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THE ONTOGENY OF MATERNAL BEHAVIOR IN RATS:
MATURATIONAL, HORMONAL AND EXPERIENTIAL INFLUENCES

By

Susan Anne Brunelli

A dissertation submitted to the Graduate Faculty
in Psychology in partial fulfillment of the
requirements for the degree of Doctor of Philosophy,
The City University of New York.

1986

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

THE ONTOGENY OF MATERNAL BEHAVIOR IN RATS: MATURATIONAL, HORMONAL AND EXPERIENTIAL INFLUENCES

by

Susan A. Brunelli

Advisor: Dr. Myron A. Hofer

The present investigation utilized several approaches to examine issues associated with the ontogeny of maternal behavior in prepubertal rats. First, individual 18, 24 or 30 day-old male and female Wistar prepubertal rats (juveniles) were continuously exposed to 3-8 day-old pups for 5 days (sensitization) to determine developmental differences in the expression of maternal and other behaviors. Exposure to pups facilitated the emergence of some maternal behaviors in juveniles but not others, e.g., retrieving, and 24 day-olds retrieved more than at other ages. Few gender differences were apparent. Juvenile play behaviors (charge, pounce) were related to retrieving behavior in the context of exposure to pups. Second, individual 18 or 30 day-old male and female rats were sensitized after infusion of maternal blood plasma, dextrose, 5% in water as a control for handling, or no handling. Plasma from parturient females selectively increased retrieving behavior in 30 day-olds, and more powerfully in females than in males. On the first day both plasma and infusion-control 30 day-olds showed higher frequencies of retrieving, crouching, anogenital licking and contact than nonhandled juveniles. Thirty day-olds receiving maternal blood plasma continued to retrieve pups, with a latency peak at 72 hr., not seen in controls. Eighteen day-olds did not generally show effects. I.V. infusion procedures led to a persistent dissociation of retrieving behavior from play in older plasma and infusion-control animals. Retrieving behavior was associated with play behaviors in younger juveniles regardless of treatment, and in older nonhandled handled animals, with no integration of retrieving with other maternal behaviors. Finally, in 24 day-old juvenile rats early maternal separation and social isolation depressed retrieving behavior, but increased pupcarrying and play. As adults, early-weaned and isolated animals showed persistent alterations in play behaviors, and previously sensitized males showed integration of maternal behaviors. All female groups showed equal facilitation and integration of maternal behaviors. Overall, the results suggest that early maternal behaviors are distinct developmentally, but come under control of hormonal mechanisms, which serve to facilitate integration.

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I also thank my Dissertation Committee members, as a group and separately. Dr. Gerald Turkewitz encouraged those insights which took the form of inspired hunches and helped translate them into testable hypotheses. Dr. Cheryl Harding lent her considerable expertise in hormones and behavior and critical questioning of aspects of the dissertation. Dr. Michael Myers contributed a contagious enthusiasm for science in general, and developmental processes in particular. Dr. Jay Rosenblatt, whose laboratory generated the original data which inspired this dissertation, and whose work was my first introduction to maternal behavior, provided thoughtful comments and suggestions through the years.

Thank you to Richard Shindledecker for many fruitful discussions of statistics, data analysis and SAS, and for the hours spent slaving over a hot terminal. Thank you also to "Mr. Gus" Romano for help in maintaining a large rat colony, for data collection and analysis above and beyond the call of volunteering, and for occasional "muscle work".

Finally, I thank my husband and colleague, Harry Shair, for running me around Grand Army Plaza (and all that that implies); my parents, Louis and Sylvia Brunelli, for providing a retreat (complete with gourmet meals) when the going got tough; and my daughter Carolyn, who participated in the process of writing this dissertation, even before she was here.

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INTRODUCTION

Maternal Behavior as Maternal Behaviors

In the rat, as in other mammals, the term "maternal behavior" subsumes a number of activities directed toward newborn young by a parturient, lactating female. Four index behaviors are commonly recognized as "maternal" in the rat: 1) building a "maternal" nest having distinctive parameters to enclose and protect young; 2) retrieving stray pups to the nest; 3) assuming a nursing ("crouch") posture for feeding; and 4) licking and cleaning of pups, especially in the anogenital region (Rosenblatt and Lehrman, 1963; Sturman-Hulbe and Stone, 1929; Wiesner and Sheard, 1933) Responsiveness to intruders is often included as a maternal behavior, since it is displayed in the period from late pregnancy to several weeks after parturition (Erskine, Barfield, and Goldman, 1978; 1980; Sturman-Hulbe and Stone, 1929; Wiesner and Sheard, 1933); and, in addition, placentophagia (i.e., eating of the placenta), which occurs during parturition (Kristal, Peters, Franz, Whitney, Nishita and Steuer, 1981; Rosenblatt and Lehrman, 1963; Sturman-Hulbe and Stone, 1929; Wiesner and Sheard, 1933). Other behaviors could also conceivably be thought of as maternal, for example, the inhibition of pup-killing and cannibalism at parturition (Peters, 1980). All of these behaviors appear at, or a few days before parturition, as a well-integrated sequence elicited immediately by interaction with neonates (Rosenblatt and Lehrman, 1963; Rosenblatt and Siegel, 1975; Sturman-Hulbe and Stone, 1929; Wiesner and Sheard, 1933) with the timing of their onset dependent upon hormone actions occurring at the end of pregnancy (Moltz, Lubin, Leon, and Numan, 1971; Rosenblatt and Siegel, 1975; see also, reviews by Moltz, 1971; Rosenblatt, Siegal, and Mayer, 1979).

In addition to direct measures of maternal behavior, other, biobehavioral activities are also involved in events occurring during the pre- and postnatal period. Among them are: alterations in threshold for thermoregulatory behaviors and increased core temperature (Leon, Croskerry and Smith, 1978; Thoman, Wetzel and Levine, 1968; Woodside and Leon, 1980), alteration of pituitary-adrenal response to stress (Thoman, Wetzel and Levine, 1968; Zarrow, Schlein, Denenberg, and Cohen, 1972), increased caecotropic activity (Leon, 1974; Moltz and Lee, 1981), increased food intake (Fleming, 1976), and others. These activities occur in addition to hormonal and physiological adjustments accompanying pregnancy, parturition and lactation (see Rosenblatt and Siegel, 1981; Tindal, 1978 for reviews).

Nonpregnant, nonparturient/lactating animals which have been "sensitized" (Noirot, 1972) to exhibit maternal behavior by continuous exposure to infants have been observed to engage in maternal behaviors (Fleming and Rosenblatt, 1974a; Rosenblatt, 1967, Wiesner and Sheard, 1933). The expression of maternal behaviors in sensitized rats has been shown to be nonhormonally based (Rosenblatt, 1967), and seems to result from the activation of underlying processes present in all species members, irrespective of parity history.

Qualitative differences exist between lactating and sensitized maternal retrieving however, in for example, the selectivity of retrieving (Beach and Jaynes, 1956; Rosenblatt, 1975; Wiesner and Sheard, 1933,) in the immediacy and constancy of the response when retrieved objects are pups (Wiesner and Sheard, 1933), as well as the severity of obstacles which a mother will surmount in order to retrieve pups (Bridges, Zarrow, Gandelman and Denenberg, 1972; Nissen, 1930). Sensitized animals also show deficits in nestbuilding and attacking of intruders (Fleming and Rosenblatt, 1974a; LeRoy and Krehbiel, 1978), and in measures of maternal motivation (Bridges, Zarrow, Gandelman and Denenberg, 1972; Stern and MacKinnon, 1976).

However, as Rosenblatt et. al. (1979) have pointed out, many of the activities that are thought of as "maternal" can occur separately and in other contexts as well. They use the example that retrieving and nestbuilding may be less specialized than nursing and pup licking. Thus, retrieving can be pressed into the service of nestbuilding or food carrying by nonpregnant/nonlactating animals.

The execution of nestbuilding does not require the presence of pups, and is the normal response of adult and juvenile rats to drops in ambient temperature below thermoneutral (Kinder, 1927). Nestbuilding in nonpregnant/nonlactating animals can also be eliminated by raising ambient temperature, whereas this is not true of pregnant and lactating females nursing pups (Kinder, 1927). Also, rats groom each other in other contexts, and anogenital licking is common in rats that are strange to one another (Grant and McIntosh, 1963). On the other hand, licking pups may be a specialized maternal activity and the crouch posture seems to be uniquely adapted to nursing pups, and is not seen in any other context (Rosenblatt, et. al., 1979).

Thus, with some exceptions, the components of maternal behavior represent biobehavioral processes which are uniquely altered in pregnant or lactating females, but which can be observed in sensitized animals, and animals in other contexts. Presumably, the processes underlying them are shared in common among species members, and therefore are subject to the same general regulatory constraints. The fact that maternal behaviors can occur separately as well as together, and in nonmaternal as well as maternal animals, suggests that each behavior is capable of being studied independently of the others.

Studying the Development of Maternal Behavior

If maternal behavior represents the functional organization of physio-behavioral systems around care of young, how should the developmental origins of maternal behavior be studied? One potential problem with studying development of maternal behavior is that, as noted above, it is a functional entity, probably comprised of many discrete systems. The second is that maternal behaviors are not normally manifested as an organized pattern until adulthood, and generally only in adult females. Unlike feeding behavior, for example, there is no "infantile form" of maternal behavior shown normally in young animals, which develops into an adult pattern (Hall & Williams, 1983). Also, factors influencing maternal behaviors during ontogeny may be difficult to detect because they may involve developmental processes not recognizable as maternal behaviors *per se*. Rather, these may contribute in a general way to physiological, behavioral and social development, and thus to the expression of maternal behaviors in adulthood, when optimal internal and external conditions are met. Similarly, because maternal behavior is actually a collection of behaviors, factors influencing the expression of one of the behaviors could affect others, and would, therefore, be difficult to separate causally. This calls for the identification and isolation of components of maternal behavior during development, and perhaps, possible clues as to the mechanisms by which they are ultimately expressed in adulthood. One of the aims of the set of studies in this dissertation is to examine the expression of discrete components of maternal behavior, under a variety of conditions, before puberty as well in adulthood, as a first step toward that end.

Factors Influencing the Development of Maternal Behaviors

Gregariousness

Recently, an approach based on the strategy of studying maternal behavior during development, has been utilized by several investigators. Bridges, Zarrow, Goldman and Denenberg (1974) showed that juvenile rats at 24 days of age could be sensitized to show retrieving behavior with very short latencies (about two days), regardless of sex. Mayer and Rosenblatt (1979b), in observations of groups of rats between 18 and 30 days old, found that up until 24 days of age juvenile rats appeared to perceive one-week old stimulus pups as social objects. Juveniles sniffed, licked, manipulated pups and spent significant amounts of time huddling in contact with them. With extended exposure, components of maternal behavior (retrieving and nestbuilding) appeared. After 24 days there was a marked decrease in contact and a corresponding increase in latencies for induction of maternal behaviors; this change was attributed to the advent of adult neophobic responses to novel objects. They also studied cue stimuli such as warmth, and age-related responses to pup stimulus properties. In preference tests younger juveniles (20-21 day-old) preferred infants, whereas older juveniles (>24 day-old) spent a greater amount of time with age-mates. Both groups preferred stimulus pups over warm, inanimate objects and older (8 day-old) stimulus pups over younger (2 day-old). Mayer, Freeman and Rosenblatt (1979) found that handling facilitated sensitization at 30 days for both sexes, but by 45 days only males responded to handling, and gender differences in latencies were present thereafter. After 45 days anosmia induced by zinc sulphate treatments were most effective in sensitization, rather than handling. The authors interpreted these results as indicative of separate processes whereby a generalized timidity, on the one hand, and olfactory-based avoidance on the other hand, inhibit maternal behavior, differentially in the sexes.

Mayer and Rosenblatt have suggested that the behavior of juveniles exposed to neonates is "...a mixture of customary responses to age-mates and responses to the special properties of the pups..." (p.422). Their conclusion regarding the attraction and repulsion of juveniles for pups addresses mechanisms whereby rats come to be maternal either in the context of parturition or sensitization. These same mechanisms have been explored in adults by a number of workers, especially the roles of olfaction, and timidity or fear of novelty, in inhibiting maternal behavior (Bridges, Zarrow, Goldman, and Denenberg, 1972; Fleming and Luebke, 1981; Fleming and Rosenblatt, 1974b,c; Fleming, Vaccarino and Luebke, 1980; Mayer et. al., 1979; Mayer and Rosenblatt, 1975, 1977; Terkel and Rosenblatt, 1971). Thus both early and later determinants of this aspect of the regulation of maternal behavior have been explored, and an ontogenetic relationship between them has been established to some extent. As Mayer and Rosenblatt (1979) have proposed with regard to the development of these responses, they "...arise ontogenetically from gregarious behavior...which itself undergoes developmental changes in relation to the stimuli involved." (p.422.)

However, another area of gregarious juvenile behavior which has not been researched in relation to maternal behavior is play. Since social play is the primary juvenile behavior from approximately weaning to sexual maturity (Thor and Holloway, 1984), this would appear to be a fruitful area of inquiry when investigating ontogenetic antecedents of maternal behavior. Play has no apparently explicit function, and definitions of play generally consist of descriptors, such as the following: 1) behavioral acts in play are similar, but not identical to those that occur in adult functional contexts; 2) A single play sequence may include acts from several functional contexts; 3) play sequences lack consummatory acts and biological consequences; 4) the form of play sequences are exagerrated, repeated frequently, and variable (unpredictable) (Fagen, 1981). Various hypotheses have been advanced for the role of play in development, such that play functions to provide practise, exercise, flexibilility, socialization and pleasure (see reviews by Fagen, 1981; Panksepp, Siviy, and Normansell, 1984; Smith, 1982; Thor and Holloway, 1984). In a recent discussion of play, Fagen (1981) has suggested that one of its hypothesized functions is to transform fragmented sequences of behavior into complex, organized behavioral patterns. In the same vein, Hofer (1981) has likened play to individual movements found in the prenatal period in promoting neurobiological development, in terms of neural integration "..of systems capable of complex, goal-directed, sequences that allow adaptation to the social and inanimate environment." (p. 274). The present work begins with the premise that play is involved in the early organization of maternal behavior. It will thus focus on the investigation of relationships between play and maternal behaviors in different juvenile age-groups and under different conditions.

Responsiveness to Hormones

There have been several investigations of the development of hormonal regulation of maternal behavior as well (see reviews by Mayer, 1983; and Rosenblatt et. al., 1979). While these studies have examined the relationships of perinatal and pubertal hormonal influences on adult maternal behavior, relatively few have attempted to observe the impact of hormonal manipulations on maternal behaviors expressed prior to puberty. One of these, by Koranyi, Lissak, Tamasy, and Kamaras (1976), reported briefly as part of a review article that they administered blood plasma from newly-parturient females via tail vein injection to 18 to 24 day-old male and female rats. In response, 90-100 percent of injected animals were showing retrieving, nursing and crouching, nestbuilding, and startle and orienting responses to pups within 12 hours of initial exposure to pups, as opposed to 0-12 percent of saline-injected animals. Mayer and Rosenblatt (1979a) have found that prior administration of pregnant mare's serum (PMS) to females at 21 days to induce premature cycling, produced 30 day-old animals with latencies to become maternal midway between cycling adult females (3-6 days) and 30 day-olds (>6 days). Handling also had less of an effect in reducing the latencies of these animals than on 30 day-old control females injected with saline. Bridges et. al. (1974) found that testosterone administered beginning at 21 days produced no change in either the latency to sensitization (0.4 - 2 days) or the number of males that were sensitized at 24 days of age (64 - 88%). This is in contrast to findings that the presence of endogenous or exogenous testosterone in male rats produces long latencies, as well as marked inferiorities in maternal behaviors when tested <u>after</u> puberty (Bridges, Zarrow, and Denenberg, 1973; Fleischer, Kordower, Kaplan, Dicker, Smerling and Ilgner, 1981; LeRoy and Krehbiel, 1978; Lubin, Leon Moltz and Numan 1972;

McCullough, Quadagno, and Goldman, 1974).

Together, these results produce a somewhat confusing picture of the role of hormones in affecting maternal behaviors shown during the prepubertal period. Koranyi et. al.'s review suggests that the all-or-none maternal responsiveness found in adult females is present and capable of activation before puberty. This activation, occurring in both males and females, appears to exist prior to the maturation of sex differences in these same behaviors. Mayer and Rosenblatt's and Bridges et. al.'s results, on the contrary, indicate that certain characteristically juvenile responses are expressed during sensitization, despite manipulation of hormones which should result in the expression of adult responses. This would imply that neural substrates are, apparently, not wholly responsive to hormone action at this age. However, all of these studies do underline the fact that developmental changes are occurring in relation to hormone regulation in juveniles, and that these may represent, interact with, or be tied to, the development of parallel systems.

The second of the present set of studies will attempt to resolve some of the difficulties that have arisen in observing the effects of hormone manipulations on juvenile rats. Although Koranyi et. al. claim to have prsented hormones in the same manner as Terkel and Rosenblatt's (1968; 1972) original studies with adult females, their review paper presents few details of methodology or results.

Therefore, this study will replicate that method of presentation, i.e., via infusion of maternal blood plasma, and in a strain of rats that has never received this type of manipulation.

Early Experience and Maternal Behavior

One strategy for studying the development of maternal behavior has involved looking at the effects of early experience, particularly maternal and social deprivation, on adult performance of maternal behaviors after parturition, and during lactation. For example, Thoman and Arnold (1968) found that maternally-deprived animals had significantly higher pup mortality than mother-reared, and weight and growth parameters in surviving pups were adversely affected as well. In two different studies, premature weaning at 14 or 15 days produced selective deficiencies in nestbuilding behavior (Novakova, 1976) or resulted in less contact and nursing behavior shown by prematurely-weaned mothers (Skolnick, Ackerman, Hofer and Weiner, 1980). Reiss (1954) reared female rats in social isolation, and in an environment without manipulable objects: after parturition these animals did not build nests, and also had high pup mortality rates, largely the result of leaving nestbuilding materials and pups scattered throughout the cage. Eibl-Eibesfeldt (1955) and Wehmer (1965) showed that object-deprived animals could build nests and rear pups adequately in a familiar, as opposed to a novel environment. Object-deprived animals still showed delays in nestbuilding before parturition, but not afterward. Rearing in unisexual litters (Sharpe, 1975) also adversely affected the amount and quality of nestbuilding, compared with rearing in bisexual litters.

Finally, Gray and Chesley (1984) found that 22 day-old male and female rats were able to differentiate between rat pups or pup-sized toys in the same manner as adults (Rosenblatt, 1975), but did not show the full range of maternal behaviors during sensitization. Retested as adults with pups, females and males preexposed to pups as juveniles showed more retrieval and licking of pups than those exposed only to toys. Thus, while juveniles are capable of responding to pups in the same "maternal" manner as adults, they differ from adults in the range and consistency of maternal behaviors shown. Moreover, exposure to pups during the juvenile period apparently increases susceptibility to sensitization in adulthood.

These studies demonstrate that some aspect of each of those early experiences produced alterations in particular behaviors, which, in turn, affected the general quality of maternal performance. One could conclude from these that there are discrete processes or mechanisms which are intrinsic to the separate development of each maternal behavior, and that these processes are probably different for different maternal behaviors. However, what is missing from most studies which have looked at the effects of early social experience on maternal behavior, is an examination of the impact of their manipulations at various points in development. As illustrated by the Gray and Chesley study, juvenile experiences with infants in and of themselves can affect maternal responsiveness to pups later in life. Thus, it is necessary to observe, and to establish relationships between forms of maternal behavior patterns as they are affected by early experience at different times in life. The third set of experiments in this dissertation will attempt to do so, placing particular emphasis on looking at the effects of premature weaning or social isolation on maternal behavior patterns both during the juvenile period and in adulthood.

Aims of This Dissertation

The foregoing studies are important because they provide the basis for the design of an ontogenetic approach to the study of maternal behavior in the rat. The present work will utilize this approach to investigate issues associated with the ontogeny of maternal behavior. Since, as discussed above, juveniles can be sensitized, behaviors elicited will be analyzed to: 1) observe the separate components of maternal behavior in their early expression; 2) observe changes in the expression of maternal behaviors by manipulations which alter both the internal (hormonal) and external environment; 3) make comparisons between juvenile and adult expression of maternal behaviors; and 4) observe the effects of early experience both acutely, and later in life.

Development of Maternal Behaviors
in Prepubertal Rats at Three Ages:
Age-Characteristic Patterns of Responses

When adult female and male rats are continuously exposed to infant rats they will, in time, exhibit components of maternal behavior (retrieving and gathering pups; licking; nestbuilding; crouching in nursing position), similar to that shown by parturient, lactating females (Rosenblatt, 1967; Fleming and Rosenblatt, 1974a; Reisbick, Rosenblatt and Mayer, 1975). This phenomenon, whereby maternal behavior is elicited in the presence of pups, termed "sensitization", has been reliably reproduced in a variety of laboratory strains by numerous investigators (see review by Rosenblatt, Siegel, and Mayer, 1979).

Bridges, Zarrow, Goldman and Denenberg (1974) showed that prepubertal rats could also be sensitized, with 24 day-olds showing an average latency to sensitization of about two days. Juvenile latencies rise thereafter to about 6 days in 30 day-olds (Mayer, Freeman and Rosenblatt, 1979), approximating adult latencies. Unlike adults, prior to 45 days of age, prepubertal juveniles show little evidence of gender differences during sensitization (Mayer et. al., 1979). However, weanling males do show more retrieval behavior than females (Gray and Chesley, 1984), which is not true of adult males (Rosenblatt, 1967; Lubin, Leon, Moltz and Numan, 1972).

Differences have also been observed between prepubertal animals and adult females in the quality of maternal behavior shown during pup exposure (see Mayer, 1983, for review). First, juvenile retrieving appears to have a "piecemeal" quality. For example, juveniles do not consistently retrieve pups from day to day, or even within a single test period. Second, while all components of maternal behavior have been observed at times in juvenile rats, retrieving and licking tend to be the predominant maternal activities shown during sensitization (Gray and Chesley, 1984). Thus, in juvenile rats retrieving may not be representative of other maternal behaviors, because of its apparent lack of association with them.

The first major aim of the present investigation was to address some of the questions regarding the "piecemeal" quality of maternal behaviors shown by juvenile rats. Because at roughly 18, 24 and 30 days of age juveniles respond in characteristically different ways to initial exposure to infants (Mayer and Rosenblatt, 1979b), we chose these ages to observe behaviors systematically during continuous pup exposure in our strain. We were interested in tracing the occurrence and frequency of each of the maternal behaviors (retrieving; licking; nestbuilding; crouching) to determine: 1) whether this procedure would selectively facilitate some maternal behaviors better than others; and 2) whether these would vary with respect to each agegroup. Since previous work has indicated that juveniles do not commence organized maternal behavior in the same all-or-none fashion as adult females, our hypothesis was that before puberty, maternal behaviors are not well correlated. We also wished to determine whether increasing integration of maternal behaviors would emerge as a function of age.

A second major aim of this study was to further characterize gregarious behaviors directed at pups by juveniles at 18, 24 and 30 days of age. It has been suggested that for juveniles maternal behavior may arise from a generalized gregariousness which changes both ontogenetically and with relation to pup stimuli (Bridges et. al., 1974; Mayer, 1983; Mayer and Rosenblatt, 1979b). In this context, the term "gregarious" has been used to describe a general affinity for body contact with pups on the part of juveniles. However, in the litter situation juvenile gregariousness is also expressed as highly specific sets of behaviors ("play behaviors") performed with siblings and which vary in occurrence and frequency by age and sex (Meaney and Stewart, 1981). On the basis of pilot observations of juveniles during both pup exposure and sibling play bouts we chose to observe a few easily-recognizable behaviors as "play" markers (Poole and Fish, 1975; Meaney and Stewart, 1981; Pellis and Pellis, 1983). Using these, we proposed to test the hypothesis that play behaviors form the "substrate" from which some maternal behaviors arise in juveniles. By looking at the occurrence and frequency of all behaviors separately, we hoped to observe relationships between specific forms of gregarious behaviors, such as play, and specific maternal behaviors. A subsidiary hypothesis was that if such relationships existed between juvenile gregarious behaviors and maternal behaviors, then they might change with age.

Methods

Subjects and Housing

The subjects were 18 (n=17: 14 females/3 males), 24 (n=18: 10 females/8 males) and 30 day-old (n=17: 10 females/7 males) Wistar strain rats (Rattus norvegicus) born in the laboratory to dams received at 18-19 days of pregnancy from Marland Farms (New Jersey). Dams were housed in terraria (40 x 20 x 24 cm) with wood shavings as bedding material. Food (Purina Rat Chow #5008) and water were available ad libitum. Animals were maintained under conditions of regulated temperature (23° ± 2°) and a reversed 12 hr dark/light cycle with lights off at 0700 and on at 1900 hrs. All litters were culled to 9-10 pups within 72 hr of birth and left undisturbed until either the day prior to testing or until 24 days of age (weaning), whichever came first.

Testing Conditions

Subjects were all tested in the maternal room in which they were raised. Throughout the sensitization period animals were housed individually in terraria of the same dimensions as home cages but which were modified with fine, wire mesh (guage = 0.3 cm square) false bottoms placed over shavings. The false bottoms facilitated seeing nest construction more easily, but allowed urine to pass through to the shavings below. A handful of shavings, two shredded paper towels, rat chow and a small amount of litter from the home cage were scattered about the false bottom, and a water bottle was suspended from the cage top. During the dark cycle the room was illuminated by a 75W red bulb suspended from the ceiling over the test area. A lamp with a 25W red bulb placed in front of cages (approximately 0.5 meter away) provided additional illumination during testing.

Observation Procedures

On Day 0 of testing, after two hours of habituation to the new cage, presentation of 2 freshly-suckled pups (test pups) provided by a donor female, began. Testing was done between 1300 and 1800 hrs. Test pups ranged in age from 3 to 8 days old. For the first test (Day 0) test pups were placed in the front of the cage; thereafter they were placed in the front corner opposite the subjects' sleeping nests. At the time of presentation, time-samples of behavior were recorded over a 15-min period. An observation consisted of noting in a 5-sec period the occurrence of the behaviors described below. Each animal was observed at 1-min intervals for a total of 16 times per 15-min session (Time 0 - Time 15 = 16 samples). It was from these time samples that we derived our frequency data. The test pups remained with each subject for 24 hr, at which time they were replaced with a fresh pair of test pups, and another 15-min observation session began. We followed this procedure for 5 days (Days 0-4) of exposure to pups.

In addition to the 15 minute test, beginning on Day 1, 15-30 min prior to presentation of new test pups, we noted the positions of subjects and test pups in the cage, as well as the occurrence of retrieving, crouching, nestbuilding or licking. We also rated the quality of subjects' nests using a rating system developed after Mayer and Rosenblatt (1979a).

Variables Recorded

There was a total of 16 behaviors included in our behavioral observation checklist. They can roughly be divided into the following four categories: A) Maternal Behaviors; B) Play & Exploratory Behaviors; C) Other (Noncontact) Behaviors; D) Passive Contact. Maternal Behaviors (A) included: 1) Retrieve - juvenile picks up pup and carries into nest or sleeping area; 2) Pupcarry -

juvenile pickes up or moves (pushes/pulls) pup, but not any appreciable distance to nest or sleeping area; 3) Nestbuild - juvenile picks up nesting material and places it in nest or arranges placement of nesting material in nest; 4) Ventral Crouch - juvenile crouches over pup with back raised in an arch and legs splayed, exposing its ventral area (nipple line) to pup(s); 5) Anogenital lick - juvenile engages in licking pup's anogenital area for a 2-5 sec period. Play & Exploratory Behaviors (B) which were directed at pups included: 1) Charge - juvenile rapidly approaches pup with bounding, leaping or hopping motions, behavior normally seen in context of sibling play bouts (after Poole and Fish, 1975); 2) Pounce - juvenile hops in an arc, landing with front paws and muzzle on pup, motion usually directed toward nape of the neck in juvenile play bouts (Poole and Fish, 1975; Pellis and Pellis, 1983). 3) Sniff/lick/paw - sniffing, licking and/or pawing pup, exclusive of anogenital area, usually seen in juveniles in first 5 min of exposure (after Mayer and Rosenblatt, 1979b). Other Behaviors (C) included all of the following behaviors performed while not in contact with pups: 1) Self-groom - juvenile grooms face or body; 2) Rest - juvenile sits, lies, reclines; 3) Eat/Drink; 4) Locomote - any locomotor action involving four limbs, including walking, running, hopping, digging, not directed at pup; 5) Sniffing - sniffing cage, objects in cage, air; 6) Pica - chewing on objects not food, e.g., shavings; 7) Rear - juvenile rears on hind legs, or climbs water bottle. Passive Contact (D) - juvenile engages in any vegetative activity in body contact with pup(s), e.g., self-grooming, lying/huddling, eating, drinking, pica, etc.

Observer Reliability

Three observers, in addition to the first author, were involved in data collection. Observer reliability was evaluated by conducting individual recording sessions with each of the three observers for 2-6 days of testing (8 animals tested per day). Agreement of each of the observers with the first author was found to average 82%.

Data Analysis

Observation data were analyzed with three questions in mind. The first question addressed whether or not retrieval behavior could be correlated with other maternal behaviors for individual juveniles within each agegroup. Criterion for inclusion in within-individual associations was the first occurrence of two consecutive days of one or more of the four maternal variables. We also did between-agegroup comparisions of percents of animals maternal; latencies to become maternal were also compared between agegroups. For this measure, we defined as "maternal" animals who retrieved at least one pup during a 15 min test for two consecutive days, a criterion that has been used in previous studies with adult animals.

Since their distributions were not necessarily normal, latency and percent data were analyzed using nonparametric tests (Kruskal-Wallis repeated measures; Mann-Whitney U; Siegel, 1956). Within-individual associations were analyzed using point-biserial correlations (Welkowitz, Ewen and Cohen, 1971).

For the second question frequency data derived from time-samples were used to determine differences among the three agegroups in levels of performance of each behavioral variable. For this measure, no criterion for performance was utilized. Daily percent occurrences of each behavioral variable were also examined and were found to be congruent with frequency data; therefore frequency data were used, due to the increased sample sizes which they afforded, and the more powerful analyses possible on normally distributed data (Winer, 1971). A nested design blocked on replications with repeated measures was employed to evaluate main effects and interactions of agegroup, sex and days of testing for each behavior (ANOVA: AGE x SEX x DAY). The Newman-Keuls test was utilized to make specific comparisons between individual means. In addition, linear regression analyses were performed (based on 3-way ANOVA main and interaction effects, above) to determine whether or not behavioral frequencies changed in an orderly fashion within each agegroup over the five days of testing. Frequencies on Day 0 were then separately analyzed to determine the relative contributions of maturational and sensitization processes. For statistical tests of frequency data, litter effects were tested using individual within litter as the error term.

The third question addressed how juvenile gregarious behaviors relate to maternal behaviors. For this measure principal component analyses (with rotations) were utilized to determine whether or not there were underlying relationships among these behavioral variables within each of the agegroups. These analyses also provided another means to assess integration of the maternal behaviors within agegroups, though not within individuals.

Questions two and three were analyzed using SAS (Statistical Analysis System, Cary, N.C.) on Montefiore Medical Center's IBM 370/30810 computer.

