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The effects of separation on the mother-infant interaction during reunion in ten to twelve day old rat litters

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THE EFFECTS OF SEPARATION ON THE MOTHER-INFANT INTERACTION DURING REUNION IN TEN TO TWELVE DAY OLD RAT LITTERS

by

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INTRODUCTION

I. Goals

Parental care is almost always required in mammals to ensure the survival and growth of the young. The amount or type of parental care varies among species due to, for example, maturity of infants at birth, food supplies, or social group born into, ie. family or colony, etc. For some altricial young, the mother-infant interaction is the most critical relationship for survival, and disruptions of this interaction have been shown to have profound behavioral effects.

Separation between a mother and her infants can and does happen in nature. Sometimes this experience is expected. Rat dams and their pups normally experience periodic separation-reunion periods during the first few weeks postpartum as the pattern of nursing entails frequent nesting bouts throughout the day. These separation periods between nesting bouts increase in length as the pups develop, ranging from .5 to 33 minutes during the first week postpartum, to 5 to 86.5 minutes during the third week (Grota and Ader, 1969). It is also possible although rare for longer unexpected periods of separation to occur in nature. The dam, while foraging for food, can become injured or threatened by a predator thereby delaying her return (Calhoun, 1962).

The first goal of this dissertation was to characterize the mother-infant interaction upon reunion after an 18 hour separation period on postnatal day 10 to 12. On a continuum of possible separation period lengths, an 18 hour separation period between a rat dam and her pups would be an extremely long time to be apart, possibly either approaching or having reached a period after which the interaction upon reunion would be altered tremendously or disrupted completely. Very few studies have systematically investigated an interaction after such a separation period, and no extensive, behavioral study has been done in the rat.

The second goal of this dissertation was to determine the relative contributions of the dam and pups to the interaction seen upon reunion after separation. We wanted to know if the changes seen due to separation were primarily the result of changes in the dam, changes in the pup, or an interaction between the two.

As will be described in the following literature review, the selection of an 18 hour separation period on postnatal day 10 to 12 was based on literature available describing the separation changes seen in pups, and the fact that many of the changes seen plateau after a separation period of this length. Later, after 24-48 hours, recovery processes return vital systems such as cardiac and respiratory rates, thermogenesis and growth back toward preseparation levels. By characterizing the interaction upon reunion after an 18 hour separation period, we can explore the possibility that the plateau of separation changes prior to their recovery serves a function for the renewal and maintenance of that relationship. In other words, the temporary delay that is present until the pup's intrinsic ability to control the physiological systems listed above becomes effective, might make a reunion more easily successful as the control of these systems might still be open to the external control normally experienced by the pup within the mother-infant interaction.

It is also possible that the separation changes seen could be adaptive to the separated state and may actually interfere with the reunion causing the interaction to appear disrupted. Or perhaps the two possible functions of the separation changes, adaptive for reunion or adaptive for separation, may not be mutually exclusive. Perhaps the separation changes will not result in a unified process of reunion because the many systems involved could be at different stages of adaptation to separation upon reunion which could result in a combination of efficient and disrupted behaviors. Of course it is also possible that the separation effects seen serve no function at all, but is seems logical that the state of the pups and dam after separation has been selected for by evolution presumably to make the response adaptive to reunion because separation can and does occur in nature.

Before describing how we approached the above goals, selective literature will be described.

II. Selective Review of Separation Literature as it Applies to Reunion

A. Effects of Separation

Many rat studies have helped us to clarify the behavioral and physiological processes that occur in the rat pup upon separation, and to understand what had been regulating these processes prior to separation within the dam-pup interaction. Two different psychophysiological response patterns have been described by Hofer (1975; 1983): the immediate protest response due to the interruption of attachment, and the delayed responses due to withdrawal of multiple regulators provided by the dam.

The immediate responses to separation in the pup include high levels of ultrasound vocalizations, locomotion, and self-grooming (Hofer and Shair, 1978). The distress vocalizations were found to be comforted by an anesthetized dam or other littermates, and is of a decreased rate if the pup is left in the home cage, rather than placed in a novel cage (Hofer and Shair, 1978). Thus, this acute separation response can be prevented by the presence of siblings and home cage nest cues. The delayed responses to separation, on the other hand, are not an immediate reaction to the situation, but rather a longer term adaptation to a new circumstance.

The dam's provision of nutrients to the pups has been shown to be associated with the slower-developing changes that occur in pups after separation. Withdrawal of the nutritional aspects of maternal care has been found to decrease the pup's heart rate and respiratory rate, and alter its sleep-wake state patterns. These changes in the physiological systems of the pups plateau after an 18 hour separation period. The decrease in heart rate and respiratory rate seen in a two week old pup separated from its dam can be maintained by nutrient intake of milk through gastric cannulae, and neither warmth nor tactile stimulation influence these functions of the pup (Hofer, 1973). The increase seen in time awake and decrease in REM, can be prevented post-separation if nutrition was delivered periodically, simulating the frequent nursing bouts of a normal interaction and temperature was determined to have a subsidiary role in regulating the sleep disturbances (Hofer and Shair, 1982). The heart rate, respiratory rate, and sleep/wake states therefore are open to external control, and are normally regulated within the mother-pup interaction. These findings led to the theory of multiple regulators which will be described later and whose utility in describing the mother-infant interaction upon reunion will be tested. As mentioned before, the fact that the changes seen in the systems just described plateau after an 18 hour separation period raises an interesting question of whether these now newly maintained levels of functioning are adaptive in some way.

B. Effect of Separation on Nursing Measures in the Pup Upon Reunion

1. Latency to Attach and Time Attached

The latency to attach to the dam's teats by the pups after separation, and the amount of time they remained attached, have been used as measures of motivation to nurse. However, it has been shown that deprived and nondeprived pups younger than 10 days of age show no difference in latency to attach to the nipples, and once attached, they remain on the teats for hours even when they receive no milk (Hall, Cramer, and Blass, 1975; 1977). Therefore, in pups less than 10 days of age, latency to attach and time spent attached would not be accurate measures of intake.

When pups are 10 days of age or older their latencies to attach to the dam's teats decrease after several hours of separation (Drewett, Statham and Wakerley, 1974; Hall, Cramer, and Blass, 1977). However, it is not until postnatal day 20 that they have been shown to detach in order to limit their ingestion (Lorenz, Ellis, and Epstein, 1982). Therefore, latency to attach on postnatal day 10-12 would reflect the pup's deprivation level, but time attached would not. Whether pups can control their intake before day 20 without adjusting time spent nursing has been the subject of many studies.

2. Control of Intake

Hall and Rosenblatt (1978) demonstrated that pups younger than 15 days of age showed no evidence of gastric or nutritional control when milk was delivered via a tongue cannula directly into the back of the mouth of the suckling infant. In fact these rats consumed milk until their stomachs were so grossly distended it threatened their respiration. Another study demonstrated that five and ten day old pups did not regulate their intake when given a limitless supply of milk from a series of milk laden dams (Cramer and Blass, 1983). Anesthetized dams were given pitocin injections to elicit milk letdown and attached pups continued to consume milk until they were so full they could no longer maintain their suckling posture. In both of these studies, the way in which pups were provided milk, either through a tongue cannula or through a series of dams which cumulatively supplied excess milk, has been shown to have overwhelmed any ability the pups' had to control their intake.

In a natural suckling situation, pups younger than 15 days of age do have some control over the amount of milk they consume while attached. Pups have been shown to increase their intake post-deprivation in the natural suckling situation, and decrease their intake following preloads (Brake, Sager, Sullivan, and Hofer, 1982; Cramer and Blass, 1983; Drewett, 1978; Friedman, 1975; Houpt and Epstein, 1973; Houpt and Houpt, 1975). This is true even though, as mentioned before, attachment time to the teat is not increased (Lorenz, Ellis, and Epstein, 1982). Hence, multiple controls exist to regulate pup's intake of milk, including the milk supply of the dam, deprivation level and/or gastric cues.

3. Sucking Behavior and Sleep-Wake States

Alterations in sucking and sleep-wake states have been shown to be involved in changes in intake after deprivation. These behaviors contribute to our understanding of how separated pups consume more milk upon reunion without increasing their time attached. It has been demonstrated that pups separated from the dam for a lengthy period of time engage in more frequent and vigorous sucking, but these increased levels decrease if the pup receives no milk while suckling (Brake and Hofer, 1980; Brake, Wolfson, and Hofer, 1979). Also, although no significant differences were found in the force of sucking during the first ME, deprived pups were shown to suck longer and with greater force than nondeprived pups during the second and third ME (Brake, Shair, and Hofer, 1988). Therefore the number and force of sucks are greater in hungry that sated pups.

As mentioned earlier, the increase seen in sucking postdeprivation decreases if milk is not available. This modulation by nutritive factors has been further defined by Brake, Sager, Sullivan, and Hofer (1982). Deprived pups given preloads decreased sucking, but only if milk was made available through a tongue cannula, therefore gastric preloads inhibited feeding but did not affect nonnutritive sucking. On the other hand, oral satiation, provided to pups by a dam without milk during the deprivation period, decreased sucking in the absence of milk, but had no affect on sucking if milk was available. Thus, oral satiation inhibited nonnutritive sucking, or sucking that occurs in the absence of milk, but did not affect sucking responses associated with feeding. Therefore sucking appears to be mediated by at least two different motivational systems- the feeding system and the suckling system.

Maternal deprivation of two week old rat pups increases time spent awake and in slow wave sleep and decreases time in paradoxical sleep (Hofer, 1976; Shair, 1986). The changes in sucking behavior due to separation have been shown to be embedded in these post-separation changes in sleep-wake states. Through changes in their sleep-wake state patterns, these deprived pups are increasing the time spent in states with higher sucking rates (awake and slow wave sleep) and decrease time spent in states with lower rates (paradoxical sleep) (Shair, 1986). During the actual let down of milk, deprived pups have been shown to remain awake longer and engage in more sucking (Brake, Shair, and Hofer, 1988). Therefore, the increase in amount and force of sucking within the changes in sleep-wake states might contribute to the increased intake seen in deprived pups.

An interesting feature of the changes seen in sucking force post-deprivation is the enormous variability displayed by hungry pups. This variability was also seen in the amount of milk consumed during a ME, which was shown to be correlated with the force of sucking during it. Brake et al. (1988) concluded that the stress of separation affects pups differently. Some compensate well and become good feeders, and others do not. Therefore, the weight gain of maternally deprived pups upon reunion could be more variable than the weight gain of nondeprived pups, as the environmental challenge that these pups were subjected to brought out differences between them.

C. Effect of Separation on Nursing Measures in the Dam Upon Reunion

As can be seen from the literature, separated pups are altered upon reunion in their interaction with the dam. Decreases in the pups' latency to attach and increased milk consumption accompanied by alterations in sleep-wake states and sucking behaviors appear to be attempting to compensate for the hours of deprivation. But the ability of the pups to increase their milk intake is not the only factor regulating their weight gain. The dam can impose limits on the amount of milk the pups obtain by behaviorally limiting the suckling opportunities available, or by the physiological limitations of the milk supply.

1. Initiation of Nursing

During early development, the dam plays a major role in initiating the feeding sequence by approaching the young in the nest, stimulating them to activity by picking them up and licking them, and making her ventrum available for nipple attachment. By day 12 to 14, the pups have improved locomotor ability and crawl to the dam for feeding. Eventually, over the next week, the pups become mainly responsible for the initiation of feeding until the process of weaning occurs between four and five weeks of age

(Rosenblatt and Lehrman, 1963; Leon, 1979). Therefore, around day 12 both the dam and pups make a contribution to the initiation of nursing, and separation at this time may result in changes in both the dam and pups behavior upon reunion.

2. Duration of Nursing Time

Once attached, the length of time spent nursing has been said to be controlled by the dam's need to maintain her thermal homeostasis (Leon, Croskerry and Smith, 1978). The temperature of the dam rises during nursing due to the increased heat generated by the litter and her decreased ability to dissipate heat due to her occupied ventrum, and upon a critical rate of temperature increase, the bout is terminated. Factors that affect maternal heat load, such as age, size, or temperature of the litter, alter the length of the contact bout. Contact time between a dam and her pups is initially 20 hours a day. By day 14 this time has decreased to 10 hours a day, and on day 21 it is about 7 hours (Grota and Ader, 1969). As pups get older, their heat load increases, and the bouts, although occur just as frequently, are shorter (Grota and Ader, 1969; Woodside, Pelchat, and Leon, 1980). Litters of a greater number of pups have been shown to experience shorter nest bout durations than smaller litters (Ader and Grota, 1970) consistent with the fact that their surface area to mass ratio is reduced, providing less opportunity for maternal heat dissipation. And the initial temperature of the pups, although had no effect on the dams latency to enter the nest, increased contact time with the dam if pups were cool (Jans and Leon, 1983). Separated pups due to their deprivation experience are smaller and possibly cooler, and whether these pups showed an increase in contact or nursing time with the dam was examined.

Not only is temperature a critical factor in regulating the duration of nursing time, Stern (1985) has shown that specific pup stimuli are needed to elicit maternal responding. The ventral somatosensory stimulation of the dam by the pup's nosing and probing, attaching and suckling behavior has been shown to be necessary for the dam to assume a nursing posture over her pups (Stern and Johnson, 1990).

3. Release of Milk

Bouts can vary in length from 10 minutes to 3 hours, but regardless of this tremendous variability, adequate nutrition is maintained. Hofer, Zmitrovich, and Shair (1989) showed that dams given only 10 minutes per hour to nurse on postnatal day 10-12, over a period of 24 hours, can produce a pup growth rate equivalent to those pups with dams who nurse longer. Although it was believed that MEs rarely occur in bouts of 10 minutes or less (Jans and Leon, 1983;

Lincoln, Hill, and Wakerley, 1973), this study demonstrated that the latency to the first ME can be reduced to under 10 minutes if that is all the time available. Jans and Woodside (1987) demonstrated that older litters, with naturally shorter nursing bouts, have a shorter latency to the first ME, however they do not have shorter inter-ME-intervals (IMEIs). Therefore, the pattern of MEs during a nursing bout are affected by multiple factors, including environmental circumstances and age of litter, but this flexibility insures that the pups will be adequately fed under multiple conditions. Since this flexibility exists, we could expect to see alteration in the number of MEs, and intervals between them due to a separation experience. In fact, an 18 hour separation period was shown to effect both: Lincoln, Hill, and Wakerley (1973) separated a dam from her pups for 18 hours on postnatal day 10 and upon reunion there was a decreased latency to the first ME and shorter IMEI than nonseparated litters.

4. The Effects of Separation on the Milk Supply

The size of the dam's milk supply is an obvious determinant of the amount of milk consumed by the pups. Separation from pups has been shown to cause an increase in the available milk supply stored in the dam's mammary glands (Friedman, 1975). Friedman (1975) demonstrated there is an increase in milk build up after 8 hours of separation when compared to 2, which increases the pups' intake upon reunion, and this increase in intake is seen in both deprived and nondeprived pups. There is a possibility that dams separated from the litter for relatively long periods of time are so milk laden on their return to the nest that small amounts of milk seep into the terminal galactophores of the teat so that pups have access to milk in the absence of a milk ejection (Wakerley, Clarke, and Summerlee, 1988).

The milk build up due to separation is released rapidly upon reunion, with decreasing milk obtained as nursing continues (Lau and Henning, 1984; Lincoln, Hill and Wakerley, 1973). Although pups are able to get more milk from a separated dam during the immediate reunion period, it has been shown that less milk becomes available over time because lactation had not been maintained by suckling. Prolactin appears to be the major hormone secreted by the hypothalamus that regulates the secretion of milk. Suckling cues from the pups have been shown to have a direct affect on prolactin release, causing its release throughout lactation. If a dam is separated from her pups on postnatal day 4, serum prolactin levels were found to decrease and accumulation of prolactin occurred in the pituitary (Amenomori, Chen, and Meites, 1970). When the pups were returned to the dam after 12 hours of deprivation, the serum prolactin level increased rapidly to levels exceeding the preseparation baseline, and the pituitary prolactin decreased.

Grosvenor and Mena (1973) found that a time delay of 8 to 12 hours exists between the release of prolactin stimulated by the pups' suckling behavior, and the stimulatory effect of this hormone on milk secretion. This time lag would mean that, upon separation, milk would build up in the dam as a result of the sucking stimulation previously experienced, and 8 to 12 hours after sucking had stopped, milk secretion would cease. Therefore large supplies of milk would be available upon reunion as previously described, however, once depleted, there would be a considerable delay until more milk is available. The length of this delay depends upon the length of separation and the lactational age of the litter.

There are competing pressures present within the dam during a separation experience: to adapt to the separated condition, or to maintain the ability to nurse her pups if reunion should occur. Physiological constraints affect the meeting of either of these goals. Little is known about the length of time necessary from the removal of the pups until the dam's hormonal status is altered, terminating lactation and establishing estrus or receptivity, but some studies have shown that it varies with different lactational ages of litters. Removal of the pups from their dam on day 13 of lactation is followed by ovulation 3 days later, as opposed to an average of 8 days later on day 8 of lactation (van der Schoot, Lankhorst, de Roo, and de Greef, 1978). However, even once estrus is reestablished, lactation can be resumed: after extreme separation periods of up to 9 days beginning on the 12th day of postpartum, lactation can be resumed if dams are placed with a foster litter, although there is a delay of many days until the mammary glands become fully functional again (Ota and Yokayama, 1965). This latter study is a demonstration of the lability of the nursing system in the dam rather than an application to circumstances that could arise in nature, for the delay of days is severe and most likely fatal to developing pups of any age unless they had already begun weaning. However, it does show that competing pressures compromise; physiological constraints create delays in the termination of maternal behavior upon separation as well as in reestablishing maternal behavior upon reunion.

5. Control of the ME reflex

The interplay between physiology and behavior is clearly seen in the study of the ME reflex. The regulation of the ME reflex has been described as a reflex arc, with the ascending pathways transmitting the suckling stimulus from the nipples to the hypothalamus forming the afferent limb, and the oxytocin neurons projecting to the posterior pituitary forming the efferent limb (Ely and Peterson, 1941; Wakerley, Clarke, and Summerlee, 1988). The suckling stimulus activates the oxytocin neurons in the hypothalamus which project to the posterior pituitary to release oxytocin, which then circulates to the mammary gland where it acts to contract the myoepithelium resulting in a ME.

The secretory tissue of the mammary glands is composed of alveoli. Although in some mammals, such as the cow, modification of this duct system provide storage space for milk, there are no such modifications in the rat. Therefore, the alveoli are mainly responsible for milk storage (Cross, 1977). If separated, the rat dam can store about 12 grams of milk in the alveoli of her mammary glands, which is equivalent to about 30 % of her daily milk yield (Lincoln, Hill, and Wakerley, 1973).

The myoepithelial cells provide the ejection force of the mammary gland. Oxytocin receptors, which develop on the myoepithelial cells appear shortly before the onset of parturition and disappear during weaning, and the appearance of these receptors correlate with development of sensitivity to exogenous oxytocin (Soloff, Alexandrova, and Fernstrom, 1979; Soloff and Wieder, 1983). Although no difference was found in the amount of oxytocin needed to elicit a ME between dams at 9-13 days postpartum, and dams at 20-24 days (DeNuccio and Grosvenor, 1971), a difference in the amount of oxytocin released due to suckling varied across lactation. The maximum frequency and amount of oxytocin released occurred on postnatal day 10, and subsequently declined (Sutherland, Aizlewood, and Wakerley, 1986). On postnatal day 10, this higher rate of oxytocin release decreased the latency to the first ME and increased the frequency of MEs as compared to postnatal day 2 or 20.

The oxytocin neurons controlling the ME reflex are in the supraoptic and paraventricular nuclei of the hypothalamus. During lactation, the oxytocin cells become greatly enlarged, and the synaptic contacts and glial relations of these cells are reorganized (Wakerley, Clarke, and Summerlee, 1988). During suckling the oxytocin neurons fire before each ME. The burst of activity lasts 2 to 4 seconds, and has a refractory period of 5 to 25 seconds. It takes 12 to 15 seconds after the burst of activity in the oxytocin neurons, for the oxytocin to travel from the hypothalamus through the blood to the myoepithelium and cause a contraction (Wakerley et al., 1988). The range of the refractory period of the oxytocin neurons is associated with the possible IMEIs that could occur. Although no correlation has been demonstrated between an individual pup's sucking and the associated milk release, Lincoln and Wakerley (1975) have shown that the reduction of the suckling stimulus by leaving only 2-3 pups on the dam's nipples reduced the magnitude of the bursts of firing in the oxytocin neurons.

What the reflex arc can tell us is that separation could have its effects on many levels of the dam's physiology. An increase in milk let down in the separated dam could be due to an increase in sensitivity to suckling, an increase oxytocin release from the hypothalamus due to suckling, an increase in sensitivity of the myoepithelial cells to the oxytocin released, or a decrease in contraction of the mammary gland needed to expel milk. In fact, DeNuccio and Grosvenor (1971) studied the effect of separation of the sensitivity of the mammary gland to oxytocin and found a progressive reduction in the dose of oxytocin required to elicit MEs as the volume of milk in the gland increased as separation progressed. Wakerley et al. (1988) hypothesized that this was probably related to the fact that the force of contraction was increased by the higher resting tension of the myoepithelium.

6. Control of the Dam's Neurochemical and Hormonal State

Suckling stimulation does not just influence the milk-ejection reflex. It has been shown to also change the dams sensitivity to ultrasonic vocalization (USV), increase food intake, and increase aggressiveness toward conspecifics.

Smotherman, Bell, Hershberger, and Coover (1978) determined that lactating mothers show a decreased response to USVs after a period of separation from their pups. The effect of separation was not immediate, but the longer the separation the greater the effects. After a five day period without pup stimulation, these mothers showed no orientation to the USVs other than by chance. Changes in the maternal responsiveness was hypothesized to be due to changes in the lactating females endogenous condition resulting from the loss of litter stimulation.

A lactating dam has a higher food consumption than nonlactating dams, which helps to compensate for the greatly enhanced energy expenditure associated with lactation (Fleming, 1976). Also, in contrast to the behavior of virgin females toward other rats, a lactating dam will immediately attack intruder rats released into her home cage (Erskine, Barfield, and Goldman, 1978). This increased aggression functions to protect the litter from potentially hostile conspecifics. This increase in food intake and aggressive behavior are all dependent upon the presence of pups, as removal of the pups decreases food intake (Fleming, 1976) and aggression (Erskine et al., 1978). Hansen and Ferreira (1986) demonstrated that the sucking stimulation provided by the pups maintains these behaviors. After lesioning the peripeduncular area of the lateral midbrain which blocked the ME pathway, mother rats ate less and interacted more peacefully with intruders.

Therefore, upon separation, the deprivation of the dam from the pups' suckling stimulus is the withdrawal of that which regulates and maintains much of her maternal behavior. How quickly maternal behavior can be resumed upon reunion depends upon the length of the separation and the lactational age of the litter. Just as the separation changes alter the pup, the dam is an altered organism due to the separation experience. The study of how these separation changes affects the interaction upon reunion will be described.

III. Theoretical Framework for Understanding Effects of Separation Upon Reunion

From a theoretical point of view, we would like to consider three time dependent processes as a framework for understanding the separation changes seen: attachment, nursing and suckling motivation, and multiple regulators.

A. Attachment

Attachment refers to a special relationship, as in the mother-infant relationship, that is specific in focus and endures over time (Gubernick, 1981). A successful attachment of the infants to their mother helps ensure that the infants will be provided with nourishment, protection, and appropriate stimulation necessary for their physiological and behavioral development. However, the attachment within the mother-infant relationship could be considered more complex than providing just these components because by the definition provided by Gubernick (1981) it is a special relationship with a specific individual, thereby entailing an emotional component.

Criteria frequently used as indicators of attachment include the preference for one individual and the seeking and maintaining of proximity to that individual (Gubernick, 1981). The degree of attachment present is often studied by measuring the intensity of attachment behaviors present upon separation. In work that helps us to understand the nature of attachment, Bowlby (1969) has described three stages that occur in the human infant as a response to separation from its mother. Despite the wide variations that exist in mother-infant relationships, such as in age, experience, or environment, the response to separation was observed to be remarkably uniform. The first stage, labeled "protest", is one in which the infant is agitated and vocalizes frequently. Obviously distressed at having lost his mother, he expends all his energy protesting her absence while constantly expecting her return. The protest response can be characterized by an intensification of motivation toward the mother when seen in the face of frustration. The second stage is one of "despair" and is characterized by the passivity of the child. All behavior is reduced and the child appears sad, has an apathetic posture and weeps easily. The child is still preoccupied with its missing mother, however now appears to feel hopeless. "Detachment", the final stage, is characterized by the rejection of the mother upon reunion. The child appears to have adjusted to the separation since upon reunion at this point the infant appears either not to recognize or obviously ignore its mother figure. Because these stages are seen in the child separated from its mother but given adequate substitute care, Bowlby depicts the mother-infant interaction as going beyond the need for food, warmth, and protection and entailing complex psychological processes.

We do not know if an attachment process as described by Bowlby helps us to understand the separation changes seen in the rat. The first stage of protest, seen immediately after separation, can be analogous to the rat pup's increased vocalization and locomotion when removed from the dam. The function of the ultrasonic distress calls by the pups is thought to be to elicit maternal retrieving behavior (Allin and Banks, 1972; Smotherman, Bell, Starzec, Elias, and Zachmam, 1974), and the increased locomotion may be increase the chances of the pup coming across its mother. These immediate protest responses to separation appear to be an intensification of attachment behaviors attempting to maintain proximity to the mother upon separation. Can the slower developing separation changes related to the withdrawal of regulators fit into the later stages of separation as described by Bowlby? Specifically, can the plateau of changes in heart rate, respiratory rate, and sleep wake state patterns somehow be related to the next stages of despair or detachment? Perhaps the interaction upon reunion will contain signs of the pups being passive toward the dam, an equivalent to the despair stage, or even rejecting the dam as an equivalent to the detachment stage. Of course it is also possible that these terms are simply not useful in describing the rat pup's long term separation response and their effect upon reunion.

