recently, other investigators have adopted an intermittent feeding schedule for the AR animals. Smart, Stephens and Katz (1982, 1983a) fed AR animals 12 times a day and found similar body weights at 21 and 25 days PN when compared to mother-reared pups. Despite the similar body weight gains AR animals had shorter bodies and tibias and lighter gastrocnemius muscles, brains, and adrenals than mother-reared pups. Livers and epididymal-fat-pad weights and small intestine lengths were greater in the AR animals (Smart et al., 1983a; Smart, Tonkiss, Stephens, Edmond, & Auestad, 1983b). Similar results on body, brain, and liver weights were found by Diaz, Moore, Petracca, Schacher and Stamper (1981b) in 18-day old AR pups fed on an intermittent schedule. In addition, Diaz et al. (1981b) reported heavier kidneys and spleens in the AR pups. Some of the differences between AR and mother-reared animals including adrenal and brain weights and body lengths, are still evident at 224 days PN (Smart et al., 1982, 1983a). In addition to the differences in somatic growth, Stamper, Petracca, Houghton, and Diaz (1982) found decreased serum levels of the thyroid hormone, T4, and Smart et al. (1981, 1983a) reported accelerated eye opening in AR pups.

It is clear that even though relatively normal body weights can be attained by AR pups on an intermittent feeding schedule, other organ weights do not show normal growth. Reasons for these differences may include the low protein and high carbohydrate content (Diaz et al., 1981b; Smart et al., 1982) or the presence of toxins or "inappropriate components" (Diaz, Moore, Stamper & Petracca, 1982a) in the Messer et al. (1969) diet. Other possibilities include differences in the amount of food delivered to AR pups compared to normal pups (Smart et al., 1983a) and in the patterning and timing of the food delivery. Also, lactating rats exhibit a circadian rhythm in nesting bouts (includes nursing bouts) with their offspring (Croskerry, 1976; Grota & Ader, 1969) and it is possible that this rhythmicity is important for healthy physical development. For example, cyclicity of the liver enzyme, tyrosine transaminase, may be dependent on rhythmic food intake in neonatal rats (Honova et al., 1968).

In the following studies, animals were reared artificially using a technique similar to Hall's (1975). Organ weights, which are useful indices of physical health and development, were gathered from animals kept under different light and feeding cycles and temperature conditions. This information, in turn, might aid in the interpretation of the behavioral measures discussed in Chapter 3.

Methods

Subjects

With the exception of one group to be described later, animals were Long-Evans hooded rats born and raised in our colony. Nulliparous

females between 4 and 6 months old were bred with males of approximately the same age. Vaginal smears were obtained daily between 0900 and 1130 hrs and the presence of sperm was used to determine the day of conception (Day 0). Males and females were separated between 2 and 5 days after conception. Colony lights were on from 0800 to 2000 hrs and off from 2000 to 0800 hrs (12:12 LD) unless otherwise specified.

The ages of the rat pups were calculated from the day of conception so that at birth a rat would be approximately 21 days old post-conception (PC). The reason for using PC ages is twofold; some animals are born after a 22-day gestation and the PC age adjusts for this difference. (In the present studies 61.7% were born after 21 days, 30.8% after 22 days, and the remaining dates were unknown.) Second, the appearances of some developmental indices, such as eye opening, depend on the PC age rather than the PN age (Croskerry, 1976).

Surgical Procedure

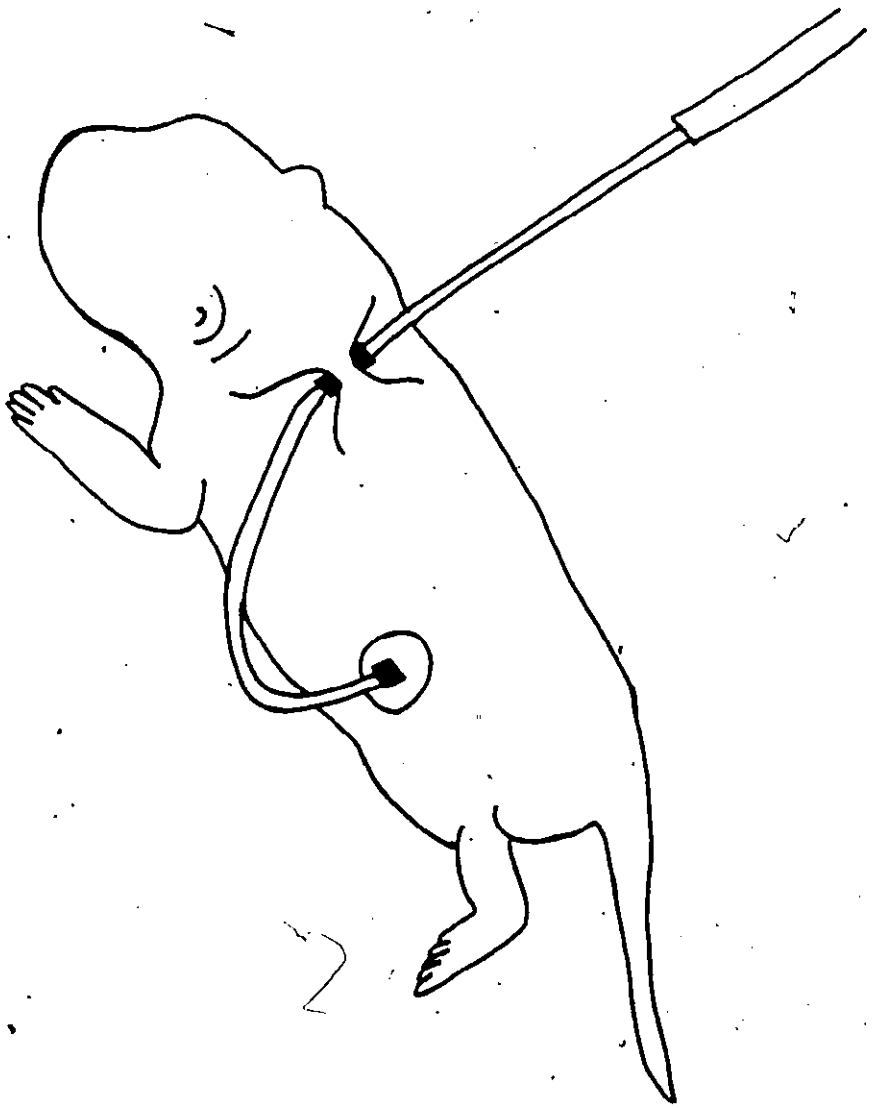
At 24 days PC rat pups were surgically implanted with gastric cannulas (Hall, 1975). Due to relatively poor survival rates in earlier groups, later groups were operated at 25 days PC. Gastric cannulas were constructed from a 15 cm length of polyethylene tubing (PE-10, Clay Adams) heated at one end to form a small flange. This flange held a small (1.6 mm diameter) flexible plastic washer in place. For anesthetization, rats were placed in a glass container with

ether-soaked cotton balls for 3 to 3.5 min. A 6.2 cm piece of silastic tubing (Silastic, Medical-Grade Tubing, Dow Corning Corp., .012 in inner diameter by .025 in outer diameter) was lubricated with corn oil and threaded down the rat's esophagus. A slightly curved and lubricated piece of guitar string wire (8.3 cm long, 0.254 mm diameter) was threaded down the silastic tubing. The tip of the wire was then pushed out through the gastric and peritoneal walls and the skin. The silastic tubing was gently pulled over the wire and out of the mouth. The gastric cannula (also lubricated with corn oil) was then threaded onto the tip of the wire protruding from the mouth. These were then pulled slowly and gently down the esophagus and through the skin until the plastic washer was against the inside stomach wall. A rubber washer (1.6 mm diameter) and a small polyethylene washer (PE-50) were threaded onto the cannula and pushed against the pup's flank. To prevent the cannula from being pulled out the wire and cannula were pulled through a small fold of skin on the back of the neck and secured with two PE-50 washers (see Figure 1). The surgery took approximately 5 min.

Care and Maintenance

Following surgery the pups were individually placed in styrofoam bowls (80 mm diameter, 40 mm deep, size 10S, Fibracan, Inc.) containing wood shavings and covered with clear plastic lids with six or seven 10 mm holes. Each bowl was then placed in two more styrofoam bowls one of which had metal weights attached to the bottom to prevent

Figure 1. A rat pup with the gastric cannula in place. The black squares represent the PE-50 washers.



tipping. They were then placed in a water bath incubator (National Appliance Co., Model 230) which was divided into eight compartments by plastic partitions. A 2 mm layer of mineral oil was added to prevent the evaporation of the water. In some studies Halazone tablets (Abbott, 4.0 mg) were added to the water to inhibit microbial growth. Unless otherwise indicated, the water temperature was kept between 45 and 49 degrees C which maintained the temperature inside the cups at approximately 29 degrees C. Humidity varied with the outside weather conditions and was determined from a wet bulb-dry bulb thermometer.

The pups were fed a diet similar to that described by Messer et al. (1969; see Table 1). The diet was delivered by 10 cc plastic syringes (Becton-Dickinson) with 23-gauge needles. The syringes were placed on infusion pumps (Harvard Apparatus Co., Inc., Model 907) which were housed in a refrigerator. One pump could hold 8 syringes. PE-50 tubing was attached to the syringes at one end and the gastric cannula at the other end. By the time the diet reached the pups' stomachs it was equilibrated with the room temperature. On the day of surgery (24 days PC) the infusion rate was 2.0 ml/24 hrs and was increased by approximately 0.62 ml to 1.00 ml a day. The amounts of food infused daily from 24 to 39 days PC were approximately 2.0, 2.6, 3.1, 3.8, 4.3, 4.9, 5.6, 6.4, 7.0, 7.8, 8.5, 9.6, 10.7, 11.6, 12.4, and 12.4 mls.

Two types of feeding schedules were used. The first was designed to minimize possible entrainment to the feeding schedule (referred to as the noncyclic schedule). Intervals of 25, 30, 35, 40,

Table 1

Artificial diet

Ingredient	Amount(per 1 liter)
Evaporated milk(Carnation)	750.0 ml
Distilled water	170.0 ml
Corn oil	66.0 ml
Vitamins[a]	10.0 ml
$FeSO_4$(BDH Chemicals)	27.0 mg
$CuSO_4$(Fisher Scientific)	15.0 mg
$ZnSO_4$(McArthur Chemical)	16.0 mg
DL-Methionine(BDH, Ltd.)	1.0 g
L-Tryptophan(BDH Chemicals)	0.5 g
Riboflavin(Grand Island Biological)	10.0 mg
Pyridoxal HCl[b]	10.0 mg

Note. Ingredients were mixed in a blender for 2 to 3 min, degassed, and then frozen in 100 ml glass containers until needed.

[a]Polyvisol (Mead-Johnson) or Infantol (Horner) liquid vitamin mixtures were used.

[b]Initially a powdered form (Grand Island Biological) was used. Later diets contained a liquid form (Parke-Davis).

or 45 min were randomly assigned as alternately feeding or nonfeeding times such that the total feeding time in 24 hrs was approximately 12 hrs. The mean duration of an interval in a 24-hr period was approximately 34 min and there were 10 to 11 feeding intervals between 0800 to 2000 hrs and an equal number between 2000 to 0800 hrs. The second feeding schedule (referred to as cyclic) was used for the feeding entrainment groups. As in the noncyclic schedule, the distribution of feeding intervals was similar between 0800 and 2000 hrs and 2000 to 0800 hrs. However, the proportion of 24-hr feeding time differed between the two 12-hr periods. Animals could receive 65.3% (470 min) of their feeding time during one of the 12-hr intervals and 34.7% (250 min) during the remaining 12-hr interval. These proportions were based on data on the distribution of nest/nursing bouts during the day and night (Croskerry, 1976). When two pumps were used for feeding the intervals were counterbalanced such that one group of animals was fed while the other was not fed. The feeding schedules were controlled by an automatic timer (AMF Paragon, Model 23002-00S). See Appendix A for detailed information on the feeding schedules.

To minimize possible entrainment to the care schedule, daily care occurred at one of four randomly assigned times: 0600, 1200, 1800, or 2400 hrs. Daily care of the rat pups consisted of weighing, checking for eye opening, and gently massaging the abdominal area with a cotton ball to stimulate micturition and defecation. The washers on the cannulas were adjusted to accommodate growth. The gastric cannulas

and the tubing from the pumps were flushed daily with physiological saline to prevent clogging by the liquid diet. The water bath temperature and humidity were recorded. Diet and the wood shavings in the bowls were renewed every other day. As the rats became older it was necessary to change the diet syringes twice a day, once during the scheduled care period and once between 0900 and 2200 hrs. Control littermates were weighed during the same care period as the AR animals. The intensity of the light during the light period of the LD cycles or the LL conditions in the artificial rearing room was approximately 516 to 688 lux at the level of the cups in the incubators (measured with a Sekonic Studio Deluxe exposure meter, Model L-28c2). Due to the lids, the intensity inside the cups was less, 473 to 602 lux. A 25W red light was used for all routines which occurred during the dark period.

Description of Groups

All animals were cared for as described previously. Pups were weaned at approximately 39 days PC unless otherwise stated. Table 2 summarizes information on litter sizes, sample sizes, water temperatures and humidity for each group to be described below.

Artificially reared on 12:12 LD (ARLD). These animals were conceived and born in the colony room under a 12:12 LD cycle (lights on from 0800 to 2000 hrs). They underwent surgery at 24 days PC and were transferred to the artificial rearing room which was kept on the same LD cycle as the colony room and were fed with the noncyclic feeding schedule. Two groups were tested, one in August and one in September, 1982.

Artificially reared on continuous light (ARLL). These animals were conceived while their dams were on the 12:12 LD colony cycle. On the day before expected parturition the dams were placed on LL. At 24 days PC the pups were cannulated and then artificially reared under LL conditions with a noncyclic feeding schedule. Two groups were run, one in December, 1982 and one in January, 1983.

Artificially reared in constant darkness (ARDD). These animals were conceived by dams on the 12:12 LD cycle. Dams were moved to DD conditions one day before parturition. Except for a 45-min exposure to light during surgery (24 days PC for February and March, 1983 and 25 days PC for June, 1983) and equipment adjustment these pups only experienced DD conditions from birth to weaning. The noncyclic feeding schedule was used.

Artificially reared on 12:12 DL (ARDL). Hooded female rats were received from Blue Spruce Farms (Altamont, N.Y.) and entrained to a 12:12 DL cycle (lights on from 2000 to 0800 hrs) for one month before mating with DL-entrained males from our colony (April, 1983 group). Another group tested in May was the offspring of our colony females entrained to the DL cycle. They were maintained on the DL cycle throughout pregnancy. Surgery on their offspring occurred early during the dark period at 24 days PC in April and 24 or 25 days PC in May. Except for the reverse light cycle these animals were similar to the ARLD group in treatment.

Artificially reared on 12:12 LD with cyclic feeding. These animals were conceived to dams on a LD (July, August, and September, 1983 groups) or DL (June, 1983) cycle. Surgery occurred at 25 days PC and animals were kept on the respective light cycles throughout rearing. In addition to the light cycle, half of the animals in each group were also kept on one of two cyclic feeding schedules. On one schedule 65.3% of the feeding time occurred during the light period and 34.7% occurred during the dark period (this group will be referred to as ARLD-LF). The other feeding schedule was the reverse with feeding occurring mainly during the dark period (ARLD-DF group).

Artificially reared on LL with cyclic feeding. These animals were similar to the groups on LD with cyclic feeding except that they were born and raised under LL conditions. Their dams were kept on 12:12 LD during pregnancy. Surgery occurred at 25 days PC. Again, two cyclic feeding schedules were used; one with feeding mainly during 0800 to 2000 hrs (ARLL-LF group) and one with feeding mainly during 2000 to 0800 hrs (ARLL-DF group). Two groups were tested, one in late September and one in October, 1983.

Artificially reared on 12:12 LD with a feeding and temperature cycle (ARLD-Temp). These animals were similar to the ARLD-LF group except that they were also exposed to an environmental temperature cycle. During the light period the mean water temperature was 59.5 degrees C (sem=0.14) and 32.9 degrees (sem=0.16) inside the cup. During the dark the water temperature was 45.7 (sem=0.36) and 27.3

(sem=0.16) inside the cup. These values were based on observations by Leon, Crosskerry, and Smith (1978) who found that the rat's nest temperature decreased about 3.2 degrees C when the dam left. To increase the incubator temperature the temperature knob was manually adjusted at 0700 hrs, 1 hr before light onset. To lower the temperature the knob was adjusted at 1715 hrs, 2.75 hrs before dark onset.

Mother-reared on 12:12 LD (MRLD). Seven groups of four animals each were randomly chosen at birth from five litters with the stipulation that all four animals were of the same sex and from the same litter. The animals were marked with a black felt-tip pen on the right or left hindpaw or forepaw. One pup from each of the seven groups was transferred to the artificial rearing room, weighed and then placed in the water bath incubator at the beginning of one of three 8-hr intervals: 0600 to 1400, 1400 to 2200, or 2200 to 0600 hrs. The pups were left undisturbed and were not fed during these intervals. At the end of the 8 hrs the pups were again weighed and then returned to the colony room where they were allowed to suckle ad lib with their dams. A new pup was then chosen and the routine repeated until all four pups had been in the incubator. This rotation meant that each pup in a group was food and mother-deprived for 8 out of every 32 hrs. Also, each pup experienced all three 8-hr intervals over a 3-day period. These animals were conceived, born and reared until weaning on the 12:12 LD cycle during October, 1982. Two pups from two different

litters were replaced; one drowned and the other became ill.

Control littermates. Control littermates of all AR and MRLD pups were reared by their dams in the colony room. Control pups for the ARLD, ARDL, ARLD-LF, ARLD-DF, ARLD-Temp, and MRLD groups were reared under 12:12 LD or DL (ARDL group). Control pups for the ARLL, ARLL-LF, ARLL-DF, and the ARDD groups were kept on LL or DD, respectively, 3 to 4 days after birth. Thereafter, they were raised on 12:12 LD.

Organ Weights

Most of the rat pups were weaned and immediately sacrificed at about 39 days PC in the evening or early the next morning. MRLD pups and five AR pups were weaned in the early evening and sacrificed the following morning. Mother-reared pups were weaned and sacrificed at the same time as their AR littermates. Body weights were taken and the animals were decapitated. The forebrain, cerebellum (including part of the brainstem anterior to the medulla), liver, spleen, and kidneys were dissected out and immediately weighed to the nearest 0.0001 grams on a Sartorius balance (Type 2442).

Results

Survival Rates

The proportion of animals that survived with their gastric cannulas intact four days before weaning was 43.3% (total number operated was 217). Surgery at 25 days PC (n=117) as compared to 24 days PC (n=100) improved the survival rate, 47% versus 39%. Deaths

which occurred within 2 days after surgery (n=32) were probably the result of internal bleeding and/or problems in recovering from the anesthesia. Sixteen pups died from bloating or stomach distension. Some animals (n=36) died from "unknown causes"; this category may include some surgery-related or bloating deaths. Of the remaining 39 pups, 33 pulled the cannulas out of their stomachs, 5 were killed because of severe weight loss (clogged or leaking cannulas) and 1 drowned. There was not a significant linear correlation between the survival rate and the mean humidity of each AR group but there was a trend for AR groups with the warmer water bath temperatures to have higher survival rates (see Table 2).

Some of the animals had sores on their paws as indicated by cracked and swollen skin. The occurrence of these sores seemed to be related to low relative humidity. Dry skin on the back and stomach was also observed in some animals. Seven out of the 94 surviving animals exhibited cataracts in the eyes (a cloudy white film obscuring the lens). Five of the seven animals were under conditions of constant light.

Body and Organ Weights

Organ weights were gathered from all groups excluding ARDL and AR-Temp. Unless otherwise stated statistics on organ and body weights were performed on litter values rather than values for individual rat pups. Due to possible intra-litter similarities, the litter values provide more conservative estimates for statistical purposes (Croskerry, 1976).

Table 2

Sample sizes, humidity, incubator temperatures, and survival rates

Group	Litter size	Number of litters	Humidity (%)	Incubator Temperature	Survival Rate
ARLD	4-15	8(15)[a]	52.4	46.9	53.6
ARLL	3-11	7(10)	34.7	47.8	41.7
ARDD	5-13	7(12)	43.7	47.5	30.8
ARDL	7-15	7(11)	43.6	47.2	37.9
ARLD-LF	7-14	10(14)	59.1	46.6	45.2
ARLD-DF	4-17	8(10)	58.1	47.0	38.5
ARLL-LF	9-13	4(7)	51.6	47.4	50.0
ARLL-DF	11-15	5(6)	52.9	46.7	46.1
ARLD-Temp	9-13	5(9)	45.9	52.6	69.2
MRLD	8-14	5(7)[b]	50.2	47.4	92.3

Note. The litter size refers to the range of litter size at birth. For the AR pups survival rate refers to the proportion of total individual animals operated on that had intact cannulas at least 4 to 5 days before weaning. In the MRLD group two pups died but were replaced with littermates. Humidity and incubator water temperature values are the average over all days.

[a]Numbers in parentheses are the numbers of individual animals.

[b]The value in parentheses refers to the number of groups, each having four animals (see text).

The mean body weights for the combined AR groups, control littermates, and the MRLD group are plotted as a function of age in Figure 2. Each litter value in the control group was made up of two to eight pups with a mode number of four (where possible, equal numbers of males and females were included). Although the AR animals did not differ significantly from control littermates at 39 days PC their weights were less between 27 and 38 days PC. The MRLD animals were relatively similar to AR animals up to about 35 days PC and thereafter their average weight gain was less. In fact, at 39 days PC there was a slight loss in weight in the MRLD animals although they did not significantly differ from their control littermates. The MRLD control littermates weighed less at weaning than the other control littermates.

The means and standard errors of the organ weights at approximately 39 days PC for the AR groups, the MRLD group, and their control littermates are given in Table 3. The body weights given here only include animals which were sacrificed for organ weight measurements. There were no significant differences (t-tests) between females and males in any of the measures for both the artificially reared animals and their control littermates. Females and males were combined for subsequent analyses.

Compared to the control littermates AR animals (all AR groups combined) had significantly smaller cerebellums ($t(52) = 4.84$, $p<.01$) and forebrains ($t(52) = 8.07$, $p<.01$). In contrast, the livers of the AR animals were significantly heavier ($t(53) = -5.45$, $p<.01$).

Figure 2. Mean body weights (± sem) of the AR pups (squares, $\underline{n}$ = 24 to 61), MRLD pups (triangles/solid line, $\underline{n}$ = 5), AR control littermates (dashed line, $\underline{n}$ = 37), and MRLD control littermates (open circle, $\underline{n}$ = 5). Sample sizes are based on the number of litters.

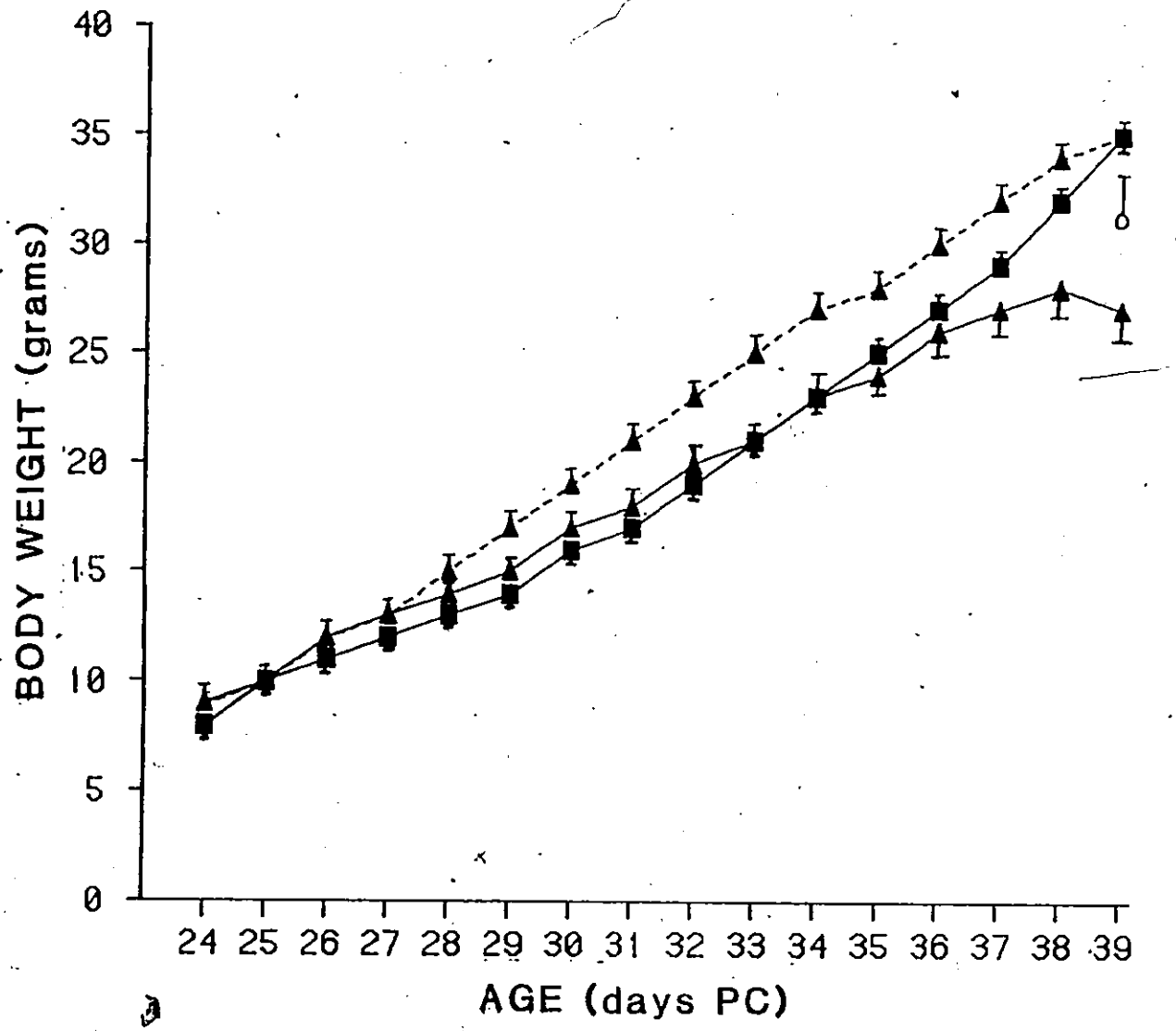

BODY WEIGHT (grams)
40
35
30
25
20
15
10
5
0
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
AGE (days PC)

Table 3

Mean organ weights

Group	Body	Cerebellum	Forebrain	Spleen	Liver	Kidneys
ARLD;2[a]	32.7	228.8	940.1	172.6	1668.4	407.0
	(0.9)	(6.1)	(1.4)	(5.1)	(124.8)	(35.9)
ARLL;5	38.2	224.1	945.2	188.5	1801.2	451.3
	(1.4)	(4.2)	(30.9)	(18.5)	(44.3)	(19.8)
ARDD;2	30.3	190.7	923.8	103.5	1455.4	402.2
	(1.2)	(9.3)	(3.8)	(24.2)	(41.8)	(15.6)
ARLD-LF;5	35.8	198.7	934.0	156.0	1437.4	424.2
	(1.9)	(8.3)	(15.2)	(13.7)	(129.0)	(29.9)
ARLD-DF;5	33.7	189.2	889.9	162.1	1493.3	403.5
	(1.7)	(12.0)	(12.5)	(18.5)	(94.5)	(18.5)
ARLL-LF;3	38.3	222.2	981.5	211.7	1535.4	458.3
	(1.9)	(12.7)	(23.7)	(64.4)	(135.8)	(43.3)
ARLL-DF;2	34.2	212.1	930.3	195.7	1419.0	444.7
	(3.3)	(2.1)	(2.1)	(26.9)	(320.6)	(22.5)
All AR;24	35.3	207.9*	932.4*	171.3	1556.4*	420.5
	(0.8)	(4.6)	(9.3)	(10.7)	(49.9)	(14.3)
Controls;26	36.2	242.6	1064.8	168.7	1265.5	396.1
	(0.9)	(5.7)	(10.4)	(7.3)	(34.1)	(10.8)
MRLD;5	27.5	215.2	991.2	88.8	925.5	312.3
	(2.3)	(3.7)	(16.4)	(18.1)	(77.7)	(28.2)
MRLD Controls;5	31.1	236.7	1030.9	114.4	985.1	364.5
	(2.5)	(11.2)	(57.9)	(15.3)	(88.4)	(27.0)

Note. Values are the means and the numbers in parentheses are the sem. Body weights are in grams and the other weights are in milligrams.

[a]The number beside the group label indicates the number of litters.

*Significantly different from controls at $p<0.01$, t-test.

Differences between the groups were not seen in the remaining organ weights or the body weights. The same pattern of results was obtained when the organ weight: body weight ratios were analyzed. That is, AR animals had significantly smaller cerebellum and forebrain: body weight ratios ($t(52) = 4.42$ and 3.81, respectively, $p<.01$) and significantly larger liver: body weight ratios ($t(53) = -7.86$, $p<.01$). In contrast to the AR pups the MRLD pups, each of which had been food deprived for 8 out of every 32 hrs did not differ significantly on any measure from their littermate controls (Mann-Whitney U-tests). There were trends towards lighter organ and body weights in the MRLD group but these failed to reach significance probably due to the small sample size. Similarly, the organ weight: body weight ratios did not differ between MRLD animals and their control littermates.

The AR rats described above were exposed to different light and feeding cycles and different humidity conditions. Also, some of the animals experienced intervals of accidental food deprivation. The main cause of this problem was that the tubing leading from the diet syringes to the gastric cannulas, or the cannulas themselves, became clogged with food. This, of course, prevented delivery of the diet to the rat pups. In an attempt to determine the contribution of these factors to the observed organ and body weights a BMDP stepwise multiple regression analysis (Dixon, 1983, program 2R) was done on the values from individual rat pups (total n= 33). Each of the weight measurements was regressed on the following five variables: humidity, light conditions (cyclic- LD or noncyclic- DD/LL) feeding schedule

(cyclic or noncyclic), amount of food delivered during "light" period (34.7, 50.0 or 65.3%) and the total number of 12-hr periods (starting with 2000 to 0800 hrs) throughout rearing during which an interval of accidental food deprivation occurred. Since some of the animals were reared for 16 days, the last variable (food deprivation) could range from 0 to 32. The independent variables were entered into the analysis in the following order: light conditions and feeding schedule, food deprivation and humidity, and finally, amount of food.

Table 4 presents the means and standard errors of the organ and body weights for each of the independent variables. For simplicity, the two continuous variables, humidity and food deprivation, were divided into two and three categories, respectively. The multiple R's and the partial correlations for each dependent variable are presented in Table 5. The best predictors for body weight were the type of feeding schedule, humidity, and the amount of "light" period feeding (R^2 = variance = 26.6%). In other words, heavier body weights were correlated with low humidity, a cyclic feeding schedule, and more feeding during the light period. These results can also be seen in Figure 3 which summarizes the weaning body weight data for each of the AR groups (all animals). Overall, animals with a cyclic feeding schedule and those fed mainly during the light period (ARLD-LF and ARLL-LF) had heavier body weights. The results in Figure 3, however, should be viewed with caution since accidental food deprivation was not considered and some of the groups have small sample sizes. The AR control littermates and MRLD animals are included for comparison.

Table 4
Mean organ weights for the independent variables in the regression analysis

Variable		Body	Cerebellum	Forebrain	Spleen	Liver	Kidneys
Light:							
	Cyclic;18	34.1	205.0	922.5	164.0	1560.1	416.5
		(0.7)	(7.3)	(9.1)	(7.6)	(55.6)	(98.2)
	Noncyclic;15	36.2	218.5	952.2	189.7	1595.7	444.9
		(1.1)	(4.5)	(13.0)	(18.9)	(71.1)	(13.5)
Feeding							
	Cyclic;19	35.4	202.4	933.4	182.0	1486.6	428.7
		(0.9)	(6.3)	(11.1)	(14.9)	(54.2)	(13.5)
	Noncyclic;14	34.4	233.0	939.6	167.0	1697.9	430.4
		(1.0)	(5.3)	(11.9)	(10.4)	(60.0)	(10.3)
"Light" Feeding	34.7%;8	33.8	192.3	902.4	177.3	1478.0	415.1
		(1.3)	(9.5)	(12.6)	(14.9)	(84.9)	(13.7)
	50.0%;14	34.4	233.0	939.6	167.0	1697.9	430.4
		(1.0)	(5.3)	(11.9)	(10.4)	(60.0)	(10.3)
	65.3%;11	36.7	209.8	956.0	185.5	1492.9	438.6
		(1.2)	(8.9)	(13.5)	(24.1)	(73.9)	(21.2)
Humidity	30-49%;10	36.6	222.0	955.9	200.4	1687.5	452.9
		(1.6)	(6.5)	(19.0)	(27.4)	(81.9)	(19.6)
	50-60%;23	34.4	206.4	927.4	164.9	1527.8	419.2
		(0.6)	(5.7)	(7.7)	(6.6)	(49.6)	(8.8)
Food Deprivation	1-3;11	36.5	220.4	955.4	193.7	1636.5	440.8
		(1.3)	(5.5)	(18.7)	(23.5)	(64.9)	(19.9)
	4-6;13	34.8	218.6	929.8	174.9	1576.9	441.5
		(1.0)	(5.9)	(10.0)	(8.6)	(86.4)	(11.5)
	7-9;9	33.5	189.1	921.3	154.7	1501.5	397.9
		(1.1)	(9.9)	(10.4)	(15.9)	(65.4)	(8.7)

Note. Values represent means (sem). The numbers to the right of the semicolons are the number of individual rat pups.

Table 5

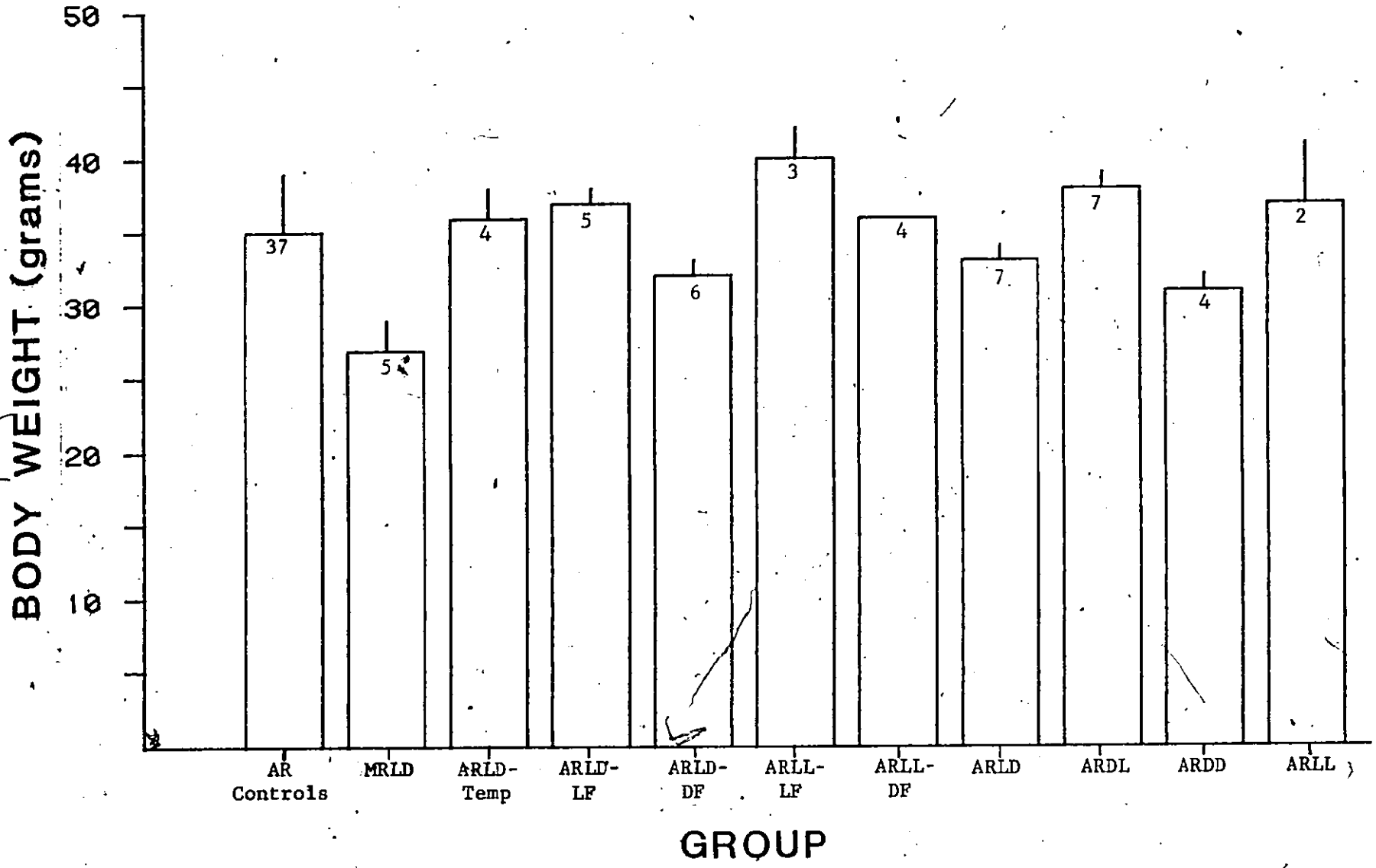
Stepwise regression results
for organ weight data

Organ	Independent Variable: Light Conditions	Feeding Conditions	"Light" Feeding	Humidity	Food Deprivation	Multiple R^a
Body		0.36	0.28	-0.43		0.515
Cerebellum		-0.43			-0.45	0.573
Forebrain	-0.30		0.41			0.509
Liver		-0.42				-0.422
Spleen		0.39		-0.45		0.463

Note. Values under the independent variables are the correlations of each independent variable with the dependent variable after the effects of the other variables have been taken into account. Blanks signify nonsignificant variables.

[a]All multiple R's were significant at $p<0.05$ except for the cerebellum which was significant at $p<0.01$. R^2 is the amount of variance accounted for by the given combination of independent variables.

Figure 3. Mean body weights (+ sem) at weaning (38.5 days PC) for the MRLD group, each of the AR groups, and the AR control littermates. Values shown underneath the sem bar are the number of litters used in each calculation.


BODY WEIGHT (grams)
50
40
30
20
10
37
5
4
5
6
3
4
7
7
4
2
AR Controls
MRLD
ARLD-Temp
ARLD-LF
ARLD-DF
ARLL-LF
ARLL-DF
ARLD
ARDL
ARDD
ARLL
GROUP

The feeding schedule and amount of food deprivation accounted for 38.2% of the variance in cerebellar weight indicating that lighter cerebellums were associated with a cyclic feeding schedule and more intervals of food deprivation. Heavier forebrain weights were correlated with DD/LL conditions and higher amounts of "light" period feeding (25.9% of the variance). Heavier spleens correlated with a cyclic feeding schedule and low humidity (21.5% of the variance). Only one variable correlated significantly with liver weight, the feeding schedule (17.8% of the variance) with lighter livers associated with a cyclic feeding schedule. None of the variables correlated significantly with kidney weight.

Eye Opening

The age of eye opening was defined as the age when both eyes were open. Litter values were used for the following Mann-Whitney U-tests. Except for the animals reared on DD, all other AR rat pups opened their eyes significantly sooner than their control littermates (see Table 6). On the average, AR pups opened their eyes 1.2 days sooner with a range of .3 days (ARDD) to 2.1 days (ARLL). In contrast, there were no significant differences in eye opening age between MRLD animals and their control littermates.

Discussion

The relatively normal body weights of the AR pups at weaning observed here have been reported by other researchers (Diaz et al., 1981b; Smart et al., 1983a; Sonnenberg, Bergstrom, Ha & Edmond, 1982).

Table 6

Average age at eye opening (Days PC)

Group	Experimentals	n	Controls	n	p[a]
MRLD	36.1(0.66)	5	36.3(0.51)	5	0.421
ARLD	35.6(0.19)	8	36.4(0.94)	7	0.036
ARDL	35.1(0.45)	7	36.8(0.86)	6	0.002
ARLL	34.9(0.92)	7	37.0(0.41)	6	0.001
ARDD	35.6(0.89)	7	35.9(0.60)	6	0.314
ARLD-Feed	35.0(0.82)	17	35.9(0.56)	17	0.002
ARLL-Feed	35.2(0.69)	7	36.8(0.58)	7	0.002
ARLD-Temp	34.7(0.45)	5	35.9(0.39)	5	0.008

Note. Values in parentheses are the sem.

[a]The significance level based on Mann-Whitney U-tests.

In contrast to these reports, daily body weight gains of my AR pups were not always comparable to those of mother-reared pups throughout rearing partly due to accidental food deprivation and other reasons to be discussed later. Also, frequent disturbances to the control littermates of the MRLD group may account for their low weight gains relative to other control animals (Lee & Williams, 1977a).

Despite the normal body weights at weaning, AR animals differed from their control littermates in the weights of other organs. The finding of lighter brains in the AR animals when compared to mother-reared pups is supported by Diaz et al. (1981b) and Smart et al. (1983a) although other researchers report no differences (Messer et al., 1969; Sonnenberg et al., 1982). These discrepant results might be due to different feeding schedules or other experimental methodologies and/or different strains of rats. The brain weights of the AR pups reported by Messer et al. (1969) were slightly smaller than mother-reared pups but the difference was not significant. In the Sonnenberg et al. (1982) study no brain weights are given for comparison. Deficits in cerebellar and cerebral weights are consistent with malnutrition or undernutrition during the early postnatal period. For example, in the undernourished rat whole brain or cerebral weights show deficits of between 11.5% and 29.3% near or at the time of weaning (Barnes & Altman, 1973; Culley & Lineberger, 1968; Dobbing & Sands, 1971; Fish & Winick, 1969; Rajalakshmi, Ali & Ramakrishnan, 1967; Sobotka, Cook & Brodie, 1974; Wehmer & Jen, 1978). The deficits

observed in the AR animals tend to be less or at the lower end of this range; 6% (Smart et al., 1983a), 10.1% (Diaz et al., 1981b), and 12.4% (my studies).

Due to some postnatal development of the cerebellum, the weight deficits (compared to cerebral weight deficits) are usually greater as a result of undernutrition, ranging from 15% to 41% (Barnes & Altman, 1973; Fish & Winick, 1969; Sobotka et al., 1974; Wehmer & Jen, 1978). As with the cerebral weights, the AR cerebellar weight deficits are less than those seen in "severely" undernourished pups; 10% (Diaz et al., 1983a) and 14.3% (my studies). The sensitivity of the cerebellum to undernutrition, however, is evident in the observation that the cerebellar weights correlated negatively with the number of accidental food deprivation intervals.

Another similarity between postnatally undernourished and AR rat pups is the persistence of the brain weight deficits into adulthood, even when ad lib feeding is introduced after weaning. Mid-forebrain weight deficits of 14% (Culley & Lineberger, 1968) and brain deficits of 8% (Dobbing & Sands, 1971) have been observed at 110 and 196 days PN, respectively. In AR animals a decrease of about 9% from control animal values was reported at 224 days PN (Smart et al., 1983a).

Despite these similarities between AR pups and undernourished pups there are some important and interesting differences. Undernourished pups usually show very low body weights at weaning and this results in brain weight:body weight ratios which are higher than

those seen in well-nourished pups (Forbes, Tracy, Resnick & Morgane, 1977; Rajalakshmi et al., 1967; Sobotka et al., 1974). Dobbing and Sands (1971) have referred to this as "brain sparing." An example of this can be seen in the MRLD group (Table 4) which is mildly undernourished relative to rats in most undernourishment studies. Although nonsignificant, the MRLD pups had slightly higher forebrain:body weight ratios than their nondeprived littermates (.0369 versus .0338). In contrast, the AR animals had relatively normal body weights, making their brain weight:body weight ratios less than those of mother-reared pups (see also, Diaz et al., 1983a).

The most likely reasons for the brain weight differences seen in AR animals may be found in the composition of the artificial diet. Relative to rat milk obtained from 0 to 9 days after parturition (Dymsza et al., 1964; Keen, Lonnerdal, Clegg & Hurley, 1981), the artificial diet contains less protein, more carbohydrates, and an abnormal composition of dietary fat (Diaz, Clark, Petracca, & Schacher, 1981a; Smart et al., 1983a; Sonnenberg et al., 1982). As discussed earlier, during the first 21 days PN the rat brain is very susceptible to protein malnourishment (Winick, Brasel & Rosso, 1972). Increasing the amount of protein and balancing the amounts of the different fatty acids, however, did not get rid of the deficits in brain weight (Diaz et al., 1981a, 1982a, b, 1983a). In addition, the deficits in brain weight were observed within 24 hours after surgery (Diaz et al., 1983a). Attempts to feed AR pups rat's milk (Diaz & Stamper, Note 1)

and an artificial diet more similar to rat's milk (Diaz, Stamper, Auestad & Edmond, 1983b) also did not alleviate the brain weight deficits.

The preceding results suggest that an essential nutrient may be absent in the artificial diet. In addition, the importance of other feeding factors as well as non-feeding factors should be considered. For example, Diaz et al. (Note 2) found normal brain:body weight ratios in 18-day old AR pups that had received 2 hrs of "social stimulation" daily since surgery. The weight gain of AR pups (fed by infusing diet down the esophagus, Miller & Dymsza, 1963) as well as the levels of brain ornithine decarboxylase in normal rat pups (Butler, Suskind & Schanberg, 1978) can be regulated by the presence of a nonlactating or teat-ligated dam. The latter finding is particularly interesting since ornithine decarboxylase may be involved in mediating tissue growth and differentiation. On the other hand, 1 hr of access to a lactating, anesthetized dam did not increase the total brain weight in the AR pups (Stamper, Diaz & Petracca, Note 3), indicating that the mere presence of a dam is insufficient in ameliorating the brain deficit.

A very interesting result in the present studies indicates that the distribution of feeding throughout the day may influence forebrain growth. Pups receiving most of their food during the "light" period had the heaviest mean forebrain weights (Table 5). Since mother rats nurse mainly during the light period (Croskerry, 1976; Grota & Ader, 1969) it is possible that if the AR feeding schedule approximates the

more normal feeding schedule then brain growth will be facilitated. A similar relationship was seen for body weight. Relevant to this is a finding by Saito and Noma (1980) who found lower weight gains in adult rats kept on an adiurnal, or noncircadian, feeding schedule. These results indicate that abnormal feeding schedules (acyclic or reversed from normal) may adversely affect the growth of some organs. One puzzling result was that heavier cerebellums were associated with a noncyclic feeding schedule. This may be the result of some extreme values in the noncyclic groups. It would be worthwhile to examine the effects of feeding schedules and lighting conditions on brain growth with much larger sample sizes.

The weights of other organs also distinguish AR pups from the usual undernourished pups. For example, Winick, Fish and Rosso (1968) found that 9-day old undernourished pups had lighter hearts, lungs, livers, kidneys, spleens, and thymus glands than control pups. Also, the mildly undernourished MRLD pups in the present studies tended to have lighter spleens, livers, and kidneys. In contrast, heavier kidneys (Diaz et al., 1981b; Messer et al., 1969) and spleens (Diaz et al., 1981b) have been found in the AR animals at weaning when compared to mother-reared pups. Spleen weight differences, however, were not observed in the present studies (see also, Messer et al., 1969). There was a tendency for some of the AR pups to have heavier kidneys but this was not significant (see also, Smart et al., 1983a). A consistent finding, however is that the AR pups have heavier livers than

mother-reared pups. The mean increase in liver weight varies from 11.6% (Diaz et al., 1981b) and 22.9% (present results) at 18 days PN to 18% at 21 days PN (Smart et al., 1983a).

Hepatomegaly, or enlarged livers, can be caused by many factors, among them abnormal fat deposits in the liver (Edmondson & Schiff, 1975). Abnormal fat deposits in turn can be caused by several factors, including high carbohydrate or low protein diets (Alpers & Isselbacher, 1975; Elias & Sherrick, 1969). Smart et al. (1983a) did find a higher percentage of fat in the livers of AR animals but this alone could not account for the heavier livers. Also, feeding AR pups a more normal diet did not alleviate the enlarged livers (Diaz et al., 1983a, b). In fact, liver weight in AR pups was found to increase significantly within 48 hours after surgery (Diaz et al., 1983a). Another possibility may involve the abnormal fatty acid composition of the artificial diet. Since the liver controls the fatty acid cycle (Emery, 1969), the improper balance of fatty acids in the diet accompanied by the abnormal ketone body metabolism in the AR pups (Sonnenberg et al., 1982) may be toxic, resulting in an enlarged liver. However, correcting the fatty acid in the AR diet still resulted in enlarged livers (Diaz et al., 1981a). Of course, other components in the diet may also be producing a toxic response in the liver (Diaz et al., 1983a).

As with the brain weights, other factors may be involved. Heavier spleen and lighter liver weights were observed in animals whose

feeding more closely approximated the normal nursing situation (i.e., unequal distribution of feeding during the two 12-hour periods, or cyclic feeding). The cyclic feeding did not normalize the liver weights although it did decrease the mean deviation from control values (16.4% for cyclic feeding versus 33.9% for noncyclic feeding). Heavier spleen and liver weights were also associated with low humidity conditions. It is possible that high relative humidity (i.e., greater than 50%) is detrimental to growth of young rats but better controlled studies are needed. Heavier livers have also been seen in rats raised in "impoverished conditions" as compared to "enriched conditions" for varying time intervals soon after weaning. The increases in liver weight, however, are not as great as those seen in the AR pups- 10% after 16 days (Geller, 1971) and 9% after 32 days in "impoverished conditions" (Uphouse & Brown, 1981).