Results

Sensitization in Juveniles: Retrieving and Other Maternal Behaviors

Eighteen, 24 and 30 day-olds which were sensitized did not differ with respect to latencies to retrieve: median latencies were 2, 1 and 2 days, respectively. However, as shown in Table 1, there were significant differences among agegroups in the percentage of animals that retrieved ($H=8.26$, $df=2$, $p=0.02$; two-tailed).

Post hoc tests showed that 24 day-olds accounted for these differences with significantly higher cumulative percentages retrieving than either 18 or 30 day-olds. There were no sex differences within any of the agegroups for this measure.

Table 2a shows that nine 18 day-olds, seventeen 24 day-olds and eleven 30 day-olds met criterion for point-biserial analysis, showing at least one maternal behavior for two consecutive days. We found that significantly more 24 day-olds met criterion than either 18 or 30 day-olds, this measure paralleling results for retrieving. None of the groups showed a significant correlation between the occurrence of retrieving and the appearance of one or more of the other maternal behaviors in individual animals. When we included individuals showing any maternal behavior without imposing a criterion for consistency (Table 2b) we found that individuals at 30 days did show a significant correlation. These data suggest that animals of this age can show all of the maternal behaviors at least once during testing, but there is little evidence of integration or consistency in their expression. However, as Tables 2a and b show, the direction and amount of change in coefficients across ages suggest the possibility of development of an integrative capacity with increasing age.

Maternal Behaviors: Frequency Data - Age-Related Differences

Given that little if any, integration of maternal behaviors occurs in juvenile rats, we next looked at the frequency of occurrence of each behavior, to further distinguish our agegroups.

It should be noted that frequencies shown are relative to the total number of observations possible based on time-samples over the entire five-day period of testing (16 per day times 5) for each behavioral variable. The mean frequencies over five days for maternal behaviors and contact are shown for each agegroup in Figure 1.

The main effects of Age and Sex for the maternal behaviors and for contact over the five days of testing are as follows: 1) 24 day-olds showed higher frequencies of retrieving than either 18 or 30 day-olds (F=11.56, df=2,46, p=0.0001); 2) 18 day-olds engaged in more contact than the older two groups (F=22.6, df=2,46, p=0.0001); 3) 24 and 30 day-olds engaged in more anogenital licking of pups (F=4.17, df=2,46, p=0.02) than 18 day-olds. We found no differences among the agegroups for ventral crouching, which occurred at very low frequencies throughout testing. There was an Age x Sex interaction for nestbuilding (F=5.72, df=2,46, p=0.0061): this was accounted for by the 18 day-old males, which showed higher frequencies of nestbuilding than either 24 or 30 day-old males, or 18 day-old females. These data should be interpreted with caution, however, due to the few animals (n=3) which comprised this group. Otherwise, frequency distributions for 18 day-old males were congruent with that of 18 day-old females for the other behavioral measures. There was also a tendency for 18 and 24 day-old females to engage in more passive contact than males (F=3.21, df=1,46, p=0.07); this was not true of 30 day-olds, which showed no sex difference for this measure.

These data indicate that there is selective facilitation of some maternal behaviors but not others, depending upon the age of juveniles tested: for example, retrieving in 24 day-olds, licking in 24 and 30 day-olds. We next attempted to determine whether or not patterns of maternal behaviors expressed over 5 days of exposure to pups also varied with age.

Maternal Behaviors: Linear Regression Analyses - Age-Related Patterns of Change

For those variables for which we found main effects of age, day or interactions of the two, we next looked for orderly changes in frequencies of behaviors over the five days of testing, using linear regression analyses. The changes over time for the three agegroups for retrieving, nestbuilding, ventral crouching, and anogenital licking are shown in Figure 2; contact is shown in Figure 3. We also did comparisons between agegroups (18 versus 24; 24 versus 30) on Day 0 to determine whether or not the initial levels of these behaviors could be attributed to either maturational (i.e., age-related) or sensitization (i.e., exposure to pups) processes.

For retrieving (Figure 2a), estimates for changes in slope over the five days indicated an increasing function for all three agegroups (F=12.51, df=4, p=0.0001): for 24 and 30 day-olds this represented a significant increase ($t=4.78$, $p=0.0001$; $t=3.43$, $p=0.0006$) in retrieving; for 18 day-olds this represented a tendency ($t=1.71$, $p=0.08$). Individual comparisons showed no differences on Day 0 levels of retrieving, indicating no particular initial preferences to retrieve as a function of age.

Like retrieving, pupcarrying increased over time for all three agegroups ($F=7.55$, $df=4$, $p=0.0001$); this was significant for 18 and 30 day-olds only ($t=3.50$, $p=0.0005$; $t=3.14$, $p=0.0017$, respectively). Also like retrieving, we found no Day 0 differences relating to initial preferences by age.

There were no changes in either nestbuilding or ventral crouching over time for any of the three agegroups. For nestbuilding this was probably the result of the nonlinear appearance of the data (Figure 2b). For crouching, levels of frequencies were low for all of the groups (Figure 2c).

Anogenital licking showed the same general pattern as retrieving (Figure 2d), with the function increasing over time ($F=7.88$, $df=4$, $p=0.0001$): this was significant for 24 and 30 day-olds ($t=4.08$, $p=0.0001$; $t=2.06$, $p=0.039$), but not for 18 day-olds. Day 0 comparisons showed no age-related baseline differences in anogenital licking: that is, none of the groups differed in the initial amount of licking shown on Day 0.

The analysis of contact (Figure 3) showed some interesting maturational differences among the three agegroups ($F=17.60$, $df=4$, p=0.0001). For 18 day-olds contact decreased significantly over the five days of testing (t=2.85, p=0.0044), whereas for 24 and 30 day-olds it increased as a function of testing (t=3.99, p=0.0001; t=5.36, p=0.0001). Day 0 between-group comparisons showed that 18 day-olds had significantly higher baseline frequencies of contact than both 24 and 30 day-olds (p=0.01; p=0.01). Twenty-four day-olds also had higher Day 0 frequencies than 30 day-olds (p=0.01), indicating that for this measure spontaneous, age-related differences were present: i.e., younger animals showed more of an initial propensity to engage in contact than older animals, without previous exposure to pups. These data, like the analyses in the previous section, indicate both differences and similarities in the patterns of maternal behaviors expressed in response to pups over time. Retrieving and licking showed facilitation, but not crouching or nestbuilding. With the exception of contact, we found no intrinsic differences in preferences to express these behaviors at any age.

Other Behaviors: Frequency Data and Linear Regression - Differences and Patterns

Having considered maternal behaviors in some detail we analyzed other behavioral variables to determine whether or not these would yield age-related differences among the agegroups. Summaries of mean frequencies over five days for nonmaternal variables are shown for each ageyroup in Figure 4.

The direction of main effects for each variable, briefly summarized, are as follows (Table 3): 1) 30 day-olds charged test pups more than either 18 or 24 day-olds, they also reared more than 18 or 24 day-olds; 2) 24 and 30 day-olds self-groomed and sniffed more than 18 day-olds; 24 day-olds also reared more than 18 day-olds; 3) 18 day-olds did more sniffing/licking/pawing of pups, and ate more than 24 and 30 day-olds.

Linear regression analyses on these behaviors indicated the following changes in functions over time (Table 3b). There was a significant increase in pouncing behavior (Figure 5a) on the part of all three agegroups over the five days of testing, with 18 day-olds showing the greatest change, 30 day-olds showing the least and 24 day-olds having a value midway between the two. Day 0 comparisons for this variable indicated that there were no differences in baseline levels among the three agegroups.

For sniff/lick/paw (Figure 5b), all groups showed significant decreases (Table 3b) with time. A similar pattern was shown for locomotion (Table 3b); however, only 24 day-olds showed a significant decline. Day 0 comparisons showed no spontaneous differences among the three agegroups upon initial exposure.

There were indications of developmental differences in other behavioral variables as well (Table 3b). For self-grooming 18 day-olds showed a significant increase with time, 30 day-olds a decrease, with 24 day-olds showing no change. Rearing was similar in form: 18 day-olds showed a significant increase, 30 day-olds a significant decrease, 24 day-olds no change. Resting showed a significant decrease on the part of 18 day-olds and significant increases by 24 and 30 day-olds. Overall, the data suggest a developmental break between older and younger juveniles with respect to these noncontact behaviors, with 18 and 30 day-olds generally at two extremes, and with 24 day-olds having values midway between.

Nest Ratings

We found no differences among any of our agegroups, nor between the sexes for nest ratings. The average ratings ranged from 1.8 - 2.45. We also found no changes in nest ratings over time for any of the three groups.

Principal Component/Factor Analyses: Relationships Among Behavioral Variables

Since we were interested in the interrelationships among many behavioral variables within and between categories of behaviors, we next subjected the frequency data to principal component analyses. This is a method of transforming a given set of variables into a new set of composite variables (principal components) that are orthogonal (uncorrelated) with one another. The first principal component is the best single descriptor of the linear relationships in the data. Subsequent components best summarize remaining relationships in the data after variance from each preceding component is removed, until all the variance in the data is accounted for. Each of the original variables is represented on a component by a coefficient, called a "loading", and the total amount of variance accounted for by a given factor is expressed as an "eigenvalue" (the summation of squares of the loadings on each factor) (Nie, Hull, Jenkins, Steinbrenner and Bent, 1975). With this technique we were attempting to look for underlying sets of relationships among all of the variables, i.e., to see which behaviors would cluster together as factors. This was done for each of the three agegroups to determine whether or not the relationships would prove to be the same, or would vary with age. We decided to examine those factors which accounted for maternal and play variables, as well as passive contact and its associated behaviors. With one exception only the first two factors accounted for relationships among only maternal and play variables. These accounted for approximately 25% of the total variance for each agegroup. Other factors were excluded from interpretation because they had eigenvalues of less than 1.0 or were not relevant to our analysis. Still other factors reflected the unique contribution of a particular maternal variable and hence did not explain a pattern of relationships among variables, but rather, the existence of a separate effect. The results of the principal component analyses are shown in Table 4.

We found that four behaviors, retrieve, pupcarry, charge and pounce loaded most heavily onto Factor 1 for all three of the agegroups, indicating that there is an underlying relationship between these "maternal" and "play" variables, regardless of the age of juveniles tested. Contact and resting (i.e., resting in contact) loaded onto Factor 2 for the three agegroups, indicating that this set of quiet behaviors is uncorrelated with active maternal and play behaviors. Factor 4 showed an interesting developmental difference in that only ventral crouch loaded heavily onto this factor for the 18 day-olds, whereas both ventral crouch and anogenital lick loaded for the older two groups (for 18 day-olds anogenital lick appeared alone on Factor 9). Moreover, these maternal behaviors are unrelated to retrieving behavior loading on Factor 1 because they appear on orthogonal factors. Nestbuilding appeared alone on Factor 8 for 18 day-olds with an extremely high loading; the same variable loaded heavily onto Factor 9 for the 24 and 30 day-olds, indicating that this maternal behavior is uncorrelated with all other maternal behaviors loading onto other factors. For the 24 day-olds retrieving loaded onto the same factor with nestbuilding; the significance of this association is unclear.

Taken together, these data indicate that an association exists between retrieving behaviors and play behaviors regardless of age, and show the emergence of an association between crouching and

and licking in the older juveniles. However, the data point, overall, to a fragmentation of maternal behaviors during the juvenile period.

Discussion

Maternal Behavior of Juveniles: Sensitization and Integration

While it is possible to "sensitize" juveniles, that is, to promote the appearance of any or all of the maternal behaviors by exposing them to pups, we found that mere exposure was insufficient to achieve full integration of maternal behaviors. However, both our individual analyses and the principal component analyses on group data indicated that, as animals get older they show an increasing capacity toward integration, apparently irrespective of gender. We would predict this trend to continue for females as they approach puberty, since it is thought that one of the necessary conditions for optimal performance of maternal behavior in adult females is the presence of circulating ovarian hormones (Krehbiel and LeRoy, 1979; Mayer and Rosenblatt, 1979a), established at puberty (Mayer et. al., 1979). On the other hand, maternal responsiveness tends to be less well organized in adult males (Fleischer, Kordower, Kaplan, Dicker, Smerling and Ilgner, 1981; LeRoy and Krehbiel, 1978; Lubin et. al., 1972; Quadagno and

Rockwell, 1972), and there is evidence that this difference appears at, or just after puberty (Mayer et. al., 1979).

It would be interesting to compare behavioral events in prepubertal rats to that of adult lactating females raising litters. The sudden appearance and organization of maternal behaviors at parturition in the rat has long been noted by investigators and appears self-evident to the observer. However, in their classic monograph, Wiesner and Sheard (1933) commented upon the independence of maternal activities both in terms of their causitive factors and with respect to the order of their disappearance as lactation progressed. Rosenblatt and Lehrman (1963) found that retrieving, nursing and nestbuilding were uniformly present in a high percentage of mothers in the first two weeks of lactation, but then declined with some variation of occurrence in the next two weeks. No attempt was made in this study to determine the extent of correlation among maternal behaviors. Slotnik (1967a) found that in newly-parturient mothers measures of pup-licking and nursing correlated with one another and with time in nest, but showed no relationship to retrieving or nestbuilding measures. Thus, there is evidence to suggest the independence of maternal behaviors in lactating rats as well.

When retrieving was used as the only measure of maternal behavior, the 24 day-old group emerged as the most "maternal", given that a large percentage of individuals of both sexes retrieved pups over the 5 days of testing. These results are consistent with data from animals of other strains of the same age or slightly younger (20 - 24 day-olds) (Bridges et. al., 1974; Mayer and Rosenblatt, 1979b; Gray and Chesley, 1984), and therefore this propensity to retrieve seems to be characteristic of weanling animals in general. However, the latencies shown by our animals, particularly the 18 and 30 day-olds, cannot be viewed as representative since so few of these met the two-day criterion in the time period alloted. Had we extended the period of exposure, we might have observed more animals retrieving but would have defeated the purpose of the study, which was to track developmental skills or propensities at specific points in time during exposure to pups.

There also seems to be no doubt that retrieving and anogenital licking were elicited from all three groups as a result of their exposure to pups, as indicated by significant increases in these functions over time. Apparently the propensity to engage in these behaviors as a response to young pups exists across the three ages.

While crouching in nursing posture does appear in the behavioral repertoire of juvenile rats, it was rarely seen in any of our animals in this context. Neither did Gray and Chesley (1984), observe any crouching in weanling animals, using a 60 minute test period. Thus, frequencies of crouching show no apparent increase with exposure to pups, and show no correlation to retrieving in prepubertal animals, as shown by principal component analyses.

Nevertheless, crouching becomes linked with anogenital licking in older juveniles, suggesting that, irrespective of low frequencies shown, exposure to infants does facilitate some functional connection between these two behaviors. Prolonged contact with pups also exposes an apparent developmental difference between older juveniles and younger animals, for whom this connection does not yet exist.

Nestbuilding appears to be least affected by exposure to pups as well as unconnected with other maternal behaviors. Like Gray and Chesley, we observed that juveniles' nest qualities were generally quite good from Day 1 on and did not change over testing. We also informally noted that younger juveniles sometimes built new nests in response to pups' locations rather than retrieving pups to sleeping nests. In addition, trends for nestbuilding were nonlinear over the five days of testing and were apparently differently distributed among the three groups. This is not surprising since a number of studies have shown the independence of this set of behaviors from other maternal behaviors in sensitized (Fleming and Rosenblatt, 1974a; LeRoy and Krehbiel, 1978), hormonally-induced, sensitized (Terkel and Rosenblatt, 1972) and parturient adult females (Slotnik, 1967a). In nonpregnant females and males nestbuilding is a function of temperature regulation: if ambient temperature is lowered or raised, nestbuilding increases or decreases accordingly (Denenberg, Taylor and Zarrow, 1969; Kinder, 1927). Thus, one likely possibility is that nestbuilding is subserving thermoregulatory demands (see below) as opposed to being a "maternal" response to pups.

Contact and Other Behaviors: Age-Related Patterns

Like Mayer and Rosenblatt (1979b), we found that the other behaviors that we looked at fell into a rough "behavioral profile" separating 18 day-olds from the older two groups. Younger animals spent most of their time in passive contact with pups; older juveniles engaged in other behaviors away from pups. The fact that we also found that passive contact actually decreased over time in 18 day-olds while increasing in the older two groups suggests a few possible interpretations, none exclusive. Mayer and Rosenblatt's (1979b) work has shown that younger juveniles are attracted to pups on the basis of a variety of cues (movement, warmth, vocalizations, smells, taste), and suggests that as juveniles grow older such stimuli become less effective in evoking approach responses, and, in fact, come to elicit avoidance, beginning at about 24 days of age.

Another interpretation that has occurred to us relates to the thermoregulatory capacities of younger juveniles, and may apply to nestbuilding as well. At 18 days of age huddling as a form of contact has presumably made the transition from "physiological", i.e., thermal control to "filial", or social control for which other cues become more salient (Alberts, 1978; Alberts and Brunjes, 1978).

Ackerman (1981) has shown, however, that separation from the mother and litter at about this age impairs or retards the development of homeostatic temperature regulation, occurring as it does at a time when thermoregulatory patterns are not yet autonomous and show instability (Conklin and Heggeness, 1971). In fact, Alberts (1978a) has shown that rats up to 20 days of age utilize huddling to reduce heat loss. Therefore, our 18 day-olds may have been responding to the need for thermal homeostasis by spending a great deal of their time huddling with pups, perhaps in combination with nestbuilding. This does not mean that other attractions, as in filial huddling, were not present, but that they may not have been primary in eliciting contact.

Exploratory and Play Behaviors: Relationships Among Maternal and Play Behaviors

With increasing exposure to pups the behaviors sniff/lick/paw decreased from relatively high levels for all three agegroups. Thus these behaviors, used as a means of exploring pups as novel stimuli (Mayer and Rosenblatt, 1979b) seem to decline with the same pattern during this period regardless of age. The data suggest that generalized sniffing, licking and pawing of pups then gives way to more directed licking of the anogenital area, since this behavior increases with exposure. If this is true, then we can infer that the sequence: sniff/lick/paw - anogenital lick probably facilitates maternal responses in juveniles. We do know that anogenital licking is an important facilitator of maternal responsiveness in adult females (Stern, 1983).

The play behavior pounce, on the other hand, increased with exposure to pups, also regardless of age. Meaney and Stewart (1981) found that pouncing increases from 21-30 days of age, and thereafter remains relatively constant. While there are obvious differences between the two studies, our data showed a similar pattern to theirs, in that the increase in pouncing became less steep with age, so that 18 day-olds showed the greatest change and the 30 day-olds the least. We cannot say, therefore, whether the increases in pouncing behaviors over time were the result of changes in responsiveness to pups, or were due solely to maturational changes. However, the similarities among patterns of retrieving, pupcarrying, anogenital licking as well as pouncing do suggest that the presence of pups may have some influence on the expression of this behavior, particularly in older animals.

The strongest evidence that there is a relationship among play and maternal variables comes from the principal component analyses. Although retrieving and perhaps pupcarrying are considered "maternal" while charging and pouncing are considered "playful", these four behavioral variables nevertheless shared an underlying statistical commonality. This suggests some sort of continuity between these specific forms of juvenile gregariousness and maternal behaviors, and hypotheses as to the nature of the relationships among them. Can play behaviors be considered generalized ontogenetic precursors of maternal behaviors similar to Mayer and Rosenblatt's (1979b) characterization of contact-affiliative behaviors, or are they more specific to this context?

Our interpretation of this phenomenon takes as its model Poole and Fish's (1975) study of the temporal relationships among the elements of social play in juvenile rats. Their study showed that the behaviors: charge - pounce - aggressive groom - wrestle followed one another with high probabilities, with charge and pounce serving to initiate play bouts. Since our data are in the form of time-samples they do not lend themselves readily to such analyses, being measures of "state" rather than of sequences (Altmann, 1969). However, they are not inconsistent with the idea that sequences may exist as either "microsequences" within each time sample of observations, or as "macrosequences" occurring over the five days of testing. As an example of the former, one hypothesis based on this, and our own, informal observations is that charge and pounce serve as sequential initiators to retrieving behavior in a behavior chain. Pupcarrying may well be a transitional behavior to retrieving either sequentially as part of a microsequence, or as an example of the latter, a transitional element during the process of sensitization. Such a model would predict that the crucial element in the transition between play and maternal retrieving would be the disparity between cues provided by infants versus those normally encountered in sibling play bouts. Young rats can apparently discriminate between "responsive" and "unresponsive" play partners and establish partner preferences based on a variety of cues (Humphreys and Einon, 1981). Moreover, differences in partner responsiveness can affect the sequential structure of play itself (Poole and Fish, 1976). Thus, it is not unlikely that stimulus properties of pups could influence or modify this otherwise highly stereotyped behavior sequence (Fassino, unpublished data).

One of the hypothesized functions of play is that it serves as a mechanism through which social attachments are formed and maintained; as part of the socialization process it may acquaint young animals with the stimulus-response contingencies involved in social interactions (Bekoff and Byers, 1981). As Pellis and Pellis (1983) have pointed out, the pounce serves to initiate play-fighting in a variety of rodent species, and thus serves an immediate, proximate, social function. In the context of sensitization this initiatory sequence, once modified, could be pressed into the service of caretaking behaviors.

Although at this point the idea is speculative, it is possible that individual maternal behaviors may be coupled with other age-specific patterns of behavior, such as the relationship we found between retrieving and play. Whether or not there is any relationship between these patterns and the development of maternal behaviors has yet to be established. However, such relationships have been demonstrated between a variety of early social behaviors and the development of various aspects of sexual behavior, in a number of species (Moore, 1984).

General Discussion

In summary, our results have shown that in prepubertal rats a complex interaction exists between sensitization manipulations and the ages at which those manipulations were introduced, depending upon which behaviors were examined. Our primary finding is that the components of maternal behavior are apparently distinct in prepubertal animals; this may perhaps account for the fact that sensitization does not elicit an integrated pattern of mothering as it does in adult females. The facts that: 1) 24 day-olds selectively retrieve more than at other ages, 2) for all agegroups anogenital licking and retrieving are selectively increased in response to exposure to pups, and that 3) all three groups show relatively little change in nestbuilding or ventral crouching, all underline Bridges et. al.'s (1974) caution that retrieving is not representative of all maternal behaviors in animals of this age. These data, and our finding that retrieving is coupled with play behaviors, argue that maternal behaviors constitute independent response systems in juveniles. Indeed, our data suggest that for prepubertal animals there are probably different mechanisms controlling different maternal behaviors, perhaps even at different prepubertal ages. We have mentioned a few possible mechanisms; in each case, they could be tied to other functions suited to the particular demands of each agegroup, or of prepubertal animals in general. We are aware that there may be any number of alternative explanations for our results, but this view of maternal behaviors in juveniles is consonent with the idea that ontogenetic adaptations can subserve the immediate or proximate functions of any particular stage of development (Oppenheim, 1980). And, as Oppenheim has pointed out, this can occur without necessarily obviating developmental traits as antecedents of adult characters. Such may be the case with early maternal behaviors.

Taken together, our results suggest that "maternal behavior" does not develop in a unitary fashion, but that maternal behaviors may have separate and distinct ontogenies.

Behavioral Responses of Juvenile Rats to Neonates After Infusion of Maternal Blood Plasma or Handling

The previous study investigated the expression of maternal behaviors in juvenile rats at three ages (18, 24 and 30 days) during exposure to pups. The results showed that juvenile maternal behaviors appear to be relatively independent during development, as well as fragmented and poorly integrated in their expression. Neither were there any gender differences in our strain for any of the behaviors examined. Another intriguing finding was that juvenile play behaviors (charge, pounce) were related to retrieving but not other maternal behaviors in this context.

Given these findings and those of previous studies, it would seem that the developmental significance of early maternal behaviors is an open question. Are they an early sign of the activation of neural substrate specific to maternal behavior in both sexes? Or, rather, do they represent generalized "precursor" systems of social behavior that may be utilized for various functions, depending upon the context in which they are elicited? And, if so, are they tied to other behavioral-neural systems specific to that ontogenetic period, such as play?

In a series of studies Terkel and Rosenblatt (1968; 1972) showed that blood plasma from newly-parturient mothers can facilitate the appearance of maternal behaviors in adult virgin female rats with significantly shorter latencies than controls during sensitization. In the first experiments blood was injected into the femoral vein in the upper thigh, resulting in 48-hr latencies (Terkel and Rosenblatt, 1968). In the second experiment cross-transfusion via jugular vein implants joining mothers and virgins resulted in latencies of about 14 hours (Terkel and Rosenblatt, 1972). In these studies, all maternal behaviors except nestbuilding were facilitated. The implication of their findings is that hormones present in maternal blood plasma were acting to induce maternal behavior.

Investigating the same phenomenon in juveniles , Koranyi, Lissak, Tamasy, and Kamaras (1976) found that within 12 hr of injection of maternal blood serum, weanling rats 18-24 days of age showed all components of maternal behavior except nestbuilding, suggesting neurological responsiveness at this age to hormones in maternal blood. However, Koranyi et. al. reported their data in a summary article, and consequently their reportage leaves a number of unanswered questions particularly regarding procedures attendant to tail vein injection.

Aside from these considerations, such an investigation has never been carried out in a Wistar-derived strain. The present study was an attempt to replicate the essential elements of Terkel and Rosenblatt's, as well as Koranyi et. al.'s studies, to determine how juveniles of our strain of rats would respond to hormones present in maternal blood plasma.

Using behavioral criteria developed for our previous study, we were interested in determining whether juveniles would show integration of maternal behaviors in response to maternal blood plasma, and if so, whether we could characterize, at two different ages, the processes by which integration came about. If all maternal behaviors showed similar facilitation this would indicate, first, that the entire complex is capable of activation; secondly, that organizational effects begun prenatally are complete and mature before puberty. Alternative are that: a) hormonal manipulations would have no effect on facilitating or integrating maternal behaviors, at both ages indicating immaturity of the neuroendocrine complex during the juvenile period, and of the overriding importance of hormonal changes at puberty in activating maternal behaviors, (Mayer et. al., 1979a; 1979b); b) there could be selective facilitation of all or some maternal behaviors by age, with older animals presumably showing more facilitation and perhaps more integration; c) some behaviors could be selectively facilitated but not others, indicating partial maturation as well as the continuing independence of maternal behaviors from one another.

We also wished to determine whether or not the relationships observed in the previous study between play and maternal behaviors would be maintained, disrupted, or extended to other maternal behaviors, as the result of hormonal manipulations. These results could elucidate the nature of relationships among different classes of behaviors during development.

Methods

Subjects and Housing

The subjects were 18 and 30 day-old Wistar strain rats (Rattus norvegicus) born in the laboratory to dams received at 18-19 days of pregnancy from Marland Farms (New Jersey). Housing and maintenance of litters were the same as in Study 1. All litters were culled to 9-10 pups within 72 hr of birth and left undisturbed until either the day prior to testing or until 24 days of age (weaning), whichever came first. Eighteen day-olds were placed with their own mothers to nurse for two hours on the first day (Day 0) of testing) between 0900 and 1100 hrs.

On the day before isolation and testing, litters at 17 and 29 days of age were weighed and ear punched, with no more than two same-sex siblings were randomly assigned to experimental groups in order to control for litter effects. Subjects were divided into three groups: PL animals received plasma taken from maternal donors (30 day-olds: n=17, 11 females/6 males; 18 day-olds: n=15, 8 females/7 males); D5W animals received 5% dextrose in water as a control for handling received during surgery and infusion procedures (30 day-olds: n=13, 8 females/5 males; 18 day-olds: n=13, 6 females/7 males); NHC (nonhandled control) animals received no handling except for initial weighing and habituation to test cages (30 day-olds: n=18, 11 females/7 males; 18 day-olds, n=14, 11 females/4 males).

Cannulation and Infusion Procedures

After weighing and assignment to groups, all subjects to be tested were placed into test cages with a sibling partner to habituate for 2 hrs. NHC group subjects and all partners were then returned to the home cage; PL and D5W subjects were each implanted with a chronic jugular cannula under ether anesthesia. Insertion of the catheter was performed in the following way. A blunt dissection was done with forceps from the front of the mandible to the back of the neck where a small incision was made. A 5-6 cm catheter of silastic tubing filled with dilute (5%) heparin solution was then inserted into the back of the neck and brought forward along the plane of the blunt dissection to to the region of the jugular vein. This was slipped into the jugular vein, passing into the ventricle and secured with a loop of silk around the vein. The remaining length of catheter extending from the vein was occluded at the opposite end with a loop of silk thread, and coiled under the skin at the back of the neck. The wound was closed with a single suture which allowed the end of the silk securing the catheter to protrude. After recovery from anesthesia subjects were returned to the home cage.

The next day (Day 0 of testing at 18 or 30 days of age), blood was withdrawn from donor dams within an average of 10 hours after parturition (+ 4.8 hr). Only those dams judged to be maternal (with pups having milk bands and/or gathered together and clean) were used as donors. Withdrawal of blood was accomplished via heart puncture under ether anesthesia, using a 16-guage needle and 10 cc syringe containing 15-20 units full-strength heparin to prevent blood clotting. 8-10 cc of blood was withdrawn via this method. Blood was then transferred into heparinized test-tubes ("Vacutainer") and centrifuged for 3-5 min. The plasma collected from this was transferred to a 10-cc syringe and placed in an infusion-withdrawal pump (Harvard Apparatus).

PL and D5W subjects were placed into a plastic restraining device, the cannula was withdrawn from beneath the skin via the silk suture and attached to the infusion syringe. PL animals received .1cc/10 gram body weight of maternal blood plasma at a rate of .051 cc per minute. Each litter of experimental subjects received plasma from a single donor female. D5W animals received the same amount of

5% dextrose in water, simultaneously with the PL group. After infusion each subject's cannula was replaced back under the neck skin and the wound sutured. Subjects in all conditions were then placed alone into the same test cages occupied on the day before to habituate for ninety minutes before being tested with pups. Subjects remained in test cages for the remainder of the experiment.

Autopsy at the end of the experiment indicated that for all subjects, tips of cannuli were within the lumen of the jugular vein for the entire experiment.

Testing Conditions and Behavioral Testing

The testing conditions and observational procedures were identical to those described in the previous study. The list of behavioral variables recorded were those recorded in the previous study.

Data Analysis

Observation data were first analyzed to determine the percents of animals that became maternal during the five days of exposure to pups. Between-age-group as well as between-treatment comparisons were made of percents of animals maternal; latencies to become maternal were also compared. We employed several measures of "maternal" in order to determine whether or not our manipulations altered either the number of maternal activities carried out other than retrieving, as well as to test for consistency in carrying out maternal activities. For one measure, we defined as "maternal" animals that retrieved at least one pup during a 15 min test for two consecutive days. We also used a modification of more rigorous criteria used for adult animals (e.g., Bridges, Feder and Rosenblatt, 1977; Stern, 1983). Those animals which: 1) retrieved; 2) crouched over; and 3) licked the anogenital region of pups for two consecutive days were considered maternal, though these behaviors did not necessarily have to occur concurrently with one another. Since preliminary data analysis had indicated that few juveniles of either age-group consistently met full adult criteria, or consistently retrieved, we also analyzed the number of animals that displayed a given behavior at least once in five days. Since their distributions were not necessarily normal, latency and percent data were analyzed using nonparametric tests (Chi-Square or Fisher Exact for treatment of percent data dichotomized into yes/no categories; for latency data, Kruskal-Wallis one-way ANOVA by ranks; Mann-Whitney U; Siegel, 1956).