B. Nursing and Suckling Motivation

Perhaps the framework of nursing and suckling motivation will help to understand the separation effects seen. The presence of a specific drive or motivated behavior in the mother-infant relationship would ensure the infants survival by eliciting an interaction in which the infant obtained necessary resources and the mother provided them. Attachment, as just described, is a motivated behavior, as the goal of attachment behaviors such as vocalization, is to achieve and maintain proximity. In theory, a driven or motivated behavior increases in intensity after deprivation, and therefore we would expect to see an enhancement of the motivated behavior post-separation.

An example of a motivated behavior in the rat pup is suckling, the means by which pups consume essential nutrients. Rat pups can locate and attach to a nipple, suckle and withdraw milk from the dam from the moment they are born. And in accordance with the definition of a motivated behavior, sucking rate and force have been shown to increase after a deprivation period (Brake and Hofer, 1980; Brake, Shair, and Hofer, 1988). In a series of studies, Brake and colleagues have shown that sucking is actually directed by two motivational systems: the motivation to feed, and the motivation to suckle or attach (for review see Brake, Shair, and Hofer, 1988). In the feeding system, suckling is influenced by nutritive factors therefore a gastrointestinal preload would decrease the drive to suckle. In contrast, the suckling system or the desire to attach is influenced by nonnutritive factors or perioral sensation and therefore the drive to suckle could be satiated by simply sucking a teat that does not supply milk. These systems interlock to control ingestion and probably insure that the pup is consuming the maximum amount of milk available, as well as ensuring that lactation is maintained by the stimulation of suckling.

Will the labeling of the motivational systems of feeding and sucking by the rat pup help to understand the separation effects seen? As described earlier in the section on the specific measures of changes in behavior and physiology due to separation in the rat pup, 10 to 12 day old pups who have been separated have a decreased latency to attach upon reunion (Drewett, Statham and Wakerley, 1974; Hall, Cramer, and Blass, 1977) and increase the time and force of their suckling (Brake, Shair, and Hofer, 1988; Shair, 1986), thereby possibly increasing their milk intake. These studies suggest that the motivational systems of the pup to attach and feed are not turned off or redirected but rather increased in strength after a separation period. If such behaviors are observed upon reunion, consistent with the motivational processes of the pup increasing in intensity, the construct of drives is useful in understanding the separation effects.

The evidence for the dam's motivation to interact with the pups is less clear. Leon (1979) has described in detail a thermoregulatory hypothesis that controls the nesting behavior of the dam. Leon's work suggests that the return of the dams to their pups has to do with the maintenance of maternal thermal homeostasis. The temperature of the dam rises during nursing due to the increased heat generated by the litter and her decreased ability to dissipate heat due to her occupied ventrum, and upon a critical rate of temperature increase, the bout is terminated. Dams would then leave the nest when it became necessary, and would return to the nest when they are again able to sustain an increase in body temperature. The longer they were away from the nest, the more likely it was that they would return. It was found that dams who experienced an immediate, rapid decline in core temperature after bout termination quickly returned to their pups perhaps because heat dissipation had occurred so rapidly. Conversely, dams who continued to have elevated body temperature after bout termination had much longer latencies to return to the nest (Leon, 1979).

Rosenblatt and colleagues (Rosenblatt, Siegel, and Mayer, 1979) have described a change in regulation of maternal behavior from the hormonal onset present before parturition to the nonhormonal maintenance after parturition. Nonhormonal maintenance entails pup stimulation which has been shown to be necessary immediately after parturition to sustain the maternal behavior and in its absence maternal behavior declines. Rosenblatt et al. (1979) states that once the nonhormonal regulation of maternal behavior has been established, mothers are more able to withstand separation periods with less disastrous consequences for the young than if separation occurred at parturition. The timing of the separation period during the lactational period is most likely a critical variable in addition to the length of separation. For example, at the lactational age of 10 to 12, the effects of separation will probably not cause total disruption of the mother-infant interaction upon reunion because for over a week the dam has been repeatedly exposed to the pup stimulation to firmly establish and maintain her maternal behavior. However, the thermoregulatory function of nesting behavior has most likely been disrupted by the separation period. Upon reunion it may be useful to understand that the dam's thermohomeostasis plays a role in bout duration and see if it is still active or if other motivational systems take over. As mentioned in the literature review, Stern and colleagues (Stern, 1985; Stern and Johnson, 1990) have shown that ventral somatosensory stimulation elicits nursing behavior by the dam, and this stimulation may contribute to the motivation of the dam. It is possible that separation may enhance or detract from the dam's sensitivity to this stimulation, altering the nursing motivation of the dam.

C. Multiple Regulators

Another framework that may help us untangle the separation effects seen is that of multiple regulators. In this framework, as described by Hofer (1978), the mother interacts with her young through stimulation of all the sensory systems, which in turn plays a role in directing and controlling the behavior and physiology of the infant. In applying the theory of multiple regulators to the rat, when the dam is separated from her pups, the withdrawal of the stimulation normally provided by the dam results in various behavioral and physiological changes which each can be restored by supplying the specific regulator or regulators that control it. Therefore, not only are certain behaviors and physiological functions controlled by specific aspects of maternal stimulation, an observed behavior may not be the result of a single regulator but rather an outcome of many regulators (Hofer, 1981).

An example of multiple regulators is found in the pups' suckling behavior which is regulated by two systems which were described earlier. First, there is regulation of nonnutritive sucking, or the attachment of the pup to a nipple in the absence of milk delivery, by perioral stimulation provided by the nipple itself. And second, there is the regulation of nutritive sucking by gastrointestinal fill or milk consumption while attached to the nipple (Brake, Shair, and Hofer, 1988). Another example of multiple regulators is seen in pup activity level as described by Hofer (1983). Pups display an increasing level of hyperactivity as separation progresses and the dam has been shown to be regulating this activity level by the combination of types of stimulation. The dam's presence modified the activity response when the pups could smell her pup not touch her. When the olfactory cue was eliminated by making the pups anosmic hyperactivity resulted. Intense tactile stimulation delivered in a schedule which mimicked the mother's periodic visits to the litter of pups also prevented hyperactivity. Therefore the dam is regulating the pups' behavioral level by both olfactory and tactile stimulation.

Theoretically, these multiple determinants of a single behavior provide the organisms with the ability to respond to separation stress as they provide the flexibility to adapt to changing environments. If one regulator is not present, another can substitute, and the behavior adapts to a new condition. It seems logical that there would be evolutionary selection for animals with such stress responses as these animals would have an increased chance for survival in a changing environment.

The concept of multiple regulators might be useful for the understanding of the separation effects seen. In addition to the existence of multiple regulators for certain processes within the mother-infant interaction, there may exist differences in the time course of change in the variable after withdrawal. Therefore different processes in the pup might be at either early or advanced stages of adaptation to separation upon reunion, resulting in an interaction which contained easily-resumed behaviors as well as disruptive behaviors. An alternative way to describe the mother-infant interaction upon reunion would be as a unitary phenomenon, with all behaviors holding together and the relationship being renewed as a whole, as would be predicted by attachment theory.

In summary, this dissertation will attempt to characterize the mother-infant interaction upon reunion in 10 to 12 day old rat litters after an 18 hour separation period. It will attempt to determine if the changes seen are primarily due to changes in the dam's behavior, or changes in the pups' behavior. And finally, it will look at how the separation effects seen upon reunion fit into the theoretical frameworks of attachment, nursing and suckling motivation, and multiple regulators of the mother-infant interaction.

IV. Strategy for Accomplishing Goals

To characterize the effects of separation on the mother-infant interaction upon reunion after separation, an observational study was done on postnatal day 10-12 comparing the interaction upon reunion of a dam with her pups who had been separated for 18 hours, to the interaction of a nonseparated litter. These two litter types being compared will be referred to as the Ss group, or separated dam with separated pups, and the Nn group, or nonseparated dam with nonseparated pups. Detailed observations were made of the initial two hours of interaction to be sure to include the entire first nursing bout, the period during which we hypothesized the effects of separation upon reunion would be most apparent.

Goal one of this dissertation was to characterize the mother-infant interaction upon reunion after a lengthy separation period. This was done by directly comparing the separated litter (Ss) to the nonseparated litter (Nn). All measures taken of these two groups were compared using an independent t-test. Results of this analysis determine what alterations occur in the interaction as a result of the separation experience. (Note: the specific measures taken will be described in detail in the methods section).

Goal two of this dissertation was to determine the relative contributions of the dam and pups to the interaction seen upon reunion after separation. To do this, two more litter conditions were observed, a nonseparated dam with separated pups (Ns), and a separated dam with nonseparated pups (Sn). With the four groups present in this study, the Nn, Ns, Sn, and Ss, we were then able to ask if the separation effects seen between the Nn and Ss groups can be attributed to changes in the dam, changes in the pups, or an interaction between the two. For example, if we found that the Ss and Nn groups were significantly different in a particular measure, and this the change in the behavioral measure was seen in the Ss and Ss groups, in contrast to the Nn and Ns groups, we could conclude that the alteration in the interaction due to separation was the result of changes in the dam condition, as these changes were seen in the separated dam groups regardless of what pup type they were with. Or, if the change in the interaction due to separation was seen in the Ss and Ns groups, in contrast to the Nn and Sn groups, we could conclude that the separation effect was the result of an alteration in the pup condition, as this change was seen in the groups with separated pups regardless what dam type they were with. The four groups were compared using a two way analysis of variance (ANOVA: Dam X Pup). Seven possible models were applied to the results of the two way ANOVA to aid in their interpretation (see Figure 1).

ANOVA models with one main effect include Models 1 and 2. These models changes due to separation in which alterations in either the dam or pup condition were responsible. The ANOVA Model 3 has no main effects but a significant interaction and represents changes due to separation in which the alterations in both the dam and pup were responsible. These effects were only seen in the groups in which dam and pup condition were disparate (Ns and Sn), as there was no significant difference between the Ss and Nn group. The ANOVA models with two main effects include Models 4 and 5. These models represent changes due to separation as a result of alterations in both the dam and pup condition. Model 4 is an additive model, in which changes in the dam and pups were in the same direction. Model 5 is a subtractive model, in which changes in the dam and pups were in opposite directions. The ANOVA models with an interaction effect and a pup and/or dam effect are Models 6 and 7. Model 6 represents a unique interaction in a similar-condition group (Nn or Ss), and Model 7 represents a unique interaction of a disparate condition group (Ns or Sn).

Figure 1: Models of possible results of the 2 way ANOVA across all four groups (Dam condition=separated or nonseparated, X Pup condition=separated or nonseparated), testing for changes in the mother-infant interaction due to separation.

I. Model 1 and 2: These models have one main effect and represent changes due to separation in which alterations in either the dam or pup condition were responsible.

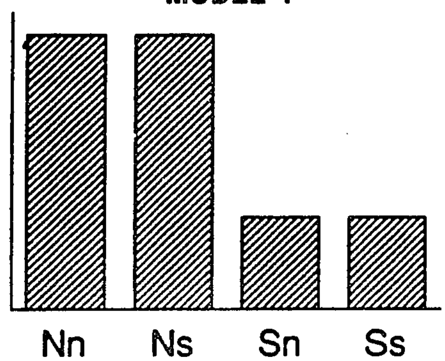
II. Model 3: This model has no main effects but a significant interaction and represents changes due to separation in which the alterations in both the dam and pup were responsible. These effects were only seen in the groups in which dam and pup condition were disparate (Ns and Sn), as there was no significant difference between the Ss and Nn group.

III. Model 4 and 5: These models have two main effects and therefore represent changes due to separation as a result of alterations in both the dam and pup condition. Model 4 is an additive model, in which changes in the dam and pups were in the same direction. Model 5 is a subtractive model, in which changes in the dam and pups were in opposite directions.

IV. Model 6 and 7: These models have an interaction between the dam and pup condition, and a pup and/or dam effect. Model 6 represents a unique interaction in a similar-condition group (Nn or Ss). Model 7 represents a unique interaction of a disparate condition group (Ns or Sn).

I. Anova Models With One Main Effect

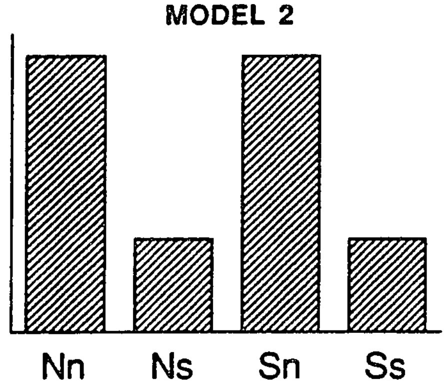
MODEL 1



DAM EFFECT

Separation effect:
Change in dam responsible

MODEL 2

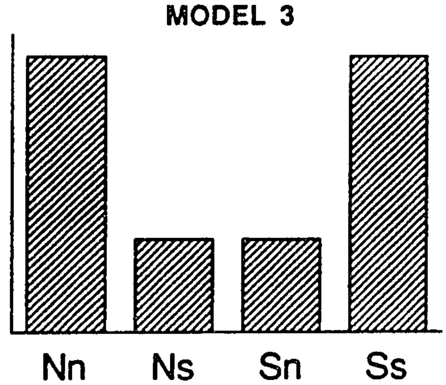


PUP EFFECT

Separation effect:
Change in pup responsible

II. Anova Model With No Main Effects and an Interaction

MODEL 3

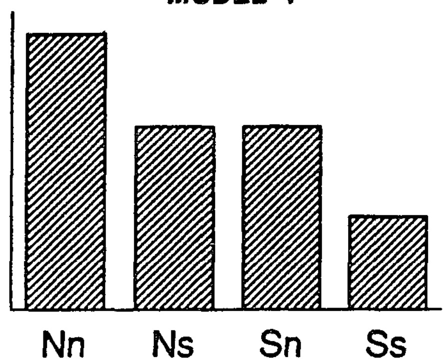


INTERACTION EFFECT

Separation effect:
Change in dam and pup responsible

III. Anova Models With Two Main Effects

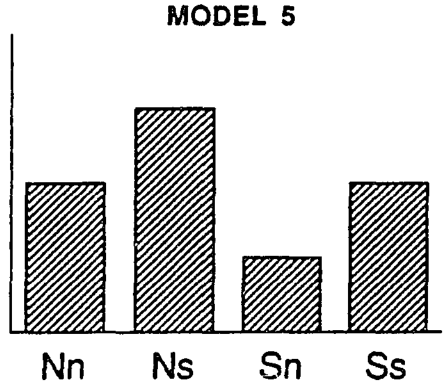
MODEL 4



DAM AND PUP EFFECT

Separation effect:
Change in dam and pup in the same direction responsible (additive model)

MODEL 5

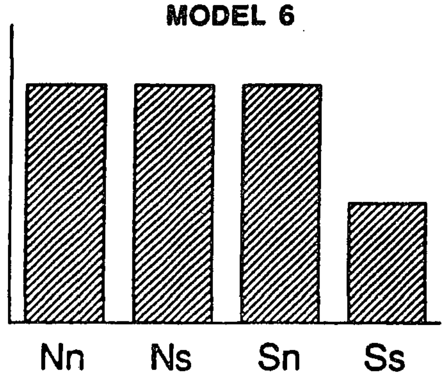


DAM AND PUP EFFECT

Separation effect:
Change in dam and pup in opposite directions responsible (subtractive model)

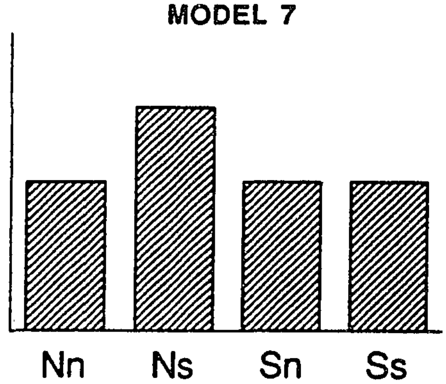
IV. Anova Models With an Interaction Effect and Pup and/or Dam Effect

MODEL 6



Separation effect:
Unique interaction of
similar condition

MODEL 7



Separation effect:
Unique interaction of
disparate condition

These models are not all inclusive of the possible ANOVA results, but represent types of outcomes that could occur which are interpretable in terms of the relative contributions of the dams and pups. These results can then be applied to the separation effects seen in the interaction upon reunion of the separated litters when compared to nonseparated litters.

GENERAL METHODS

Subjects: Four to five primiparous Wistar rats (Hilltop) were delivered timed-pregnant each week for a total of 18 weeks spread out over a period of a year and a half. These test litters were born and raised in the laboratory on a 12:12 light-dark cycle, with lights on at 7:00 am. Animals were housed singly in clear polycarbonate cages (46 X 24 X 20 cm) with grill tops holding chow and water bottles, and with wood shavings as bedding. Food (Agway Prolab; Rat, Mouse, Hamster, #3000) and water was available to the dams ad lib. Cages were checked for births each morning and afternoon, and the day of birth was labeled as Day 0-1. Litters were culled to 9 pups 1-3 days after birth with a sex ratio as equal as possible. Litters were reared in the testing room so as to accustom them to the room and to the presence of laboratory personnel and ambient noise in that area prior to the experiment.

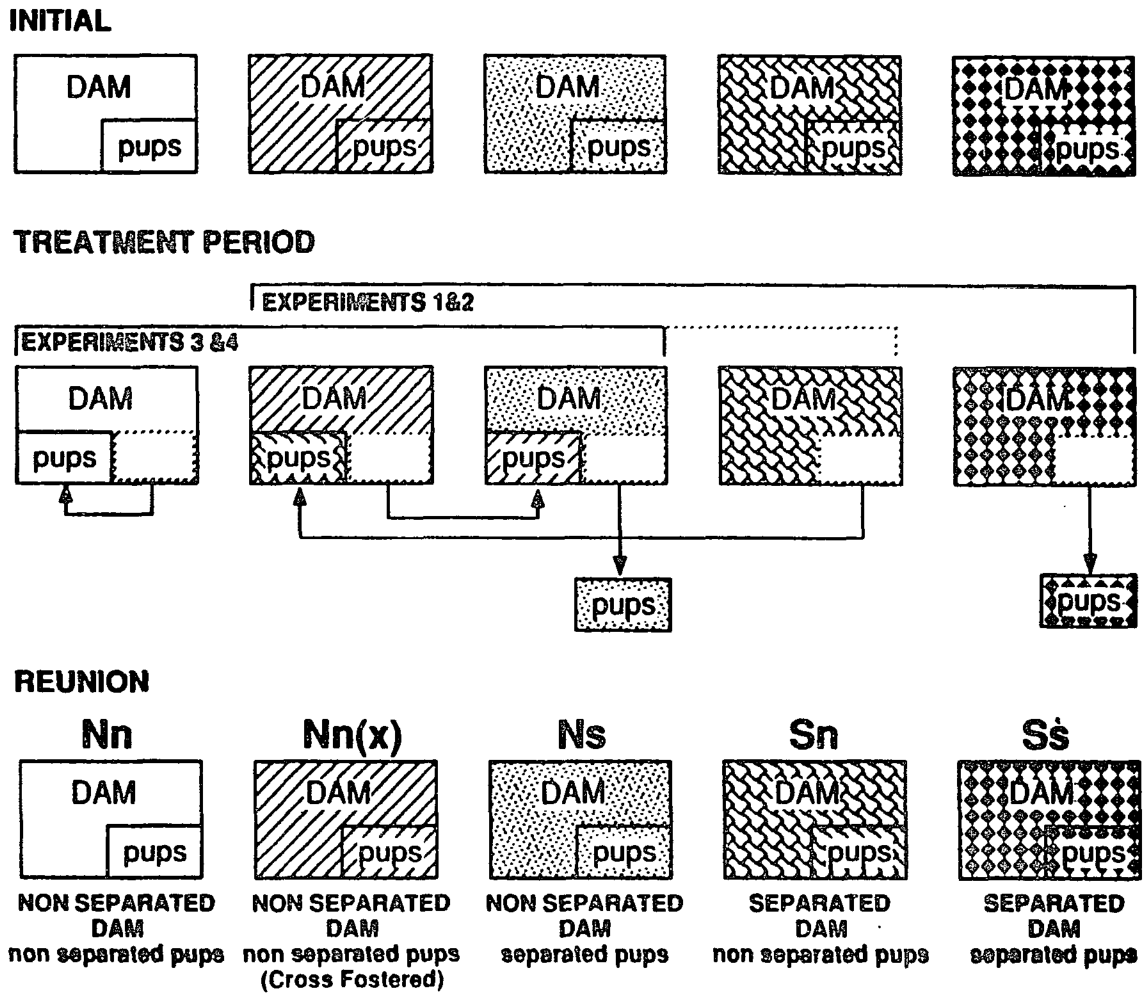
<u>Experimental Set-up</u>: For the experiments, cages were on stands with mirrors positioned underneath to allow the litters to be observable at all times, even during nursing. Two days prior to testing, the litters were placed on these stands in the testing positions in order to reduce the novelty of this condition. Also, the amount of shavings was reduced prior to the test period to allow visibility of the dam nursing her pups. To aid in reducing the stress of this manipulation, the shavings were reduced as gradually as possible without increasing the amount of handling of the animals already necessary. Approximately 50 % of the home cage shavings were removed on the day before testing during the separation or cross fostering of litters, and another approximate 40 % on the day of testing immediately prior to the reunion. Note: the effects of the lack of nesting material on the thermal characteristics of the nursing behavior will be taken into consideration in the discussion of the results.

Litters were tested three or four at a time, one in each condition (the number and type of conditions depending upon the experiment), approximately 25-30 cms apart. This presented a methodological problem, for it has been shown that exteroceptive stimuli, such as a nearby lactating litter, can release prolactin in the dam (Deis, 1968), although the effect is minimal at 10-14 days post-partum, as compared with 21 days postpartum (Mena and Grosvenor, 1972). The advantages of testing litters simultaneously in the same environment outweighed these possible effects, but to balance them out across the groups the position of each condition in relation to the others was rotated for each repetition of the experiment.

Procedure: On the day prior to testing, dams and pups were either separated or cross fostered for 18 hours as shown in Figure 2. These 18 hours will be called the treatment period. To place the dams and pups into their appropriate condition for the treatment period, all dams were removed from their pups and placed in holding cages. The home cage shavings were reduced the first time, and the pups were either placed in holding cages with fresh and home cage shavings on heating pads in another room or placed in the home cages of the dams' who were also in the nonseparated condition. Once the pups were in position for the treatment period, the dams were returned to their home cages. Note: none of the pups in Experiment 1 or 2 were positioned with their actual dam, so that the reunion of the litters for the test period the next day was a return to natural partners for all conditions. This was controlled for by a litter that was not cross fostered in Experiment 3 and 4.

The placement of separated pups on heating pads, set at 36.5°C, during the treatment period functioned to keep them warm. As previously described, dams build nests that maintain pups at a warm temperature (Jans and Leon, 1983), but the procedure described above disrupted the natural nest of the litter. The study presented here was designed to look at separation effects that would occur if pups became separated from their dam as a group in an insulated nest,

Figure 2: The procedure, which consists of three separate periods: 1) the initial period, with all litters identical in agé and experience, 2) the treatment period, on postnatal day 10-12, during which the dams and pups experience 18 hours of either separation, cross-fostering, or just handling, and 3) the reunion period, during which all dams and pups were returned to their natural partners and the reunion observed.


INITIAL
DAM
pups
TREATMENT PERIOD
EXPERIMENTS 1&2
EXPERIMENTS 3 &4
REUNION
Nn
Nn(x)
Ns
Sn
Ss
NON SEPARATED DAM non separated pups
NON SEPARATED DAM non separated pups (Cross Fostered)
NON SEPARATED DAM separated pups
SEPARATED DAM non separated pups
SEPARATED DAM separated pups

therefore they were artificially kept warm. Note: the known effects of pup temperature during separation and upon reunion will be taken into account when discussing the results. During the separation period the pups remained huddled together in a pile sleeping and burrowing as is typically observed when the dam is away from the nest only briefly.

The total time to position the litters for the treatment period was approximately 15 minutes, and was completed between 4:00 and 5:00 pm. The treatment period ended 18 hours later, and the reunion test began. All tests commenced between 10:00 and 11:00 am, no more than 4 hours after the beginning of the light cycle in the test room. The circadian pattern of maternal behavior that has been demonstrated to exist is as follows: the dams spend almost twice as much time with the pups during the light portion of the cycle (Grota and Ader, 1969) during which time the pups increase their milk intake. The inverse of this pattern takes place during the dark portion of the cycle and consists of an increase in the dam's general activity and food and water intake (Stern, 1989). Therefore the timing of the experiment consists of separating the dams and pups during the period when they naturally spend the least amount of time nursing, and then reuniting them for the period in which they naturally spent the most time together. This is not to say that we minimized the disruption, but rather that the reunion was observed during the portion of the light-dark cycle in which the dam and pups spend the most time together.

The following morning, an hour prior to the reunion, all dams were placed in holding cages, and the shavings in their home cages were reduced again to an amount that allowed viewing of the interaction from underneath. Pups from each litter, separated and nonseparated alike, were stimulated to urinate and defecate, then weighed and marked with an odorless marker.

One male pup in each litter was randomly selected to be the focal pup and was marked uniquely. The method of observing the litters upon reunion (described later in detail) included noting the specific behaviors of the focal pup, such as dam directed, other directed and quiet behavior. The observation of these behaviors were made more specific by focusing on one pup. The selection of a male focal pup presented a possible methodological problem because dams differ in their behavior toward their offspring depending upon their sex. Studies have shown that dams lick the anogenital (AG) regions of male offspring more frequently and for greater durations than female offspring (Moore, 1982; Moore and Morelli, 1979; Richmond and Sachs, 1984). However, the focal pup alone was not used for all measures. The dam's behavior toward any pup was noted, including the rate of AG licking of pups by the dam. Also, the contact and attachment of all the pups of the litter was noted, not just that of the focal pup.