Other contributions to the abnormal somatic growth seen in AR pups could be abnormal amounts of food intake (Smart et al., 1983a) and the invariant nutritional composition of the diet. Several nutrients in rat's milk undergo changes as weaning approaches, such as decreases in iron and copper levels, increases in protein levels, and fluctuations in the carbohydrate levels (Keen et al., 1981). Also, the AR pups receive food directly into their stomach and are lacking the normal opportunities for suckling and early oral ingestive behaviors.

In addition to the differences in brain and liver weights the AR pups opened their eyes significantly sooner than their mother-reared

littermates (see also, Smart et al., 1981, 1982, 1983a). Premature eye opening was also observed by Messer et al. (1969) but they stated that the addition of more riboflavin, pyridoxal, methionine, and tryptophan to the diet ameliorated this condition. Since the present diet and the diet used by Smart et al. (1983a) contained these nutrients it is likely that other factors are involved. Precocious eye opening has been observed following injections of salivary gland extracts (may contain epidermal growth factor) in mice (Carlson, 1969) and ACTH analogues in rats (van der Helm-Hylkema & de Wied, 1976). It has also been observed after rearing in small, as opposed to large, postnatal litters (Ryan, 1977) although this finding may be a result of retarded eye opening in large litters due to undernourishment (Wehmer & Jen, 1978). Some investigators have suggested that extra "stimulation" or certain stressful experiences during the early postnatal period may accelerate the maturation of some developmental indices. For example, the relatively mild stress of ear punching in mice (Barnett & Burn, 1967) and daily 2-min separation from the dam with or without "shaking" stimulation in rats (Levine, 1959) accelerated eye opening. The type and duration of the stimulation as well as the changes it induces in maternal behavior (i.e., Barnett & Burn, 1967) may be important since early weaning (Hofer, 1975b), daily open-field testing (Lee & Williams, 1977b) and intermittent maternal separation (MRLD group in the present studies) do not induce premature eye opening. It is interesting that the groups which deviated the least from their littermates (with the

exception of the ARDL pups) were the ones on a LD cycle. The animals on constant light (ARLL) were the most accelerated and those on constant darkness (ARDD) did not differ from their control littermates, suggesting that lighting conditions may influence eye opening.

Messer et al. (1969) reported the presence of cataracts in some of their AR pups but stated that changes in the diet prevented this problem. The diets used in the present studies and by Sonnenberg et al. (1982) were based on the modified diet described by Messer et al. (1969). However, cataracts (or an eye condition resembling cataracts) were still observed in 7.4% (present studies) and 20% (Sonnenberg et al., 1982) of the AR pups (see also, Rusiniak, Garcia, Palmerino & Cabral, 1983). One possible explanation may be related to the finding of abnormally high plasma galactose levels in AR pups by Sonnenberg et al. (1982). Adult rats fed a diet high in galactose develop cataracts (Korc, 1974) and lowering the lactose levels in another artificial diet ameliorated this problem (Dymsza et al., 1964). Of course, other metabolic disorders may induce cataracts (Haddad, 1974).

The survival rate of the AR pups observed in the present study was low relative to reports by Diaz et al. (1981b) and Smart et al. (1983a). Sonnenberg et al. (1982) also commented on the relatively high mortality rate in their AR pups. Aside from the surgery-related deaths, there are several possible explanations for the low survival rates. Bloating, or abdominal distension (which often results in low weight gains and sometimes death), has been noted by several researchers using an artificial diet with rat pups (Diaz et al., 1981b;

Miller & Dymsza, 1963; Smart et al., 1983a; present results). Intermittent feeding attenuated the incidence of bloating in my studies but did not eliminate the problem. Diaz, Samson, Kessler, Stamper, Moore, Robisch, and Hodson (1980) found that the addition of deoxycholic acid, a bile salt, decreased the incidence of abdominal distension (Diaz et al., 1981b) although some AR animals still became bloated (see also, Smart et al., 1983a). Related to the problem of bloating might be the observation by Sonnenberg et al. (1982) of negligible amounts of the amino acid, taurine, in the plasma of AR pups. In rats, taurine is important in forming conjugates with bile salts, resulting in taurocholic acid (Spaeth & Schneider, 1976). Bovine milk, which is used in the artificial diets, contains about 15 times less taurine than rat milk from the later preweaning period (Sturman, Rassin & Gaull, 1978). Since the major source of taurine in the young rat is from maternal milk (Huxtable & Lippincott, 1982) the AR pups may not be getting adequate amounts of this amino acid. This may interrupt normal bile salt metabolism which in turn may interfere with lipid absorption in the intestinal tract. Other problems might include the high osmolarity of some of the artificial diets (Miller & Czajka, 1967; Sonnenberg et al., 1982), the postulated inefficiency of the infant rat's stomach in digestion (Naismith, Mittwoch & Platt, 1969), and a possible lack of maternally-transmitted immunities (Auerbach & Clark, 1975).

Another possible reason for the mortalities could be inappropriate temperatures inside the styrofoam cups. Thermoregulatory

abilities of rats do not develop until the second to third weeks postnatally (Fowler & Kellogg, 1975; Hahn, Krecek & Krecková, 1956) and before that time the pups rely on maternal heat (Leon et al., 1978) and huddling with their littermates (Alberts, 1975). Supplying infant rats separated from their dam and siblings with an external heat source (30 to 35 degrees C) increases survival rate (Oswalt & Koch, 1975) and attenuates retardation of some growth indices such as fur development (Stone, Bonnet & Hofer, 1976). The temperatures used in the present studies, on the average, were around 29 degrees C and may have been inadequate. It is worth noting that raising rats under cool temperatures has been found to accelerate the development of some aspects of thermoregulation (Krecek, Krecková, Martinek, 1957). Also, frequent forays from the nest by the dam (Croskerry, 1976) result in temperature fluctuations which may influence growth and health. In fact, the animals with the temperature cycle had the highest survival rate of all the AR groups. Whether this is due to the cyclic changes in temperature, the higher daily mean temperature, or other factors is not clear.

To summarize, the growth of AR animals differs in several ways from the growth of mother-reared pups. The normal body weights seen at weaning in the AR pups are not indicative of normal growth of the brain, liver and, in some studies, other peripheral body organs. Perhaps the major contributor to the abnormal growth is the artificial diet although improvements in the diet have not been entirely successful in improving the growth of AR pups (Diaz et al., 1983a, b;

Smart et al., 1983b). The type of feeding schedule, which was found to contribute to growth in the present studies, as well as other factors, such as surgical trauma, the effects of direct intragastric feeding, and the prolonged maternal separation should be pursued further.

Chapter 3

The Development of Circadian Activity Rhythms

Movement and locomotor activity go through several changes during the early postnatal period. During the first week after birth rats and mice can crawl and move their heads as well as make rudimentary face washing and grooming movements (Blanck, Hard, & Larsson, 1967; Bolles & Woods, 1964; Fox, 1965). Early in the second postnatal (PN) week grooming becomes more adult-like and the pups exhibit what has been referred to as pivoting movements, or circular movements of the whole body using the abdomen and one of the hindlegs as a pivot (Blanck et al., 1967). Near the end of the second week and the beginning of the third week the pups are walking, rearing, running, and jumping as well as showing an auditory startle (Bolles & Woods, 1964; Fox, 1965). Exploration of a novel area occurs during the third week PN (Goodrick, 1974) as does what Bolles and Woods (1964) refer to as hopping behavior.

The rhythmicity of locomotor activity under "natural" conditions is not well understood in the young animal. Bolles and Woods (1964) and Norton, Culver, and Mullenix (1975) did not observe circadian activity rhythms until after the third postnatal week in the rat. Ultradian activity rhythms with periods around 2 hrs were observed during the second to third weeks by Richter (1927). In a cross sectional study, Teicher and Flaum (1979) studied the locomotor activity of rat pups isolated from their mother for 24 hrs. Ultradian

rhythms with periods of 1 to 2 hrs were detectable in 6-day old pups and became clearer between 9 and 15 days PN. Unlike the results of Norton et al. (1975) a nocturnal distribution of activity was present at 15 days PN (Teicher & Flaum, 1979). Greater nocturnal activity in 15-day old rats separated from their dams for 16 hrs was also observed by Campbell and Raskin (1978) and Randall and Campbell (1976). This rhythmicity was not observed when pups were housed with an anesthetized adult rat (Randall & Campbell, 1976) or in a warm home cage (Campbell & Raskin, 1978).

Under certain circumstances, locomotor activity is sensitive to the effects of acute social isolation (24 hrs or less). Rat pups isolated from their mother and littermates for 6 or 16 hrs show an increase in activity during the isolation which peaks around 15 days PN and thereafter declines (Campbell & Raskin, 1978; Randall & Campbell, 1976). Similar results were found by other investigators using different lengths of isolation although the age at which the peak occurred varies among studies (Buelke-Sam & Kimmel, 1980; Oakley & Plotkin, 1975; Shaywitz, Gordon, Klopper, Zelterman, & Irvine, 1979). Testing the isolated pups in a familiar environment greatly attenuates the activity peak (Campbell & Raskin, 1978) although there may be sex differences in this response (Buelke-Sam & Kimmel, 1980; Buelke-Sam, Sullivan, Kimmel & Nelson, 1984). Some of these results are supported by the studies of Hofer (1973a, b, 1975a) on 2-week old rat pups separated from their mothers for 4 to 18 hrs. He also found that

anosmic pups with their dams were similar to isolated pups in terms of activity suggesting that maternal odor cues may influence the young pups' activity (Hofer, 1975a).

Studies on the effects of prolonged social isolation, particularly after weaning, are abundant. Locomotor activity, as measured in open fields or photocell cages, is usually enhanced in rats reared after weaning in social isolation (Einon & Morgan, 1977; Einon & Sahakian, 1979; Segal, Knapp, Kuczenski, & Mandell, 1973; Sahakian, Robbins, Morgen, & Iversen, 1975; Weinstock, Speiser, & Ashkenazi, 1978) although some investigators observe the opposite effect (Gardner, Boitano, Mancino, D'Amico, & Gardner, 1975). Prolonged maternal and/or litter isolation (i.e., more than 24 hrs) also influences the locomotor activity of preweanling rats. Baenninger (1967) reported that between 13 and 21 days PN pups reared singly, as opposed to those reared with littermates, spent more time in exploratory locomotion when observed without the dam. Maternally isolated pups reared with their littermates in an incubator between birth and 15 days PN, and hand-fed every 3 to 4 hrs did not differ from mother-reared animals in the amount of open field locomotor activity shown at 68 days PN (Thoman & Arnold, 1968b). Goldenring et al. (1982) found that artificially reared (AR) rats were more active than mother-reared at 12 days PN but not at later ages. In contrast, Diaz et al. (1982a) observed that at 18 days PN AR animals crossed fewer squares in an open field than mother-reared pups. A higher protein diet (Diaz et al., 1982a) as well

as daily, 1-hr nursing with the dam for 10 days (Stamper et al., 1980) attenuated the differences between the open field behavior of the AR and mother-reared pups.

The effects of maternal isolation on the offspring's biological rhythms are less well known. The distribution of sleep-states during maternal isolation has been investigated. Changes in the duration of time spent in paradoxical, or active, sleep have been seen in AR rat pups (Juvancz, 1981), pups isolated from their dams for 24 hrs. (Hofer, 1976), and infant rabbits reared in almost complete isolation from their does (DeSantis, Waite, Thoman & Denenberg, 1977). Maternal isolation per se may not be unique in affecting sleep-state patterns since similar changes in sleep states are observed in rats (Tagney, 1973) and kittens (McGinty, 1972) isolated after weaning. Also, the distribution of sleep-states in maternally isolated monkeys may not differ substantially from mother-reared monkeys (Reite & Short, 1977). Information on circadian rhythms is very scanty. Ten days of maternal separation increased the amplitude and delayed the acrophase of the body temperature rhythm of pigtailed monkeys (Reite, Seiler, Crowley, Hydinger - Macdonald, & Short, 1982).

One of the major purposes of the present experiments was to study the effects of prolonged maternal isolation during the early postnatal period on the ontogeny of the rat's locomotor activity, particularly the rhythmic aspects of activity. The other major purpose was to put back into the artificial rearing environment some of the

aspects of the early postnatal period which may influence rhythm ontogeny. The following questions formed the basis for the experiments presented in this chapter:

1. When do mother-reared pups show a circadian rhythm in locomotor activity? A within-litter, rotating design (see Chapter 2, Methods) was used to study the activity of mother-reared pups (MRLD group) throughout the first two PN weeks. The MRLD group served as a control group, albeit an imperfect control group, against which the artificially reared animals were compared.
2. Is a LD cycle alone sufficient for entraining a young rat's rhythm? For example, some studies indicate that the circadian rhythms of sighted rat pups will entrain to a LD cycle when the dam is freerunning (Deguchi, 1979; Takahashi et al., 1979). AR pups were reared with only a 12:12 LD or DL cycle (ARLD and ARDL groups).
3. What are the effects of an arrhythmic environment on the development of the locomotor activity rhythm? Many earlier investigations examined the effects of LL and DD from birth but did not account for maternal factors which may remain rhythmic under those lighting conditions. AR pups were reared under LL or DD and constant temperatures, and were fed on a noncyclic schedule (ARLL and ARDD groups).

4. Can a cyclic feeding schedule synchronize rat pups' activity rhythms? There is evidence supporting maternal synchronization of the offspring's rhythms. Specific aspects of the mother-offspring interactions responsible for this synchronization have not been identified. However, manipulations of the nursing rhythm result in shifts in the acrophases of rat pups' circadian pineal NAT (Deguchi, 1977) and plasma corticosterone rhythms (Hiroshige et al., 1982c; Miyabo et al., 1980). These results suggest that the nursing rhythm may be capable of synchronizing the offspring's rhythms. AR pups experienced a cyclic feeding schedule under conditions of LL to determine whether a feeding cycle alone could synchronize activity (ARLL-LF and ARLL-DF groups). Since rats nurse mainly during the light, it was also of interest to examine whether predominantly diurnal (ARLD-LF group) or nocturnal (ARLD-DF group) feeding would augment or interfere with, respectively, synchronization to the LD cycle.
5. Would the addition of a temperature cycle improve synchronization of the AR pups' activity? During the preweaning period, there are daily fluctuations in the rat's nest temperature (Croskerry, Smith & Leon, 1978) and the dam shows circadian rhythms in body temperature

(Kittrell, Note 4) during the preweaning period. Also, environmental temperatures affect the activity of rat pups (Hofer, 1973a). It seems plausible, then, that daily temperature changes may contribute to the synchronization of the pups' locomotor activity rhythm. AR pups were reared on a LD cycle with predominantly diurnal feeding and high daytime temperatures (ARLD-Temp group). It was reasoned that since most of the dam's nesting time occurs during the light period, the nest temperature, on average, would be highest during that time.

Methods

Groups

Activity data were obtained from all of the groups described in Chapter 2, Methods. Some of the experimental conditions (see Table 7) consisted of groups of animals which were tested at different times during the year due to mortality and restrictions on the number of animals that could be run at one time.

The number of individual animals and litters whose data were used for each age in the statistical analyses are given in Table 8. Some of the recorded data were not used due to deaths and technical problems (e.g., poor transducers). Data from animals whose gastric cannulas came out later than 3 to 4 days after surgery were included if the recordings were good and the animals were not ill. Activity data

Table 7

Experimental conditions
and water bath temperatures

Group	Experimental Conditions		Temperature Dark	Temperature Light
MRLD	Mother-reared; 12:12 LD.		47.5(0.2)	47.2(0.3)
ARLD-Temp	AR;Light feeding; Temperature cycle; 12:12 LD.		45.7(0.4)	59.5(0.1)
ARLD-LF and DF	AR;12:12 LD(except for June-12:12 DL; Mainly light-fed(LF) or dark-fed(DF).	June	48.8(0.9)	46.8(0.5)
		July	47.2(1.2)	46.0(0.1)
		Aug.	47.7(0.9)	46.9(0.4)
		Sept.	46.5(0.3)	46.3(0.2)
ARLL-LF and DF	AR;LL;Mainly light-fed (LF) or dark-fed (DF).	Sept.	47.7(0.5)	47.7(0.4)
		Oct.	46.4(0.1)	46.4(0.1)
ARLD	AR;12:12 LD;Noncyclic feeding schedule.	Sept.	48.0(0.3)	47.6(0.2)
		July	46.0(0.2)	46.2(0.3)
ARDL	AR;12:12 DL;Noncyclic feeding schedule.	April	47.3(0.3)	47.9(0.2)
		May	46.8(0.2)	47.2(0.2)
ARDD	AR;DD;Noncyclic feeding schedule.	Feb.	47.3(0.3)[a]	
		Mar.	47.5(0.2)	
		June	47.6(0.2)	
ARLL	AR;LL;Noncyclic feeding schedule.	Dec.	47.8(0.2)	
		Jan.	47.7(0.1)	

Note. Values represent the means (sem). "Dark" and "light" period values for the ARLL-LF and ARLL-DF groups refers to 2000 to 0800 hrs and 0800 to 2000 hrs, respectively.

[a]Temperatures for ARDD and ARLL groups are the mean daily temperatures (degrees C).

Table 8

Sample sizes
for the activity data

Group		Age(days PC) 26	27	28	29	30	31	32	33	34	35	36	37	38
MRLD	In	7	7	7	7	7	7	7	7	7	7	7	7	7
	Lit	5	5	5	5	5	5	5	5	5	5	5	5	5
ARLD-Temp	In	6	8	8	8	8	8	8	7	7	6	6	6	6
	Lit	4	5	5	5	5	5	5	5	5	4	4	4	4
ARLD-LF	In	12	13	13	13	13	13	13	13	13	11	6	6	4
	Lit	9	10	10	10	10	10	10	10	10	10	6	6	4
ARLD-DF	In	8	9	9	9	9	9	9	9	9	8	8	7	4
	Lit	7	8	8	8	8	8	8	8	8	7	7	6	3
ARLL-LF	In	5	6	7	7	7	7	7	7	7	7	6	6	4
	Lit	3	4	4	4	4	4	4	4	4	4	4	4	3
ARLL-DF	In	5	5	5	5	5	5	5	4	4	4	4	4	3
	Lit	4	4	4	4	4	4	4	3	3	3	3	3	2
ARLD	In	8	12	12	12	12	12	12	12	12	12	12	10	10
	Lit	5	8	8	8	8	8	8	8	8	8	8	7	7
ARDL	In	9	9	9	10	10	10	10	10	10	9	7	7	3
	Lit	6	6	6	7	7	7	7	7	7	7	6	6	2
ARDD	In	10	11	11	11	11	11	11	11	11	11	9	7	5
	Lit	6	7	7	7	7	7	7	7	7	7	6	4	3
ARLL	In	8	8	8	9	9	9	9	9	9	8	7	6	5
	Lit	6	6	6	7	7	7	7	7	7	6	5	5	4

Note. "In" refers to the number of individual rat pups and "Lit" is the number of litters.

from 54 female and 37 male pups were combined for the following analyses. This was done for two reasons; first, the sample sizes of some of the groups were too small to divide them up by sex and second, a $\underline{X^2}$ analysis on the frequency of significant peaks in the autocorrelation plots of the AR animals indicated no effects due to sex.

The mean water temperatures in the incubators during the "light" and "dark" periods for each group are given in Table 7. The mean relative humidity (averaged over all recording days) is plotted as a function of the time of year in Figure 4. There were clear seasonal variations in humidity. These variations were examined in one of the analyses.

Recording Equipment

As described in Chapter 2, the rat pups were individually housed in styrofoam bowls in one of two water bath incubators. Each incubator was divided into 8 compartments by plastic barriers. Movements of the animal were transmitted to the surface of the water in its own compartment. Water movement was sensed with a simple transducer held in place on the side of the incubator by a metal bracket. It was made from a 1 in diameter fisherman's float on which was mounted a 10 cm length of wooden dowel bearing a piece of photographic film exposed with a 0.5 mm horizontal grid (see Figure 5). The grid moved vertically in the slit of a solid state optical switch (Clairex, type CL1-210). The dowel was housed in a length of glass tubing

Figure 4. Mean relative humidity in the water bath incubators as a function of the time of year. Each mean is based on data from 9 to 18 days.

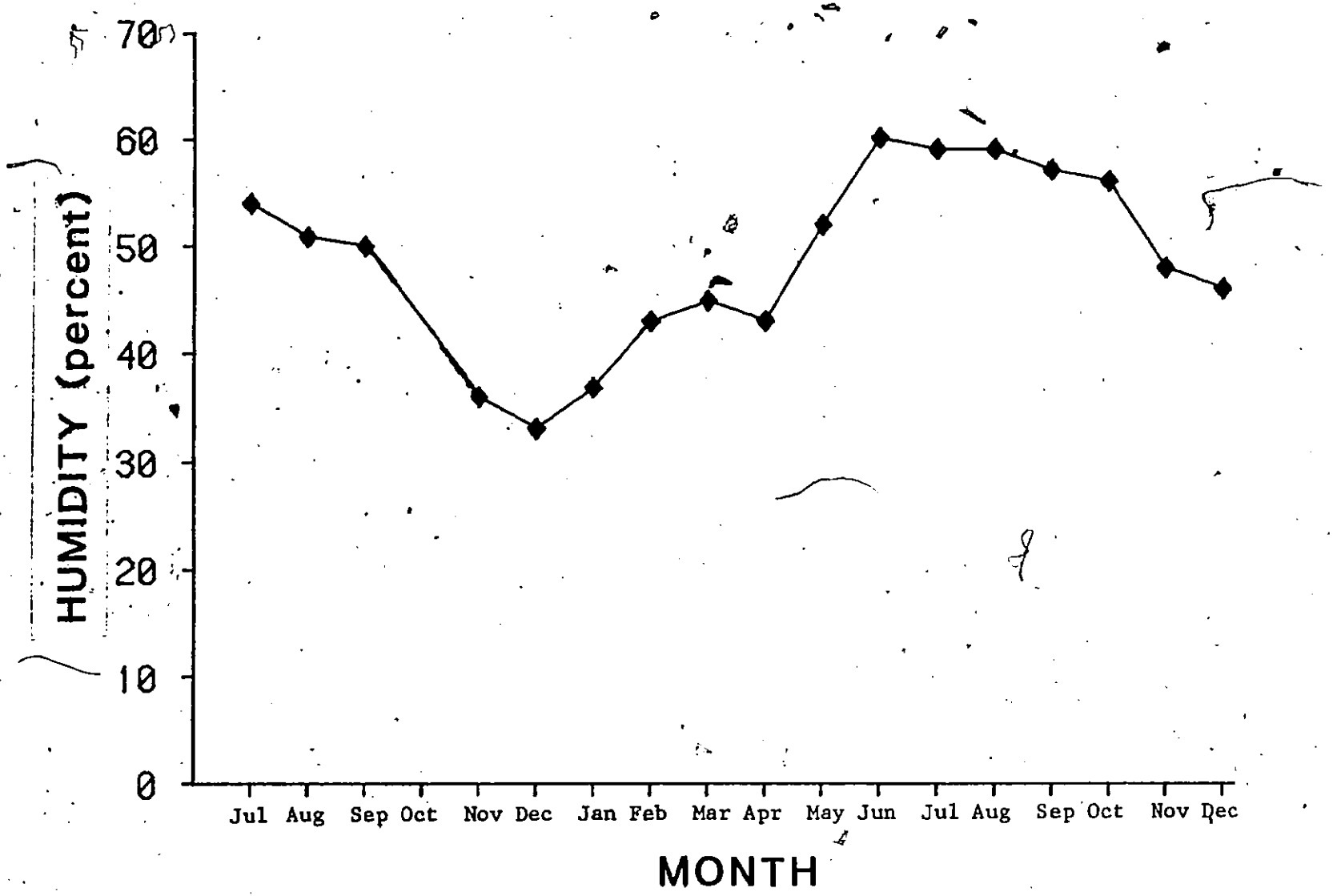
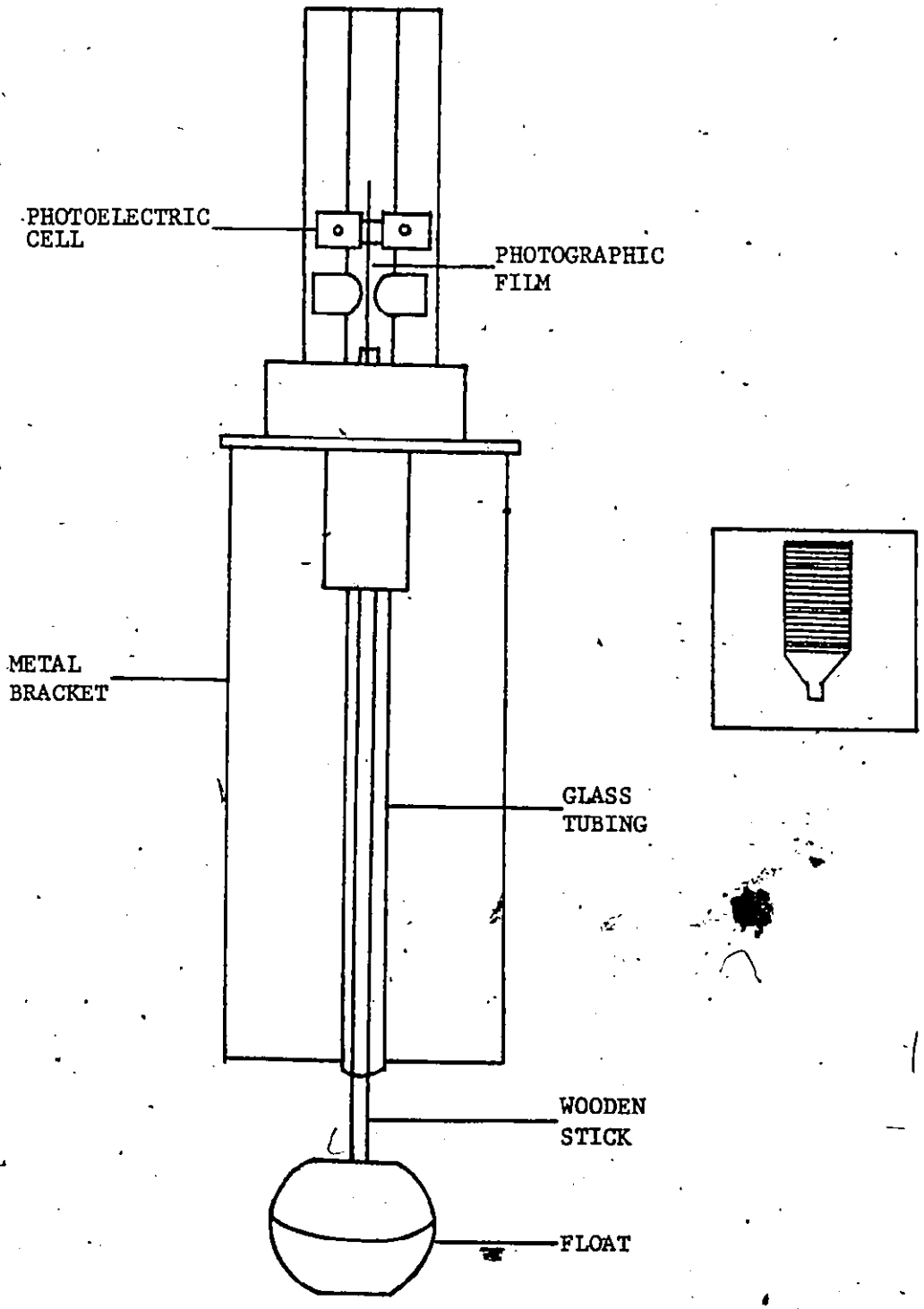

HUMIDITY (percent)
70
60
50
40
30
20
10
0
Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
MONTH

Figure 5. Drawing of the activity transducer. Inset - A drawing of the photographic film with the grating pattern.


PHOTOELECTRIC CELL
PHOTOGRAPHIC FILM
METAL BRACKET
GLASS TUBING
WOODEN STICK
FLOAT

constraining its movements to the vertical. The output of the optical switch was shaped by a schmitt trigger feeding a seven bit binary counter. Relatively little of the motion was transmitted to adjacent compartments. The principal mode of the bowl is a rocking motion, the vertical component being quite small. Since the bottom of the plastic barrier is below the surface of the water the resulting wave motion is damped quite efficiently. The grid spacing was chosen so that the transducer was sensitive to distinct locomotor movements of the pup in the compartment but relatively insensitive to the smaller motions transmitted from adjacent compartments. It was difficult to develop a reliable method of calibrating the sensitivity of the transducers so comparison of the absolute amount of movement between compartments is not valid. However, the sensitivity appears to be stable over time within a compartment. Further, the transducer was independent of the depth of water in the incubator over a wide range.

The counts generated by as many as 16 animals (two incubators) were logged by a Commodore 2001 computer controlling a custom built interface. The IEEE port of the computer was used alternately to set up the address of a 16-channel, 7-bit multiplexer, read the count through an array of three state gates and finally to clear the count. The occupied compartments were sampled once every 15 sec, a period too short to allow the count to exceed the maximum of 127 under normal recording conditions. Counts were cumulated over 15 min, stored in the computer memory, processed, and printed out as required.

Data Analyses

Activity which occurred during the daily care intervals (i.e., when animals were weighed, etc.) or other times of disturbance (i.e., changing the diet syringes) was handled in the following way. The 24-hr mean activity (2000 to 2000 hrs for pups born to LD-entrained dams and 0800 to 0800 hrs for those born to DL-entrained dams) was calculated and the data falling during disturbed intervals were replaced by that mean (Chatfield, 1980). This procedure was repeated and the final values were used in the statistical analyses. For the autocorrelation analyses a 3-day mean (72 hrs) was used instead of the 24-hr mean since these analyses were based on 3 days of data. Each of the seven groups of four MRLD pups was treated as a single animal.

Time series analysis was used in order to detect rhythms in the data which may or may not be synchronized to zeitgebers. It also allowed for the quantification of the rhythm's period and significance. A technique similar to that described by Binkley, Adler, and Taylor (1973) was used for the detection of circadian rhythmicity. Overlapping 3-day blocks of data were subjected to an autocorrelation analysis. For example, days 1, 2, and 3, then days 2, 3, and 4 and so forth were analyzed. For the present data there were 11 3-day blocks. At least two successive 24-hr intervals with circadian rhythms had to occur in order to detect significant rhythmicity. Since there were 96 samples in each 24-hr interval, a total of 288 samples were analyzed for each 3-day block. For some animals the last 15 min to 1 hr of data on the last day of testing were not recorded so only 284 to 287 samples were analyzed.

The autocorrelation program was run on a Commodore Pet computer (Model 2001). The autocorrelations were calculated in the following way:

$$\sum_{i=1}^{N-k} ((x_i - \bar{x})(x_{i+k} - \bar{x})) / \sum_{i=1}^{N-k} (x_i - \bar{x})^2$$

"N" is the total number of samples to be analyzed (in this case 288), "x_i" is a data point, and "$\bar{x}$" is the 3-day mean. The data were analyzed for rhythms with periods between 15 min and 30 hrs in steps of 15 min, or for 120 time lags. In the present analyses "k", the lag, took on values between 1 and 120. The autocorrelation values were printed out and plotted against the time lags. For circadian rhythms, the time lags between 20 and 28 hrs were inspected for significant autocorrelation values and peaks. In general, significant autocorrelation peaks imply the presence of rhythms with the corresponding periods indicated by the time lags. An autocorrelation value was significant at the .01 level if it was greater than or equal to $c = \pm 1/(N-1) + 2.326/\sqrt{N+1}$ (Ezekiel & Fox, 1959) or approximately .134 and -.140 for the present data where N = 288. Only the peaks ($c > .134$) were of interest here. There were relatively few significant troughs ($c < -.140$).

Two parameters were derived from the autocorrelation plots and calculated for each 3-day block. First, the area under a peak made up of significant autocorrelation values may be indicative of the overall significance of a rhythm. The following formula was used for the "area" calculations:

$$\text{Area} = \sum_{i=1}^{n} (r_i - 0.134)$$

where "r_1" to "r_n" are consecutive autocorrelation values of the same sign, greater than or equal to 0.134. This "area" calculation is a conservative estimate of the area under the peaks. Second, a weighted mean of the period for each peak was calculated using the following equation:

$$\text{Period} = \sum_{i=1}^{n} ((r_i - 0.134)(p_i))/\text{Area}$$

where "p_i" is the time lag of a significant point on the autocorrelation plot. In some cases more than one significant peak occurred in a 3-day block. When this happened the peak which was closest to 24.0 hrs was used in the analyses. Appendix B includes a more detailed description and an example of these calculations.

Results

The group means shown for the 24-hr and day-night data are based on litter values. These litter values were calculated from 96 activity samples (the number of 15-min samples in a 24-hr period). Some of the activity samples at 38.5 days PC were missing for a few animals. Means for this age were based on the following number of samples: 94 to 95 (ARLD-Temp), 93 to 94 (ARLD-DF), 92 to 96 (ARLD), 86 to 96 (ARLL-LF), 93 (ARLL-DF), and 96 for the remaining groups.

The reason for the particular pairings of groups in some of the following figures is that the paired groups were most similar to each other in the kinds of external synchronizers which were present (i.e.,

LD only, feeding cycle only, etc.). The ARLD-Temp group differs in many ways from the MRLD group, but the two were paired since the ARLD-Temp group did have more cyclic cues than the other AR groups.

24-hr mean activity

The 24-hr activity means for each AR group and the MRLD group are presented in Figures 6 to 10. With the exception of the ARLL-LF group (Figure 8), the activity of the mother-reared animals (Figure 6) is less than the activity of the AR animals between 26 and 31 days PC. After 31 days PC the activity of the MRLD group either exceeds, equals or remains slightly less than the activity of the AR groups. Also, the mean increase in activity with age in the MRLD group is smooth compared to the changes seen in the AR groups. In fact, some of the AR groups show little increase in 24-hr activity (i.e., the ARLD-Temp group after 26 days PC, Figure 6).

To investigate the age-related changes in daily activity, the 24-hr group means were regressed on age using a BMDP polynomial regression analysis (Dixon, 1983, program 5R). This was done for each experimental group. The results from the linear regression are presented in Table 9. The multiple R^2 represents the amount of variance in the data which is accounted for by the linear equation. The data from the ARLD-LF, ARLD-DF, ARLD, and ARLL groups significantly fit a linear function. The data from the ARLD-Temp, ARLL-LF, ARLL-DF, ARDL, and MRLD groups were best fit by higher-degree polynomials. Data from the ARDD group were not described significantly by any of the polynomials examined. Although the MRLD group's data were fit best by

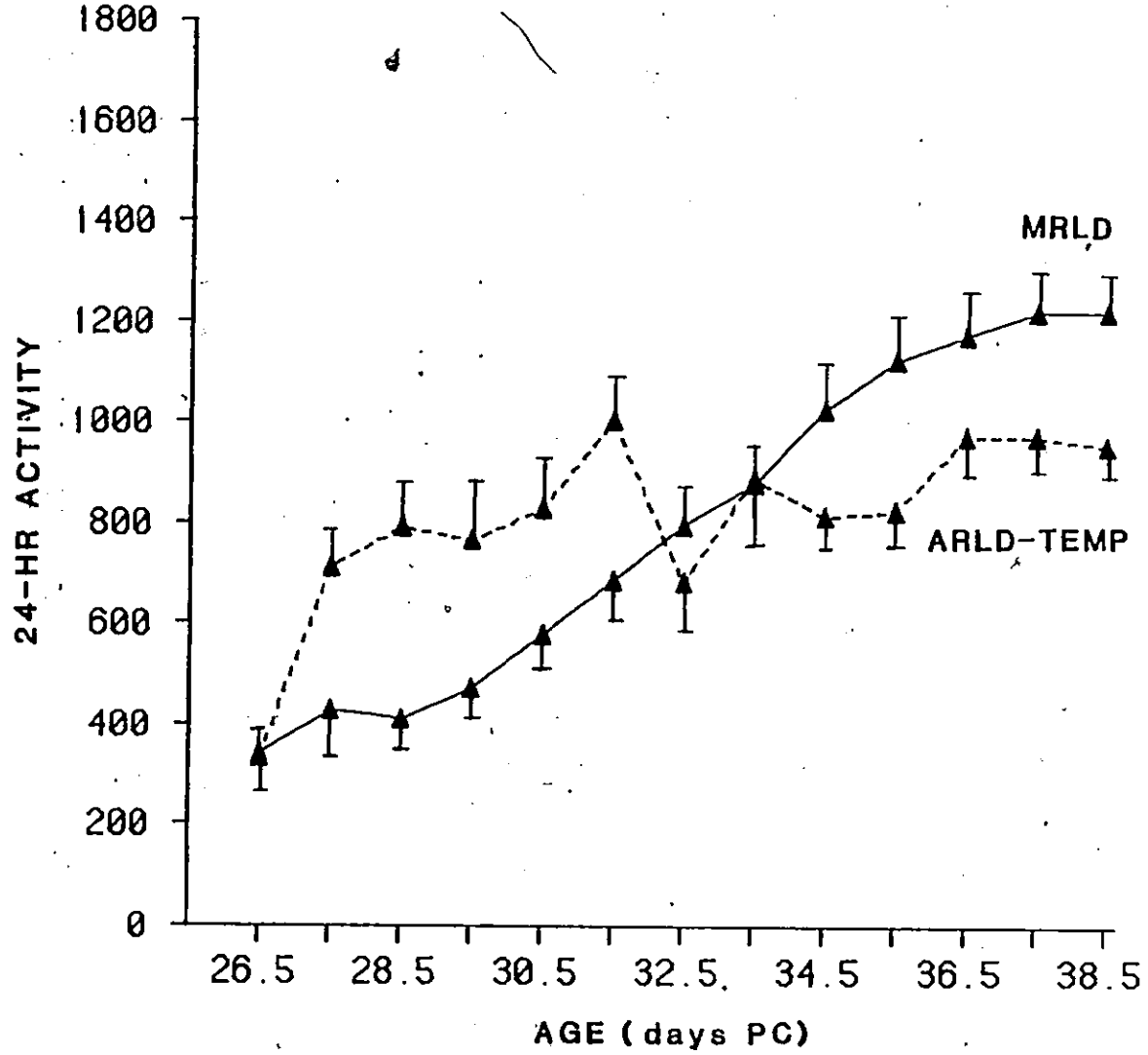
Figure 6. The 24-hr mean (± sem) activity for the MRLD (solid line) and ARLD-Temp (dashed line) groups as a function of age. Values are based on the number of litters (see Table 8).

Figure 7. The 24-hr mean (± sem) activity for the ARLD-LF (dashed line) and ARLD-DF (solid line) groups.

Figure 8. The 24-hr mean (± sem) activity for the ARLL-LF (dashed line) and ARLL-DF (solid line) groups.

Figure 9. The 24-hr mean (± sem) activity for the ARDL (solid line) and ARLD (dashed line) groups.

Figure 10. The 24-hr mean (± sem) activity for the ARDD (solid line) and ARLL (dashed line) groups.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1600
1400
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200
0
24-HR ACTIVITY
MRLD
ARLD-TEMP
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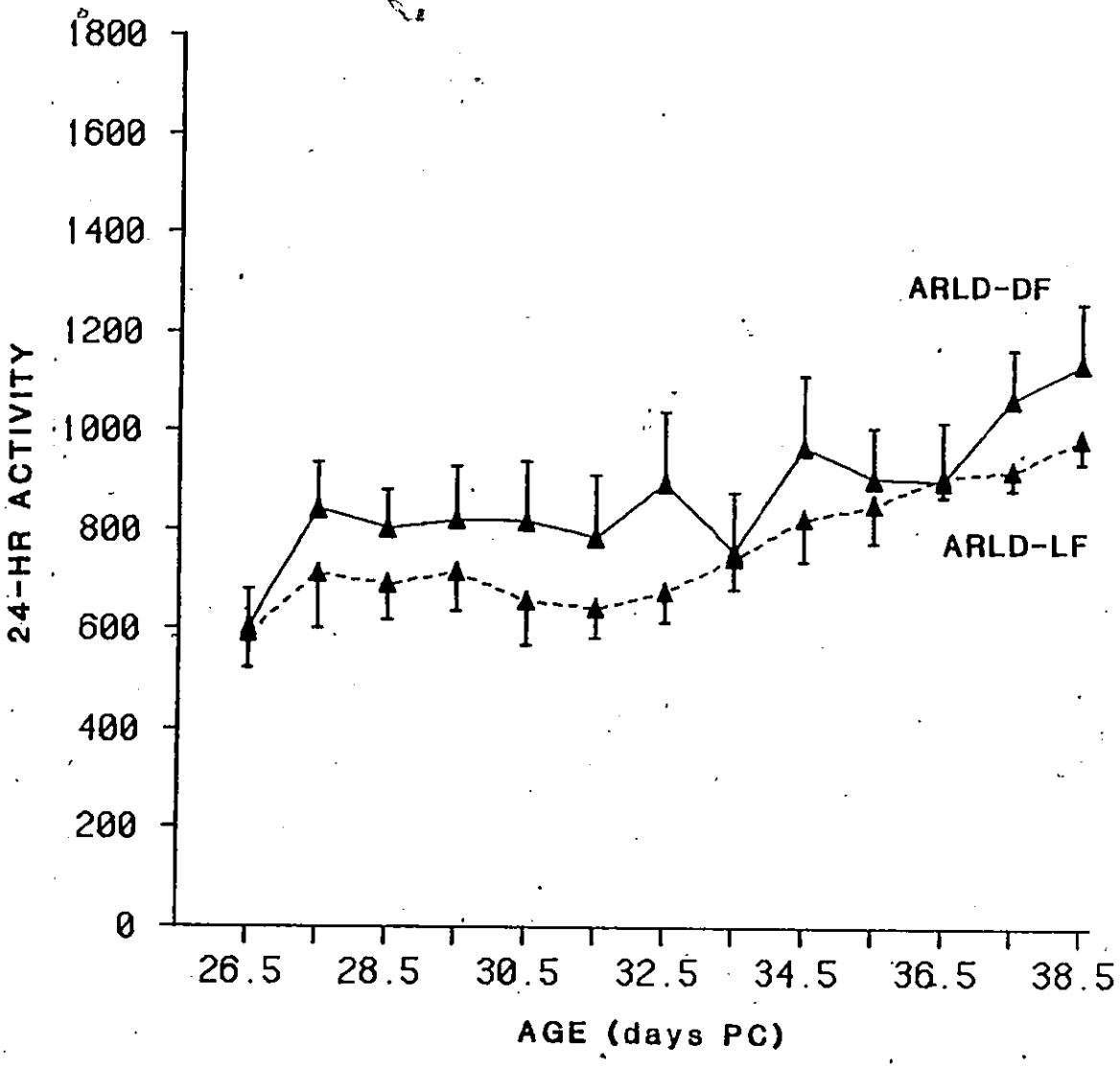
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 6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24-HR ACTIVITY
ARLD-DF
ARLD-LF
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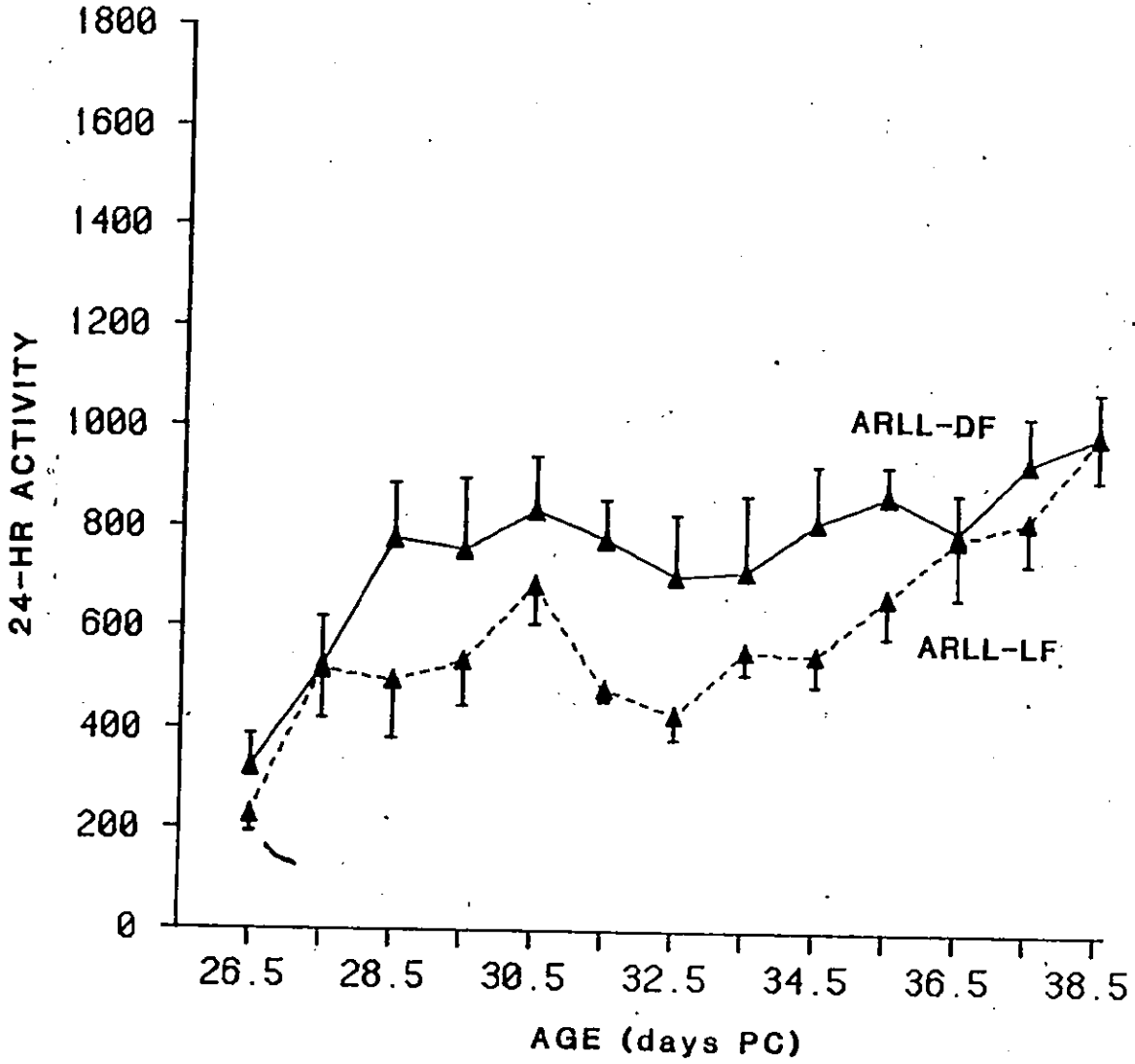
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 7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24-HR ACTIVITY
ARLL-DF
ARLL-LF
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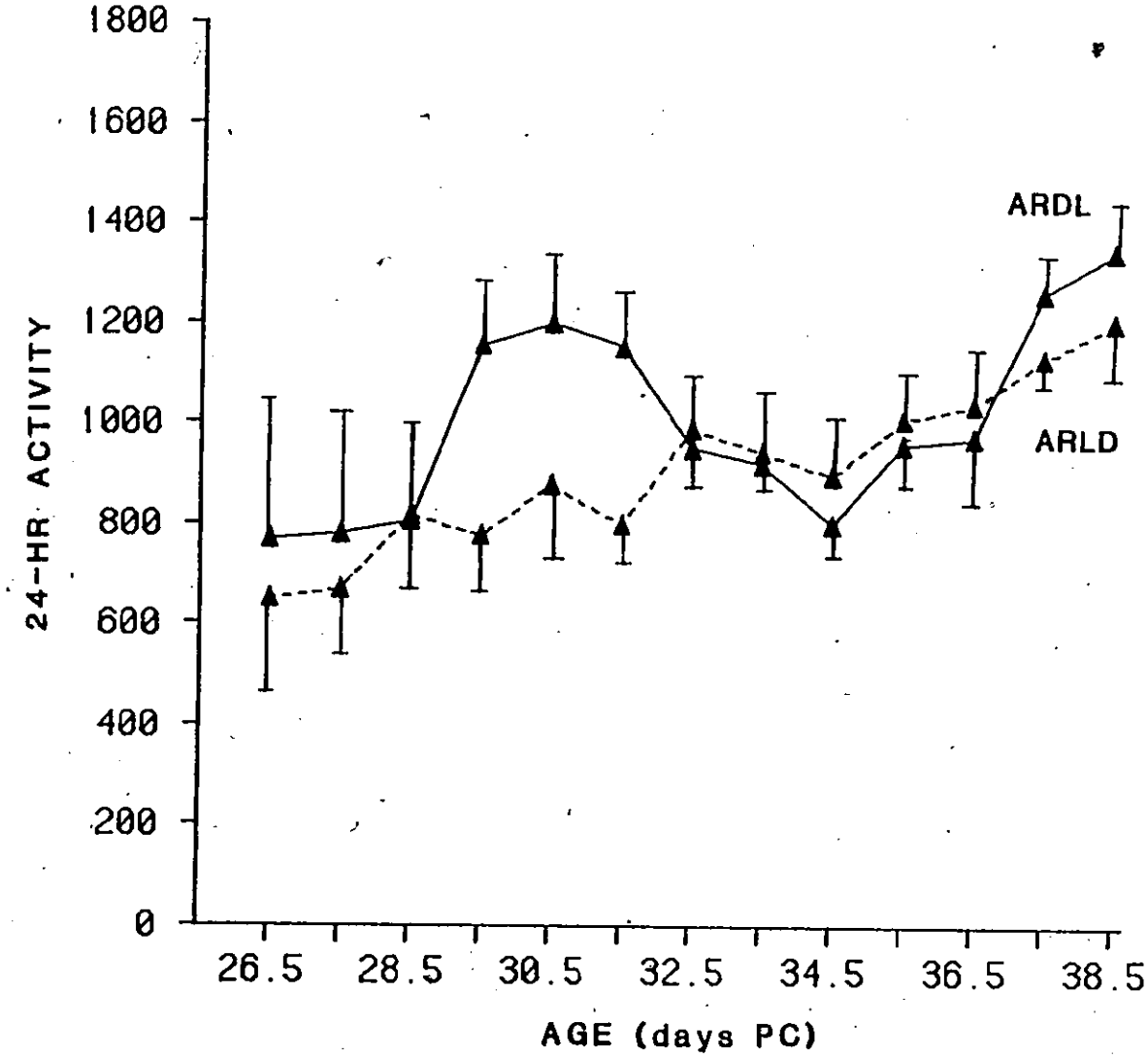
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 8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24-HR ACTIVITY
ARDL
ARLD
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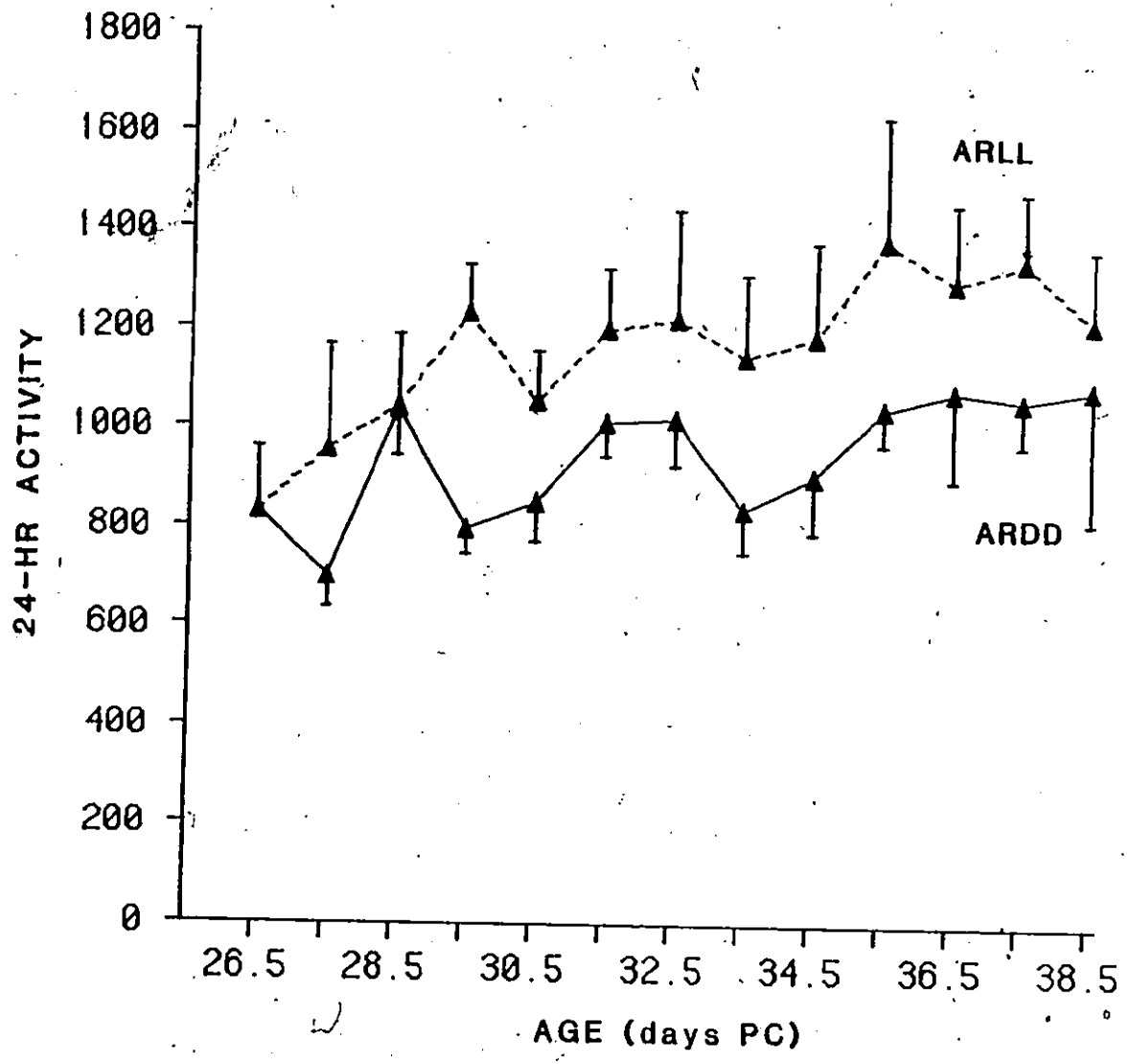
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 9


1800
1600
1400
1200
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800
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200
0
24-HR ACTIVITY
ARLL
ARDD
26.5
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 10

Table 9

Multiple R^2s and slopes for the linear regression on 24-hr activity

Group	R^2	Slope
MRLD	.975	84.3
ARLD-Temp	.491	31.7
ARLD-LF	.802*	28.2
ARLD-DF	.670*	28.7
ARLL-LF	.669	40.9
ARLL-DF	.636	36.1
ARLD	.897*	40.4
ARDL	.281	26.7
ARLL	.664*	32.3
ARDD	.485	22.8

Note. An asterisk indicates that the first degree polynomial was the best fit for the data at $p<0.05$, $df = 11$.

a nonlinear function, the linear correlation coefficient for this group (.987) was significantly higher than the linear correlation coefficients of each of the AR groups ($p<.02$ using the "Fisher r to z transformation," Winkler & Hays, 1975, p. 653).