As in Study 1, frequency data derived from time-samples were used to determine differences among age and treatment groups in levels of performance of each behavioral variable. For this measure, no criterion for performance was utilized. Analyses of these data were the same as for Study 1.

A principal component factor analysis was utilized to determine: 1) whether maternal behaviors would become more integrated (i.e., correlated with one another) as a result of our treatments; and 2) whether the pattern of relationships between juvenile play behaviors and maternal behaviors would be changed by our treatments.

Results

Retrieving and Other Maternal Behaviors - Differences, Consistency, and Integration

As Figure 6 shows, hormones in maternal blood plasma selectively increased retrieving in 30 day-olds. This was true when the data were analyzed to compare the number of animals that retrieved at least once in five days of testing (Table 5a). By this criterion, a higher percentage of 30 day-old PL animals retrieved than did the combined D5W and NHC groups. This was not true when PL and D5W groups were combined and compared with NHC animals, nor when PL and NHC groups were combined and compared with D5W animals. Individual comparisons showed a trend toward more PL animals retrieving than either D5W or NHC groups (Table 6); this was not true of comparisons between D5W and NHC groups. There was also a trend among female groups toward more PL retrieval than combined D5W and NHC females (Table 6). There were no differences among male groups. There were also no sex differences in retrieving for this measure, either when males and females were compared within treatment groups, or when males from all treatment groups were compared with females from all treatment groups.

By imposing a criterion for consistency of at least two consecutive days of retrieving (Table 5b), differences among groups were obscured. These data show that few animals of either age-group retrieve consistently, but that hormones in blood plasma increases the number of 30 day-olds that retrieve at least once during exposure to pups. This was not true of 18 day-olds, in either case.

When other maternal behaviors were analyzed separately, there were no differences among treatment groups within each age group, nor were there differences between sexes (Table 6).

In a comparison between combined groups of 18 and 30 day-olds, a higher percentage of 30 day-olds than 18 day-olds retrieved for two consecutive days (Table 5b), but not for retrievals on at least one of five days. Thirty day-olds also had significantly lower latencies to retrieve than did 18 day-olds in either case.

Table 6 shows that more 30 day-olds crouched than did 18 day-olds, whereas more 18 day-olds exhibited nestbuilding in the presence of pups.

Table 7a shows that few of the animals of either age met adult criteria (engaging in retrieving, crouching, and anogenital licking) for two consecutive days. The same was true when the two-day criterion for behavior was relaxed so that any one instance of each maternal behavior exhibited within five days was counted. Chi-square tests showed that there were no differences within each age-group for this measure. Nor were there differences in latencies to onset of these three behaviors. However, more 30 day-olds engaged in retrieving, crouching and licking at least once during the five days of testing than did 18 day-olds (only one 18 day-old was seen to engage in the three maternal behaviors). Therefore, despite age or treatment, few, if any animals perform three or more maternal behaviors consistently, but that as animals get older they do perform them sporadically in the presence of pups.

Thus, in 30 day-old individuals the infusion of maternal blood plasma selectively elicited retrieving behavior such as is seen in adults. However, while retrieving was performed by individual animals sometime during the five days of testing, it did not necessarily occur concurrently with other maternal behaviors, or consistently over time. There were no sex differences for any behavioral measures.

Maternal and Other Behaviors: Frequency Data - Immediate and Later Effects of Treatments

In this analysis, the frequency of occurrence of each behavioral variable was examined to further determine the effects of our manipulations. Summaries of main and interaction effects are shown in Table 8.

Age Differences

Overall, 30 day-olds showed higher frequencies of most of the maternal and other categories of behaviors than 18 day-olds (Table 8). Of the maternal behaviors 30 day-olds showed more retrieving, crouching and anogenital licking. Thirty day-olds also charged more than 18 day-olds. Eighteen day-olds, on the other hand, engaged in more passive contact, as well as nestbuilding and exploratory sniffing, licking and pawing of pups.

Within Age-Groups: Treatment Effects

Within age-groups the majority of effects of administration of plasma or handling were shown in GROUP x DAY interactions. In order to determine specifically what those interactions signified, orderly changes in frequencies of behaviors over the five days of testing were determined, using linear regression analyses on interaction terms. The results of regression analyses are shown in Table 9, for 30 day-olds (9a), and for 18 day-olds (9b). F-values indicate whether the interaction term: DAY (AGEGR x GR) was significant or not. For each group the results of t-tests for significant changes in slope over time, and their direction are also shown. Specific comparisons (t-tests based on least-square means) then determined daily differences between treatment groups, and complete the table.

There were immediate (Day 0) effects, and effects that occurred slowly over the next 4 days of testing. These varied according to age, treatment and the behavior examined. Since levels of some behaviors were very high on Day 0 compared with other days, linear regression analyses were done beginning on Day 1 for those behaviors for which Day 0 was significantly higher than Day 1. This allowed for the disparity between Day 0 and Day 1, which would otherwise have contributed to nonlinearity in the data between Days 0 and 5. These are shown for overall F-values, as well as t-tests.

Thirty Day-Olds

The changes in behavior over time for the three 30 day-old treatment groups are shown in Table 8a and Figure 7 (a-d).

Initial Effects

As shown in Figure 7, both 30 day-old PL and D5W animals showed significantly higher levels of retrieving (7a), crouching (7b), anogenital licking (7c) and contact (7d) on Day 0 than NHC animals; this was also true for Day 1 levels of retrieving, crouching (PL only), licking and contact. Since PL and D5W groups could not be differentiated on these days, the transient, high levels shown for these maternal behaviors in both must be attributed to the handling that these groups received during the cannulation-infusion processes. On Day 0, levels of sniffing-licking-pawing were depressed in PL and D5W animals below that of the NHC animals, again suggesting a transient handling effect in these two groups.

Continuing Changes with Exposure

Table 9a and Figure 7a show that PL animals showed a significant increase in retrieving from Day 1 on; this was reflected in significantly higher levels retrieving on Day 3, compared with both D5W and NHC groups. The other two groups also showed some increase over time: for the NHC group, this reflected a tendency. Thus in 30 day-olds the infusion of maternal blood plasma seems to have accelerated frequencies of retrieving over the test period, compared with more modest changes in retrieving shown by the two control groups, and suggesting a hormone-facilitated response for this behavior.

Crouching (7b), increased from Day 1 on for all three groups. This was significant for the D5W group and was a trend for the PL animals. The D5W animals as a group crouched more than PL and NHC animals on Days 2 and 3, but by Day 4 both D5W and PlASMA groups were showing higher levels of crouching than NHC animals. Both handling and maternal blood plasma, therefore, facilitated slow-acting changes, leading to an increase in crouching, as represented by the D5W group, and to a lesser extent, by the PL group. Whether these represent the same or different underlying processes is unclear.

All three groups increased anogenital licking over time from

Day 0 levels: these were significant for the D5W and NHC groups. D5W animals showed higher levels of anogenital licking than PL and NHC groups on Day 2 and Day 3, respectively. Levels of licking in PL animals were only as high as that of control animals on these days. Apparently the elevations in licking produced on Days 0 and 1 by handling were lost in PL animals on subsequent days, suggesting some selective effect counteracting the effects of handling.

Analyses of daily changes for the three groups showed that there were positive changes in contact over the five days of testing. NHC animals showed a significant increase from Day 1 levels, while PL and D5W animals showed no change. The NHC group had higher levels of contact than PL animals on Day 3. As with the maternal behaviors observed, handling intiially facilitated contact, but thereafter nonhandled controls attained similar levels with increasing exposure to pups.

None of our manipulations affected nestbuilding in 30 day-olds, nor did they affect pupcarrying for either age-group.

Sniffing-licking-pawing decreased significantly for NHC's, due mainly to the very high levels shown on Day 0 for this group. Moreover, NHC animals showed higher levels of this behavior than D5W's on Days 2 and 3 , but not PL animals. Thus the acute effects of handling (in this case, suppression of the behavior) seen in both PL and D5W animals was counteracted somehow in the PL group, precipitating frequencies to control levels. Charging and pouncing showed no changes either in the short- or long-term, indicating that these behaviors are not affected by either hormones or handling in 30 day-olds.

Eighteen Day-Olds

Table 9b is a summary of immediate and slower changes in 18 day-old behaviors, as analyzed by linear regression. Figure 3 shows nestbuilding and contact, the only maternal behaviors for which 18 day-olds showed group differences. For 18 day-olds, handling produced higher levels of nestbuilding in the D5W group than both PL and NHC animals on Days 0 and 1 (Figure 8a). The regression analysis showed that all three groups also significantly decreased their levels of nestbuilding from Days 0 through 4. Thus, in 18 day-olds, handling produced acute elevations in nestbuilding, but this was apparently negated by the effects of infusion of maternal blood plasma. Thereafter, nestbuilding declined in all three groups, irrespective of treatment.

The analysis of contact (Figure 8b) showed an interesting maturational difference between the younger and older animals. Whereas for 30 day-olds (see Figure 7d) levels of contact either increased or remained the same during exposure to pups, for 18 day-olds contact decreased significantly over the five days of testing. Another difference between 18 and 30 day-olds is that handling of PL and D5W animals had no effect on initial (Day 0) levels of contact in the younger animals, whereas it did for the older group (Figure 6d). Daily between-group comparisons for 18 day-olds showed no differences until Days 2 and 3, when PL and D5W animals had significantly higher frequencies of contact than NHC animals. On Day 4 the situation was reversed, with the NHC group showing the higher frequencies of contact than either PL or D5W animals. Apparently handling prevented the depression contact shown in control animals on Days 2 and 3.

While pupcarrying increased for all three groups over the five days of testing, this was only significant for the NHC group. The play variables charge and pounce also increased in all three 18 day-old groups, unlike the 30 day-olds. This increase in charging was represented as a trend for the D5W and NHC groups. Pouncing increased significantly in all three 18 day-old groups. Like the 30 day-old controls, sniffing-licking-pawing also decreased significantly in the 18 day-old NHC group; this was not true for either the PL or D5W groups. Thus, the decrease in this exploratory behavior shown by controls of both ages was prevented by handling.

Gender Differences - Maternal Behaviors

One of the hypotheses tested by this experiment was that if hormones present in maternal blood plasma were acting on neural substrate, then there would be some selectivity in behavioral response by gender. However, selective gender responsiveness to handling has already been shown (Mayer et. al., 1979; Mayer and Rosenblatt, 1979a), and so could conceivably account for differences between the sexes. Our results show that, in fact, for most maternal behaviors, the two manipulations produced effects which can be differentiated, and which appear to interact. These are listed in Table 10, and are shown for 30 day-olds in Figure 9 (a-d).

Thirty Day-Olds

As shown in Figure 9a, PL females retrieved more than D5W and NHC females. PL females also retrieved more than NHC males, but not more than PL or D5W males. These two groups of males did significantly more retrieving than NHC males, as well as D5W and NHC females. This latter result accounted for the sex difference which showed males retrieving more than females. Moreover, these results are most interesting because they differentiate by sex the group effects noted above. The fact that PL females retrieved more than D5W females strongly suggests that the mechanism facilitating retrieving in females is via hormonal activation of neural substrate. On the other hand, since PL and D5W males cannot be differentiated, the mechanism which seems to have activated retrieving in males is handling. This presumably involves some other mechanism not accessible to females, as shown by the lack of any effect on D5W females' levels of retrieving.

Figure 9b shows that PL and D5W females crouched more than all male groups. There were no differences between PL and D5W females, both of which groups also exhibited higher levels of crouching than NHC females. Thus, in females, hormonal effects cannot be differentiated from the effects of handling alone, which also facilitated crouching. Males, on the other hand, were unresponsive to either manipulation, with both PL and D5W groups showing comparably low levels of crouching as controls. Again, for this behavior, the sexes were differentiated, but only in response to handling, not plasma.

There was a difference in anogenital licking (Figure 9c) between PL females and males which reflected the depression of PL males' performance of anogenital licking overall, relative to the other treatment groups. PL and D5W females engaged in more anogenital licking than NHC females. There were no differences between D5W and PL females, suggesting that handling, and not hormone activation, was the factor in promoting this behavior in females. For the male groups, D5W and NHC males licked pups more that PL males. Apparently hormones present in plasma selectively decreases licking in males, whereas handling has no effect.

Figure 9d shows the depression of sniffing-licking-pawing in both PL and D5W males relative to their female counterparts, as well as to NHC females and males. There were no differences between these two male groups, suggesting that handling was responsible for the change. For females there were no differences among groups, which all showed high levels of this behavior. These data indicate that frequencies of sniffing-licking-pawing are unaffected by either hormone or handling manipulations in females, but that handling alone can depress this exploratory behavior in 30 day-old males, and suggesting the basis of another gender difference.

Figure 10 shows differences in contact among 30 day-olds, which indicated that, while there were no differences among females, handled males (D5W) were in contact more than all female groups. This was not true of males that received plasma in addition to handling (PL group). Levels of contact in PL males were the same as for the NHC males, suggesting that plasma may suppress contact in these males.

We found no gender differences among any of our 30 day-old groups with regard to pupcarrying, nestbuilding, charge and pounce.

Eighteen Day-Olds

As predicted by a hypothesis postulating maturational processes as a mechanism for hormonal effects, 18 day-olds showed few gender differences in maternal or in any other behaviors. However, 18 day-old males showed more nestbuilding than 18 day-old females. There were also some intriguing differences for levels of nestbuilding by group (Figure 11). As Group x Sex interactions (Table 8) indicated, nestbuilding behavior (11a) in females was apparently unaffected by either handling, or hormones in maternal blood plasma. However, for males plasma hormones appeared to have suppressed nestbuilding in the PL group, relative to D5W males, which, in contrast to female data, suggests a difference in the effects of plasma according to sex at this age. For contact (11b), there was a significant sex difference; otherwise no differences were observed. This indicates that in males levels of contact are lower than that of females, and confirms a trend for 18 day-olds observed in Study 1.

Nest Ratings

Although we found no effect of age or treatment per se on nest ratings, we did find AGEGR x GR interactions (Table 11: F=24.14, df=2, p=0.0001) which indicated that handled 30 day-olds (PL, D5W) had higher average nest ratings than all 18 day-olds, as well as 30 day-old controls. All 18 day-olds had higher nest ratings than the 30 day-old NHC group. Handling had no effect on 18 day-old nest ratings. Thus, handling improves the quality of nests in 30 day-olds but not those of younger animals.

Principal Component Analyses: Relationships Among Maternal and Play Variables

One of the purposes of this study was to examine the relationships among maternal behaviors for changes in response to hormone or handling manipulations. Presumably, if neural substrate were capable of response to hormones present in plasma, principal component analyses would show more integration in PL animals relative to controls. That is, maternal behaviors: retrieving, crouching and licking would show a higher degree of relatedness to one another by virtue of appearing on the same factor, for PL animals but not for controls. In addition, because a relationship had been established in Study 1 between play and retrieving behaviors at all ages, another question that arises is: how would these relationships be affected by hormonal manipulation? And, would these relationships be affected by age?

The results of the principal component analysis for the 30 day-olds are shown in Table 12a. These indicate that our first hypothesis was disconfirmed: to our surprise, handling produced as much integration as when plasma and handling were combined. This is shown by the fact that primary loadings for retrieving, crouching and anogenital licking appeared together on Factor 2 for both the PL and D5W groups, and is in contrast to the NHC group, for which only crouching and anogenital licking appeared together on a factor (3), but not in association with retrieving.

An interesting cluster of behaviors occurred for D5W animals on Factor 3: crouching, anogenital licking and contact. this awas similar to the structure of Factor 3 for the NHC group, which consisted of crouching, anogenital licking, and contact with a negative loading. The significance of this similarity in structure is unclear, especially given that the primary loadings for the D5W group for crouching and anogenital licking lie on Factor 2 with retrieving, in a virtual identical configuration to the PL group. However, one interpretation of the fact, that for the D5W group, these same behaviors appear on separate factors corresponding to that of both PL and NHC groups, suggests that this group may share patterns common to both.

For the PL and D5W groups the association between retrieving and pupcarrying, charging and pouncing was considerably weaker than for the NHC group, in that the primary loading for retrieving did not occur on this factor for the two handled groups. As in the NHC's, the latter three behaviors appeared strongly on Factor 1 for the PL and D5W animals, but with the addition of sniff-lick-pawing. This produced a play-exploratory variable, leaving the status of pupcarrying as a maternal behavior in some doubt, but cementing its association with the play behaviors. As the magnitude of loadings on Factor 1 show, this variable has more in common with play-exploratory behaviors than it does with maternal behaviors.

As can be seen in Table 12b, for 18 day-olds there was little differentiation among the three groups with respect to relationships among behavioral variables. For all three 18 day-old groups, as with the 30 day-old NHC's, Factor 1 consisted of retrieve, pupcarry, charge and pounce, all with high positive loadings, and indicating a high degree of association among these maternal and play variables. This suggests that this may be an immature, "juvenile" pattern which is relativly insensitive to manipulation, as compared with 30 day-olds. Passive contact also appeared on Factor 1 with a negative loading, indicating that this behavior is absent in the presence of the active maternal-play complex. However, for 18 day-old D5W and PL groups sniff/lick/paw also loaded positively onto Factor 1, producing a new association of this exploratory behavior with the maternal-play variables. For these behaviors, 18 day-olds could be manipulated in the same way as 30 day-olds, for whom handling (as represented by the undifferentiated D5W and PL groups) produced the same pattern. And, as in the 30 day-old NHC group, nestbuilding and passive contact loaded positively together on Factor 2 for the 18 day-old NHC group, indicating that this may also be a "juvenile" pattern of behavior, in unmanipulated animals.

As Table 12b shows, for the 18 day-old D5W group, crouching and anogenital licking appeared together on Factor 4, indicating that handling facilitated the association between these two maternal variables. Otherwise, for 18 day-old PL and NHC animals, crouching, licking, nestbuilding and contact appeared singly, and apparently at random on various factors. This is interesting since the PL group showed no such association, and in light of the fact that 30 day-olds show this pattern, reinforces the notion that handling and hormones may be acting to trigger separate processes promoting integration. If that were the case, then it would mean that 18 day-olds are susceptible to the effects of handling, at least for certain behaviors, but showed no effects of plasma, in addition.

Discussion

Our results show complex interactions between hormone and handling manipulations which depend upon the sex and age at which those manipulations were introduced, as well as the behaviors which are examined. First, individual juveniles did not reliably or consistently perform maternal activities. However, 30 day-old PL animals showed an increased tendency to perform three characteristic maternal behaviors, retrieving, crouching and anogenital licking.

Secondly, the administration of hormones present in maternal blood plasma, or manipulation by handling, selectively increased maternal behaviors in juveniles, depending upon the age or sex of the animals. Differential effects on maternal behaviors via plasma or handling were as follows: a) maternal blood plasma selectively increased retrieving in PL 30 day-olds; b) there were immediate (Day 0) effects of handling, as shown in increased retrieving, crouching, anogenital licking, contact, and sniffing-licking-pawing by PL and D5W over NHC 30 day-olds, and nestbuilding in PL and D5W 18 day-olds; c) there were also longer-lasting effects of handling, as shown in the facilitation of crouching, anogenital licking and pupcarrying in 30 day-olds, in nestbuilding, sniffing-licking and pawing, and a depression of pupcarrying in 18 day-olds.

A number of interactions of gender differences with different treatments became evident. In 30 day-olds: a) maternal blood plasma, but not handling, selectively facilitated retrieving in females, but not males, accounting for the effect on 30 day-olds noted above; b) for males, handling, but not plasma, facilitated retrieving; c) handling alone facilitated crouching in females, and neither plasma nor handling affected crouching in males; d) handling increased anogenital licking in females but not males; e) plasma depressed anogenital licking in males but not females; f) handling depressed sniffing-licking-pawing in males, but had no affect on females; g) handling increased contact in males, but not females, and plasma lowered contact to control levels in males but not females. In 18 day-olds: a) plasma hormones appear to have suppressed nestbuilding behavior in males; b) while differences were not clearcut, D5W and NHC males did more nestbuilding than did D5W and NHC females, suggesting a possible sex difference; c) females showed more passive contact than males, irrespective of group affiliation.

Third, we found that retrieving behavior is associated with play behaviors in younger juveniles irrespective of treatment, and in older control animals. Handling younger juveniles (PL or D5W) produces a coupling of crouching with anogenital licking characteristic of older juveniles, whereas handling older juveniles (PL or D5W) produces the dissociation of retrieving behavior from play, and an integration of the same behavior with crouching and licking. These occur whether or not hormones are administered during handling. However, there was some suggestion from these data that the integration processes evoked by hormone infusion were different from those elicited by handling.

In contrast to our findings, the data reported by Koranyi et. al. (1976) indicated a more general behavioral responsiveness to hormones by juveniles, which clearly differentiated animals given plasma from those given saline. In addition, the latency for the performance of all behaviors by plasma-injected animals was extremely sudden (an average of 12 hours). There are a number of possible reasons why our results could have differed from theirs, ranging from details of methodology to the nature of juvenile maternal behavior.

First, as indicated in the introduction, because Koranyi et. al.'s paper was in the nature of a review, both their methodology and their results were sketchily presented, making it difficult to interpret the differences between their results and ours. For example, the description of their method of injection does not include such details, but the difficulties involved in injecting serum versus saline could have played a role in determining the performance of maternal behavior in plasma- versus saline-injected animals. Unless the tail is dissected (under magnification, ether anesthesia and/or restraint) it is difficult to tell whether or not a bolus of plasma has been actually injected into the vein.[1] If the tail vein were not accurately injected, saline would be absorbed quickly into capillaries of the tail. Plasma, on the other hand, could remain in interstitial tissues for hours to days due to its high molarity, and act as an irritant to tissues, much the same as a tail-pinch. Work with adult females has shown that tail-pinch can facilitate the onset of maternal behavior during sensitization (Szechtman, Siegel, Rosenblatt, and Komisaruk, 1977), as well as other stimulus-bound behaviors in adult and infant rats (Sahakian and Robbins, 1977; Szechtman and Hall, 1980). Thus, the amount of tail irritation experienced by plasma-injected animals in the

Koranyi et. al. study could by itself have accounted for the differences between their plasma- and saline-injected animals. It is reasonably certain that the method of infusion used in the present study guaranteed systemic circulation of maternal blood plasma in our subjects.

Equally striking was the extremely short latency (12 hours) with which Koranyi et. al.'s plasma-injected animals performed maternal behaviors, shorter than Terkel and Rosenblatt's (1968) average of 2.25 days for a similar method of injection into the femoral vein, and comparable to the 14.5-hour latency obtained by Terkel and Rosenblatt's (1972) more effective cross-transfusion method. However, our data indicate that this striking initial effect may have been an artifact of handling, or perhaps of a tail-pinch in the case of Koranyi et. al.'s study, and that actual plasma effects may be slower-acting. Several investigators have shown that juvenile rats can be induced to be maternal with extremely short latencies (Bridges et. al, 1972; Gray and Chesley, 1984; Mayer and Rosenblatt, 1979), and particularly handled juveniles (Mayer, et. al., 1979). Indeed, as the results of this study have shown, handled animals show high frequencies of maternal behaviors as soon as 90 minutes after infusion. It has been suggested that tail pinch may accelerate the onset of maternal behavior by inducing behavior patterns that facilitate it, such as licking and increased contact with pups (Szechtman et. al., 1977);

handling also promotes increased contact in juveniles (Mayer et. al., 1979). Thus the two procedures may have the same functional outcome, that is the facilitation of maternal behaviors with very short latencies in juveniles.

Interactions of Hormones, Handling and Sex - Evidence for Neuroendocrine Responsiveness

The fact that 30 day-old PL females showed more retrieving by the third day (72nd hour) of testing than either D5W or NHC females shows that these animals were responding to the hormones present in maternal blood plasma, and not merely to nonspecific effects of plasma. The latency with which females in this study reached their peak of retrieving is comparable to latencies of adult females injected with blood plasma (Terkel and Rosenblatt, 1968), which supports this conclusion. However, other behaviors such as crouching, were not similarly facilitated with such specificity. This differential responsiveness to hormones by 30 day-old females may have its basis in the imminent onset of puberty. The first estrus in female rats generally takes place around postnatal day 34-40, whereas for males onset of puberty does not occur until about 10 days later (Ojeda, Andrews, Advis and Smith White, 1980). Moreover, maturation of the neuroendocrine reproductive axis is nearly complete in female rats by about 30 days of age, as evidenced by a number of indicators: increased uterine weight, increasing serum estradiol levels, increasing sensitivity of central components to steroid positive feedback, etc. (Ojeda, et. al., 1980; Parker and Mahesh, 1976). Thus, enhanced retrieving by 30 day-old PL females is probably a sign of a growing responsiveness to hormones, not present in males or younger females. This evidence agrees well with reports that for females adultlike responses are established sometime around puberty (Mayer, et. al., 1979). However, the lack of integration of maternal behaviors observed in individual females, especially in contrast to adult female responses to plasma hormones (Terkel and Rosenblatt, 1968;1971), suggests incomplete maturation of mechanisms underlying these responses. The differential facilitation of maternal behaviors either by plasma or by handling is probably also reflective of the same lack of hormonal maturity.

In addition, these data are in agreement with Gray and Chesley's (1984) finding that juvenile males retrieve more than females, although in our strain it required handling to achieve this effect. While the two studies are not precisely comparable with respect to ages of animals or experimental manipulations, we think that the similarity may lie in the fact that their animals were, in effect, handled when they were removed and returned to cages each day of exposure to pups. Mayer and Rosenblatt (1979b) have reported that brief periods of handling from Days 21-24 increased 24 day-old contact with pups, suggesting that weanlings at that age do react to handling.

Mayer, Freeman and Rosenblatt (1979) found that handling (from Day 21 through 30) differentiated 30 day-old males and females, in that males required more handling to maximize maternal behavior, as defined by pup retrievals. Our results extend their findings for other maternal behaviors: for example, 30 day-old handled males retrieve more than females, females crouch more than males and handled males explore pups less. Similarly, 30 day-old PL males lick pups less, and 18 day-old PL males engage in less nestbuilding, suggesting a hormonal effect on males. These kinds of interactions accounted for variations seen in the major effects of our treatments. Given their complexity and behavioral specificity, they suggest that differences elicited by administration of plasma hormones and by handling are indicative of gender dimorphisms which are very likely to be emerging along more than one behavioral dimension. Again, this could reflect partial maturation of the neuroendocrine axis, which in turn could mean the activation of organizational influences laid down during the perinatal period.

There is general agreement that organizational effects of perinatal androgens influence the expression of differences between male and female rats in maternal behaviors expressed during sensitization (Bridges, Zarrow, and Denenberg, 1972; Lubin, Leon, Moltz, and Numan, 1972; McCullough, Quadagno, and Goldman, 1974;

Quadagno, MCullough, Ho, and Spevak, 1973; Quadagno and Rockwell, 1972). However, gender differences noted for a wide range of behaviors also have their roots in the perinatal period, including emotionality and exploratory behavior (as measured by open field activity), and learning behavior (e.g., passive avoidance) (see Archer, 1973, 1975; Beatty, 1979; Goy and McEwen, 1980; Gray, 1971; Quadagno, Briscoe, and Quadagno, 1977 for reviews). As Mayer et. al. (1979) have pointed out, differences in responsiveness to handling have been demonstrated for these classes of behavior as well, suggesting a role for them in the expression of maternal behaviors. This suggests the concommitant development of other neural and behavioral systems that might effect responsiveness to pups, including those related to arousal, learning and timidity (see Bronstein and Hirsch, 1976; Campbell and Raskin, 1978; Spear and Brake, 1983; Stehouwer and Campbell, 1980).

Facilitation by Age

Older juveniles were differentiated from younger juveniles primarily by the behaviors with which they responded to cohabitation with pups: 30 day-olds showed more retrieving, crouching, and anogenital licking; 18 day-olds showed more nestbuilding and passive contact. As in the last study, nestbuilding activity declined in all 18 day-olds, irrespective of group affiliation, indicating that this behavior undergoes a maturational change over time that does not take place in older juveniles. Moreover, 18 day-old males appeared to show a suppression of nestbuilding activity in response to plasma hormones. What this suggests is that the neuroendocrine complex associated with the performance of maternal behaviors is, perhaps, only begining to mature in 18 day-old juveniles, relative to that of 30 day-olds. It also appears that, at least for some behaviors, the processes whereby maternal behaviors are faciltiated in older animals are very likely not present in juveniles of 18 days of age, or represent different functions altogether.

The fact that 30 day-olds responded to handling by increasing levels of contact on the first day of testing, whereas the 18 day-olds did not, supports Mayer and Rosenblatt's (1979b) work indicating that the growth of a generalized timidity after 24 days plays a greater part in sensitization of older juveniles. On the other hand, the high levels of contact shown by 18 day-olds were not also accompanied by high levels of maternal activities. This suggests that passive contact may play a different role in the sensitization of younger juveniles from that of older juveniles and adults, although it does not preclude contact as a precondition for the expression of maternal behavior (Fleming and Rosenblatt, 1974a,b; Fleming and Luebke, 1981; Mayer and Rosenblatt, 1979a; Terkel and Rosenblatt, 1971).

Interrelationships Among Maternal and Play Behaviors

In the present study the administration of plasma hormones or handling altered the relationships between play and maternal behaviors, and amongst maternal behaviors themselves. One of the more intriguing aspects of this study is the fact that for 30 day-olds retrieving could be coupled with or uncoupled from maternal or play behaviors, depending upon which manipulation was used. For both PL and D5W groups retrieving became associated with crouching and anogenital licking, suggesting that handling may have achieved the integration of these behaviors, at the expense of an association of retrieving with play. On another factor, the D5W group also showed an association between crouching and anogenital licking - but without retrieving, and instead with contact, characteristic of the NHC group. Thus, while the D5W and PL groups showed similar patterns of integration among maternal behaviors, the nature of these associations may be based on different processes. It also suggests that the behaviors themselves can reflect different underlying processes.

While this is only one way of interpreting the factor structure, it does agree with our analyses of groupings of the three maternal behaviors presented initially in the results. In these, more PL animals were shown to engage in retrieving, crouching and anogenital licking than either of the control groups. Thus, in these analyses, the prehoc grouping of behaviors was a more powerful tool for determining differences than were the factor analyses, which were posthoc in nature. On the other hand, the factor analyses may have disclosed two distinct populations amongst the D5W animals: those that retrieved in combination with crouching and anogenital licking, and those that only crouched and licked.

The previous study found that crouching and anogenital licking were associated in older (24, 30 day-old) juveniles, but not 18 day-olds. In this study the same association was produced in the 18 day-old D5W group, but not in the PL group. There is no ready explanation for this, especially since there were no corresponding group differences for these behaviors among the 18 day-olds. However, this finding would at least tentatively indicate that handling promotes a pattern of behavior characteristic of older juveniles, perhaps by accelerating underlying processes responsible for integration. Since 30 day-old controls (NHC) showed the same correlation between retrieving and the play variables as the younger animals, the same explanation may hold: i.e., that handling promotes a more adultlike pattern of behavior. It would be interesting to know if handled 30 day-olds were more competent at retrieving, perhaps facilitating the transition to crouching and anogenital licking once in the nest. Our impression was that as animals became sensitized, they did in fact, become more competent at retrieving pups to the nest.