Once stimulated, weighed, and marked, pups were placed in their home cage in a pile, and axillary temperatures taken on two of the pups, including the focal pup. For the test period, the dams were returned to their natural pups in their home cage and observations were made, as described below, for 2 hours. After this observation period, the dams were removed, axillary temperatures taken on the same two pups as before, and all the pups were weighed again. Depending upon the experiment, it was either terminated at this point, or the pups were immediately returned to their dam, and observations and/or weights were taken again at later times. Variations of the procedure will be described below in the individual descriptions of the experiments.

Behavioral Observations: Immediately upon the return of the dam to her pups, behavior was sampled at one minute intervals and continued for two hours. All observations were made by me from approximately 2 meters away. I either called out the positions and behaviors of the test litters to an assistant who recorded them, or I recorded them myself directly onto the data sheets. Although the animals were accustomed to the presence of laboratory personnel as it occurred regularly during the checking of births, feeding schedules, and running of experiments, all communication was done as quietly as possible.

Observations of behaviors were made using the snapshot technique: each minute the mother and her pups were observed during a brief period (2-5 seconds) and recorded on a checklist according to the categories and criteria described below. This time sampling technique has been utilized in previous studies from this laboratory (Brunelli, Shindledecker, and Hofer, 1984; Hofer, Zmitrovich, and Shair, 1989).

Nursing Behaviors

Contact was noted as whether more or less than half the pups were in contact with the dam.

Attachment was noted as whether more than more or less than half the pups were attached to teats. The descriptions of attachment were often referred to in terms of nursing: Full nursing was when more than half the litter was attached, and Partial nursing was when less than half the litter was attached.

Latency to attachment was determined in three ways: 1) initial attachment is the first time that more than half the pups are attached, 2) stable attachment is the beginning of the time that more than half the pups are attached for 3 or more consecutive observation times, and 3) solid attachment must continue for 10 or more consecutive observations.

Milk Ejections (MEs) were the only event recorded whenever they occurred during the 2 hour observation period, in contrast to the other periodic snapshot observations. MEs were noted when the dam was observed to arch her back abruptly and extend her legs into a splayed position while her pups were concurrently activated to treadle, stretch, push against her ventrum, and often nipple switch. This patterned behavioral activation characteristically lasted 10-20 seconds. A ME was rated as being a Definite ME if the observer noted all of the above mentioned components of the ME pattern. A ME was rated as being a Possible ME if the pups were seen to be activated as a group and treadling, but either there was no distinct stretch response, and/or the dam did not fully arch. In this latter case, the observer was not fully convinced that a ME had occurred.

Dam's Behavior

Pup directed behavior, included a) anogenital (AG) licking: the dam licked the anogenital area of the pup, b) body licking: the dam licked any other area of the pup excluding the AG area, and c) retrieving of the pups.

Other directed behavior, included a) self-grooming, b) rearing, c) burrowing, d) eating or drinking, and e) changing of position which is generalized movement.

Quiet epochs included times of inactivity.

Nursing positions noted included

a) arched, when the dam is arched over the pups with legs splayed, or b)on top, when the dam is over the litter but does not have an arched back, or splayed legs. The on side nursing posture is also described in the literature as occurring when the dam is lying on the side of the pile of pups. This posture occurred in only one litter and only briefly so it was not included in the analysis.

Pups' Behavior

The contact and attachment of the majority of the litter was noted and the dam's behaviors directed toward the litter and any pup was noted as described above. All other behavioral observations listed below were of the single focal pup.

Dam directed behavior included a)locomotion toward the dam, b)nosing the ventrum, c) mouthing and licking a teat during the process of attachment, and d)treadling or stretching while on the teat.

Other directed behavior included changes of position or generalized movement not directed toward the dam, such locomotion away from the dam or burrowing in the pile.

Quiet epochs included periods of inactivity, regardless of attachment.

The location of the focal pup was also noted. The pup was either in contact or away from the dam, and in or out of the litter pile.

Behaviors were analyzed for both the dams and the pups prior to the first nursing bout which was the time before solid attachment, and once attached during the first nursing bout. The nursing bout was defined as beginning at solid attachment and ending when more than 3 minutes of less than half or zero pups were attached. The behavior of the dam and the focal pup of each litter was noted every minute, and the number of behaviors within each category (dam or pup directed, other directed, or quiet) were totaled and divided by the time available for them to occur. Analysis compared these rates of dam and pup behaviors between dam and pup condition.

To understand preattachment behavior further, the number of transitions between the above mentioned categories of behaviors were also analyzed.

Postattachment analysis, or analysis during the first nursing bout was done a)excluding the time of definite MEs, and b)excluding the time of definite and possible MEs combined. Definite MEs are known to be the behavioral response due to milk let down, and these were eliminated in behavioral analysis because differences between the conditions can be seen better by eliminating the effects of different rates of MEs. Possible MEs were included in the first analysis because it is not clear whether possible MEs are signs of an actual let down of milk, or increased activation and this latter was not necessary to eliminate.

It is important to note that the division of behaviors into the distinct categories of dam or pup directed, other directed, and quiet was done in order to better understand the overall direction of the dam or pup behavior. However, the behaviors were interrelated. For example, the dam's self grooming behavior was categorized as an other directed behavior, yet could also be seen as a pup directed behavior if it was being done by the dam in order to make her ventrum more desirable for nursing, as her saliva has been shown to facilitate attachment. To help account for the complexities of the behaviors, the behaviors will be described not only within their overall category, but also they will be looked at separately and possibilities of their function will then be described.

EXPERIMENT 1

Methods

This experiment compared four conditions:

1) A nonseparated dam with nonseparated pups (Nn)
2) A nonseparated dam with separated pups (Ns)
3) A separated dam with nonseparated pups (Sn)
4) A separated dam with separated pups (Ss)

The experimental set up and procedure were as described in the general methods. Immediately after the weights and temperatures were taken at hour 2, the pups were reunited again with their dam and fresh shavings were added to the home cage. The next day weights of all pups were taken again at hour 24, and the experiment was terminated. The four conditions listed above were tested simultaneously, in six trials, creating a total of six litters per condition.

ANALYSIS

Frequency and rates of behavioral measures were analyzed for the full two hour observation period, the first vs second hour, preattachment, and during the first nursing bout. As described in the introduction, to characterize the effects of separation on the mother-infant interaction upon reunion, an independent t-test was run on each measure to determine if differences existed between the Nn and Ss groups. A two way analysis of variance was then used to compare the Nn, Ns, Sn and Ss groups for main effects of dam and/or pup condition, and interactions of dam and pup condition (ANOVA: Dam X Pup). Referring back to Models 1 through 7 of Figure 1, the ANOVA results can be interpreted to understand the relative contributions of the dam and pup to the separation effects seen. If necessary, a post-hoc F test with pooled variance was utilized to make specific comparisons between individual condition means.

An analysis of covariance was done to determine if differences between groups in certain measures still existed when means were adjusted for by axillary temperatures of the pups, and if a relationship existed within the groups between the pups' temperatures and the nursing behaviors observed upon reunion (TREATMENT= condition, COVARIATE= temperature). The pup's axillary temperature immediately prior to reunion was the covariate for ANCOVA of latency to attach, latency to the first ME, total number of MEs over 2 hours, total time spent nursing over 2 hours, rate of MEs, latency to the first nursing bout, and length of the first nursing bout. The change in temperature over the two hour reunion period was the covariate for the analysis of differences between groups in total number of MEs over two hours, total time spent nursing over 2 hours, rate of MEs, and length of the first nursing bout.

RESULTS

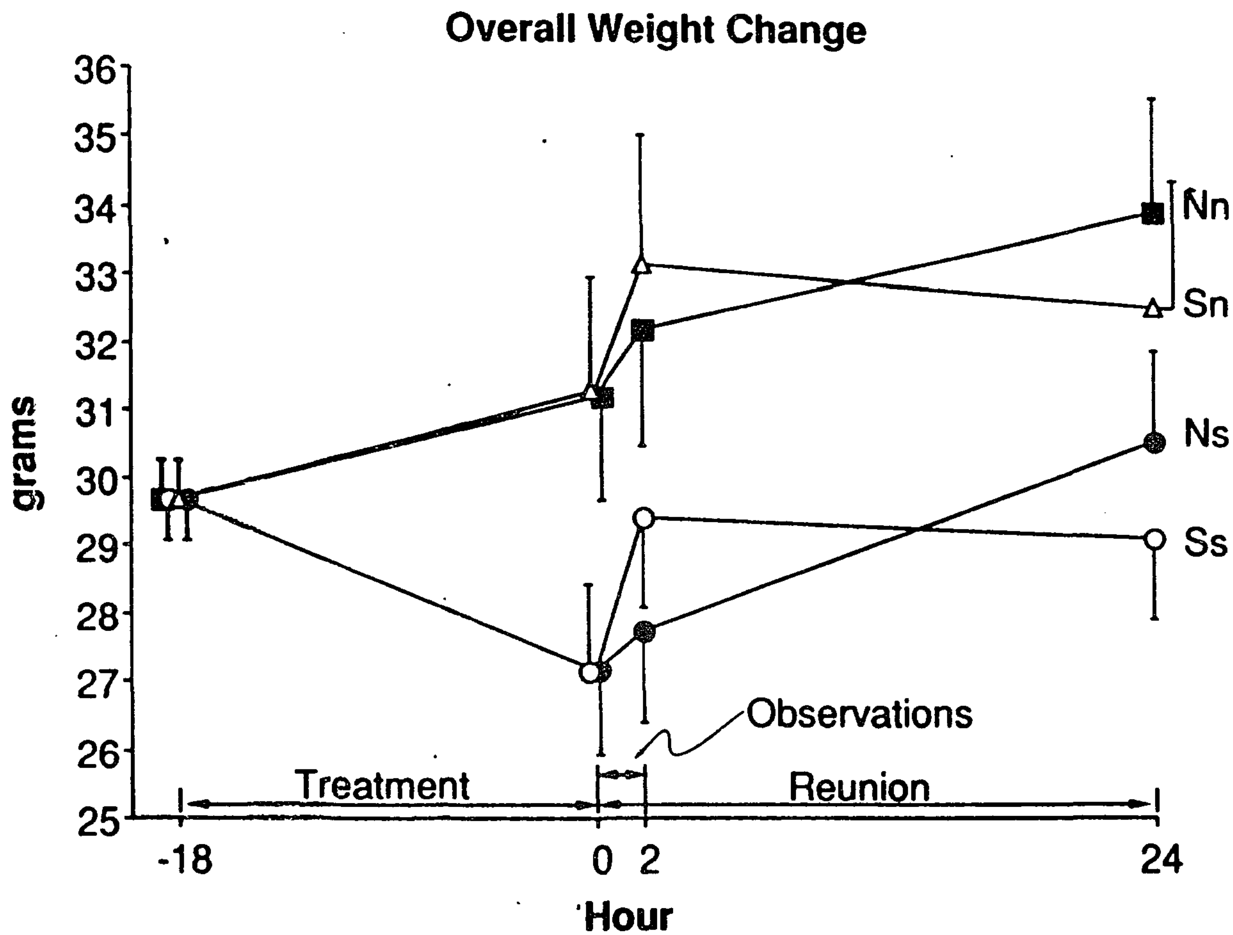
Weight

The overall weight changes of the pups are shown in Figure 3. Over the 18 hour treatment period separated pups lost weight and nonseparated pups gained. Once the reunion occurred, all pups gained weight, but at different rates and in different patterns over time in the different conditions.

Over the initial two hour period of reunion, pups of the separated litter (Ss) gained more than pups of the nonseparated litter (Nn) ($t(10)=-8.928, p<.001$). The results of the 2 way ANOVA comparing all four conditions (Nn, Ns, Sn, and Ss) demonstrated that this was a result of separation changes that occurred in the dams' condition; pups with a separated dam gained significantly more weight than pups with a nonseparated dam regardless of their deprivation condition (Dam effect: $F(1,20)=57.03, p<.001$; Pup and Interaction effects nonsignificant) (see Figure 4). The 2 hour weight change is thus an example of Model 1 (Figure 1) with a single main effect of the dam condition, although in the opposite direction than that shown in the model.

Between hours 2 and 24, pups of the Nn group gained weight and pups of the Ss group lost weight ($t(10)=7.157, p<.001$). The results of the 2 way ANOVA comparing all four conditions demonstrated that this difference was again the result of changes that occurred in

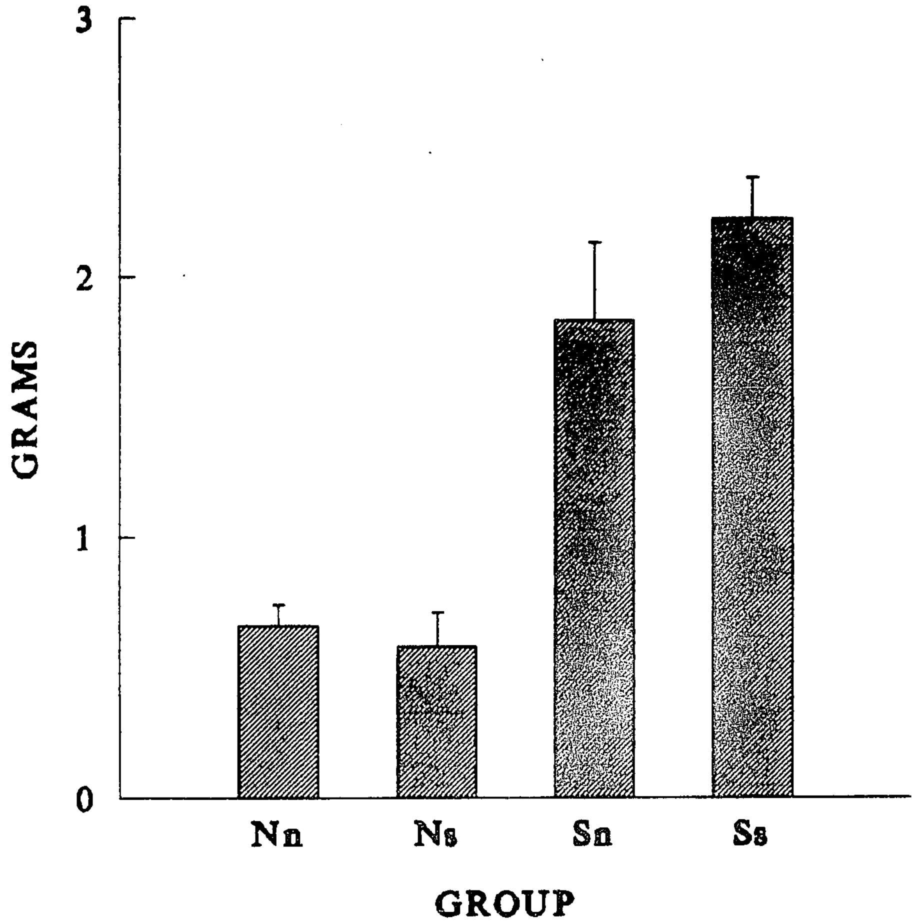
Figure 3: The mean weight of the 6 litters per group immediately prior to the treatment period, prior to the reunion, 2 hours after reunion, and 24 hours after reunion.



Overall Weight Change
grams
36
35
34
33
32
31
30
29
28
27
26
25
Nn
Sn
Ns
Ss
Observations
Treatment
Reunion
-18
0
2
24
Hour

Figure 4: The mean weight change for the 6 litters in each group during the first two hours of reunion. An independent t-test demonstrated that the Nn and Ss groups were significantly different ($p<.001$). The t-test result can be explained by a significant dam effect found in the 2 way ANOVA (Dam condition X Pup condition) across all four groups. There was no effect of the pup condition, nor an interaction between the dam and pup condition. These results fit a Model 1 of separation effects.

WEIGHT CHANGE, HRS 0-2



the dam condition in addition to changes occurring in the pup condition; pups with a separated dam lost weight and pups with a nonseparated dam gained (Dam effect: $\underline{F}(1,20)=242.00, p<.001$), and separated pups gained significantly more than nonseparated pups (Pup effect: $\underline{F}(1,20)=9.39, p<.007$) (see Figure 5). These results are an example of Model 5, with a dam and pup effect seen in the separation changes, but occurring in opposite directions from each other. Separation decreases the weight gain of pups with separated dams, but increases the weight gain of separated pups. These changes were not of equal magnitude because when combined in the Ss group, they did not balance each other completely and result in an equal weight gain to the pups of the Nn group. Since the Ss pups gained less, the decrease in weight due to the dam condition must be greater than the increase in weight gain due to the pup condition.

As shown in Figure 6, over the entire 24 hour period, pups of the Nn group gained more than pups of the Ss group ($t(10)=2.180, p<.055$). Further analysis revealed that this was a result of pups with a separated dam gaining less weight (Dam effect: $\underline{F}(1,20)=41.40$, $p<.001$) and separated pups gaining more weight (Pup effect: $\underline{F}(1,20)=10.28, p<.005$). These results were also an example of Model 5, with separation effects being a result of changes in both the dam and pup condition which occur in opposite directions

Figure 5: The mean weight change for the 6 litters per group during hours 2 to 24 of reunion. An independent t-test demonstrated that the Nn and Ss groups were significantly different ($p<.001$). The 2 way ANOVA across all four groups found a significant dam and pup effect. These results fit a Model 5 of separation effects, as the changes in dam and pup condition were in opposite directions.

WEIGHT CHANGE, HRS 2–24

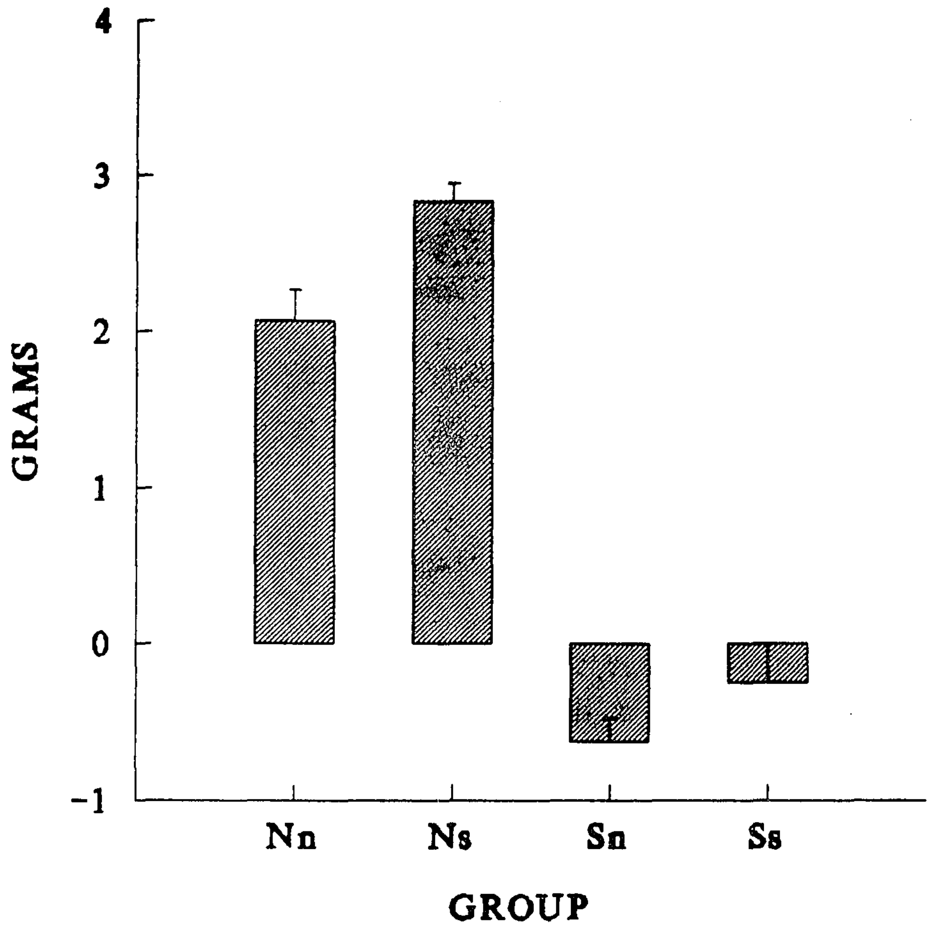
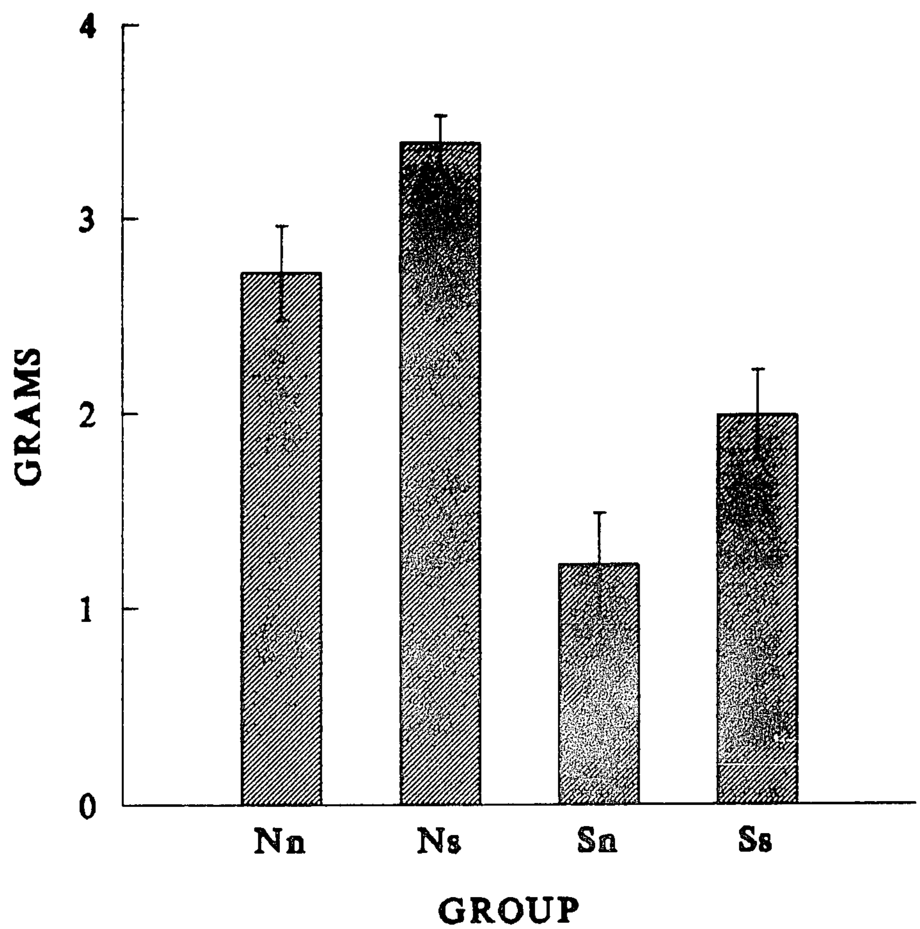


Figure 6: The mean weight change for the 6 litters per group during hours 0 to 24. An independent t-test demonstrated that the Nn and Ss groups were significantly different ($p<.055$). The 2 way ANOVA across all four groups found a significant dam and pup effect. These results fit a Model 5 of separation effects, as the changes in dam and pup condition were in opposite directions.

WEIGHT CHANGE, HRS 0–24



from each other. Again, these effects of the two conditions were not of equal magnitude as pups of the Ss group gained less than pups of the Nn group, thus the decrease in weight gain due to the dam condition was greater than the increase in weight gain due to the pup condition.

Post-hoc analysis on the 24 hour weight change revealed that the separated pups with a nonseparated dam (the Ns group) gained the most (Post-hoc, Ns vs. Nn, $\underline{F}(1,20)=4.540, p<.047$; Ns vs. Sn, $\underline{F}(1,20)=46.463, p<.001$; Ns vs. Ss, $\underline{F}(1,20)=19.478, p<.001$). Therefore, the nonseparated dam was able to supply more milk over time than the separated dam, and separated pups were able to obtain more from this dam than nonseparated pups.

As described in the Introduction, variability has been seen in the amount of milk pups consumed after deprivation. Some pups became good feeders and sucked with greater force and others did not (Brake et al., 1988). To determine if the stress of separation had increased the variability of pup weight gain in this experiment, the coefficient of variation (standard deviation of the mean weight divided by the mean weight) for each litter was calculated at hour 2 and at hour 24 and compared across groups. No significant differences were found between the Nn and Ss groups at hour 2 or 24. To determine if the similar variability between these groups was the result of different processes (see Models 3, 5, or 7), the 2 way ANOVA was run to determine the relative contributions of the dams and pups. However, there were no significant differences between dam condition, pup condition, or an interaction between the two. Therefore, separation did not affect the variability of weight changes in pups.

Summary and Discussion

To summarize the weight data, pups with a separated dam gained more weight over 2 hours than those with a nonseparated dam. This is consistent with the literature showing that there is milk build up in the separated dam which is released rapidly upon reunion (Lau and Henning, 1984; Lincoln, Hill and Wakerley, 1973). Concurrent with this milk build up, it has also been shown that a separated dam's threshold to oxytocin is decreased (DeNuccio and Grosvenor, 1971) and the force of contraction of the myoepithelium increased due to its higher resting tension (Wakerley et al., 1988) thereby increasing the amount of milk the pups obtain.

Although pups with a separated dam gain weight during the first 2 hours of reunion, they then lose weight over the next 22. Because we only have weights from these reported times, we cannot be sure of the curve of the weight change. However, this longer term weight loss upon reunion after a separation period is consistent with the literature showing that although there is milk build up which is released rapidly upon reunion, less milk is obtained as nursing continues (Lau and Henning, 1984). Grosvenor and Mena (1973) found that a time delay of 8 to 12 hours exists between the release of prolactin stimulated by the pups' suckling behavior and the stimulatory effect of this hormone on milk secretion. Therefore, 8 to 12 hours after suckling had stopped, milk secretion also ceased. Large supplies of milk would be then be available upon reunion as previously described, because the milk produced from previous suckling experience built up in the dam's mammary glands. However, once depleted, there would be a considerable delay until more milk was available as the new suckling stimuli must begin anew to stimulate prolactin release to stimulate milk release. Therefore after separation upon reunion, as seen in the literature, we have found an increase in the short term supply of milk to pups and a decrease in the long term supply of milk.