Day-night Differences

Day-night or light-dark differences in locomotor activity are crude indicators of circadian rhythmicity. However, they do provide some information on whether the activity is synchronized to the LD cycle or one of the 24-hr feeding cycles used in the present studies.

The mean proportions of dark-period activity for the groups with a LD and/or feeding cycle are shown as a function of age in Figures 11 to 14. For the ARLL-LF and ARLL-DF groups the term "dark period" actually refers to the "maternal dark period," or the dark period of the dam's prepartum LD cycle.

The AR group means shown in Figures 11 to 14 were combined and then subjected to a BMDP stepwise regression analysis (Dixon, 1983, program 2R) in order to determine the relative contributions of the following independent variables to the observed proportions of dark-period activity: age, mean water bath temperature, mean humidity, light conditions (LL or LD/DL), feeding schedule (noncyclic or mainly light- or dark-fed), and the proportion of animals accidentally food deprived during the light and dark periods. In addition, interactions between the light conditions and feeding schedules were analyzed. The MRLD group means (Figure 11) and the data from the ARLL and ARDD groups were not included in this analysis.

Figure 11. The proportion of dark-period activity (± sem) as a function of age for the MRLD (solid line) and ARLD-Temp (dashed line) groups. Values are based on the number of litters (see Table 8).

Figure 12. The proportion of dark-period activity (± sem) for the ARLD-LF (dashed line) and ARLD-DF (solid line) groups.

Figure 13. The proportion of maternal dark-period activity (± sem) for the ARLL-LF (dashed line) and ARLL-DF (solid line) groups.

Figure 14. The proportion of dark-period activity (± sem) for the ARLD (dashed line) and ARDL (solid line) groups.

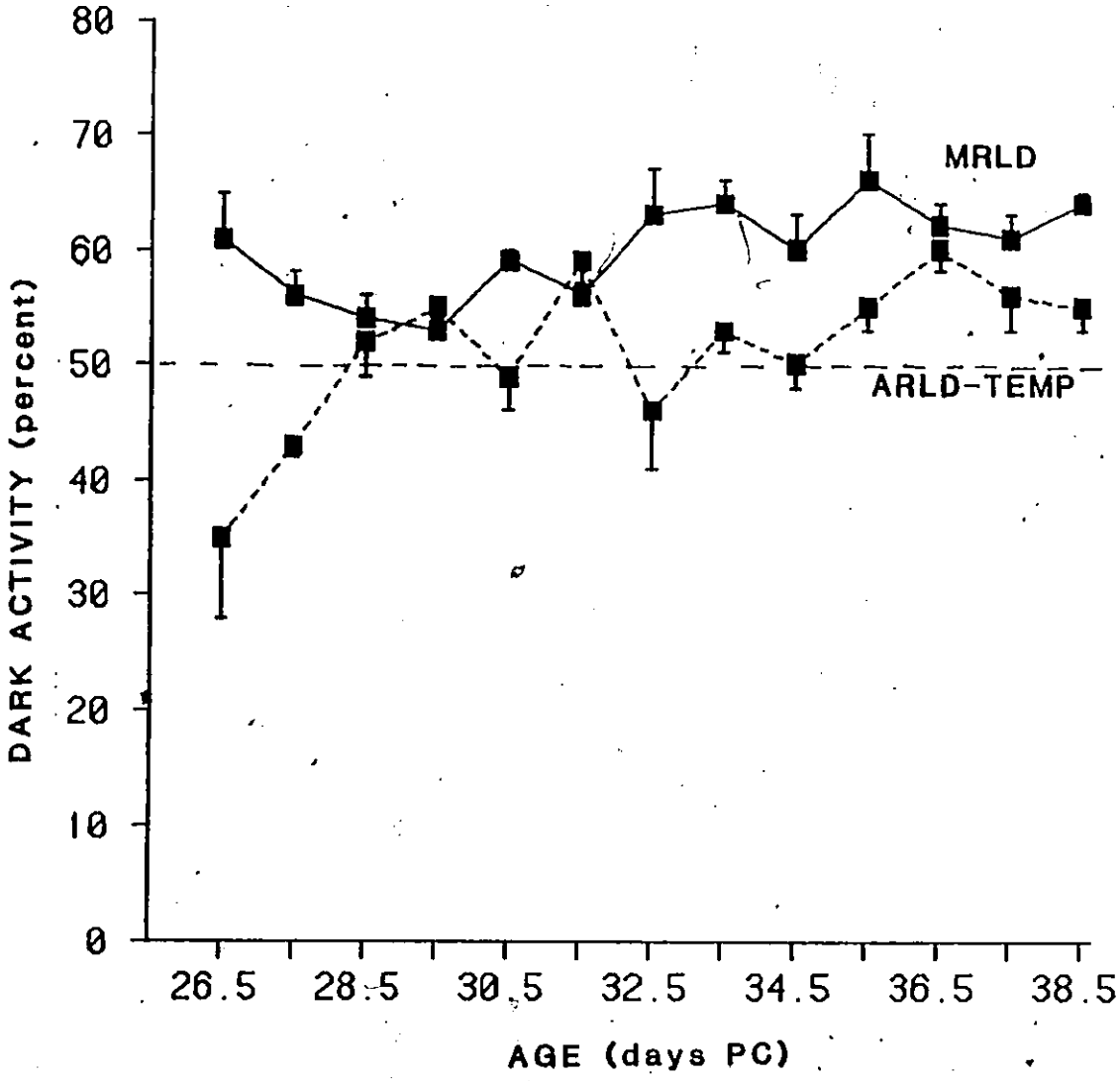

80
70
60
50
40
30
20
10
0
DARK ACTIVITY (percent)
MRLD
ARLD-TEMP
26.5
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 11

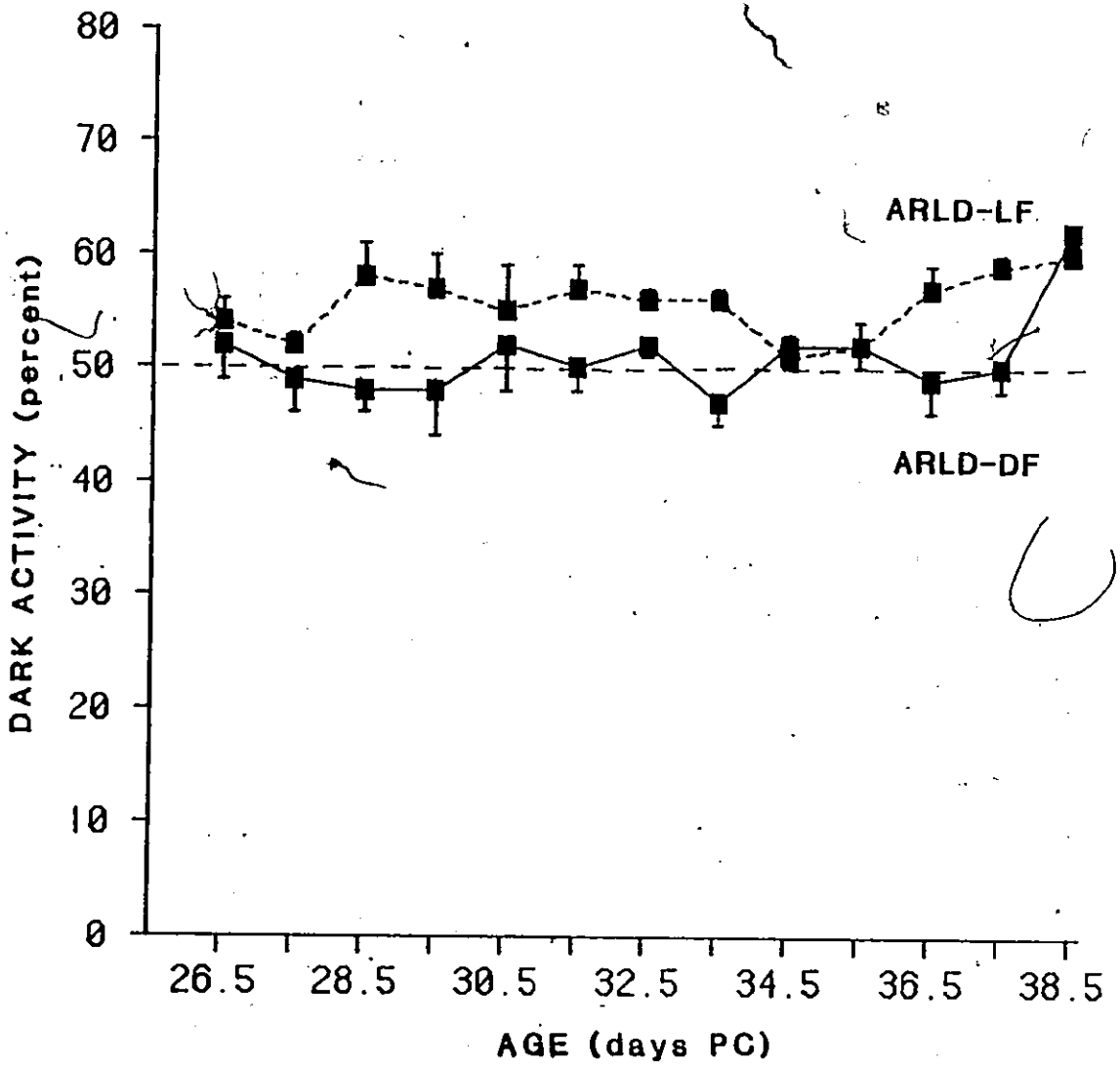

80
70
60
50
40
30
20
10
0
DARK ACTIVITY (percent)
ARLD-LF
ARLD-DF
26.5
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 12

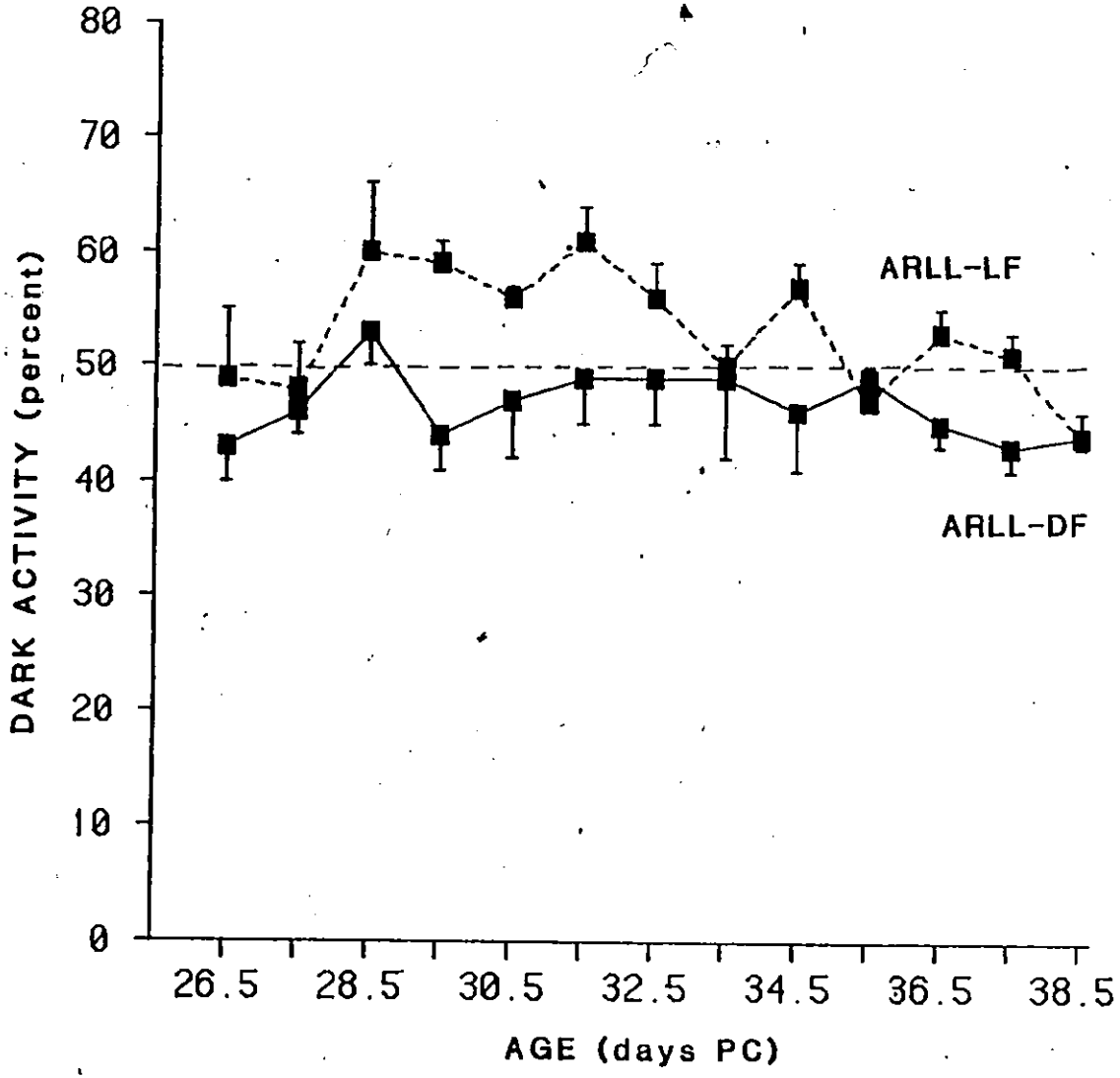

80
70
60
50
40
30
20
10
0
DARK ACTIVITY (percent)
ARLL-LF
ARLL-DF
26.5
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 13

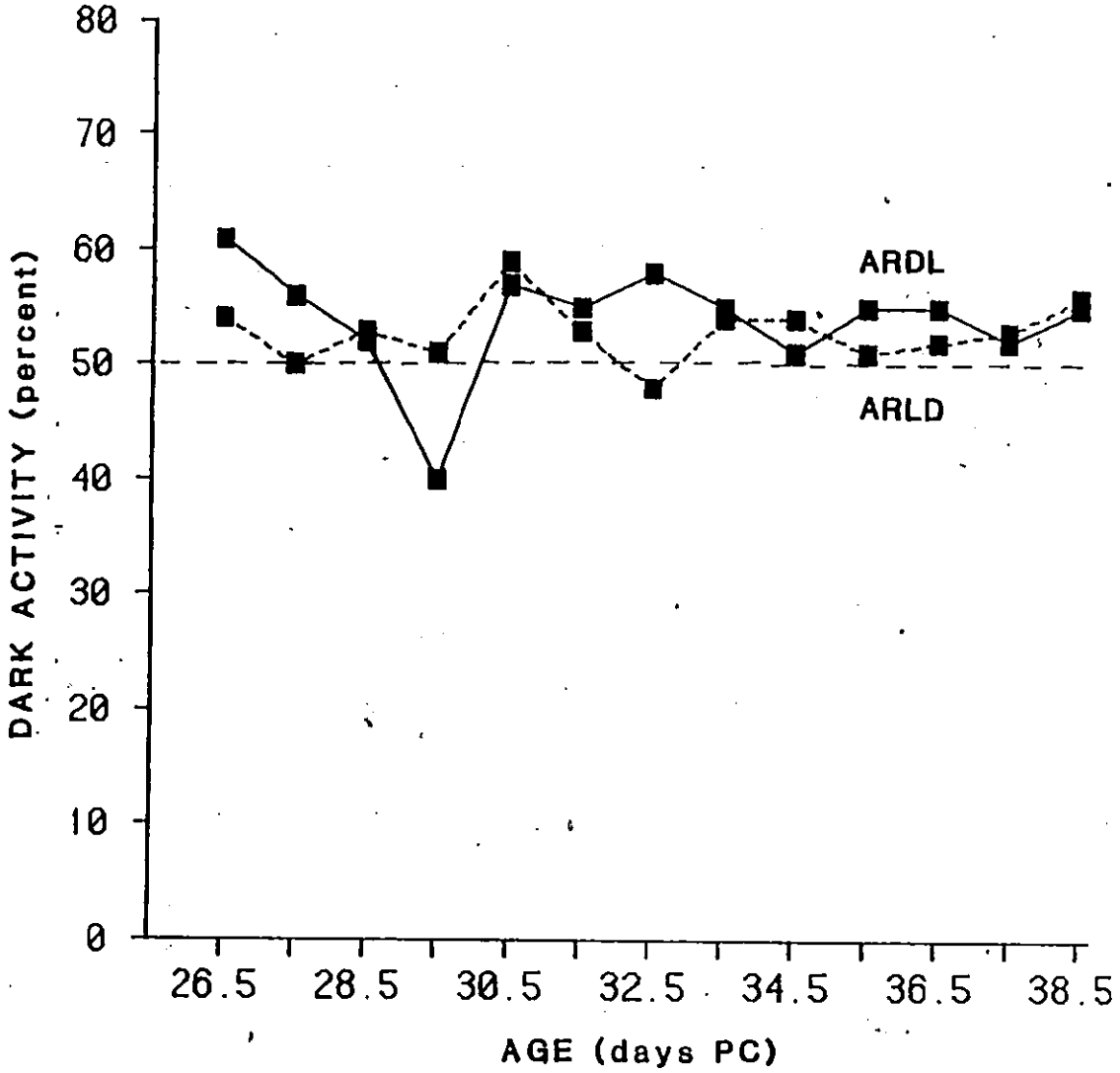

80
70
60
50
40
30
20
10
0
DARK ACTIVITY (percent)
ARDL
ARLD
26.5
28.5
30.5
32.5
34.5
36.5
38.5
AGE (days PC)

Figure 14

The partial correlations and the associated F-ratios for each of the independent variables are shown in Table 10. The partial correlations are the correlations of each of the independent variables with activity after the effects of the other independent variables in the regression equation have been taken into account. The F-ratios are approximate tests of the significance of each of the independent variables' contributions to the multiple R.

Water bath temperature and humidity did not correlate significantly with the proportion of dark-, or maternal dark-, period activity. In a preliminary analysis humidity correlated negatively with activity such that high activity counts were associated with low humidity. This was largely due to the ARLL group which was run during the time of the lowest humidity. Since this was the only group which experienced the low humidity conditions it is not clear whether humidity alone or some other factors in the ARLL group (i.e., the constant light) were responsible for the high activity. Age was also not significantly correlated with the proportion of dark-period activity. This is in contrast to the MRLD pups (Figure 11) whose mean proportions of dark-period activity increased significantly during 32 to 38 days PC (Mann-Whitney $U(6,7) = 1$, $p<.001$; median proportions are 55.7% for 26 to 31 days and 62.9% for 32 to 38 days PC).

In order to clarify the effects of the feeding and light cycles on dark-period activity the mean daily deviations from 50% of the mean proportion of dark-, or maternal dark-, period activity were calculated for each group (Table 11). The ARLL and ARDD groups are included here

Table 10

Stepwise regression results for the proportion of dark-period activity

Independent Variable	Partial Correlation	F-ratio
Age	0.141	1.73
Water Bath Temperature	0.091	0.71
Humidity	0.133	1.53
Dark food-deprived	0.211	4.00*
Light food-deprived	-0.258	6.13*
Light condititons	0.029	0.07
Light-period feeding	-0.022	0.04
Dark-period feeding	-0.469	24.23*
Light x Light-period feeding	0.002	0.00
Light x Dark-period feeding	0.267	6.59*

Note. Dark and light food-deprived refer to the proportion of animals accidentally food deprived during the dark and light period, respectively. Light conditions were either cyclic (LD or DL) or noncyclic (LL). In light or dark-period feeding animals received 65.3% of their food during the light or dark period, respectively. The last two variables in the table are the interactions between the light conditions and the type of feeding schedule.

* Significant at $p<0.05$.

for comparison. The dark-period activity did not correlate significantly with the lighting conditions (Table 10). This indicates that overall, animals on a feeding cycle only (i.e., ARLL-LF group) had similar proportions of dark-period activity (or deviations from 50%) as animals only on cyclic light (ARLD and ARDL groups). Also, animals with both a LD cycle and feeding cycle (ARLD-LF) had higher deviations from 50% than animals on only a feeding cycle (ARLL-LF) or light cycle (ARLD and ARDL) but this was not significant in the regression analysis.

The proportion of dark-period activity correlated significantly with four of the independent variables (Table 10) and together they accounted for 30.64% of the variance (multiple R= .5535, $F(4,86)$ = 9.50). First, the proportion of dark-period activity was decreased when feeding occurred mainly during the dark (ARLD-DF and ARLL-DF groups). The mean of the means shown in Figures 11 to 14 for the two dark-fed groups was 48.9% as compared to 53.5% for the groups fed mainly during the light or on a noncyclic feeding schedule. This variable alone accounted for about 16.9% of the variance. The second variable which correlated significantly was the interaction between the light conditions and dark-period feeding. Animals on a LD cycle with dark-period feeding (ARLD-DF) had higher proportions of dark-period activity (mean = 51.1%) than the animals on constant light with "maternal dark period" feeding (ARLL-DF, mean = 46.7%). Another way of looking at this result is that the deviations from 50% were greater and negative for the ARLL-DF group (Table 11). Higher proportions of

Table 11

Deviations of the proportion of dark-period activity from 50%

Group	Deviation[a]
MRLD	10.00(1.14)
ARLD-Temp	1.37(1.90)
ARLD-LF	5.79(0.79)
ARLD-DF	1.09(1.05)
ARLL-LF	3.19(1.55)
ARLL-DF	-3.34(0.84)
ARLD	3.07(0.75)
ARDL	4.08(1.37)
ARLL	-1.32(0.98)
ARDD	-0.08(1.09)

Note. The MRLD, ARLL, and ARDD groups were not included in the regression analysis.

[a]The deviations are the mean deviations from 50% (averaged over 13 days) of the mean percent of dark period activity. The numbers in parentheses are the sem.

dark-period activity were also observed when more animals were food deprived during the dark period (third significant variable) or fewer animals were food deprived during the light period (fourth significant variable).

To summarize, the overall proportions of dark-period, or maternal dark-period, activity were similar between the groups of AR pups with only a LD cycle (ARLD or ARDL) or feeding cycle (ARLL-LF). Feeding mainly during the dark-, or maternal dark-, period attenuated the proportion of activity occurring during the dark. There was an interaction between the lighting conditions and the feeding schedules such that the group with only a predominantly "nocturnal" feeding schedule (ARLL-DF) had lower proportions of maternal dark-period activity than the group with a LD cycle and nocturnal feeding (ARLD-DF). Accidental food deprivation during the dark period increased the proportion of dark-, or maternal dark-, period activity. Unlike the age-related increase in dark-period activity in the mother-reared animals, age did not contribute significantly to the activity of the AR pups. Water bath temperatures and humidity also did not make significant contributions.

Time Series Analysis

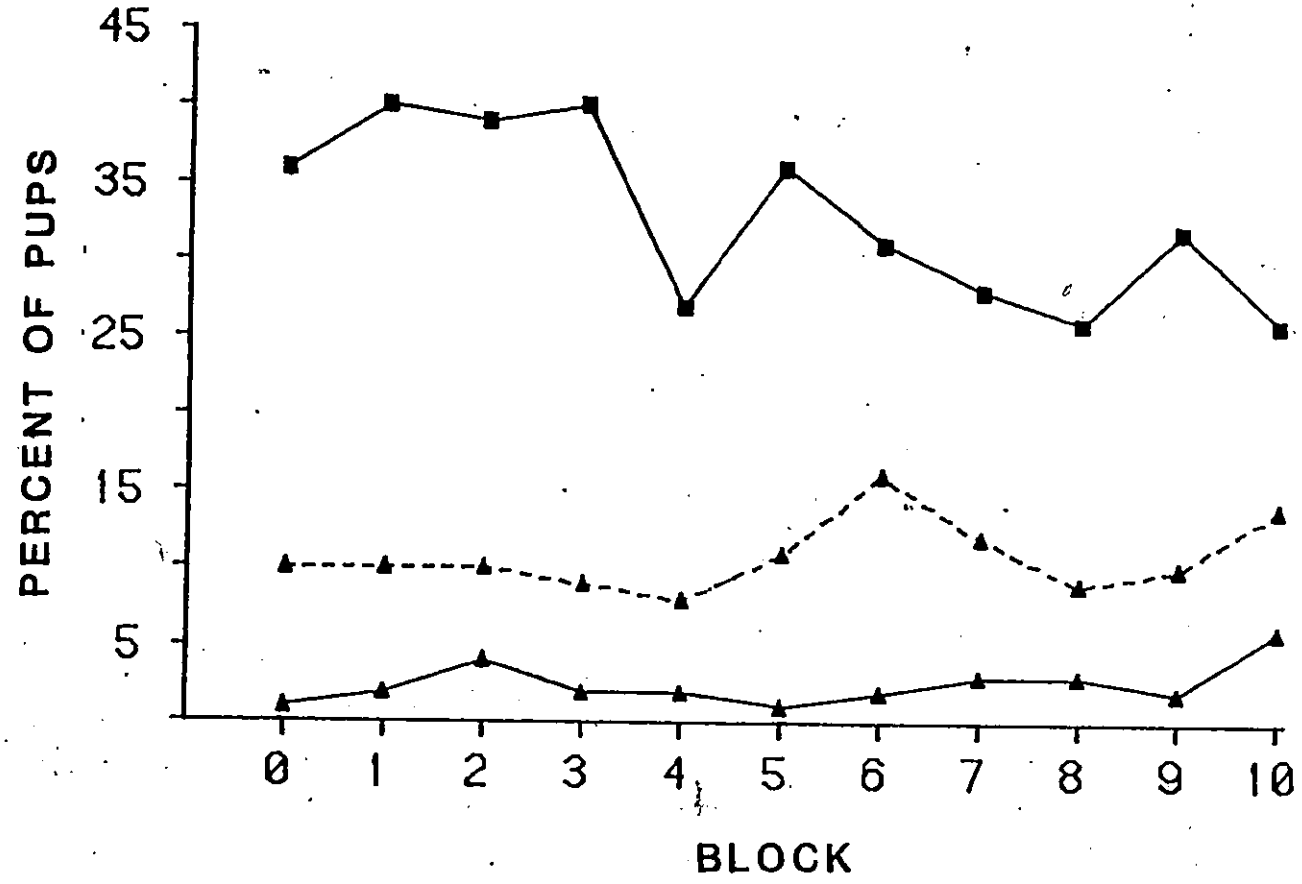
All of the autocorrelation analyses were performed on data from individual animals (see Appendix C for these data). As mentioned previously, there were no sex differences in the frequency of AR animals showing significant autocorrelation peaks. Similarly,

gestation length (21 versus 22 days) and the number of days spent with the dam before surgery (range of 2 to 4 days depending on the gestation length and age at surgery) did not influence the number of animals showing significant autocorrelation peaks.

In Figures 16 to 25 and 27 to 29 the abcissa represents the 3-day block of the autocorrelation plots (see Methods section). For all cases block 0 refers to 26.5 to 28.5 days PC, block 1 to 27.5 to 29.5 days, and so on to block 10 which is 36.5 to 38.5 days PC. Ninety three of the 130 significant autocorrelation peaks for the AR pups fell during times of accidental food deprivation. (Five of these peaks fell during intervals when the timer controlling the infusion pumps was not working properly in the ARLD-LF group.) The upper graph in Figure 15 illustrates the proportion of all AR animals which were food deprived during each 3-day block. In the middle plot, each point represents the proportion of the animals shown in the upper plot which showed significant autocorrelation peaks. A single pup may be represented in one or more blocks. The lower plot shows the same calculation for the pups that were not food deprived. It is evident that a higher proportion of pups showed significant autocorrelation peaks during food deprivation as compared to non-food deprivation intervals. In the following analyses food deprivation (FD) and non-FD autocorrelation peaks were treated separately.

Occurrence of peaks. The proportions of pups in each AR group showing non-FD peaks during each block are shown in Figures 16 to 20.

Figure 15. The proportion of total animals food deprived during each 3-day block (squares). The two lower lines represent the proportion of animals showing significant peaks during FD (dashed line) and non-FD (solid line) intervals for each 3-day block. Block 0 represents 26.5 to 28.5 days PC and so on up to Block 10 which is 36.5 to 38.5 days PC.


45
35
25
15
5
PERCENT OF PUPS
0
1
2
3
4
5
6
7
8
9
10
BLOCK

None of the AR groups approached the mean proportion (averaged over all blocks) of the MRLD group, approximately 87% (Figure 16). In addition, 100% of the MRLD pups showed significant peaks at some time during the 13 days of recording. Four of the seven MRLD groups showed significant peaks between 26 and 28 days PC. Table 12 presents the percentage of pups in each group that showed non-FD peaks at any time. (This is the percentage based on the total number of animals for which there were activity data.) The ARLD-Temp and ARLL-DF groups had the highest proportions and the ARDL group the lowest. Also shown in this table are the mean number of peaks per animal (number of peaks divided by number of animals showing significant peaks) for non-FD and FD intervals. This is a crude measure of the mean duration of the rhythm. Aside from the fact that none of the AR groups approached the mean of the MRLD group there is no obvious pattern. However, when the non-FD and FD peaks are combined the ARLD-Temp group has the highest mean.

There were also age-related changes in the frequency of occurrence of significant peaks. Significantly more animals in the MRLD group (six out of seven) had more peaks during the last five blocks (32 to 38 days PC) as compared to the first five blocks (26 to 32 days PC; Cochran Q-test, $Q(2) = 8.86$, $p<.02$). Age-related changes were also observed in the non-FD peaks for the AR groups combined; twice as many AR pups had peaks during 31 to 38 days PC as during 26 to 32 days PC (14 versus 7; $X^2(2) = 7.75$, $p<.05$). Three pups had peaks occurring during both time intervals.

Period. The mean periods of the significant peaks for each block for each group are shown in Figures 21 to 25. Small dots

Figure 16. The proportion of total MRLD (solid line) and ARLD-Temp (dashed line) animals showing significant circadian (20 to 28 hrs) peaks in the autocorrelation plots for each 3-day block. Only non-FD peaks are shown for the AR groups. Values are based on the number of individual rat pups.

Figure 17. The proportion of ARLD-LF (dashed line) and ARLD-DF (solid line) pups showing significant circadian autocorrelation peaks.

Figure 18. The proportion of ARLL-LF (dashed line) and ARLL-DF (solid line) pups showing significant circadian autocorrelation peaks.

Figure 19. The proportion of ARLD (dashed line) and ARDL (solid line) pups showing significant circadian autocorrelation peaks.

Figure 20. The proportion of ARLL (dashed line) and ARDD (solid line) pups showing significant circadian autocorrelation peaks.

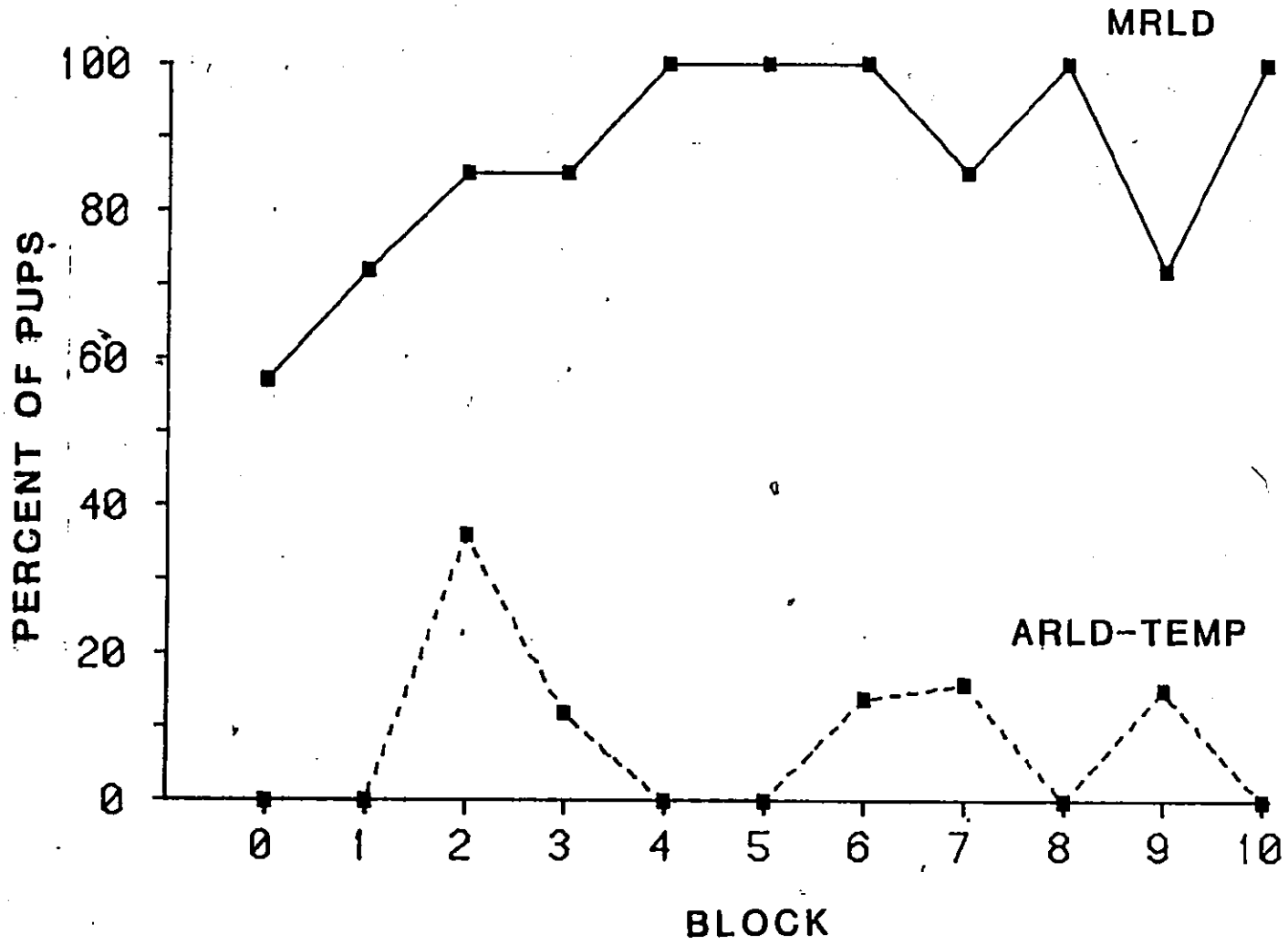

MRLD
ARLD-TEMP
PERCENT OF PUPS
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
BLOCK

Figure 16

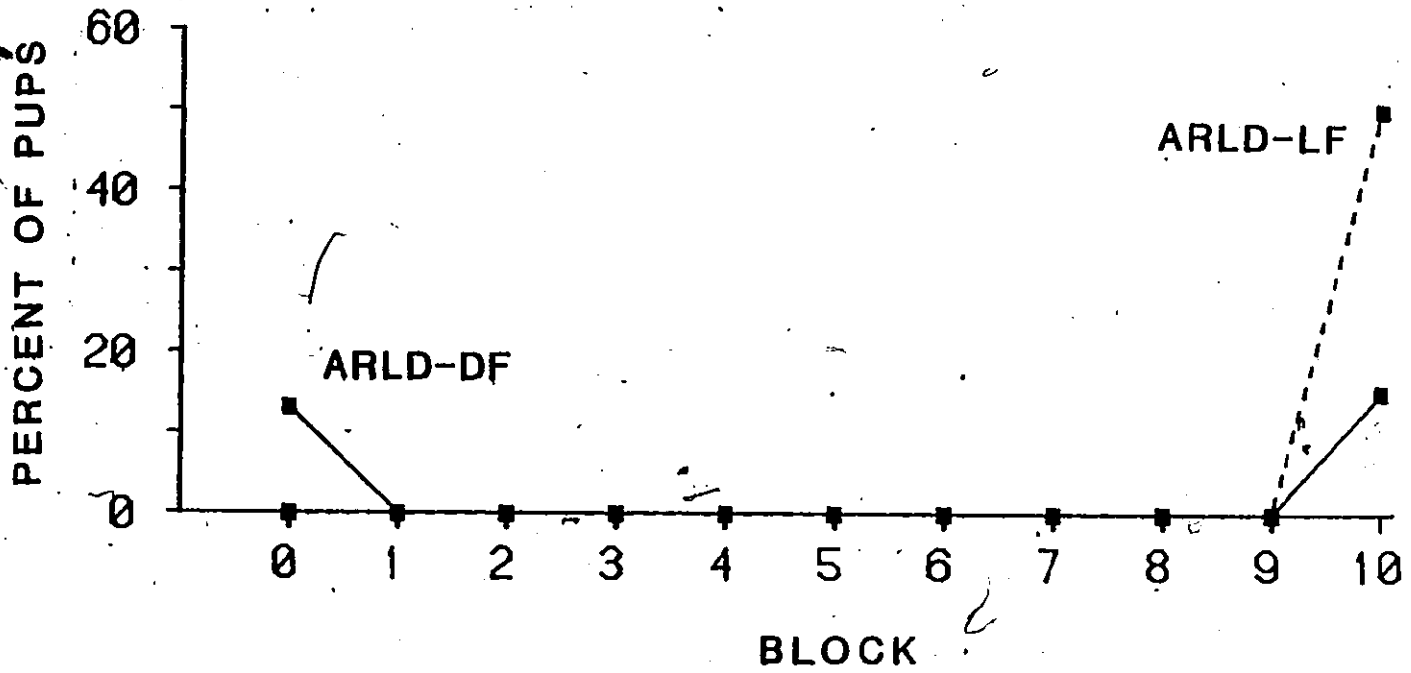


Figure 17

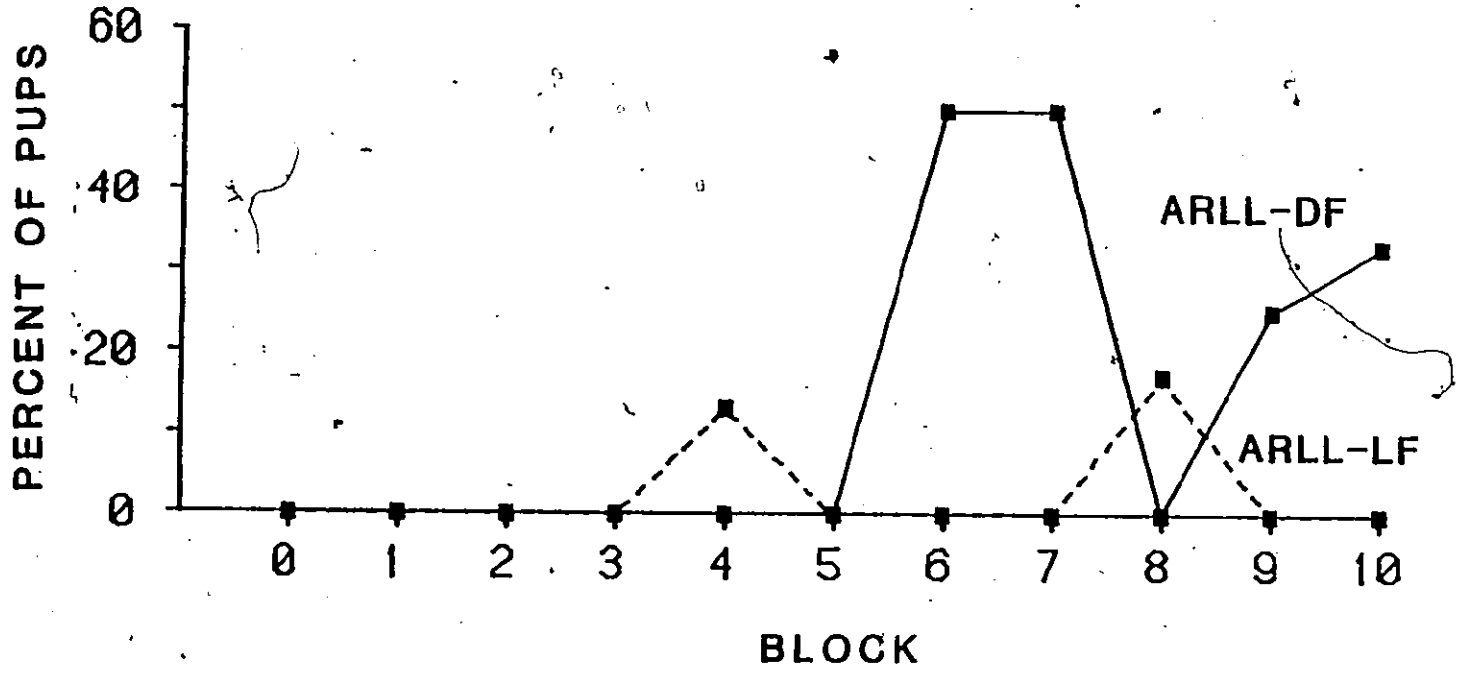


Figure 18

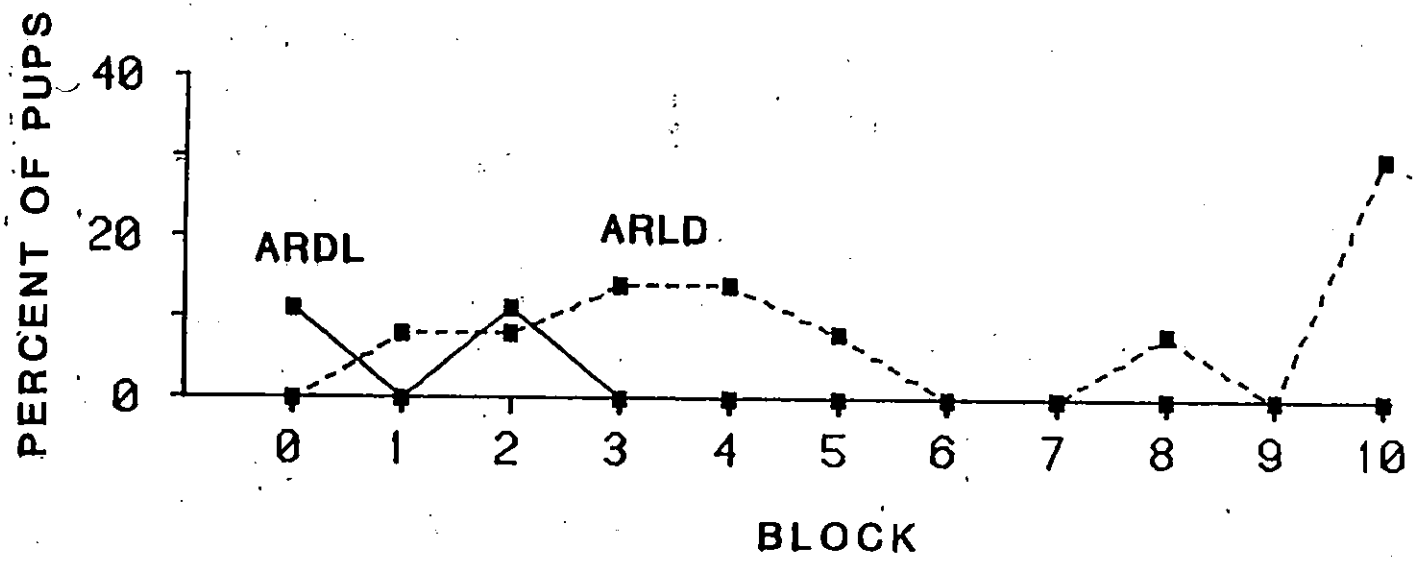


Figure 19

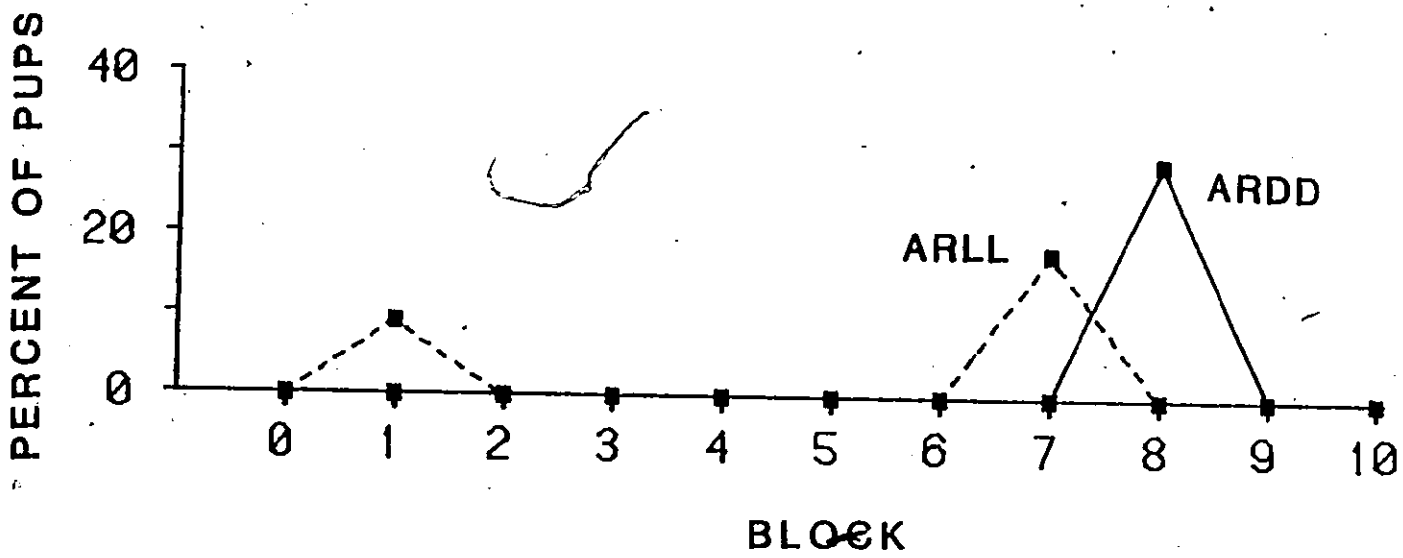


Figure 20

Table 12

Occurrence of autocorrelation peaks

Group	Percent with peaks[a]	Mean peaks per animal non-FD	FD	Total
MRLD	100.0	9.6(67)[b]		
ARLD-Temp	62.5	1.4(7)	2.6(13)	3.3
ARLD-LF	15.4	1.0(2)	2.4(26)	2.5
ARLD-DF	22.2	1.0(2)	1.7(7)	1.8
ARLL-LF	14.3	2.0(2)	2.2(9)	2.2
ARLL-DF	60.0	2.0(6)	1.0(1)	2.3
ARDL	10.0	2.0(2)	2.0(6)	2.0
ARLD	41.7	2.2(11)	2.4(19)	2.7
ARLL	22.2	1.0(2)	1.0(3)	1.2
ARDD	27.3	1.0(3)	1.5(9)	1.5
AR Total	28.5	1.5(37)	2.1(93)	1.8

[a]These values are based on the total number of animals with activity data.