In the last study, the main finding of principal component analyses was the discovery of a relationship between retrieving and pupcarrying and the play behaviors charge and pounce, in juveniles exposed to infants at 18, 24, or 30 days of age. In this study, for all 18 day-old groups and for the 30 day-old controls, this relationship remained strong, whereas for 30 day-old PL and D5W animals it was weakened. Thus, the relationship between retrieving and the play behaviors may comprise a pattern which is characteristic of juvenile rats, and is retained in younger animals, irrespective of other changes brought about by our manipulations. In older juveniles the basic pattern appears to be more malleable, perhaps due to underlying maturational changes.

It was hypothesized in the last study, that pupcarrying, or incomplete retrieving, serves as a transitional element, perhaps sequentially as part of a behavior chain, during sensitization. Although not directly tested, the present data support that hypothesis, in that, in all of our groups, pupcarrying was still associated with the play behaviors, regardless of age. In addition, in the plasma-injected and handled groups (PL, D5W) at both ages, sniffing-licking-pawing, used as a means of exploring pups as novel stimuli (Mayer and Rosenblatt, 1979) became linked with the maternal - play variable created as Factor 1, perhaps becoming incorporated into the hypothetical behavior chain. Our own informal observations indicate that sniffing-licking-pawing, pupcarrying or retrieving may all serve as alternative endpoints to the sequence: charge - pounce - (?) behavior. Which behavior occurs as the endpoint might depend, perhaps, on pup stimulus cues, whether or not juveniles were in the early or later stages of sensitization, or, for example, on whether or not it was the first five minutes of exposure, as Mayer and Rosenblatt (1979) found for the occurrence of sniff-lick-paw.

Together, these data indicate that the processes of neuroendocrine maturation in juvenile rats occur slowly, and in a piecemeal fashion for different maternal behaviors. The differential facilitation of maternal behaviors by sex reinforces this notion as well. However, they also indicate the development of processes whereby, as juveniles get older, they demonstrate an increasing capacity toward integration of maternal behaviors.

(1) C. Sawyer, personal communication.

Acute and Long-term Effects of Premature Weaning or Early Social Isolation on the Expression of Maternal Behaviors in Sensitized Rats

Introduction

Although the social environment is an all-pervasive influence from the moment of birth (and earlier), little work has been done to delineate the ways in which the early environment interacts with maturational processes during development to produce normal patterns of maternal behavior in adult rats. Most investigations of the ontogeny of maternal behavior have emphasized the examination of the effects of early manipulations, particularly maternal and social deprivation, on adult performance of maternal behaviors. For example, Thoman and Arnold (1968) subjected rats to maternal deprivation and/or social isolation from birth by artificially rearing them: the maternally-deprived animals were reared either in groups or singly, in incubators. Two control groups were reared by mothers either with siblings or as singletons. At maturity, following mating and parturition, the investigators found that deprivation had not disrupted performance of maternal behaviors, and concluded that by most of their direct measures, mother-deprived animals made adequate mothers. In contrast to their direct measures, incubator-reared mothers had a significantly higher incidence of pup mortality than mother-reared groups, due primarily to isolate-reared offspring deaths. Isolate-reared offspring also showed retardation in weight gain and eye-opening, indicative of deficient mothering.

Studies examining the long-term effects of premature weaning on maternal behavior have also found that prematurely-weaned females can provide adequate maternal care, but with some important differences. Novakova (1977) found that early-weaned (15 days) mothers built rudimentary nests compared with those built by normally-weaned (30 days) mothers, although on other measures of maternal behavior, early-weaned mothers were comparable to normally-weaned. Skolnick, Ackerman, Hofer and Weiner (1980), working with our own strain of Wistar rats, also found no deficits in retrieving in their mothers weaned at 15 days, and did not observe nestbuilding activities, but in daily observations found that these females, whether rearing own or cross-fostered litters, spent less time nursing and in contact with their pups, and engaged in more activities outside the nest than normally-weaned (21 days) mothers.

Finally, Sharpe (1975) examined the maternal behavior of rats reared in unisexual or bisexual litters, and found that unisexually reared mothers engaged in less nestbuilding activity and built less elaborate nests than rats reared in bisexual litters, indicating another experiential influence on maternal behavior.

While these studies demonstrate that the early environment can impact upon the performance of maternal behavior at a later period, they provide little information about how these experiences exerted their effects on the development of the behaviors. Their results are difficult to interpret because no knowledge of the processes involved has been gained. This difficulty in interpretation is largely the result of the gap in time between the early event and its later consequences.

The present set of experiments has attempted to fill in that gap by utilizing a longitudinal approach. In this study we looked at the effects of a brief early experience: early weaning or social isolation from 14 to 24 days of age on maternal behavior expressed at 24 days, and then later at 100 days, during sensitization. In this way we thought to establish a relationship between the acute and long-term effects of early experience, as a first step toward delineating the processes by which those experiences are transduced.

In addition, this study may answer some questions regarding the continuity of maternal behaviors expressed by juveniles with those expressed in adulthood. Because of the fragmented and inconsistent nature of the maternal behaviors observed in juveniles during sensitization and their relationship to play behaviors, demonstrated in the previous two studies, the question arises as to whether these bear any direct relationship to the adult form. Two recent studies suggest that early behaviors may be continuous with adult behavior. Taylor (1980) has found that juveniles tend to maintain the same position in a hierarchy of aggressiveness from 20-30 days of age to adulthood at 100 days, showing high positive correlations between juvenile and adult behavior. More pertinent to this study, Gray and Chesley (1984) found that females and males exposed to pups as juveniles showed more retrieving and licking of pups in adulthood than those which had not been preexposed. Significant positive correlations showed that those females that retrieved most as juveniles also retrieved most as adults; this was not true of males. Their study demonstrates that, at least for retrieving behavior (they did not compare other maternal behaviors), rats do show continuity between the juvenile and adult form; moreover, the experience of early exposure to pups had long term effects, in the form of group differences.

Experiment 1

The previous two studies addressed issues of the relatedness of various components of juvenile maternal behaviors, their relationships with other classes of behavior during development and to the maturation of the neural substrate underlying them. The present experiment was directed at examining the role of early experience on development of maternal behaviors, in the maturing rat.

The aims of this experiment were: 1) to determine whether early-weaning or early social isolation would selectively affect maternal behaviors expressed by 24 day-old juvenile rats during sensitization; 2) to determine whether early-weaning or early social isolation would selectively affect gregarious behaviors, e.g., contact and play behaviors (charge, pounce) expressed during sensitization; and 3) to determine whether the relationship between juvenile maternal behaviors and play behaviors would change as a result of early experience manipulations.

Methods

Subjects and Housing

Subjects were 33 female and 30 male 24 day-old juvenile rats born and raised as described in Experiments 1 and 2, until the age of 14 days.

Procedures

Eleven litters of rats were utilized in a split litter design. At 14 days of age all pups in each litter were weighed, earpunched and matched by sex and weight to one of three weaning conditions. Animals in the early-weaned group (EWG) were separated from mothers and housed in mixed-sex groups of 3-4 from each litter; One male and one female sibling from each litter were also early-weaned, but were placed into social isolation (early-weaned isolate group: EWI). The rest of the litter, consisting of 4-6 male and female siblings, were returned to mothers where they remained undisturbed until weaning and testing at 24 days of age. All early-weaned subjects were placed in terraria identical in size and shape to home cages with a layer of home cage shavings; for EWI subjects terraria were divided in half by a sheet of plexiglass, so that each subject in the EWI group occupied approximately half the area of that of the EWG group. Thermoregulated heat was supplied through the terrarium floor to maintain body temperature of the separated animals. Separated animals were fed ad libitum with a mash consisting of powdered rat chow and warm water, which was changed daily. Water was also available ad libitum, from bottles suspended into cages over mash. Care was taken not to handle or otherwise disturb separated animals at the time of feeding; these feedings were matched by frequent feeding of mothers and litters.

One subject of each sex from each weaning group per litter was utilized for testing at 24 days of age: EWG: n=21 (11 females/10 males); EWI: n=21 (11 females/10 males); NW: n=21 (11 females/10 males). Due to procedural error, one male had to be dropped from each treatment condition, accounting for the unequal n's between males and females.

The testing conditions and observational procedures were identical to those described in Studies 1 and 2. All observers were blind to animals' treatment status at all times.

Data Analysis

In the previous two experiments frequency data derived from time-samples provided a means for fine-grained analyses of changes in behaviors and interrelationships among behaviors. In this study we were primarily interested in distinguishing among our groups: we therefore analyzed daily percent occurrence of each behavioral variable for each animal (dichotomous data), rather than the total frequency of behaviors for each animal. The F-statistic is robust with respect to violation of assumptions of normality and homogeneity (Kirk, 1968; Winer, 1971), therefore, parametric statistics were used to accomodate the factorial design of the experiment (3-way ANOVA: Treatment Group x Sex x Days with repeated measures on C; where 3-way ANOVA indicated tendencies toward Group effects but no Group x Day interactions, data were collapsed to perform 2-way ANOVA: Treatment Group x Sex; posthocs were done with Newman-Keuls). Where distributions departed sufficiently from normality to affect the F-statistic appropriate transformations were utilized (Kirk, 1968; Quenouille, 1950; Winer, 1971). Each behavioral variable was analyzed separately.

Frequency data were utilized, however, for principal component (factor) analyses for EWG, EWI and NW groups, to determine underlying relationships among behavioral variables, as in the previous two experiments.

Results and Discussion

Table 13 shows mean weights of the three groups at 24 days of age, just prior to testing. A two-way ANOVA revealed a significant group, but no sex difference in weights ($F(2,76)=16.7254$, $p<0.01$). Posthoc tests showed that NW animals weighed more than both EWG and EWI groups. There were no weight differences between the latter two groups, nor were there differences between sexes.

Behavioral Differences - Maternal Behaviors

A preliminary analysis showed that few (total=6) 24 day-olds met adult 2-day criteria for maternal behavior (see Study 1: Methods). Fisher-Exact tests between individual groups failed to find differences, either with or without a 2-day criterion for consistency. Similarly, comparisons of group latencies to show retrieving/crouching/anogenital licking with either a criterion or no criterion for consistencies revealed no differences among groups or sexes. Therefore, as an approximation to this criterion, the groups were compared with respect to the combined frequencies of retrieving, crouching and anogenital licking shown by individuals throughout the five days of testing. There were significant differences among groups (F(2,57)=3.92, $p<0.05$) but not between sexes. Post hocs indicated that NW animals showed more of these three behaviors than EWI animals ($p<0.05$), but not EWG animals. There were no differences between EWI and EWG groups on this measure. These data indicate that, while 24 day-olds do not show maternal behaviors with the degree of consistency or integration of adults, early isolation can reduce the the frequency with which these behaviors, taken as a group, are elicited by pups during exposure.

Table 14 shows each behavioral variable analyzed and results of ANOVA tests of significance for individual frequencies of behaviors. As illustrated in Figure 12, fewer animals in the EWI group retrieved than the NW or EWG groups; in addition, NW animals retrieved more than animals in the EWG groups. These data indicate that retrieving behavior is most powerfully affected by the combination of early-weaning and social isolation, but also by early weaning per se. As the data will show, these two conditions may be separable by the mechanisms whereby they affect retrieving.

Figure 13 shows crouching behavior amongst the groups. Crouching usually occurs at low levels in 24 day-olds, therefore it not surprising that there were neither group nor sex differences for this behavior. There was a tendency for EWG females to crouch more than other females, and males as well, but Fisher-Exact tests comparing the number of EWG females crouching to other groups did not confirm this tendency.

There were no significant group differences in ano-genital licking, nor were there group differences for nestbuilding. There were also no differences in overall nest ratings. Similarly, all groups showed equivalent percents of passive contact, which were quite high for all.

Induction Effects

There were also Days effects that cut across groups (Table 14), indicating that all three groups were increasing maternal behaviors in response to pup exposure, despite overall differences in mean performances. These included retrieving, crouching, and nestbuilding, and anogenital licking. There were also a significant and Group x Day effects for this maternal measure. Posthoc tests showed that the NW and EWG animals significantly increased licking over the five days of testing, whereas the EWI group did not. Contact also increased over testing, but a Sex x Day effect (F(2, 228)= 2.45; $p<0.05$) indicated that males increased more than females, having started with lower baselines.

Other Behaviors

We next looked at other social behaviors directed at infants by

24 day-olds. Our manipulations affected three of these behaviors: pupcarrying, charging and pouncing. As shown in Figure 14, more of both EWI females and males showed these behaviors than their NW or EWG siblings. Males had a slight tendency to pupcarry more than females, but this only constituted a trend. There was no Day effect for any of these three behaviors, indicating that they did not increase in any of the three groups with exposure to pups, unlike retrieving, crouching or licking. The fact that changes in distributions for these three behaviors were all in the same direction suggest some functional connection among them.

We also found differences among groups in levels of activity (Figure 15): a significantly higher percentage of both EWI and EWG animals engaged in activities while not in contact with pups, compared with NW's. Other behaviors, including the exploratory sniff-lick-paw showed no differences, either by group, sex or days. Significant Days effects showed that activity and rearing decreased with testing for all groups, suggesting that prolonged contact with pups lessened these avoidance activities.

Weight and Retrieving Performance

A question that can be raised with respect to retrieving and pupcarrying in this study is whether or not the performance of these behaviors were related to the weight of juveniles. Since there was a weight difference between normally-weaned and early-weaned groups, it is possible that the lighter EWG and EWI animals retrieved less than NW animals and attempted more pupcarries because they were physically unable to retrieve pups. Therefore, individual weight was correlated with each individual's retrieving and pupcarrying mean over five days of testing. Table 15 shows the results of these correlations. There was no relationship between weight and retrieving for NW animals combined, or males and females separately, nor was there any relationship found for EWI animals, together or separately. However EWG animals did show a positive correlation: when this was broken down further by sex, EWG females showed a significant positive correlation between weight and retrieving, but not males. Therefore, for the combined group of EWG animals, apparently weight contributes to retrieving, and this is specifically true for females. This is interesting in light of the fact that there were no weight differences between EWG and EWI animals. Is it possible that different mechanisms contribute to retrieving deficiencies in these two groups? The suggestion is that for early-weaned isolates other factors (discussed below) were probably responsible for depressing retrieving below that of both normally-weaned and early-weaned group levels. Moreover, elevated levels of pupcarrying in EWI animals appear to be unrelated to weight differences. When female and male groups were combined, there was no relationship between weight and retrieving for females, but a significant correspondance between these for males. Thus for males, within each treatment group, there was no relation between weight and the ability to retrieve, but taken for a range of weights, weight becomes an important factor. For females, weight apparently does not contribute toward retrieving performance, except in the case of EWG females. There were no similar correlations shown for pupcarrying, for either combined or separate groups.

The weight and retrieving results of this study are in partial disagreement with that of Gray and Chesley (1984), who found that for weanling females weight and retrieving performance were uncorrelated, whereas for males there was a negative correlation between the two measures, indicating that heavier animals retrieve less. Our data show the opposite effect for the males. The discrepancy between the two studies may be due to several factors. One is that there was probably more variability in weight in this study than in theirs, since weight was affected by early weaning in this study. Thus a greater range in weight from highest to lowest could have acted to alter the direction of the relationship between retrieving and weight that seems to be present males. A second factor may lie in differences in measures of retrieving: the two studies had different scales of measurement for retrieving, as well as different length test periods (10 days versus 5 days) within which to measure retrieving, and these could have accounted for differences. Nevertheless, irrespective of the direction of differences between the two studies, they both agree that, as a general rule, weight appears to be a predictor of retrieving efficiency in weanling male rats, but not in females. The fact that EWG females (and possibly males) are affected in their performance thus appears to be specifically related to their condition.

Factor Analyses - Relationships Among Behavioral Variables

Table 4 shows the results of three separate principal component (factor) analyses for EWG, EWI and NW groups. Only the first two principal factors are shown, since they had eigenvalues greater than two, a more conservative criterion for inclusion of factors than in previous analyses. Using only the first two factors provides the simplest way to consider the factor analysis data for these groups.

The factor analyses supported data from our comparisons between groups. That is, compared with the NW and EWG groups, which showed Retrieving, Pupcarrying Charging and Pouncing together on Factor 1, for the EWI group Retrieving was no longer correlation with Pupcarrying-Charging-Pouncing group of behaviors. Thus, social isolation effectively reduced the frequency of retrieving to the extent that it was no longer associated with the play behaviors. In addition, these data replicate the findings of the previous two experiments of a Retrieving - Play connection in control animals, and animals which, though early weaned, were able to play in a group situation.

Factor 2 was identical for the three groups: consisting of

Sniff-Lick-Paw, Activity, and Rear, and suggesting that pup exploration and activity, as represented by these three behaviors share some common relationship.

Taken together, the data suggest a number of patterns and possible interactions among early experiences and different classes of behaviors. The first pattern that emerges is that retrieving behavior appears to be functionally related in a reciprocal fashion to three other social behaviors, pupcarry, charge and pounce, shown at this age in the presence of neonates. The changes effected by social isolation are consistent with our previous reports that these four social behaviors share a statistical commonality. As in the previous experiment, retrieving could be disengaged from the other behaviors, in this case by changing the social, as opposed to the hormonal milieu. Social isolation produced a decrease in the number of animals retrieving. Pupcarry, by definition an incomplete retrieval, provided us with a measure of "disorganization" of retrieving, and increased with isolation, providing the connection between the two.

The fact that the social isolates charged and pounced more is consistent with studies which indicate that juveniles will display increased play behaviors following a period of social isolation (Panksepp, 1981; Panksepp and Beatty, 1980; Stevens and Alberts, 1981). The isolates' behavior cannot simply be attributed to hyperactivity, a phenomenon known to occur in social isolates

(Baumal, DeFeo and Lal, 1969; Einon and Morgan, 1977b), as well as in early-weaned animals (Hofer, 1975). More EWG animals were active as well, but did not engage in pupcarrying, charging or pouncing, and their levels of retrieving were midway between controls and isolates. Moreover, at least in the case of females, their retrieving behavior may have been more tied to the ability to physically carry pups, associated with their weight. It is likely, therefore that isolation in 24 day-olds disorganizes retrieving behavior as the result of a deprivation of social interactions, and perhaps specifically of play interactions.

While the mechanism whereby the deprivation of social interaction could disrupt retrieving behavior was not addressed in these experiments, Einon and Morgan (1976) have observed that socially isolated rats tend to have difficulty in inhibiting responding, or in switching between different patterns of behavior. As result of the fact that one hour of social contact a day alleviates such deficits in isolates (Einon and Morgan, 1978), they have suggested that characteristics of social exposure facilitating the ability to switch between patterns of behavior may arise from the rapid alternation of roles and behavior patterns encountered in play. Similarly in domestic cats, juvenile social isolates show an inability to modify behavior in response to social cues during play (Guyott, Bennett, and Cross, 1980). Thus, it is possible that socially isolated rats may be unable to change from a "playful" pattern of behavior to an appropriate caretaking behavior such as retrieving on the basis of cues encountered in association with week-old pups.

While the data do not support firm conclusions, they suggest an intriguing possibility that crouching behavior may be under the control of different mechanisms from retrieving, as evidenced by the tendency for more EWG females to engage in this behavior. It is reasonable to suppose that the mechanisms underlying crouching in juveniles may involve thermal factors as it does in lactating adult females (Woodside and Leon, 1980). Prematurely weaned animals are known to have thermoregulatory deficits (Ackerman, 1981; Greenberg, 1983) and for this reason may show selective effects on crouching behavior. On the other hand, it is by no means certain that thermoregulatory-endocrine relationships underlying crouching behavior in sensitized animals are isomorphic with crouching associated with lactatation and nursing. Nor is it clear why only EWG females should be selectively affected, as opposed to other prematurely-weaned groups, for example EWI females, or EWG males. Thus, while any conclusions regarding this effect must be only tentative, especially given the numbers of animals crouching overall, nevertheless, the phenomenon bears future experimental attention.

As in the previous experiment neither nestbuilding nor anogenital licking were affected by any of our treatments, suggesting that these behaviors are also under separate control from both retrieving and crouching. Their increases over five days does suggest, however, that they are susceptible to influence by pup exposure, and therefore show potential for integration with other maternal behaviors.

In summary, these results support data from our previous experiments which have suggested that early maternal behaviors are distinct during development. They have also shown environmentally-induced lability among maternal behaviors early in life, with respect to the degree that different maternal behaviors can be facilitated or inhibited by differential early experience. Thus, these experiments can be considered a model for naturally-occurring environmental factors which could influence their development.

Experiment 2

This experiment had two major aims: 1) to test for the persistence into adulthood of differences engendered by early maternal separation or early social isolation on maternal behaviors shown at 24 days of age; and 2) to test the hypothesis that individual differences in maternal and other behaviors exhibited early in life will be predictive of individual differences in adults on those same behaviors. In order to control for the effects of previous exposure to pups at 24 days, an additional group was added, the NT, or Normally Weaned Not Tested group.

Methods

Subjects

Subjects were 33 females and 30 males from the previous experiment which constituted the EWG, EI and NW groups, with the addition of the Normally-Weaned Not Tested (NT) group (n=22,) which included 11 females and 10 males. For the most part, these were littermates (one of each sex per litter) of animals in the former three groups. These were reared by their mothers until 24 days of age, at which time they were placed in mixed-sex groups into aquarium cages identical to the cages of siblings being tested, in the same test room. Because some NT animals from the original 11 litters became unavailable for testing at 100 days, animals from an additional three to four litters were included in the NT male/female groups: no more than one individual of each sex per litter was used for each group. These were siblings of juveniles also tested with pups at 24 days of age in another experiment: weaning and housing conditions for these NT group animals were identical to NT animals derived from litters used in Experiment 1.

Procedures

After 5 days of exposure to infants from 24 to 28 days of age for the EWG, EWI and NW groups, or group living in test cages for the NT group, all subjects were placed into same-sex groups in standard wire-mesh hanging cages in the colony room. When subjects were an average of 13 weeks they were reweighed and placed into test cages identical to those used at 24 days in the same room in which they were originally tested. Subjects were then habituated for 7-10 days, since they went from a 12-hr standard light cycle in the colony room to a 12-hr reversed light cycle in the test room. It had been previously noted that it required approximately this amount of time for activity to be readjusted to reversed conditions.

At 14 weeks of age (96 to 100 days), all subjects were exposed to pups for eight days. Testing conditions and observational procedures were as described in Experiment 1. Unlike at 24 days of age, some cannibalism was exhibited at 100 days of age. We therefore established a criterion, wherein an animal was removed from the study after showing two consecutive days of cannibalism. By this criterion, although a total of three animals cannibalized once, only one, an EWI male, was excluded from analyses. The other two animals were a NW female and NT female.

Data Analysis

Data analyses of separate behavioral variables were the same as for Experient 1. For comparisons of adults meeting maternal behavior criteria (see Study 1: Methods) nonparametric statistics were used for analyses, including Chi-Square or Fisher Exact Tests when subjects were dichotomized into those that showed the requisite behaviors and those that did not. Correlations between behavioral measures at 24 and 100 days were calculated by the method of Spearman, with the use of t-tests to determine significance for n's of over 30 (Siegal, 1956, p. 202). For weight data, the Pearson "r" correlation was calculated with one-tailed probabilities, as these effects were in the expected direction.

As in the previous experiment, frequency data were utilized, for principal component (factor) analyses on EWG, EWI, NW and NWNT groups, to determine underlying relationships among behavioral variables.

Results

Weight Data

A 2-way ANOVA comparing weight in 100 day-old NW, NT, EWG and EWI males and females showed no differences among groups, nor was there any interaction of group and sex. As expected, however, there was a marked sex difference: i.e., males weighed more than females (F(1,69)=279.7, p<0.01). Correlations of weight at 24 and 100 days of age showed a significant positive correlation for NW females (r=.61, p<0.025, df=9) and for NW males as well (r=.84, p<0.005, df=8). Of the early-weaned groups, EWI animals showed significant positive correlations: for EWI females (r=.65, df=9, p<0.025) and EWI males (r=.84, df=8, p<0.0005); neither EWG females nor EWG males showed significant correlations between 24 day-old and 100 day-old weights. These data indicate that the effects of early-weaning on weight present at 24 days had disappeared by 100 days, indicating the recovery of function for this parameter of early experience. In the NW and EWI groups individual animals maintained their relative weights with respect to other individuals, but this was not true for the EWG group.

Maternal Behaviors

Few adult females and no adult males met criteria for maternal behavior, showing retrieving, crouching, and anogenital licking together for two consecutive days within the eight-day period of this testing. There were no differences between female groups, as shown by separate Fisher Exact Tests, for either the number of females meeting criteria, or in their latencies. A greater number of animals showed all of these behaviors at least once during testing, and therefore as an approximation to criteria, the groups were compared with respect to the combined frequencies of retrieving, crouching and anogenital licking shown by an individual at any time during the five days of testing, as had been done with juveniles. The results of this analysis revealed no differences among treatment groups for either sex, but did show a strong sex difference (2-way ANOVA: $F(1,75)=5.70$, $p<0.05$) as expected, with females showing higher frequencies of these behaviors than males.

Table 17 shows the results of 2-way ANOVA's of individual maternal behaviors. The 3-way analyses showed Days effects for retrieving ($F(7,525)=11.06$, $p<0.01$), crouching ($F(7,525)=4.32$, $p<0.01$), anogenital licking ($F(7,525)=4.98$, $p<0.01$), and contact ($F(7,525)=7.78$, $p<0.01$) over the eight days of testing, indicating that, for these behaviors, exposure to pups was inducing sensitization equally in all groups. This was not true for nestbuilding behavior: there was no increase over time for any groups of either sex over days. There was a Sex x Day interaction for retrieving ($F(7,525)=3.16$, $p<0.01$): posthoc analyses showed that there was an increase in retrieving behaviors for both males and females. A 2-way ANOVA of collapsed data (Group x Sex) indicated that females retrieved more, overall than males (see below), accounting for the interaction.

Two-way analyses of collapsed data (Group x Sex) revealed that neither early weaning nor early isolation affected the expression of retrieving, crouching, licking, nestbuilding or amount of contact shown by 100 day-olds, in comparisons among the four treatment groups. Nor was there any effect of prior contact with pups at 24 days old when compared with animals receiving first contact with pups at 100 days old (NW versus NWNT). This was true of both males and females.

As expected, there were strong sex differences for all maternal behaviors (Figures 16 - 19): that is, 100 day-old females retrieved (Figure 16), crouched (Figure 17), licked pups' anogenital areas (Figure 18), and did more nestbuilding (Figure 19) than 100 day-old males. Females also showed a tendency to remain in contact with pups more than did males, though this effect did not attain significance.

In contrast to findings for maternal behaviors, the effects of early experience were expressed in pouncing, activity, and rearing behaviors. The EWG and EWI groups pounced more than both NW and NT groups (Figure 20); males also pounced more than females. Though no Group x Sex interaction was found differentiating the groups, the EWG and EWI males appeared to account for the greater amount of pouncing. Figures 21 and 22 show that EWI animals also were more active and reared more than NW, NT and EWG animals, indicating a specific effect of isolation on these behaviors. Males and females were equally active and reared equally across groups, although there was a tendency for females to rear more. These data indicate that longterm behavioral changes can be produced by early weaning and early isolation which are expressed in the context of exposure to pups.

Nest Ratings

A 2-way ANOVA (Group x Sex) showed that there was no main effect for nest ratings, or interactions between treatment groups or sex. However, female mean nest ratings were significantly higher than males ($F(1,75)=26.1833$, $p<0.01$)1

Correlations: Juvenile and Adult Behaviors

To test the hypothesis that individual differences in maternal and other behaviors exhibited early in life will be predictive of individual differences in adults, correlations were done to determine the extent to which individual animals occupied the same relative ranks for individual frequencies of the same behavioral variables. Table 18 shows the results of these correlational analyses at 24 and 100 days of age. As expected, there were few correlations between composite measures of maternal behaviors, since so few animals at either age simultaneously showed retrieving, crouching, licking or nestbuilding. The exception to this was the EWG male group, which showed a significant positive correlation for individual frequencies of combined retrieving, crouching and anogenital licking (r_s=.63, $p<0.05$). When these were broken down into component behaviors, the analyses showed that the relationship was the result of high correlations for retrieving and anogenital licking, indicating that individual EWG males were consistent in rankings across time for these behaviors. Similar consistency in ranking for crouching was shown by EWI females. Neither NW nor EWG females showed any relationship between levels of crouching at 24 and 100 days. There were no other significant relationships shown between maternal behaviors performed at 24 and 100 days.

Other behaviors of interest which showed significant correlations across time were pouncing, activity, and rearing. Since the early-weaned groups had shown higher levels of these three variables in various combinations at both ages, they were combined together for correlational analysis at 24 and 100 days for each group. The results were somewhat startling and counterintuitive: all groups showed negative correlations, and these were significant for both EWI females and males. Trends for these were shown by NW males, and EWG females. These behaviors were then broken down into the combination of activity and rearing to parallel adult group differences (EWI groups showing higher levels of these than other groups: see above) with pouncing treated separately. The results showed that only the EWI males retained a significant negative correlation for pouncing and for the combination of activity and rearing. These negative correlations are somewhat difficult to interpret. However, they do demonstrate that animals which showed this behavior most at 24 days of age performed it least at 100 days of age, and vice-versa. This may show a systematic developmental transformation wherein an acute change in pouncing, for example, in one direction or another induced by early isolation is predictive of long term changes in the opposite direction.

Factor Analyses - Relationships Among Behavioral Variables

In this analysis, only the first two factors, or factors with eigenvalues of 2.00 or greater were included for interpretation. These criteria conformed to the "scree test" advocated by Cattell (see Kim and Mueller, 1978 for reviews of factor analysis).

In contrast to factor analyses at 24 days of age, factor analyses at 100 days showed that females from all groups were capable of integrating maternal behaviors in response to exposure to pups. As shown in Table 19, Factor 1 consisted of Retrieving, Crouching, and Anogenital Licking, and also Contact for all four groups, with the addition of Nestbuilding for the EWG females. This occurred irrespective of weaning history or exposure to pups. However, whereas females were not differentiated in maternal behaviors, Factor 2 indicated that play-exploratory behaviors were retained in females previously exposed to pups. Combinations of Charging, Pouncing, Pupcarrying and Sniff-Lick-Paw loaded onto this factor for NW, EWG and EWI females, whereas for NWNT females Other and Contact dominated Factor 2.

To our surprise, a similar profile was shown by all male groups previously tested with pups at 24 days: that is, all showed full integration of maternal behaviors, with loadings for Retrieving, Crouching, Anogenital Licking and Contact on Factor 1. EWG males, like their female siblings, also showed Nestbuilding on Factor 1. This suggests that some interactive aspect of early weaning in a group and previous experience with infants produces an integration of nestbuilding behavior into the pattern of maternal behavior in early-weaned group animals. However, unlike female groups with previous experience with pups at 24 days, only the EWI males retained the play behaviors, Charge and Pounce, along with Sniff-Lick-Paw on Factor 2. In contrast to males that had previous experience with pups, NWNT males showed Retrieving on Factor 1, and Crouching, Licking and Pouncing on Factor 2, suggesting that males with no experience do not integrate maternal behaviors.