The ability of separated pups to increase their own intake was not detected until after the 2 hour reunion. During the first two hours of reunion there were no significant differences seen between pup conditions. It is possible that the separated dam's milk build up and the resulting increased intake by her pups masked any differences between separated and nonseparated pups. This is consistent with Friedman (1975), who has shown that the milk build up in the separated dam increased the intake of pups upon reunion in the first 90 minutes, regardless of their deprivation experience.

Over the next 22 hours, after the milk build up had been released, differences between separated and nonseparated pups appeared: separated pups gained more weight than nonseparated pups when with a nonseparated dam, and lost less weight when with a separated dam. Separated pups with a separated dam did not gain as much as separated pups with a nonseparated dam, thus the decrease in milk supply in the separated dam must be a limiting factor.

In addition to the supply of milk available to the pups, another possible contributing factor to the differences in weight gain could be an alteration in the metabolic processes of the pups due to separation. However, a minor pilot study demonstrated that the weight loss due to metabolic processes alone are minimal and do not differ between separated and nonseparated pups. Half of a 10 to 12 day old litter of pups (n=5) were taken from their dam, stimulated to urinate and defecate and then their AG area was sealed with collodian to prevent further elimination of either urine or feces. Weights were then taken to the nearest .01 gram before and after a two hour period during which time the pups were in their home cage without their dam on a heating pad. The next day, an identical procedure was performed on the other half of the litter (n=5) which had been previously separated for 18 hours on a heating pad.

Both conditions of pups lost less than 1% of their body weight over this 2 hour period, and there was no difference between the separated and nonseparated conditions in amount lost. Therefore separation did not appear to alter rate of weight loss, nor do metabolic processes acount for much weight change in comparison to milk consumption or elimination.

There were no changes in weight gain variability as were predicted from the research of Brake et al. (1988). They had noted that pups responded differently to the stress of separation making some better feeders than others, as measured by the rate and amplitude of sucking. However, there were no differences seen in weight gain variability between separated and nonseparated pups over 2 hours, or over 24.

Temperature

Prior to reunion, pups of the Ss group were cooler than pups of the Nn group ($t(6)=4.814, p<.004$), as measured by their axillary temperatures. During the 2 hour reunion period, pups of both groups became warmer, but the Ss pups had a greater increase in temperature ($t(6)=3.146, p<.025$) resulting in no differences between these groups in pup temperature at the end of the 2 hour reunion. When all four groups were examined, the axillary temperatures of the separated pups were significantly cooler than nonseparated pups prior to reunion (Pup effect only, pre: $F(1,12)=23.865$, $p<.001$)(see Figure 7). Thus the lower pre-reunion pup temperatures of the Ss group were due to the effect of separation on the pups, representing a Model 2 separation effect. It is important to note that during the separation period, separated pups were in a cage with shavings and a nest area, on a heating pad thermoregulated to maintain nest temperature. This was the standard laboratory procedure and we can confidently assume that the pups were of nest temperature during this time. However, their temperature was not taken immediately after being in this situation, but rather was taken after all pups of all groups were stimulated to urinate and defecate, and weighed, and placed back into their home cage in the test room. During these manipulations, we attempted to maintain the temperature of pups of all groups by keeping them on the heating pad at all times, or manipulating them off the heating pad for the shortest time possible. We also attempted to stimulate the nonseparated pups for a comparable amount of time although their bladders were not as full. Why pups who had been separated from their dams for 18 hours were cooler upon reunion despite their being maintained at nest temperature during separation and manipulated as much as nonseparated pups prior to reunion will be discussed below.

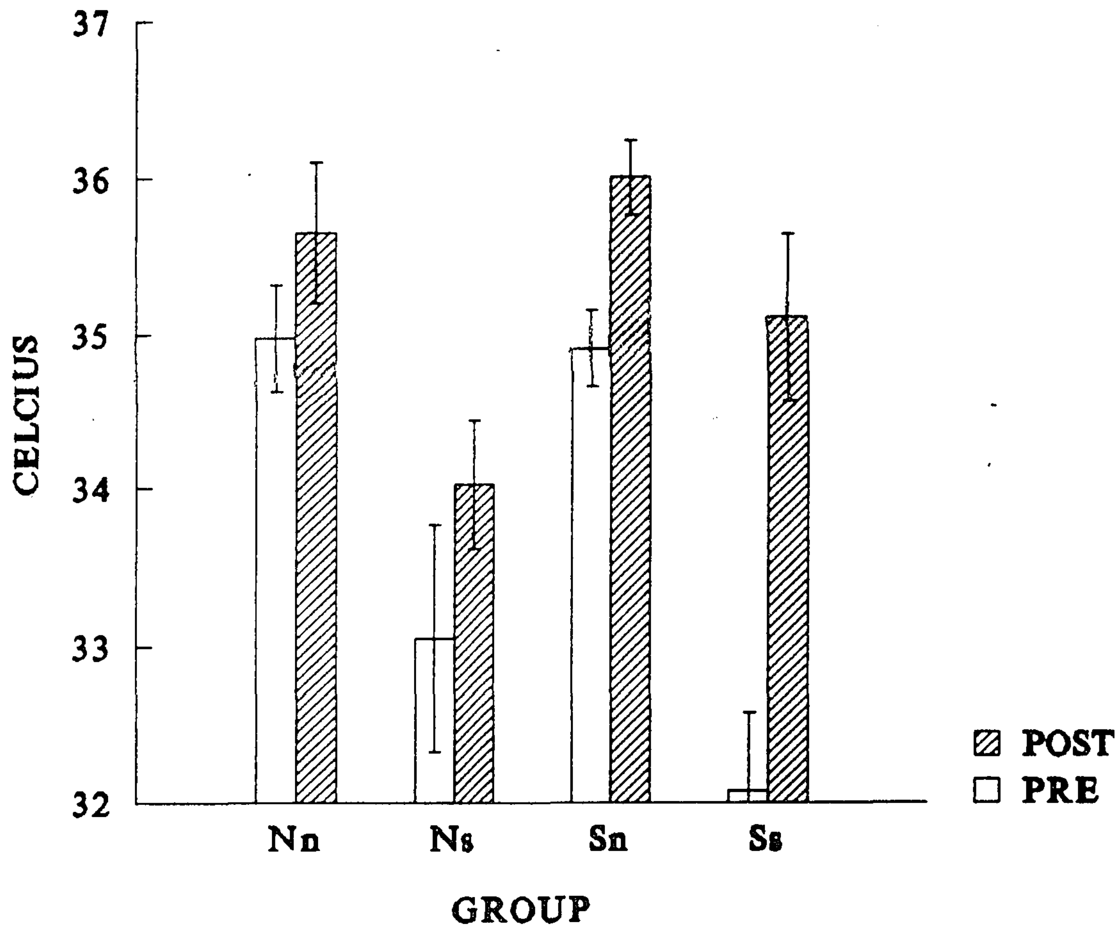
Figure 7: The mean pup axillary temperatures for 4 litters per group prior to reunion, after 2 hours of reunion, and the change in temperature over the 2 hours of reunion.

There was a significant difference between the Nn and Ss groups prior to reunion (t-test, $p<.004$) which can be explained by the pup effect seen by the 2 way ANOVA. Pup axillary temperature prior to reunion represents a Model 2 of separation effects.

There was no significant difference between the Nn and Ss groups after reunion. The 2 way ANOVA found a pup effect, therefore the pup axillary after reunion represents a Model 2 of separation effects.

There was a significant difference between the Nn and Ss groups in change of temperature over the 2 hour reunion period (t-test, $p<.021$) which can be explained by the dam effect and pup trend seen by the 2 way ANOVA. The change in pup temperature over the 2 hour reunion period represents a Model 4 of separation effects.

PUP TEMPERATURE



Even though no difference was found between the axillary temperatures of the pups in the Nn and Ss groups after the 2 hour reunion period, when the Ns and Sn groups were included in the analysis, separated pups were found to be significantly cooler after the 2 hour reunion period (Pup effect, post: $\underline{F}(1,12)=8.817$, $p<.013$). This effect of separation is represented in Model 2. Pups of the Ns group were cooler than pups of the Nn and Sn group, and tended to be cooler than pups of the Ss group (post hoc Ns vs Nn, $\underline{F}(1,12)=7.303, p<.020$; Ns vs Sn, $\underline{F}(1,12)=10.788, p<.008$; Ns vs Ss, $\underline{F}(1,12)=3.196, p<.100$) contributing to this effect.

The increase in temperature over the two hours was significantly greater in pups with separated dams (Dam effect: $\underline{F}(1,12)=5.257, p<.042$), and there was a trend that the increase in temperature was greater in separated pups (Pup trend: $\underline{F}(1,12)4.249$, $p<.063$). Therefore, the greatest increase in temperature in the Ss group was due to the separation effects in both the dam and the pups. This data supports a Model 4 of separation effects, in that in the uniformly separated condition (Ss), there was an additive effect of the separated dam and separated pup conditions.

Summary and Discussion

Although during separation, the separated pups were kept in a cage with shavings and a nest area and warmed on heating pads, they were cooler upon reunion. We can assume that they became cooler, not during the separation period, but during the manipulation of stimulating and weighing the pups after separation prior to reunion. The fact that this procedure affected the separated pups' temperature more than the nonseparated pups' was probably due to a few things. First, it has been stated that without the periodic ingestion of warm milk which reaches the core of the body, pups do not maintain normal temperatures (Leon, 1979). And second, when deprived of their dam's milk and warm ventrum, pups of this age have no capacity to respond to a cold challenge (Stone, Bonnet, and Hofer, 1976). There is evidence that cold induced thermogenesis is massively inhibited by the central nervous system of the maternally deprived pup, and that this suppression of metabolism actually prolongs the pup's ability to survive periods of deprivation at low temperatures (Bignall, Heggeness, and Palmer, 1974; 1977). Therefore, while being stimulated and weighed, the separated pups could not increase their metabolism to combat the cold challenge and their body temperature dropped quickly. Another way in which these pups probably lost temperature was in evaporative heat loss; as they became slightly wet from urine during AG stimulation, the evaporation of this moisture would cool them down. The evaporative heat loss would be likely to affect the separated pups more than the nonseparated pups as they had considerably more urine to eliminate, and thus potentially more moisture to evaporate. Although it would have been of interest to confirm that their body temperatures were normal prior to manipulation, having an accurate record of their body temperatures immediately prior to the reunion was critical to determine if the pup temperature affected the behaviors that followed. It also must be mentioned that the core temperature of the pups would have been a more accurate measure of their true temperature. However, the taking of a core temperature has been shown to activate or stress animals and raise their temperature temporarily and this was necessary to avoid due to the behavioral observations being done. Therefore, the cooler temperatures of the separated pups were measured by inserting a probe in the pups' axilla with minimized handling at the cost of losing accuracy in the measure.

Separated pups tended to warm up more over the 2 hour period, but, in accordance with the Law of Initial Values, they also had a lower starting point and therefore had the opportunity to show a greater increase in temperature over time. The Ss condition was the optimal condition for an increase in temperature, due to this tendency in separated pups toward a greater increase, and also, the presence of a greater increase in pups with separated dams. Separated pups with a nonseparated dam do not increase as much as those in the Ss condition as shown in Figure 7, and this cannot be due to a ceiling effect because the other pups increased much more. Perhaps separated dams are maintaining contact longer thereby raising the pups' temperatures.

The temperature of the pups has been shown to affect amount of contact with the dam (Leon, Croskerry and Smith, 1978) and latency to attach (Henning, Chang and Gisel, 1979). An ANCOVA was utilized to determine the effects of temperature on these measures as well as on others. This analysis determined if differences between conditions existed when differences in pup temperature were accounted for, and if there was a relationship within the groups between the measure and the pup temperature. These results will be presented in the various sections dealing with the specific measure tested.

The Interaction Upon Reunion

Analysis of the dam-pup interaction was done over different time periods. The reunion was observed for two hours because we thought this time period was likely to contain the first nursing bout of all groups. The total two hour observation period was analyzed for complete quantification of behaviors that apply directly to the two hour weight change. Then the first nursing bout was analyzed because this was a complete natural unit of interaction that can be seen in all litters of each condition. The nursing bout was the period during which we hypothesized the effects of separation upon reunion would be most apparent because effects might dissapate over time.

A. THE FIRST 2 HOURS OF REUNION

Analysis of the first 2 hours of reunion included the time the litter spent nursing, the number and length of nursing bouts, and ME information. One might predict that incidence of increased weight gain of pups with a separated dam over two hours was produced by nursing longer, more frequent nursing bouts, and/or by having more milk ejections (MEs).

1. Time Spent Nursing

The time spent nursing was calculated by the time the pups were attached. There was no difference between the Nn and Ss groups in amount of time spent full nursing, which is when more than half the litter was attached. However, analysis across all four groups resulted in a significant pup effect: separated pups spent more time full nursing than the nonseparated pups over 2 hours ($\underline{F}(1,20)=7.25, p<.015$) (see Figure 8). These results are an example of a Model 2, however, the effect was contributed to solely by the disparate groups (Ns and Sn), as there was no difference between the like-condition groups (Nn and Ss). In fact, it appears that the pup effect was mainly created by the increase in full nursing in the Ns group. Perhaps the separated pups have an increased motivation to nurse which the nonseparated dam tolerates.

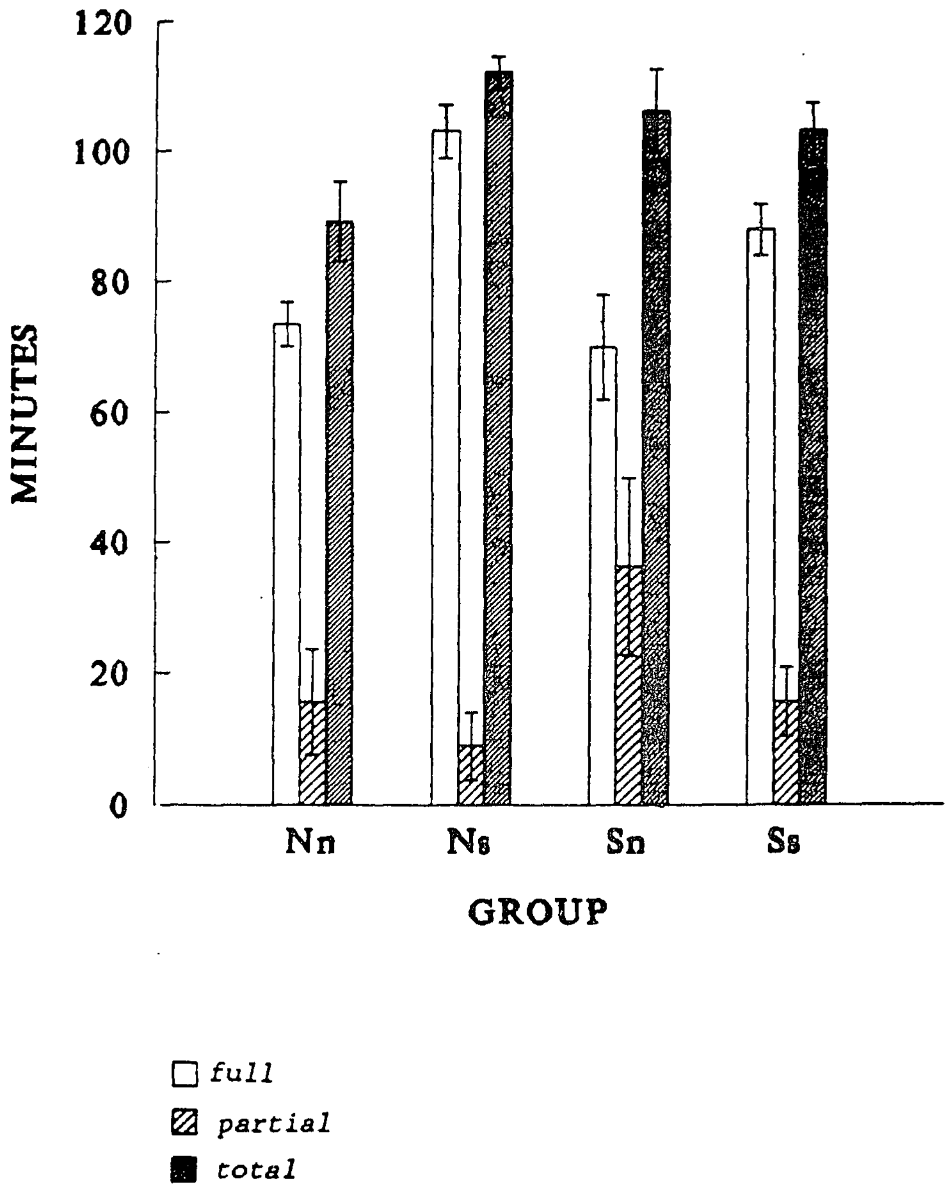
<u>Figure 8</u>: The mean time of the 6 litters per group spent full nursing, partial nursing, or full and partial nursing combined.

There was no significant difference between the Nn and Ss groups in time spent full nursing. The 2 way ANOVA found a pup effect. These results fit a Model 2 of separation effects. (Note: without litter Q, analysis found a pup effect and interaction trend, and the results still fit a Model 2).

There was no significant difference between the Nn and Ss groups in time spent partial nursing. The 2 way ANOVA found both a dam and pup effect. These results fit a Model 5 of separation effects.

There was a trend for the Nn and Ss groups to be different (t-test, $p<.088$) in total time spent nursing. The 2 way ANOVA found an interaction effect and a pup trend. These results fit a Model 6 of separation effects, the Nn group being unique. (Note: without litter Q analysis found only an interaction effect, the results now best fit a Model 3).

NURSING OVER 2 HOURS



The Sn group had a litter, labeled Q, in which full nursing occurred for only 9 consecutive minutes over the entire 2 hour period. Because other behavioral measures were also quite different in this litter from all other litters of all groups, analysis was done on all measures with and without litter Q to determine its effect on the statistical results. For all the following analyses this outlier litter will be included, and results that differ without it will be noted. This litter will be discussed in detail later.

When analysis of full nursing excluded litter Q, the pup effect was still present (Pup effect: $\underline{F}(1,19)=7.009, p<.017$), however, now there was also a trend for the disparate groups to full nurse more than the similar-condition groups (Interaction trend: $\underline{F}(1,19)=3.374, p<.083$). This was due to the increase in the mean of full nursing in the Sn group from 69.83 minutes (SEM=13.65) to 82.00 minutes (7.58). When separation effects include an interaction trend and a significant pup effect, Model 6 or 7 might be represented, however this was not the case. There was no one group which was different from the others contributing to these effects. Therefore, the data of full nursing without litter Q also best fit the Model 2 of separation effects. The trend for disparate groups to full nurse more was the result of the high Ns rate of this behavior. The Sn rate, although increased without

Q was still not higher than the Ss rate.

Partial nursing time, when less than half the litter was attached, was not significantly different between the Nn and Ss groups. However, analysis across all four groups revealed that separated dams spent more time partial nursing their pups (Dam effect: $\underline{F}(1,20)=6.905, p<.017$) and separated pups spent less time partial nursing with their dams (Pup effect: $\underline{F}(1,20)=6.905, p<.017$) (see Figure 8). As in Model 5, when combined in the Ss group, these effects balance each other out and result in no difference in time spent partial nursing from the Nn group. The dam effect was created by the increased time spent partial nursing seen in the Sn group, which could be due to the separated dam's attempt to nurse while the nonseparated pups are not interested. And the decrease in time spent partial nursing and increase in time spent full nursing in the Ns group suggests that if separated pups have the opportunity to nurse they do so. Results of analysis were similar when litter Q was excluded.

To take into account the differences between groups in time available for partial nursing to occur due to time spent full nursing, the <u>rate</u> of partial nursing time was also computed (time available/time spent partial nursing). As before, there was no difference between the Nn and Ss groups in rate of partial nursing. Analysis across all four groups revealed that separated dams tended to have higher rates of partial nursing (Dam effect:

$\underline{F}(1,20)=3.262, p<.090)$, and a significant interaction between the groups existed (Interaction effect: $\underline{F}(1,20)=4.358, p<.051)$. Both the dam trend and interaction effect were contributed to by a high rate of partial nursing in the Sn group. As was stated in the previous paragraph describing the time spent partial nursing, perhaps the increased rate of partial nursing in the Sn group was due to the separated dam's attempt to nurse while the nonseparated pups were not interested.

When both forms of nursing were combined, also shown in Figure 8, there was a trend for the Ss group to nurse more than the Nn group ($t(10)=-1.850, p<.095$). When analysis was done across all four groups, there was a trend for separated pups to nurse more (Pup trend: $\underline{F}(1,20)=3.768, p<.067$) and there was a significant interaction (Interaction effect: $\underline{F}(1,20)=6.59, p<.019$). These effects were contributed to by the Nn group which spends less time nursing than the Ns and Sn groups, and tends to spend less than the Ss group (post-hoc Nn vs. Ns, $\underline{F}(1,20)=10.157, p<.006$; Nn vs. Sn, $\underline{F}(1,20)=5.630, p<.029$; Nn vs. Ss, $\underline{F}(1,20)=3.728, p<.069$). This is an example of Model 6 ANOVA results, as there is a unique interaction in the similar-condition group Nn which decreases time spent nursing. Or perhaps, separation increased nursing in the other groups, whether it was separation of the dam or pup, and tended to increase nursing when both the dam and pup were separated. It is interesting to note that the Ns group spends almost all of the 2 hours nursing.

When litter Q was excluded from analysis of total nursing, the trend for separated pups to nurse more disappeared, and the interaction effect remained (Interaction effect: $\underline{F}(1,19)=10.848, p<.005$). The Nn group was still significantly lower than the other groups (post-hoc, Nn vs. Ns: $\underline{F}(1,19)=12.493, p<.003$; Nn vs. Sn: ($\underline{F}(1,19)=10.384, p<.005$; Nn vs. Ss: $\underline{F}(1,19)=4.585, p<.046$), but the increase in total nursing in the Sn group created a greater contrast between the disparate and similar-condition groups. Now the results best fit a Model 3, the disparate groups nursing more over the two hour period than the similar condition groups.

Through an ANCOVA, using either the pup pre-reunion axillary temperature or the change in temperature over 2 hours as the covariate, the difference between groups in total nursing time was still found to exist when the means were adjusted by pup temperature (ANCOVA, treatment=groups, covariate=pups' pre-reunion axillary temperature, trend across groups: $\underline{F}(3,11)=3.239, p<.065$; treatment=groups, covariate=change in temperature over 2 hours, effect across groups: $\underline{F}(3,11)=3.567, p<.052$). No relationship was found within groups between temperature and time spent nursing. Therefore, the pups' temperature was not a critical determinant of the amount of time the litters spent nursing over the 2 hour period.

2. Number and Length of Nursing Bouts

The time the litters spent nursing can be broken down into the number and length of nursing bouts over the 2 hour period. A nursing bout was defined as being at least 10 minutes long and having breaks in attachment no longer than 3 minutes. This criterion was so that the first nursing bout of the reunion would be sure to include MEs. Although attachment periods of 3 to 9 minutes occurred in all conditions they were not counted as bouts, and were rarely observed to contain a ME.

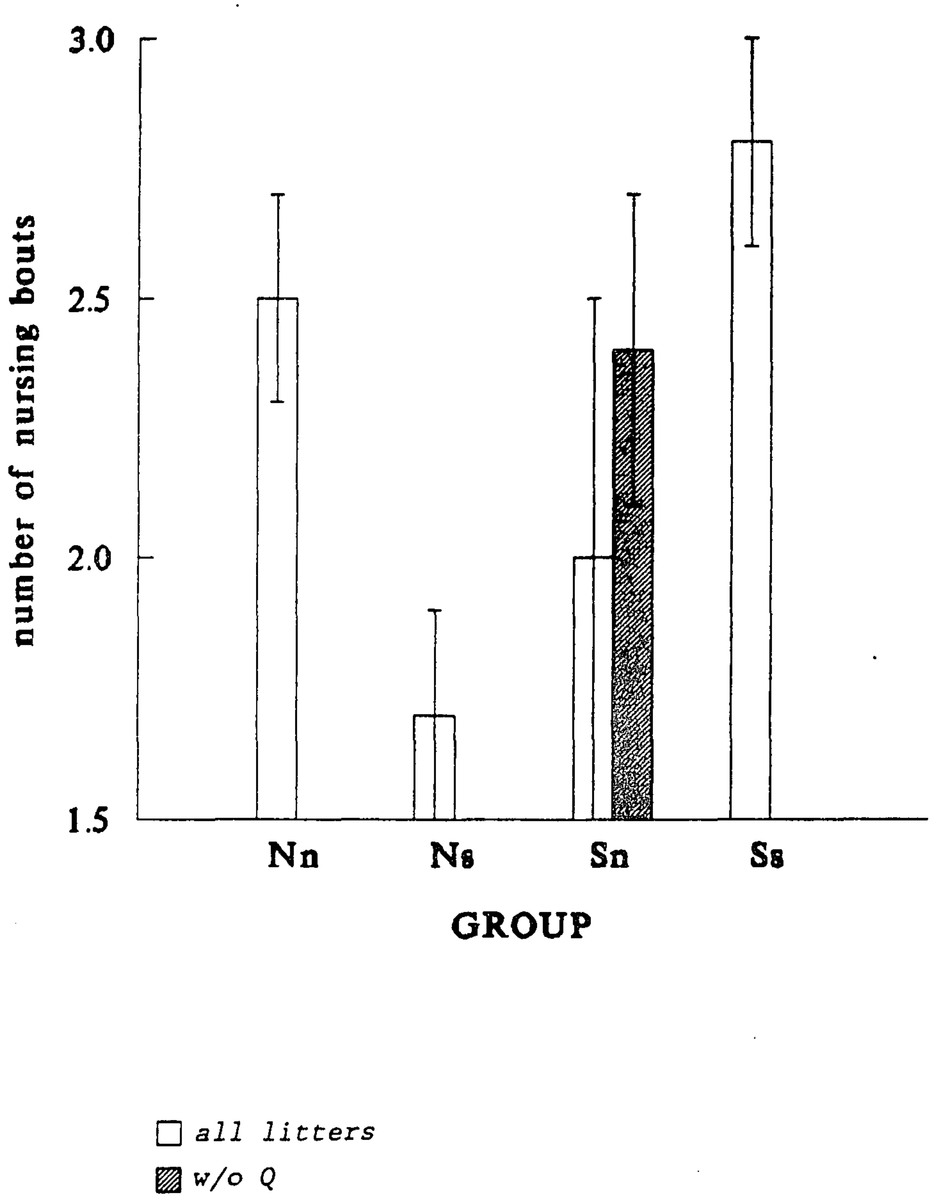
There was no significant difference between the Nn and Ss groups in the number of nursing bouts observed over the 2 hour period. These results are an example of Model 3, as there was an effect of separation effect on the interaction between the dam and pup conditions (Interaction effect: $\underline{F}(1,20)=8.621, p<.009$); the same-condition groups, Nn and Ss, had more nursing bouts over two hours than the disparate condition groups, Ns and Sn (see Figure 9). Therefore, although the Nn group spent the least time nursing, it did not decrease its number of nursing bouts.