[b]The numbers in parentheses are the number of significant peaks.

represent a non-FD peak and large dots are two or more peaks. The asterisks are the FD peaks. One of the most striking observations is the large variability of the periods in the AR groups as compared to the MRLD group. This is shown in Table 13 which presents the mean periods (averaged over all peaks and blocks) and the standard errors of the means for each group. The large variability is evident in both the non-FD and FD peaks (with the exception of the ARDL non-FD peaks). Also, the ARLL and ARLD-DF non-FD period means deviate the most from 24 hrs (21.75 hrs and 25.62 hrs, respectively). Comparisons between groups and between non-FD and FD peaks are difficult because of the small number of peaks in some of the groups. When AR groups are combined the mean periods of the non-FD and FD peaks are slightly less than the mean for the MRLD group. The distributions of the non-FD and FD periods are similar (Figure 26) although about 9% more of the non-FD periods fall within 23 to 25 hrs. As a comparison, 62 out of the 67 peaks (92.5%) in the MRLD groups had mean periods between 23.5 and 24.5 hrs.

The mother-reared pups showed significant increases in the mean period during 31 to 38 days PC (median = 24.13 hrs) as compared to 26 to 32 days PC (median = 23.91 hrs; Wilcoxon test, $T(7) = 0$, $p<.01$). This increase, however, was largely due to the occurrence of four extreme values during blocks 8 to 10 (Figure 21). A comparison of AR pups showing peaks during the same time intervals did not reveal any age-related changes.

"Area". The area calculations for individual animals are plotted as a function of the autocorrelation block in Figures 27 to 31.

Figure 21. The mean period of the activity rhythms for the MRLD (A) and ARLD-Temp (B) groups plotted as a function of the 3-day block. Small dots are individual pups and large dots are two or more pups. For the AR pups, dots represent non-FD periods and asterisks are FD periods.

Figure 22. The mean period of the activity rhythms for the ARLD-LF (A) and ARLD-DF (B) pups.

Figure 23. The mean period of the activity rhythms for the ARLL-LF (A) and ARLL-DF (B) pups.

Figure 24. The mean period of the activity rhythms for the ARLD (A) and ARDL (B) pups.

Figure 25. The mean period of the activity rhythms for the ARLL (A) and ARDD (B) pups.

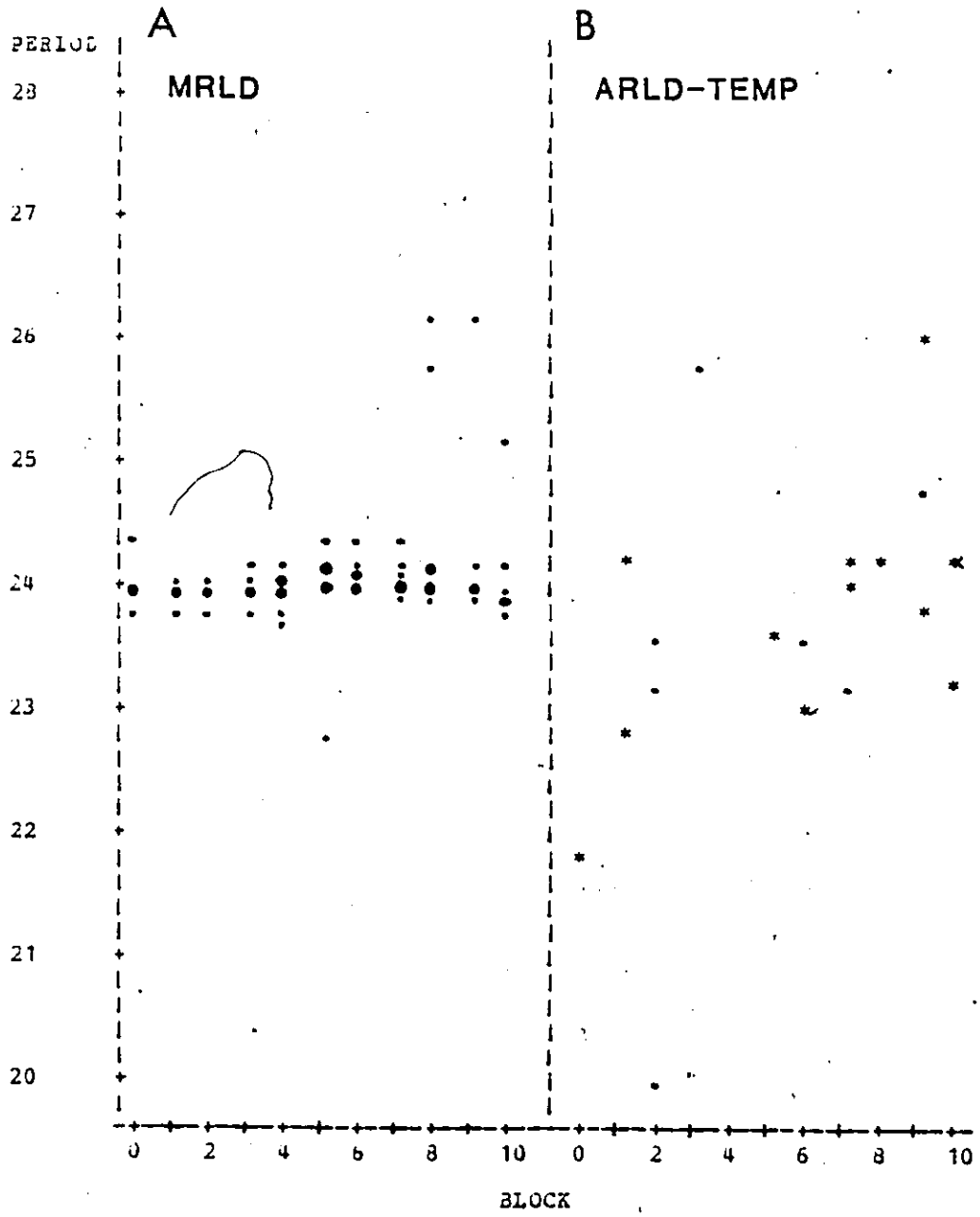

A
MRLD
B
ARLD-TEMP
PERIOD
28
27
26
25
24
23
22
21
20
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 21

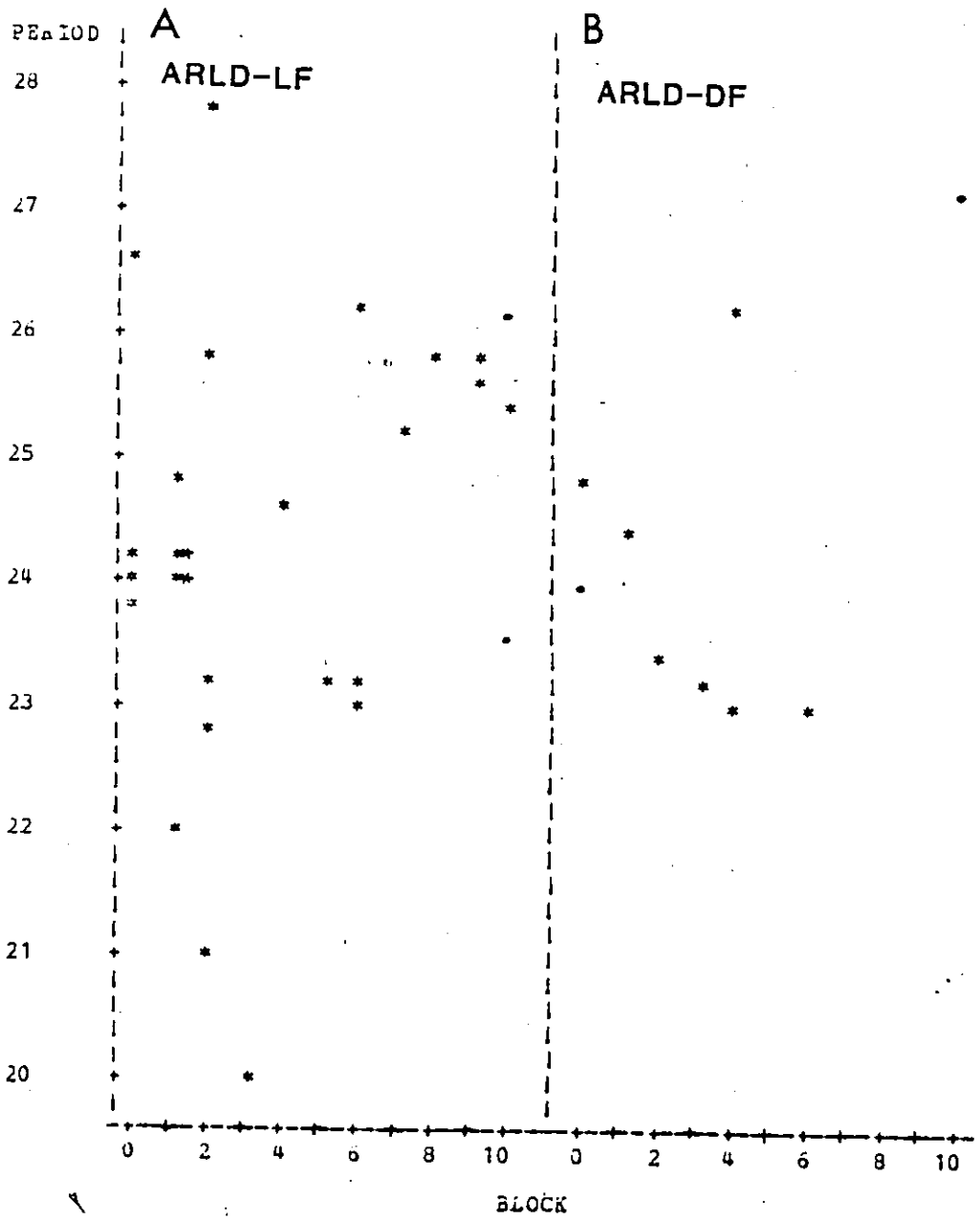

PERIOD
A
ARLD-LF
B
ARLD-DF
28
27
26
25
24
23
22
21
20
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 22

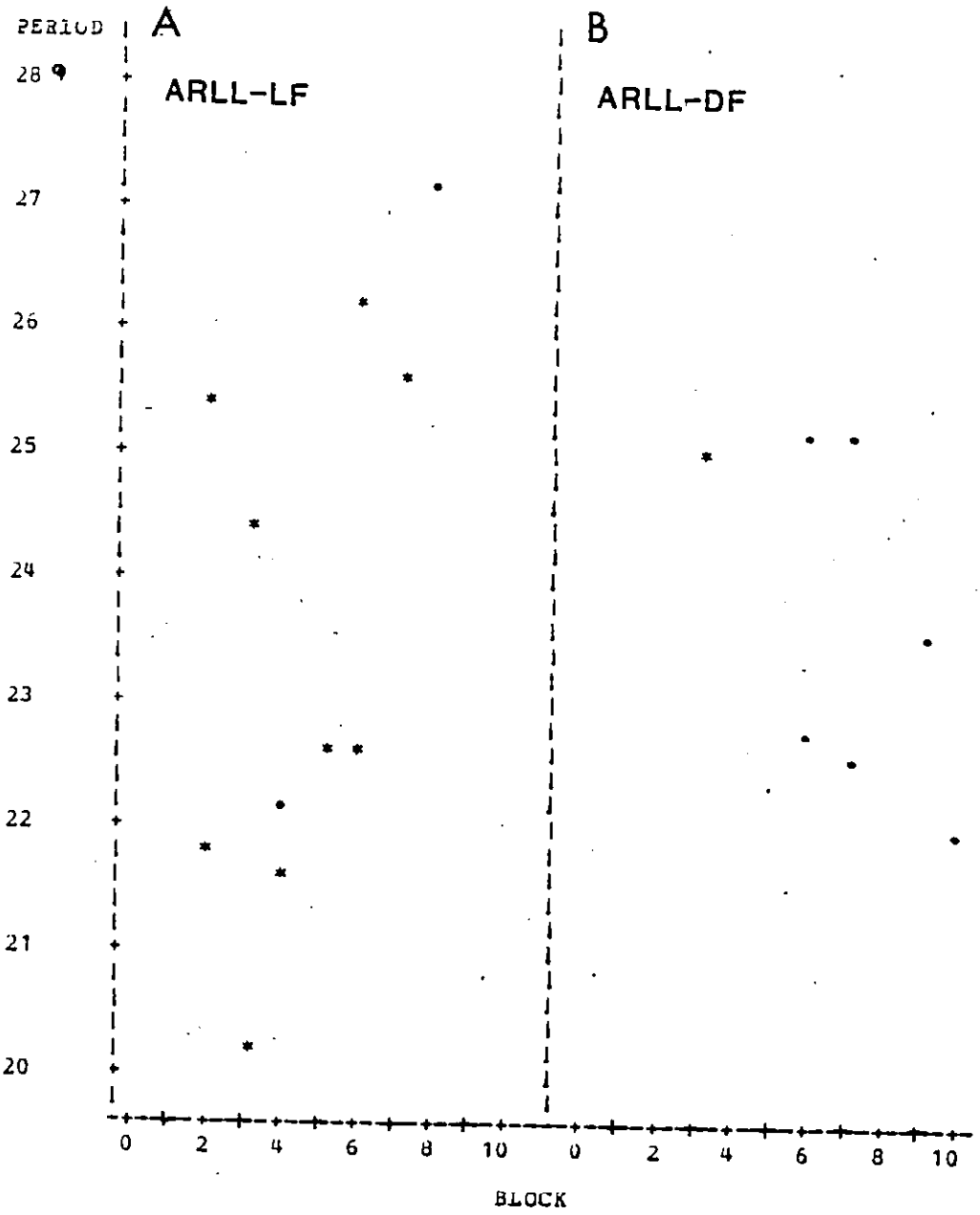

PERIOD
A
ARLL-LF
B
ARLL-DF
28
27
26
25
24
23
22
21
20
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 23

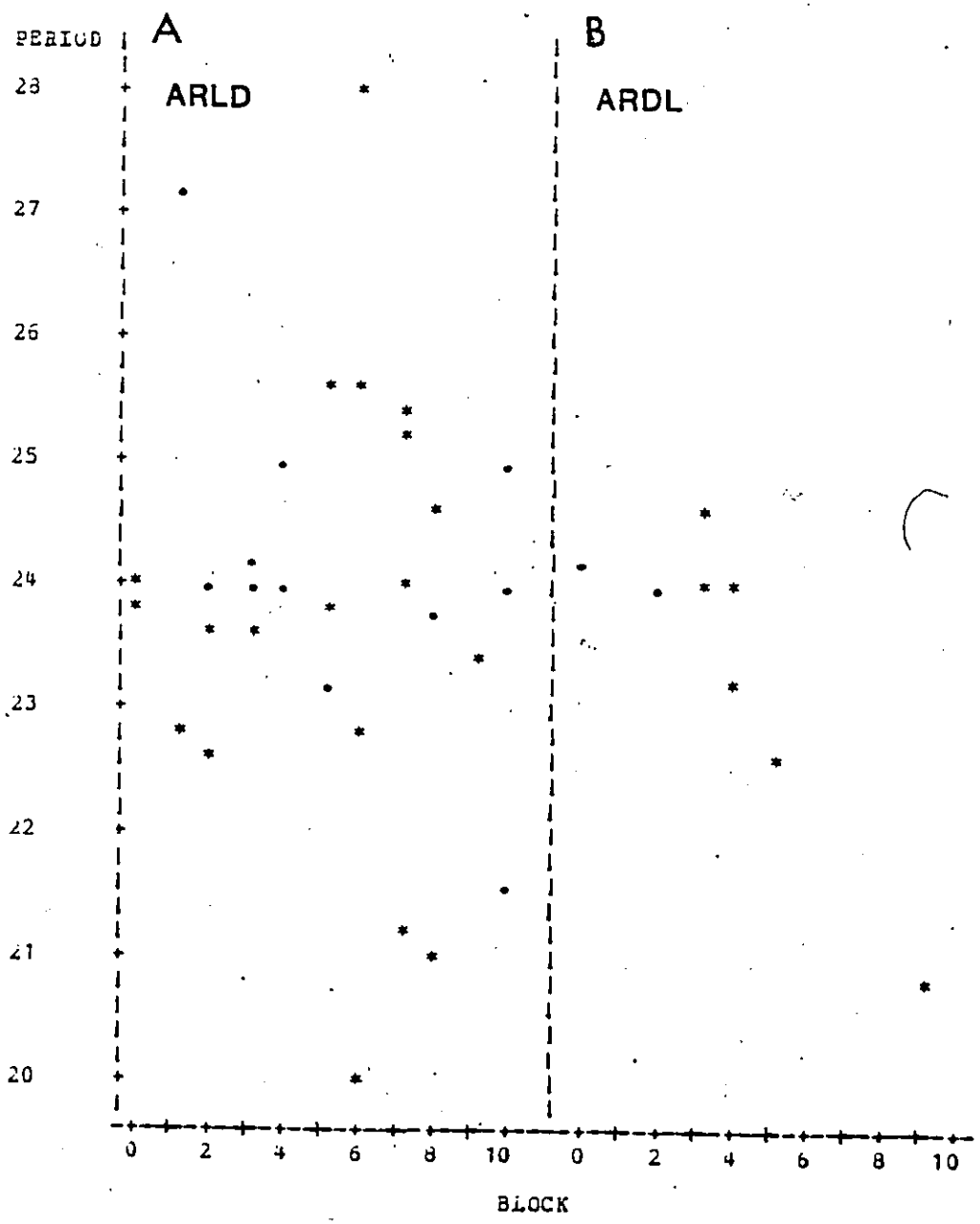

PERIOD
A
ARLD
B
ARDL
28
27
26
25
24
23
22
21
20
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 24

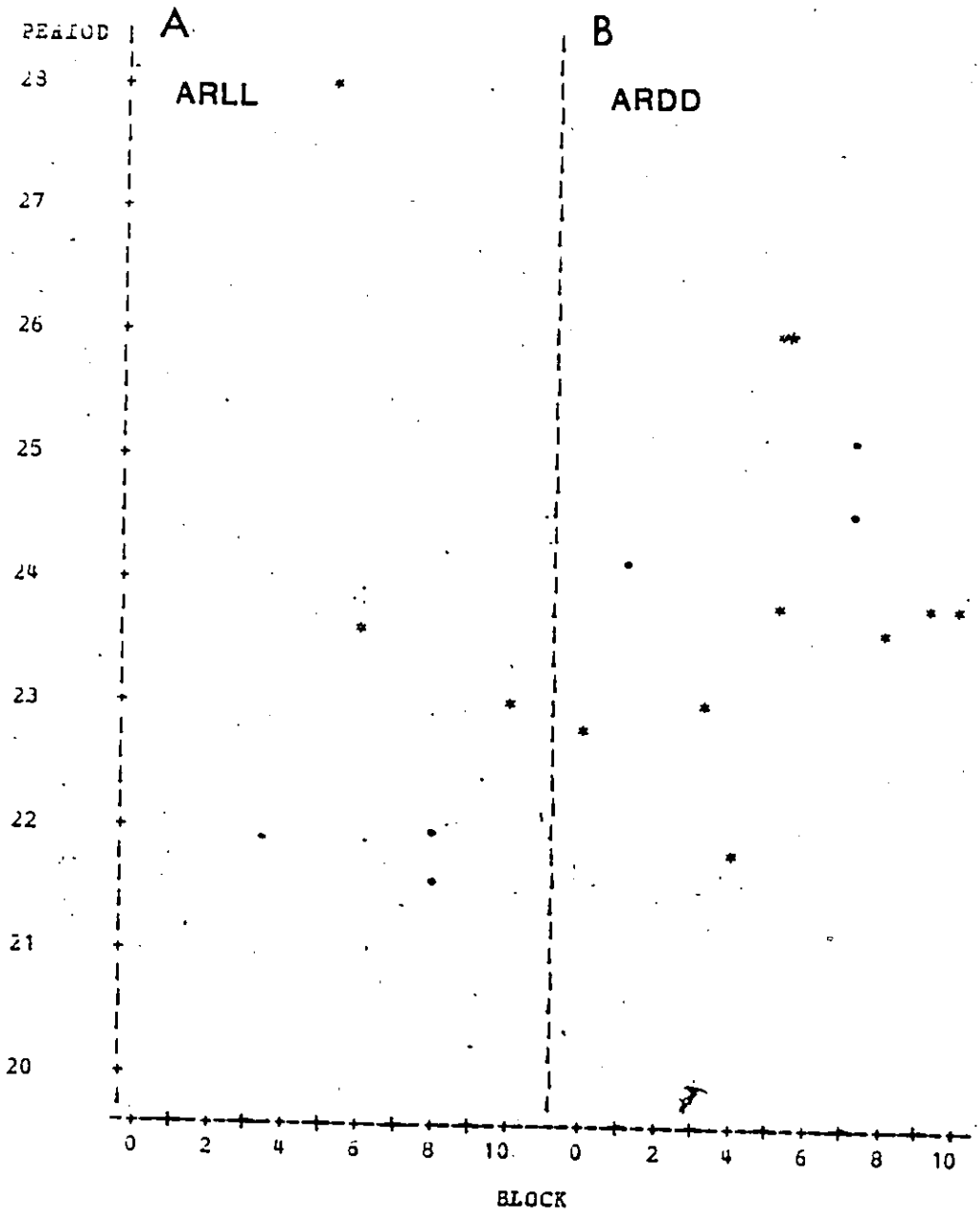

PERIOD
A
ARLL
B
ARDD
28
27
26
25
24
23
22
21
20
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 25

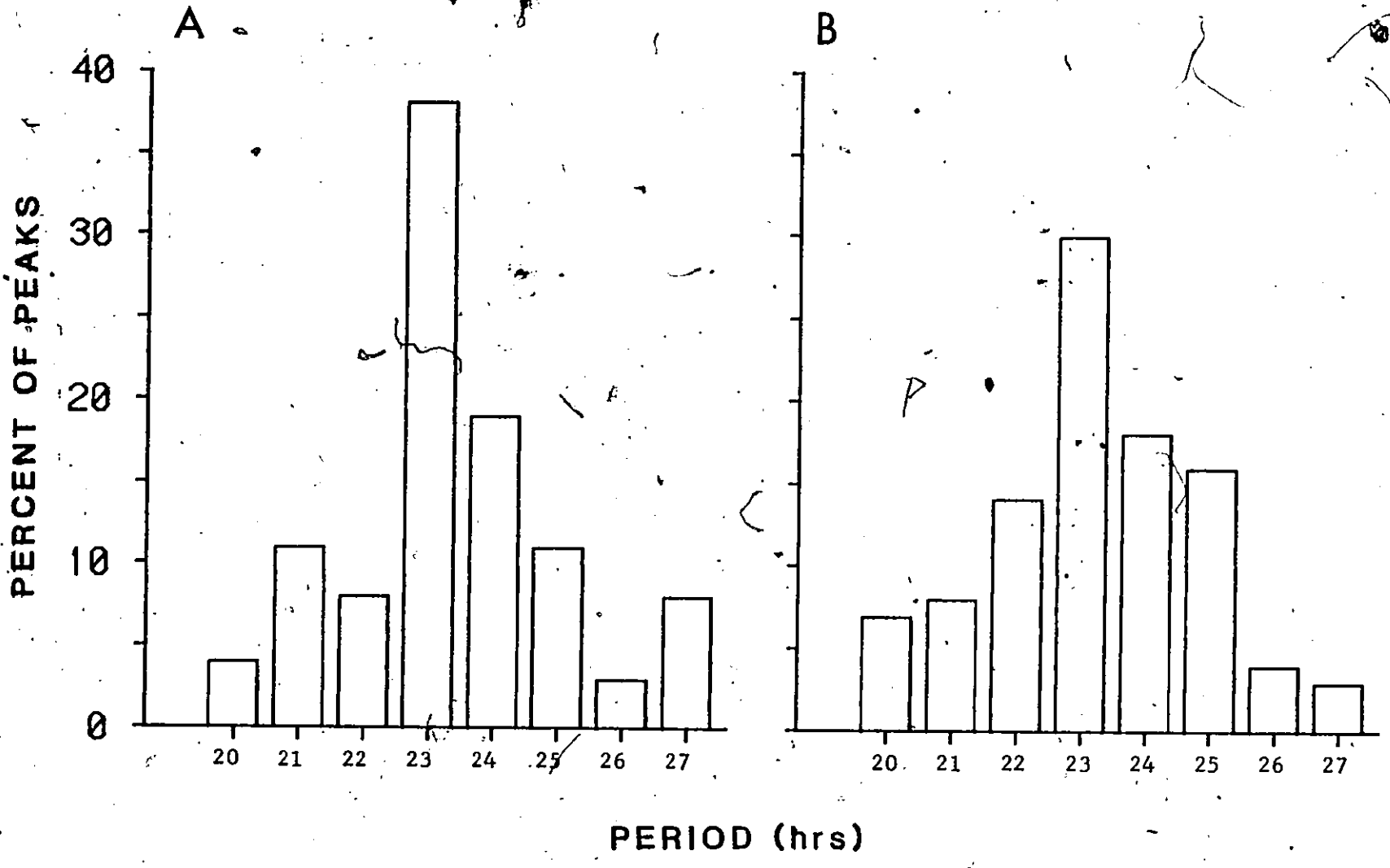
Table 13

Mean periods for non-FD and FD autocorrelation peaks.

Group	non-FD	FD
MRLD	24.09(0.06)	
ARLD-Temp	23.43(0.67)	23.77(0.28)
ARLD-LF	24.85(1.35)	24.26(0.34)
ARLD-DF	25.62(1.62)	23.99(0.45)
ARLL-LF	24.66(2.59)	23.34(0.70)
ARLL-DF	23.53(0.58)	25.00(0.00)
ARDL	24.05(0.05)	23.17(0.56)
ARLD	24.17(0.41)	23.70(0.42)
ARLL	21.75(0.25)	24.83(1.59)
ARDD	24.67(0.30)	23.80(0.47)
AR Total	23.97(0.27)	23.87(0.17)

Note. Numbers in parentheses are the standard errors of the means. See Table 12 for the number of peaks in each group.

Figure 26. Frequency distributions of the periods (hrs) of the activity rhythms falling during non-FD (A) and FD (B) intervals for the AR pups. The categories of the periods are 20 to 20.9 hrs, 21 to 21.9 hrs and so forth.


A
B
PERCENT OF PEAKS
40
30
20
10
0
20
21
22
23
24
25
26
27
PERIOD (hrs)

The mean areas and sems (averaged over all the blocks) for the non-FD peaks (dots) and FD peaks (asterisks) are given in the figure captions. It is obvious that the areas for the peaks in the MRLD group (Figure 27A) are greater than for any of the AR groups. The overall means for the AR groups were .0255 (sem = .0041) for non-FD peaks and .0453 (sem = .0113) for FD peaks. These means are approximately 8.4 (non-FD peaks) and 4.7 times (FD peaks) smaller than the overall MRLD mean (.2154, sem = .0237) indicating that the rhythmic activity in the AR pups is attenuated compared to the rhythmicity seen in mother-reared pups. The two groups on constant conditions, ARDD and ARLL, had the lowest means for the non-FD peaks (.0164 and .0158, respectively).

The areas did not change significantly with age in the AR or MRLD groups. As mentioned in the preceding paragraph, the FD peaks had a larger mean area than the non-FD peaks. This was largely due to the high FD areas observed in the ARLD-DF and ARLL-LF groups. Twelve AR animals had both non-FD and FD peaks. Of these 12, seven showed greater mean areas during FD intervals and 5 during non-FD intervals.

To summarize, fewer AR animals had significant autocorrelation peaks falling between 20 and 28 hrs. The mean number of significant autocorrelation peaks per animal for the AR groups (non-FD and FD combined) was, on the average, 5.3 times less than the mean number per animal in the MRLD group. With one exception, the variability of the mean periods of the autocorrelation peaks was higher in the AR groups.

Figure 27. The mean area of the autocorrelation peaks falling within 20 to 28 hrs for the MRLD (A) and ARLD-Temp (B) groups, plotted as a function of the 3-day block. Small dots are individual pups and large dots are two or more pups. For the AR pups the dots represent non-FD areas and asterisks are FD areas. The overall mean areas are : MRLD = .2154; ARLD-Temp non-FD = .0224 and FD = .0262.

Figure 28. Autocorrelation peak areas for the ARLD-LF (A) and ARLD-DF (B) groups. Overall mean areas are: ARLD-LF non-FD = .0511 and FD = .0380; ARLD-DF non-FD = .0185 and FD = .1169.

Figure 29. Autocorrelation peak areas for the ARLL-LF (A) and ARLL-DF (B) groups. Overall mean areas are: ARLL-LF non-FD = .0176 and FD = .1513; ARLL-DF non-FD = .0288 and FD = .0045.

Figure 30. Autocorrelation peak areas for the ARLD (A) and ARDL (B) groups. Overall mean areas are: ARLD non-FD = .0234 and FD = .0197; ARDL non-FD = .0512 and FD = .0180.

Figure 31. Autocorrelation peak areas for the ARLL (A) and ARDD (B) groups. Overall mean areas are: ARLL non-FD = .0158 and FD = .0067; ARDD non-FD = .0164 and FD = .0221.

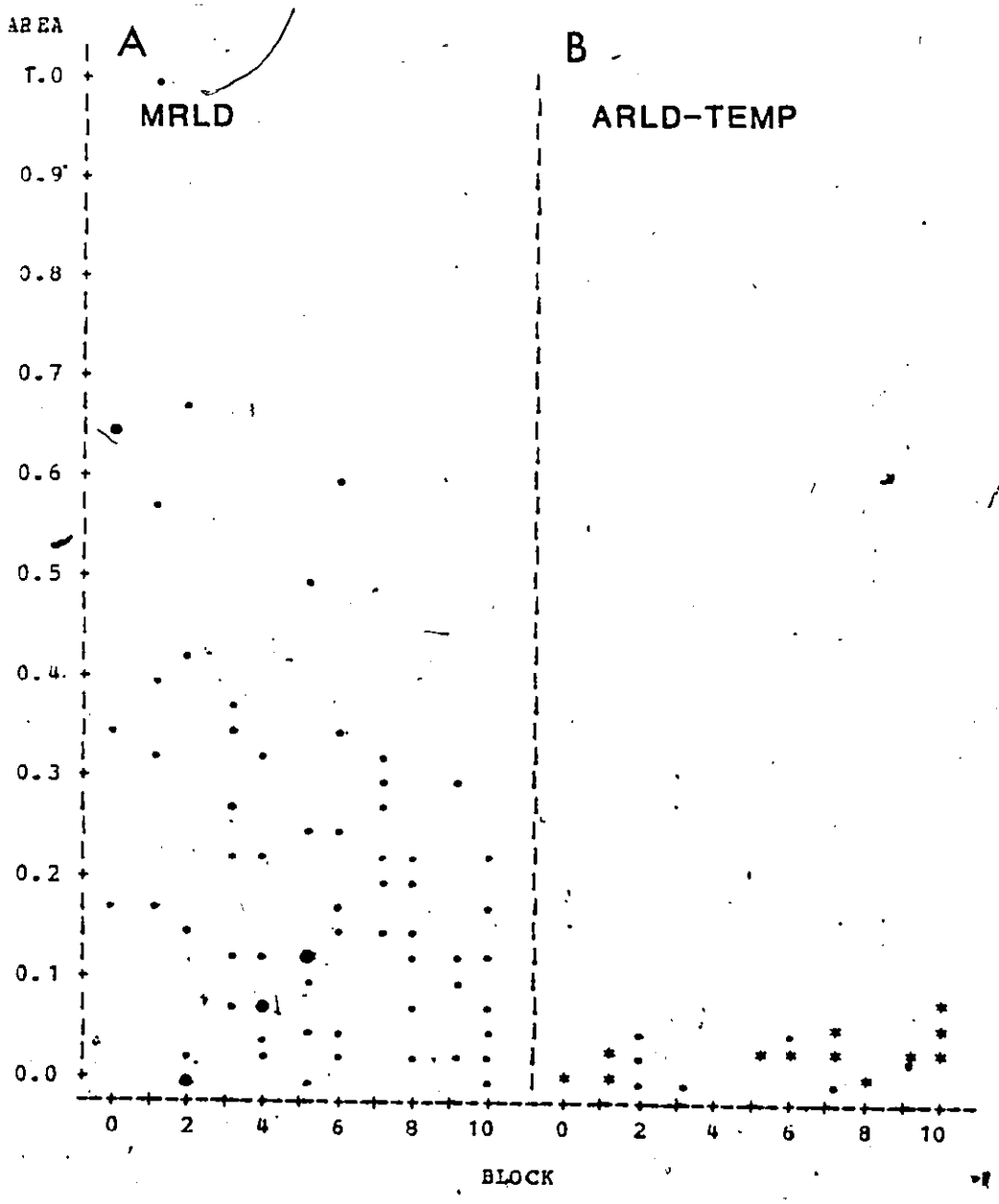

AREA
A
B
MRLD
ARLD-TEMP
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 27

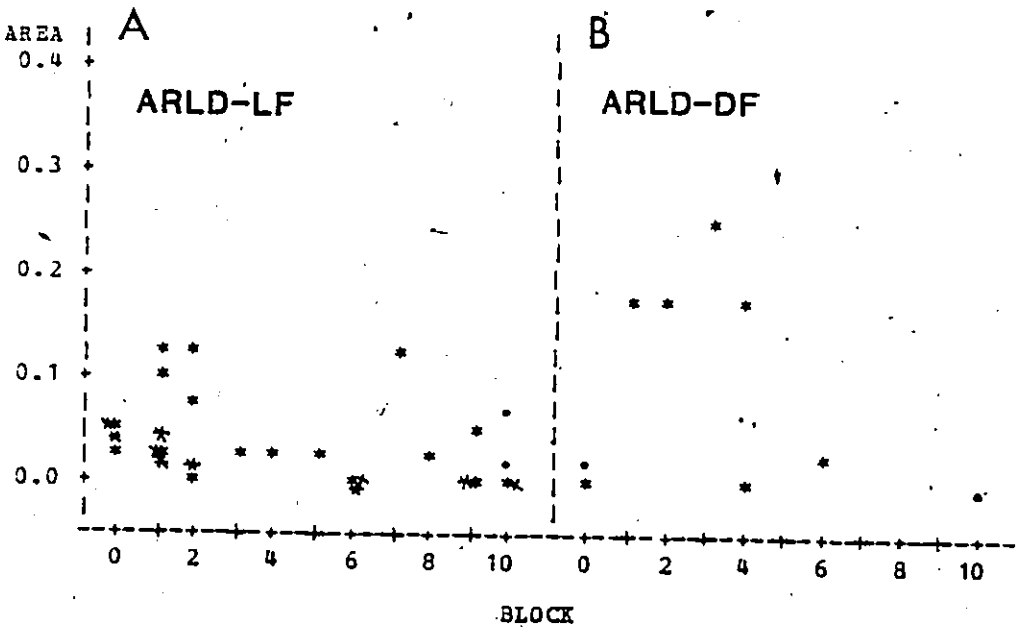


Figure 28

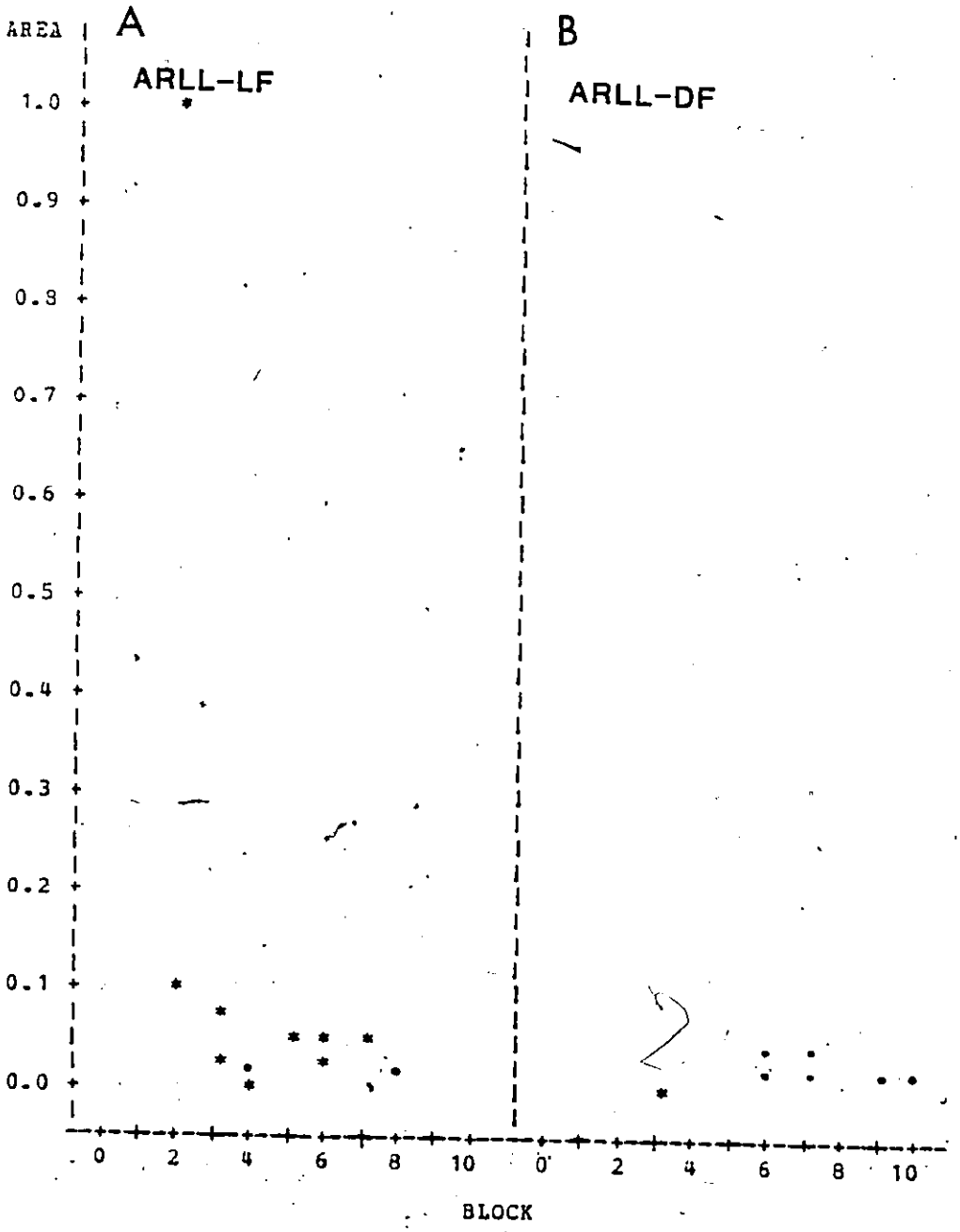

AREA
A
ARLL-LF
B
ARLL-DF
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
0
2
4
6
8
10
BLOCK

Figure 29

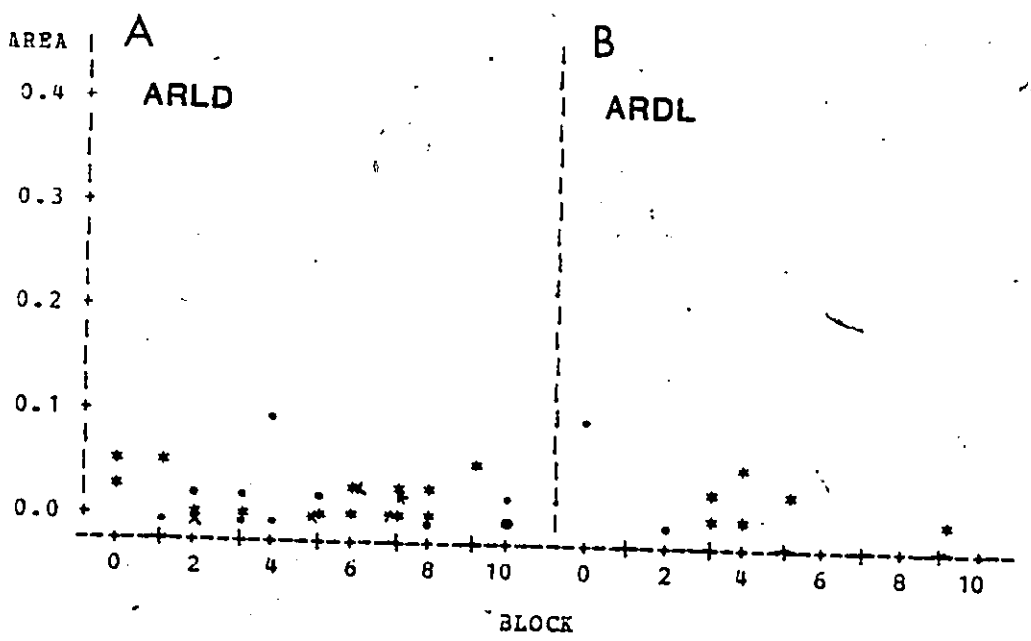


Figure 30

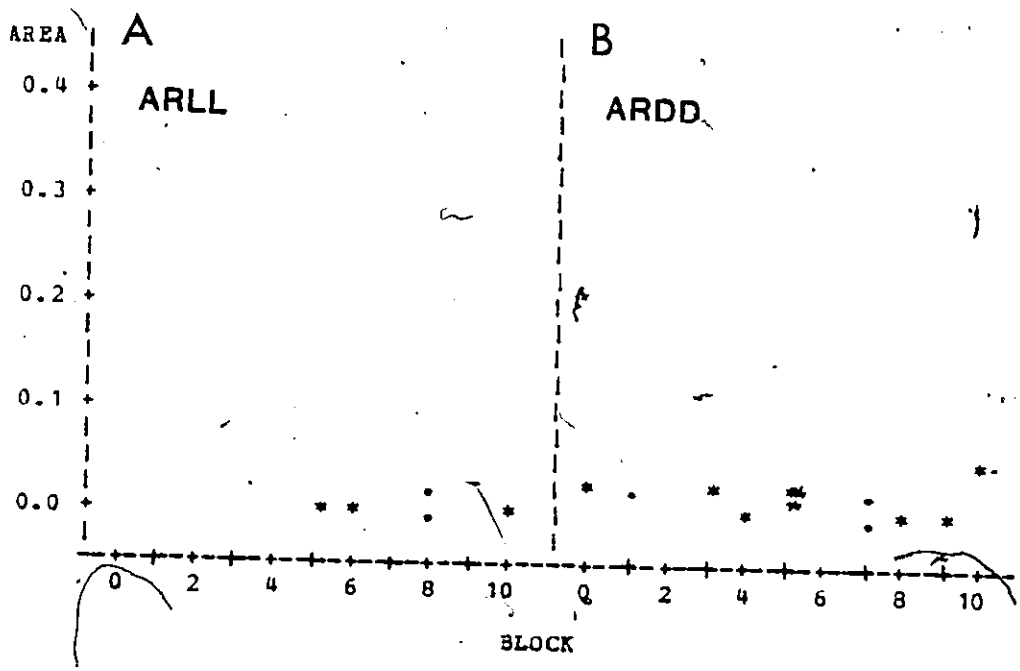


Figure 31

However, the AR mean periods were within 1 hr of 24.0 hrs. The mean areas of the autocorrelation peaks from the AR groups were much lower than the mean area for the MRLD group. Both AR and MRLD groups had more significant autocorrelation peaks during the last 7 to 8 days of recording than during the first 7 days of recording.

Three-hr Mean Activity

Three-hr peaks. The peak of a circadian rhythm is usually used as a reference point for determining the phase relationship with a zeitgeber. In the present case 3-hr means were used to determine when the peak amount of activity occurred within a 24-hr period. Three-hr peaks were calculated for each day that fell within each 3-day block which showed a significant peak in the autocorrelation analysis. For example, if the first block had a significant autocorrelation peak the 3-hr peak was determined for 26.5, 27.5, and 28.5 days PC. Since only two days with peaks of activity were needed to produce a significant autocorrelation peak, the calculation of 3-hr peaks for each of the three days in a block may be an overestimation of the number of 3-hr peaks which actually represent the peak of a circadian rhythm.

All of the 3-hr peaks in the MRLD group (n=87) and all but one 3-hr peak in each of the AR non-FD (n=96) and FD (n=220) categories were greater than or equal to the daily 24-hr mean plus 2 sem. This is an indication that these 3-hr peaks represent significant levels of activity. Due to the overlapping nature of the 3-day blocks some of the non-FD 3-hr peaks are also represented in the FD 3-hr peaks.

Figures 32 to 36 illustrate the individual 3-hr peaks for each group as a function of age. On the ordinate, "1" refers to the first 3 hrs of the dark or maternal dark period and "5" is the first 3 hrs of the light or maternal light period. Only the non-FD intervals are included here for the AR groups. In the MRLD group the 3-hr peaks appear to be concentrated in the dark period (Figure 32A). This is also the case for the ARLD-Temp (Figure 32B), ARLD-LF (Figure 33A), ARLD (Figure 34A), ARDL (Figure 34B), and ARLL-LF groups (Figure 35A). The 3-hr peaks for the ARLL-DF group (Figure 35B), in contrast, occur slightly more frequently during the maternal light period. In the remaining groups, ARLD-DF, ARLL, and ARDD, the 3-hr peaks tend to be more scattered throughout the day. Frequency distributions of the 3-hr peaks summed over age are shown in Figure 37. In the mother-reared group (Figure 37A), the mode of the 3-hr peaks (39%) fell during 0200 to 0500 hrs. Relatively few fell during the light period although an increase in frequency was observed during the three hrs before dark onset. In contrast, the mode 3-hr peak for the non-FD intervals (Figure 37B) for the combined AR groups (28%) fell during the first three hrs of the dark or maternal dark period. As with the MRLD group, an increase in frequency was seen during the three hrs before dark onset. The distribution for the combined AR FD intervals (Figure 37C) was similar to that for the non-FD intervals except that the 3-hr peaks were more evenly distributed throughout the dark or maternal dark period. In the MRLD group 92% of the 3-hr peaks were accompanied by a

Figure 32. The time of occurrence of the peak 3-hr mean of activity as a function of age (days PC) for the MRLD (A) and ARLD-Temp (B) groups. Values are for individual pups. On the ordinate, "1" refers to the first three hrs of the dark period, or 2000 to 2300 hrs. The "5" refers to the first three hrs of the light period, or 0800 to 1100 hrs. Only non-FD intervals are shown for the AR pups.