Discussion

Upon first glance, comparisons between group data would seem to indicate that the experience of premature weaning at 15 days, or a period of isolation at the same age exerts no effect on the expression of maternal behaviors at a later time. Neither does the experience of being exposed to pups at 24 days of age have any influence on maternal behaviors upon reexposure at 100 days, in group comparisons to experimentally naive animals.

However, correlations and factor analyses add a significant dimension to this story. Thus, there were significant positive correlations among early-weaned animals for some maternal behaviors: for EWG males, in individual levels of retrieving and anogenital licking between 24 and 100 days; and for EWI females in individual levels of crouching. That no other groups of either sex showed these correlations may be an indication that longterm perturbations in these behaviors result from early weaning and isolation. While these did not show up as group differences, the correlational data suggest that individual differences in patterns of maternal behaviors early in life may be predictive of individual differences in adults for the same behaviors. Perhaps the experience of early weaning and social isolation serve to preserve the "juvenile" quality of maternal behaviors, thus preserving individual differences.

The factor analyses indicated that all adult females are able to integrate maternal behaviors in the context of sensitization, regardless of weaning or experience with pups. This is evidenced by the fact that Factor 1 consisted entirely of maternal behaviors for all groups. However, the presence of juvenile play behaviors on Factor 2 for all females previously exposed to pups demonstrated that in fact some aspect of the experience of exposure to pups at 24 days promotes the retention of these characteristics in the same situation, later in life. Moreover, the presence of nestbuilding on Factor 1 for both female and male EWG groups suggests that some aspect, probably thermoregulatory, promotes the integration of this behavior with other maternal behaviors. Indeed, the fact that this behavior has been shown to be uncorrelated in previous studies with lactating and sensitized animals (Fleming and Rosenblatt, 1974; Slotnick, 1967) reinforces this suggestion.

Most striking was the retention and integration of maternal behaviors in males previously exposed to pups at 24 days of age. This is in contrast to males with no previous exposure, which showed licking and crouching as separate from retrieving behavior. These males resembled those from previous studies which have shown that intact adult males show great variability and little integration of maternal behaviors (e.g., Lubin et al, 1972). Moreover, these data are in partial agreement with Gray and Chesley (1984), who found that early exposed animals are more responsive to pups in adulthood than those not previously exposed to pups.

On the other hand, the lack of differentiation among groups may be due to several factors. For example, in the present study only 30 to 40 percent of females, and fewer males, were retrieving within eight days, irrespective of treatment, indicating that a longer time period was necessary for sensitization of the remaining animals. In comparable sensitization studies, the period of exposure to pups generally varies from 8 or 9 days to over two weeks, with 10 days being the approximate average, and with 60 to 80 percent of females showing retrieving by eight days (e.g., Bridges, Zarrow, Gandelman, and Denenberg, 1972; Fleming and Rosenblatt, 1974; Gray and Chesley, 1984; Stern, 1983; Stern and MacKinnon, 1978). This discrepancy is difficult to explain in terms of procedures used: in the present study test conditions, habituation time, age and number of stimulus pups, and age of adult subjects were all well within standard parameters of sensitization. However, some strains of rats are noted to be more "susceptible" to sensitization; there is evidence to indicate that the Wistar strain is more "resistant" (Jacobowski and Terkel, 1985). Perhaps in our strain of Wistar rats a longer sensitization period could have exposed differences inherent in groups, once a larger baseline of sensitized animals had been established.

A second design feature which contributes to difficulty in interpreting comparisons between groups is that, while there was the NT group to act as a control for the effects of early exposure to pups in normally-weaned animals, for logistical reasons there were no comparable controls for EWG and EWI groups. Presumably, if the effects of exposure to infants and sensitization at 24 days of age were to act to reduce differences due to early weaning or early isolation, untested controls would be a sensitive indicator of this. Without such controls it is difficult to determine whether or not the absence of differences shown between weaning groups at 100 days is due to the mere fact of exposure and sensitization at 24 days, or to some variable occurring during the intervening period.

Some intervening variables which could account for performances at 100 days immediately come to mind. First, while a number of studies have found that virgin female rats sensitized to show maternal behavior are more easily sensitized later (Fleming and Rosenblatt, 1974a; Bridges, 1975, 1977; Cohen and Bridges, 1981), others have shown that a factor governing the expression of maternal behaviors during sensitization in female rats is the presence of circulating hormones, either ovarian or testicular (Bridges, Zarrow and Denenberg, 1973; LeRoy and Krehbiel, 1978; Mayer, Freeman and Rosenblatt, 1979; Mayer and Rosenblatt, 1979a; McCullough, Quadagno, and Goldman, 1973; Quadagno, McCullough, Ho and Spevak, 1973). Since animals were left gonadally intact throughout this experiment, it is not inconceivable that for females hormonal factors could have overridden the effects of early experience in the ten intervening weeks between the first and second exposures to pups.

Second, the deleterious effects of early weaning or isolation have been shown to become attenuated in the interval between an early and later period, without therapeutic intervention of any kind (Ackerman, Hofer and Weiner, 1975; Hofer, 1975b), presumably through maturational processes or the environmental milieu. In this study, an important aspect of the milieu, particularly for social isolates, was group housing after 28 days, affording the opportunity for social interactions with peers, in addition to experiencing the sights, sounds, and smells of the colony. While socialization subsequent to social isolation does not affect some classes of behavior in rats (see below) it can alleviate deleterious effects of social isolation in others, such as some learning tasks (Einon, Morgan and Kibbler, 1978) and sexual behaviors (Gerall, Ward and Gerall, 1967; Hard and Larsson, 1968).

Long-Term Effects

Some behaviors which showed continuity between their juvenile and adult expression, persisting as group differences, were higher levels of activity, rearing and pouncing behaviors. This finding is consistent with studies showing that social isolation prior to 50 days produces persistent hyperactivity in rats even if animals are subsequently rehoused in social groups (Einon and Morgan, 1978).

Early social isolates also show more rearing and object contact and are slower to emerge into a novel environment as adults (Einon and Morgan, 1976; 1977). With respect to the present experiment, these studies suggest that such characteristics generalize to novel situations, which the daily introduction of pups could be considered to be. Our data provide no evidence that the higher levels of activity or rearing observed at 100 days were particularly related to the process of sensitization. However, these behaviors could interfere with the expression of maternal behaviors in other contexts, for example, under different test conditions (see below). This is not unlikely, since hyperactivity has been shown to interfere with performance of sexual behavior in isolated male rats (Gerall, Ward, and Gerall, 1967) as well as in other species (Gerall, 1963; Mason, 1960).

Unlike rearing and activity, pouncing behavior was directed at pups, although the specificity of pups as objects of this behavior was not tested by placing another object, for example a mouse, or another adult, into the cage. Pouncing behavior exhibited by adults could be an expression of prey-killing behavior (Karli, 1956; Paul and Kupferschmidt, 1975), especially since the EWI male which cannibalized did pounce before killing pups. However, this was not true for the other two animals that cannibalized, nor did any of the other animals which pounced kill pups during the test period. An alternative possibility is that this represents the intrusion of juvenile pouncing behavior into the adult behavioral repertoire. Juvenile pouncing may have been elicited by stimulus properties of pups, previously encountered at 24 days of age, and reflect some altered threshold of responsiveness to pup cues. A careful comparison of the morphology or motor pattern of the adult pounce could determine whether or not its function was juvenile, i.e., playful, or adult, i.e., predatory, since their motor patterns are dissimilar in form (Paul and Kupferschmidt, 1975; Pellis and Pellis, 1983; Potegal, personal communication.).

The hypothesis that pouncing is the juvenile form is derived from studies investigating the development of sexual behavior. Several investigators (Folman and Drori, 1965; Gerall, Ward and Gerall, 1967; Hard and Larsson, 1968; Kagan and Beach, 1953) have noted that male rats reared in social isolation display behavior patterns such as headshaking, tunnelling, leaping and climbing responses in the presence of receptive females. Hard and Larsson (1971) have shown that increased frequency of climbing responses is the result of social isolation between 17 and 66 days of age, and not a variety of other factors. Gerall et. al., and Hard and Larsson (1971) have hypothesized that these behavior patterns, observed in the prepubertal play of rats but disappearing normally by puberty, compete in social isolates with responses involved in normal sexual behavior.

In the present study a number of indicators showed that pouncing behavior was an important component in the retesting of animals previously exposed to pups, including its appearance in the factor analysis for female groups, and in the high levels shown by EWG and EWI animals. Thus it is possible that some aspect of previous exposure at 24 days in combination with early weaning promoted the reemergence of this behavior in adults during sensitization. In this respect, the social isolation model may apply to resensitization. It should be recalled that all animals were, in effect, in social isolation for a period of 5 days at their initial testing at 24 days. While they had pups as companions providing thermal, tactile and other cues, these probably differed from those normally encountered in the litter situation. In addition, cues encountered during social play, which is at its peak at this age (Meaney and Stewart, 1981) were quite specifically missing. Thus the experience of isolation from play interactions, and in addition, early weaning, may be a factor in predisposing pouncing in adults during sensitization. Whether pouncing in adults bears any relation to the expression of maternal behaviors during sensitization other than contextual, remains to be determined.

The negative correlations shown for pouncing, activity and rearing suggest that the mechanisms mediating their development in early-weaned and isolated animals are quite separate from those mediating development of maternal behaviors. Moreover, if these correlations are a true reflection of developmental changes occurring in individual animals, they indicate that those animals which were most active, pounced and reared most at 24 days of age were those that did so least at 100 days, and vice-versa. These results are difficult to interpret with respect to mechanisms involved. However, one could speculate that the expression of these behaviors at 24 days could have forestalled the appearance of these behaviors at 100 days. Or, those animals which showed these behaviors at 100 days are those for whom the maturation of these systems did not occur until sometime between 24 and 100 days. In any case, it would be interesting to know if the emergence or disappearance of these behaviors at 100 days were in any way the consequence of the experience of exposure at 24 days.

Potential Differences Between Groups - Relationship to Testing Conditions

If, in fact, there were differences among adult treatment groups in maternal behaviors which were unexpressed at 100 days of age, what conditions could have facilitated their emergence? Le Roy and Krehbiel (1978) have pointed out that standard homecage tests (familiar environments, absence of intruders, etc.), and standard measures of maternal behaviors (e.g., latencies to retrieve, presence or absence of maternal behaviors) provide very favorable conditions for obtaining homogeneity of results in studying maternal behavior. They thus tend to emphasize similarities rather than differences among various classes of subjects. However, when test parameters and response measures have been varied experimentally, qualitative differences in maternal behavior have been detected. For example, whereas a lactating female will attack, and engage in pupcarrying and/or nestbuilding in response to an adult intruder, previously sensitized intact and ovariectomized virgins and castrated males will not (LeRoy and Krehbiel, 1978). Bridges, Zarrow, Gandelman and Denenberg (1972) and Stern and MacKinnon (1976) have shown that lactating mothers can be distinguished from sensitized intact virgins or males by their superior pup-retrieval performance from a T-maze. Mayer and Rosenblatt (1979a), using the same test, found differences between intact and ovariectomized sensitized females, and even between females ovariectomized early versus later in life.

As has been noted, our groups did vary on a number of nonmaternal behaviors which could have influenced the expression of maternal behaviors under conditions other than the standard paradigm which was utilized in this study. Equally significant were indications that certain experiential effects were persistent. By utilizing measures other than the standard sensitization test, it might be possible to show that such behaviors as rearing, activity and pouncing compete with maternal behaviors in the context of sensitization, in animals having the experience of early-weaning and isolation. In this way, perhaps the relationships and interactions among early experiences, early maternal behaviors and the expression of maternal behaviors later in life could be better delineated.

General Discussion

The introduction to this paper began with the premise that maternal behavior in the rat is not a unitary phenomenon. Rather, behaviors considered "maternal" in this species occur separately under other circumstances, and that under particular sets of conditions these behaviors become organized functionally around the care of young. If maternal behaviors consist of separate, independent response systems, then it should be possible to study their separate development, using various research strategies. In the present study several lines of evidence emerged suggesting that maternal behaviors do undergo separate developmental courses.

First, maternal behaviors can be selectively facilitated by sensitization during the prepubertal period, such that some maternal behaviors are expressed more frequently than others. In the first study maternal behaviors were expressed more or less frequently as a function of age, e.g, juveniles retrieved more at 24 days than at either 18 or 30 days, whereas 18 day-olds engaged in more nestbuilding. Administration of maternal blood plasma or handling also had selective effects on the frequency of behaviors shown:

these interacted with both the age at which they were administered, and with sex. Early-weaning or isolation manipulations altered the expression of retrieving and crouching but not other maternal behaviors at 24 days of age.

The coupling of maternal and play behaviors in juveniles, and their uncoupling by various manipulations, also argues for the separate development of maternal behaviors. Moreover, they point to the possibility that during ontogeny, these behaviors are linked with systems not usually thought of as "maternal". Similarly, there were tentative indications that a connection may exist between thermoregulatory competence and crouching and nestbuilding behaviors in juveniles, suggesting the linkage of these early systems during development.

These findings raise fundamental questions about the nature of the development of maternal behaviors. The basic similarity of cues needed to elicit responsiveness between adults and juveniles (Mayer, 1983; Mayer and Rosenblatt, 1979b) as well as the responses themselves suggest a degree of continuity between early and later maternal behaviors. However, another conceptualisation, suggested by Williams (Note 1), for the development of aspects of sexual behavior, may better represent the complexity of relationships indicated by the present work. For example, precocious lordosis-like responses can be elicited in neonatal and weanling rats (Sodersten, 1975; 1978; Sodersten, Damassa and Smith, 1977;

Williams, 1982; Williams and Baron, 1983, Williams, Walther and Yoo, 1983). This behavior is capable of hormone facilitation in very young animals, although special conditions are necessary to elicit it (Williams, 1982; Williams and Baron, 1983; Williams, et. al., 1983). As cited by Williams (1), Beach (1966) has suggested that the motor patterns involved are manifestations of infantile activities, like elimination or stretching, unrelated to adult behaviors. Komisaruk (1978) cites evidence for the incorporation of the lordosis response into a range of adult and infantile reflex behaviors, suggesting that lordosis is a component of a general neural system which in the adult is hormone sensitive (1). These two views could easily be applied to the manifestation of early maternal behaviors, in that components may be utilized for other functions in early life, which would not preclude the integration of the same components into a complex adult pattern cabable of hormonal activation. As such, they could represent "ontogenetic adaptations" (Oppenheim, 1981) early in life, as discussed in Study 1.

Although increasing maturity, as well as administration of plasma and handling at 30 days of age, promoted the integration of maternal behaviors, there was little indication of organization in individual animals, as indicated by their inconsistency and unreliability in performing three maternal behaviors. An inference to be made from these findings is that maternal behaviors are not fully capable of integration in juveniles. In addition, the qualitative differences between juveniles and adults shown by the present as well as previous studies argue strongly for this conclusion. In particular, the effects of the administration blood-bourne hormones in plasma on juveniles suggest that complete organization of maternal behaviors must occur against a background of neuroendocrine and other physiological changes that take place during puberty, as discussed in Study 2. Thus, the majority of evidence indicates that maternal behavior, like other hormone-mediated behaviors (Goy and McEwen, 1980; McEwen, 1981), undergoes an "activational" period during puberty, whose chief influence appears to be to promote an increased level of responsiveness to pups in females, as shown by a variety of measures. This follows a perinatal organizational period, during which differentiation of maternal responsiveness is believed to occur (Bridges, Zarrow, and Denenberg, 1973; Ichikawa and Fujii, 1982; Quadagno, McCullough, Ho, and Spevak, 1973).

However, neither this study, nor any others to date, have determined the extent to which maternal behaviors are dependent upon hormones prior to puberty: that is, just how responsive to hormones the juvenile neural system mediating maternal behaviors is. While both Koranyi et. al.'s study and Study 2 of the present series both suggest that there is some responsiveness present, the two studies are in disagreement with respect to the extent to which there is neuroendocrine functioning. Indeed, Study 2 indicates behavioral heterogeneity in response to plasma, suggesting the relative insensitivity, and thus immaturity, of some neuroendocrine components in the system, whereas Koranyi et. al.'s study suggests rather more complete development.

It is therefore desirable to approach the problem in such a way as to avoid future ambiguities in interpreting results. Specifically, more rigorous control of hormone actions, such as are used in adult studies (e.g., Siegel and Rosenblatt, 1975), should be incorporated into the design of experiments testing the hormone specificity of maternal behaviors prepubertally. This includes gonadectomy or exogenous hormone treatment during the perinatal period or later to determine the effects of perinatal hormones on juvenile maternal behaviors, as well as controlled dosages of ovarian steroids, to determine dose-responses. With these tools it would be possible to address a number of questions regarding the mechanism of hormone action in juveniles, among them: 1) determining the hormonal and behavioral specificity in juvenile rats, for maternal behaviors both individually and collectively; and 2) charting the developmental time-course of steroid-dependency at different ages in juveniles, if such exists.(2)

On the other hand, while infusion of plasma hormones may be less rigorous and controlled than administration of exogenous hormones, it does have the advantage of being more "naturalistic". Presumably, blood plasma of parturient females represents "physiological" levels of hormones, and this may account for the complexity of effects seen in the present set of studies. Exogenous hormones, unless carefully titrated, may only have "pharmacological" results, which may mask subtle effects, not otherwise seen.

If maternal behaviors undergo relatively separate ontogenies, either as generalized precursors of adult forms or as mechanisms tied to other behavioral systems, then an analytical approach could be taken to studying the early development of individual maternal behaviors. This includes investigating the impact of specific deficits brought about by early deprivation studies, in order to isolate mechanisms involved. Experiment 1 of Study 3 of the present investigation examined the relationship of two modes of early deprivation to the emergence of both maternal and nonmaternal behaviors during sensitization. The fact that early maternal separation and social isolation produced alterations in different behaviors suggests that some aspect of each experience corresponded to processes underlying the particular behavior affected. Therefore a next step would be to identify the mechanisms whereby early maternal separation and isolation exerted their effects on behaviors shown by sensitized juveniles. A research strategy that has been particularly useful in this regard has been to selectively provide missing elements of the mother-infant or sibling relationship in the absence of others and observe the impact on the behaviors changed by separation (Hofer, 1978; 1981; 1983). Thus provision of elements known to affect weight loss and thermoregulatory abilities in early-weaned animals (Ackerman, 1981; Ackerman and Shindledecker, 1978; Greenberg, 1983) could permit the investigation of nutritional and thermoregulatory contributions to behavioral changes shown by early-weaned animals during sensitization. Similarly, providing either handling or play-partners to isolates (Einon and Morgan, 1978; Stevens and Alberts, 1981) could reverse selective alterations in retrieval and play behaviors shown by these animals during sensitization. If these manipulations successfully reversed changes brought about by early separation or isolation they would provide clues to mechanisms involved in the development of individual maternal behaviors, at least those shown early in life.(3) The next question which would have to be answered is: Are these early behaviors and their underlying processes representative of the same processes and behaviors later in life?

The persistence of behaviors shown by early-weaned and isolated animals suggests that these experiences constituted significant life-history events which imposed an organization to maternal and other behaviors, continuing into adulthood. The study by Gray and Chesley illustrates an example of this in normally-reared animals, in that, early exposure to infants was in and of itself a significant event capable of altering a maternal response (retrieving) which persisted into adulthood. The factor analyses in the Experiment 2 of Study 3 indicated that this was true of maternal behaviors shown by males previously exposed to infants, and of play behaviors shown by females also previously exposed to infants. While group differences shown by juveniles subjected to early weaning conditions had virtually disappeared, on the other hand small but persistent effects of early weaning and social isolation did show up in individuals later in life. Thus, early life history interacts with later events to produce an organism, which on the whole, resumes a normal course of development, but given the right circumstances shows the effects of early interventions in particular sets of behaviors. Evidence from other areas of developmental inquiry show similar phenomena in animals which have had some special type of treatment early in development, e.g., early stimulation and brain development (Rosenzweig and Bennett, 1978; Walsh and Greenough, 1976). Thus, an event such as early-weaning, occurring against a relatively random background of actions of typical developmental experiences, may provide continuity between the early and later expression of behaviors where none would otherwise exist.

In adult females some factor or factors appeared to promote the integration of maternal behaviors which to a great extent overwhelmed the effects of early experience, at least in group comparisons and in factor analyses based on group data. In all probability, the most crucial event is the onset of puberty, and with it normal functioning of hormones in a cyclical fashion.

However, individual differences in maternal behaviors expressed either during sensitization or in the behavior of lactating females could reveal modulations introduced by the impact of early experience. This is an area of inquiry which deserves further experimental investigation.

If there are different levels at which early processes are continuous with those later in life, then it is important that the levels at which these early processes operate be identified. Analytical investigation can serve to identify correspondances between early and later events which might not be immediately obvious. Moreover, there is some indication that an early insult, such as early weaning in rats, may not show its effects under "normal" laboratory conditions, but rather, needs a "stressor" appropriate to the nature of the insult to show up later on life (Ackerman, 1981). In the early deprivation studies cited in the introduction, discrete behaviors seemed to be affected, suggesting that something about the processes of pregnancy, parturition, or rearing infants was "stressful" enough to show up deficits brought about by early deprivations. The next step is to pinpoint irregularities in discrete behaviors and investigate hypotheses as to the mechanisms underlying them, in order to relate the nature of deficits to the nature of early insults.

(1) C.L. Williams, grant proposal, personal communication.

(2) J.S. Rosenblatt, grant proposal, personal communication.

(3) M.A. Hofer, grant proposal, personal communication.

APPENDIX I: TABLES

Table 1

Cumulative Percents of Each Agegroup Retrieving (Two Consecutive Days) Over 5 Days of Testing

Agegroup	%	(#/n)[1]
18	6	(1/17)
24	77	(14/18)** ***
30	24	(4/17)

** Significantly greater than 30 day-olds: $p=0.028$
*** Significantly greater than 18 day-olds: $p=0.004$

[1] 18 day-olds (n=14 females/3 males);
24 day-olds (n=10 females/8 males);
30 day-olds (n=10 females/7 males).

Table 2

a. Point-biserial Correlation of Any Maternal Behaviors (Criterion=Two Consecutive Days) with Retrieving

Agegroup	(#/n) Showing Any Behavior	rho=
18	(9/17)	-0.3162
24	(17/18)+	0.1157
30	(11/17)	0.4183

b. Point-biserial Correlation of Any Maternal Behaviors Shown with Retrieving (No Criterion)

18	(15/17)	-0.3796
24	(18/18)	0.2091
30	(16/17)	0.4247*

* p=0.05 (one-tailed)

\+ p=0.05 Significantly greater than 18/30 day-olds (Chi Square)

Table 3
a. Analysis of Behaviors[1]

	Source of Variance		
Behaviors	Age	Sex	Day
	F=(df=2,46)	F(df=1,46)	F(df=4,46)
Charge	5.17**	0.58	0.76
Pounce	0.10	1.61	8.19****
Sniff/Lick/Paw	4.08*	3.04	6.75***
Self-groom	23.42****	0.01	1.46
Rest	0.98	3.22	0.77
Eat/Drink	10.52***	0.06	4.06**
Locomote	1.84	0.05	1.51
Sniff	4.78*	0.03	2.23
Pica	9.80***	1.26	2.66*
Rear	9.91***	2.02	0.17

b. Linear Regression Analyses: Changes in Nonmaternal Behaviors Over 5 Days of Testing

		Agegroup		
Behaviors		18	24	30
	F(df=4,46)	t=		
Charge	1.34	1.33	1.29	0.76
Pounce	9.15****	3.46***	3.28***	2.17*
			3****	4.09****

Table 4
Principal Component Analysis[1] of Behaviors: 18, 24 and 30 Day-Olds[2]

	18	24	30
Principal Component	Percent of Variance		
Factor 1	14.2%	13.5%	13.2%
Retrieve	0.38*	0.35	0.56
Pupcarry	0.80	0.74	0.59
Charge	0.73	0.66	0.74
Pounce	0.84	0.85	0.85
Factor 2	10.8%	9.9%	9.9%
Contact	0.82	0.77	0.74
Resting	0.69	0.89	0.84
Factor 4	7.4%	7.3%	7.4%
Crouch	0.42	0.58	0.65
Anogenital Lick	----	0.81	0.81
Factor 8	6.4%	6.5%	6.4%
Nestbuilding	0.92	----	----
Factor 9	6.4%	6.4%	6.4%
Nestbuilding	----	0.89	0.99
Retrieving	----	0.41	----
Anogenital Lick	0.98	----	----

[1] Table represents rotated solution (Nie, et. al., 1975).

[2] Based on a total of 4160 observations derived from daily time-samples over 5 days.

*Numbers are coefficients: Express relationship of each behavioral variable to the factor.

---- Indicates that variable had loading of <0.30 onto factor for designated age-group.

Table 5
Percent of Animals in Each Group Retrieving

	On at Least 1 of 5 Days			For 2 Successive Days		
	#	(%)	Latency[1]	#	(%)	Latency
30 Day-Olds						
PL(n=17)	14	(82)[a,b,c]	1	8	(47)	1
F(n=11)	8	(73)[d]	0.5	4	(36)	0
M(n=6)	6	(100)	2	4	(66)	2
D5W(n=13)	6	(46)	0	4	(31)	0
F(n=8)	3	(38)	4	1	(13)	4
M(n=5)	3	(50)	0	3	(50)	0
NHC(n=18)	9	(50)	2	6	(30)	2
F(n=11)	4	(36)	2	3	(27)	2
M(n=7)	5	(71)	2	3	(43)	2
Combined(n=48)	29	(60)	1	18	(38)[f]	1
Comb. F(n=30)	15	(50)	2[e]	8	(27)	2[g]
Comb. M(n=18)	14	(48)	2	10	(56)	2
18 Day-Olds						
PL(n=15)	6	(40)	3	3	(20)	3
F(n=8)	5	(63)	3	2	(25)	3.5
M(n=7)	1	(14)	3	1	(14)	3
D5W(n=13)	6	(46)	3	1	(8)	2
F(n=6)	1	(50)	2	1	(17)	2
M(n=7)	3	(43)	3	0	(0)	>4
NHC(n=14)	6	(43)	2.5	0	(0)	0
F(n=11)	4	(36)	2.5	0	(0)	0
M(n=3)	2	(66)	2.5	0	(0)	0
Combined(n=42)	18	(43)	3	4	(9.5)	3
Comb. F(n=25)	12	(48)	3	3	(12)	2
Comb. M(n=17)	6	(33)	3	1	(6)	2

[1] In days.

[a] Significantly greater than D5W/NHC combined (X_2=3.97, df=1, p<0.05).
[b] Greater than D5W (X_2=2.87, df=1, p<0.10).
[c] Greater than NHC (X_2=2.75, df=1, p<0.10).
[d] PLF > D5WF + NHCF (Combined): X_2=2.29, df=1, p<0.10.
[e] Significantly less than 18 day-olds (Z=3.18, p<0.001).
[f] Significantly less than 18 day-olds (Z=2.37, p<0.01).
[g] Significantly greater than 18 day-olds (X_2=8.04, df=1, p<0.01).

Table 6
Percent of 30 Day-Olds Engaging in Individual Maternal Behaviors
a. Within Age Comparisons

	PL		D5W		NHC	
Behavior	%(a)	%(b)	%(a)	%(b)	%(a)	%(b)
Retrieve	82[1,2,3]	47	46	31	50	33
Females	73[4]	36	38	13	36	27
Males	100	67	60	60	71	43
Crouch	59	6	69	38[5]	39	6
Females	64	9	88	38	36	9
Males	50	0	40	40	43	0
A-g Lick	82	53	92	46	94	72
Females	91	55	100	38	100	73
Males	67	50	80	60	86	71
Nestbuild	53	24	62	15	44	11
Females	64	27	75	0	55	18
Males	33	17	40	20	29	0

b. Comparisons Between Age Groups

Retrieve (b):	30 Day-Olds (38%) > 18 Day-Olds (10%) (X_2=8.04, df=1, p<0.01)
Crouch (a):	30 Day-Olds (54%) > 18 Day-Olds (5%) (X_2=23.26, df=1, p<0.001).
Anogenital Lick (b):	30 Day-Olds (58%) > 18 Day-Olds (26%) (X_2=8.16, df=1, p<0.01).
Nestbuild (a):	18 Day-Olds (76%) > 30 Day-Olds (52%) (X_2=4.62, df=1, p<0.05).

(a) At Least Once in Five Days (b) For 2 Consecutive Days in Five

[1] Significantly greater than D5W/NHC combined (X_2=3.97, df=1, p<0.05)

[2] Greater than D5W (X_2=2.87, df=1, p,0.10)

[3] Greater than NHC (X_2=2.75, df=1, p<0.10)

[4] PL Females greater than D5W/NHC Females Combined (X_2=2.29, df=1, p<0.10)

[5] D5W significantly greater than PL/NHC Combined (X_2=5.74, df=1, p<0.02)

Table 7

Percent of Animals Engaged in Three Maternal Behaviors[1]

	On at Least 1 of 5 Days			On Two Successive Days		
	#/n	(%)	Latency[2]	#/n	(%)	Latency
30 Day-Olds						
PL=	6/17	(35%)	0.5	1/17	(6%)	0
D5W=	3/13	(23%)	0	1/13	(8%)	0
NHC=	5/18	(28%)	2	1/18	(5%)	2
Combined=	14/48	(29%)[a]	1.5	2/48	(4%)	0
18 Day-Olds						
PL=	0/15	(0%)	4	0/15	(0%)	4
D5W=	1/13	(8%)	0	0/13	(0%)	4
NHC=	0/14	(0%)	4	0/14	(0%)	4
Combined=	1/42	(2%)	4	0/42	(0%)	4

[1] Retrieve/Crouch/Anogenital Lick

[2] Median Latency, in days.

[a] Significantly greater than 18 Day-Olds: $X^2=9.72$, df=1, $p<0.01$.