As shown in Figure 9, analysis done without litter Q of the Sn group, which was never observed to have a nursing bout, found that litters with a separated dam had more nursing bouts (Dam effect: $\underline{F}(1,19)=6.346, p<.022$), and the

Figure 9: The mean number of nursing bouts of the 6 litters per group over the 2 hour period. There was no difference between the Nn and Ss groups in mean number of nursing bouts over the 2 hour period. The 2 way ANOVA across all groups found an interaction effect. These results fit a Model 3 of separation effects.

When litter Q of the Sn group was excluded from analysis, the 2 way ANOVA across all groups found a dam effect and interaction effect. These results fit a Model 7 of separation effects, the Ns group being unique.

NUMBER OF NURSING BOUTS

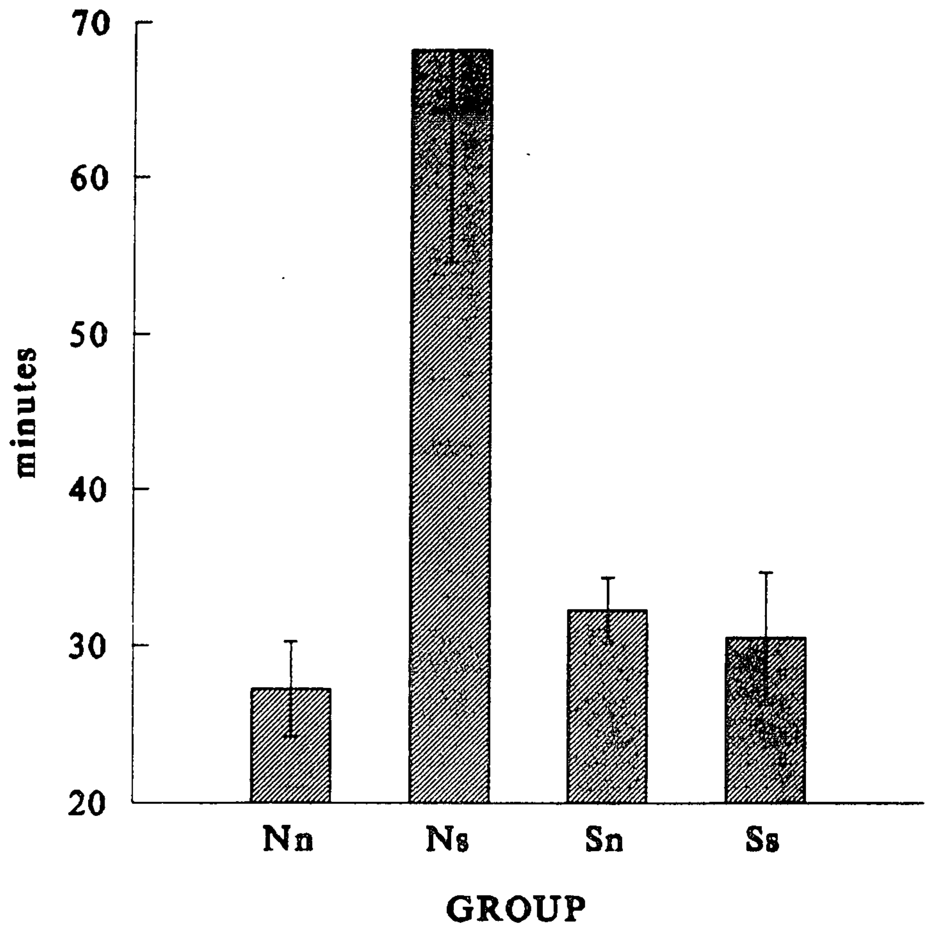


Ss and Nn groups had more nursing bouts over the first 2 hours of reunion than the disparate groups (Interaction effect: $\underline{F}(1,19)=8.948, p<.009$). This latter analysis represents Model 7 ANOVA results, in which a disparate condition, in this example the Ns group, expressed changes due to the experience of separation. The unique combination of a nonseparated dam with separated pups resulted in fewer nursing bouts over the 2 hour period than all other groups (post-hoc Ns vs. Nn, $\underline{F}(1,19)=8.134, p<.011$; Ns vs. Sn, $\underline{F}(1,19)=5.726, p<.028$; Ns vs. Ss, $\underline{F}(1,19)=15.942, p<.002$). Therefore, although the Ns group spent almost the entire two hour period nursing, it did not increase the number of nursing bout in order to do so.

As shown in Figure 10, there was no difference between the Nn and Ss groups in the mean length of nursing bouts over the 2 hour period. However, analysis across all four groups revealed that nonseparated dams had longer nursing bouts (Dam effect: $\underline{F}(1,19)=4.499, p<.048$) and separated pups had longer nursing bouts (Pup effect: $\underline{F}(1,19)=6.482, p<.021$) and a significant interaction between dam and pup condition also existed (Interaction effect: $\underline{F}(1,19)=7.678, p<.013$). These results represent a Model 7, as the very high value for the Ns group was responsible for all of these significant effects; the Ns group had significantly longer nursing bouts than all other groups

Figure 10: The mean length of nursing bouts of the 6 litters per group over the 2 hour period. There was no difference between the Nn and Ss groups. The 2 way ANOVA found a dam effect, pup effect and an interaction effect. These results fit a Model 7 of separation effects, the Ns group being unique.

MEAN LENGTH OF NURSING BOUTS



(post-hoc Ns vs. Nn, $\underline{F}(1,19)=14.842, p<.002$, Ns vs. Sn, $\underline{F}(1,19)=10.396, p<.005$, Ns vs. Ss, $\underline{F}(1,19)=12.565, p<.003$). Therefore, the length of nursing bouts varied across the groups with the Nn, Ss, and Sn groups having a higher frequency of shorter nursing bouts. In the Ns group, the relationship between the dams and pups included less frequent, longer nursing bouts. In fact it appears that separated pups with a nonseparated dam tend to nurse continuously, rather than in separate bouts.

3. Number of MEs Over 2 Hours

There was no significant difference between the Nn and Ss groups in the number of definite MEs over the first two hours of reunion. However, when analysis was done over all four groups, separated dams were shown to deliver fewer definite MEs than nonseparated dams (Dam effect: $\underline{F}(1,20)=6.22, p<.023$) (see Figure 11). These results are an example of Model 1 separation effects, as the changes seen due to separation are the result of alterations in the dam condition. Since there was no difference between the Nn and Ss groups, the dam effect must be contributed to by the disparate groups, specifically the low number of definite MEs seen in the Sn group.

The Ss group delivered more possible MEs than the Nn group over the 2 hour period ($t(10)=-3.894, p<.007$). Analysis across all four groups revealed that this was a result of separated dams delivering more possible MEs (Dam effect: $\underline{F}(1,20)=8.95, p<.008$), as well as a tendency toward separated pups to elicit more possible MEs (Pup trend: $\underline{F}(1,20)=3.529, p<.076$). These effects are an example of Model 4 ANOVA results, as they are clearly additive. As can be seen in Figure 11, there was a clear progression of number of possible MEs from the Nn, Ns, Sn groups to the Ss group, the optimum condition being the Ss group which had the greatest number of possible MEs.

To determine the effect of these possible MEs on the weight gain of the pups, correlations of the number of possible MEs and the weight gain over two hours were done within groups and across groups. Although there were no significant correlations within any of the groups, a positive relationship was found when all the groups were combined (Pearson $r=.640(24), p<.01$).

When analysis of the number of possible MEs excluded litter Q, the trend for separated pups to elicit more possible MEs disappeared, but separated dams still delivered significantly more possible MEs ($\underline{F}(1,19)=11.262, p<.004$). The results fit a Model 1 of separation effects, representing an increase in the number of possible MEs over the 2 hour period, due to alterations in the dam condition only. The mean of possible MEs in the Sn group was increased from 4.00 (SEM=1.48) to 4.80 (1.53), Therefore, the increase in number of possible MEs elicited by separated

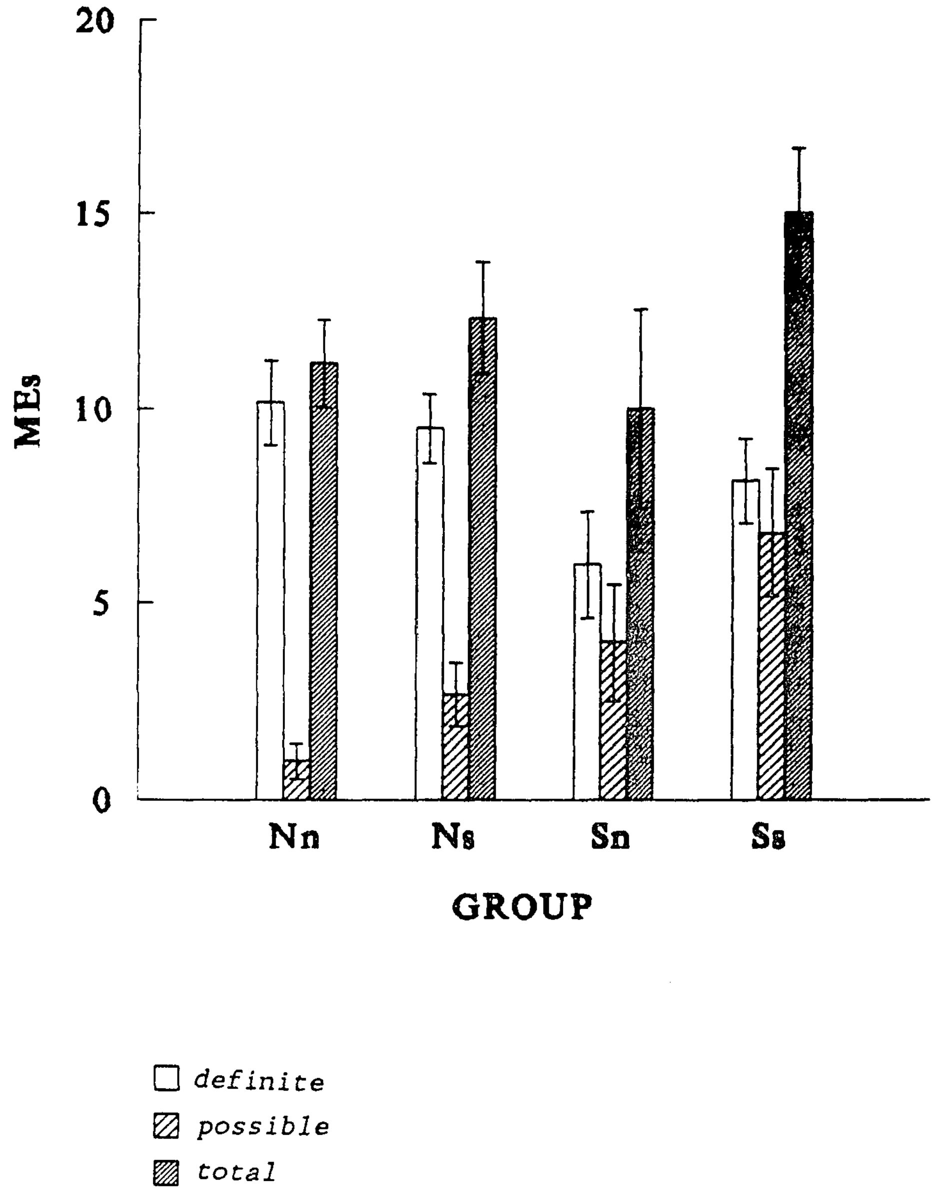
Figure 11: The mean number of MEs of the 6 litters per group over the 2 hour reunion period, including definite MEs, possible MEs, and definite and possible MEs combined.

There was no significant difference between the Nn and Ss groups in number of definite MEs during the 2 hour period. The 2 way ANOVA found a dam effect. These results fit a Model 1 of separation effects.

There was a significant difference between the Nn and Ss groups in number of possible MEs over the 2 hour period (t-test, $p<.007$). The 2 way ANOVA found a significant dam effect, and pup trend. These results fit a Model 4 of separation effects. (Note: without litter Q, analysis found only a dam effect. These results best fit a Model 1 of separation effects).

There was a trend for the Nn and Ss groups to be different in the total number of MEs over the two hour period (t-test, $p<.088$). The 2 way ANOVA found a pup trend. These results fit a Model 2 of separation effects. (Note: without litter Q, analysis found no effects).

NUMBER OF MEs OVER 2 HOURS



pups seen earlier in the analysis was the result of the depressed rate in the Sn group caused by litter Q.

As shown in Figure 11, when definite and possible MEs were combined, there was a trend for the Ss group to have had more MEs than the Nn group ($t(10)=-1.894, p<.088$). The ANOVA across all four groups revealed that this was a result of a trend for separated pups to elicit more MEs than nonseparated pups ($\underline{F}(1,10)=3.033, p<.098$). Although it is just a trend for there to be more MEs due to the alteration in pup condition postseparation, this is an example of Model 2 separation effects. When analysis excluded litter Q, the trend for a pup effect was lost, and no effects of separation were found on the number of MEs over the two hour period. Therefore, the trend for separated pups to elicit more MEs over 2 hours was contributed to by the decreased number of MEs in the Sn group when litter Q was included. This decreases the importance of the pup trend seen in the earlier analysis.

An ANCOVA using pup pre-reunion axillary temperatures as the covariate was unable to confirm if there was a difference groups in total number of MEs when temperature was accounted for, because the data failed the assumption often called the "homogeneity of slopes" assumption. In other words the data could not be analyzed using the ANCOVA model because there was a significant interaction between the two variables, and the regression line of the dependent variable onto the covariate was not the same in each of the four groups.

4. ME Rate

A. ME rate over the first two hours of reunion

To determine if separation affected the number of MEs per time spent nursing, the rate of MEs was calculated by dividing the total number of MEs by the time spent full nursing. (Note: there were only five MEs, out of 291 total from all litters of all groups, that occurred during partial nursing and these 5 MEs were eliminated from the calculations). There were no significant differences in ME rate between the Nn and Ss groups, and analysis across all four groups revealed that there were no separation effects on ME rate due to dam or pup condition. When pup pre-reunion axillary temperature and change in temperature over 2 hours were used as covariates in an ANCOVA, no significant differences were seen between groups, nor was there a relationship between ME rate and pup temperature. Therefore pup temperature was not related to the rate of milk delivery seen over the full two hour period.

B. ME rate of hour 1 vs. hour 2 within groups

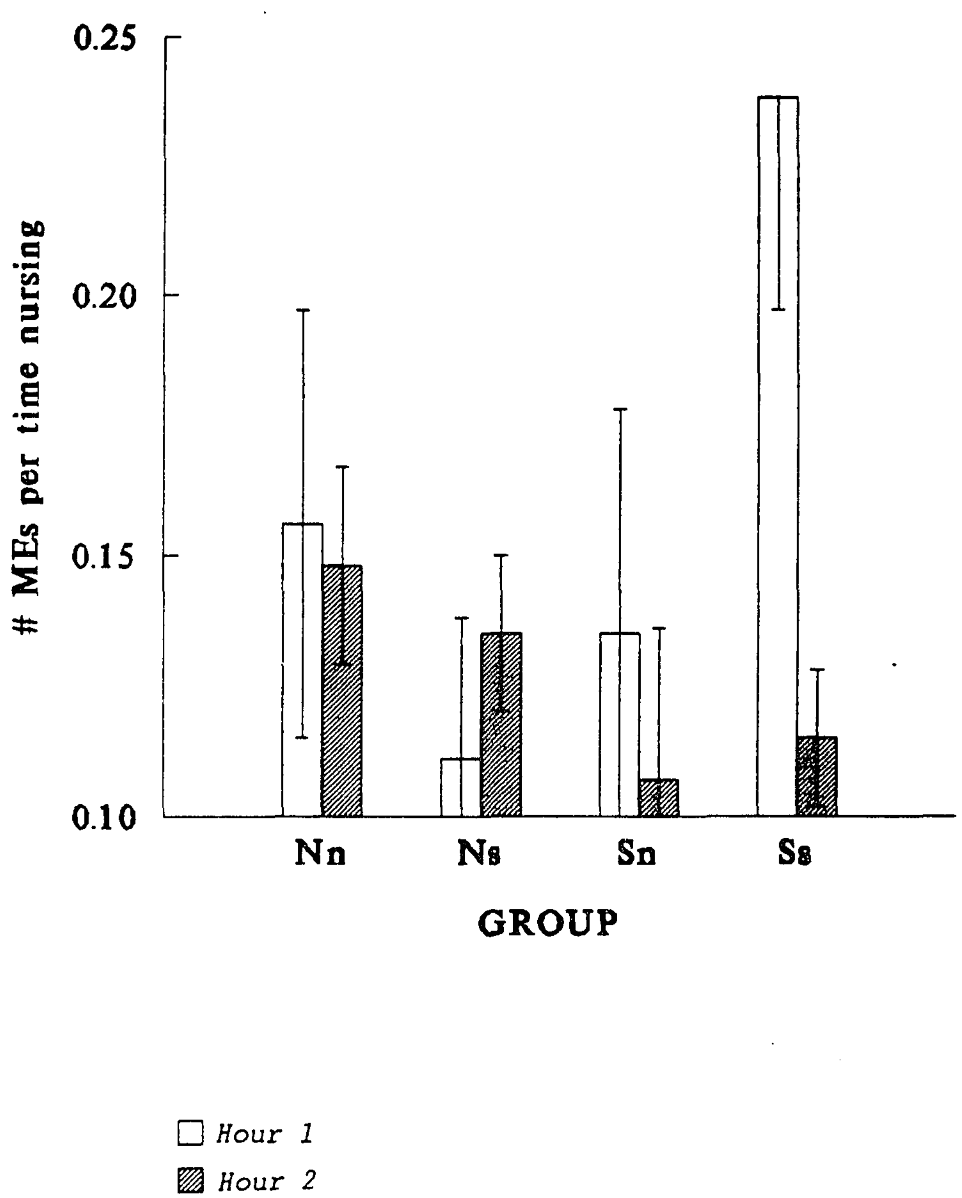
Although there were no effects of separation seen on the rate of ME delivery over the 2 hour period, it is possible that there was a difference in rate of MEs over time. From the weight gain data previously reported, in addition to the literature, it is believed that initially upon reunion the milk supply of the separated dam is greater than that of the nonseparated dam, but that it then decreases as nursing progresses. The proposed hypothesis is that this decrease in milk supply would be reflected in a decreasing ME rate, while the nonseparated dam's milk let down would be more consistent over time. To determine if this was true, the ME rates of hour 1 and hour 2 were compared within groups.

The Ss group was as predicted - there was a decrease in ME rate over time (dep.t(5)=3.02, p<.030) (see Figure 12). This could be reflecting a decrease in milk supply, supporting the above mentioned hypothesis. It is also possible that the ME rate decreased as the pups became satiated, but this is inconsistent with the 24 hour weight loss of pups with separated dams; it seems unlikely that pups would be so satiated from the first two hours of nursing showing a decrease in ME rate, that they would lose weight over the next 22 hours.

As shown in Figure 12, there were no significant differences over time in ME rate in the Nn, Ns, or Sn group. Therefore we can conclude that these groups, and therefore the nonseparated dams, appear to have had a uniform pattern of ME delivery over 2 hours, although it must be emphasized that we know nothing about the amount of milk released in

Figure 12: The ME rate (total number of MEs/ time spent full nursing) of the 6 litters per group in hour 1 and hour 2. There was no significant difference between the Ss and Nn group in their ME rate during hour 1 or hour 2. Only the Ss group was significantly different between hour 1 and hour 2 (t-test, $p<.030$).

ME RATE, HOUR 1 & HOUR 2



each ME. The Sn group showed a decrease in ME rate from hour 1 to hour 2, which is consistent with the above hypothesis, however this was not significant.

The change over time of the ME rate in the Ss group appears to be due to their elevated rate of ME delivery during hour 1. The Ss group's rate of ME delivery during hour 1 was not significantly higher than the Nn group, although was higher than the Ns group (post hoc, $\underline{F}(1,20)=5.435, p<.031$) and tended to be higher than the Sn group (post hoc, $\underline{F}(1,20)=3.552, p<.075$). Because the Sn group was not also elevated in hour 1, the Ss group must have a tendency toward a higher ME rate during the first hour of reunion not only due to milk build up during the separation period, but also due to the separated pups' nursing behavior. When types of MEs and time spent nursing were compared separately between hour 1 and hour 2 in the Ss group, the decrease in ME rate was found to be a result of a significant decrease in the number of possible MEs (dep.$t(5)=3.57, p<.017$), as there were no changes in the number of definite MEs and time spent nursing over time.

Summary and Discussion

The critical components of the mother-infant interaction include the dam's provision of warmth and nutrients to the pups, and her ability to protect the litter from possible predators. The latter component was not observed in this study, however, the dam's provision of warmth and nutrients were. As noted in previous sections, the pups of the Ss group, in comparison to the Nn group, gained more weight and increased their temperature more over the first two hours of reunion. The increased weight gain was the result of alterations in the dam's condition due to separation, and the increase in temperature was the result of alterations in both the dam's and pup's condition. It appears that the dams and pups within the mother-infant interaction were attempting to compensate for the deprivation period.

Analysis of the initial two hours of reunion revealed that there were few differences between the interactions of the Ss and Nn groups which could possibly explain how the differences in weight and temperature measures were achieved (see Table 1 for a list of all results of Experiment 1, including the 2 hour analysis). The amount of time the litters spent nursing would be an obvious means to achieve these goals. Data from Grota and Ader (1969) suggests that litters of this age spend approximately 40 to 50% of their time together and that the dams spend more time nursing their young during the day. Woodside and Jans (1988) found that during the day, the percent of time spent nursing on postnatal day 12 averages around 70%. Our percentages of total nursing time ranged from 74% to 93%, which could be considered average to high but the literature cited to which

TABLE 1
Summary of Experiment 1 Results

Weight			
2 hour weight change:	Ss>Nn	Mod 1:	Dam effect, SD>ND
Hour 2 to 24:	Nn>Ss	Mod 5:	Dam effect, ND>SD Pup effect, sp>np
Hour 0 to 24:	Nn>Ss	Mod 5:	Dam effect, ND>SD Pup effect, sp>np
Coeff. of variation:	Nn=Ss	No effects	
Temperature			
pre-reunion:	Ss<Nn	Mod 2:	Pup effect, sp<np
post-reunion:	Ss=Nn	Mod 2:	Pup effect, sp<np -Ns coolest
change in temp.:	Ss>Nn	Mod 4:	Dam effect, SD>ND Pup effect, sp>np -Ss greatest
2 Hour Reunion			
Nursing			
full nursing:	Nn=Ss	Mod 2:	Pup effect, sp>np -contributed to by Ns
w/oQ		Mod 2:	Pup effect, sp>np Interaction trend -no unique group
partial nursing:	Nn=Ss	Mod 5:	Dam effect, SD>ND Pup effect, sp<np
total nursing:	trend:Ss>Nn	Mod 6:	Pup trend, sp<np Interaction effect -Nn the lowest
w/oQ		Mod 3:	Interaction effect, Ns, Sn>Ss, Nn -Nn the lowest

ANCOVA: -total nursing: difference between groups still there when temp (pre or delta) taken into account, and no within group relationship.

<u>Number and Length of NBs</u>

number of bouts:		Nn=Ss	Mod 3:	Interaction effect, Ns, Sn<Nn, Ss
	w/oQ		Mod 7:	Interaction effect Dam effect -Ns is the lowest
length of bouts:		Nn=Ss	Mod 7:	Dam effect Pup effect Interaction effect -Ns greatest
number of MEs:				
definite		Nn=Ss	Mod 1:	Dam effect, SD<ND
possible		Ss>Nn	Mod 4:	Dam effect, SD>ND Pup trend, sp>np
	w/oQ		Mod 1:	Dam effect, SD>ND
total	trend	Ss>Nn	Mod 2:	Pup trend, sp>np
	w/oQ			No effects
rate of MEs:		Nn=Ss		No effects
hour 1-hour2				Ss decreased over time

Litter Q (Sn group outlier)
- never had a nursing bout
- full nursing 9 consec. minutes only
- never observed to have had a ME
- 8/9 pups gained-although very little

<u>The First Nursing Bout</u>

Latency to Attach		
initial att.	Nn=Ss	No effects
stable att.	Nn=Ss	No effects
solid att.	Nn=Ss	No effects

ANCOVA: -lat to solid att.: still no effects across groups when temp. accounted for, w/in group trend: cooler pups attach slower.

Length of 1st Bout	Nn=Ss	Mod 3: Interaction effect Ns, Sn>Nn, Ss -Ns had 2 litters with long bouts

ANCOVA: -length of first bout: group effects still present when temp. accounted for, w/in group effect: warmer pups have longer bouts.

Disruptions	Ss>Nn	Mod 2: Pup effect, sp>np
Latency to 1st ME		
sol.att to def ME	Nn<Ss	Mod 1: Dam trend, ND<SD
sol.att to 1st ME	Nn=Ss	No effects
reunion to def ME	trend Nn<Ss	No effects
reunion to 1st ME	Nn=Ss	No effects

ANCOVA- lat to 1st ME: no group difference when temp taken into account, no w/in group relationship.