Figure 33. The time of occurrence of the peak 3-hr mean activity for the ARLD-LF (A) and ARLD-DF (B) groups. The "1" refers to 2000 to 2300 hrs.

Figure 34. The time of occurrence of the peak 3-hr mean activity for the ARLD (A) and ARDL (B) groups. The "1" refers to 2000 to 2300 hrs (ARLD group) or 0800 to 1100 hrs (ARDL group).

Figure 35. The time of occurrence of the peak 3-hr mean activity for the ARLL-LF (A) and ARLL-DF (B) groups. The "1" refers to 2000 to 2300 hrs.

Figure 36. The time of occurrence of the peak 3-hr mean activity for the ARLL (A) and ARDD (B) groups. The "1" refers to 2000 to 2300 hrs.

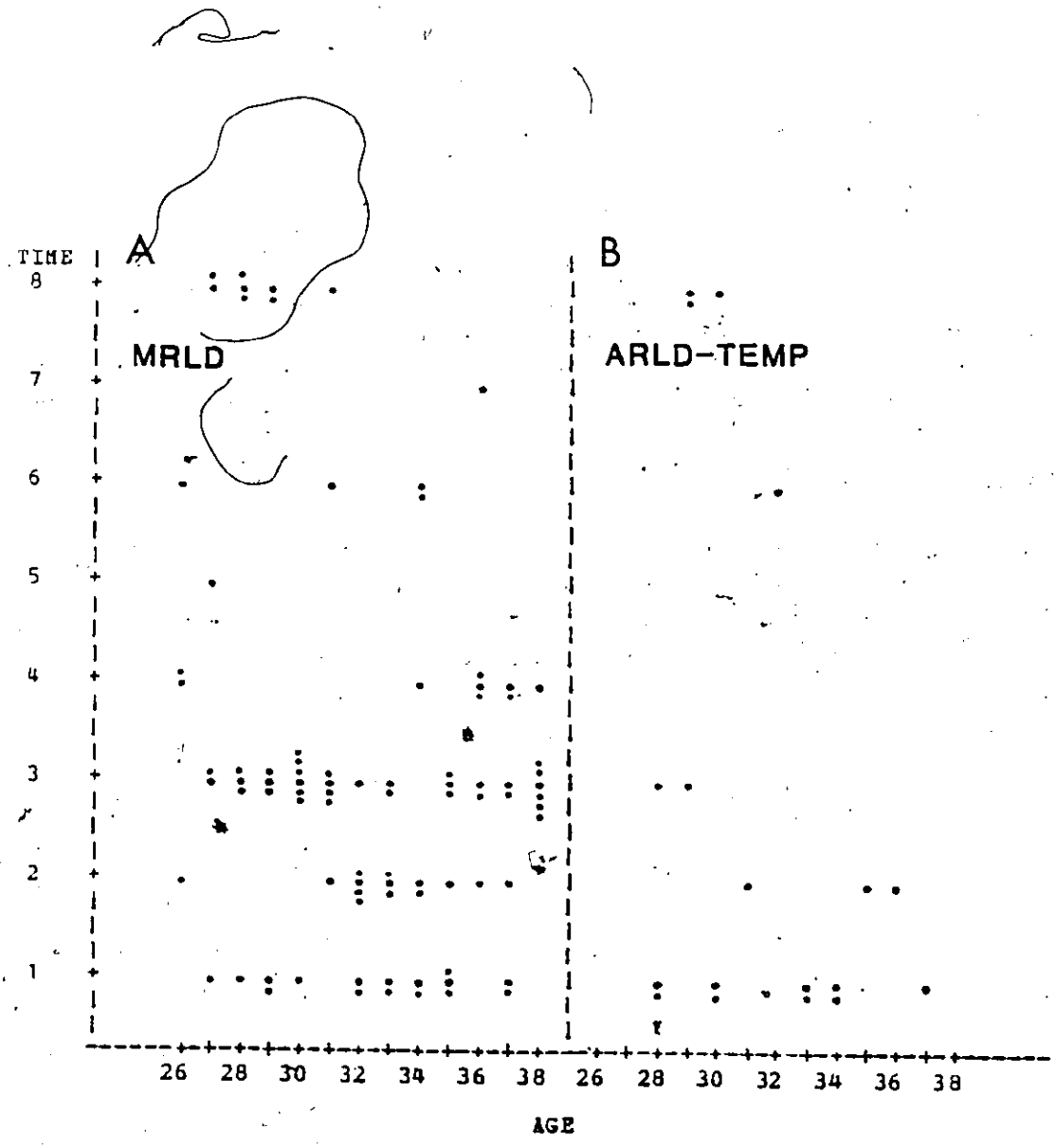

TIME
A
B
MRLD
ARLD-TEMP
8
7
6
5
4
3
2
1
26 28 30 32 34 36 38 26 28 30 32 34 36 38
AGE

Figure 32

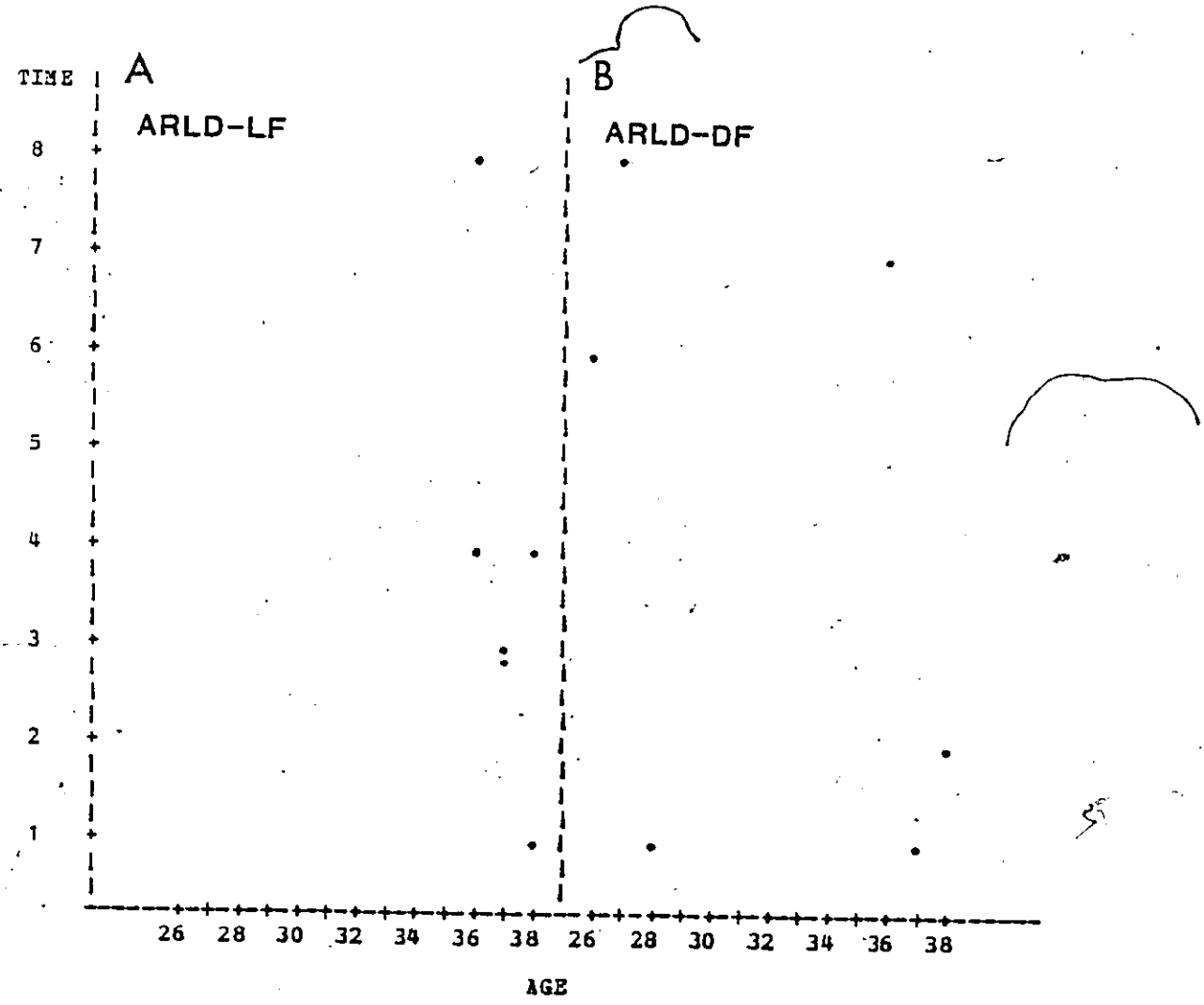

TIME
A
ARLD-LF
B
ARLD-DF
8
7
6
5
4
3
2
1
26 28 30 32 34 36 38 26 28 30 32 34 36 38
AGE

Figure 33

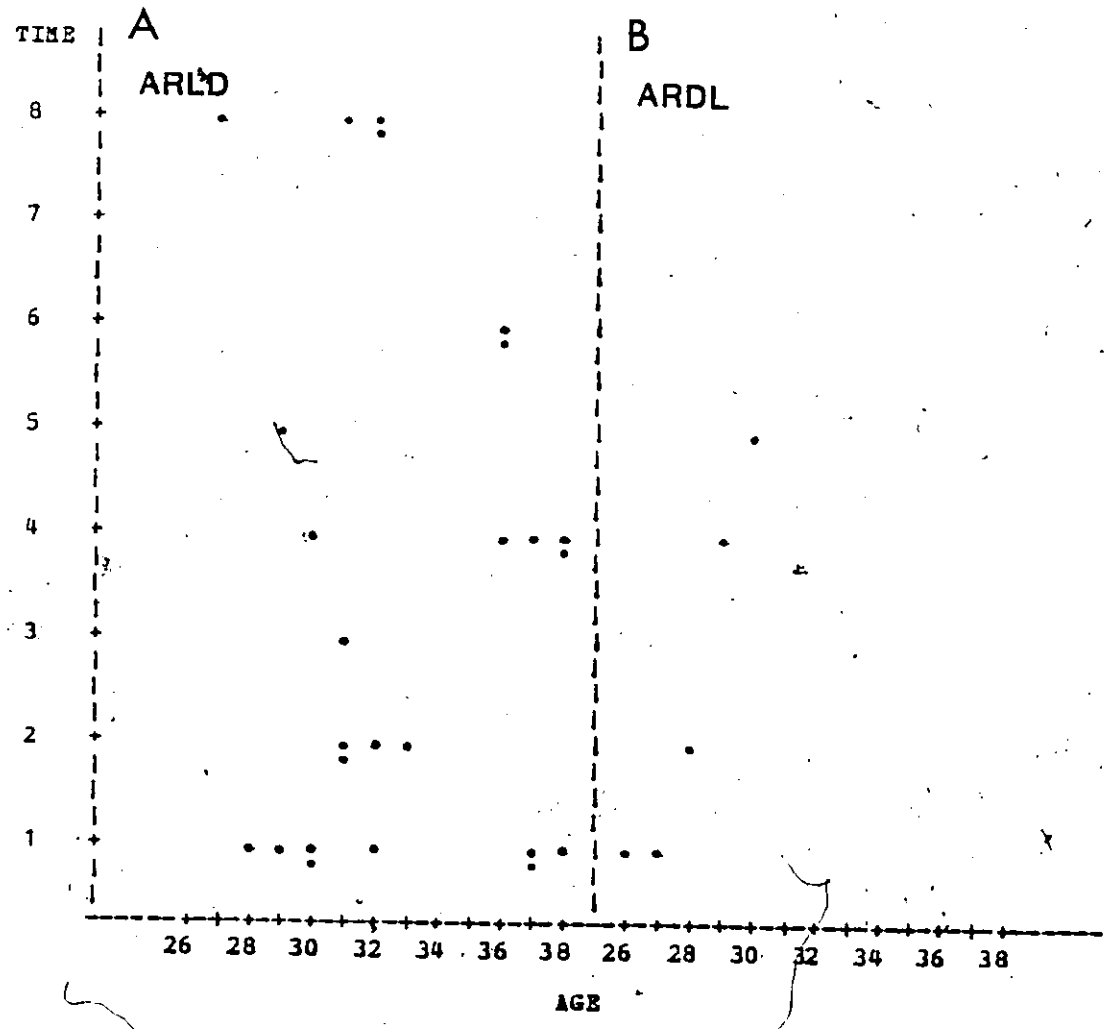

TIME
A
ARLD
B
ARDL
8
7
6
5
4
3
2
1
26
28
30
32
34
36
38
26
28
30
32
34
36
38
AGE

Figure 34

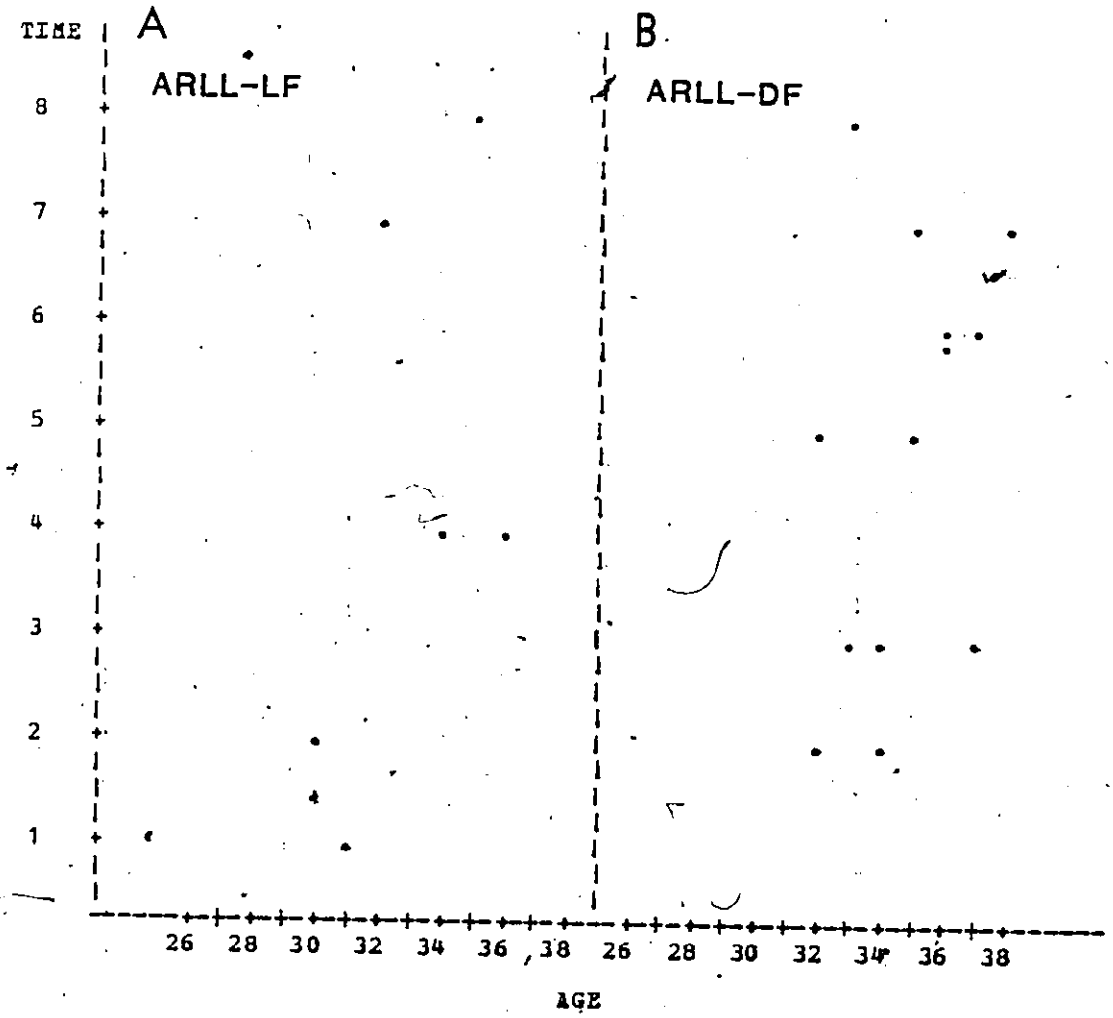

TIME
A
ARLL-LF
B
ARLL-DF
8
7
6
5
4
3
2
1
26 28 30 32 34 36 38 26 28 30 32 34 36 38
AGE

Figure 35

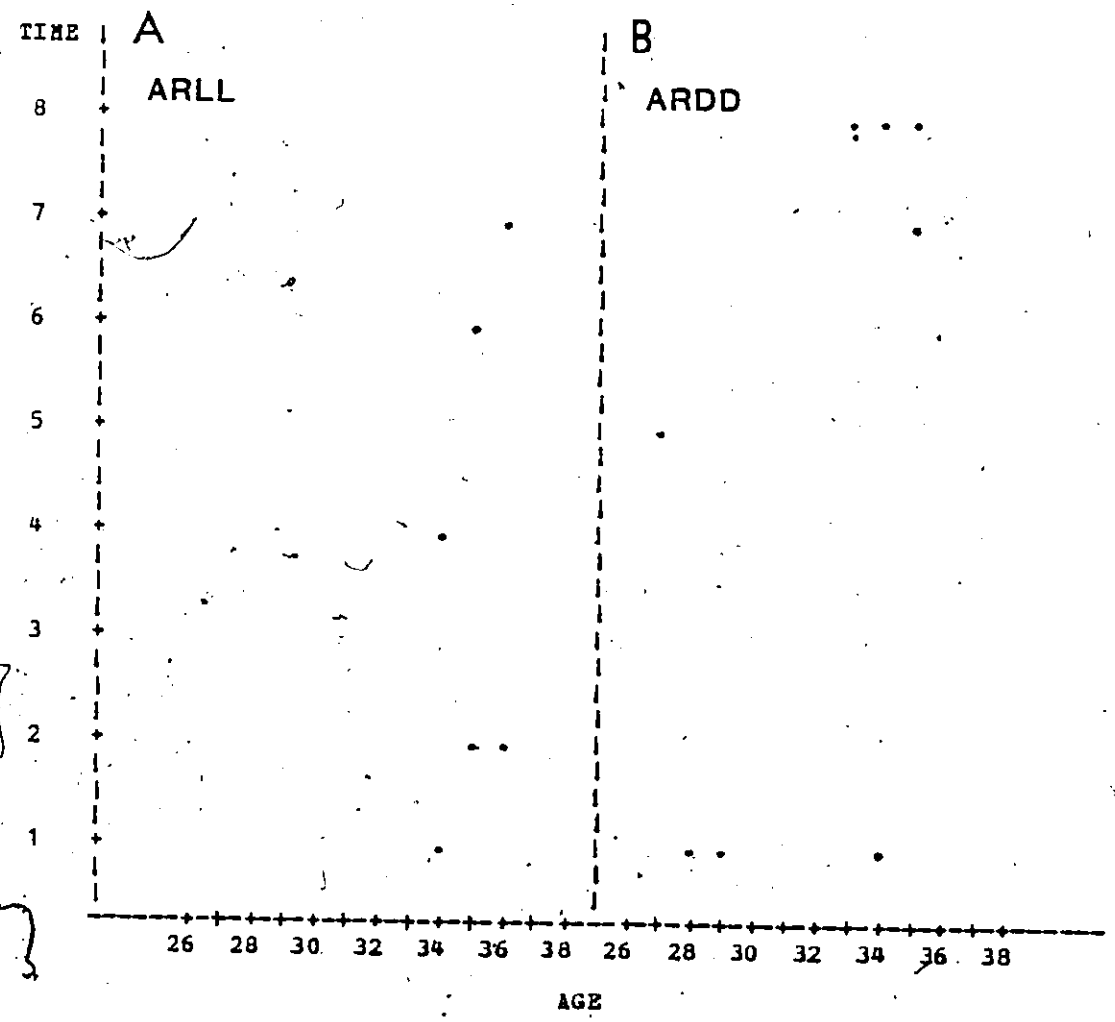

TIME
A
ARLL
B
ARDD
8
7
6
5
4
3
2
1
26 28 30 32 34 36 38 26 28 30 32 34 36 38
AGE

Figure 36

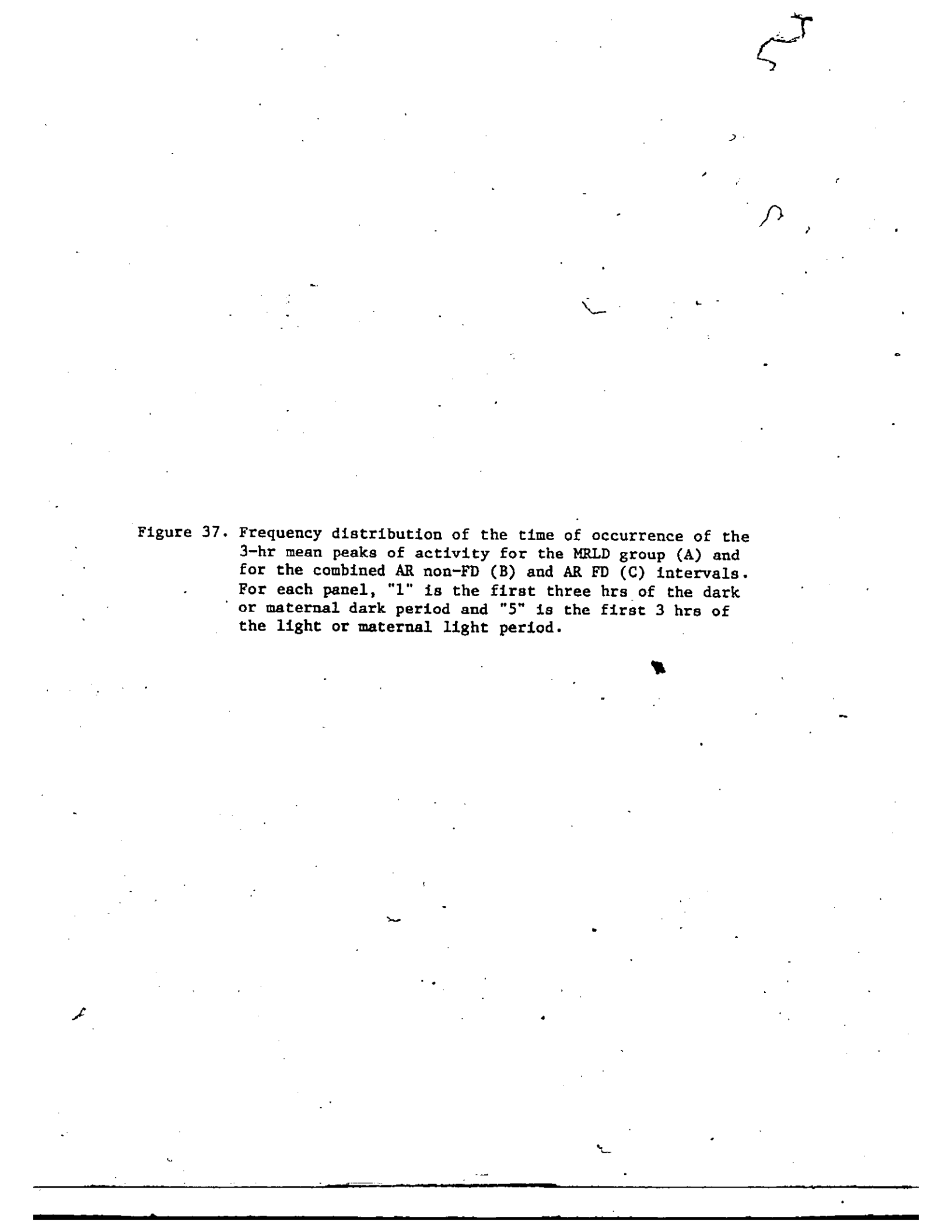
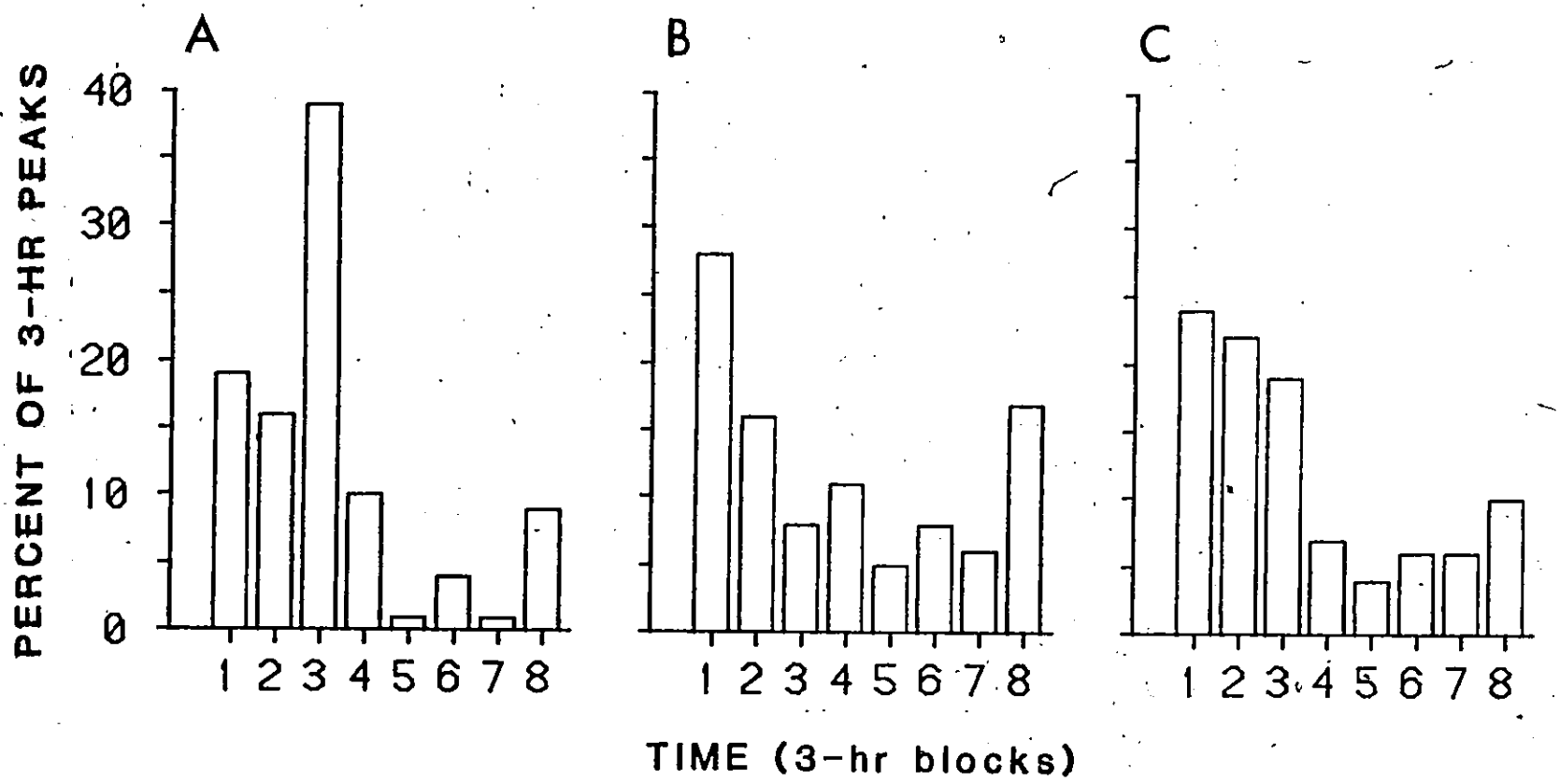


Figure 37. Frequency distribution of the time of occurrence of the 3-hr mean peaks of activity for the MRLD group (A) and for the combined AR non-FD (B) and AR FD (C) intervals. For each panel, "1" is the first three hrs of the dark or maternal dark period and "5" is the first 3 hrs of the light or maternal light period.


A
B
C
PERCENT OF 3-HR PEAKS
40
30
20
10
0
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
TIME (3-hr blocks)

second 3-hr peak of activity which was greater than or equal to the daily 24-hr mean plus 2 sem. This was also the case for 79% of the combined AR non-FD 3-hr peaks and 82% of the combined AR FD 3-hr peaks.

In the MRLD group a significantly higher frequency of 3-hr peaks occurred during the first half of the dark period (2000 to 0200 hrs) between 32 and 38 days PC as compared to 26 to 31 days PC (23 versus 7, respectively; $\underline{X^2}(1) = 8.53$, $\underline{p}<.01$). None of the AR groups showed significant age-related changes.

Daily activity. Figures 38 to 47 present the group 3-hr activity means for 27.5, 31.5, 35.5, and 38.5 days PC. The rhythmicity of the mother-reared group is relatively clear even at 31.5 days PC (Figure 38). Another striking feature of the MRLD activity is an increase with age in the amplitude of the day-night difference. This appears to be due mainly to an increase in the night activity. Group rhythmicity is less clear in the AR animals although several interesting observations can be made. The AR groups on a LD or DL cycle show troughs soon after light onset during some ages (for example, ARLD and ARLD-LF at 27.5 and 31.5 days PC, Figures 40 and 42) suggesting that these pups may be responding to the LD cues. An exception to this is the ARLD-DF group which was fed mainly during the dark period (Figure 43). Although examination of the proportion of maternal dark-period activity revealed some synchronization to the feeding schedule in the ARLL-LF and ARLL-DF groups, it is less clear in the group 3-hr plots (Figures 44 and 45). Of course, these figures present data from only 4 out of 13 days of recording.

Figure 38. Three-hr means (± sem) of activity for the MRLD group at 27.5, 31.5, 35.5, and 38.5 days PC. Values are based on the number of litters (see Table 8 for the sample sizes). The black bar represents the dark period from 2000 to 0800 hrs.

Figure 39. Three-hr means (± sem) of activity for the ARLD-Temp group. During the light period, 0800 to 2000 hrs, this group received 65.3% of its food and the environmental temperature was the highest.

Figure 40. Three-hr means (± sem) of activity for the ARLD group. The dark period was from 2000 to 0800 hrs.

Figure 41. Three-hr means (± sem) of activity for the ARDL group. The dark period was from 0800 to 2000 hrs.

Figure 42. Three-hr means (± sem) of activity for the ARLD-LF group. Two of the litters were on 12:12 DL (dark period from 0800 to 2000 hrs) during 27.5 to 35.5 days PC. The rest were on 12:12 LD (dark from 2000 to 0800 hrs). These pups received 65.3% of their food during the light period.

Figure 43. Three-hr means (± sem) of activity for the ARLD-DF group. Three litters from 27.5 and 31.5 days PC, two from 35.5 days PC, and one from 38.5 days PC were on a DL cycle (dark from 0800 to 2000 hrs). The rest were on a LD cycle. They received 65.3% of their food during the dark period.

Figure 44. Three-hr means (± sem) of activity for the ARLL-LF group. The open bar represents the time of most feeding (65.3% of the food), or the maternal light period (0800 to 2000 hrs).

Figure 45. Three-hr means (± sem) of activity for the ARLL-DF group. The open bar represents the time of most feeding (65.3% of the food), or the maternal dark period (2000 to 0800 hrs).

Figure 46. Three-hr means (± sem) of activity for the ARDD group on constant darkness. The first time point for each plot is 2000 to 23.00 hrs.

Figure 47. Three-hr means (± sem) of activity for the ARLL group on constant light. The first time point for each plot is 2000 to 23.00 hrs.

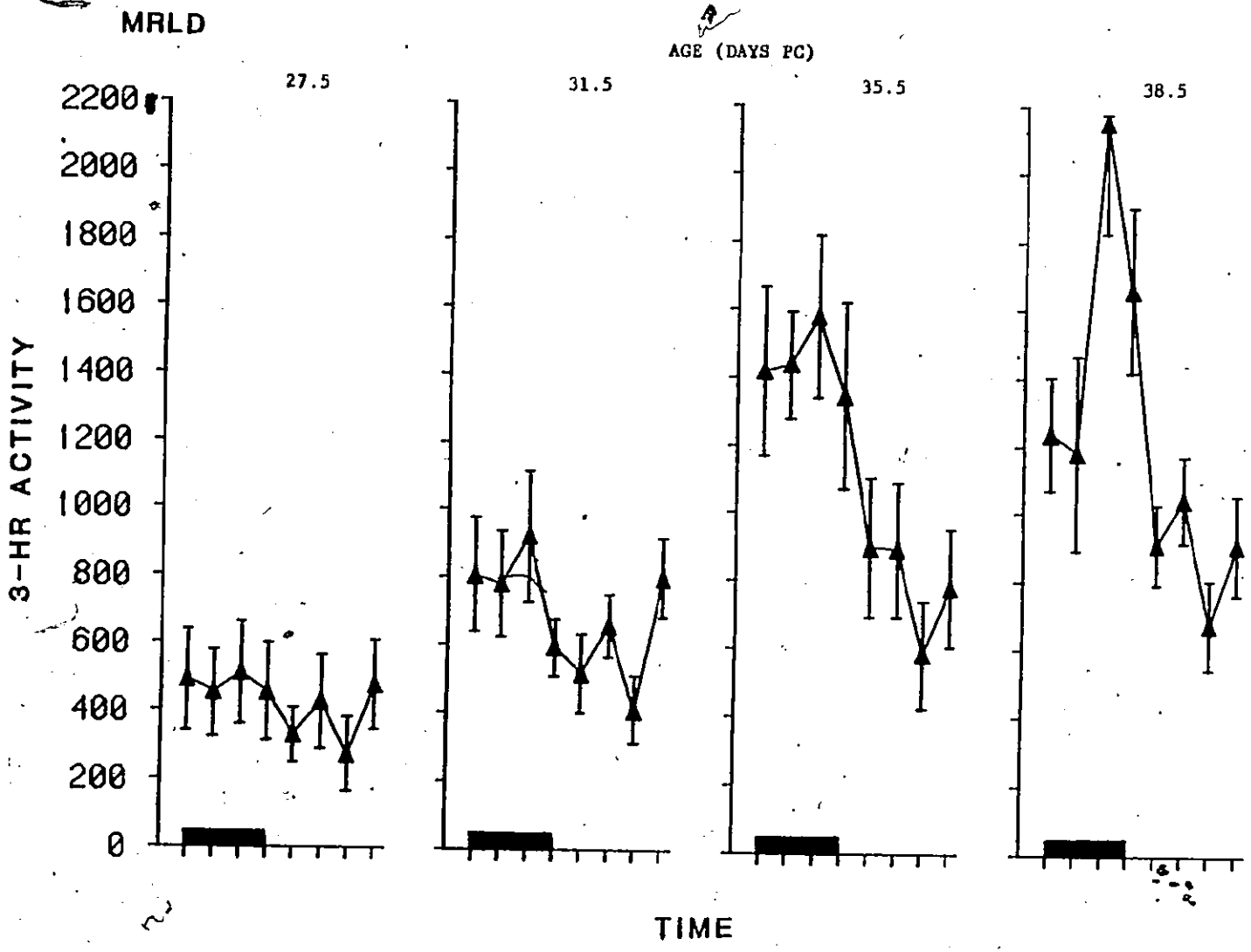

MRLD
AGE (DAYS PC)
27.5
31.5
35.5
38.5
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 38

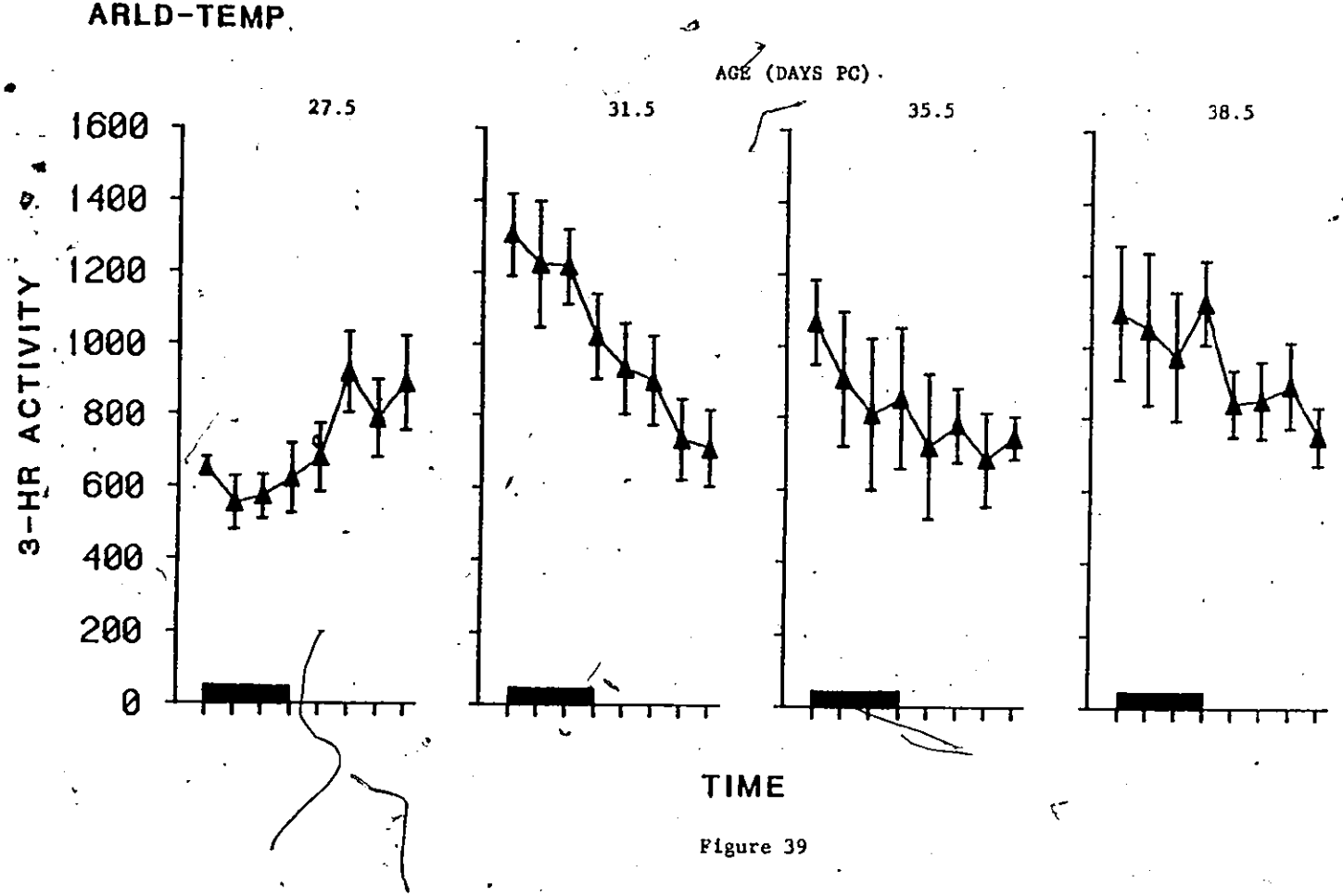

ARLD-TEMP
AGE (DAYS PC)
27.5
31.5
35.5
38.5
3-HR ACTIVITY
1600
1400
1200
1000
800
600
400
200
0
TIME

Figure 39

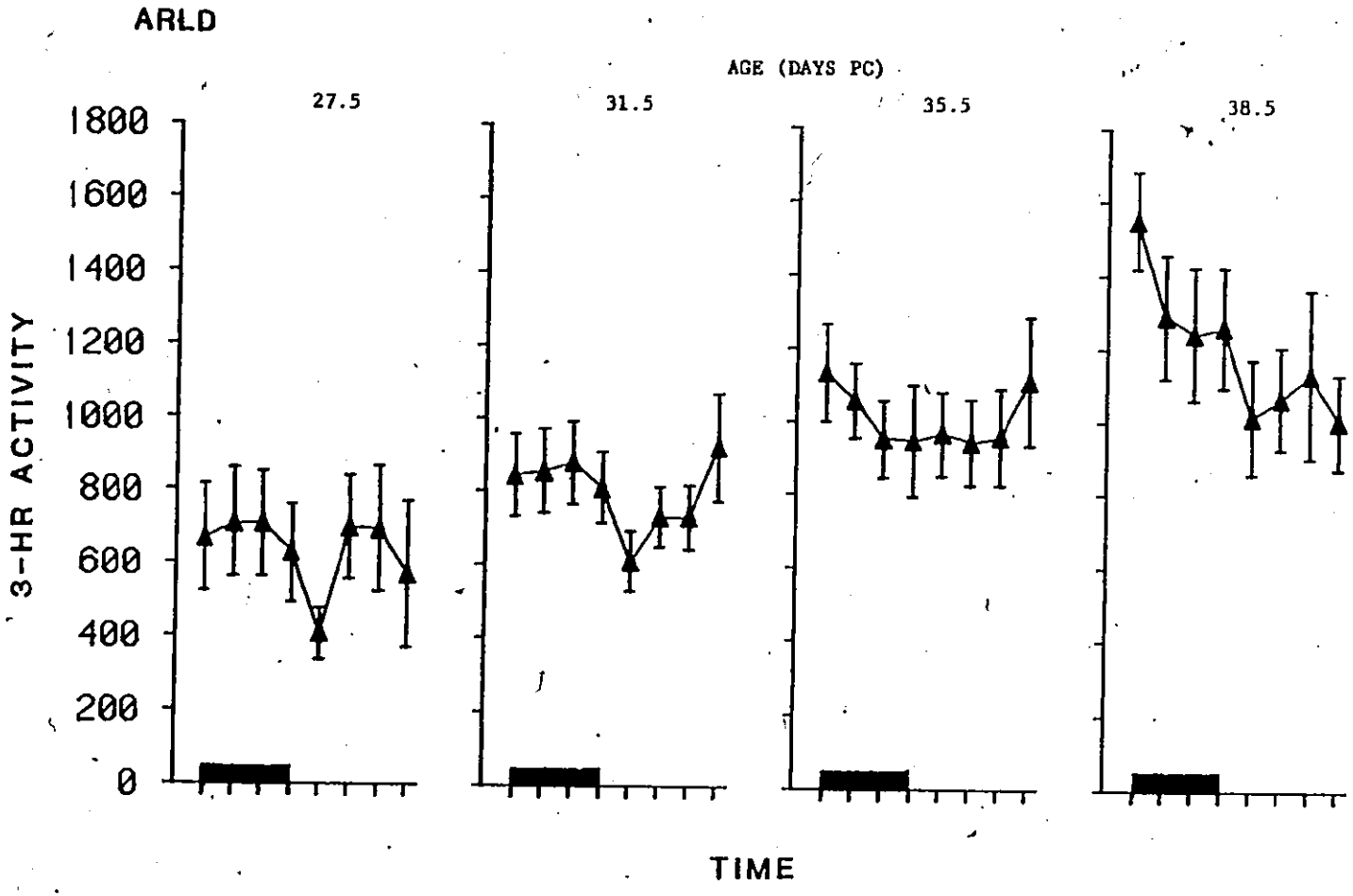

ARLD
AGE (DAYS PC)
27.5
31.5
35.5
38.5
1800
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 40

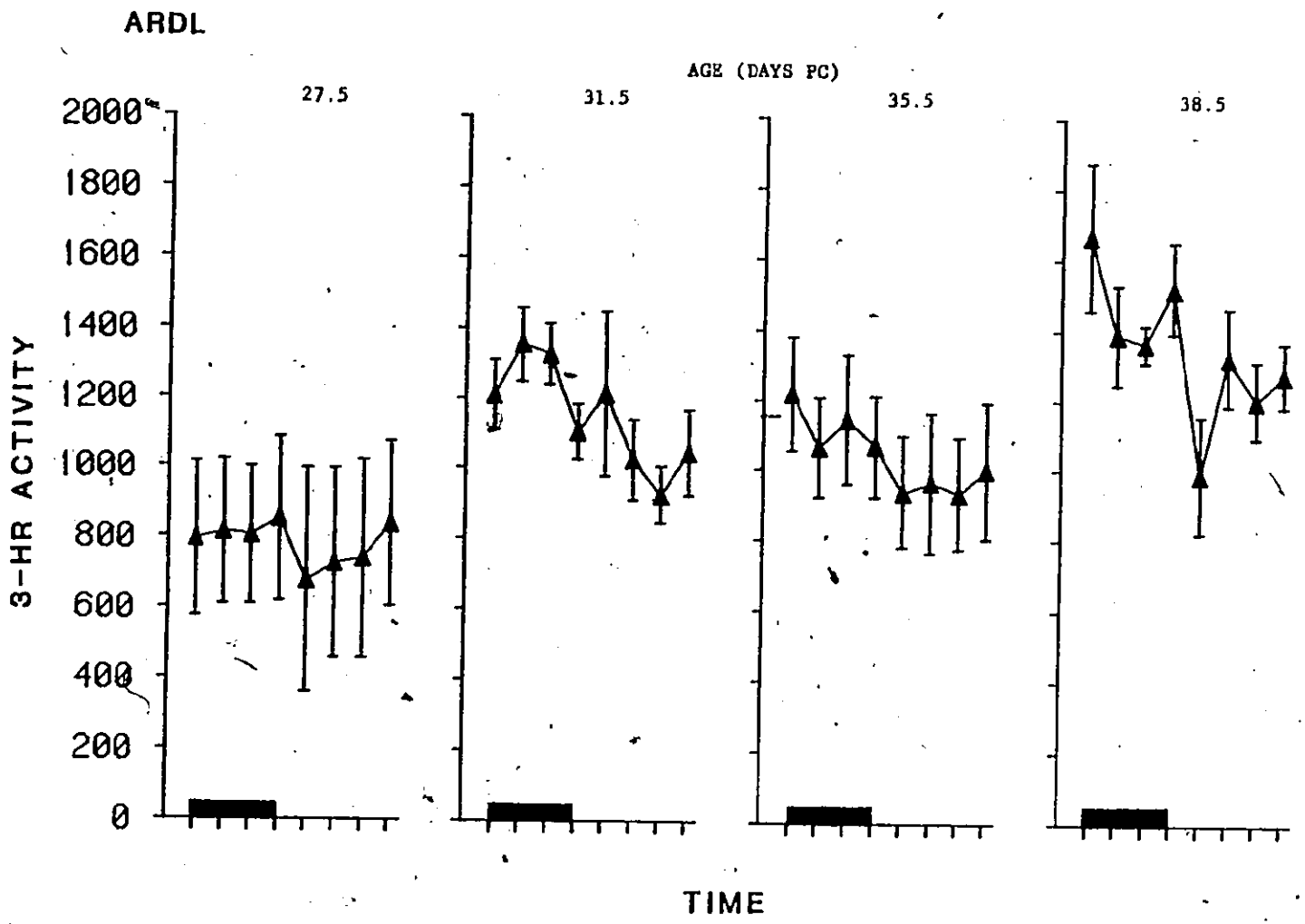

ARDL
AGE (DAYS PC)
27.5
31.5
35.5
38.5
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 41

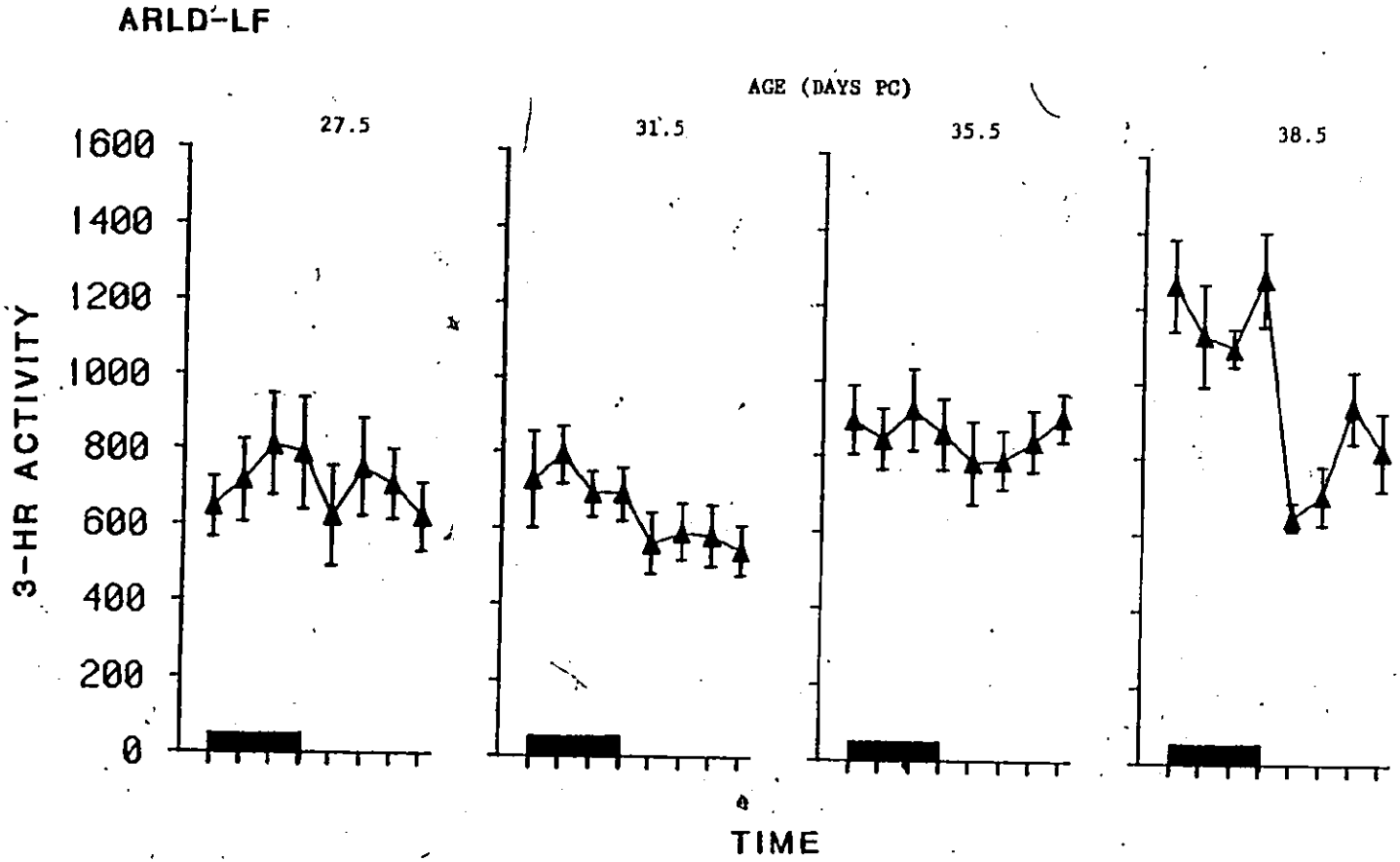

ARLD-LF
AGE (DAYS PC)
27.5
31.5
35.5
38.5
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
0
TIME