Table 8
Analyses of Behaviors

	Source of Variance				
Behaviors	Age	Group	Day	Age x Day	Group x Day
	F(1,46)=	F(2,46)=	F(4,46)=	F(4,46)=	F(8,46)=
Retrieve	19.41**	3.28+	12.33****	10.49**	3.59**
Pupcarry	0.07	2.05	8.92*	3.27**	2.78**
Crouch	7.04*	1.48	2.38*	3.02*	4.29****
Anogenital Lick	9.07*	0.35	8.60****	1.72	2.32*
Nestbuild	17.60**	1.84	4.68***	8.52****	2.09*
Contact	141.33****	4.03+	21.68****	28.52****	5.93****
Sniff-Lick-Paw	37.36***	0.77	3.98**	3.18**	2.78*
Charge	28.81**	0.87	1.57	5.40**	1.40
Pounce	2.45	0.55	6.73****	12.97****	3.68**

+p<0.10
*p<0.05
**p<0.01
***p<0.001
****p<0.0001

Table 9
Linear Regression Analysis on Behaviors

a. 30 Day-Olds

Group	Behavior (F(6,48)=)	Specific Comparison[1]
	Retrieve (4.0)	Day 0: PL/D5W>NHC (0.001/0.001)
PL	(t=3.55, p<0.001)[2]	Day 1: PL/D5W>NHC (0.01/0.05
NHC	(t=1.78, p<0.10)	Day 3: PL>D5W/NHC (0.01/0.01)
D5W	(t=0.84, p=.40)	
	Crouch (4.28)	Day 0: PL/D5W>NHC (0.001/0.001)
D5W	(t=3.33, p<0.001)	Day 1: PL>NHC (0.01)
PL	(t=1.66, p<0.10)	
NHC	(t=0.46, p=0.64)	
	Anogenital Lick (3.30)	Day 0: PL/D5W>NHC (0.80/0.01)
D5W	(t=3.17, p<0.01)	Day 1: PL/D5W>NHC (0.01/0.01)
NHC	(t=2.12, p<0.05)	
PL	(t=0.94, p=0.35)	
	Nestbuild (5.49)	None
NHC	(t=1.59, p=0.11)	
PL	(t=0.66, p=0.51)	
D5W	(t=0.01, p=0.99)	
	Contact (17.35)	Day 0: PL/D5W>NHC (0.001/0.001)
NHC	(t=6.36, p<0.001)	Day 1: PL/D5W>NHC (0.05/0.01)
D5W	(t=1.39, p=0.16)	
PL	(t=1.07, p=0.28)	
	Sniff-Lick-Paw (3.98)	Day 0: NHC>PL/D5W (0.001/0.01)
NHC	(t=4.11, p=0.001)	Day 2: NHC>D5W (0.10)
PL	(t=0.69, p=0.49)	Day 3: NHC>D5W (0.10)
D5W	(t=0.31, p=0.75)	
	Pupcarry (0.95)	None
NHC	(t=1.48, p=0.14)	
PL	(t=1.02, p=0.31)	
D5W	(t=0.69, p=0.49)	
	Charge (2.64)	None
	Pounce (2.64)	None
PL	(t=1.56, p=0.2)	
NHC	(t=1.16, p=0.24)	
D5W	(t=0.08, p=0.93)	

[1] Daily between-group comparisons based on overall F-value.

[2] T-test for zero change in slope over 5 days.

Table 9
Linear Regression Analysis on Behaviors

b. 18 Day-Olds

Group	Behavior (F(6,48)=)	Specific Comparison[1]
	Retrieve (10.22)	None
NHC	($t=4.30$, $p<0.001$)[2]	
PL	($t=0.84$, $p=0.40$)	
D5W	($t=0.70$, $p=.48$)	
	Crouch (5.35)	None
NHC	($t=2.87$, $p<0.001$)	
PL	($t=2.33$, $p=0.19$)	
D5W	($t=0.10$, $p=0.92$)	
	Anogenital Lick (3.30)	None
NHC	($t=2.36$, $p<0.05$)	
D5W	($t=1.40$, $p=0.16$)	
PL	($t=0.89$, $p=0.37$)	
	Nestbuild (5.49)	Day 0: D5W>PL/NHC (0.05/0.001) Day 1: D5W>PL/NHC (0.05/0.05)
D5W	($t=4.47$, $p<0.001$)	
PL	($t=3.68$, $p<0.001$)	
NHC	($t=2.83$, $p<0.01$)	
	Contact (18.80)	None
NHC	($t=8.76$, $p<0.001$)	
PL	($t=5.50$, $p<0.001$)	
D5W	($t=2.13$, $p<0.05$)	
	Sniff-Lick-Paw (2.77)	None
NHC	($t=3.00$, $p<0.05$)	
PL	($t=2.81$, $p<0.01$)	
D5W	($t=1.39$, $p=0.54$)	
	Pupcarry (4.62)	None
NHC	($t=3.86$, $p<0.001$	
D5W	($t=1.39$, $p=0.16$)	
PL	($t=0.95$, $p=0.34$)	
	Charge (0.70)	None
	Pounce (2.64)	Day 1: NHC>PL/D5W (0.03/0.05) Day 4: PL>D5W (0.10)
PL	($t=3.52$, $p<0.001$)	
NHC	($t=1.45$, $p=0.15$)	
D5W	($t=1.34$, $p=0.18$)	

1 Daily between-group comparisons based on overall F-value.

2 T-test for zero change in slope over 5 days.

Table 10
Gender Differences

Source of Variance

Behavior	Sex F(1,46)=	Age x Sex F(1,46)=	Group x Sex F(2,46)=	Age x Group x Sex F(2,46)=
Retrieve	2.88+	4.23*	3.67*	3.74*
Pupcarry	0.63	0.05	0.65	0.58
Crouch	25.40****	13.14**	7.95**	4.04*
A-g Lick[1]	1.03	1.85	4.65***	5.20**
Nestbuild	0.35	12.12***	8.08**	2.80+
Contact	10.60**	5.08*	1.79	4.38*
S-L-P[2]	4.38*	7.05**	8.24***	0.96
Charge	4.40*	2.25	0.67	3.21*
Pounce	0.64	0.50	2.17	3.72*

1 Anogenital Lick; 2 Sniff-Lick-Paw

+p<0.10
*p<0.05
**p<0.01
***p<0.001
****p<0.0001

Table 11
Nest Ratings

Age	Group	Mean
18	PL	1.96
18	D5W	1.94
18	NHC	1.97
30	PL	2.09
30	D5W	2.06
30	NHC	1.83

Specific Comparisons

Within 18 Day-Olds	Between Age-Groups	Within 30 Day-Olds
None	30 PL> 18 PL** 30 D5W>18 D5W**	30 PL> 30 NHC** 30 D5W> 30 NHC**
	18NHC> 30 NHC**	

* p<0.05
** p<0.01

Table 12
Principal Component (Factor) Analysis: Relationships Among Behavioral Variables

a. 30 Day-Olds

Groups	Factors (Percent of Variance)			
PL	1(18.1)	2(11.4)	3(11.0)	4(9.8)
	Pupcarry (0.57)*	Retrieve (0.54)	Contact (-0.84)	Crouch (0.34)
	Charge (0.86)	Crouch (0.45)	Other (0.74)	Contact (0.31)
	Pounce (0.90)	A-g Lick[1] (0.74)	---- ----	Rear (-0.81)
D5W	1(17.2)	2(11.6)	3(10.8)	4(9.8)
	Pupcarry (0.48)*	Retrieve (0.39)	Crouch (0.30)	Self-groom (-0.35)
	Charge (0.87)	Crouch (0.48)	Contact (0.58)	Other (0.53)
	Pounce (0.90)	A-g Lick (0.69)	A-g Lick (0.40)	Rear (-0.57)
	Contact (-0.31)	---- ----	Other (-0.78)	S-L-P[2] (-0.43)
NHC	1(16.4)	2(10.9)	3(10.4)	4(10.0)
	Retrieve (0.44)	Contact (0.70)	Crouch (0.54)	Crouch (0.43)
	Pupcarry (0.61)	Nestbuild (0.28)	Contact (-0.47)	Contact (0.33)
	Charge (0.74)	Other (-0.84)	A-g Lick (0.66)	A-g Lick (0.48)
	Pounce (0.85)	---- ----	Other (-0.35)	Self-Groom (-0.53)
	---- ----	---- ----	---- ----	S-L-P (-0.43)

*Numbers in parentheses are coefficients: Express relationship of each variable to the factor. [1]Anogenital Lick. [2]Sniff-Lick-Paw

Table 12
Principal Component (Factor) Analysis:
Relationships Among Behavioral Variables

b. 18 Day-Olds

Groups	Factors (Percent of Variance)			
PL	1(16.4)	2(13.4)	3(10.1)	4(9.4)
	Retrieve (0.37)*	Pupcarry (0.39)	Other (-0.63)	Nestbuild (-0.37)
	Pupcarry (0.58)	Contact (0.65)	S-L-P (-0.81)	Rear (0.89)
	Charge (0.73)	Pounce (-0.72)	---- ----	---- ----
	S-L-P (0.41)	---- ----	---- ----	---- ----
D5W	1(15.7)	2(12.5)	3(10.1)	4(9.1)
	Retrieve (0.34)*	Pupcarry (0.36)	Other (0.75)	Crouch (0.61)
	Pupcarry (0.69)	Contact (0.76)	S-L-P (-0.73)	A-g Lick (0.80)
	Charge (0.44)	Pounce (0.33)	---- ----	---- ----
	Pounce (0.80)	Other (-0.56)	---- ----	---- ----
	S-L-P (0.32)	S-L-P (-0.49)	---- ----	---- ----
NHC	1(18.2)	2(12.7)	3(10.4)	4(8.9)
	Retrieve (0.47)	Nestbuild (0.29)	Other (0.75)	Nestbuild (-0.31)
	Pupcarry (0.77)	Contact (0.88)	S-L-P (-0.80)	Rear (0.94)
	Charge (0.74)	Other (-0.60)	---- ----	---- ----
	Pounce (0.83)	S-L-P (0.49)	---- ----	---- ----
	---- ----	---- ----	---- ----	S-L-P (-0.43)

Table 13
NW, EWG, EWI Weights at 24 Days of Age*

Weaning Group	Females	Males
	X (SD)	X (SD)
NW	69.6 (7.6)	74.9 (9.9)
EWG	59.1** (7.8)	57.5** (11.2)
EWI	61.4* (7.9)	63.7* (8.8)

*N.B. Although weights are shown separately, there were no differences between males and females in any of the three groups, and group differences are for combined sexes.

*EWI significantly less than NW ($p<0.05$)
**EWG significantly less than NW ($p<0.01$)

Table 14
Analyses of Individual Behaviors

Behaviors	Day	Group	Sex	Group x Sex
	Source of Variance			
	F=(4,228)	F=(2,57)	F=(1,57)	F=(2,57)
Retrieve	16.9**	4.04*	1.64	0.76
Pupcarry	0.84	11.47**	2.27	0.64
Crouch	3.08*	0.66	0.11	2.45+
A-g Lick	3.57*	2.07	0.24	0.54
Nestbuild	3.05*	0.57	1.56	0.24
Contact	6.32**	0.49	2.48+	0.55
S-L-P	1.75	1.19	1.19	0.94
Charge	1.58	4.26*	0.83	0.26
Pounce	1.02	5.46**	0.09	0.01
Activity	4.78**	3.41*	0.23	1.28
Rear	2.71*	1.3	0.49	0.39

+ $p<0.10$ * $p<0.05$ ** $p<0.01$

Table 15
Correlations Between Weight and Retrieving
or Pupcarrying at 24 Days

Group	Weight - Retrieving	Weight - Pupcarrying
NW		
Combined	0.21	0.02
Females	0.25	-0.20
Males	-0.21	0.42
EWG		
Combined	0.42*	-0.14
Females	0.54*	0.38
Males	0.08	-0.50
EWI		
Combined	0.16	-0.14
Females	0.06	0.07
Males	0.35	-0.21
Combined Females	0.19	-0.13
Combined Males	0.36*	-0.04

* $p<0.05$

Table 16
Principal Component Analysis[1] of Behaviors

Twenty-four Day-Olds

Treatment Groups	Factors (Percent of Variance)	
EWG	1(18.6)	2(16.4)
	Retrieve (0.47)*	S-L-P[1] (0.63)
	Pupcarry (0.74)	Rear (0.62)
	Charge (0.53)	Activity (0.62)
	Pounce (0.86)	
EWI	1(23.8)	2(14.3)
	Pupcarry (0.80)	S-L-P (0.71)
	Charge (0.77)	Activity (0.70)
	Pounce (0.88)	Rear (0.50)
NW	1(19.1)	2(15.4)
	Retrieve (0.29)*	Activity (0.70)
	Pupcarry (0.72)	S-L-P[1] (0.61)
	Charge (0.72)	Rear (0.58)
	Pounce (0.82)	

*Numbers in parentheses are coefficients: Express relationship of each variable to the factor.

[1] Sniff-Lick-Paw

Table 17
Analyses of Behaviors

	Source of Variance		
Behaviors	Group	Sex	Group x Sex
	F=(3,76)	F=(1,76)	F=(3,76)
Retrieve	0.23	6.37*	0.24
Pupcarry	1.68	0.05	0.21
Crouch	0.47	14.54**	0.48
A-g Lick	1.63	5.13*	0.20
Nestbuild	0.61	8.67*	0.21
Contact	0.67	3.22+	0.28
S-L-P	0.22	1.40	0.37
Charge	1.25	0.69	0.07
Pounce	3.04*	3.98*	0.82
Activity	2.83*	0.16	0.13
Rear	2.30+	2.31+	0.91

+ $p<0.10$
* $p<0.05$
** $P<0.01$

Table 18
Correlations Between Individual Components of Behavior*
24 Days - 100 Days

	Females			Males		
	Weaning Group					
Behavior	NW	EG	EI	NW	EG	EI
Ret/Crouch/AgLick	.30	.26	.15	.21	.63*	.08
Retrieve	.39	.16	-.21	.07	.60+	.08
Pupcarry	-.15	.34	-.27	.37	.21	0
Crouch	.10	.45	.71*	.25	.19	-.16
Anogenital Lick	.06	.21	-.005	.07	.82*	-.05
Nestbuild	.38	.09	.21	.07	.45	.11
Contact	.06	-.19	-.02	.07	.35	-.33
Sniff-Lick Paw	-.18	.17	.20	.17	.08	.30
Charge	.08	.14	.14	0	.006	.43
Pounce	0	.11	.002	.42	.25	-.65*
Activity	.49	.21	-.51	-.05	-.09	-.43
Rear	-.55	.09	-.48	-.15	-.06	-.37
Act. + Rear	-.005	-.32	-.60+	.02	.05	-.64*
Pounce/Act/Rear	-.01	-.60+	-.70*	-.61+	-.37	-.67*

* Spearman rho (two-tailed).
* $p<0.05$
\+ $p<.10$

Table 19

Principal Component Analysis[1] of Behaviors:

a. One-Hundred Day-Old Females

	EWG	EWI	NW	NWNT
Factors		Percent of Variance		
1	23.8%	22.1%	22.1%	22.4
	Retrieve (0.76)*	Retrieve (0.72)	Retrieve (0.77)	Retrieve (0.71)
	A-G Lick (0.83)	A-g Lick (0.76)	A-g Lick (0.75)	A-g Lick (0.68)
	Crouch (0.68)	Crouch (0.67)	Crouch (0.67)	Crouch (0.59)
	Contact (0.68)	Contact (0.75)	Contact (0.59)	Contact (0.58)
	Nestbuild (0.33)			
2	16.4%	14.7%	17.4%	14.5[2]
	Pupcarry (0.84)	Charge (0.94)	Pupcarry (0.54)	
	Pounce (0.83)	Pounce (0.94)	Charge (0.86)	
	S-L-P (0.36)		Pounce (0.87)	

[1] Table represents rotated solution (Nie, et. al., 1975).

*Numbers are coefficients: Express relationship of each behavioral variable to the factor.

[2] Indicates Factor had eigenvalue of less than 2.

Table 19

Principal Component Analysis[1] of Behaviors:

b. One-Hundred Day-Old Males

	EWG	EWI	NW	NWNT
Factors		Percent of Variance		
1	20.2%	21..0	18.3%	20.3
	Retrieve (0.73)*	Retrieve (0.60)	Retrieve (0.32)	Retrieve (0.47)
	A-G Lick (0.72)	Pupcarry (0.55)	A-g Lick (0.74)	Contact (0.48)
	Crouch (0.51)	Crouch (0.72)	Crouch (0.49)	Other (0.89)
	Contact (0.73)	Contact (0.72)	Contact (0.69)	
	Nestbuild (0.52)			
2	16.5%	17.4%	13.9%[2]	15.7
	Other (0.92)	Charge (0.89)		Pounce (0.86)
		Pounce (0.92)		A-g Lick (0.48)
		S-L-P (0.71)		Crouch (0.90)

[1] Table represents rotated solution (Nie, et. al., 1975).

*Numbers are coefficients: Express relationship of each behavioral variable to the factor.

[2] Indicates Factor had eigenvalue of less than 2.

APPENDIX II: FIGURES

Figure 1

Mean frequencies of maternal behaviors and passive contact observed for each age-group over 5 days of testing. a) 18 day-olds (n=17: 14 females/3 males) denoted by open bars; b) 24 day-olds (n=18: 10 females/8 males) denoted by stippled bars; c) 30 day-olds (n=17: 10 females/7 males) denoted by hatched bars. Note that frequencies are relative to the total possible observed (N=16 per day x 5 days) for each behavior. Based on a total of 4160 observations derived from time-samples.

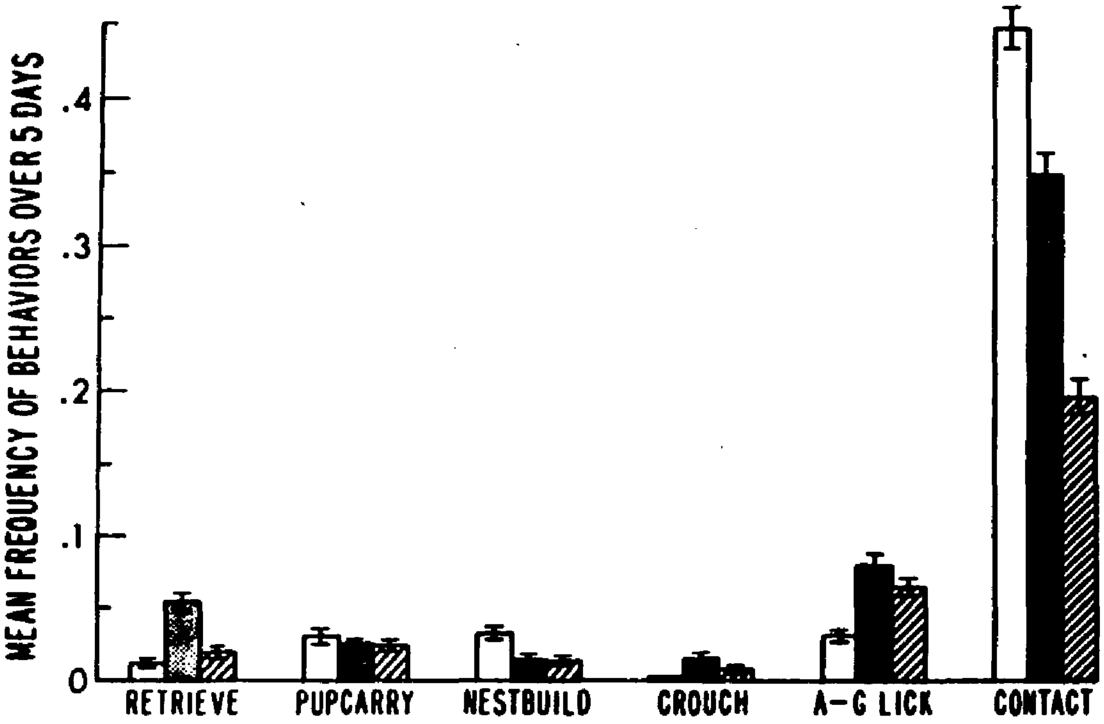

MEAN FREQUENCY OF BEHAVIORS OVER 5 DAYS
.4
.3
.2
.1
0
RETRIEVE
PUPCARRY
NESTBUILD
CROUCH
A-G LICK
CONTACT

Figure 2

Daily changes in group means over 5 days of testing for: a) retrieving; b) nestbuilding; c) crouching; d) anogenital licking. Day 0- 4 of testing for 18 day-olds (n=17: 14 females/3 males) denoted by triangle; for 24 day-olds (n=18: 10 females/8 males) by circle; 30 day-olds (n=17: 10 females/7 males) by box. Frequencies as in Figure 1.

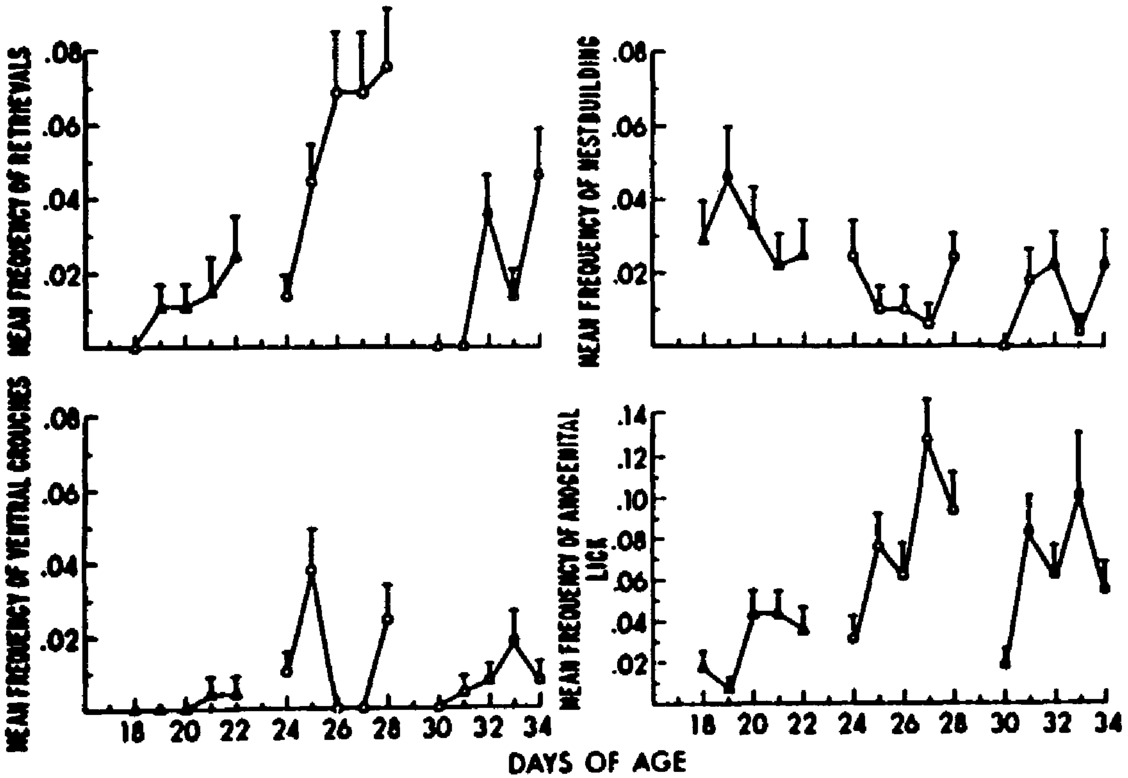

MEAN FREQUENCY OF RETRIEVALS
MEAN FREQUENCY OF NESTBUILDING
MEAN FREQUENCY OF VENTRAL CROUCHES
MEAN FREQUENCY OF ANOGENITAL LICK
.08
.06
.04
.02
.14
.12
.10
.08
.06
.04
.02
18
20
22
24
26
28
30
32
34
DAYS OF AGE

Figure 3

Daily changes in group means over 5 days of testing for contact behaviors. Days 0 - 4 of testing as in Figure 2.

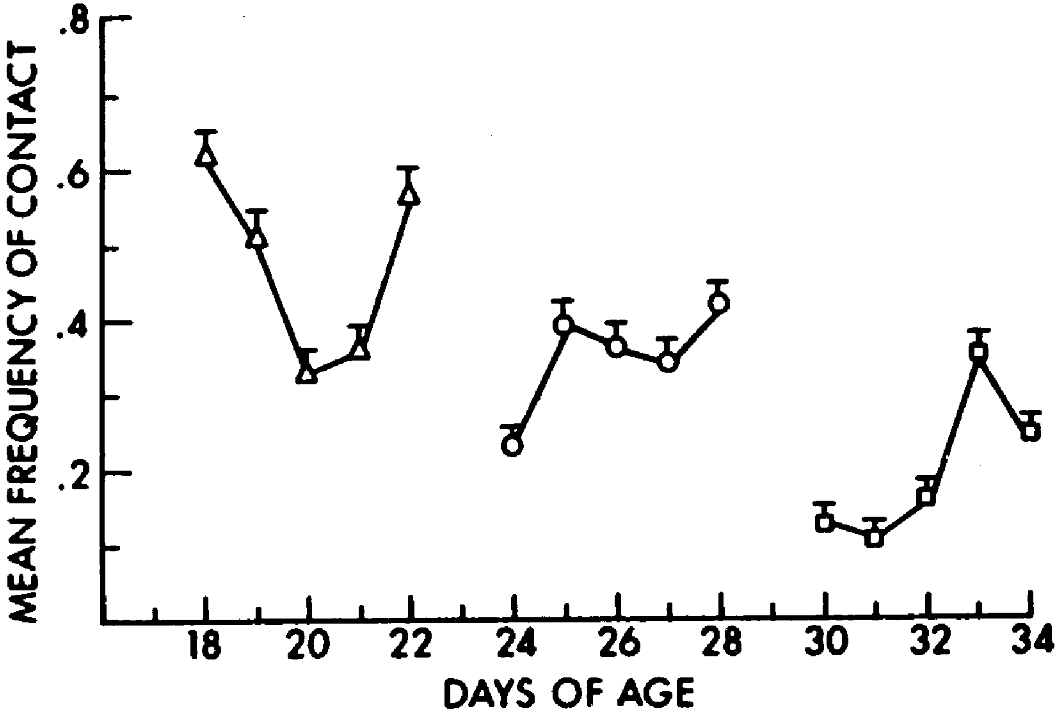

MEAN FREQUENCY OF CONTACT
.8
.6
.4
.2
18
20
22
24
26
28
30
32
34
DAYS OF AGE

Figure 4

Mean frequencies of nonmaternal behaviors observed for each age group over 5 days of testing. Frequencies as in Figure 1.

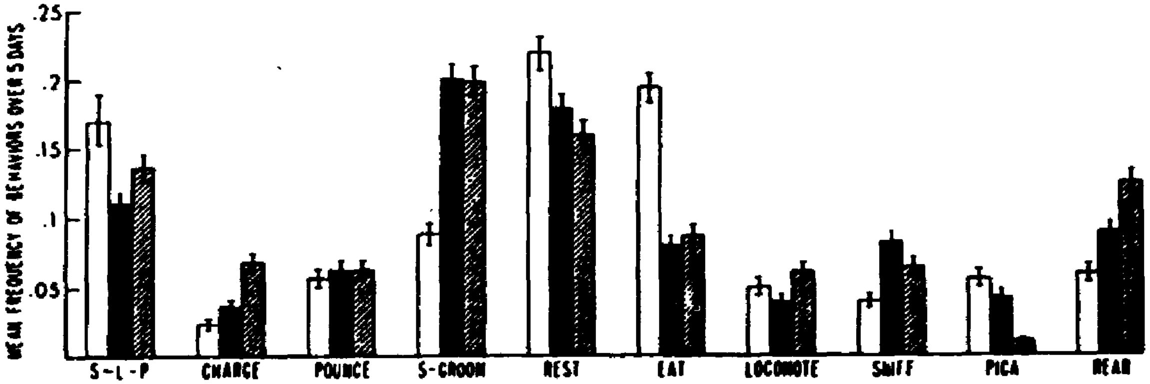

.25
.2
.15
.1
.05
MEAN FREQUENCY OF BEHAVIORS OVER 5 DAYS
S-L-P
CHARGE
POUNCE
S-GROOM
REST
EAT
LOCOMOTE
SNIFF
PICA
REAR

Figure 5

Daily changes in group means over 5 days of testing for: a) pouncing; b) sniff-lick-paw. Day 0 - 4 of testing as in Figure 2.

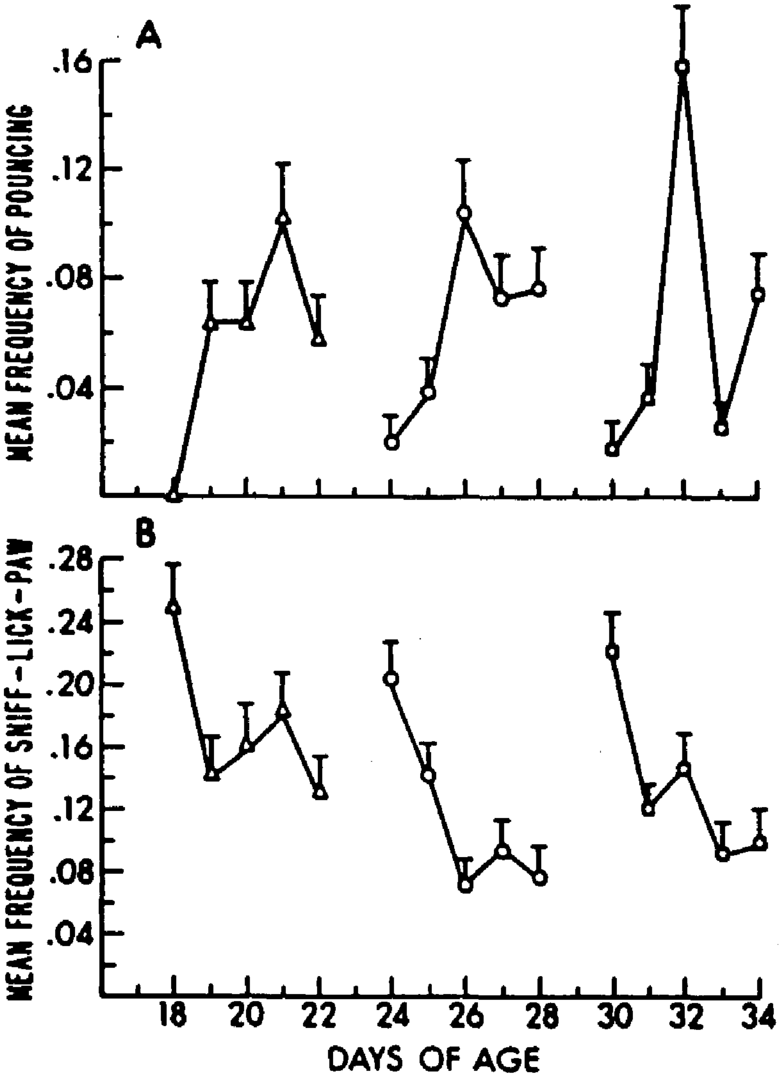

A
MEAN FREQUENCY OF POUNCING
.16
.12
.08
.04
B
MEAN FREQUENCY OF SNIFF-LICK-PAW
.28
.24
.20
.16
.12
.08
.04
18
20
22
24
26
28
30
32
34
DAYS OF AGE

Figure 6

Thirty Day-Olds. Cumulative percents retrieving at least once in five days of testing. PL (n=17: 11 females/6 males) denoted by filled circle; IC (D5W) (n=13: 8 females/5 males) denoted by open circle; NHC (n=18: 11 females/7 males) by triangle.