Number of MEs		
definite	Nn=Ss	No effects
possible	Ss>Nn	Mod 4: Dam effect, SD>ND Pup trend, sp>np
total	trend Ss>Nn	No effects
Inter-ME-intervals		
with disr.	Nn=Ss	Mod 3: Interaction trend Ns, Sn>Nn, Ss
w/o disr.	Nn=Ss	Mod 3: Interaction trend Ns, Sn>Nn, Ss

ANCOVA: -IMEI: difference across groups is lost when temp. taken into account, no w/in group relationship.

Behavior
Prior to the First Nursing Bout

Dam Behavior

Pup Directed Behavior:	Nn=Ss	No effects	
Body licking	Ss>Nn	Mod 1:	Dam effect, SD>ND
Other Dir. Behavior:	Nn>Ss	Mod 1:	Dam effect, ND>SD -Nn highest
w/oQ		Mod 1:	Dam trend, ND>SD
change postion	Nn=Ss	Mod 6:	Pup trend Interaction effect -Nn highest,even of Ss in post-hoc
Quiet:	Nn=Ss	Mod 3:	Interaction effect Ns, Sn>Nn, Ss
w/oQ		Mod 3:	Interaction trend

Pup Behavior

Dam Directed Behavior:	Nn=Ss	No effects	
Change position in contact		Mod 1:	Dam effect, SD>ND
Other Directed Beh.:	Ss<Nn	Mod 2:	Pup effect, sp<np
Quiet:	Ss=Nn	No effects	
Contact of litter:	Ss>Nn	Mod 4:	Dam trend, SD>ND Pup trend, sp>np
Attach. of litter:	Ss>Nn	Mod 2:	Pup effect,sp>np

Transitions

between dam behavior:	Nn=Ss	Mod 3:	Interaction effect Ns, Sn>Ss, Nn
between pup behavior:	Nn=Ss	No effect	

Behaviors During First Bout

Dam Behavior

Pup Directed Béhavior:	Nn=Ss	Mod 3-Best Fit Dam trend, SD>ND Interaction Nn, Ss>Ns, Sn
w/o possible MEs	Nn=Ss	Mod 1: Dam trend, SD>ND
Body lick:	Nn>Ss	Mod 6: Pup effect Interaction effect -Nn highest
Other Directed Behavior:	Nn=Ss	Mod 6: Pup effect Interaction effect -Ss highest
Change of position	Nn=Ss	Mod 6-Best Fit Pup trend Interaction trend -Ss highest (NS)
w/o possible MEs		Mod 6: Pup effect Interaction effect -Ss highest
Quiet:	trend Nn>Ss	Mod 6: Dam trend Interaction effect - Ss lowest
Arched Nursing:	trend Ss>Nn	Mod 6: Pup effect Interaction effect -Ss highest
w/o possible	Nn=Ss	No effects
On Top Nursing:	trend Ss>Nn	Mod 6: Dam trend Pup trend Interaction effect -Ss lowest
w/o possible	Nn=Ss	Mod 3: Interaction effect Sn, Ns>Nn, Ss

<u>Pup Behaviors</u>			
Dam Directed:	Ss>Nn	**Mod 4:**	**Dam effect, SD>ND** **Pup effect, sp>np**
nosing & probing	Ss>Nn	**Mod 2:**	**Pup trend, sp>np**
w/o poss	Ss>Nn	**Mod 4:**	**Dam trend, SD>ND** **Pup trend, sp>np**
mouth & lick	Nn=Ss	**Mod 2:**	**Pup trend, sp>np**
treadle	Ss>Nn	**Mod 4:**	**Dam effect, SD>ND** **Pup effect, sp>np**
Other Directed:	Nn=Ss	**No effects (mostly zero)**	
Quiet:	Nn>Ss	**Mod 4:**	**Dam effect, ND>SD** **Pup effect, np>sp**

we could compare concerns only normative data. Not only is the effect of separation and/or cross-fostering a factor to consider for differences, but also the lack of nesting material. This decrease in shavings might increase nursing time as the pups are nursed longer to keep them warm (temperature variables described below) or perhaps even to protect them as dams have been shown to build nests which cover the pups considerably if material is available and will relocate nests if disturbed.

There was a trend for the Ss group to nurse longer which would increase the time available for pups to consume milk, as well as to be warmed by their dam's milk and ventrum. However, it was shown that this trend toward nursing longer in the Ss group was a result of the Nn group nursing less, unique to the Nn interaction. Therefore, the trend toward an increase in time spent nursing was probably not the means by which weight and temperature were altered in the Ss group because nursing time was greater even in the Ns group, the other nonseparated dam group, when compared to the Nn group, and the Ns group did not show the same weight or temperature effects over the 2 hour period. Also, although there was a trend for the Ss group to nurse longer over the two hour period, there was no difference between the Ss and Nn groups in the number or length of nursing bouts.

These results were not consistent with the study by

Jans and Leon (1983) which demonstrated that contact time increased if pups were cool, hypothetically because cool pups would take longer to create the rise in maternal temperature which would curtail the contact bout. Because we did not observe an increase in contact time in groups with separated pups who were cooler upon reunion, our results do not support the theory that nest bout time is regulated by the dam's need to maintain her thermal homeostasis. However, this study did include a deprivation component, which could be affecting the time in contact, as dams' and pups' might be more motivated to nurse. In fact, perhaps the increased rates of time spent nursing seen in the Ns, Sn, and Ss groups were due to an increase in motivation to nurse by either the separated dam, or separated pup, or both. Specifically, perhaps the separated pups were able to maintain an increased level of stimulation of the dam's ventrum, and the separated dam made her ventrum available for such stimulation for a longer time, and/or was more sensitive to any pup stimulation present, for this stimulation has been hypothesized to be the critical variable in maintaining the dam's nursing posture (Stern and Johnson, 1990).

The effect of separation most likely to have contributed to the increased weight gain of pups in the Ss group was the increase of possible MEs delivered by separated dams. Normally, the transfer of milk occurs in the restricted periods of milk ejections, noted when the dams arch and the pups display the characteristic stretch response. Possible MEs were noted when the pups were seen to be activated as a group and treadling, but either there was no distinct stretch response, and/or the dam did not fully arch, and therefore the observer was not fully convinced that a ME had occurred. It is possible that no milk, or just a drop of milk, was present when these behaviors occurred. Perhaps the separated pups were sucking hard enough to extract very small quantities of milk in between full oxytocin induced MEs, and due to deprivation, were overreactive to the small amount of milk and frantically treadled. Or, perhaps the pups were overresponding to any slight movement in the dam, mixing up their cues of milk release. The dams could have been hypersensitive to the pups' suckling, and shifted positions often in an attempt to settle down. It is true that when the dam shifts her position significantly, the pups appear to be activated like during a ME, but rather they are treadling and moving with the dam in an attempt to remain attached.

Looked at in conjunction with the increased weight gain of the pups with separated dams, it is most likely that these possible MEs of the separated dams were an actual let down of milk. This is supported by the positive correlation found across all groups between the number of possible MEs and the weight gain over the 2 hour period. Perhaps during possible MEs milk was released slowly, and trickled down in quantity, but not in an isolated milk ejection response. DeNuccio and Grosvenor (1971) found that after 16 to 24 hours of separation, the passive resistance of milk entry from the alveoli to the mammary gland was overcome, and milk was available without oxytocin. They also found that the separated dam has an increased sensitivity to oxytocin, and perhaps due to this, they had an increased sensitivity to the pups' suckling and any stimulation could release milk. Also, separation of the dam has been shown to increase the resting tension of the myoepithelium, and less contractile force is needed to release milk (Wakerley et al., 1988), and Grosvenor (1965) found that mechanical stimulation by stretching the myoepithelium easily elicited milk release when the mammary glands were distended. Therefore, treadling by the pups may have helped to increase the positive pressure within the gland, mechanically inducing mammary contractions, thereby extracting more milk without the full oxytocin response. Thus, perhaps the release of milk had an indefinite beginning and end and this longer milk consumption period could have been reflected in the increase in what were called possible MEs.

It was predicted that separated pups could influence the rate of MEs by increasing the rate and amplitude of their sucking as well as the time they spent in sleep/wake state associated with increased sucking rates (Brake, Shair, and Hofer, 1988; Shair, 1986). Lincoln and Wakerley (1975) have shown that the reduction of the suckling stimulus by leaving only 2 to 3 pups on the dam's nipples reduced the magnitude of the bursts of firing in the oxytocin neurons. Therefore, perhaps the increases in the separated pups sucking rate, amplitude, and duration would increase the magnitude of the bursts of firing by the oxytocin neurons, and thereby increase the ME rate. The only support we have found thus far that separated pups can alter the rate of MEs, was the increased ME rate of the Ss group during the first hour of reunion. This is not strong support for the separated pups' ability to alter ME rate, as this increased rate in the Ss group decreased in hour 2, and was possibly due to dam's milk supply. Also, the other group with separated pups, the Ns group, did not display these effects. And last, there was no difference in the total number of MEs over the two hour period. However, perhaps the effect of separated pups on the ME rate will be seen in the analysis of the first nursing bout which includes the latency to the first ME and the inter-ME-interval. It was these measures that were shown to be decreased by an 18 hour period of deprivation on post-natal day ten (Lincoln, Hill, and Wakerley, 1973).

The Sn group outlier, litter Q

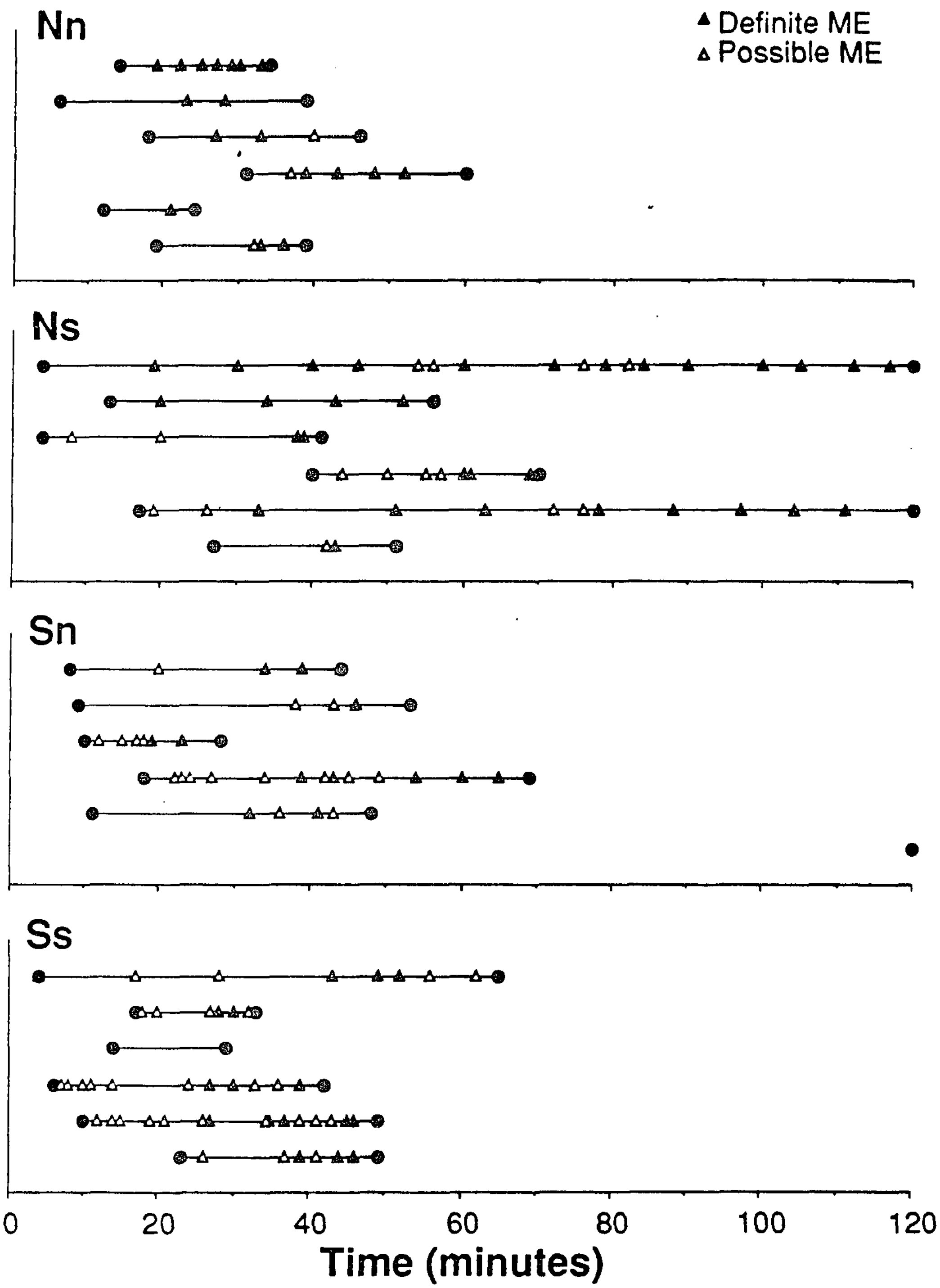
The litter Q in the Sn group was unusual. Over the first 2 hours of reunion, the pups never showed full nursing for the criterion 10 minutes. They were only observed to be attached with more than half the litter for a period of 9 consecutive minutes during the second hour. All other litters from all groups spent a long time full nursing, with a mean of 86.65 minutes (SEM=3.84). The pups of Q did spend a very long time nursing partial nursing for a total of 73 minutes, the mean of all other litters being 16.83 (2.33). Unlike all other litters of all groups, Q was never observed to have had a ME, definite or possible. What was unexpected, to say the least, was that 8 out of 9 pups in litter Q gained weight over the first 2 hours of reunion. The average litter weight gain for Q was .41 grams (.13). This was less than the average weight gain of pups with separated dams (2.17 (.10)), and of nonseparated pups (.62 (.08)). Pups therefore, must have been receiving milk without any behavioral signs of milk letdown or consistent nursing behavior.

The unusual interaction of litter Q will be explored further to determine the roles of the dam and the pups in the disruption that occurred.

B. THE FIRST NURSING BOUT

The first nursing bout was the hypothesized time period during which the effects of separation upon reunion would be most apparent. It is a natural unit of the dam-pup interaction which can be looked at across all groups. As shown in Figure 13, the first nursing bout ended as soon as 24 minutes after reunion in the one litter of the Nn group, but was still continuing in two litters of the Ns group when the interactions were terminated at the two hour mark. These two litters of the Ns group attached during the first 20 minutes of reunion and continued to nurse until separated again when weights and temperatures were taken at the end of the reunion period, thus having nursing bouts which lasted over 100 minutes. It is true that because these nursing bouts were artificially ended they were not the complete natural unit of interaction as in the other litters. However, these "bouts" were included in the analysis as they contained MEs and nursing behavior and were representative of the Ns group. Their extreme length was taken into consideration for all behavioral analysis was done on the rates of behaviors, or number of behaviors per time available for them to occur. The observed state of continual nursing was abnormal and possibly the result of the unnatural combination of a nonseparated dam with separated pups. Whether the interaction of the dam and pups

<u>Figure 13</u>: Histograms of the first nursing bout of each litter of each group. Definite and possible MEs are represented by the arrows. Only 5 litters are shown in the Sn group because litter Q was never observed to have had a nursing bout.


Nn
Ns
Sn
Ss
▲ Definite ME
△ Possible ME
0
20
40
60
80
100
120
Time (minutes)

in this group differed from others will be seen in the behavioral analysis of the first nursing bout.

The first nursing bout was not considered to begin until the first minute of at least 10 consecutive minutes when more than half the litter was attached (full nursing). The bout ended when less than half the litter was attached (partial nursing) or there was no nursing for more than 3 minutes. The analysis included the following measures: latency to attach, length of the first nursing bout, latency to the first ME, and number of MEs, and inter-ME-intervals. The number of episodes during the nursing bout in which partial nursing or no nursing occurred for 3 minutes or less were also analyzed.

1. Latency to Attach

There were no significant differences between the Nn and Ss groups in latency to attach, whether defined as initial attachment (the first minute of full nursing), stable attachment (the first minute in which full nursing continued for at least three consecutive minutes), or solid attachment (the first minute in which full nursing continued for at least ten consecutive minutes). These multiple definitions were used to screen for fragmentation of nursing activity which would be reflected in inconsistent attachment behavior.

The similarity of the latency to attach measures between the Nn and Ss groups was not the result of different processes as there were no significant differences between the four groups (Nn, Ns, Sn, Ss) in latency to attach, whether it was defined initial, stable, or solid attachment. Therefore, separation did not alter either the dams' or the pups' latency to attach.

Latency to solid attachment, which was also the beginning of the first nursing bout, is shown in Figure 14. Litter Q of the Sn group was never observed to be solidly attached, as consecutive minutes of full nursing occurred for only 9 minutes over the entire 2 hour observation period, between minutes 70 and 79. When analysis excluded litter Q, no differences were again found across all groups.

When latency to initial attachment or solid attachment was adjusted for the pups' axillary temperatures prior to reunion in an ANCOVA, there was still no significant difference between groups in latency to initial or solid attachment. There was, however, a trend within groups for pups with lower temperatures to have longer latencies to solid attachment which is the first nursing bout (ANCOVA, treatment=groups, covariate=pups' pre-reunion axillary temperature, within group trend: $\underline{F}(1,11)=3.904, p<.075$). As shown in Figure 15, a scatter plot of pup pre-reunion axillary temperature and latency to solid attachment, this trend is the result of warmer pups within groups attaching faster.

Figure 14: Latency to solid attachment. Mean latency to solid attachment for the six litters per group. This latency represents the beginning of the first nursing bout, and was the first minute of full attachment of 10 consecutive minutes of full attachment. There was no significant difference between the Nn and Ss group in their latency to solid attachment. Analysis across all groups found no effects.

Litter Q was never observed to be solidly attached. Analysis without litter Q also found no effects.

LATENCY TO SOLID ATT.

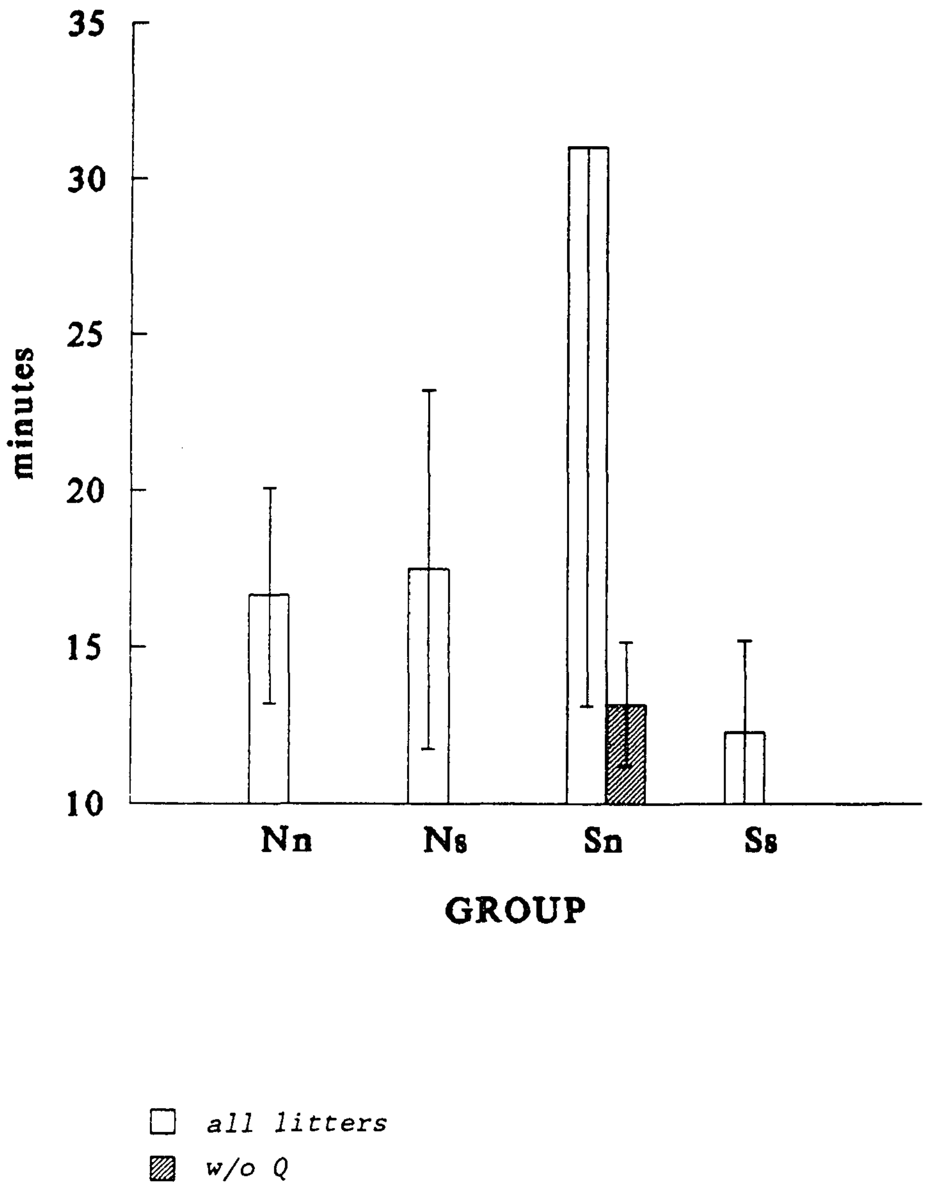
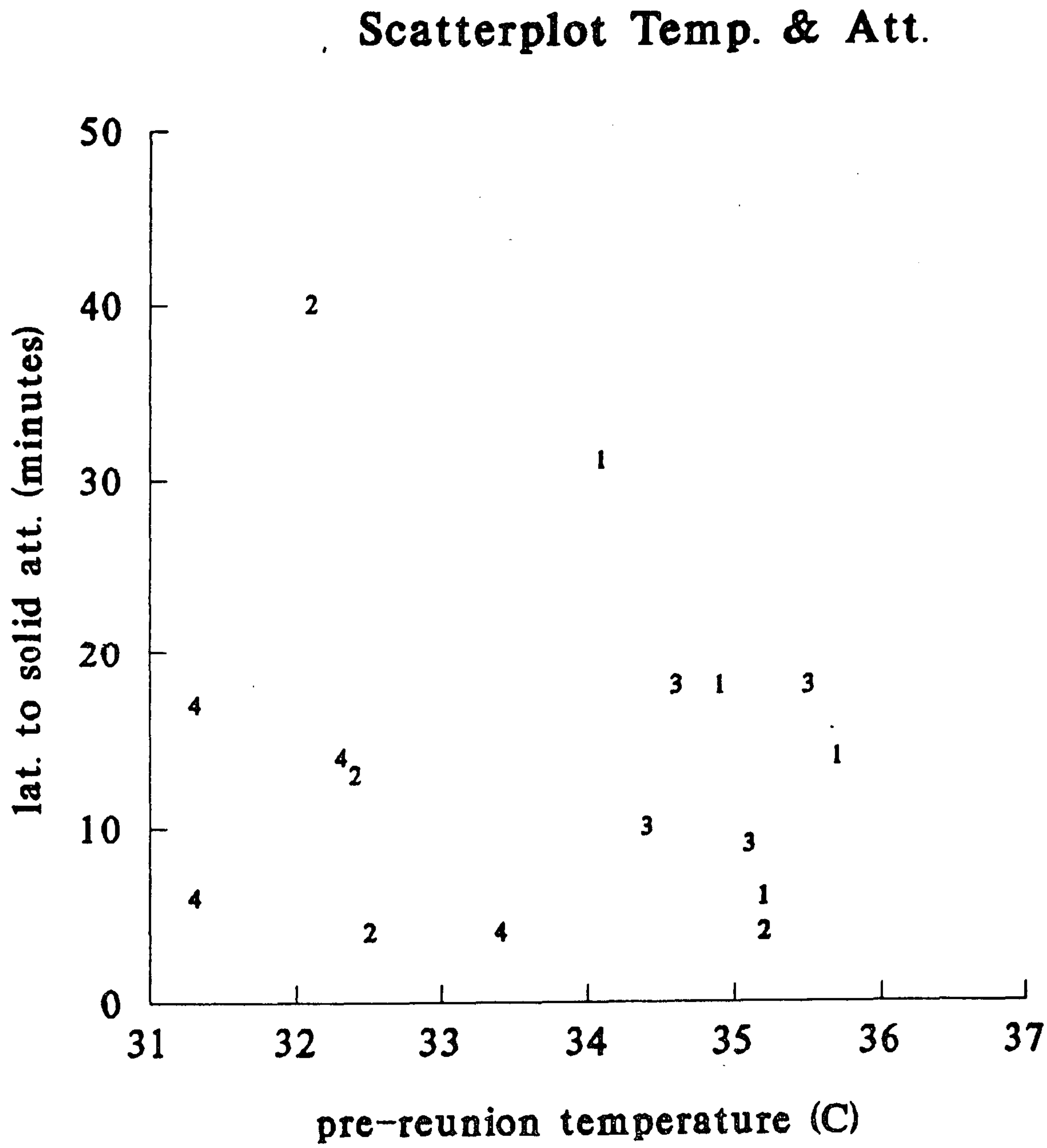


Figure 15: Scatterplot of pup axillary temperature prior to reunion, and the latency to solid attachment. The litters in the Nn, Ns, Sn and Ss groups are represented by the numbers 1, 2, 3, and 4 consecutively. There are 4 litters per group.

The ANCOVA (treatment=group, covariate=pup temperature prior to reunion) found a trend within groups for warmer pups to attach faster ($p<.075$).