Figure 42

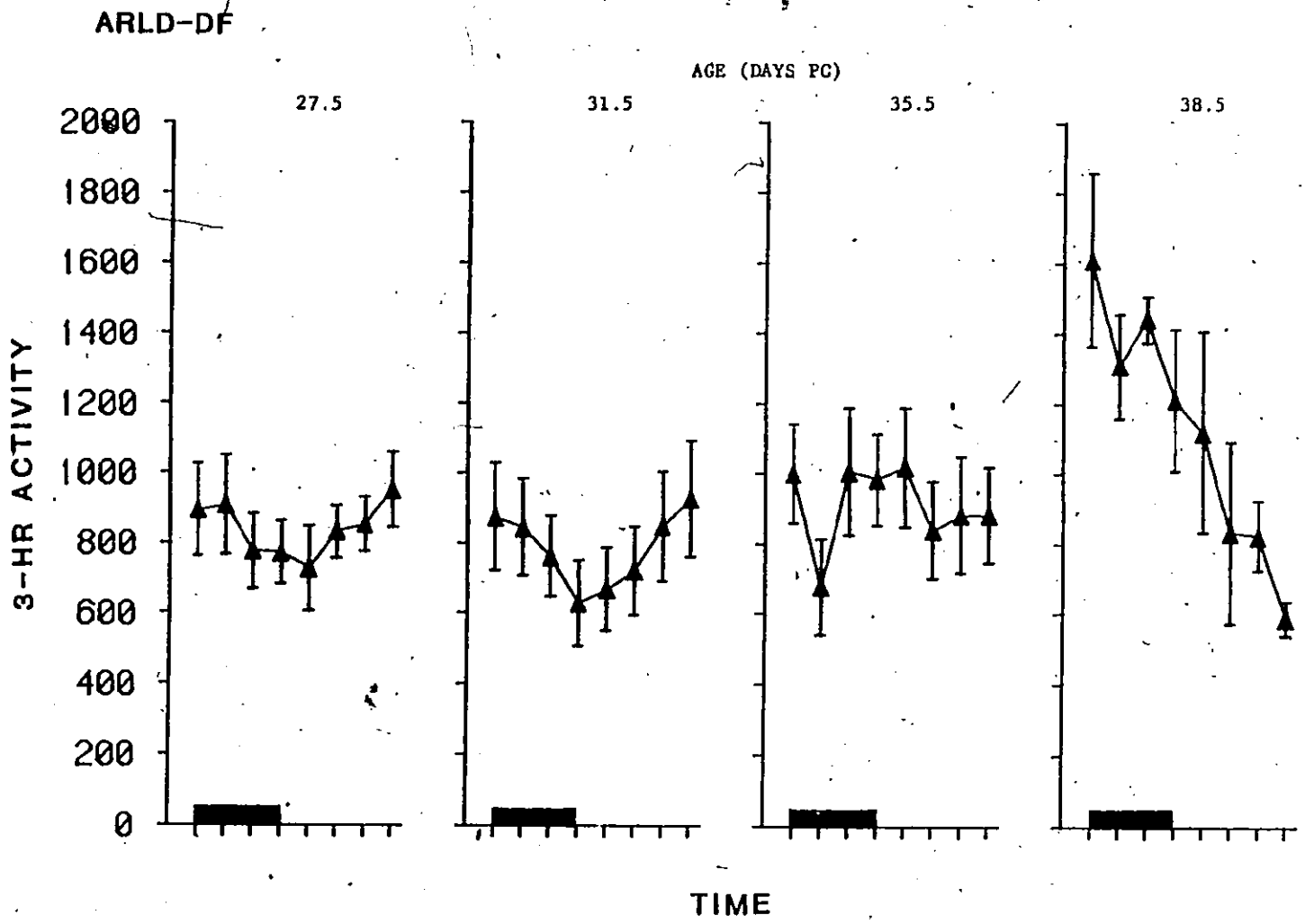

ARLD-DF
AGE (DAYS PC)
27.5
31.5
35.5
38.5
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 43

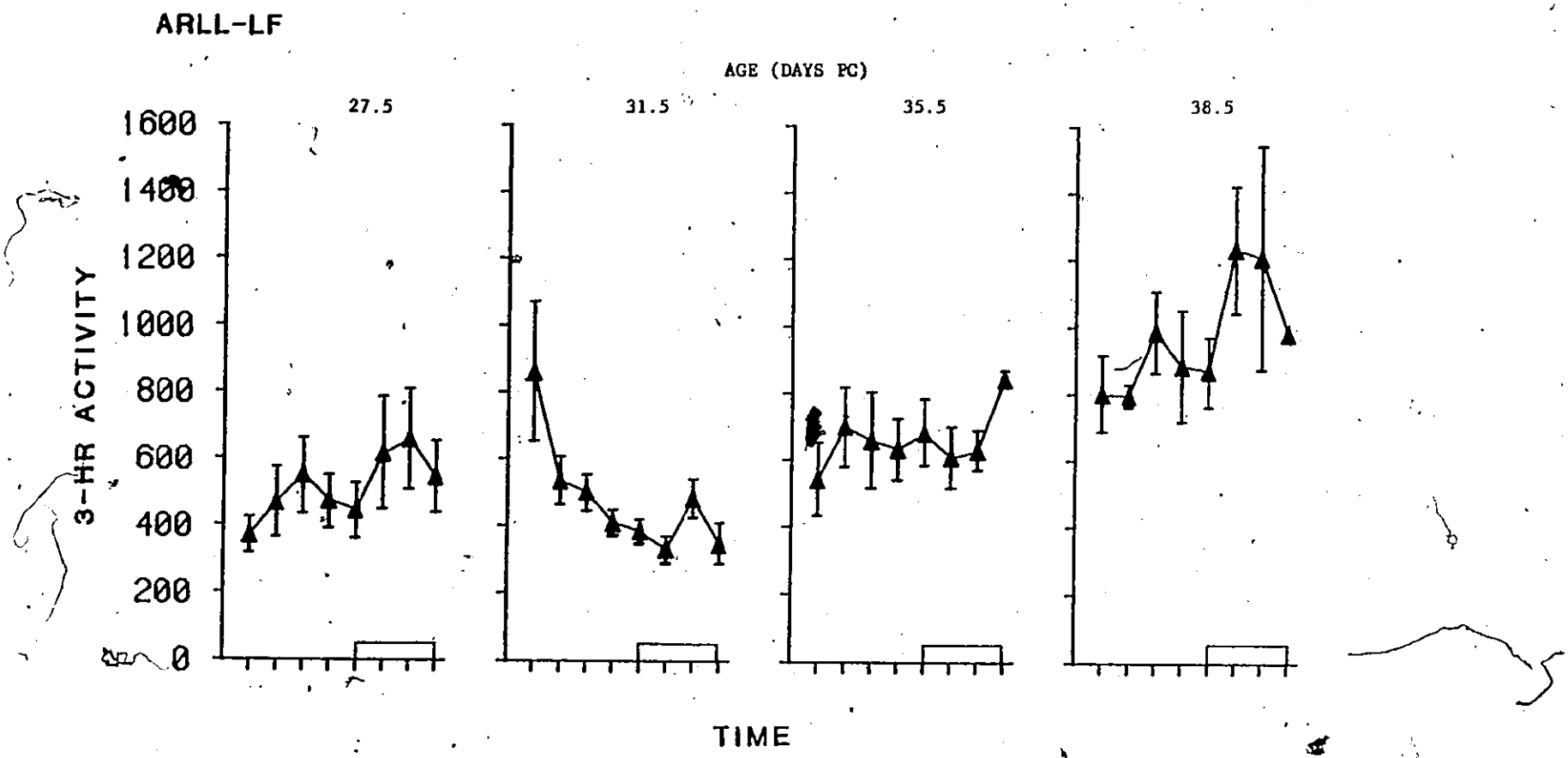

ARLL-LF
AGE (DAYS PC)
27.5
31.5
35.5
38.5
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 44

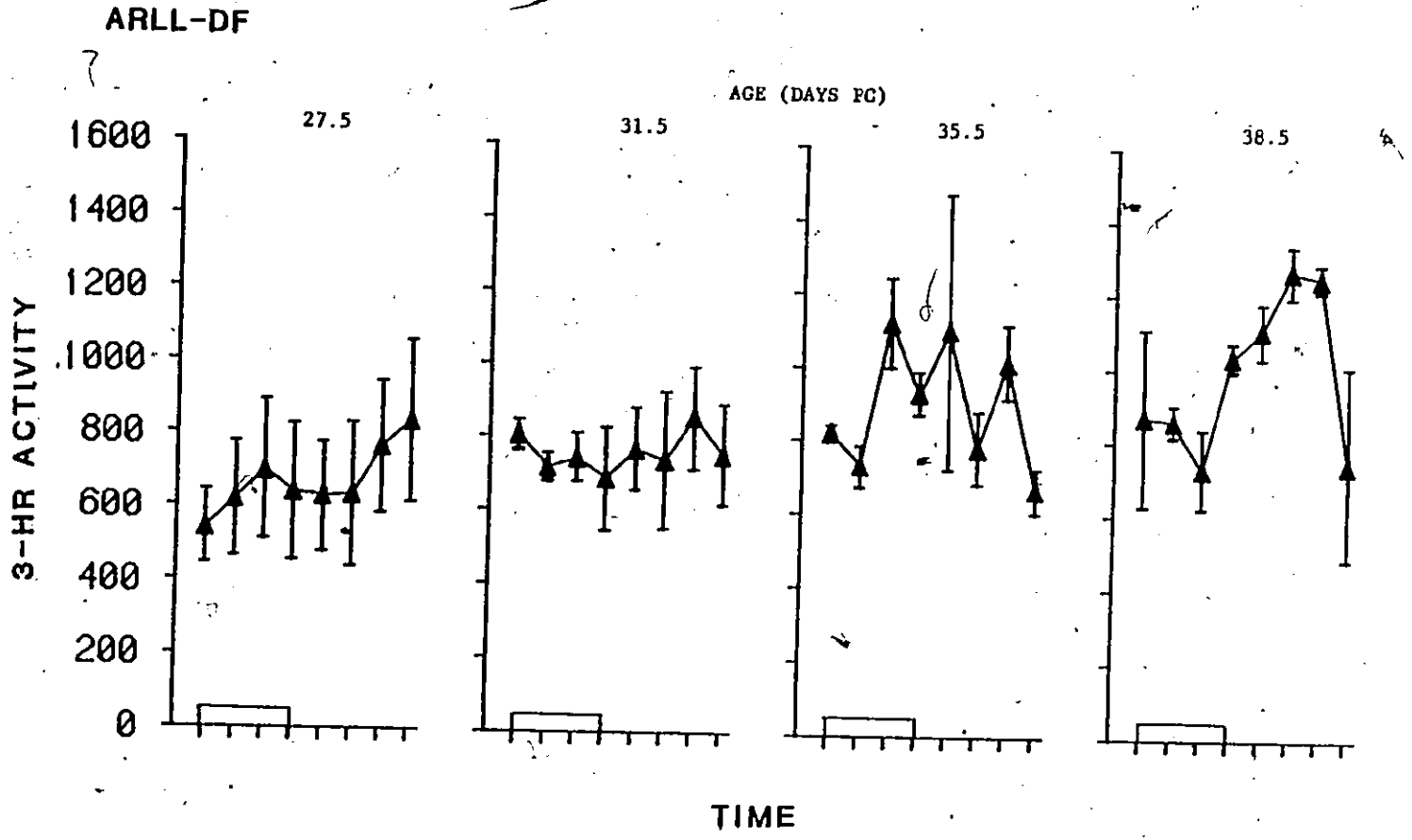

ARLL-DF
AGE (DAYS PC)
27.5
31.5
35.5
38.5
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 45

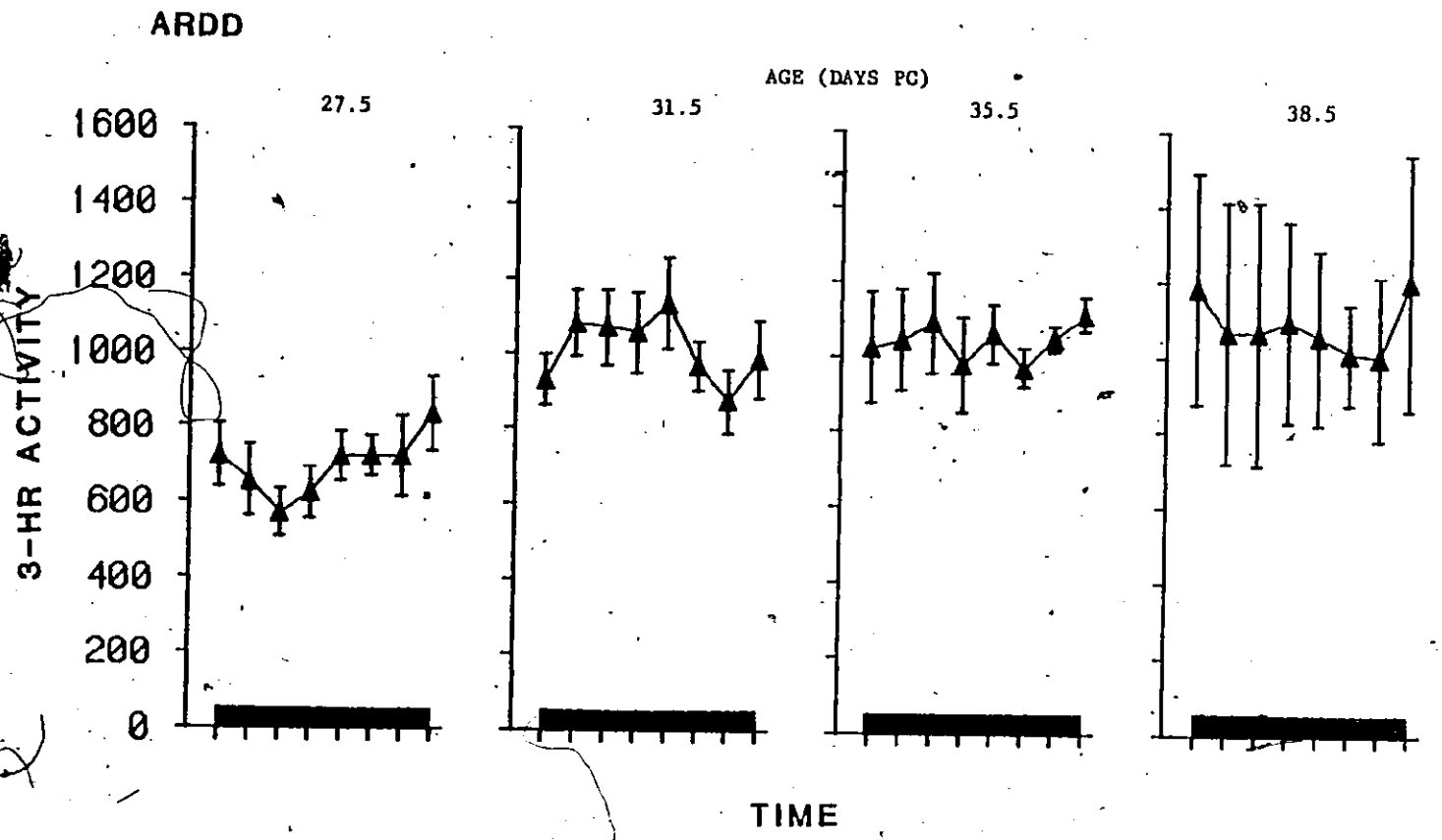

ARDD
AGE (DAYS PC)
27.5
31.5
35.5
38.5
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 46

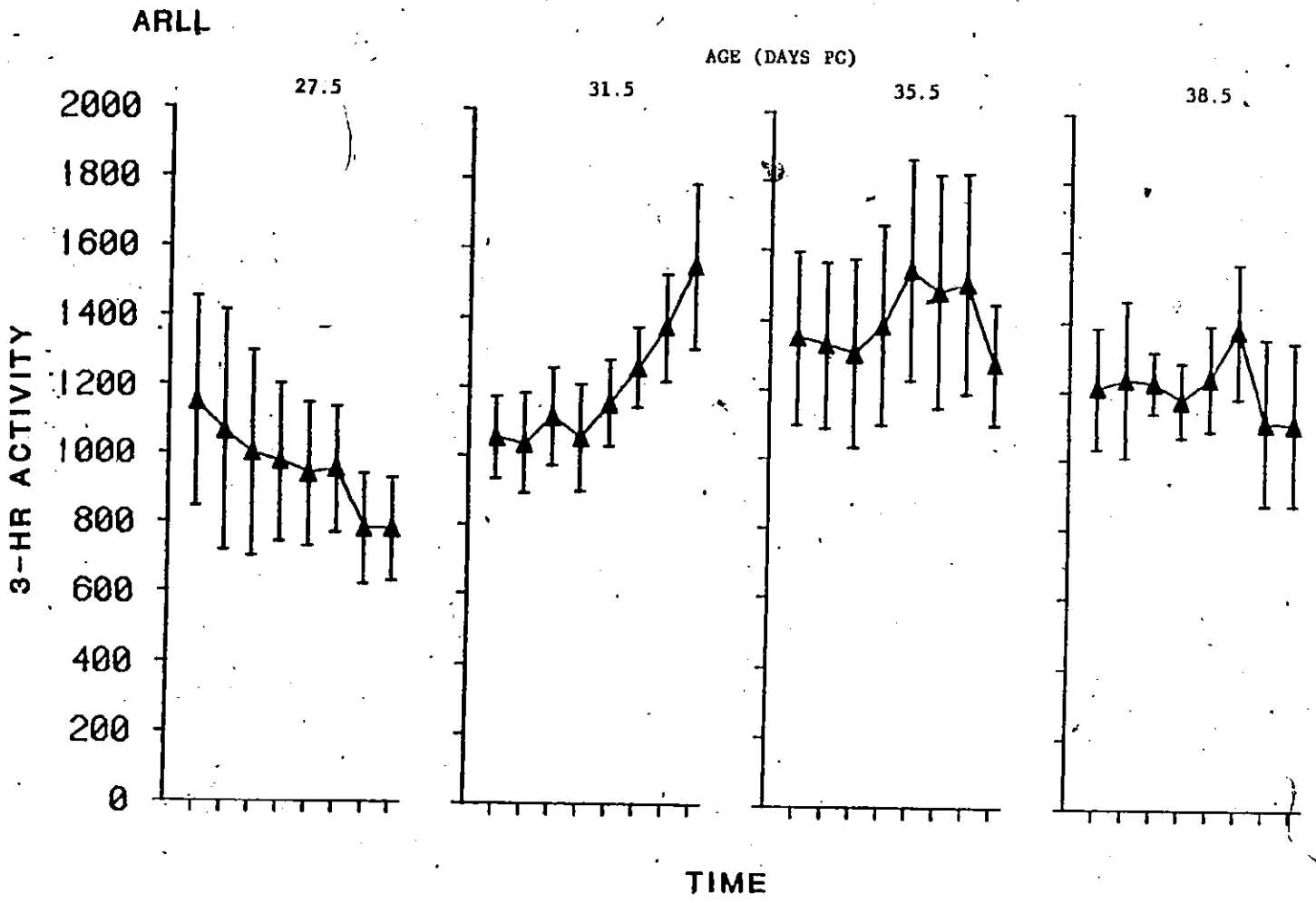

ARLL
AGE (DAYS PC)
27.5
31.5
35.5
38.5
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3-HR ACTIVITY
TIME

Figure 47

Another observation concerning the groups on a light-dark cycle was that all of them showed a greater proportion of activity during the dark period on the day of weaning, 38.5 days PC. This was not seen in the animals with only a feeding schedule, the ARLL-LF group. Examples of 3-hr activity plots over all days for individual pups in each group are presented in Appendix D.

To summarize, the peak amount of 3-hr activity occurred during the dark period for the MRLD group, all of the AR groups on a light-dark cycle (excluding the one with nocturnal feeding), and the AR group with only a diurnal feeding cycle (ARLL-LF). The AR group with only nocturnal feeding (ARLL-DF) had slightly more 3-hr peaks during the maternal light period. The AR pups on constant conditions (ARLL and ARDD) or with a LD cycle and nocturnal feeding (ARLD-DF) had peaks which tended to be more scattered throughout the day. The daily 3-hr activity means reveal clear activity rhythms in the mother-reared pups, with levels of dark-period activity increasing with age. Most of the AR groups on a LD cycle showed troughs in activity at light-onset as well as high levels of dark-period activity on the day of weaning.

Physical Growth and Activity

Data from a total of 27 AR pups with both activity and organ weight measures were used to determine if there was an association between physical development and activity rhythms. A rhythmicity score was calculated for each pup based on the following measures: 1) the number of autocorrelation blocks showing a significant peak, 2) the mean period of the autocorrelation peaks (averaged over the blocks in

1)), and 3) the mean area of the autocorrelation peaks (averaged over the blocks in 1)). Mean periods less than 23 hrs or greater than 25 hrs received a score of 1.5 and those between 23 and 25 hrs inclusive received a score of 3.0. Mean areas were scored in steps of .5; areas between 0 and .015 inclusive received a score of .5, .0151 to .030 received a 1.0 and so on up to .0751 to .090 which received a score of 3.0. The number of blocks and area measures each had a weight of 1.0 and the period measure had a weight of 1.5. For example, rat W5 from the ARLD-LF group showed significant peaks in 3 blocks, had a mean period of 23.67 hrs, and a mean area of .0131. This would give a rhythmicity score of 8.0 (or (1*3) + (1.5*3) + (1*0.5)). The scores ranged from 0 to 12 with a mean of 4.84 and a sem of .67. These scores were then regressed (Dixon, 1983, BMDP program 2R) on the body, forebrain, cerebellum, liver, spleen, and kidney weights.

The partial correlations of each organ weight with the rhythmicity score are shown in Table 14. Body, forebrain, and spleen weights contributed significantly to the variance in the rhythmicity score (multiple R^2 = .5089, $F(3,23) = 7.95$, $p < .05$). The negative partial correlation for the body weight may be related to the associations between accidental food deprivation and decreased body weight (Chapter 2) as well as higher frequencies of significant autocorrelation peaks.

Discussion

General Activity

The ontogeny of the daily activity of artificially reared rat pups obviously differs from that of mother-reared pups. Comparisons of

Table 14

Partial correlations between the organ weights and the rhythmicity scores

Organ	Partial Correlation
Body	-.7123[a]
Cerebellum	-.1809
Forebrain	.3037[a]
Liver	.0159
Spleen	.4642[a]
Kidneys	.0120

[a]These variables contributed significantly to the variance in the rhythmicity score.

absolute differences may be unreliable due to the difficulty in calibrating the activity transducers. Another possible confound is the increase in weight with age. At the heavier weights the activity transducers may be more easily triggered. However, the results from some of the AR groups generally support those of Goldenring et al. (1982). They found that AR pups were more active at younger ages than mother-reared pups but equally active at older ages. The results are at variance with those of Diaz et al. (1982a) who found lower levels of activity in AR pups tested in an open field at 18 days PN. In the Goldenring et al. (1982) and the present study most types of movement were recorded as activity. Diaz et al. (1982a) primarily investigated the number of squares entered in the open field and the number of rears. Results from a pilot study of the open field behavior of some earlier groups of AR and mother-reared pups (see Appendix E) support the possibility that the group differences may depend on the type of activity measured. The AR pups crossed fewer lines in an open field but in general spent more time pivoting than mother-reared littermates. Nutrition and social isolation factors (Diaz et al., 1982a) as well as the surgical trauma and the immediate environment of the styrofoam cups in which the AR pups are raised (Appendix E) may also contribute to an explanation of the differences in activity levels.

In addition to the levels of activity, the developmental curves describing the age-related changes in activity differed between AR and mother-reared pups. In contrast to the relatively smooth increase in activity with age seen in the MRLD group, the AR pups showed erratic or

relatively minor increases. Besides the prolonged maternal isolation other factors, including humidity, temperature, abnormal physical growth, accidental food deprivation and feeding/lighting conditions may contribute to these differences. Contrary to other studies (Campbell & Raskin, 1978), a peak of activity at 15 days PN was not observed in the intermittently isolated mother-reared pups or the AR pups. Timing and duration of the recordings, strain and sex differences as well as the types of activity measured may account for some of this discrepancy.

Activity Rhythms of the Mother-reared Pups

The early appearance of the activity rhythm in the mother-reared pups was somewhat unexpected. Although other circadian rhythms are evident during the early postnatal period, including pineal NAT activity (Deguchi, 1975a) and SCN deoxyglucose uptake (Fuchs & Moore, 1980), previous investigators have not observed circadian rhythms in locomotor activity before two (Teicher & Flaum, 1979) to three weeks PN (Bolles & Woods, 1964; Norton et al., 1975). One possible explanation for the early occurrence is that the acute (8 hrs) maternal separation (a type of stress) somehow enhanced the pups' rhythmic activity. In support of this are the results of Infurna (1981) who observed circadian rhythms in the number of squares crossed during 3 min in an open field in 7-day old rat pups. The presence of litter shavings from the home cage abolished the rhythmic activity of 15-day old pups isolated for 16 hrs from the dam (Campbell & Raskin, 1978). Other stresses may also influence rhythms; shocking and handling rat pups accelerates the appearance of day-night differences in plasma

corticosterone levels (Ader, 1969). The absence of circadian rhythmic activity before 15 days PN in the study by Teicher and Flaum (1979) is difficult to explain. Among other factors it is possible that the movement transducers and/or the time series analysis used in the present study were more sensitive detectors of rhythmicity.

In addition to rhythmicity, the activity peaked during the dark period and was relatively low during the light period indicating that the pups were detecting dark-light differences even before the age of eye opening (14 to 15 days PN). Increases with age in the amplitude of the day-night differences - due largely to increases in the level of dark-period activity - were also observed. Similar amplitude changes are seen in pineal NAT (Ellison et al., 1972) and plasma corticosterone levels (Ramaley, 1978a). Dark period peaks are also seen before eye opening in the open field activity (Infurna, 1981) and pineal NAT activity (Deguchi, 1975a) of rat pups. Similarly, pronounced dark-light differences occur in the latency of 5-day old pups to suckle an anesthetized dam (Henning & Gisel, 1980). How are the dark-light transitions detected by the rat pups? Several researchers have postulated the existence of extraretinal photoreception, primarily via the pineal gland (Henning & Gisel, 1980; Zweig, Snyder, & Axelrod, 1966). At present there is little support for this hypothesis from studies on prenatal (Reppert & Schwartz, 1983) and postnatal (Hiroshige et al., 1982b) synchronization of circadian rhythms. There is evidence suggesting that 6-day old rat pups can detect dark-light differences

through the eyelids (Routtenberg, Strop & Jerdan, 1978). In 32 hours of testing in the MRLD group (2200 to 0600 hrs), two pups were recorded from only during dark conditions (2200 to 0600 hrs), one during a dark-to-light transition (0600 to 1400 hrs), and one during a light-to-dark transition (1400 to 2200 hrs). The increases or decreases in activity following the transitions may have occurred via input through their closed eyelids.

Another possible explanation for the dark-light differences in the MRLD group is synchronization by maternal factors. Prenatal and/or postnatal synchronization to maternal rhythms occur in the plasma corticosterone (Hiroshige et al., 1982c; Takahashi et al., 1982), pineal NAT (Takahashi & Deguchi, 1983), SCN deoxyglucose uptake (Reppert & Schwartz, 1983), locomotor activity (Takahashi et al., 1984), and feeding rhythms (Levin & Stern, 1975). Specific maternal factors responsible for the synchronization have not been identified. Changes in the nursing regimen produce shifts in the offspring's plasma corticosterone peaks (Hiroshige et al., 1982a; Miyabo et al., 1980), implicating the nursing rhythm as a possible synchronizer.

The average period for all the significant autocorrelation peaks in the MRLD group was 24.09 hrs. The freerunning plasma corticosterone rhythm of blinded rat pups also has an average period slightly greater than 24 hrs (24.5 hrs, Hiroshige et al., 1982b; 24.2 to 24.4 hrs, Takahashi et al., 1982) as does the locomotor activity rhythm at 5 weeks PN (24.4 hrs, Takahashi et al.,1984). The mode frequency of 3-hr

peaks (summed over all ages) for the MRLD pups occurred between 0200 to 0500 hrs or 6 to 9 hrs after dark onset. This is later than the peak activity observed by Infurna (1981) in 7-day old pups which occurred 3.5 hrs after dark onset. It should be noted, however, that most of the MRLD animals had secondary 3-hr peaks of activity which were sometimes close in value to the primary peak. There was also evidence for age-related shifts in the time of occurrence of the peak 3-hr mean activity in the MRLD pups. For all pups, an increase in the frequency of 3-hr peaks during the first half of the dark period occurred during 32 to 38 days PC. This may indicate a shift in the timing of 3-hr peak activity for some of the rat pups. Shifts in acrophases with age also occur in hamster pineal melatonin (Rollag & Stetson, 1981) and human body temperature rhythms (Abe et al., 1978).

Activity Rhythms in Artificially Reared Pups

Aside from the studies on sleep-state organization in isolated infant rabbits (DeSantis et al., 1977) and AR pups (Juvancz, 1981), the present experiments were the first to examine the effects of early and prolonged postnatal maternal isolation on rhythm development. On the whole, relative to the MRLD pups, fewer AR pups exhibited significant circadian rhythms. When rhythms occurred they usually appeared later and were attenuated and transient. A couple of cautionary statements regarding the interpretation of the AR animals' autocorrelation plots are needed. First, it could be argued that the appearance and disappearance of significant autocorrelation peaks should not be

interpreted as rhythmicity. In the present context, rhythms were defined in a lenient manner so as to encompass cyclic changes in activity which may only last for two to three days. Using these criteria, some of the plots of 3-hr mean activity for individual AR animals indicate the presence of rhythmicity (see Appendix D).

Second, the finding of higher variability in the mean period of the autocorrelation peaks in AR pups relative to the MRLD pups may be misleading. Some of the significant autocorrelation peaks which deviate greatly from 24 hrs (i.e., less than 23 hrs or greater than 25 hrs) may be spurious due to the large number of time lags analyzed. On the other hand, some investigators such as Halberg and Lee (1974), define circadian rhythms as rhythms having periods of 24 hrs ± 4 hrs and this criterion was used in the present studies. This scatter in periods may, therefore, reflect the lack of adequate synchronizing cues in the AR environment. Despite the variability, the overall mean period for the AR pups was relatively close to that of the MRLD pups although it was slightly less than 24 hrs (23.97 hrs versus 24.09 hrs, respectively).

The results from the combined AR groups indicate a disruption of circadian rhythm development due to some aspect(s) of the AR environment. However, AR pups did respond differentially to the various lighting, feeding and temperature conditions. The remainder of the discussion will address the issue of the effectiveness of these conditions as early rhythm synchronizers and their roles in the more

natural situation. In addition, other aspects of the AR environment which may influence rhythm development will be discussed.

Light Cycles

Artificially reared animals on 12:12 LD or DL cycles do show evidence of rhythmic activity. The significant non-FD peaks detected in the autocorrelation analysis had average periods close to the MRLD pups (ARLD - 24.17 hrs, ARDL - 24.05 hrs). Furthermore, for the 3-hr mean activity, troughs were noted at the dark-to-light transitions on some days and, at 38.5 days PC, activity was clearly the highest during the dark. Postnatal maternal rhythms were suggested as possible synchronizers for the MRLD group. They may also play a role in the ARLD and ARDL pups showing rhythms before eye opening since the pups were with their dams for 2 to 4 days before surgery. Prenatal maternal synchronization is another possibility, as indicated in studies on the plasma corticosterone rhythm of blinded rat pups (Honma et al., 1984 a, b) and the locomotor activity rhythms of hamsters (Davis & Gorski, 1982, 1983).

Relative to the ARLD pups fewer ARDL pups showed significant rhythms. The reasons for this are not obvious. Yamazaki and Takahashi (1983) observed a delay in the development of the plasma corticosterone rhythm of blinded pups raised by DL-entrained dams. It is possible that in their study the nursing patterns of those particular dams were not rhythmic enough to entrain the offspring's rhythms (Takahashi, Note 5).

In contrast to the animals on a LD cycle, the animals under constant conditions exhibited fewer rhythms which also tended to be attenuated. The mean period of the non-FD autocorrelation peaks in the ARLL group deviated the most of any AR group from 24 hrs, 21.75 hrs. Unfortunately, this value was based on only two peaks. In comparison, the ARDD mean period was 24.67 hrs (three peaks), closer to values for the ARLD and ARDL pups. Circadian plasma corticosterone rhythms are not observed in rat pups raised from birth in LL (Krieger, 1973; Ramaley, 1975) although rhythms occur under DD conditions (Itoh et al., 1980; Takahashi et al., 1979). However, other circadian rhythms appear under LL conditions including the pineal NAT rhythm (Deguchi, 1975a) and sleep-wake rhythms (Astic et al., 1976; Hagino et al., 1979; Ibuka, 1984). The high levels of 24-hr activity in the ARLL group which may be related to low relative humidity conditions might be an important factor.

Feeding Cycles

Although the presence of a light-dark cycle was more conducive to rhythm development than constant conditions, the ARLD and ARDL pups did not approach the level of rhythmicity observed in the MRLD group. The use of a feeding cycle which approximated the largely diurnal nursing seen in mother-reared pups (Croskerry, 1976; Hughes et al., 1978), either alone (ARLL-LF) or in conjunction with a LD cycle (ARLD-LF), also did not increase the frequency of animals showing significant circadian rhythms. The mean periods for the two ARLD-LF

pups were 23.5 and 26.21 hrs. The mean periods for the ARLL-LF pup with significant rhythms deviated more from 24 hrs, 22.07 and 27.25 hrs. The autocorrelation peaks for the ARLL-LF pup were also of a much smaller amplitude than those for the ARLD-LF pups.

Despite the low frequency of animals showing significant circadian rhythms, the predominantly diurnal feeding cycle did influence the proportion of dark-period, or maternal dark-period, activity. Pups tend to be more quiescent during feeding (personal observations). Therefore, during longer intervals of non-feeding, activity levels might be expected to increase. Lincoln, Hill, & Wakerly (1973) also reported decreased levels of movement within a few seconds after the initiation of suckling in infant rats. Animals on 12:12 LD and a predominantly diurnal feeding cycle (ARLD-LF group) tended to show higher average proportions of dark-period activity than AR groups on only a LD cycle (ARLD or ARDL) or feeding cycle (ARLL-LF). This trend was not significant, largely due to the decreased dark-period activity seen in the ARLD-Temp group which also had the diurnal feeding cycle. The mean deviation from 50% for the ARLD-LF group was 5.79%; in combination with the ARLD-Temp group this mean deviation dropped to 3.58%. In addition, a feeding cycle alone (under conditions of LL) was, on the average, as effective as the LD cycle alone in synchronizing the pups' activity. Also, between 28 and 34 days PC the maternal dark-period activity of the ARLL-LF pups was as high as that seen in the ARLD-LF group.

The preceding results provide additional support for the hypothesis that maternal nursing rhythms may serve as synchronizers for offspring rhythms during the early postnatal period (Hofer & Shair, 1982; Takahashi & Deguchi, 1983). Unlike the pups with both a LD and diurnal feeding cycle, however, pups with only a feeding cycle (ARLL-LF group) did not show heightened maternal dark-period activity on the day of weaning. One possibility is that as the pups get older the feeding cycle becomes a less effective synchronizer in the absence of other cyclic cues. Relevant to this is the observation of Levin and Stern (1975) that blind rat pups reared by sighted dams "lose" synchronization to their sighted dam as they get older.

Additional support for the role of nursing rhythms in the postnatal synchronization of offspring rhythms comes from the data on the pups with the predominantly nocturnal feeding cycle. The range of the periods of the autocorrelation peaks in the ARLL-DF group was 22.0 to 25.25 hrs. Only one animal had a peak with a period relatively close to 24 hrs, 23.5 hrs, and this occurred between 35 and 37 days PC. These results may indicate that the lack of a LD cycle produces greater variability in the average period. Unfortunately, only two autocorrelation peaks were significant in the ARLD-DF group (the periods were 27.25 and 24.0 hrs) so a comparison is almost meaningless.

Nocturnal feeding did influence the distribution of day-night activity, resulting in a decrease in the proportion of dark-period activity. One of the more interesting results was that animals with a

LD cycle showed less synchronization to the predominantly nocturnal feeding cycle than the pups on LL. An attractive hypothesis is that since mother-reared pups nurse mainly during the light period, the nocturnal feeding in the presence of a LD cycle produces a discordant situation. If this is the case, then the issue of why the discordance arises must be addressed. The ARLD-DF pups were with their LD-entrained dams for at least 3 days before the maternal separation. It is possible that in these 3 days the pups became synchronized to the predominantly diurnal nursing rhythms. When confronted with a nocturnal feeding schedule under a LD cycle the pups could not shift their activity to coincide with the new feeding cycle. Another possibility is that this pre-separation synchronization occurred in part during the prenatal period. Under LL a similar situation may exist but the lack of a LD cycle may attenuate the discordance. Partial support for this explanation comes from studies employing the restricted nursing paradigm. Pups are restricted to either dark or light-period nursing by being maternally separated during either the light or dark period, respectively. Hiroshige et al. (1982c) reported that at 4 weeks of age blind pups on dark-restricted nursing had plasma corticosterone peaks which were more scattered and sometimes bimodal when compared to pups on light-restricted nursing. Miyabo et al. (1980) also observed bimodal peaks in some of their dark-restricted groups. However, Hiroshige et al. (1982c) took this not as evidence for a conflict with the normal nursing situation but as an indication

of the merely modifying role of postnatal maternal rhythms on offspring rhythms. What is clearly needed is an analysis of the changes produced in mother-offspring interactions in the restricted nursing procedure before definite conclusions are made. There is evidence for a complimentary role of feeding and LD cycles in the establishment of the circadian rhythm in jejunal sucrase activity. A sucrase rhythm appeared in 20-day old pups weaned from the dam if the onset of feeding was coordinated with the onset of darkness (Henning & Guerin, 1983). Pups fed mainly during the light showed no rhythm in sucrase activity.

Earlier it was suggested that the synchronizing effectiveness of a feeding cycle alone diminished as the pups became older (ARLL-LF group). This was not supported by the activity data on the day of weaning in the ARLL-DF group. They still showed high levels of activity during the time of least feeding. Unfortunately, the sample size for this day was small. Surprisingly, the ARLD-DF pups showed high dark-period activity on the day of weaning. It was not as distinct as the nocturnal activity seen in the ARLD-LF group at the same age and the sample size was small, but it may indicate that near weaning the LD cycle is a more potent synchronizer than the feeding cycle.

Temperature Cycles

The AR group with a LD, feeding, and temperature cycle had the highest proportion of pups showing significant rhythms. In contrast to the MRLD and ARLD-LF groups, the mean non-FD period for the ARLD-Temp pups was slightly below 24 hrs, 23.43 hrs. Except for the ARLD-DF

group, the pups with a temperature cycle exhibited dark-period activity which overall deviated the least from 50% of the total daily activity. The low levels of dark-period activity seen between 26 and 28 days PC may be partly due to accidental food deprivation which occurred during the light period.

The temperature cycle was designed with the assumption that since the dam proportionately spends the least amount of time on the nest during the dark period, the overall night temperature would be lower than the day temperature. Increases in activity in response to a relatively cool environment (22 degrees C) do occur in 10-day old pups (Wishaw, Schallert, & Kolb, 1979). The increases are also seen in 1- and 5-day old pups but only during the initial part of a 15 min test period; thereafter the pups become immobile (see also Hofer, 1973a, 1975b). The decline in activity is positively correlated with a decline in the pup's body temperature (Wishaw et al., 1979). It is possible that the night temperatures inside the water bath incubators were cool enough to decrease the pups' activity. Thus, despite the dark cues and the decreased feeding during this time, the overall proportion of dark-period activity would be depressed. During 36 to 38 days PC, a time when some of the thermoregulatory mechanisms in rat pups are almost fully developed (Gulick, 1937; Hahn et al., 1956), the ARLD-Temp pups did exhibit predominantly night activity although it was still at a level lower than that seen in pups without a temperature cycle.

Food Deprivation

The finding that accidental food deprivation (FD) intervals were associated with significant rhythms and increased activity was fortuitous and was one reason behind the studies on the cyclic feeding schedules. The mean periods of the FD autocorrelation peaks were relatively close to 24 hrs, even in the ARLL group. Most of the rat pups showing significant FD autocorrelation peaks experienced only one FD interval in a 3-day block. Since two successive days of rhythmic activity were necessary to detect significant rhythmicity it is possible that the FD interval enhanced rhythmicity that was already present or it induced rhythmicity which continued into the following 24 hrs. Earlier it was suggested that the acute maternal separation experienced by the MRLD pups enhanced their rhythmic activity. The acute FD intervals experienced by the AR pups may be analogous to the MRLD pups' short-term maternal separation. A few pups had two or more successive days with FD intervals - these intervals may have served as synchronizers for the activity rhythm. However, the effects of accidental food deprivation may differ in quality among the different AR groups. It may also suppress the occurrence of rhythms in certain circumstances. The mean number of FD intervals for animals showing no significant rhythms was comparable to that for animals showing rhythms only during FD intervals (4.4 and 4.5, respectively).

Other Considerations

None of the AR groups approached the prominent circadian rhythmicity seen in the MRLD pups. Previously it was suggested that in

the absence of maternal factors the MRLD pups' rhythmicity may be "disinhibited" even under noncyclic temperature conditions and food deprivation. It is also possible that feeding and temperature cycles are relatively unimportant in synchronizing the pups' rhythms under normal conditions. This seems unlikely - optimal feeding and temperature conditions are important determinants of survival and growth, particularly during early development.

Given the evidence from the present studies and other investigations, feeding/nursing rhythms may serve as synchronizers during the early postnatal period. Substances transmitted via the mother's milk, suckling experiences (Shair, Brake, & Hofer, 1984), as well as temporal parameters may be important factors in determining the effectiveness of feeding cycles. The environmental temperature cycle used in the ARLD-Temp group was not ideal for synchronizing rhythms although it did have an effect on activity. A cycle which more closely mimicked the natural situation (i.e., ultradian, as well as circadian, fluctuations) might be more successful. In addition, the environmental temperatures used in the other groups may have been inappropriate.

Along with feeding/nursing, temperature variables, and light cycles, olfactory (Hofer, 1975a) and tactile aspects of mother-offspring interactions may play significant roles. The finding of different modes of peak 3-hr activity for the AR and MRLD pups suggests that the MRLD pups may be responding to cues that were not present in the AR environment. Other cues may also be operating in the

AR environment since much of the variance in the proportion of dark-period activity was still not accounted for by linear relationships with the feeding cycles and accidental food deprivation. One possibility is humidity - low levels were associated with increases in daily activity although this was confounded by the light conditions (ARLL group). In addition to mother-offspring interactions, factors such as surgical trauma and the health of the pups should be considered. The AR pups showed abnormal growth, including smaller brains and heavier livers at weaning (Chapter 2). Although preliminary, there was evidence suggesting that measures indicative of health, such as heavier forebrains and spleens, were associated with more pronounced rhythmicity in the AR pups' activity.

A final consideration involves the definition of "activity." The repertoire of movements or activity that a rat pup can engage in is quite large (Bolles & Woods, 1964). Specific types of activity were not categorized in the present studies although incidental observations were made. For example, the AR and MRLD pups were observed to groom themselves, roll over, and walk around in the styrofoam cups. In addition, AR pups were sometimes observed to extend their bodies and flex their forelegs against the side of the cups. Interestingly, this behavior sounds similar to what Shair et al. (1984) observed in normal pups in response to maternal milk ejections during suckling. The mother-reared and AR pups may differ in the types and/or frequencies of various activities which in turn may influence the overall rhythmicity.

Summary of Main Findings

1. Clear circadian rhythms in locomotor activity with nocturnal peaks were observed in mother-reared animals starting as early as 26 to 28 days PC.
2. The rhythmic activity of the AR pups was more attenuated and of a shorter duration than that of mother-reared pups. Some synchronization to rhythmic stimuli did occur; LD cycles and feeding cycles were equally effective in synchronizing activity before weaning.
3. Exposure to a predominantly diurnal feeding cycle and a LD cycle improved synchronization of the AR pups' activity. Synchronization was less effective in the presence of a LD cycle and predominantly nocturnal feeding.
4. The addition of an environmental temperature cycle attenuated the AR pups' activity.
5. The periods of the autocorrelation peaks of AR pups on LL deviated the most from 24 hrs. Both LL and DD conditions resulted in highly attenuated autocorrelation peaks.

Chapter 4

General Discussion

Developmental rhythm research has focussed on the interrelated issues of physiological mechanisms, endogeny (Davis, 1981), and prenatal/postnatal maternal synchronization (see Chapter 1). The following discussion will emphasize the contributions of the present research to these issues. In addition, discussions of the relationships between rhythm development and growth and implications for human research are included.

Rhythm Development

Physiological Mechanisms

The present results support those from other studies indicating that open eyes and LD cues are not needed for the occurrence of circadian rhythmicity or synchronization during the early postnatal period. The partial effectiveness of feeding cycles in synchronizing rat pups' activity raises questions regarding the physiological mechanisms underlying this type of synchronization. For example, are the physiological mechanisms similar and/or related to those mediating LD synchronization? In adult animals, feeding entrains locomotor activity and hormone rhythms even in the absence of the SCN (Krieger et al., 1977; Phillips & Mikula, 1979). In addition, what factors (e.g., olfactory) are important in mediating the occurrence of rhythms in the presence of acute "stressors" such as maternal separation (MRLD group) and accidental food deprivation (AR groups)?

Endogeny

The concept of endogenous rhythmicity implies that an organism will exhibit rhythms even after having been reared from conception in the absence of external cyclic cues. Earlier investigators used the occurrence of rhythms after prolonged LL or DD conditions from conception and/or birth as evidence for the endogenous nature of rhythms. However, from the present studies and those of other researchers, non-light cues which remain rhythmic under LL or DD conditions, such as mother-offspring interactions, appear to be important to the expression of early rhythms. Attempts to achieve arrhythmicity during the prenatal period by lesioning the mother's SCN have produced questionable results (eg., Honma et al., 1984a). In the case of successful SCN lesions, effects on the offspring which may not be related to the arrhythmicity, such as growth, must always be considered.

Maternal Factors and Synchronization

In the present studies synchronization, rather than entrainment, to maternal and other environmental factors was emphasized. The definition of entrainment is more restrictive. It implies that 1) the entrained rhythm has a stable phase relationship with the zeitgeber(s), 2) when the zeitgeber is phase-shifted the entrained rhythm phase shifts accordingly, and 3) under constant conditions the rhythm begins to free run with a relatively predictable period (Enright, 1981b). These aspects of entrainment were not directly investigated in the present research but results from studies on the plasma corticosterone

rhythms of blind rat pups suggest that maternal factors serve as entrainers of the offsprings' rhythms (e.g., Takahashi et al., 1982). With the artificial rearing procedure it was possible to study some of the potential maternal and other environmental synchronizers in isolation or in combination with each other.

Some of the AR pups synchronized their activity to the cyclic feeding schedules suggesting that in the normal rearing situation the nursing rhythm may serve as an entrainer. Aspects of the nursing situation other than temporal parameters should also be studied. These would include suckling experiences and feeding via an oral rather than a gastric route. Environmental temperature, another factor partially regulated by the mother during the early postnatal period, also affected the activity levels of the ARLD-Temp pups but not necessarily in a cyclic manner. The thermoregulatory abilities of rat pups change dramatically between birth and weaning. As a result, the environmental temperature at which thermoneutrality occurs would also change. In order to keep the environmental temperature as similar as possible among the various groups (except for the ARLD-Temp group) the temperature was kept constant throughout the day and across age. More information is needed on the quantitative and qualitative aspects of the fluctuations in nest temperature which occur as a result of maternal behavior and how these fluctuations influence rhythm development. Also, the humidity in the room where the pups were tested varied with the outside weather conditions. Further investigations on

the effects of humidity on the activity of rat pups would be useful.

Light-dark cycles also serve as synchronizers during the early postnatal period. The appearance of group day-night differences at weaning in the AR pups on LD cycles suggests that as the pups get older light-dark cues may be more salient or potent synchronizers than, for example, feeding cycles or other maternal cues. In order to test this possibility pups should be born to and reared by freerunning dams on DD or dim LL. The pups could then be tested in the same way as the present MRLD group but under a DL cycle.