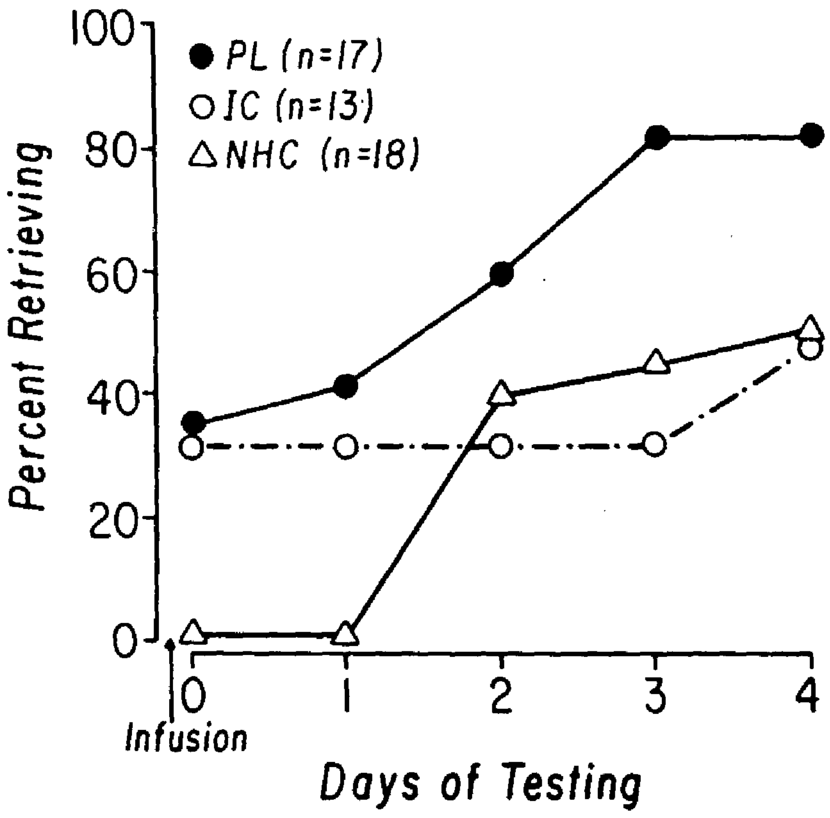

100
80
60
40
20
0
Percent Retrieving
● PL (n=17)
○ IC (n=13)
△ NHC (n=18)
0
1
2
3
4
Infusion
Days of Testing

Figure 7

Thirty Day-Olds. Daily changes in group means over 5 days of testing for: a) retrieving; b) crouching; c) anogenital licking; d) contact. Day 0 - 4 of testing for PL denoted by filled circle; for D5W by open circle; for NHC by triangle. Note that frequencies are relative to the total possible observed (N=16 per day x 5 days) for each behavior.

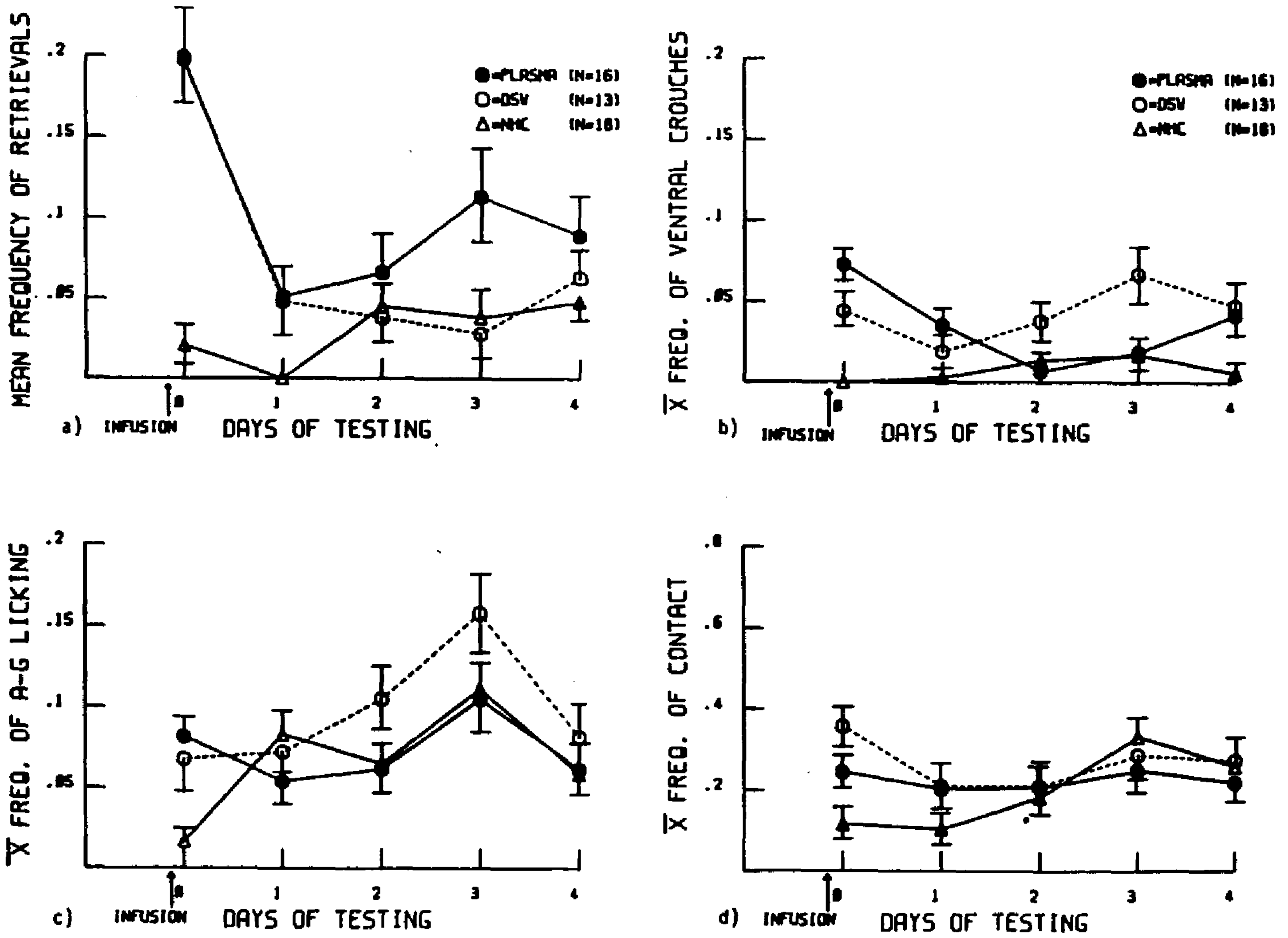

MEAN FREQUENCY OF RETRIEVALS
.2
.15
.1
.05
●=PLASMA (N=16)
O=DSV (N=13)
Δ=NHC (N=18)
a)
INFUSION
0
1
2
3
4
DAYS OF TESTING
X̄ FREQ. OF VENTRAL CROUCHES
b)
X̄ FREQ. OF A-G LICKING
c)
X̄ FREQ. OF CONTACT
.8
.6
.4
.2
d)

Figure 8

Eighteen Day-Olds. Daily changes in group means over 5 days of testing for: a) nestbuilding; and b) contact behaviors. Day 0 - 4 of testing as in Figure 7.

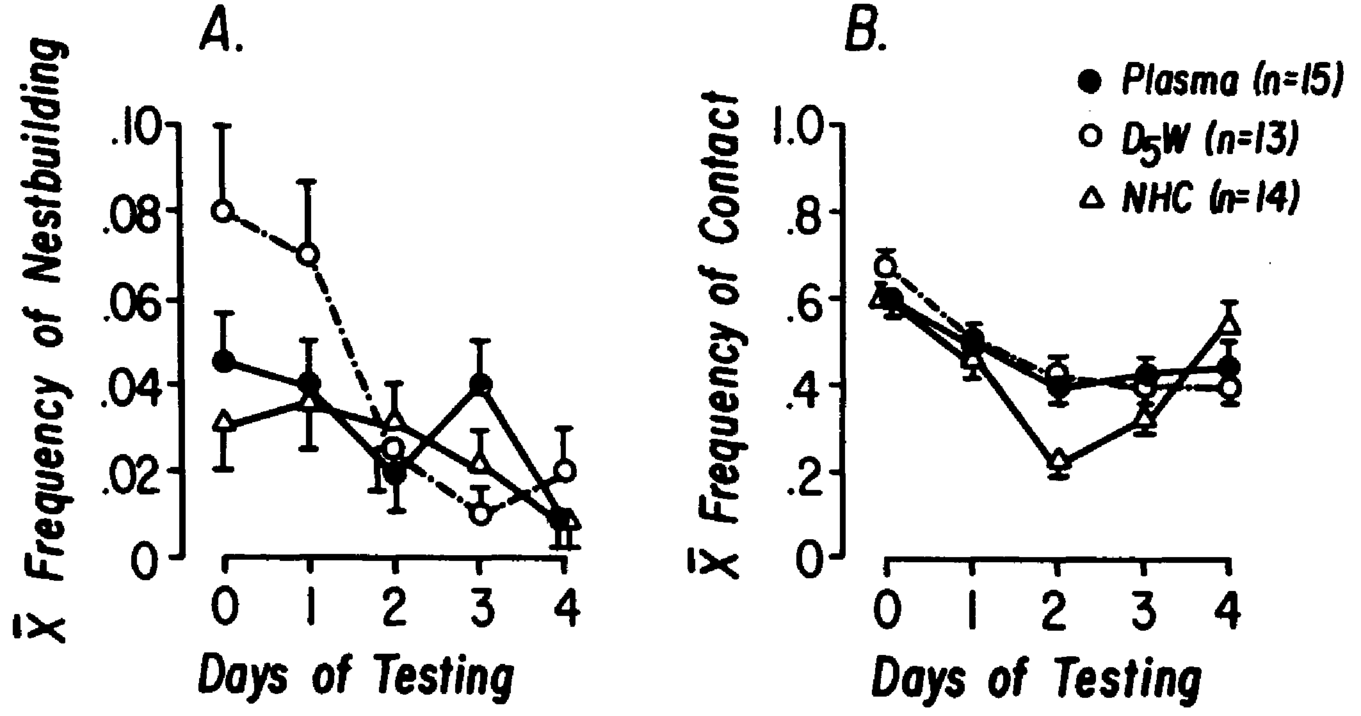

A.
X̄ Frequency of Nestbuilding
.10
.08
.06
.04
.02
0
0
1
2
3
4
Days of Testing
B.
X̄ Frequency of Contact
1.0
.8
.6
.4
.2
0
0
1
2
3
4
Days of Testing
● Plasma (n=15)
○ D5W (n=13)
△ NHC (n=14)

Figure 9

Thirty Day-Olds. Mean frequencies of each maternal behavior observed over 5 days for males and females in each treatment group. A) Retrieving: PL females > D5W, NHC females, NHC males; D5W, PL males > D5W, NHC females, NHC males. B) Crouching: PL, D5W females NHC females, PL, D5W, NHC males. C) Anogenital Licking: PL, D5W females > NHC females; D5W, NHC males > PL males. D) Sniff Lick Paw: PL, D5W, NHC females > PL, D5W, males; NHC males > PL males. Based on Neuman-Keuls post-hoc comparisons, $p<0.05$.

30 Day-Olds: 5-Day Means

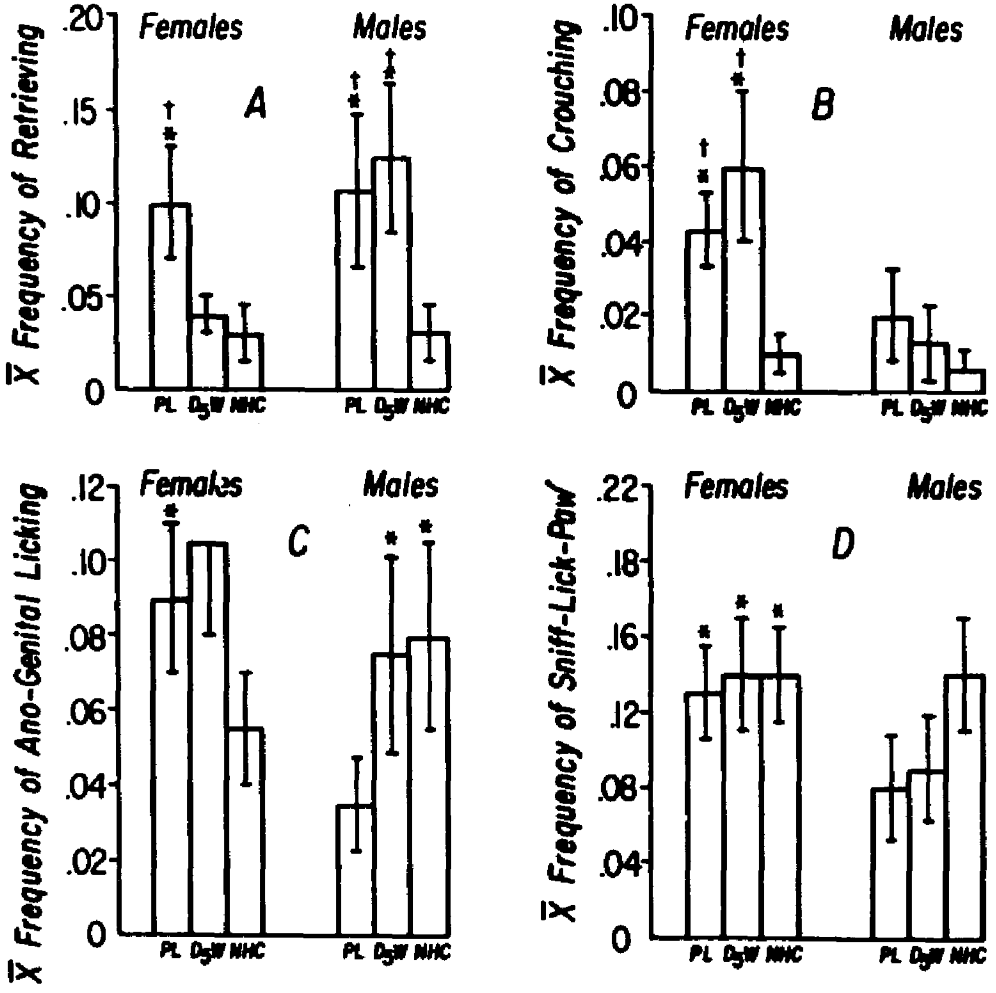


Figure 10

Thirty Day-Olds. Mean frequencies of contact behavior observed over 5 days for males and females in each treatment group. D5W males > PL, D5W, NHC females. Based on Neuman-Keuls post-hoc comparisons, $p<0.05$.

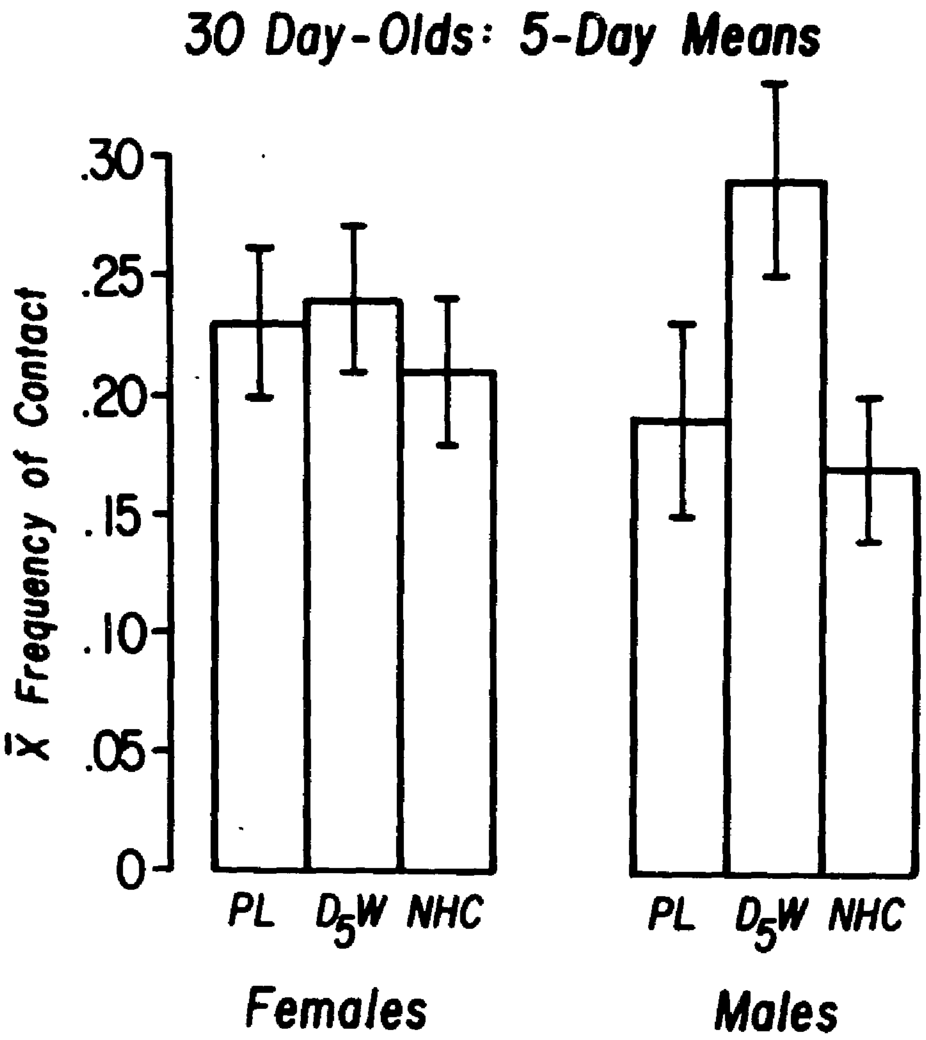

30 Day-Olds: 5-Day Means
$\bar{X}$ Frequency of Contact
.30
.25
.20
.15
.10
.05
0
PL
D_5W
NHC
Females
PL
D_5W
NHC
Males

Figure 11

Eighteen Day-Olds. Mean frequencies of maternal behaviors observed over 5 days of testing by treatment group and sex. A) Nestbuilding: D5W males > D5W, NHC females, PL males; B) Combined females > males. Based on Neuman-Keuls post-hoc comparisons: $p<0.05$.

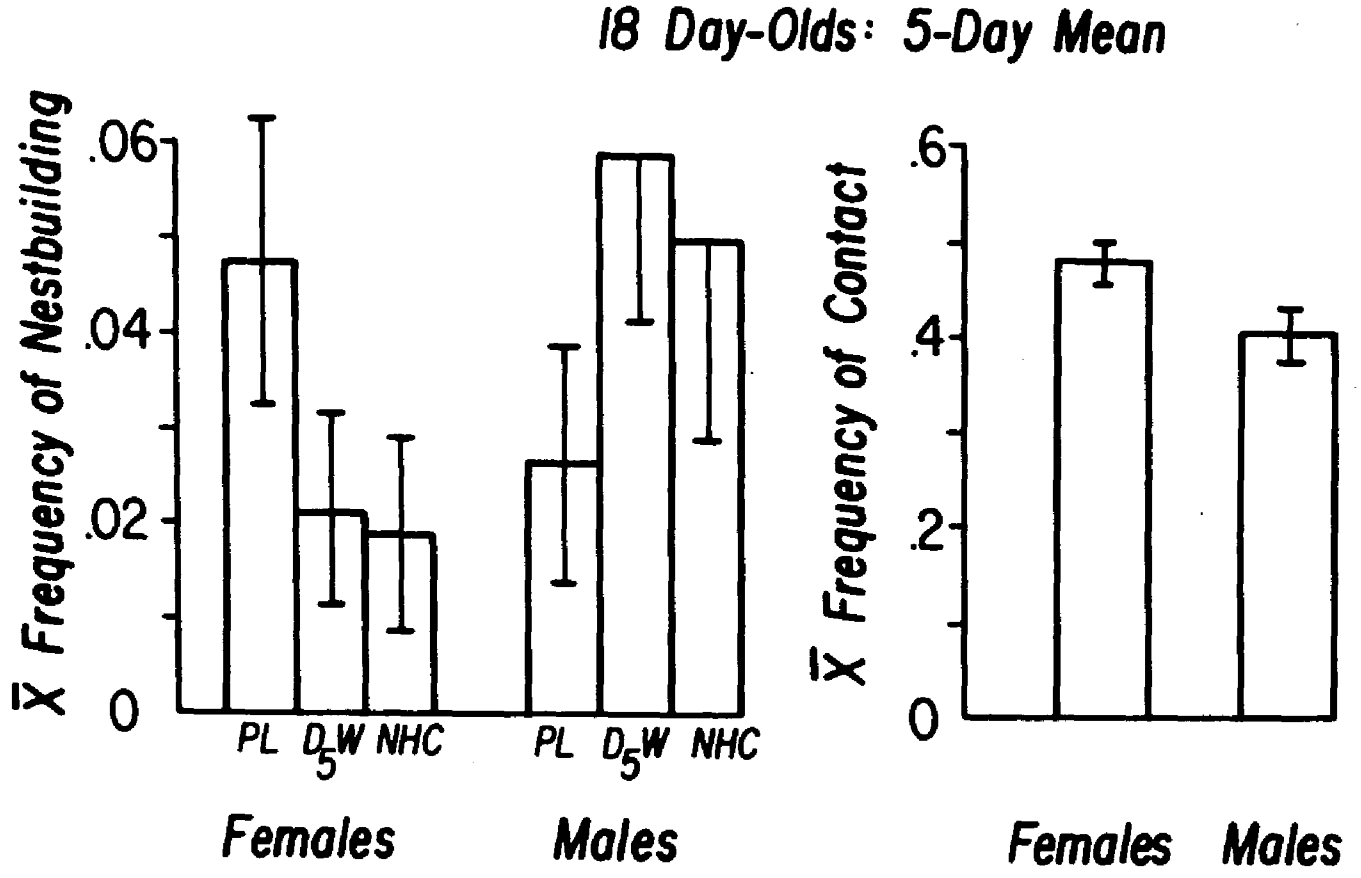
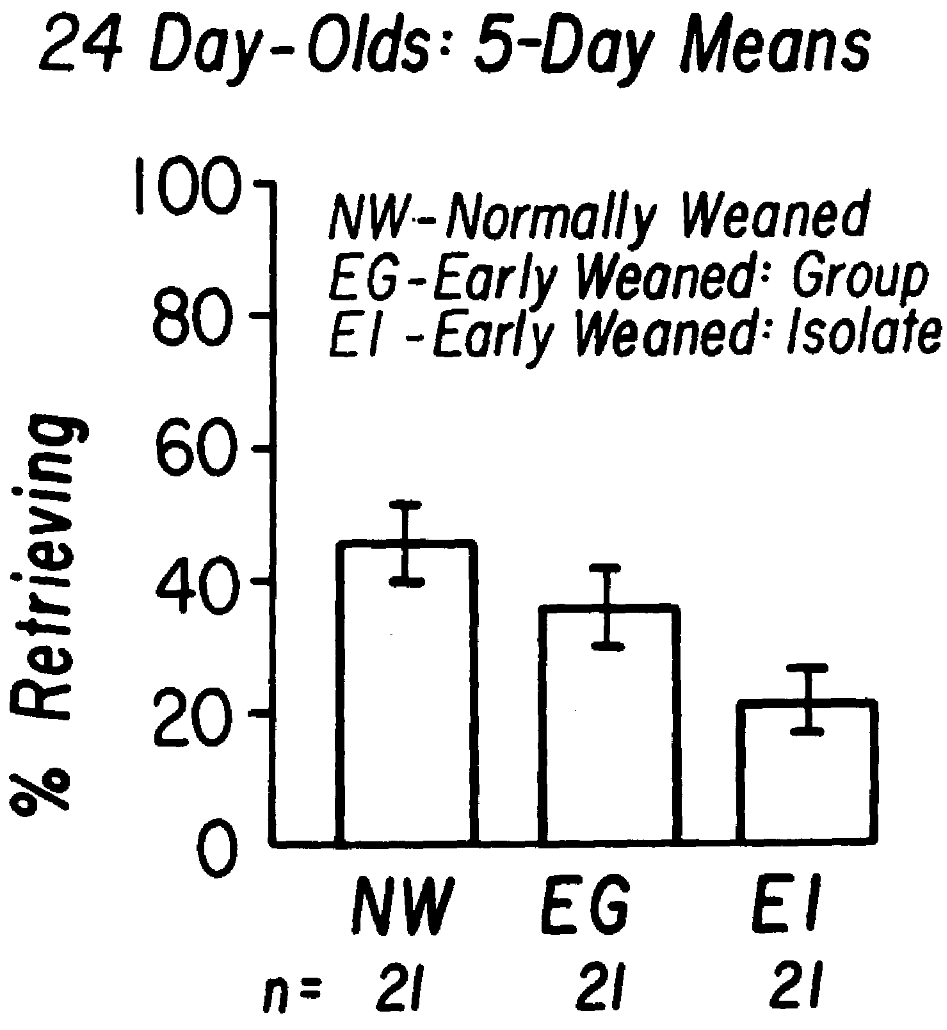

18 Day-Olds: 5-Day Mean
$\bar{X}$ Frequency of Nestbuilding
0
.02
.04
.06
PL
D_5W
NHC
PL
D_5W
NHC
Females
Males
$\bar{X}$ Frequency of Contact
0
.2
.4
.6
Females
Males

Figure 12

Mean percent of each group retrieving over 5 days of testing. Females and males have been combined due to lack of differences. Newman-Keuls posthocs: NW greater than EWI ($p<0.01$); NW greater than EWG ($p<0.05$); EG greater than EWI ($p<0.01$).

Figure 13

Mean percent of each group crouching over 5 days of testing. Females and males have been combined.


24 Day-Olds: 5-Day Means
NW-Normally Weaned
EG-Early Weaned: Group
EI -Early Weaned: Isolate
% Retrieving
100
80
60
40
20
0
NW
EG
EI
n= 21
21
21

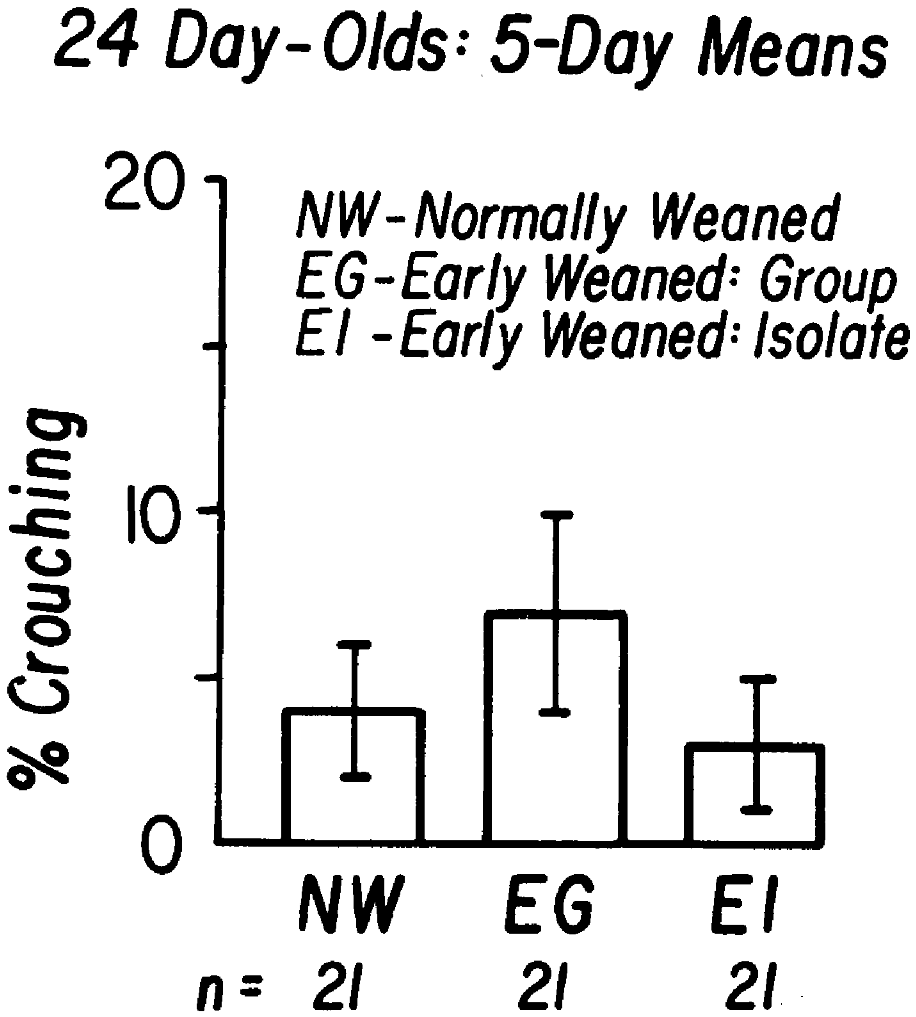

24 Day-Olds: 5-Day Means
NW-Normally Weaned
EG-Early Weaned: Group
EI -Early Weaned: Isolate
% Crouching
20
10
0
NW
EG
EI
n = 21
21
21

Figure 14

Mean percent of each group a) pupcarrying; b) charging; c) pouncing over 5 days of testing. Females and males have been combined due to lack of differences. a) Pupcarrying: EWI greater than NW ($p<0.01$); EWI greater than EWG ($p<0.01$). b) Charging: EI greater than NW ($p<0.01$); EWI greater than EWG ($p<0.05$). c) Pouncing: EWI greater than NW ($p<0.01$); EWI greater than EWG ($p<0.01$). Based on Newman-Keuls posthoc comparisons.

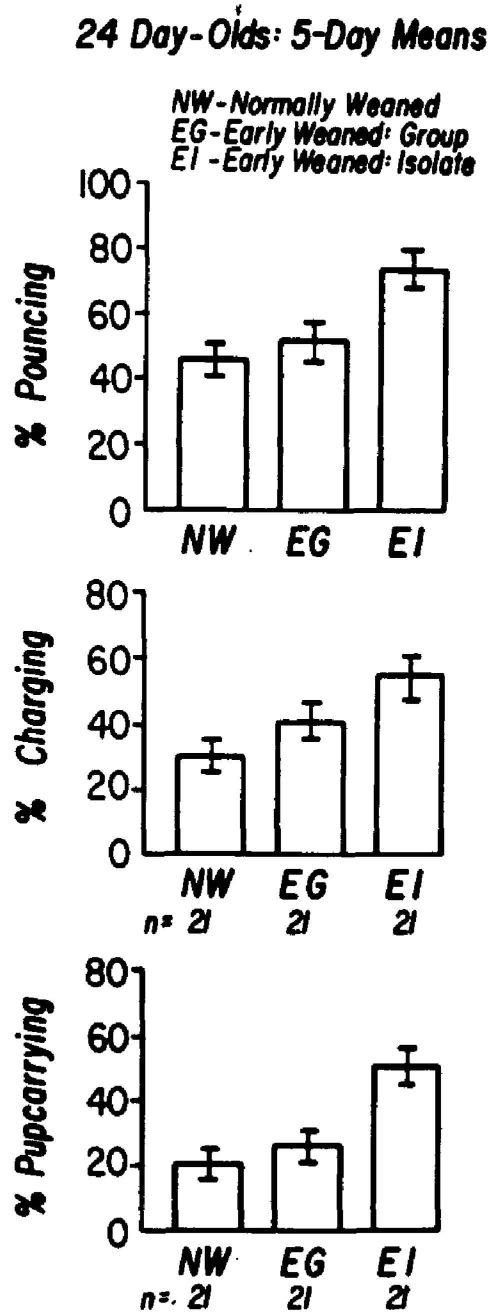
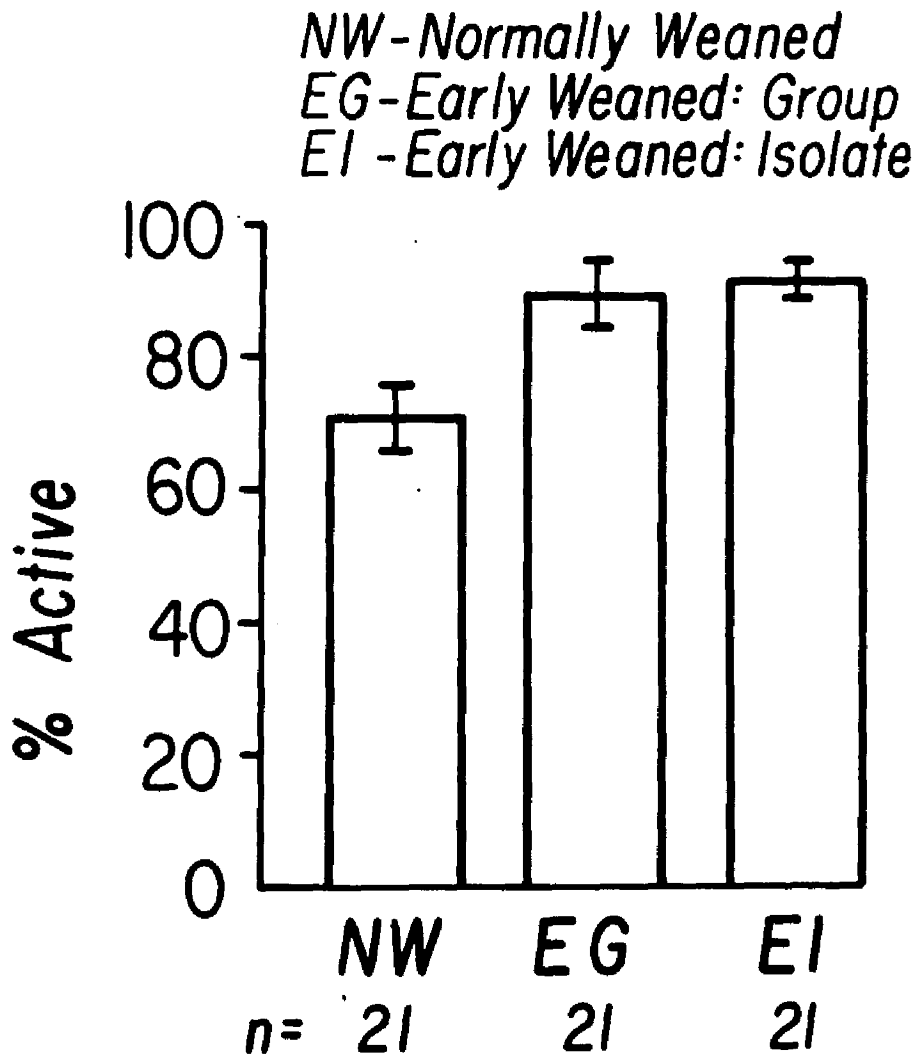

24 Day-Olds: 5-Day Means
NW-Normally Weaned
EG-Early Weaned: Group
EI-Early Weaned: Isolate
% Pouncing
100
80
60
40
20
0
NW
EG
EI
% Charging
80
60
40
20
0
NW
EG
EI
n= 21
21
21
% Pupcarrying
80
60
40
20
0
NW
EG
EI
n= 21
21
21

Figure 15

Mean percent of each group active over 5 days of testing. Females and males have been combined.