Scatterplot Temp. & Att.
lat. to solid att. (minutes)
pre-reunion temperature (C)
50
40
30
20
10
0
31
32
33
34
35
36
37

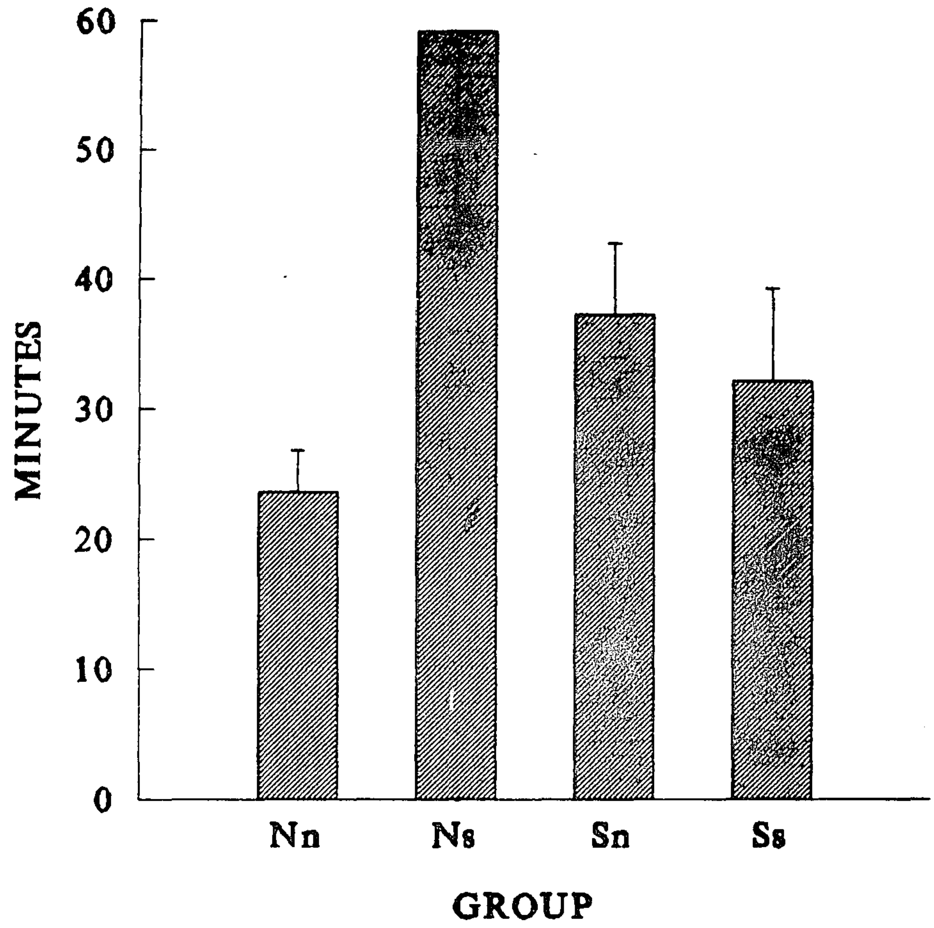
2. Length of First Nursing Bout

There was not a significant difference between the Nn and Ss groups in the length of the first nursing bout. The similarity in length of the first nursing bout was not a result of different processes, as there were no significant dam or pup effects found in the 2 way ANOVA across the four total groups (note: litter Q of the Sn group had to be excluded from analysis because this litter never had a nursing bout). However, there was a significant interaction (Interaction effect: $\underline{F}(1,19)=4.21$, $p<.055$) (see Figure 16). This data fits the Model 3 illustration of separation effects, which represents a unique interaction in the disparate groups. As mentioned earlier, the Ns group had two litters which had a first nursing bout over 100 minutes long. The longest length first nursing bout in each of the other groups was considerable shorter, being 33 min. for the Nn, 51 min. for the Sn, and 61 min. for the Ss group. Although the Ns groups appeared to have the highest mean length of the first nursing bout, it was only significantly greater than the Nn group and tended to be greater than the Ss group (post-hoc, Ns vs. Nn, $\underline{F}(1,19)=6.786, p<.018$; Ns vs. Ss, $\underline{F}(1,19)=3.925, p<.063$).

When pup axillary temperature prior to reunion was taken into account in an ANCOVA, the significant difference between the Nn, Ns, Sn and Ss groups was still present (ANCOVA, treatment=group, covariate=pups' pre-reunion

Figure 16: Mean length of the first nursing bout of the 6 litters of the Nn, Ns, and Ss group, and 5 litters of the Sn group (litter Q of the Sn group never had a nursing bout). There was no significant difference between the Nn and Ss groups in length of the first nursing bout. Analysis across all four groups found an interaction effect. These results fit a Model 3 of separation effects.

LENGTH OF 1ST NURSING BOUT

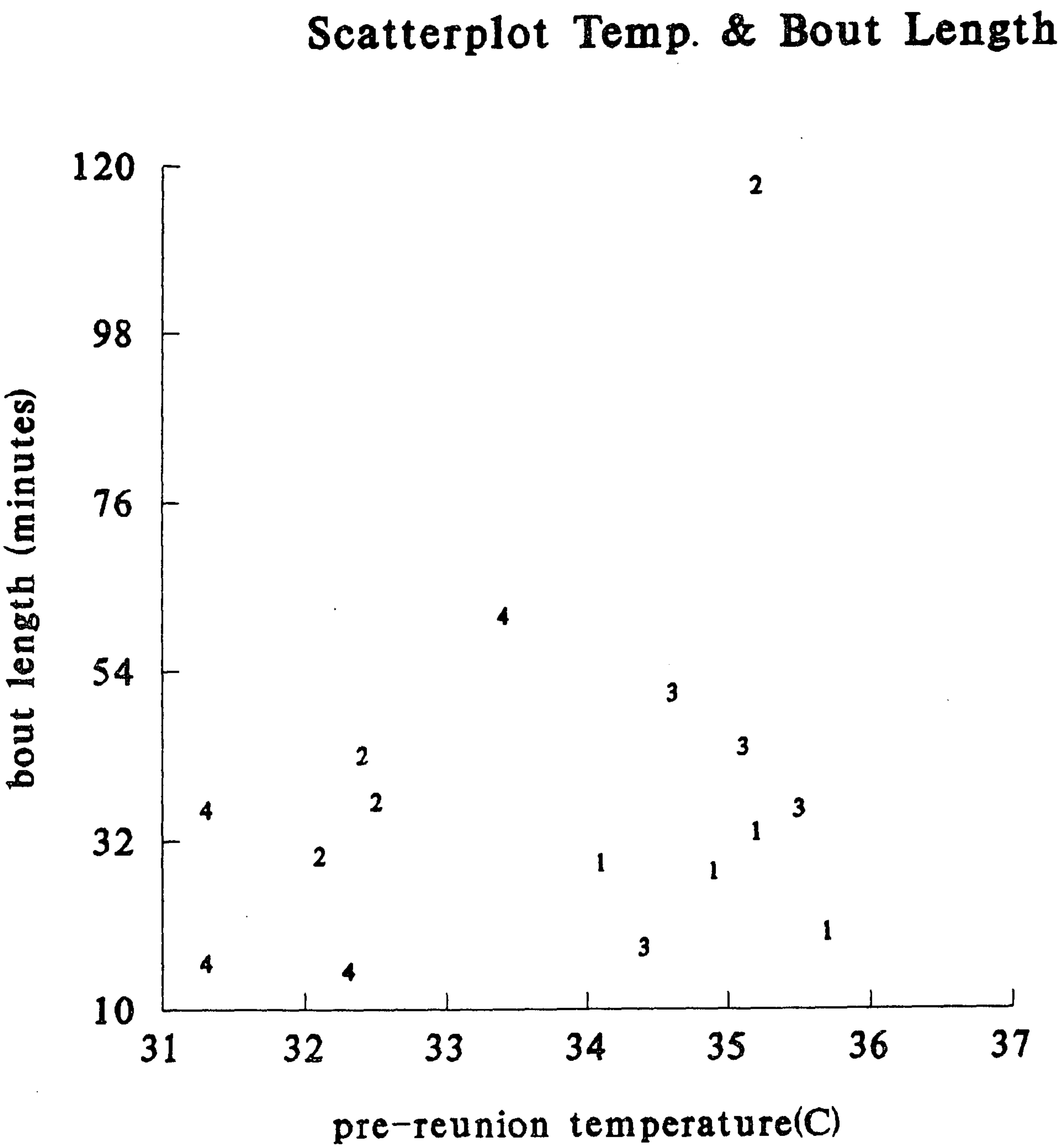


axillary temperature, across group effect: $\underline{F}(3,11)=7.342, p<.007)$. Therefore, adjusting the means by the differences in pups' prereunion axillary temperature did not eliminate the increased length of first nursing bouts in the disparate groups; in other words, the difference in prereunion axillary temperatures did not account for the entire difference found between groups.

Also, it was found that within groups, the warmer the pups' axillary temperatures prior to reunion, the longer the first nursing bout (ANCOVA, treatment=group, covariate=pups' pre-reunion axillary temperature, within group effect: $\underline{F}(1,11)=16.714, p<.003)$. As shown in Figure 17, this effect appears to be contributed to mainly by the groups with separated pups. It is important to note that no significant pup effects were found for the latency to the first nursing bout or the length of the first nursing bout, which means that separated pups who were cooler prior to reunion were not different for these measures. However, what we have found is that within groups, including the cooler separated pups, the warmer pups tend to have a shorter latency to the beginning of the first nursing bout, and have a longer bout once attached.

<u>Figure 17</u>: Scatterplot of pup axillary temperature prior to reunion, and the length of the first nursing bout. The litters in the Nn, Ns, Sn and Ss groups are represented by the numbers 1, 2, 3, and 4 consecutively. There are 4 litters per group.

The ANCOVA found a significant regression within groups ($p<.003$).


Scatterplot Temp. & Bout Length
120
98
76
54
32
10
bout length (minutes)
31
32
33
34
35
36
37
pre-reunion temperature(C)

3. Disruptions During the First Nursing Bout

As defined earlier, the nursing bout was not considered to end until at least 4 consecutive minutes of partial nursing or no nursing occurred. Episodes of 3 minutes or less of partial nursing or no nursing can therefore occur throughout this time, and be considered a measure of disruption. The rate of these episodes within the first nursing bout were figured for each litter by dividing the total number that occurred by the length of the bout. This was done to control for the variation between litters in the total amount of time available for them to occur. Occurrences of nipple switching behavior after MEs were not counted as a disruption episode.

There were no occurrences of disruption in the Nn group, and multiple occurrences in the Ss group. This separation effect was due to changes in the pups' only, because when this measure was compared across all groups, no periods of disruption occurred in litters with nonseparated pups regardless of whether they were with separated or nonseparated dams, and all disruptions occurred in litters with separated pups (Pup effect: $\underline{F}(1,20)=11.796, p<.004$). Separation changes that occur only in the pups are represented in Model 2. These disruptions were somewhat more consistently observed in the Ss group (5 out of 6 litters) than in the Ns group (2 out of 6 litters). It is interesting to note that the two Ns litters with very long first nursing bouts were the litters in which disruption occurred.

4. Milk Ejections During the First Nursing Bout

A. Latency to the First Milk Ejection

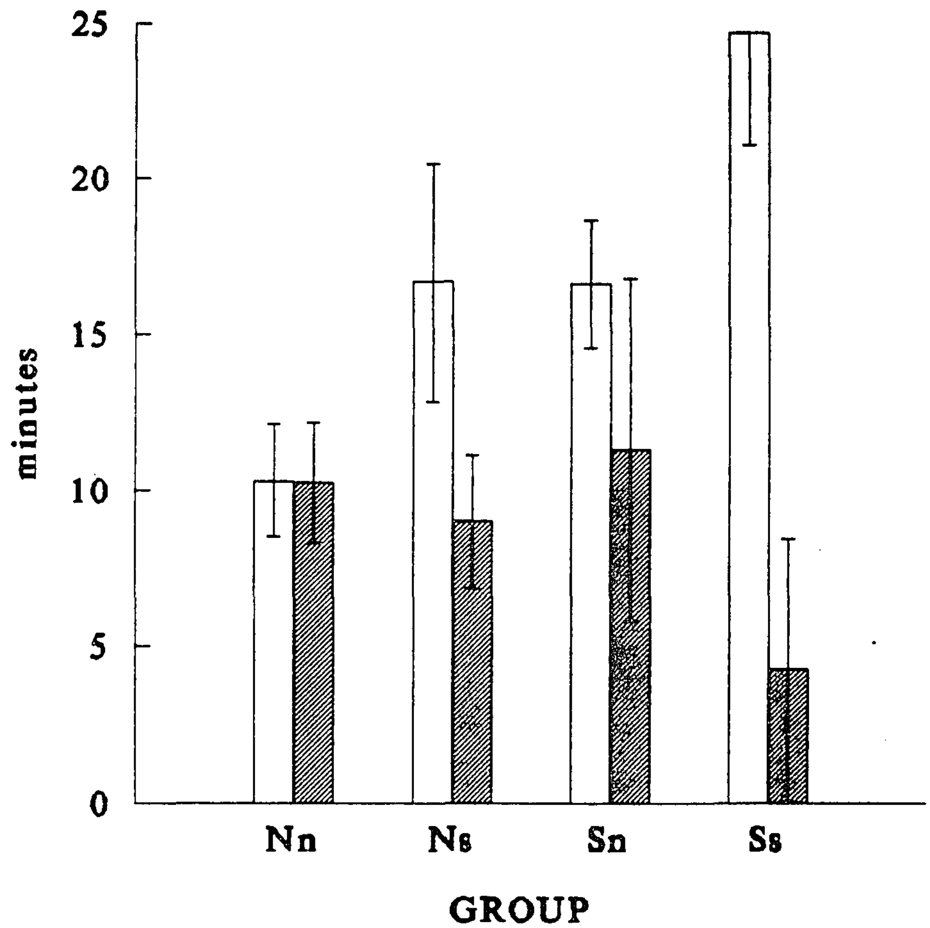
The Nn group had a significantly shorter latency to the first definite ME than the Ss group when measured from the time of solid attachment which is the beginning of the first nursing bout ($t(10)=-3.551, p<.006$). This difference can be explained by separation effects in the dam condition as the ANOVA across all groups found a trend for separated dams to have a longer latency to the first definite ME from the time of solid attachment (Dam trend: $\underline{F}(1,19)=3.004, p<.100$). This results fit a Model 1 of separation effects, as the increase in latency to the first definite ME after separation upon reunion was the result of alterations in the dam condition. However, as shown in Figure 18, when analysis was done on latency to the first definite or possible ME, the significant difference between the Nn and Ss groups was lost. Six out of six litters in the Ss group had a possible ME before a definite ME, which when included, decreased their latency to the first ME from 24.67 (SEM=3.60) to 4.25 (4.18). Only two out of six litters in the Nn group had possible MEs first which, when included, decreased the latency only slightly from 10.33 (1.82) to 10.25 (1.95) in the latency to the first ME. The ANOVA

Figure 18: The mean latency of the 6 litters of group Nn, Ns, and Ss and the 5 litters of group Sn to the first definite ME from the time of solid attachment, or to the first definite or possible ME.

There was a significant difference between the Nn and Ss groups in latency to the first definite ME from the time of solid attachment (t-test, $p<.006$). The 2 way ANOVA found a trend for a dam effect. These results fit a Model 1 of separation effects.

There was no significant difference between the Nn and Ss group in latency to the first definite or possible ME from the time of solid attachment. The 2 way ANOVA found no effects.

LAT.TO 1ST ME FROM SOL. ATT.



☐ *to def. ME*
▨ *to def. or poss. ME*

across all groups found no effects. In summary, separation increased the latency to the first definite ME from the beginning of the first nursing bout, but had no effect on latency to the first definite or possible ME.

Another way to look at the speed with which pups first get a milk ejection is to measure the latency to the first ME from when the dam and pups were first placed together. This was done to bring out possible differences between the groups by adding in the time it took for litters to become attached and begin nursing. As shown in Figure 19, the Nn group tended to have a shorter latency to the first definite ME ($t(10)=-2.088, p<.064$), but this trend was lost when the latency was measured to the first definite or possible ME. The ANOVA across all groups found no significant effects in either latency measure- to the first definite ME, or to the first definite or possible ME- possibly due to the high variability of this measure in all groups. In summary, separation tended to increase the latency to the first definite ME from the time of reunion in the Ss group, but had no effect on latency to the first definite or possible ME.

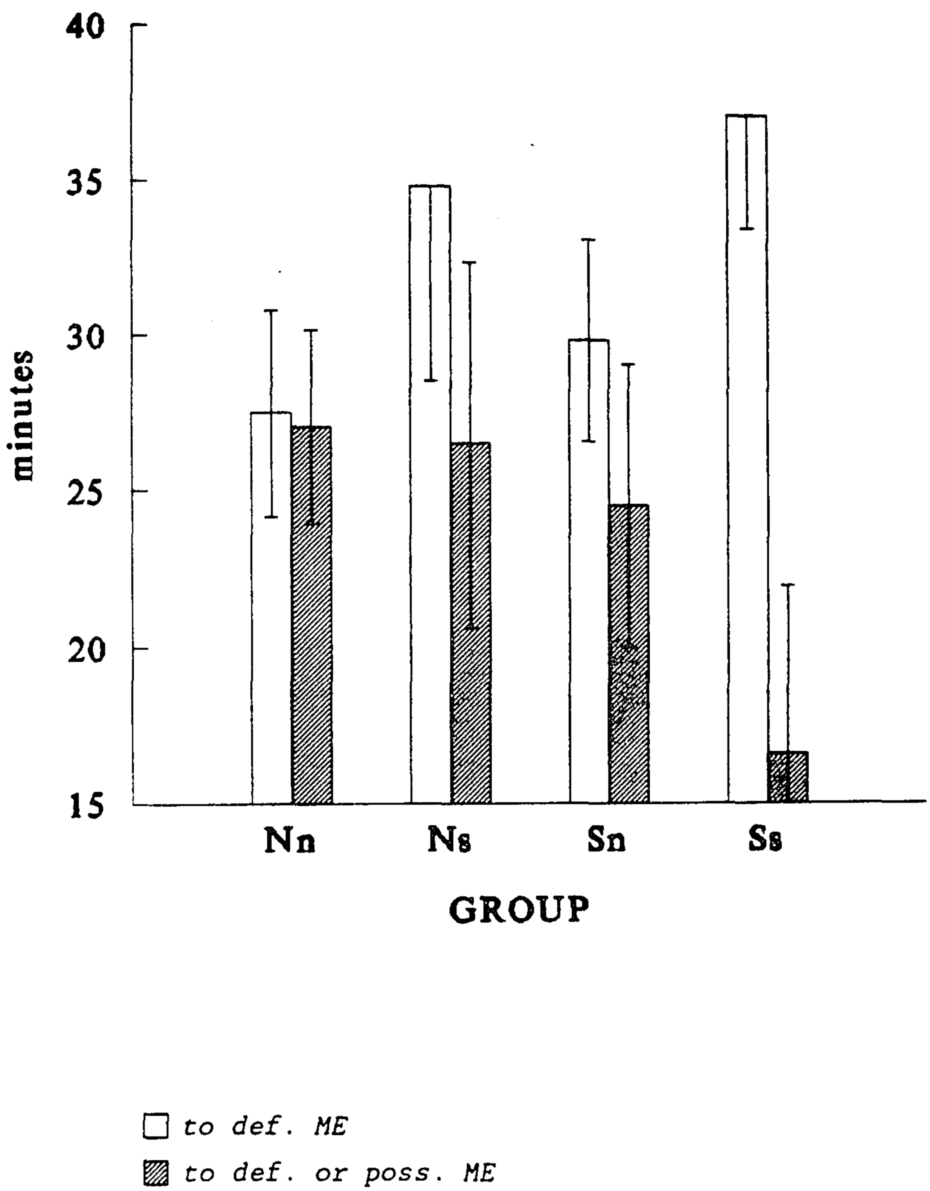
When the means of the latency to the first definite or possible ME from the time of reunion were adjusted for by the pups' pre-reunion axillary temperatures in an ANCOVA, no significant difference was found across groups. Nor was there a relationship found within groups between pup

Figure 19: The mean latency of the 6 litters of group Nn, Ns, and Ss and the 5 litters of group Sn to the first definite ME from the time of reunion, or to the first definite or possible ME from the time of reunion.

There was a trend for the Nn and Ss groups have different latencies to the first definite ME from the time of reunion (t-test, $p<.064$). The 2 way ANOVA found no effects.

There was no difference between the Nn and Ss group in latency to the first definite or possible ME from the time of reunion. The 2 way ANOVA found no effects.

LAT. TO 1ST ME FROM REUNION



temperature and this measure. Therefore, differences in pup temperature prior to reunion did not affect the measure of latency to the first ME.

B. Number of Milk Ejections

There were no differences between the Nn and Ss groups in the number of definite MEs during the first nursing bout (see Figure 20). The ANOVA across all groups also revealed no differences due to dam or pup condition, or an interaction between the two. Therefore separation did not affect the number of definite MEs during the first nursing bout.

The Ss group had significantly more possible MEs during the first nursing bout ($t(10)=-3.429, p<.007$). This was due to an increased number of possible MEs in groups with separated dams (Dam effect: $\underline{F}(1,19)=6.836, p<.018$), and a tendency for more possible MEs to occur in groups with separated pups (Pup trend: $\underline{F}(1,19)=3.130, p<.094$). These results fit a Model 4 of separation effects, an additive model. A clear progression in the number of possible MEs over the four groups can be seen in Figure 20, the Nn group having the least amount of possible MEs, and the Ss group the most.

The Ss group tended to have more total MEs (definite and possible MEs combined) than the Nn group ($t(10)=-1.894, p<.088$). This is obviously contributed to by the increased number of possible MEs seen in the separated dams' condition just described (see Figure 20). The ANOVA across all groups revealed no significant effects of separation on this measure in the dam condition, pup condition, or an interaction between the two.

C. Inter-Milk Ejection-Intervals

Inter-ME-intervals (IMEIs) were analyzed in two ways- including intervals with disruption periods and excluding them. Separated pups were shown to have disruption periods during the nursing bout, unlike nonseparated pups, and any disturbances in nursing have been shown to increase the IMEI (Lincoln, Hentzen, Hin, van der Schoot, Clarke and Summerlee, 1980; Lincoln and Paisley, 1982). We wanted to see if we would find similar results.

There were no significant differences in IMEIs between the Nn and Ss groups whether disruption periods were included or not. However, a trend toward an interaction between dam and pup condition existed with and without disruption periods; as shown in Figure 21, the Nn and Ss groups tended to have shorter IMEIs than the Ns and Sn, or the disparate conditions (with disruptions: $\underline{F}(1,15)=3.964, p<.066$; without disruptions: $\underline{F}(1,15)=3.827, p<.070$). These results fit the Model 3 of ANOVA results. Although there was no difference between the Nn and Ss groups for this measure, separation did affect the

Figure 20: The mean number of MEs during the first nursing bout of the 6 litters of the Nn, Ns, and Ss group and the 5 litters of the Sn group, including definite MEs, possible MEs, and definite and possible MEs combined.

There was no significant difference between the Nn and Ss groups in number of definite MEs during the first nursing bout. The 2 way ANOVA found no effects.

There was a significant difference between the Nn and Ss groups in number of possible MEs during the first nursing bout (t-test, $p<.007$). The 2 way ANOVA found a significant dam effect, and pup trend. These results fit a Model 4 of separation effects.

There was a trend for the Nn and Ss groups to be different in the total number of MEs during the first nursing bout (t-test, $p<.088$). The 2 way ANOVA found no effects.