One of the more interesting findings concerns an interaction between the LD cycle and the feeding cycle. The AR pups on LD with predominantly nocturnal feeding (ARLD-DF) showed less synchronization of their activity than animals on only the LD/DL cycle (ARLD and ARDL) or nocturnal feeding schedule (ARLL-DF). One possible explanation is that, since nursing is normally predominantly diurnal, the pups were receiving discordant or incompatible cues when exposed to LD and nocturnal feeding. This possibility raises several interesting questions:

1. Would the same thing occur if pups were separated from the mother soon after birth (e.g., within 12 hrs)? It is possible that, in the present AR groups, synchronization to the diurnal nursing rhythm occurred during the first 2 to 3 postnatal days. This early synchronization may have then hindered subsequent

synchronization to the novel nocturnal feeding cycle.

2. Can the discordance occur with other maternal and environmental cues? For example, temperature fluctuations, hormonal levels in the mother's milk, olfactory cues, and tactile stimulation may also be involved.
3. How does this discordance affect the "internal temporal order" among other biological rhythms (Moore-Ede & Sulzman, 1981)? "Internal temporal order" refers to the synchrony among biological rhythms. For example, the body temperature and plasma cortisol rhythms in humans are synchronized to each other (Moore-Ede, Sulzman & Fuller, 1982). Sulzman, Fuller, Hiles, and Moore-Ede (1978) found that conflicting light and feeding temporal cues resulted in the dissociation of some biological rhythms in adult monkeys.
4. Does this early discordance affect later entrainment to, for example, shifts in the light cycle? If disruptions are observed in the AR pups after weaning it would be of interest to determine the duration and age range during which maternal isolation is most disruptive. Recent observations by Sasaki, Murakami and Takahashi (1984) on the drinking rhythm in rats may be relevant. Blind pups fostered after 10 days PN to a

dam entrained to another light-cycle did not entrain to the foster dam's rhythms whereas those fostered before 5 days PN did. They suggested that there may be a critical period for postnatal maternal entrainment (see also Reppert et al., 1984).

Related to question 4 above is the issue of whether the lack of normal rhythmicity in the AR pups is due to the AR procedure (i.e., surgical trauma, inadequate nutrients) or to the absence of appropriate rhythmic cues during a critical period. One experiment which would address this problem would be to return the AR pups to the mother at various times after the beginning of maternal isolation. Their rhythms could then be studied both before and after weaning.

It was suggested earlier that the synchronization observed in the MRLD group and some of the AR pups could be due to prenatal maternal factors. To address this problem the following experiments should be done:

1. Pups conceived to dams entrained to a LD cycle should be fostered at birth to dams that are freerunning under DD or dim LL. The activity of these pups could be recorded as in the MRLD group but under dim LL or DD conditions. The phase of the pups' rhythms could then be determined and compared with those of nonfostered pups.
2. AR pups born to LD-entrained dams should be reared and

have their activity recorded under a DL cycle. They could then be compared to pups reared postnatally on a LD cycle.

3. Ideally pups should be reared soon after birth (i.e., within 12 hrs) in maternal isolation. This would be difficult given the present technique although improvements in the AR procedure, such as reducing the surgical trauma, are possible.

Rhythm Development and Growth

In the present studies there was an association between rhythm development, physical growth, and appropriate synchronizing cues. For example, there was some evidence for a relationship between rhythmicity in activity and body, spleen, and forebrain weights. In addition, the feeding schedule had some influence on physical growth. Heavier spleens and lighter livers in the AR pups were correlated with a cyclic feeding schedule and heavier forebrains were associated with a predominantly diurnal feeding cycle. None of these conditions, however, fully corrected the deviations from normal values. The role of biological rhythms and external synchronizers in regulating growth and maintaining the health of animals is not well known and should be investigated in more detail. For example, Saito and Noma (1980) found that body weight gains in young male rats were less following an adiurnal feeding schedule. In addition, the abnormal metabolism of the AR pups (Sonnenberg et al., 1982) and surgical trauma should also be

considered as possible influences on rhythm development.

Prolonged noncyclic environments, such as LL, DD, constant temperature, humidity, etc., may also affect the health of an organism (Ehret, Groh, and Meinert, 1978). For example, some rats kept under long-term LL eventually lose their circadian rhythmicity although ultradian rhythms are still prominent. Albers et al., (1981) found that after prolonged LL three rats died and it was noted that their activity rhythms were dominated by relatively short periods (2.5 hrs) about five days before death. Although highly speculative, one possibility is that the short rhythms were a precursor to poor health which then ended in death. Desynchrony among a monkey's biological rhythms was offered as an explanation for deteriorating health in a weightless environment (Hahn, Hoshizaki & Adey, 1971). In these experiments, of course, it is difficult to sort out the causes and the effects. More conclusive results come from experiments on monkeys with freerunning body temperature rhythms under LL. Fuller, Sulzman, and Moore-Ede (1978) found that, compared to LD-entrained animals, monkeys on LL failed to regulate their body temperatures normally when exposed to cold for 6 hrs. The authors favored the possibility that, in LL or arrhythmic conditions, the synchrony between the monkey's biological rhythms is lost and this somehow results in impaired thermoregulation. Two of the AR groups in the present research were not only reared in maternal isolation but they were exposed to constant light or dark and constant temperatures during a period of rapid postnatal growth. In

addition to studying the postweaning rhythms of these AR pups, studies on, for example, the development of their thermoregulatory and immune responses should be done.

Implications for Human Infants

Research on the development of rhythms in infants, particularly the ontogeny of sleep-wake behavior, has a long history. It has not been until recently, however, that more sophisticated tools were developed for long-term monitoring and analyses of rhythm data. Also, recent data from other species on, for example maternal synchronization and the relationships between rhythms and health, should inspire the examination of the roles these factors play in human development (Anders, 1982). Two related areas in need of study are suggested in the following discussion.

The first area involves the need for more data on the role of parental and other factors on the synchronization of infant rhythms (Kleitman & Engelmann, 1953). There is evidence suggesting that the feeding and care schedules may influence an infant's activity rhythms (Sander, Julia, Stechler & Burns, 1972; Sander et al., 1979) and sleep-wake patterns (Gabriel, Grote & Jonas, 1981). Other factors such as parental work and sleep schedules, the regulation of lights on-lights off, and noise levels would add to an understanding of what early postnatal synchronizing cues are available to, and effective for, the infant.

The second area arises from concerns surrounding the

environments of neonatal care units in hospitals. Lawson, Daum, and Turkewitz (1977) and Gottfried, Wallace-Lande, Sherman-Brown, King, Coen, and Hodgman (1981) found that infants in special or intensive care units did not lack stimulation (i.e., auditory, visual, tactile); however, the stimulation was not well-patterned and factors such as lighting and noise were not distributed in a diurnally rhythmic manner. Relationships between these factors and the infant's early, as well as later, development are not well documented. It is not unreasonable to hypothesize that some of the differences observed between, for example, premature and full-term infants' sleep-states (Michaelis, Parmelee, Stern & Haber, 1973) are related to the immediate environment of the hospital ward. A stressful situation, such as a laboratory setting, may disrupt day-night differences in sleep-states of healthy infants (Sostek, Anders & Sostek, 1976).

The possible association between rhythmicity and health is also applicable to humans. Benotti et al. (1976, cited in Moore-Ede & Sulzman, 1981) observed that fatty livers were avoided in adult patients fed intravenously if the diet was infused in a cyclic rather than continuous manner. This could also apply to infants kept on intensive care wards who may need to be fed intravenously.

Summary

The present data add to the growing literature on the contributions of mother-offspring interactions to the ontogeny of biological rhythms. It is tempting to postulate that maternal factors such as the nursing rhythm and temperature regulation, in conjunction

with other factors such as LD cycles, not only provide the offspring with entraining cues but also serve to maintain synchrony among the offspring's various biological rhythms. It may not be sufficient that the entraining cues are present but that they are present during certain periods of development and also have, for example, the appropriate amplitude and phase relationships with other cues. These factors in turn may influence the growth and health of the developing organism. Similar questions should also be pursued in investigations on human development.

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Appendix A
Feeding Schedules

Generally two pumps were used for infusing the diet. Hence, there were two counterbalanced feeding schedules, arbitrarily labelled here as "A" and "B." The letter "A" next to a time interval in the following tables refers to a feeding time for group "A" and a nonfeeding time for group "B" (vice versa for "B" next to a time interval).

Noncyclic Schedule- Lights were on from 0800 to 2000 hrs for pups on a LD cycle except for the ARDL when they were on from 2000 to 0800 hrs.:

A	0815-0855 hrs	B	2025-2105 hrs
B	0855-0930	A	2105-2135
A	0930-0955	B	2135-2215
B	0955-1020	A	2215-2300
A	1020-1105	B	2300-2335
B	1105-1130	A	2335-2400
A	1130-1205	B	2400-0030
B	1205-1230	A	0030-0100
A	1230-1305	B	0100-0130
B	1305-1345	A	0130-0155
A	1345-1420	B	0155-0225
B	1420-1455	A	0225-0250
A	1455-1535	B	0250-0320
B	1535-1600	A	0320-0355
A	1600-1640	B	0355-0430
B	1640-1725	A	0430-0500
A	1725-1750	B	0500-0535
B	1750-1835	A	0535-0620
A	1835-1915	B	0620-0705
B	1915-1955	A	0705-0740
A	1955-2025	B	0740-0815

	Feeding (A)	Feeding (B)
Total time	715 min	725 min
Average time	34.05	34.52
interval (sem)	(1.53)	(1.46)
Number of:0800-2000 hrs	10	10
intervals:2000-0800 hrs	11	11

Time interval (min)	Frequency
25	9
30	8
35	11
40	8
45	6
Total	42

Cyclic schedule: The following schedule was used for the ARLD-LF, ARLD-DF, ARLL-LF, ARLL-DF, and ARLD-Temp groups. For most animals on the LD cycles, 0800 to 2000 hrs was the light period. Some animals in the ARLD-LF and ARLD-DF groups were on the reverse light cycle (lights on from 2000 to 0800 hrs).

0800 to 2000 hrs		2000 to 0800 hrs	
A	0755-0840 hrs	B	1955-2040 hrs
B	0840-0905	A	2040-2105
A	0905-0950	B	2105-2150
B	0950-1020	A	2150-2220
A	1020-1100	B	2220-2300
B	1100-1130	A	2300-2330
A	1130-1215	B	2330-0015
B	1215-1240	A	0015-0040
A	1240-1320	B	0040-0120
B	1320-1345	A	0120-0145
A	1345-1425	B	0145-0225
B	1425-1450	A	0225-0250
A	1450-1535	B	0250-0335
B	1535-1555	A	0335-0355
A	1555-1635	B	0355-0435
B	1635-1700	A	0435-0500
A	1700-1745	B	0500-0545
B	1745-1810	A	0545-0610
A	1810-1855	B	0610-0655
B	1855-1915	A	0655-0715
A	1915-1955	B	0715-0755

Schedule	Feeding time (hrs) 0800-2000	2000-0800	Percent feeding from 0800-2000hrs
A	470 min	250 min	65.3
B	250 min	470 min	34.7

Mean feeding interval of 12-hr period with most feeding=42.7 min. Mean feeding interval of 12-hr period with least feeding=25.0 min. Total feeding time=720 min.

Time interval (min)	Frequency
20	4
25	12
30	4
40	10
45	12
Total	42

Appendix B
Period and area calculations from the autocorrelation analysis

Figure B1 represents an autocorrelation plot for one block or 3 days of data. In this case it is from animal N0 in the mother-reared group. The abcissa is the lag in hours and the ordinate is the autocorrelation value. The dashed line represents the significance level at $p=0.01$. Lags between 20.0 and 28.0 hrs were examined for significant autocorrelation values. In the example, there are six successive autocorrelation values between 23.25 and 24.50 hrs inclusive which exceed the significance level- 0.1771, 0.1439, 0.4284, 0.6376, 0.2454, and 0.1669. The peak formed by these values is referred to as the "autocorrelation peak" in the text.

The area value would be calculated as follows:

$$\text{Area} = \sum_{i=1}^{6} (r_i - 0.134)$$

$$= (0.1771-0.134)+(0.1439-0.134)+\ldots+(0.1669-0.134)$$

$$= 0.9953$$

where "r_i" is the autocorrelation value and 0.134 is the significance level. This is a conservative estimate of the area under the peak (hatched portion) and gives an indication of the strength of the rhythm.

The weighted period, or tau, was calculated as follows:

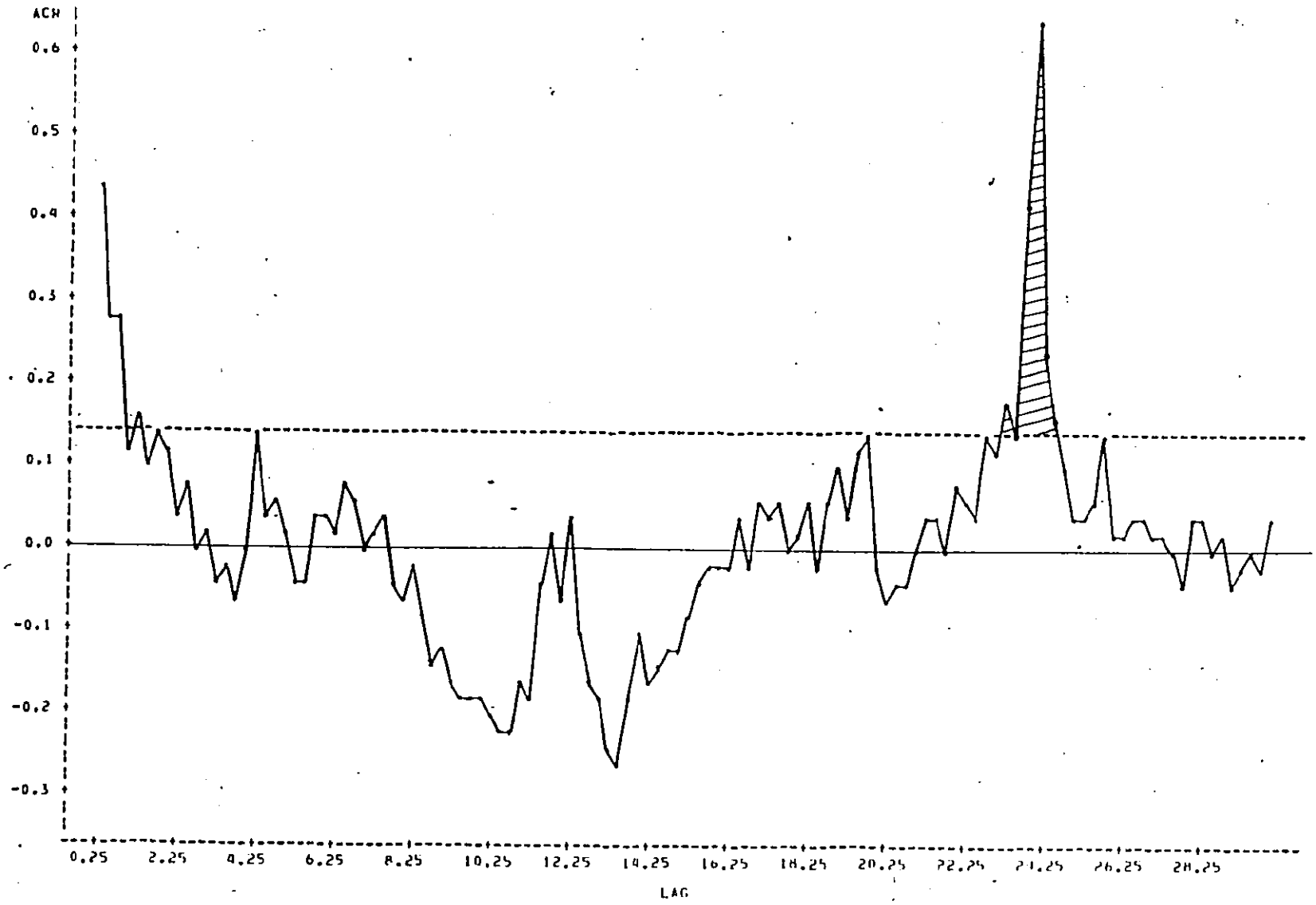
$$\text{Period} = \left(\sum_{i=1}^{6} (r_i - 0.134)(p_i)\right)/\text{Area}$$

$$= ((0.0431)(23.25) + .. + (0.0329)(24.5))/0.9953$$

$$= 23.93 \text{ hrs}$$

where "p_i" is the lag of the "ith" autocorrelation value.

These calculations were done on each block which had significant autocorrelation peaks.



Appendix C
Autocorrelation data
for individual animals

The first number following the colon refers to the autocorrelation block (0 = 26.5 to 28.5 days PC, etc.), the second number is the mean period, and the third number is the mean area of the autocorrelation peak. Additional peaks are separated by semicolons.

AR non-food deprivation peaks:

Group/Rat

ARLD-Temp
T1-1 : 6, 23.5, 0.0387
T5-3 : 7, 23.2, 0.0006; 9, 24.7, 0.0305
T11-10: 2, 20.0, 0.0249
T8-11 : 2, 23.5, 0.0467; 3, 25.7, 0.0103
T8-8 : 2, 23.2, 0.0050

ARLD-LF
W5 : 10, 23.5, 0.0149
P2 : 10, 26.2, 0.0874

ARLD-DF
Q12 : 10, 27.2, 0.0017
10-9 : 0, 24.0, 0.0354

ARLL-LF
I-7 : 4, 22.1, 0.0147; 8, 27.2, 0.0206

ARLL-DF
H-13 : 6, 22.7, 0.0186; 7, 22.5, 0.0214
H-11 : 6, 25.2, 0.0470;,7, 25.2, 0.0530; 9, 23.5, 0.0135
D-12 : 10, 22.0, 0.0191

ARLD
G1 : 10, 25.0, 0.0097
G3 : 1, 27.2, 0.0036; 2, 24.0, 0.0298; 3, 24.0, 0.0344;
10, 24.0, 0.0095
V0 : 3, 24.2, 0.0083; 4, 24.9, 0.0958
U8 : 4, 24.0, 0.0117; 8, 23.7, 0.0019
M7 : 5, 23.2, 0.0219; 10, 21.5, 0.0314

ARDL
7(Apr): 0, 24.1, 0.0983; 2, 24.0, 0.0042

ARLL
S8 : 8, 22.0, 0.0056
S12 : 8, 21.5, 0.0260

ARDD
0(Feb): 1, 24.2, 0.0305
8(Jun): 7, 25.2, 0.0044
11(") : 7, 24.5, 0.0142

AR food deprivation peaks:

Group/Rat

ARLD-Temp
T1-1 : 5, 23.5, 0.0214
T5-0 : 1, 24.2, 0.0036; 9, 23.7, 0.0170
T11-10: 7, 24.1, 0.0348; 8, 24.2, 0.0008; 10, 23.1, 0.0739
T8-11 : 6, 23.0, 0.0151; 9, 26.0, 0.0144;, 10, 24.2, 0.0310
T8-8 : 0, 21.7, 0.0029; 1, 22.7, 0.0257; 7, 24.0, 0.0622;
10, 24.2, 0.0375

ARLD-LF
P7 : 1, 24.2, 0.1048; 6, 23.2, 0.0091
W5 : 4, 24.5, 0.0137; 6, 23.0, 0.0106
X4 : 2, 27.7, 0.0061; 3, 20.0, 0.0231
P2 : 0, 23.8, 0.0462; 7, 25.1, 0.1140
G3 : 1, 24.2, 0.0152; 2, 25.7, 0.0073; 6, 26.2, 0.0012
12-1 : 1, 24.7, 0.0371; 2, 23.2, 0.0101; 9, 25.5, 0.0504
9-3 : 1, 23.9, 0.1288; 5, 23.2, 0.0126; 8, 25.7, 0.0279;
9, 25.7, 0.0079; 10, 25.3, 0.0088
7-4 : 1, 22.0, 0.0147
G1 : 0, 24.0, 0.0589
E6 : 0, 26.5, 0.0594; 2, 22.8, 0.0699
G7 : 0, 24.1, 0.0199; 1, 24.0, 0.0161; 2, 20.9, 0.1154

ARLD-DF
Q0 : 6, 23.0, 0.0287
Y6 : 2, 23.4, 0.1809; 3, 23.2, 0.2541
Q12 : 0, 24.7, 0.0006; 1, 24.3, 0.1834; 4, 23.0, 0.1625
D11 : 4, 26.2, 0.0086

ARLL-LF
5-2 : 4, 21.5, 0.0122
1-4 : 2, 25.4, 0.9948; 3, 24.4, 0.0657
F-1 : 2, 21.8, 0.1024; 3, 20.2, 0.0275; 6, 26.2, 0.0265;
7, 25.5, 0.0421
I-5 : 5, 22.5, 0.0477; 6, 22.5, 0.0426

ARLL-DF
H-13 : 3, 25.0, 0.0045

ARLD
E15 : 0, 23.7, 0.0210; 2, 23.5, 0.0026; 3, 23.5, 0.0018
F12 : 0, 24.0, 0.0511; 1, 22.8, 0.0411; 2, 22.5, 0.0087;
8, 24.5, 0.0018
V0 : 6, 20.0, 0.0255
U8 : 5, 23.7, 0.0068; 6, 22.7, 0.0251
V4 : 5, 25.5, 0.0002; 6, 25.5, 0.0233; 7, 25.2, 0.0159
W9 : 7, 21.2, 0.0079; 8, 21.0, 0.0311; 9, 23.4, 0.0555
W10 : 6, 28.0, 0.0028; 7, 24.0, 0.0292
W7 : 7, 25.3, 0.0237

ARDL
0(Apr): 3, 24.0, 0.0318; 4, 24.0, 0.0437; 5, 22.5, 0.0208
2(Apr): 4, 23.2, 0.0025
4(May): 3, 24.5, 0.0057; 9, 20.7, 0.0037

ARLL
10 : 5, 28.0, 0.0088
S12 : 10, 23.0, 0.0078
X2 : 6, 23.5, 0.0036

ARDD
3(Feb): 5, 26.0, 0.0357
7(Feb): 4, 21.7, 0.0089
11 : 5, 23.7, 0.0367
14 : 0, 22.7, 0.0200; 5, 26.0, 0.0319
8(Jun): 3, 23.0, 0.0176; 8, 23.5, 0.0011
9(Jun): 9, 23.7, 0.0076; 10, 23.7, 0.0395

MRLD autocorrelation peaks:

N12 : 2, 24.0, 0.0050; 3, 24.1, 0.2766; 4, 24.2, 0.3138; 5, 24.1, 0.1222; 6, 23.9, 0.2493; 7, 23 .9, 0.3296; 8, 23.9, 0.1285; 10, 25.2, 0.0064

N0 : 0, 23.9, 0.6547; 1, 23.9, 0.9953; 2, 23.9, 0.6652; 3, 23.8, 0.3836; 4, 23.9, 0.0188; 5, 24.3, 0.2483; 6, 24.2, 0.3582; 7, 24.0, 0.1532; 8, 24.0, 0.2208; 9, 24.1, 0.1188; 10, 24.2, 0.0638

N10 : 1, 23.7, 0.1858; 2, 23.7, 0.0040; 3, 23.9, 0.1213; 4, 23.9, 0.1153; 5, 24.0, 0.1237; 6, 24 .1, 0.1512; 7, 24.2, 0.2358; 8, 24.1, 0.1548; 9, 24.0, 0.1415; 10, 23.9, 0.1326

N2 : 1, 23.9, 0.3951; 2, 23.9, 0.4303; 3, 23.9, 0.3562; 4, 23.7, 0.2166; 5, 22.7, 0.0060; 6, 24.4, 0.0481; 7, 24.3, 0.2984; 8, 24.1, 0.1613; 9, 24.0, 0.2974; 10, 24.0, 0.2275

N8 : 0, 24.3, 0.3534; 3, 24.0, 0.0768; 4, 24.0, 0.0629; 5, 24.0, 0.0418; 6, 24.0, 0.0362; 8, 25 .8, 0.1879; 9, 23.9, 0.1014; 10, 23.7, 0.1659

N6 : 0, 23.8, 0.1764; 1, 23.9, 0.5639; 2, 24.0, 0.0316; 4, 23.7, 0.0367; 5, 24.0, 0.4937; 6, 24 .1, 0.5967; 7, 24.1, 0.2003; 8, 26.2, 0.0651; 9, 26.2, 0.0189; 10, 23.9, 0.0573

N14 : 0, 23.9, 0.6571; 1, 24.0, 0.3226; 2, 23.9, 0.1575; 3, 23.9, 0.2304; 4, 24.0, 0.0627; 5, 24 .1, 0.1015; 6, 24.0, 0.1842; 7, 24.0, 0.2671; 8, 24.0, 0.0322; 10, 23.9, 0.0354

Appendix D
Plots of 3-hr means over all days for individual animals from each group

Figures D1 to D10 are plots of the 3-hr means of activity for individual rat pups. The dark horizontal bar represents the dark period. For all figures, excluding Figure D8, the first 3-hr mean of each day represents activity from 2000 to 2300 hrs. For the ARDL pup in Figure D8 the first 3-hr mean of each day is for activity occurring from 0800 to 1100 hrs. The mean periods (tau, in hrs) for each block which had a significant autocorrelation peak are also shown. An asterisk next to the period signifies a food deprivation peak. The details of each figure are as follows:

Figure D1. Rat N0 of the MRLD group with a 12:12 LD cycle.

Figure D2. Rat T5-3 of the ARLD-Temp group with a 12:12 LD cycle. This pup also received 65.3% of its food during the light period. The incubator temperature was highest during the light.

Figure D3. Rat W5 of the ARLD-LF group with a 12:12 LD cycle. About 65.3% of its food was delivered during the light period.

Figure D4. Rat Q12 of the ARLD-DF group with a 12:12 LD cycle. About 65.3% of its food was delivered during the dark period.

Figure D5. Rat I-7 of the ARLL-LF group on constant light. About 65.3% of the food was delivered during the maternal light period (open bar).

Figure D6. Rat D-12 of the ARLL-DF group on constant light. About 65.3% of the food was delivered during the maternal dark period (open bar).

Figure D7. Rat G3 of the ARLD group with a 12:12 LD cycle.

Figure D8. Rat #7 (May) of the ARDL group with a 12:12 DL cycle.

Figure D9. Rat #11 (March) of the ARDD group on constant darkness.

Figure D10. Rat S12 of the ARLL group on constant light.

The animals in Figures D3, D5, and D7 are missing data for 26.5 days PC. Also, the ordinate scale may differ among the figures.