24 Day-Olds: 5-Day Means

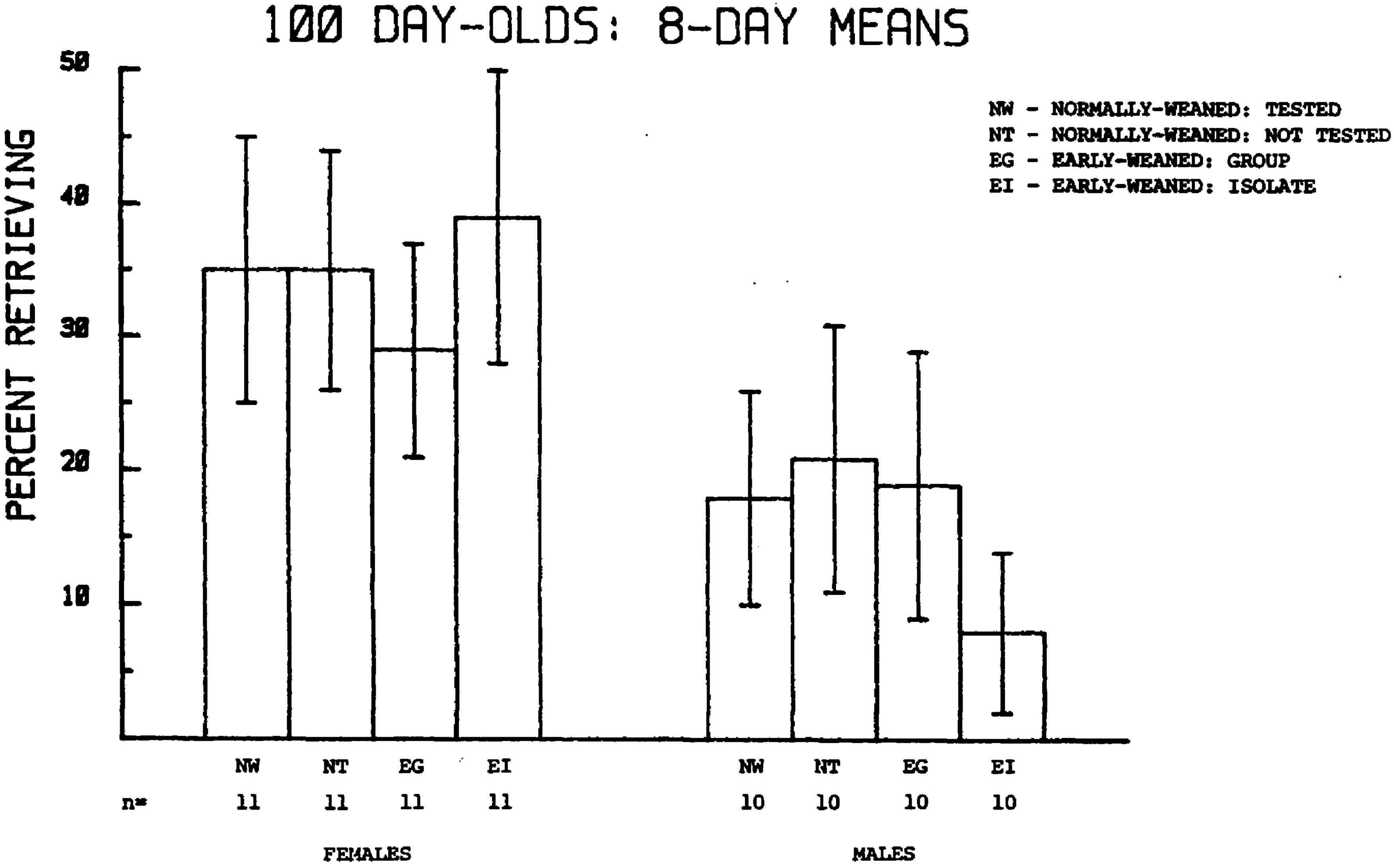
Figures 16 - 19

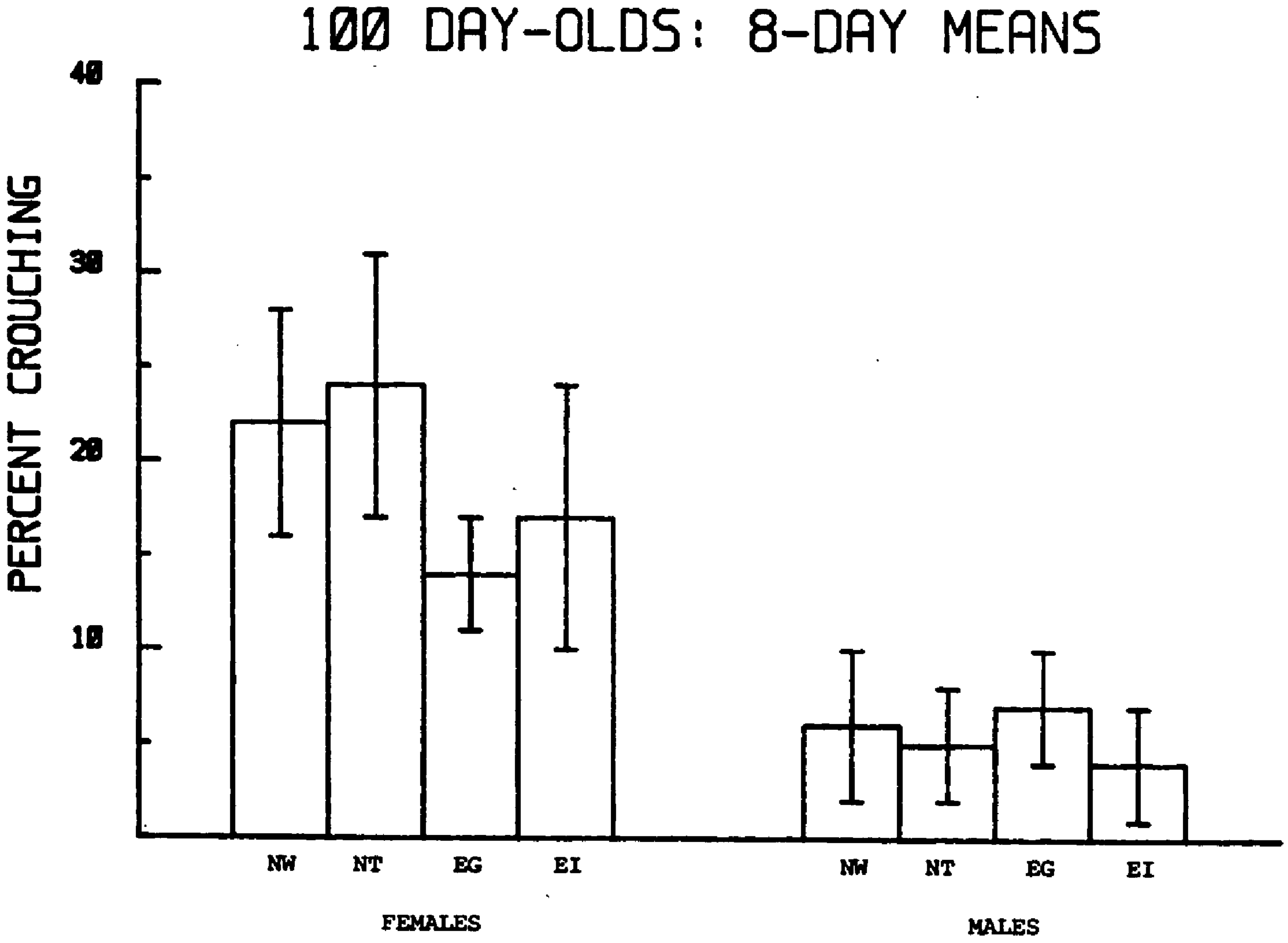
Figure 16. Mean percent of each group retrieving over 5 days of testing. Differences between combined female versus combined male groups only.

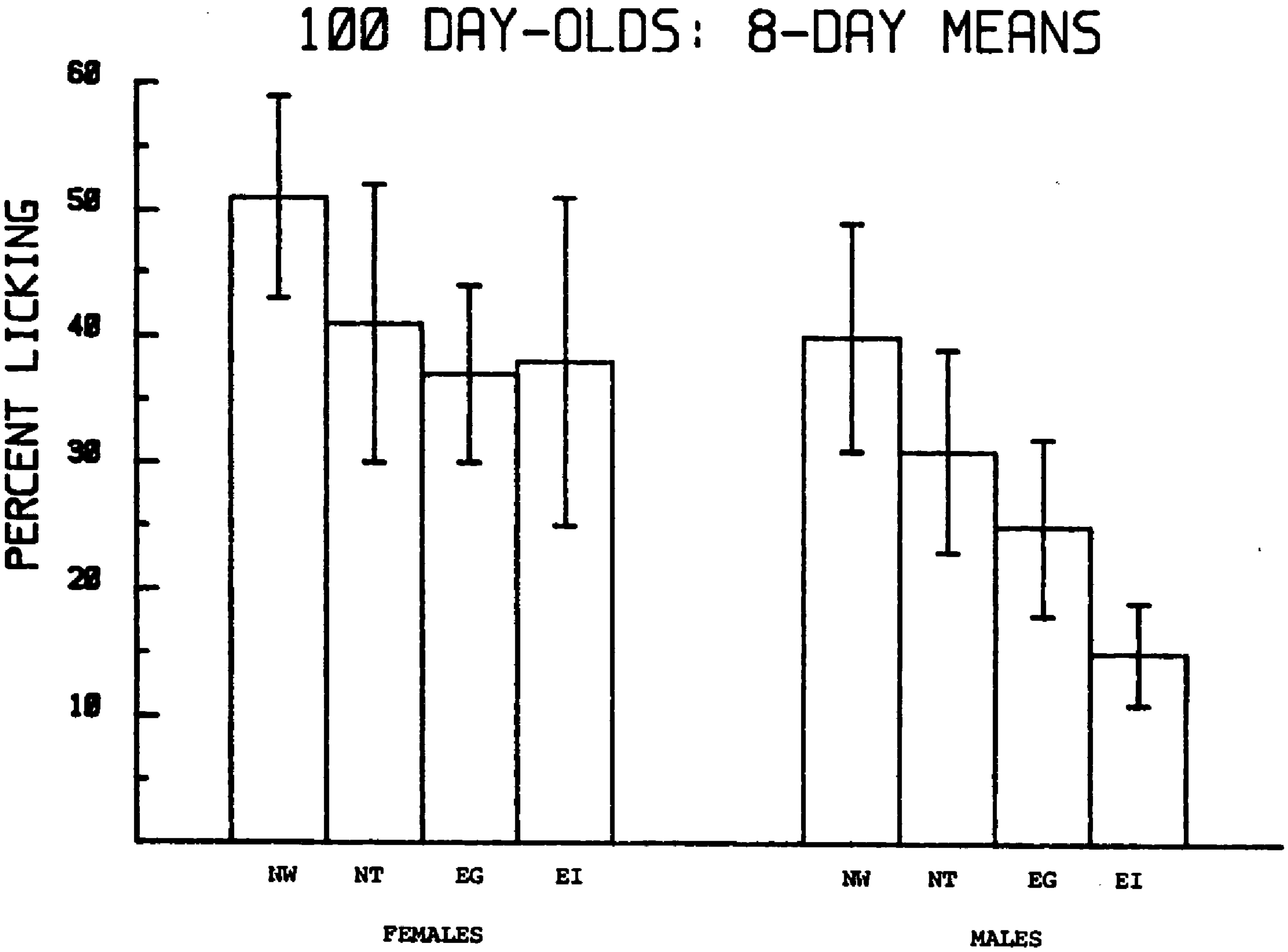
Figure 17. Mean percent of each group crouching over 5 days of testing. Differences between combined female versus combined male groups only.

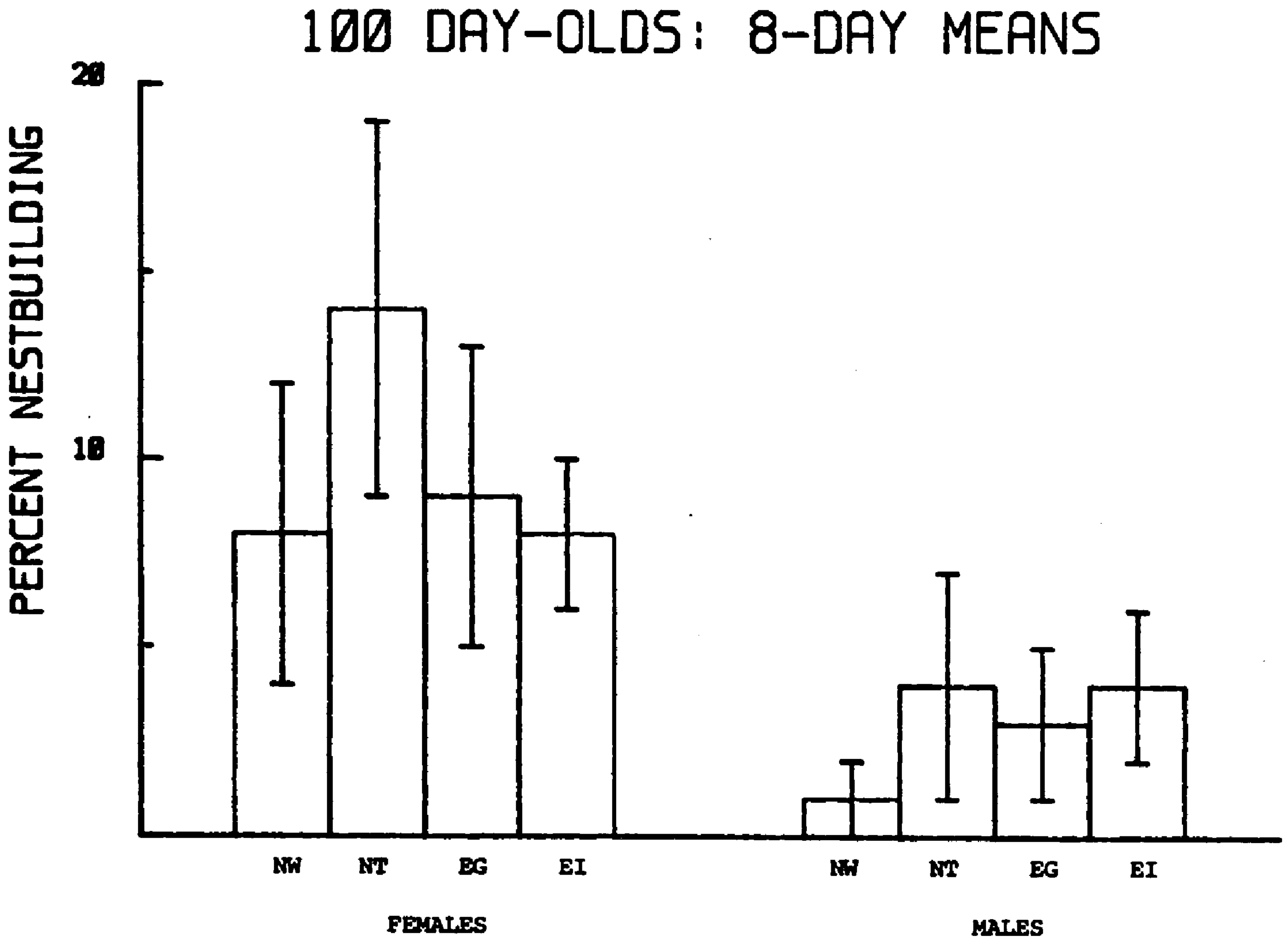
Figure 18. Mean percent of each group anogenital licking over 5 days of testing. Differences between combined female versus combined male groups only.

Figure 19. Mean percent of each group nestbuilding over 5 days of testing. Differences between combined female versus combined male groups only.


100 DAY-OLDS: 8-DAY MEANS
PERCENT RETRIEVING
50
40
30
20
10
NW - NORMALLY-WEANED: TESTED
NT - NORMALLY-WEANED: NOT TESTED
EG - EARLY-WEANED: GROUP
EI - EARLY-WEANED: ISOLATE
NW
NT
EG
EI
n=
11
11
11
11
NW
NT
EG
EI
10
10
10
10
FEMALES
MALES


100 DAY-OLDS: 8-DAY MEANS
PERCENT CROUCHING
40
30
20
10
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES


100 DAY-OLDS: 8-DAY MEANS
PERCENT LICKING
60
50
40
30
20
10
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES

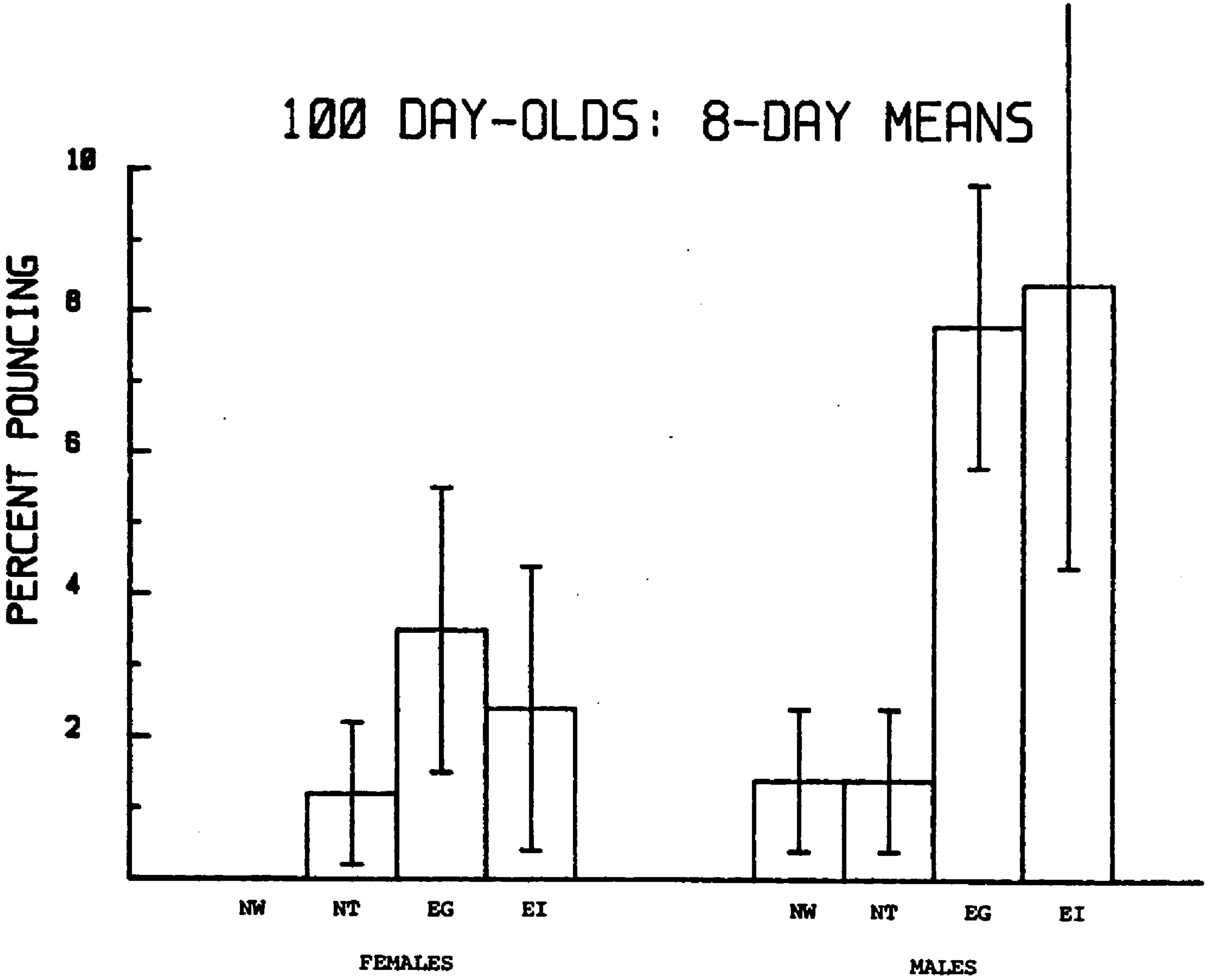

100 DAY-OLDS: 8-DAY MEANS
PERCENT NESTBUILDING
20
10
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES

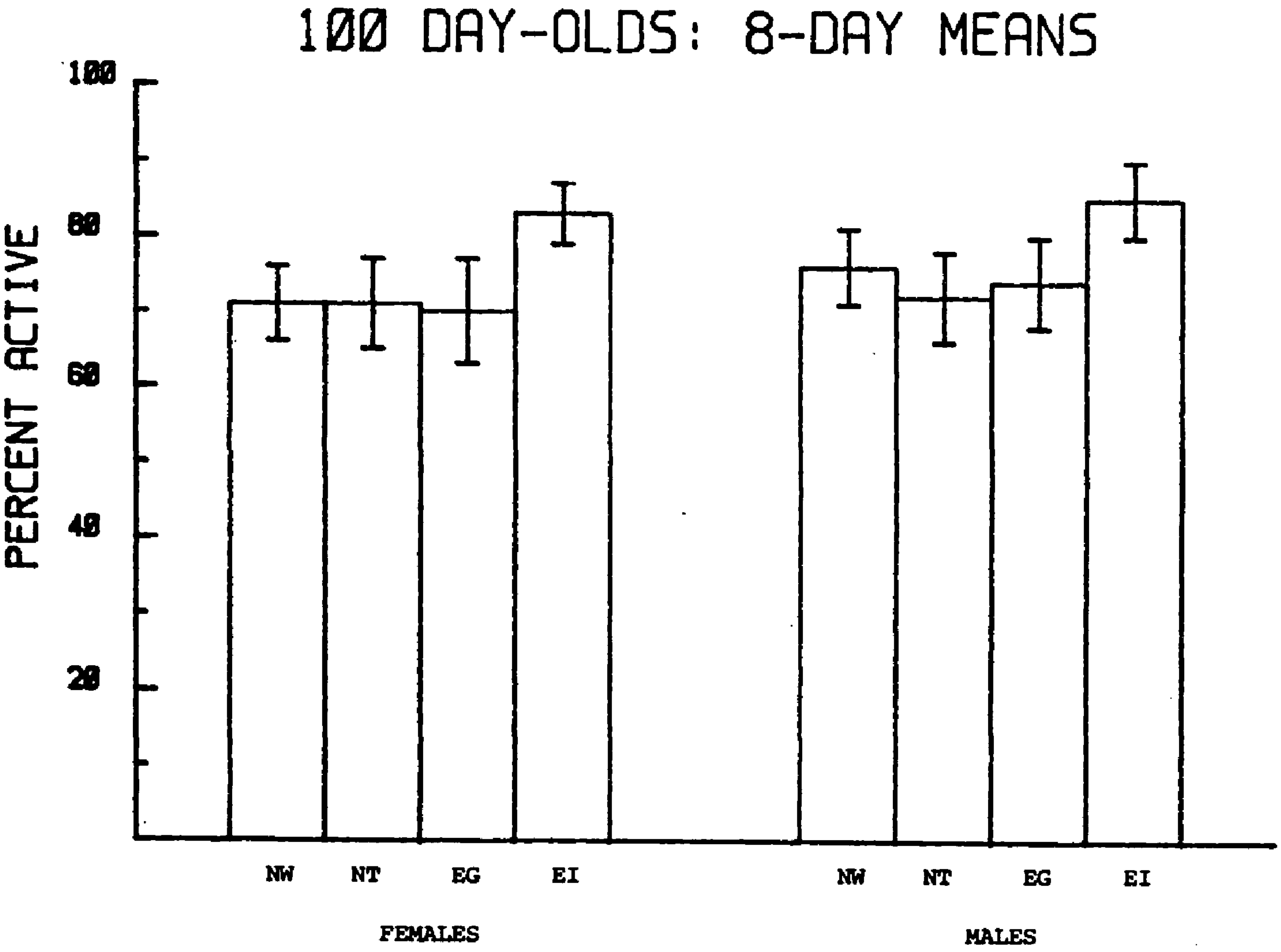
Figures 20 - 22

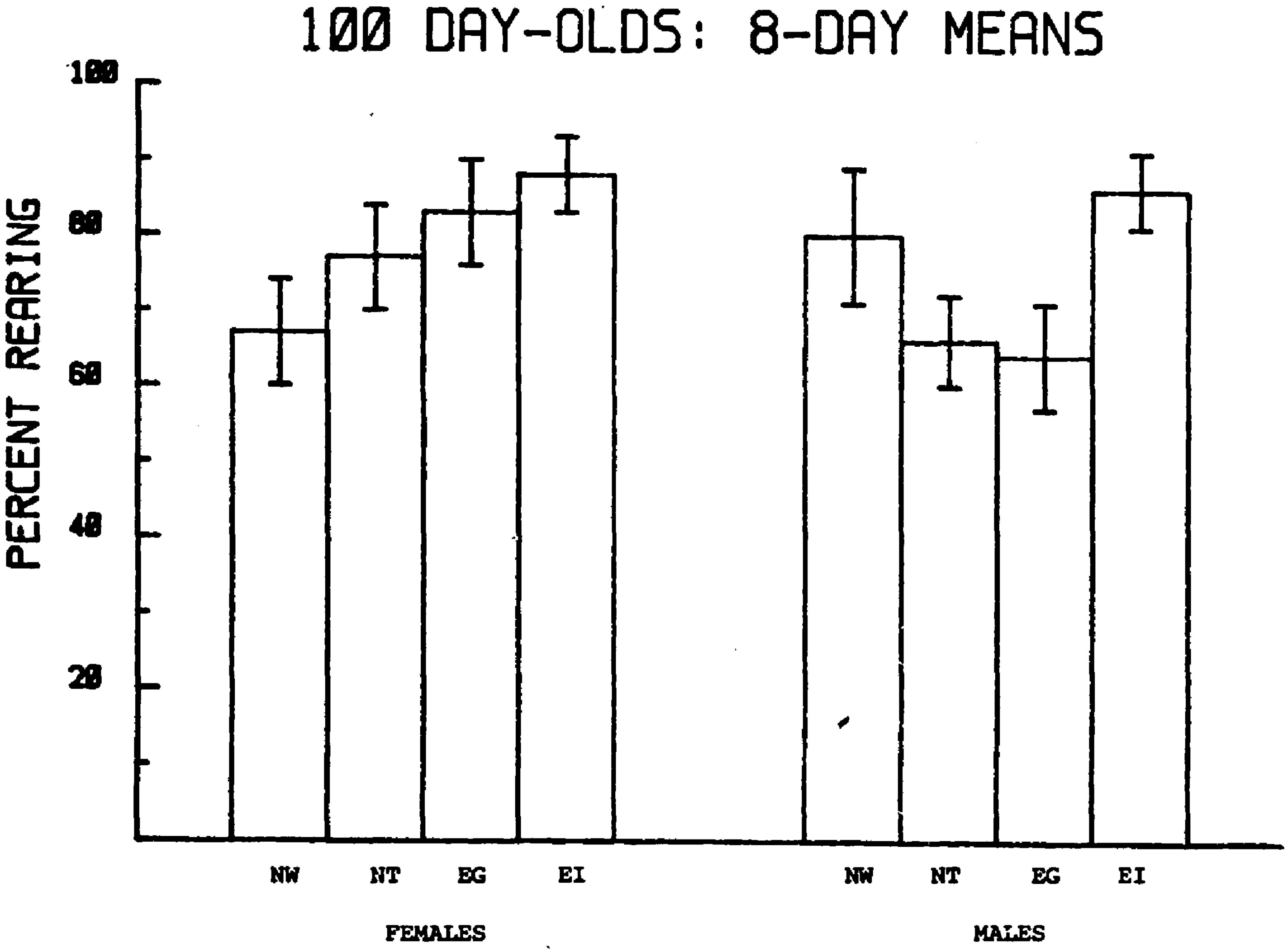
Figure 20. Mean percent of each group pouncing over 5 days of testing. Newman-Keuls posthoc comparisons: EG,EI greater than NW, NT groups (males and females combined). Also, combined male groups greater than combined female groups.

Figure 21. Mean percent of each group active over 5 days of testing. Newman-Keuls posthoc comparisons: EI greater than NW, NT, EG groups (males and females combined).

Figure 22. Mean percent of each group rearing over 5 days of testing. Newman-Keuls posthoc comparison: EI greater than NW, NT, EG groups (males and females combined).


100 DAY-OLDS: 8-DAY MEANS
PERCENT POUNCING
10
8
6
4
2
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES


100 DAY-OLDS: 8-DAY MEANS
PERCENT ACTIVE
100
80
60
40
20
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES


100 DAY-OLDS: 8-DAY MEANS
PERCENT REARING
100
80
60
40
20
NW
NT
EG
EI
FEMALES
NW
NT
EG
EI
MALES

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