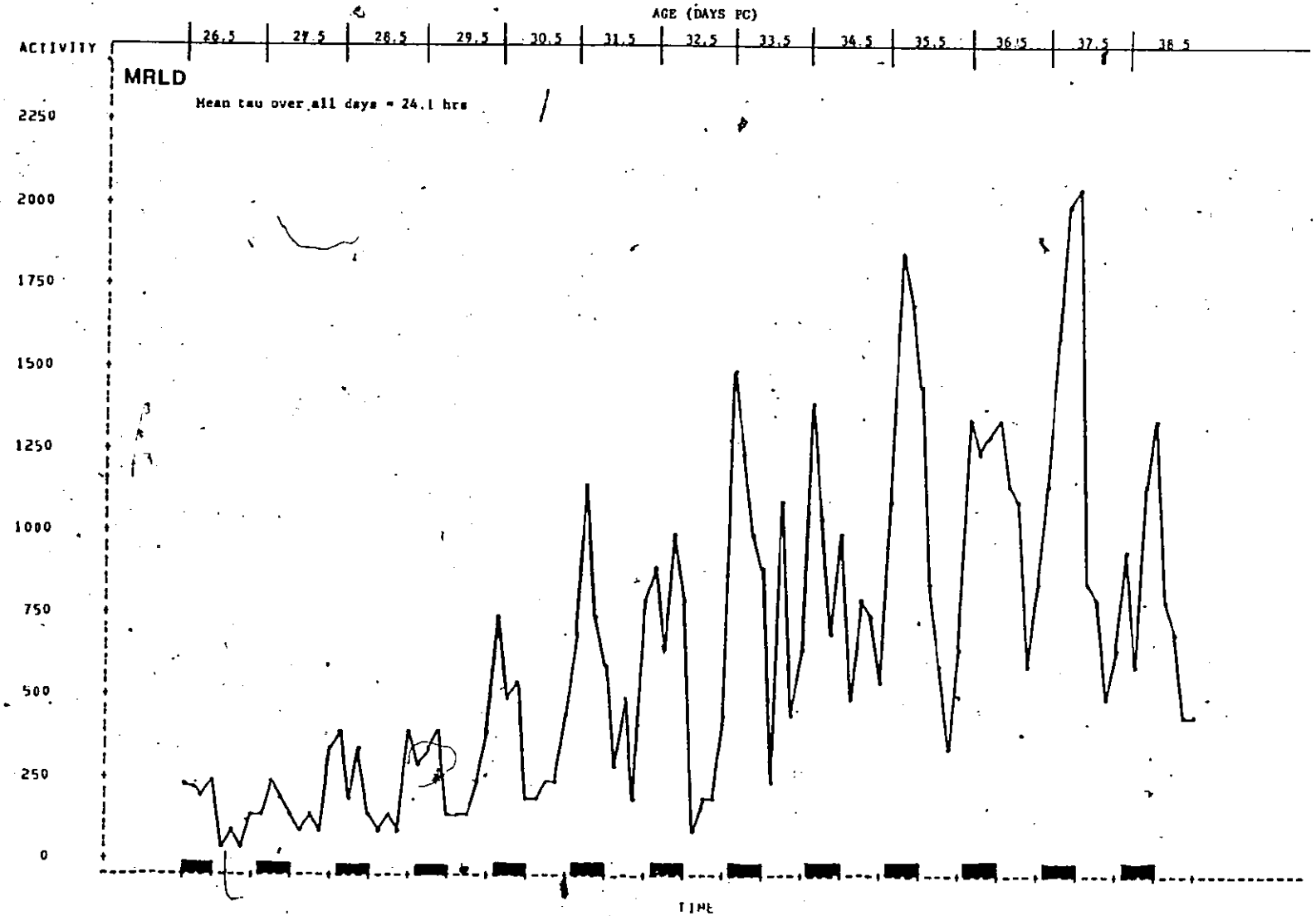


Figure D1

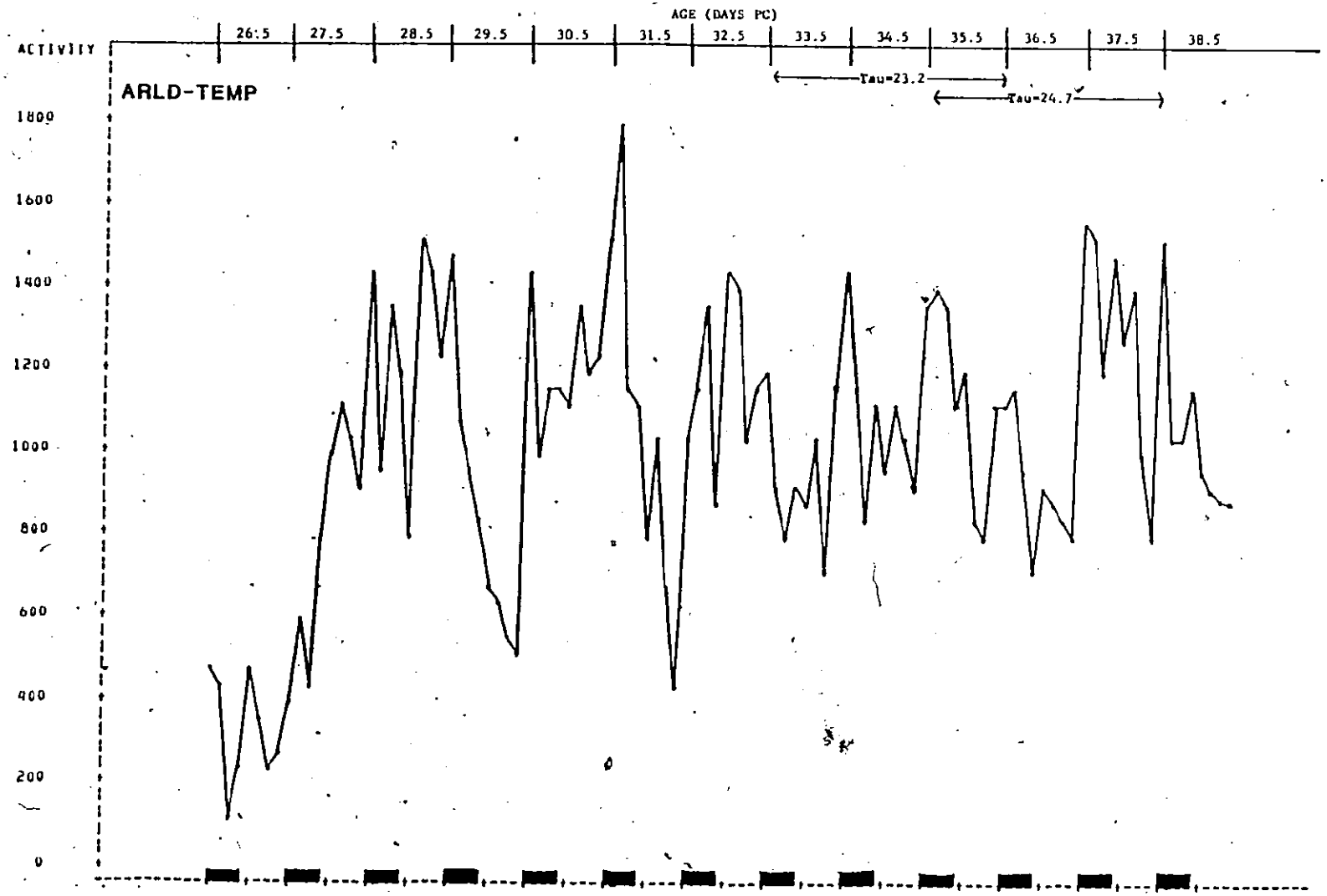


Figure D2

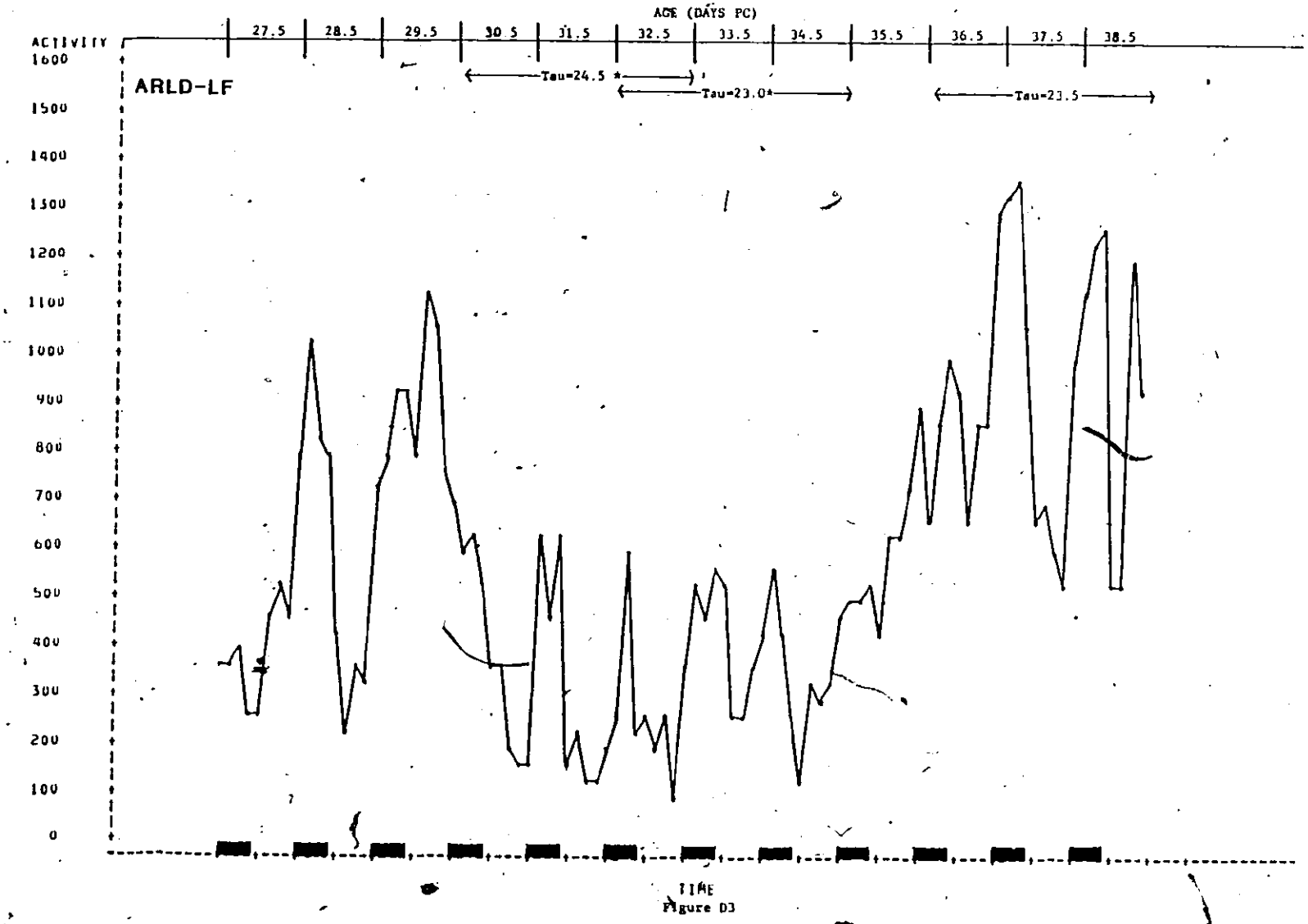


Figure D3

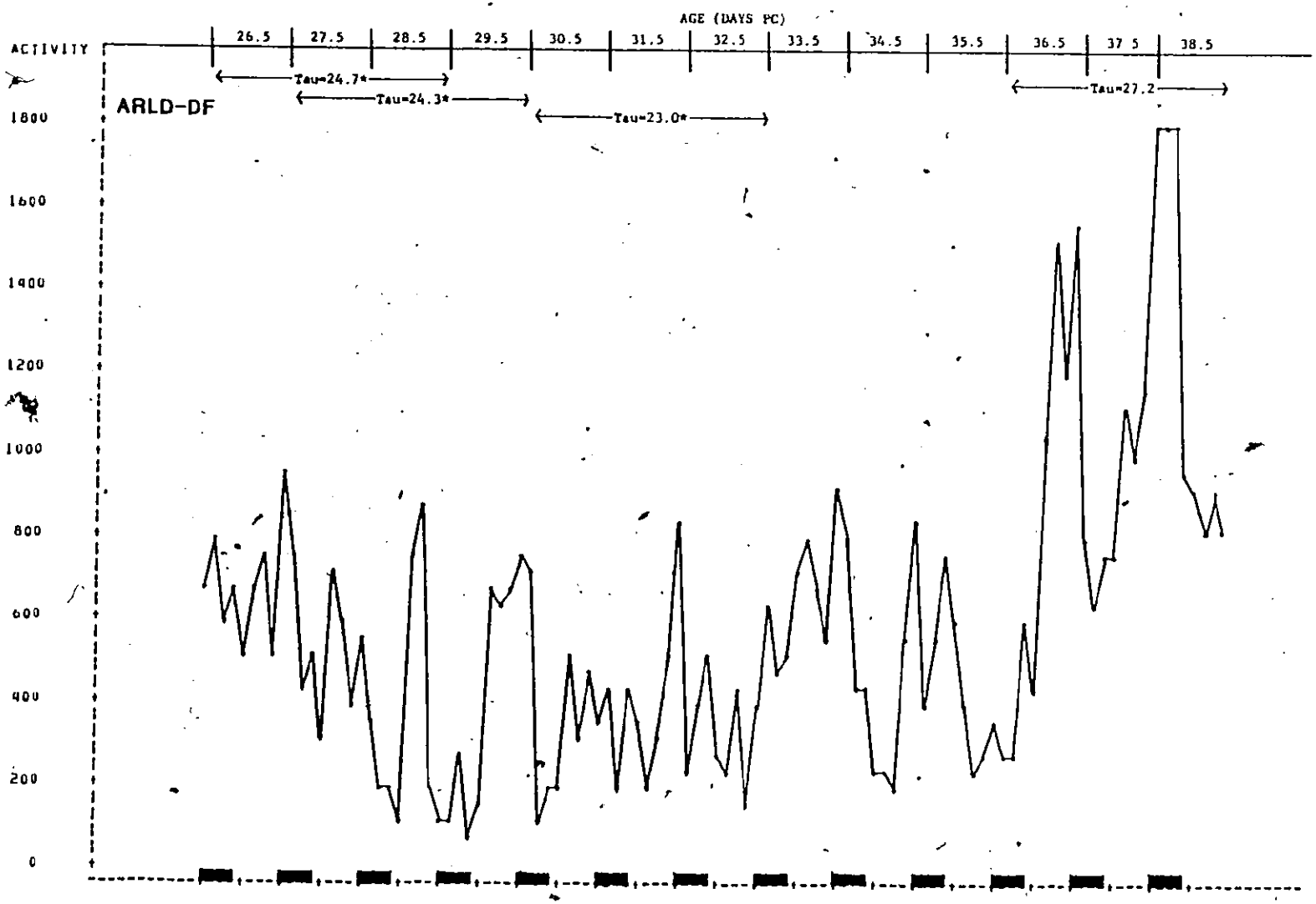


Figure D4

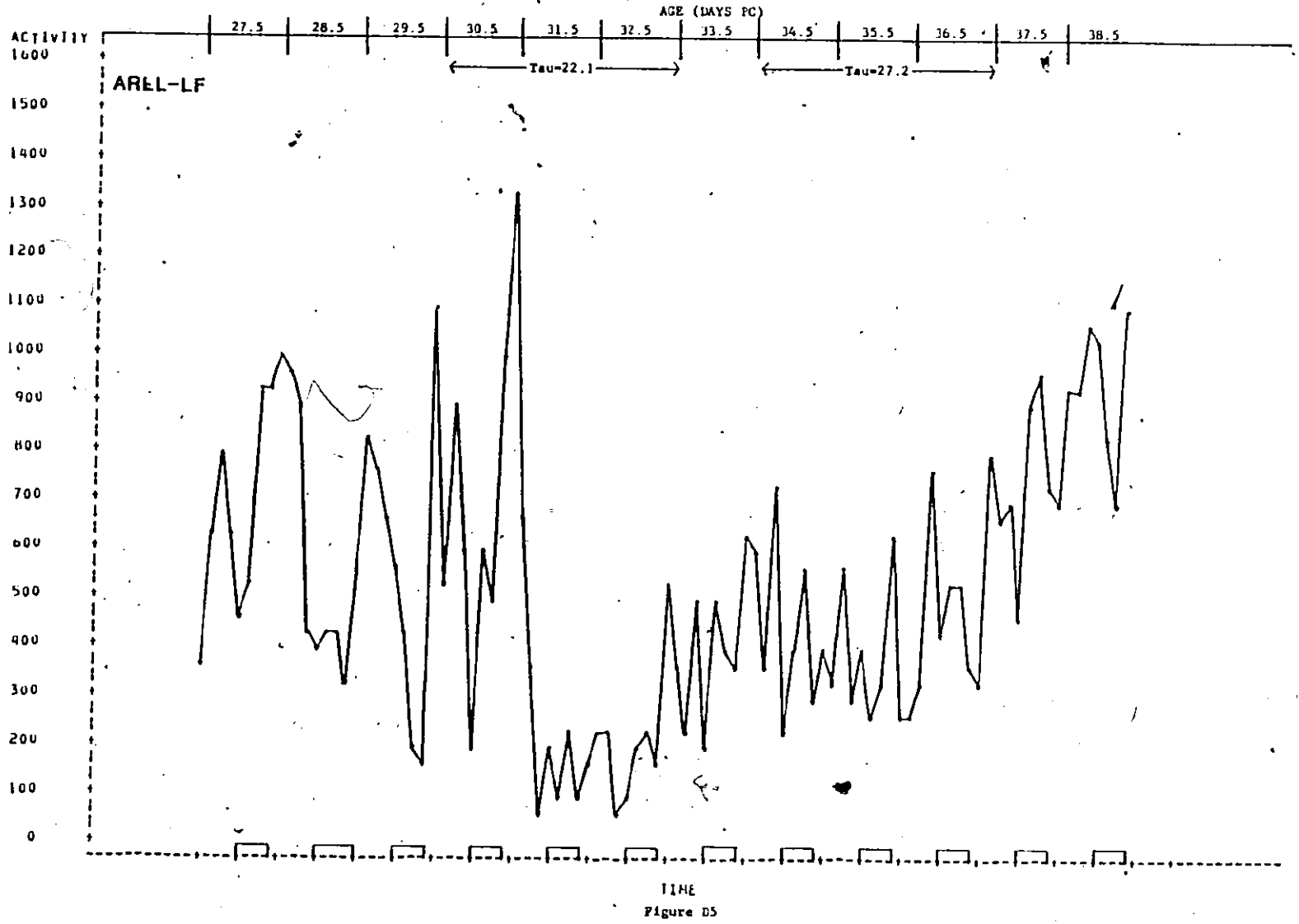


Figure D5

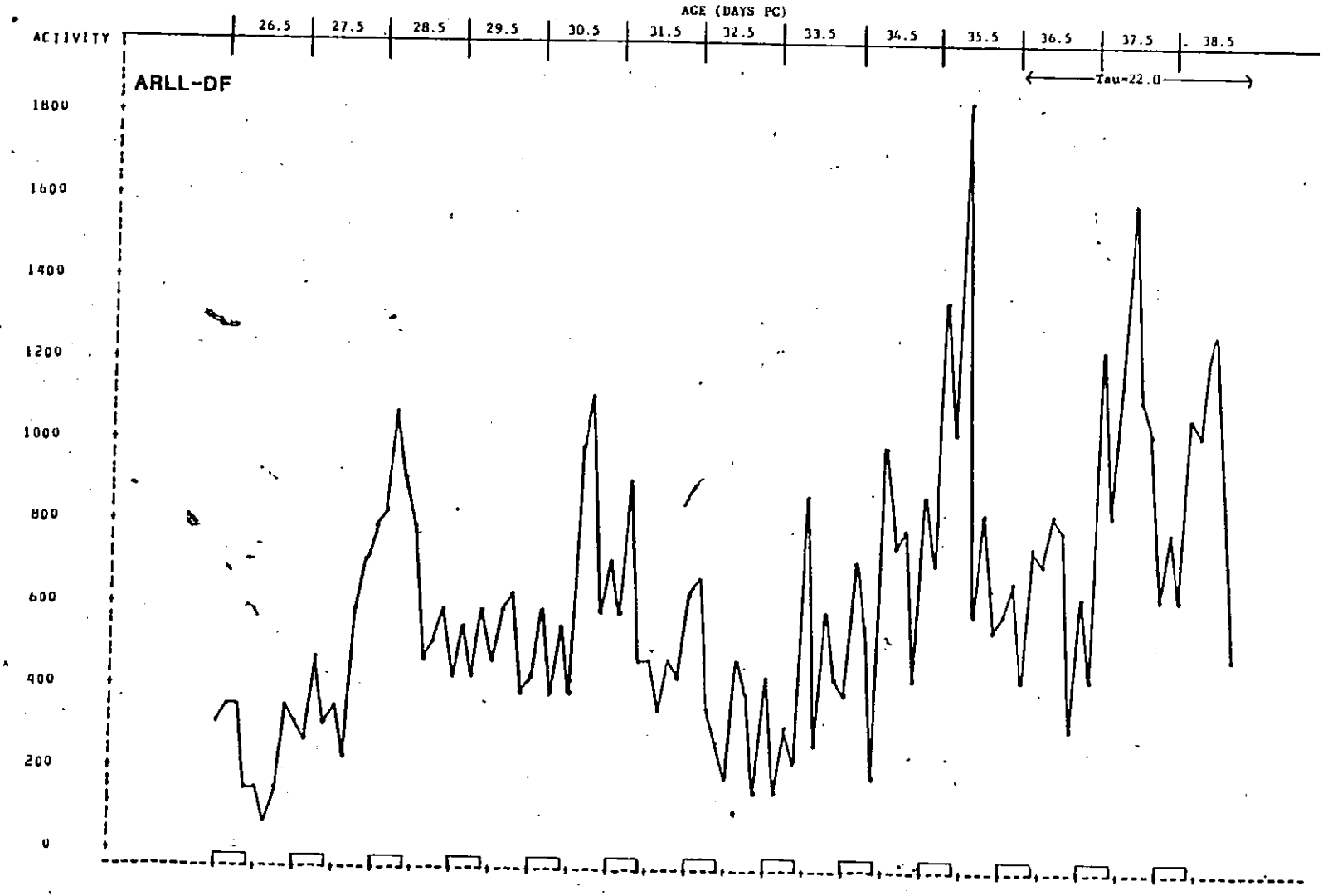


Figure D6

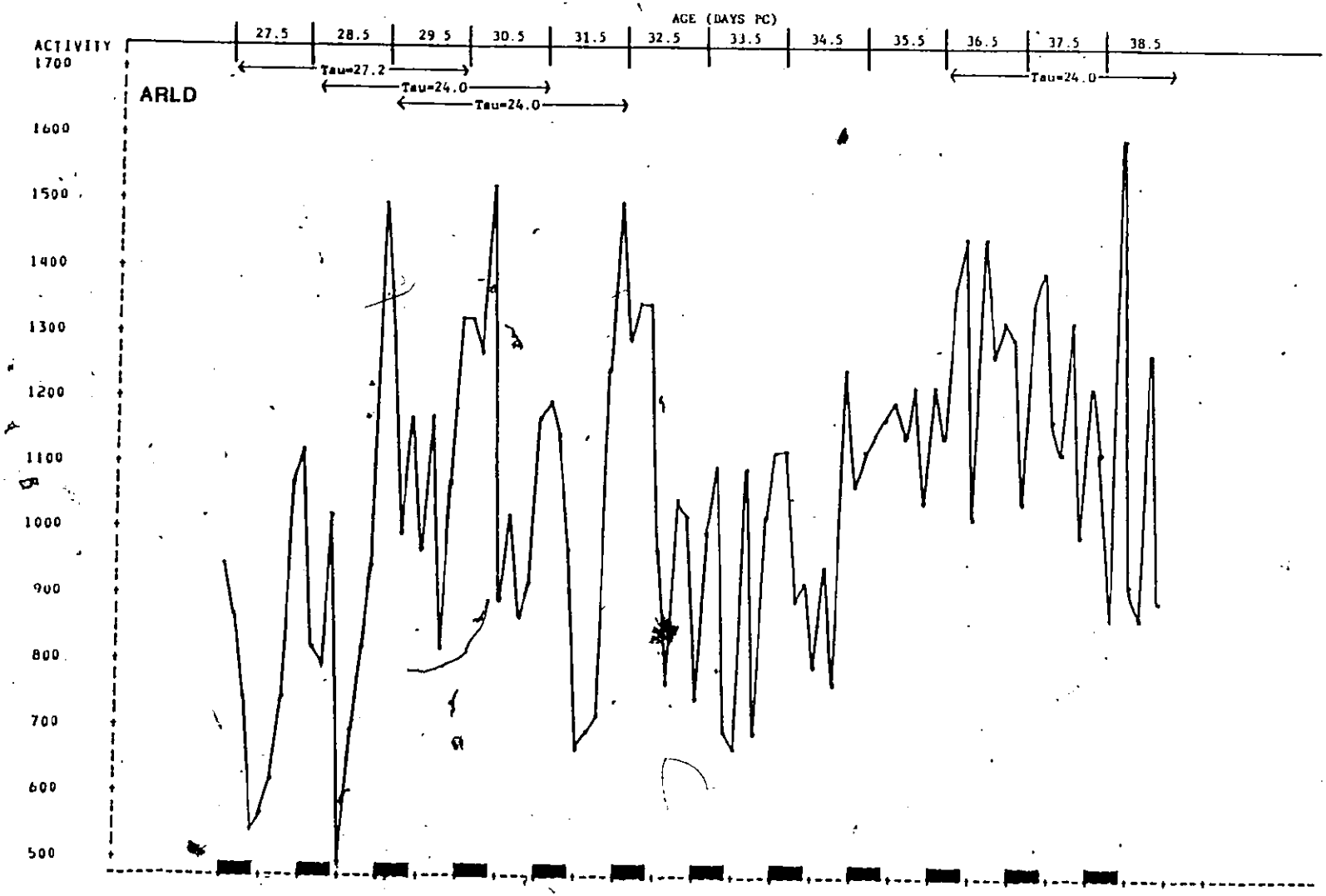


Figure D7

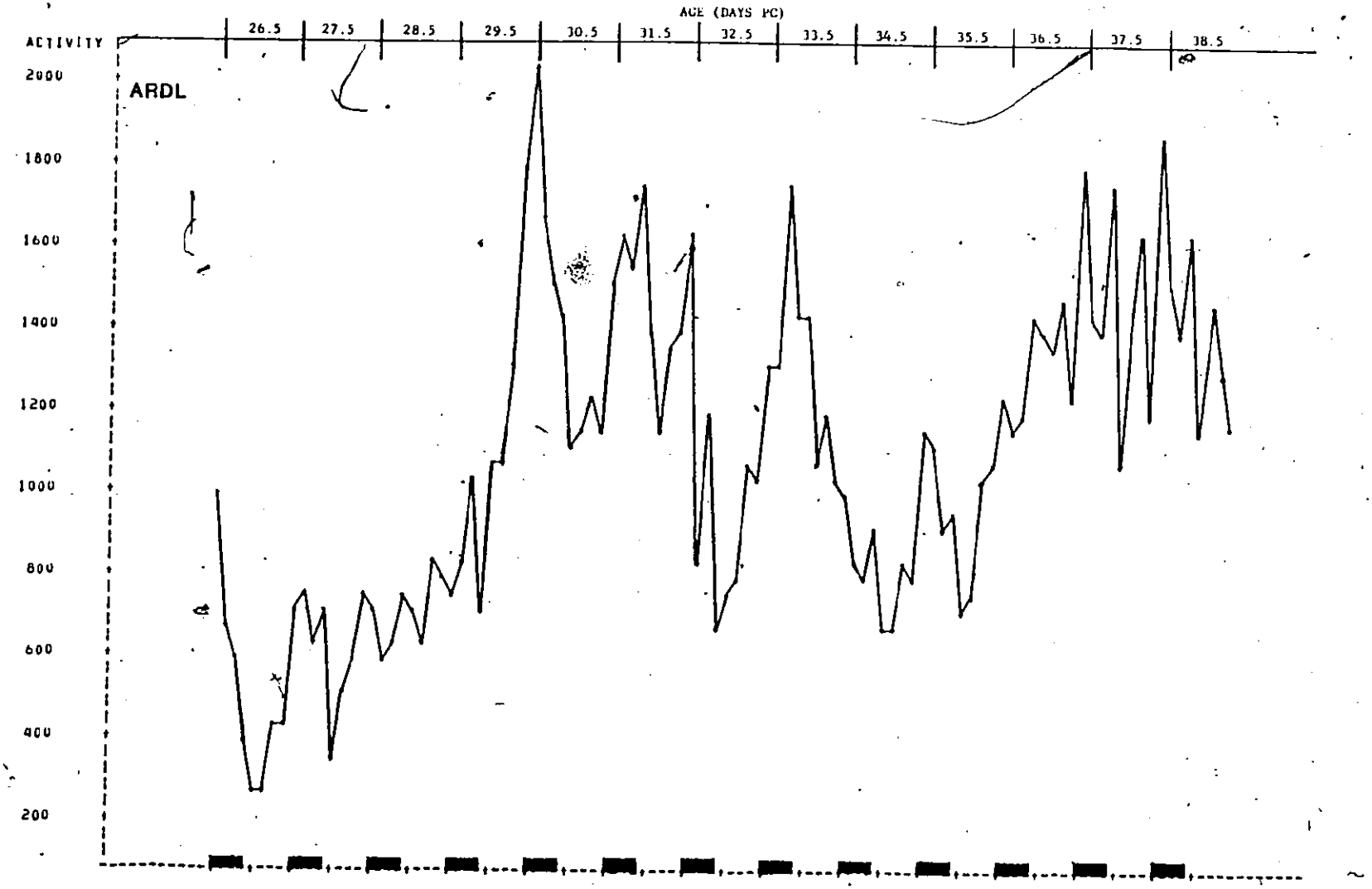


Figure D8

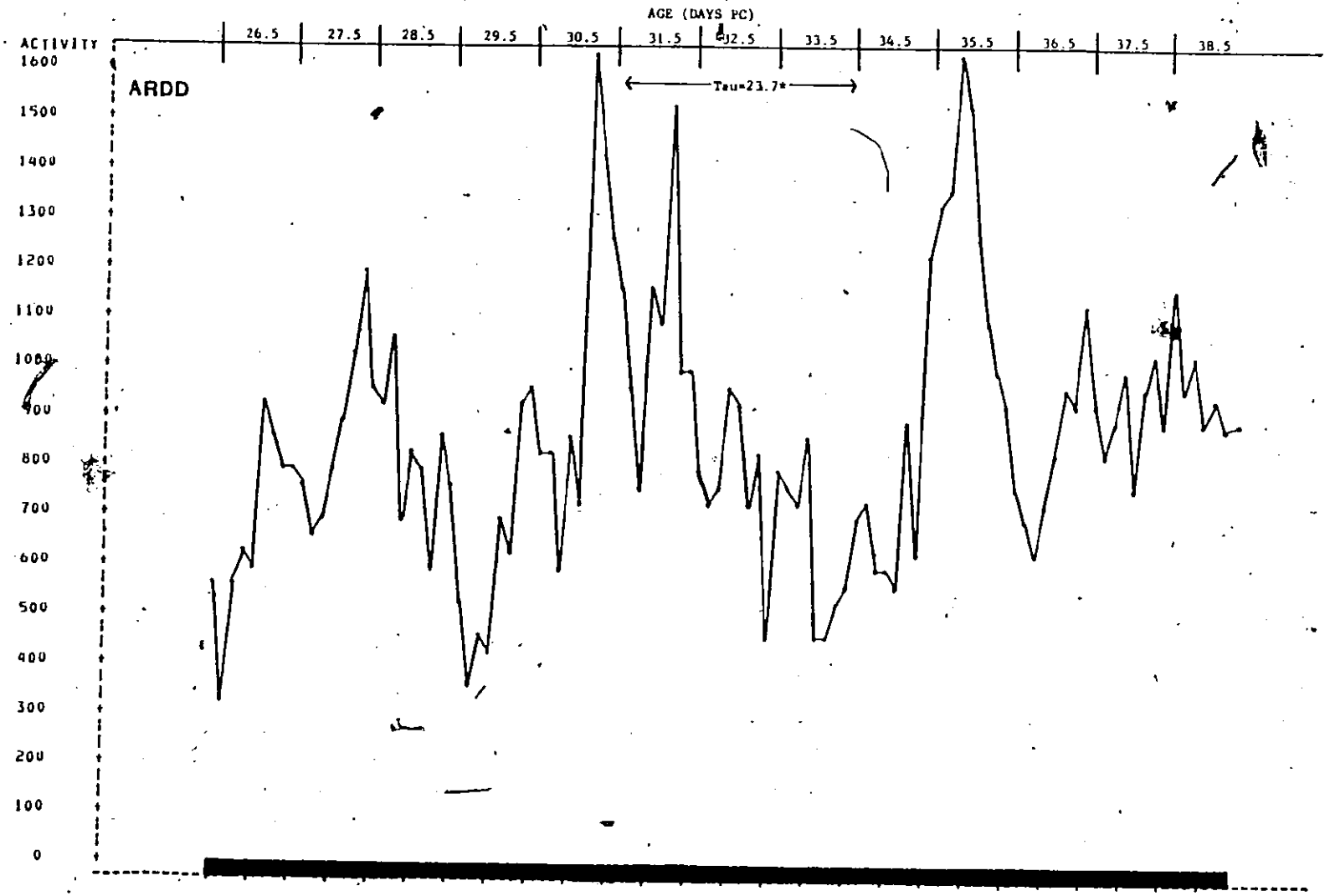


Figure D9

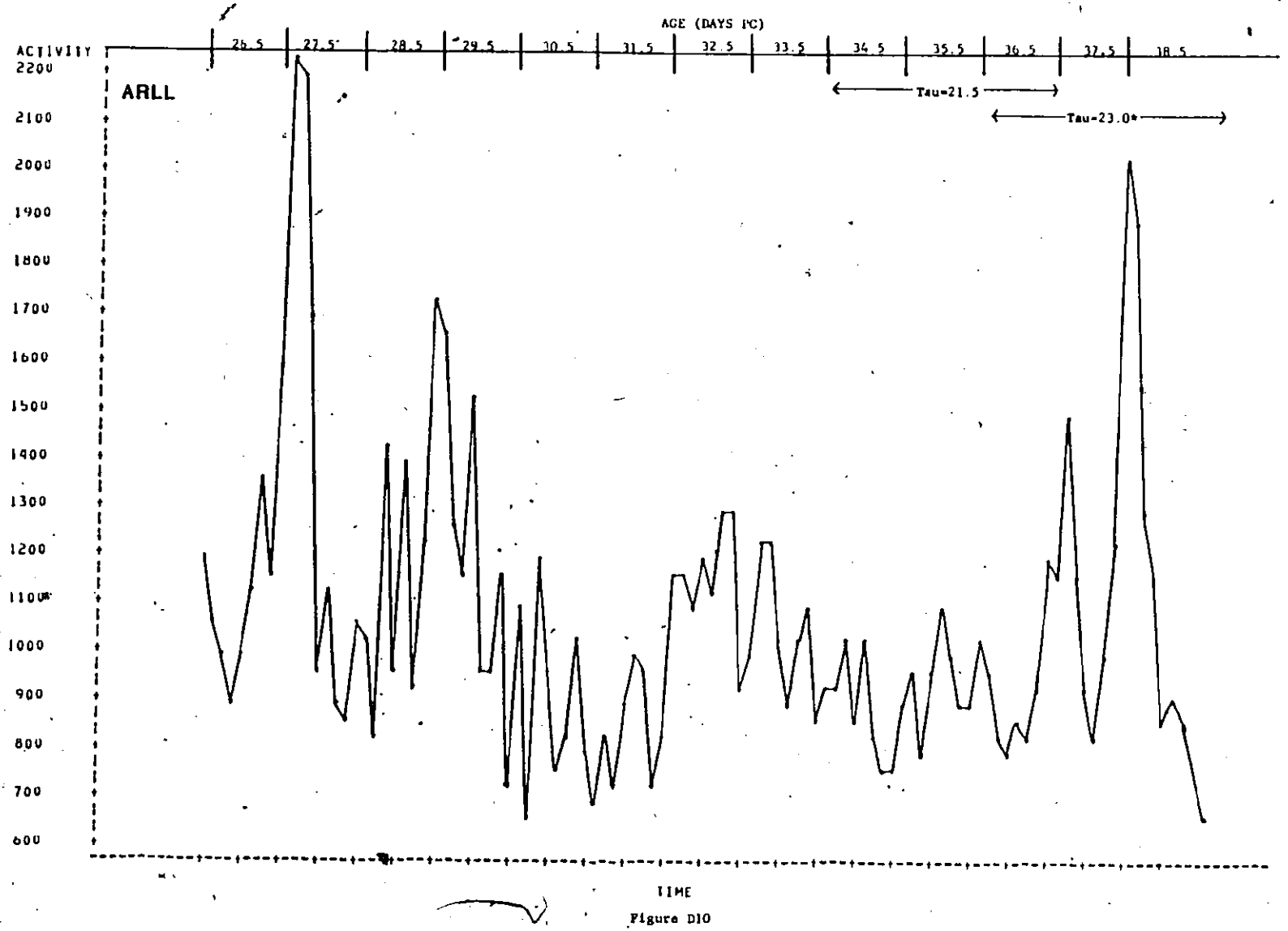


Figure D10

Appendix E
Developmental indices and open-field behavior of AR rat pups

In September and November, 1979, AR pups and their control littermates were tested for the appearance of various developmental indices and changes in their open-field behavior. One of the reasons behind this pilot study was to determine if there were behavioral changes due to the abnormal rearing environment (i.e., maternal isolation and the artificial diet) of the AR pups. Also, it was a good opportunity to compare the effects of an intermittent and continuous feeding schedule on the behavior of AR pups.

Methods

Hooded rats were implanted with gastric cannulas 3 days after birth and reared in water bath incubators until 18 days PN. One group of AR pups (September) was fed continuously (CAR group). The November group was fed "intermittently" (IAR group) such that they received 5 to 6.5 hrs of feeding time during the light period (0800 to 2000 hrs) and 9.5 to 11 hrs during the dark period (2000 to 0800 hrs). The control littermates remained with the dam. Initially, equal numbers of pups in the CAR group were tested in the open-field on alternating days beginning at 5 days PN. Starting on day 11 they were tested randomly every day until 18 days PN. Pups in the IAR group were tested randomly every day from 5 to 18 days PN. Control littermates from both groups were treated similarly.

The open-field area was a clear, plastic breeding cage (29.2 cm

wide x 34.3 cm long x 17.1 cm deep) placed on the floor and marked off in 12 equal squares with masking tape. The pup was placed in the center of the cage and its activity was recorded for 2 min. Activity measures included the amount of time spent grooming, number of lines crossed, number of head raises, and the number of pivoting movements (a pup will pivot its body in circles using a hindleg and the abdomen as support). The ages at which a straight crawl and walking on all four legs appeared were also recorded.

Open-field testing occurred after (CAR group) or before (IAR group) weighing and checking for eye opening. Erect pinnae were checked daily from birth. Testing took place between 0830 and 1230 hrs. Due to moving, the testing rooms were different for the two groups. Dams were removed from the cage immediately before testing and remained separated from the control littermates throughout testing.

Results

Sample sizes varied daily mainly due to deaths so only general trends in the group means will be discussed. Group means are based on individual pup values.

As can be seen in Figure E1, the intermittently fed AR pups consistently weighed more than the continuously fed group except at 10, 13, 14, and 16 days PN. During these days the tubings to the pump became clogged and prevented delivery of the milk formula. There were no deaths due to bloating in either AR group although many of the CAR pups appeared bloated and undigested diet could be seen in their stomachs and intestines.

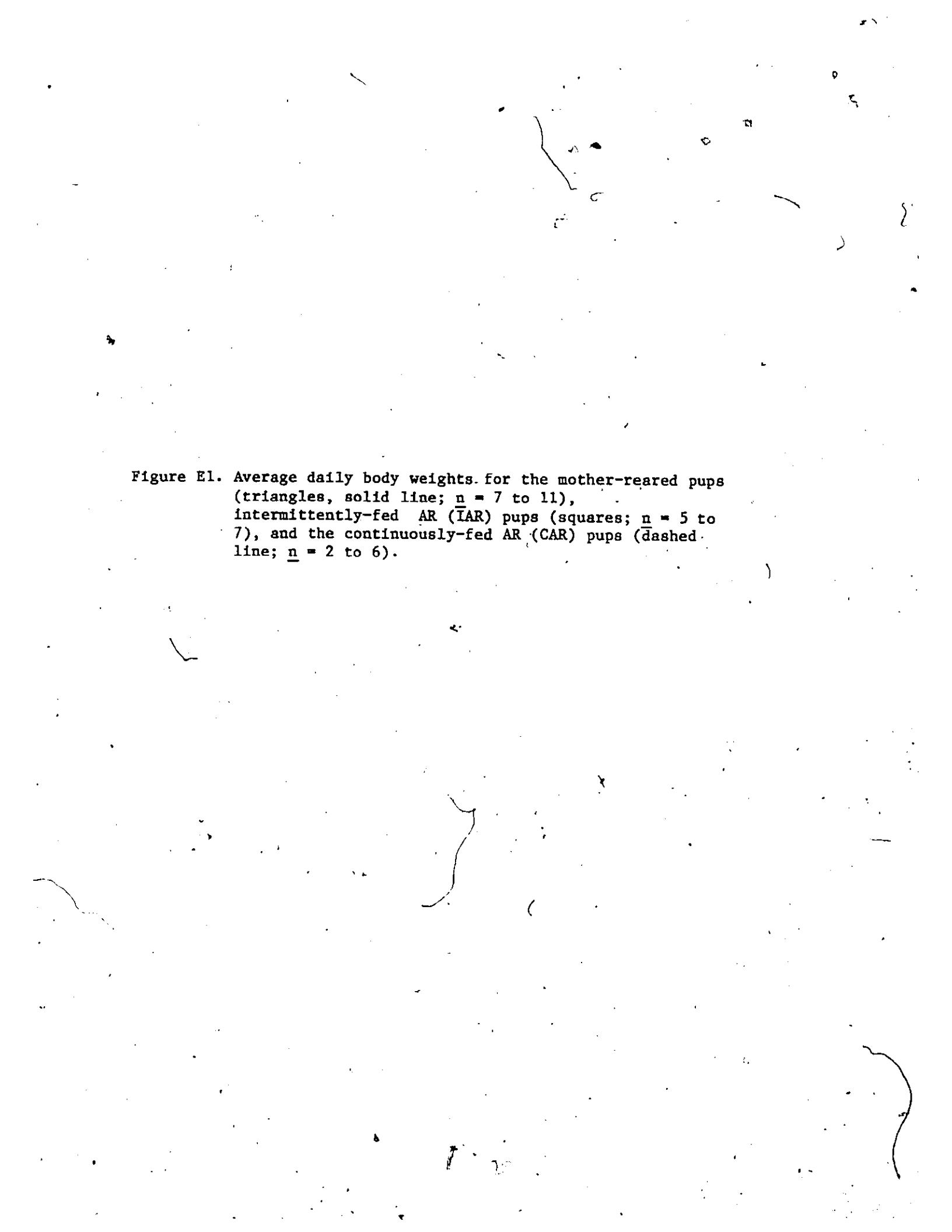


Figure E1. Average daily body weights for the mother-reared pups (triangles, solid line; n = 7 to 11), intermittently-fed AR (IAR) pups (squares; n = 5 to 7), and the continuously-fed AR (CAR) pups (dashed line; n = 2 to 6).

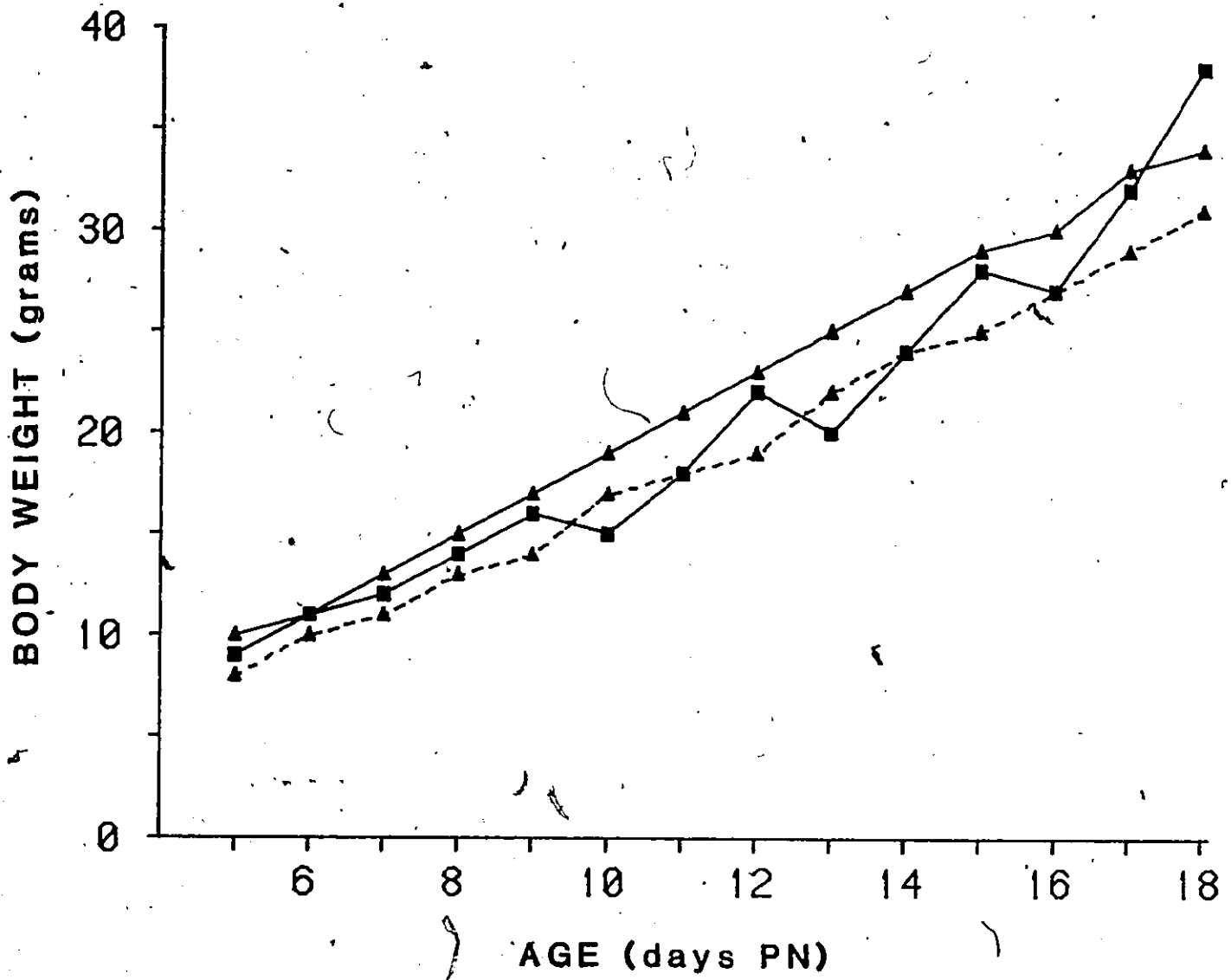


Figure E1

The appearances of some developmental indices are given in Table E1. Relative to the mother-reared pups pinnae unfurling was delayed in the CAR pups but not the IAR pups. The pups that were most delayed on this measure were those animals whose pinnae were not unfurled on the day of surgery, suggesting that surgical trauma may play a role. Eye opening was accelerated by approximately 1 day in both AR groups.

Both AR groups started grooming at an earlier age and groomed more than the control pups until 12 days PN (Figure E2). This trend was reversed after 12 days. Head raising was more frequent in the IAR pups until 11 days PN and thereafter it was more frequent in the control groups (Figure E3). The CAR pups consistently had fewer head raises than the IAR and control pups.

The number of lines crossed were similar for the IAR and control groups until 15 days PN (Figure E4). Thereafter, the control pups were more active. CAR pups had the lowest frequencies throughout the testing period and were delayed in starting to crawl straight and walk on all four legs (see Table E1).

Although both control and IAR pups showed decreasing frequencies of pivoting between 6 and 17 days PN, the IAR pups had consistently higher means than controls (Figure E5). The CAR pups had low pivoting frequencies between 7 and 9 days PN, then there was a sharp increase in pivoting at 12 days PN followed by a decline after 14 days. Qualitatively, both AR groups appeared more frantic and uncoordinated in their pivoting. They would often flop themselves on their backs

Table E1

Mean age at appearance of developmental indices

Group	n[a]	Pinnae Unfurling	Crawling Straight	Eye Opening
IAR	7	3.0	9.0	13.8
CAR	5-6	9.4	13.5	14.3
Controls	11	3.4	8.5	15.1

Note. IAR is the intermittently-fed and artificially reared group and CAR is the continuously-fed group.

[a]"n" is the number of individual animals.

Figure E2. Average time spent grooming (secs) for the control (triangles, solid line), IAR (squares), and CAR pups (dashed line).

Figure E3. Average number of head raises for the control (triangles, solid line), IAR (squares), and CAR pups (dashed line).

Figure E4. Average number of lines crossed for the control (triangles, solid line), IAR (squares), and CAR pups (dashed line).

Figure E5. Average number of pivots for the control (triangles, solid line), IAR (squares), and CAR pups (dashed line).

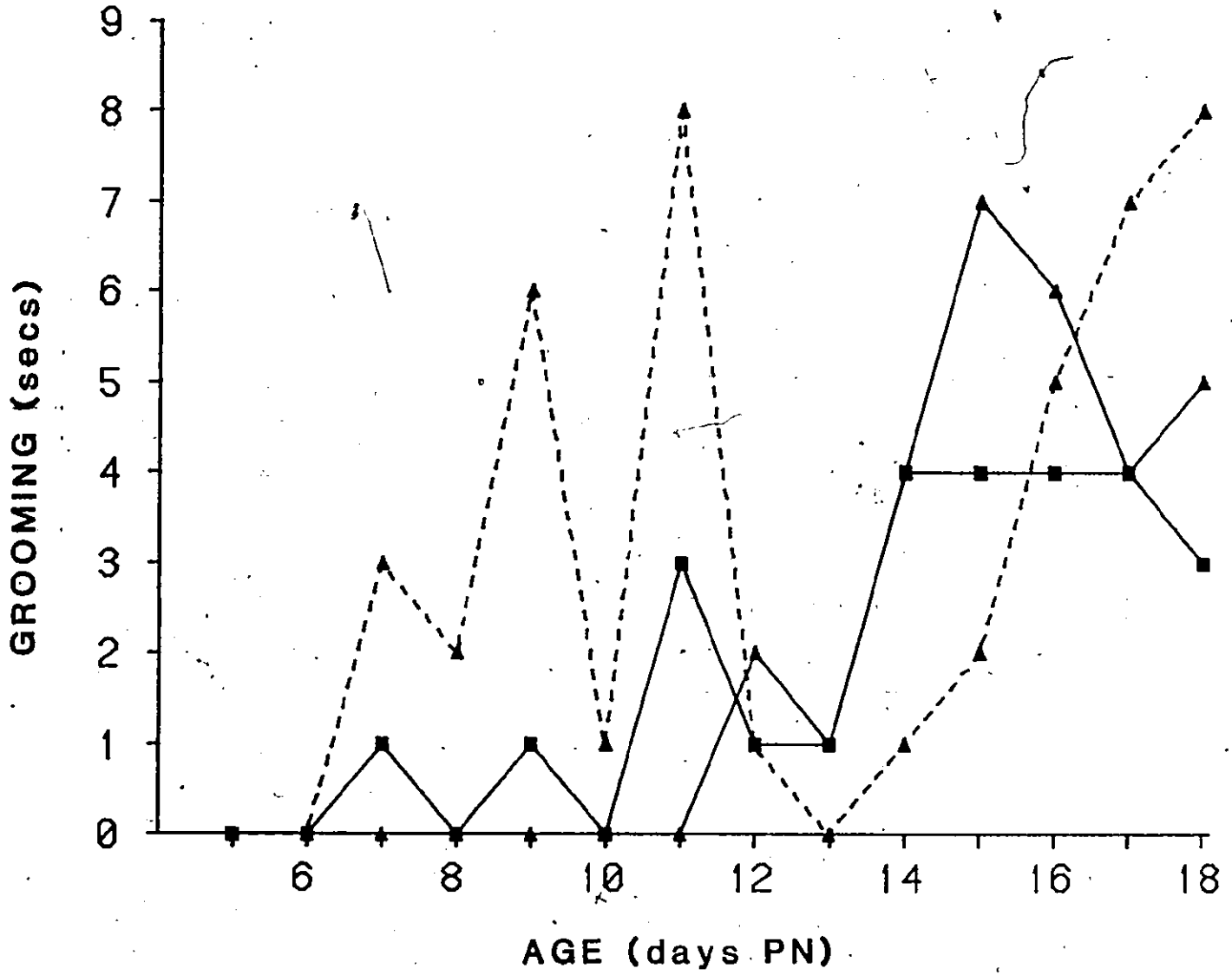


Figure E2

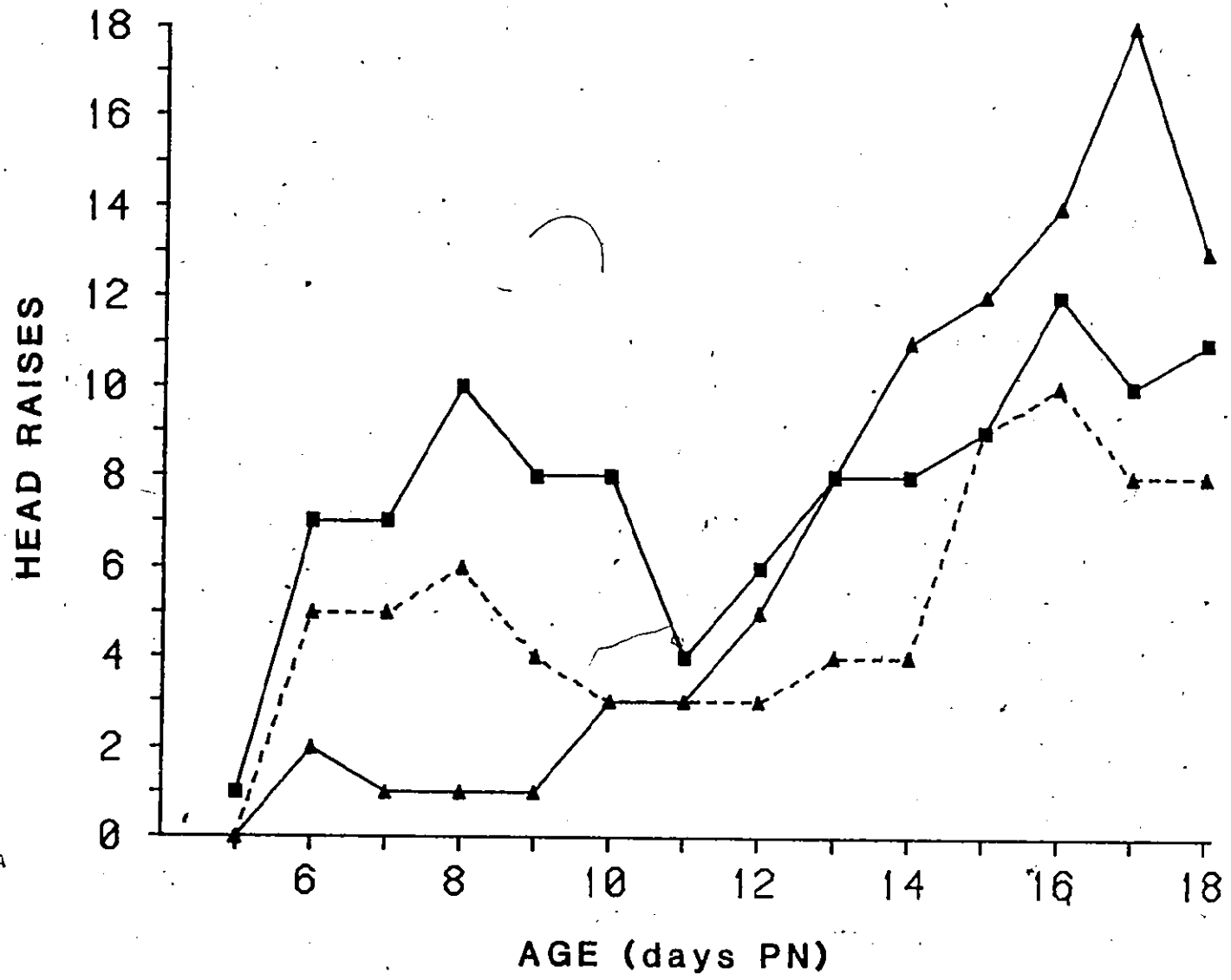


Figure E3

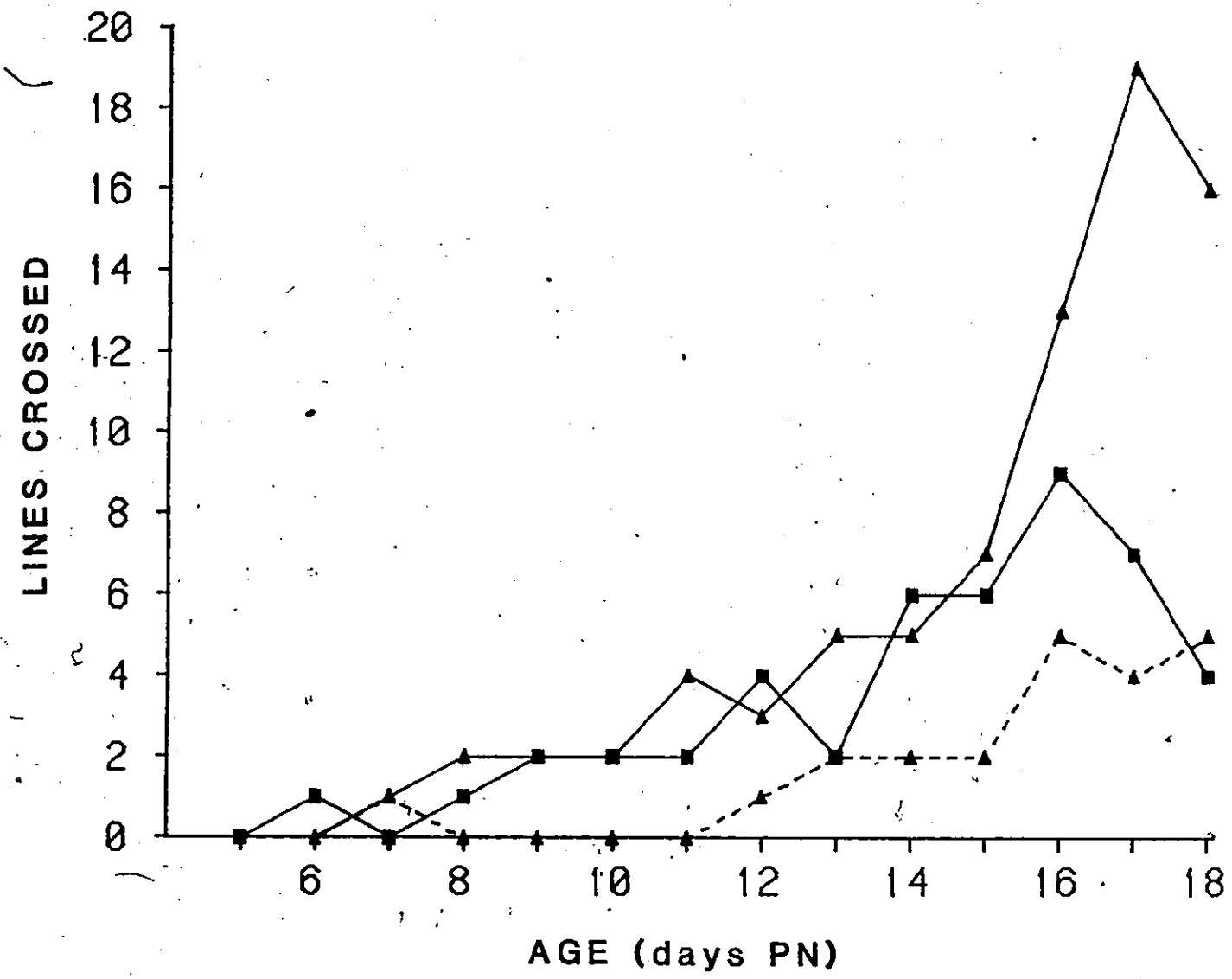

LINES CROSSED
20
18
16
14
12
10
8
6
4
2
0
6
8
10
12
14
16
18
AGE (days PN)

Figure E4

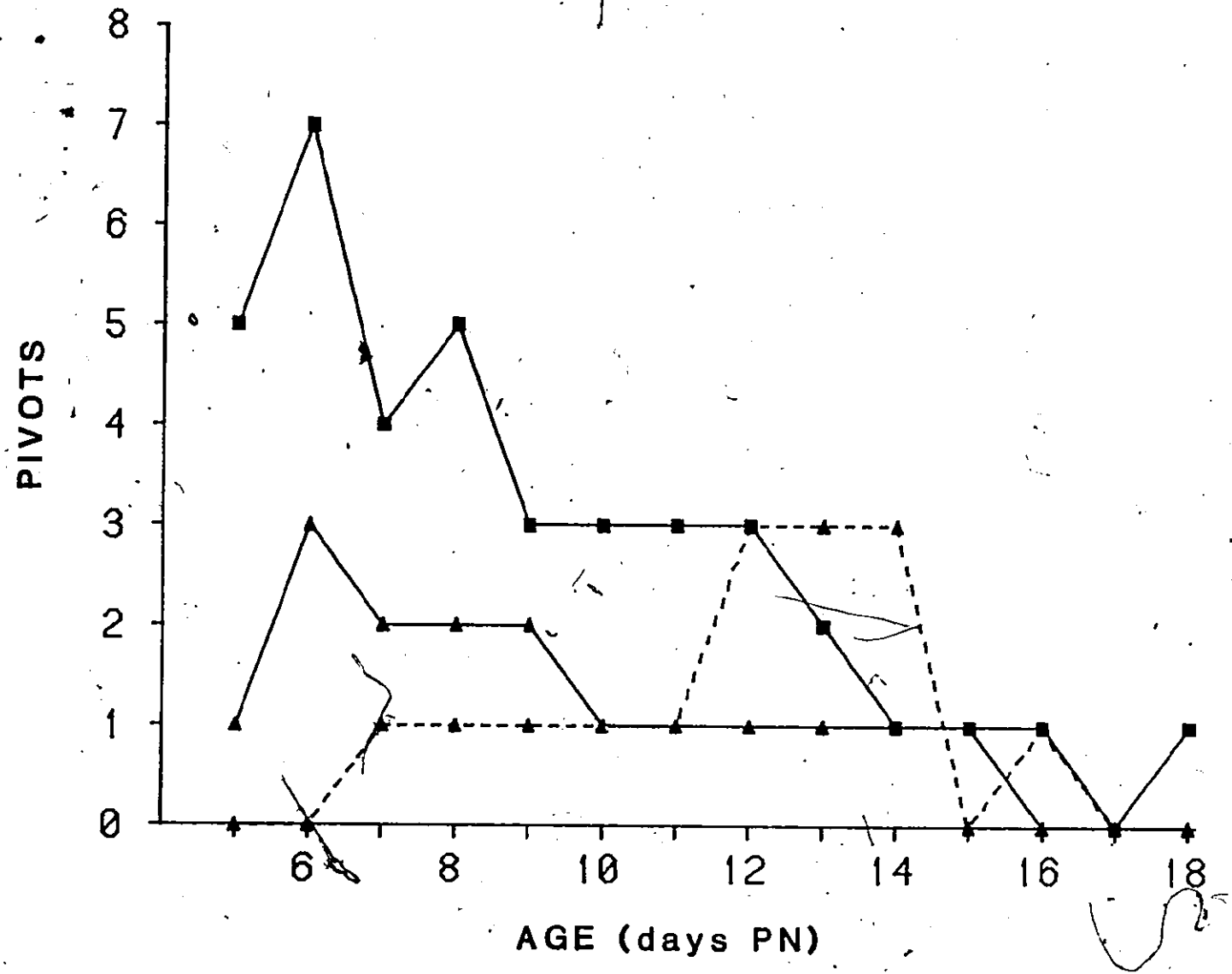


Figure E5

whereas the control pups were more cautious and rarely fell over while pivoting. In addition, the AR pups exhibited whole body tremors which lasted 2 to 3 sec.

Discussion

Relative to the continuously fed AR pups the intermittently fed pups had fairly normal body weight gains and an absence of abdominal bloating (see Chapter 2 for a more thorough discussion of this problem). As with the results reported here, pinnae unfurling tends to be delayed in undernourished rat pups (Smart & Dobbing, 1971; Wehmer & Jen, 1978). However, the observation that the most delayed pups were those whose pinnae were not unfurled on the day of surgery implicates surgical trauma or some other factor, as well as malnutrition. The premature eye opening seen in the AR pups was discussed in detail in Chapter 2.

The greater amounts of grooming seen in the AR pups up through 12 days PN are not readily explained. Hofer (1973) observed that 14-day old pups separated from their dams for 18 hours groomed more than non-separated pups during the first 5 min of open-field testing. Both AR groups in the present study lacked the normal maternal grooming so it is unlikely that a single factor is responsible for this behavior. Head-raising, a possible orientation response, is evident as early as 4 days PN in rat pups (Bolles & Woods, 1964). The effects of undernutrition on this response are equivocal; some investigators report fewer (Smart & Dobbing, 1971; Wehmer & Jen, 1978) or more head

raises (Altman et al., 1971) as a result of early postnatal undernutrition. Age and the severity of undernutrition may be important. Besides dietary factors the AR pups may be responding to the presence of the cannula through the skin on the back of the neck.

Pivoting behavior reaches a peak between 6 and 10 days of age and is then superseded by walking (Blanck et al.,1967). Severely and mildly undernourished rat pups do not pivot as frequently as well nourished pups, suggesting some type of retardation in locomotor development (Altman et al., 1971). The continuously fed AR group in the present study also had low levels of pivoting and was delayed in starting to crawl straight, suggesting that their state of nutrition may be a factor. Nutrition, however, may be secondary to the bloating which occurred in this group. In contrast to the continuously fed group the intermittently fed AR pups had high amounts of pivoting during the first 13 days of testing but they still crossed fewer numbers of lines in the open-field than the control littermates. Diaz et al. (1982a) also observed fewer lines crossed in AR pups during 5 min of open field testing. Similar to these findings is the observation that fifteen-day old rat pups show high amounts of activity when isolated in an unfamiliar environment (Campbell & Raskin, 1978). Pivoting behavior, rather than line crossing, may predominate in the IAR pups since the types of movements that are possible in the artificial rearing environment may be somewhat restricted.

In summary, the AR pups differ from mother-reared rats both

behaviorally and in the timing of some developmental indices such as ear unfurling and eye opening. Nutrition and the maternal isolation may be important but other factors probably also contribute to the observed differences. Included among these factors are surgical trauma, environmental temperature differences, feeding schedules, and the immediate environment of the styrofoam cups in the water bath incubators.

Appendix F
Development of day-night differences in pineal N-acetylserotonin (NAS) levels

Several biochemicals in the pineal gland show strong circadian rhythms including NAT, melatonin, and serotonin (Klein et al., 1981). In addition, very low levels of illumination during the dark suppress NAT activity in adult rats (Minneman et al., 1974). Pineal NAS is the immediate precursor to melatonin and is present in high levels during the dark period. The following study was designed to examine the development of day-night differences in NAS levels and age-related effects of the suppressive capacity of different levels of illumination.

Methods

One to two-week pregnant hooded rats were obtained from Blue Spruce Farms (Altamont, N.Y) in July, 1982 and placed on 12:12 LD (lights on from 0600 to 1800 hrs). One day after birth (Day of birth = Day 0) litters were culled or cross-fostered such that there were 10 to 11 pups in each litter, about half males and half females.

At birth litters were assigned to one of 30 age x light level groups. The six light levels were control dark, control light (88 lux), light level 1 (5.5 lux), light level 2 (1.4 lux), light level 3 (0.35 lux), and light level 4 (0.17 lux). Pups were killed by decapitation (guillotine or scalpel for younger ages) at 4, 6, 7, 10, or 14/15 days of age. In addition, the dams were used as adult

controls.

For the control dark and the four light levels (1 to 4) the following procedure was used on the day of sacrifice: Three female and three male pups were placed in separate compartments in a cardboard box 1 hr before dark onset (about 1700 hrs) and sacrificed about 3 hrs later (between 2000 and 2130 hrs). In the control light condition pups were placed in the box at 1300 hrs and sacrificed at 1600 to 1630 hrs. The cardboard boxes were housed in compartments with the appropriate lighting conditions. A 25W red light was used during the dark and the lower intensity light levels. After decapitation the pineals were extracted within 1 to 2.5 min, placed in plastic mini vials and frozen on dry ice. They were then transferred to a storage freezer and kept at -80 degrees C until assaying in summer, 1983.

NAS assay

A radioimmunoassay (RIA) procedure was used (Pang, Brown, & Campbell, 1981). The frozen pineal was sonicated with 200 µl of ethanol for 30 sec and then washed with 800 µl ethanol. This was centrifuged for 4 min at 8000 rpm and the supernatant was then dried down in an evacuator. Two mls of fresh phosphate buffer (pH 6.5) was added and the mixture was vortexed, then centrifuged for 20 min at 5000 rpm. Fifty µl of diluted tritiated NAS (approximately 2000 cpm) and 100 µl of antisera were added to 400 µl of the pineal supernatant. After 40 hrs of incubation at 4 degrees C, 850 µl of concentrated NH_4SO_4 was added. The samples were then incubated at 4 degrees C for 1 hr, centrifuged for 20 min at 5000 rpm and the precipitate was separated out. Five hundred fifty µl of deionized water was added to

the precipitate and then this mixture was vortexed. Five hundred µl was pipetted into scintillation vials. Five mls of scintillation cocktail were added and the vials were capped, shaken and counted (for 10 min each) in a scintillation counter. All samples were run in duplicate. Five assays were done using the above procedure.

Results and Discussion

The number of picograms (pg) per pineal of NAS was based on a standard curve obtained for each assay. Due to the relatively low correlations between the actual amount and the calculated value obtained for some of the standard curves and the high variability between the assays in the control serum samples the following results should be viewed as very preliminary.

Table F1 shows the average amount of NAS (picograms) at each age and for each light level. A day-night difference is evident at 4 days PN but is not pronounced until 10 days PN. This agrees somewhat with the pronounced day-night differences in pineal NAT at 7 days PN (Ellison et al., 1972) and melatonin at 8 days PN (Tamarkin et al., 1980) in rat pups. There is no consistent pattern to the amounts of NAS observed under the four light levels. The values for the adult animals were consistently low, even in the control dark condition. The reasons for this are unknown; assay problems, the stress due to handling the animals as well as other factors may be responsible.

Table F1

N-acetylserotonin (NAS) levels
in pineal glands

Age (days)	Light Condition: Control Dark	Control Light	Level 1	Level 2	Level 3	Level 4
4	452.1 (63.2)	310.5 (60.0)	641.5 (132.4)	439.2 (130.0)	553.0 (91.7)	365.9 (99.5)
6	657.8 (145.9)	471.2 (45.4)	612.4 (143.8)	448.0 (78.6)	593.7 (116.5)	512.3 (14.8)
7	781.6 (269.0)	581.8 (236.1)	973.6 (205.9)	1005.5 (121.9)	674.7 (163.4)	705.3 (109.2)
10	1351.7 (278.2)	72.9 (36.2)	809.5 (141.3)	902.9 (125.7)	638.5 (178.4)	1090.7 (212.6)
14-15	957.5 (85.9)	259.5 (57.7)	510.2 (46.1)	588.4 (217.7)	615.0 (90.2)	558.5 (146.6)
Adult	211.9 (78.0)	232.2 (34.9)	202.5 (59.6)	230.3 (51.1)	172.7 (17.8)	231.0 (40.3)

Note. Values represent the average amount of NAS (in picograms) of 3 to 6 pineals. Numbers in parentheses are the s.e.m. "Control light" is the brightest and "Level 4" is the dimmest.