MEs IN 1ST NURSING BOUT

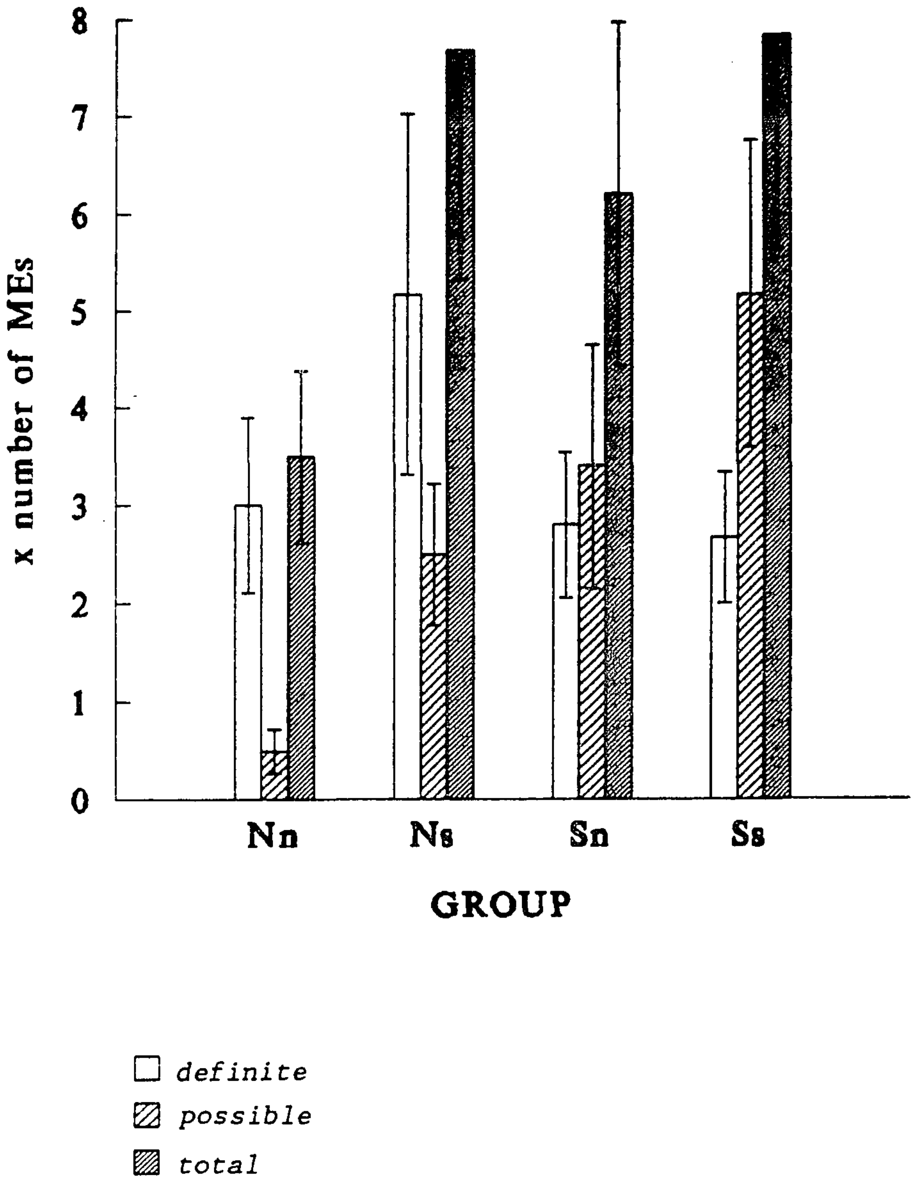
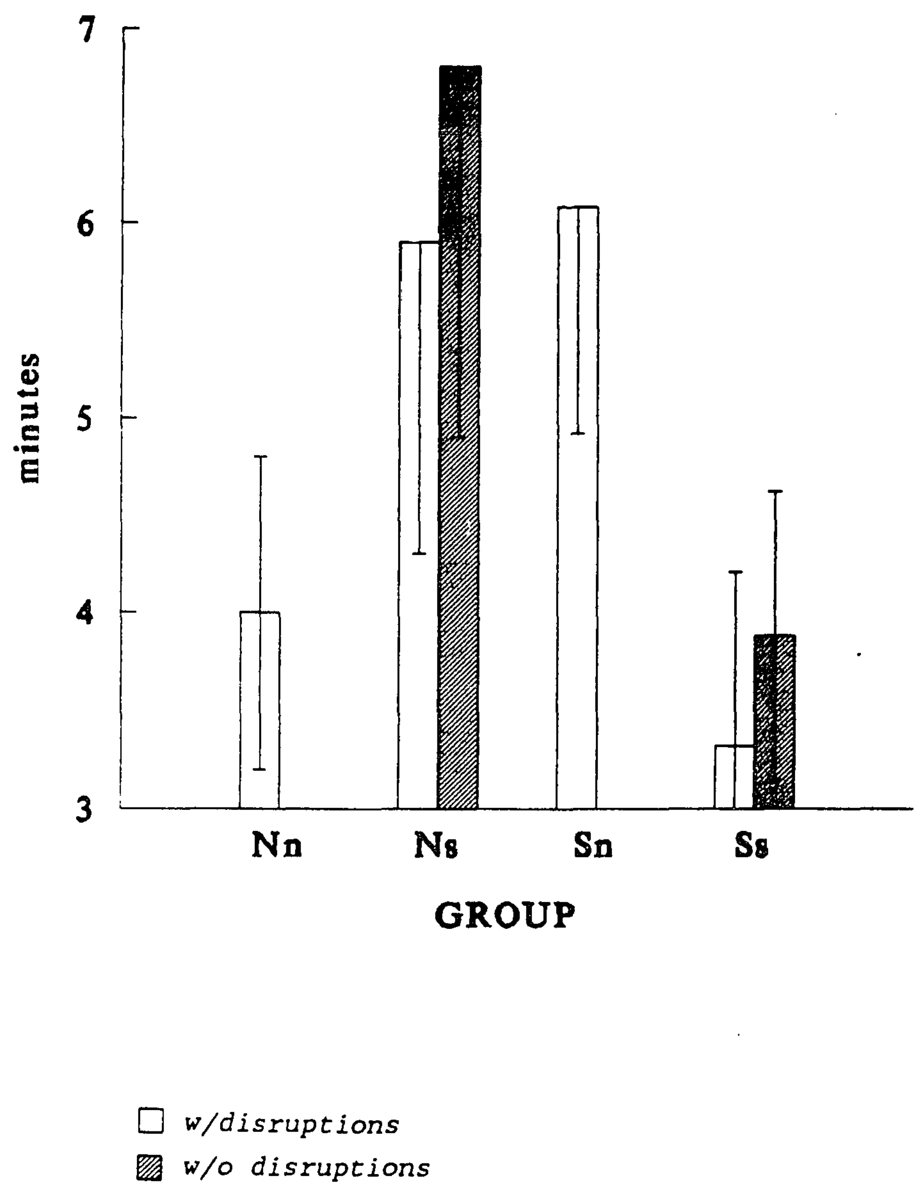


Figure 21: The mean IMEI during the first nursing bout of the 6 litters of the Nn, Ns, and Ss group and the 5 litters of the Sn group. The IMEI was calculated with and without the periods of disruption seen in groups with separated pups.

There was no significant difference between the Nn and Ss group in IMEI when times of disruption were included. The 2 way ANOVA found a significant interaction effect. These results fit a Model 3 of separation effects.

There was no significant difference between the Nn and Ss group in IMEI when times of disruption were excluded. The 2 way ANOVA found a significant interaction effect. These results fit a Model 3 of separation effects.

IMEIs DURING 1ST NURSING BOUT



IMEI as seen by the longer intervals in the Ns and Sn groups. Perhaps this was the result of the unique, unnatural combination of dams and pups in these litters which resulted in asychronous behaviors between the dams and pups.

Summary and Discussion

The first thing someone might notice when comparing the first nursing bout of dams and pups who have been separated to those who have not been, is the lack of differences. One might have predicted that the latency to attach would decrease due to the pups' deprivation (Drewett, Statham and Wakerley, 1974; Hall, Cramer, and Blass, 1977), or being cooler upon reunion (Henning, Chang, and Gisel, 1979) however there was no difference in latency to attach. And in fact, within the groups cooler pups attached slower than pups who were warmer, not replicating Henning, Chang and Gisel (1979) under these conditions. It is possible that the separated dams had retracted nipples due to the non-suckling deprivation period, and although the separated pups were working harder to attach, they had more to do and therefore did not attach faster. However, if this were true, the Ns groups would theoretically have a decreased latency to attachment, and this was not seen. Analysis of dam and pup behaviors described later include descriptions of the Nn and Ss groups prior to the first nursing bout, and could possibly illuminate if the Ss group was attempting to attach sooner but for some reason (due to dam and/or pup behavior) was unsuccessful.

Once attached, there was no difference between the Nn and Ss group in the length of the first nursing bout. This was not unexpected because analysis of the total two hour reunion period found no difference in the number or length of nursing bouts during this time. However, it is consistent with the idea that the pups of the Ss group were consuming more milk without increasing the time spent nursing.

A significant relationship was found between the pup's axillary temperature prior to reunion and nursing bout length within groups. Mainly warmer pups had longer bouts. This is not in accordance with work that has demonstrated that dams stay in contact longer with pups who are cool (Leon et al. 1978), and the hypothesis that time spent nursing is regulated by the dam's thermoregulatory behavior. Theoretically, cooler pups would take longer to raise their dams' core temperature thereby increasing the amount of time they could stay in contact. However, we found that within groups, the warmer pups tended to attach faster and have longer bouts once attached. Another factor other than warmth appears to involved in determining nursing time. Perhaps analysis of behaviors will reveal that warm and cool pups, or dams with them, have different patterns of behavior prior to nursing. Stern and Johnson (1990) have demonstrated the importance of ventral somatosensory stimulation of the dam to maintain her nursing posture, and perhaps the warmer pups were able to stimulate the dam to allow faster attachment and then to maintain it. In fact, Stern and Johnson (1990) chilled 2 day old pups and found that the dam does not actually crouch over them until the pups have been warmed by the dam's tongue, paws, and ventrum to at least close to nest temperature. Perhaps cooler pups were unable to stimulate the dam sufficiently due to a decrease in mobility.

Lincoln, Hill, and Wakerley (1973) found a decrease in latency to the first ME and a decrease in the IMEI post deprivation. However, in this study, neither of these results were seen. There was no difference between the Nn and Ss groups in IMEI during the first bout, and the Ss group actually had a longer latency to the first definite ME from time of attachment, and tended to have a longer latency to the first ME from the time of reunion. When analysis of latency to the first ME included both definite and possible MEs, there was no difference between the Nn and Ss groups in latency to the first ME. As previously stated, we believe possible MEs were times of actual milk let down which contribute to the increased weight gain of pups with separated dams. Therefore, there was no difference in latency to the first observable let down of milk. Looking at both the latency to the first ME and the IMEI, it can be stated that the pups of the Ss group were not gaining more weight by having faster MEs.

Consistent with the analysis of the two hour period, the effect of separation most likely to have contributed to the increased weight gain of pups in the Ss group was the increase of possible MEs delivered by separated dams. During the first nursing bout separated dams delivered more, and separated pups elicited more possible MEs. Although we can only speculate about what caused the increased weight gain of pups with separated dams from analysis of the first nursing bout, it likely that it was contributed to by the increase in possible MEs. Not only is this the most obvious difference between the Nn and Ss groups, it is consistent with the two hour analysis which more accurately reflects behaviors that could create the 2 hour weight change differences. In the analysis of behaviors of the first nursing bout described below, we can attempt to determine if specific dam or pup behaviors altered by separation contributed to the increase in weight gain also.

The Ss group, unlike the Nn group, did have periods of disruption, when the pups were unattached for brief periods of time during the first nursing bout. These periods of disruption might have aroused the dam which has been shown to affect the IMEIs since a prerequisite for a ME is a synchronized electroencephalogram (EEG) or sleep in the dam

(Lincoln et al., 1980). But no clear evidence of this was found as there was no change in the IMEI. What these disruption periods were due to can perhaps be explained when the analysis of specific dam and pup behaviors are looked at. They could be a sign that the separated pups were hyperaroused and unable to remain attached, or that the dam was hypersensitive and could not settle down and by moving often the pups had trouble staying attached. However, pups of the Ss group were consuming more milk despite these disruption periods.

The disparate groups were different from the Nn and Ss groups perhaps due to the dam and pup behaviors being asynchronous. Over the entire two hour period, these groups spent more time full nursing and had longer, less frequent bouts during this time. Analysis of the first nursing bout also revealed a prolonged bout duration in the disparate groups. Perhaps the nonseparated dam tolerated the separated pups and allowed them to nurse as long as they seem interested, and their interest had increased due to deprivation. As mentioned previously, there were two litters of the Ns group which had nursing bouts that lasted over 100 minutes long. Also, in the Sn group, perhaps the separated dam had an increased interest in nursing due to her distended mammary glands, and therefore allowed the nonseparated pups to nurse longer. The only sign of behavior being counterproductive or asynchronous in the disparate groups is the increase seen in IMEI suggesting that they are not efficiently receiving milk. However, the IMEI intervals found by Lincoln, Hill, and Wakerley (1973) ranged from 2 to 20 minutes with a mean of 6.6 minutes, and the IMEIs found in this experiment, whether with or without disruption periods, were within this range for all groups, the means ranging from 3.3 to 6.8 minutes. Therefore, the trend toward the Ns and Sn group having longer IMEIs possibly resulted from asynchronous behavior, yet the length was not abnormal. Perhaps analysis of the dam and pup behaviors will confirm whether the disparate groups are behaving asynchronously or not.

Behaviors Before and During the First Nursing Bout

So far, we have seen results that suggest that the main difference between separated and nonseparated litters is the increase in latency to the first definite ME, in possible MEs, in milk transfer, in warming of pups, and in nursing disruptions. To determine if there were differences in behaviors related to dam or pup condition that led to these increases, analysis was done on dam and pup behaviors. For example, pups might be sucking harder to stimulate milk release, or treadling more to mechanically induce contractions of the mammary glands and thereby release milk. An increase in behavioral activation of the separated pups could perhaps contribute to their increase in temperature over time, in addition to their contact with the dams' ventrum and consumption of warm milk. The separated dam might be more motivated to nurse due to her distended mammary glands, or perhaps due to a metabolic imbalance resulting from her not having delivered the milk being produced nor consuming the pups' urine which supplies water and electrolytes to her. Therefore the dam may be seen to clean and groom her young more prior to nursing to stimulate them to nurse. The dam might also show signs of increased sensitivity to the pups suckling, and show increased movement and delay in settling down to quiet nursing.

The behaviors analyzed can be grouped into three main categories: dam or pup directed, other directed, and quiet.

First, behaviors that occurred prior to the first nursing bout will be described. The latency to solid attachment which is the beginning of the first nursing bout (Figure 14) determines the times available for these behaviors to occur in each group. Analysis was done with and without litter Q. Because litter Q never had a nursing bout, there were 2 hours of pre-nursing bout behavior. All results presented will include litter Q, however if results of analysis without litter Q differ, this will be described.

Then the results will be described for the behaviors of the dams and the pups during the first nursing bout. The times available for these behaviors to occur in each group are shown in Figure 16, the length of the first nursing bout. Litter Q was not included in these analyses as it was never observed to have had a nursing bout. The behaviors during the first nursing bout will not include epochs in which definite MEs occurred. When analyses excluding epochs with both definite and possible MEs differ, this will be described.

PRIOR TO THE FIRST NURSING BOUT

Dam Behaviors:

The dams' behaviors prior to the first nursing bout were noted as being either 1) pup directed, which consists of anogenital (AG) licking, body licking, or retrieval of pups, 2) other directed, which consists of self-grooming, rearing, changing position, or eating and drinking, or 3) quiet, which consists of periods of inactivity. Analysis was done on the behavioral category, then on each specific behavior within it.

Pup directed behavior:

There was no significant difference between the Nn and Ss group in the general class of pup directed behaviors prior to the first nursing bout. Analysis across all groups found no significant separation effects of the dam or pups condition or an interaction between them (see Figure 22).

When individual behaviors were looked at, the Ss group was shown to body lick their pups more than the Nn group ($t(10)=-2.733, p<.022$). Analysis across all groups revealed that this was due to separation effects on the dam; separated dams spent more time body licking pups regardless of the pups' deprivation condition (Dam effect: $\underline{F}(1,20)=5.003, p<.038$), which fits a Model 1 of ANOVA results. No differences were found between the Nn and Ss groups in rate of AG licking or retrieval, and analysis

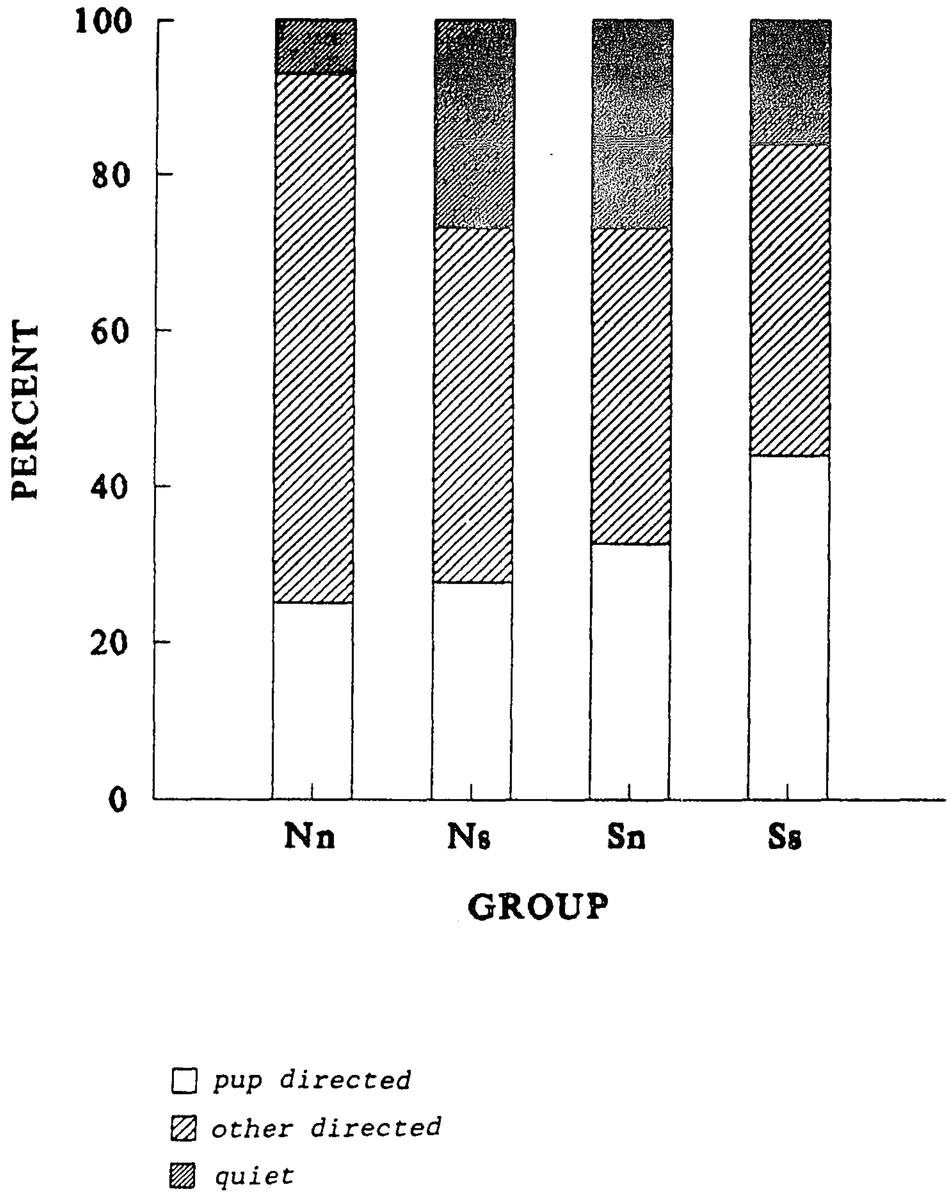
<u>Figure 22</u>: The mean percent time the dams of the 6 litters of each group spent pup directed, other directed, or quiet prior to the first nursing bout.

There was no significant difference between the Nn and Ss group in time the dam spent pup directed prior to the first nursing bout. Analysis across all groups with the 2 way ANOVA also found no effects.

There was a significant difference between the Nn and Ss groups in time the dam spent other directed prior to the first nursing bout ($p<.056$). The 2 way ANOVA found a significant dam effect. These results fit a Model 1 of separation effects because only a dam effect was found, but they also fit a Model 6 because the Nn group was significantly higher than the other groups.

There was no significant difference between the Nn and Ss group in time the dam spent quiet prior to the first nursing bout. The 2 way ANOVA found a significant interaction effect. These results fit a Model 3 of separation effects.

DAM BEHAVIOR PRE-ATT.



across all groups found no significant dam, pup, or interaction effects.

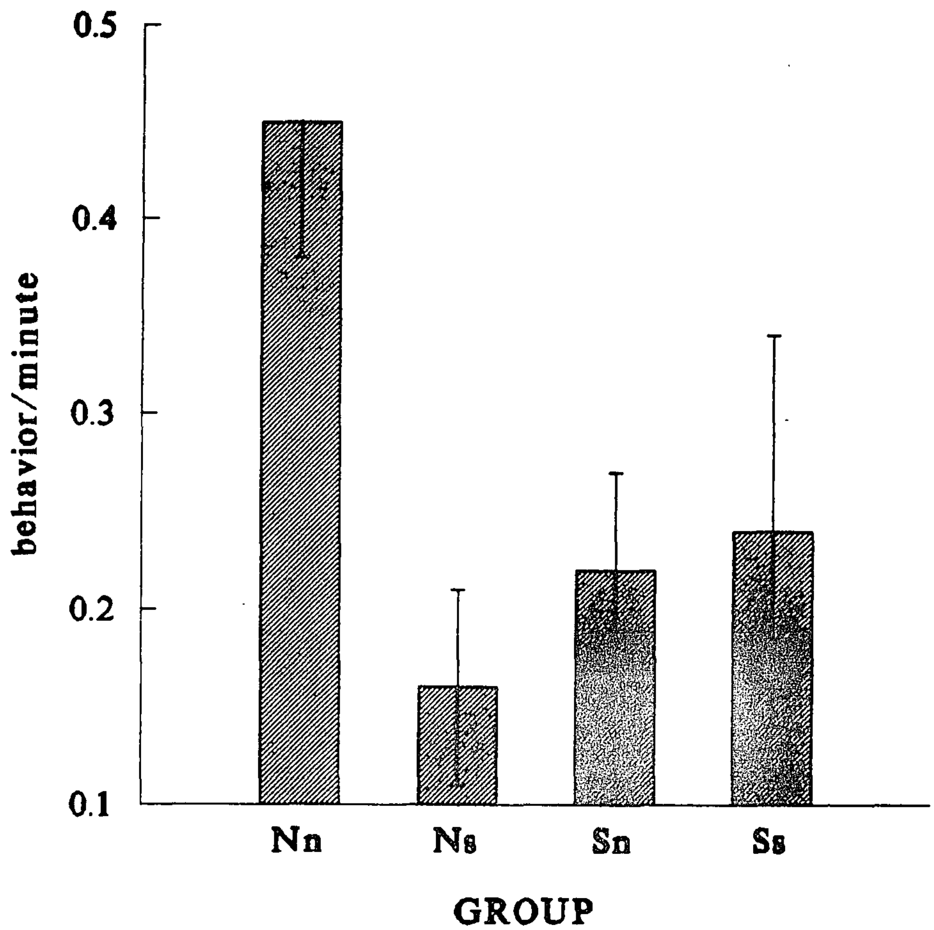
Other directed behavior:

The dams of the Ss group were less other directed than the dams of the Nn group prior to the first nursing bout ($t(10)=2.176, p<.056$). Analysis across all groups revealed that this was due to the effects of separation on the dam condition; the separated dam had a significantly lower rate of other directed behaviors than the nonseparated dam (Dam effect: $F(1,20)=4.965, p<.039$) (see Figure 22). Although this appears to be an example of Model 1 ANOVA results with a single main effect of the dam condition, there is also a reason to consider it a Model 6 (even though there is not a significant interaction effect) as the dam effect seen can be solely attributed to the Nn group having a significantly higher rate than the Ss, Sn, and Ns groups (Nn vs Ss post hoc, $F(1,20)=6.815$, $p<.018$; Nn vs Sn post hoc, $F(1,20)=6.575$, $p<.020$; Nn vs Ns post hoc, $F(1,20)=4.095$, $p<.060$). This overall effect of dam condition was only a trend when litter Q was excluded from analysis (Dam trend: $F(1,19)=3.811, p<.067$).

There were no significant differences prior to the first nursing bout between the Nn and Ss group in the rates of self-grooming, rearing, or eating and drinking, and there were no effects found when analysis was done across all groups. There was no difference between the Nn and Ss group in the rate of changes of position (t(10)=1.708,p<.120), however analysis across all groups revealed a pup trend and significant interaction effect for which the high rate of the Nn group was solely responsible (Pup trend: F(1,20)=3.421,p.080); Interaction effect: (F(1,20)=4.769,p<.042). As shown in Figure 23, the Nn group had a higher rate of this position change than all other groups. This was one of the few instances that when this post-hoc test, comparing two groups but taking into account the variability of all four groups, detected a difference between the Nn and Ss groups not previously seen with the t-test (Nn vs. Ss post hoc, F(1,20)=4.309, p<.052; Nn vs Sn post hoc, F(1,20)=5.345, p<.033, Nn vs Ns post hoc, F(1,20)=8.134, p<.011). Since the increased rate of other directed behavior seen by nonseparated dams was contributed to mainly by this measure we can see that a Model 6 applies best to the overall rate of other directed behavior also, as suggested above.

Figure 23: The mean rate of changing of position of the dams of the 6 litters of each group prior to the first nursing bout. There was no significant difference between the Nn and Ss groups. The 2 way ANOVA found a significant interaction effect and a pup trend. These results fit a Model 6 of separation effects. The Nn group was significantly higher than the other groups.

CHANGING POSITION BY DAM



Quiet:

There was no significant difference between the Nn and Ss groups in time spent quiet preattachment. Analysis across all groups revealed no main effects of the dam or pup conditions, however there was a significant interaction between the two ($\underline{F}(1,20)=5.197, p<.035$). As shown in Figure 21, the Nn and Ss group were less quiet than the disparate groups (Ns and Sn), fitting a Model 3 of separation effects. This effect was only a trend when litter Q was excluded from the analysis ($\underline{F}(1,19)=3.840, p<.066$). As we will see later, the quiet epochs of the disparate groups were erratic.

Summary and Discussion

In summary, the increase in body licking and decrease in other directed behaviors by the dam support the hypothesis that separated dams were more motivated to nurse. Analysis of behaviors prior to the first nursing bout demonstrated that separated dams licked the pups' bodies more, perhaps attempting to stimulate them to nurse. Also, Wakerley, Clarke, and Summerlee (1988) hypothesized that the grooming of the pups prior to nursing provides conditioned tactile, olfactory, and visual stimuli of the pups to ensure maximal milk release upon nursing. We also predicted that the dam might show signs of increased sensitivity to the pups' suckling, and show increased movement and delay in settling down to quiet nursing. Analysis of behaviors during the first nursing bout will attempt to confirm this.

The decrease in other directed behavior by the dams of the Ss group prior to the first nursing bout was mainly contributed to by an increase in other directed behavior by the dams of the Nn group. The dams of the Nn group were shown to have the highest rate of other directed behavior preattachment, consisting mainly of changing position, or generalized movement not directed toward the pups. Perhaps because neither the dam nor pups in this condition had been separated, there was not the urgency to be directed toward each other, but rather the dam was exploring the cage due to the disruption of the nest and handling of her pups which occurred during the set-up for test procedure. The pups of the Nn, or control condition, were cross-fostered during the 18 hour treatment period, therefore the condition of the pups was somewhat novel although they were actually the dam's natural pups. However this did not seem to elicit pup directed behavior. How cross-fostering affected the dam-pup interaction was studied in Experiment 3.

The rate of AG licking prior to the first nursing bout was unaffected by separation. The behavior of AG licking of the pups by the dam has been shown to have multiple functions. Because infant rats do not urinate spontaneously until 2 -3 weeks after birth, they depend upon being licked to stimulate reflexive urination (Capek and Jelinek, 1956-

cited in Gubernick and Alberts, 1983). Also, the dam ingests the pups' urine which functions to recycle water and electrolytes lost during lactation (Gubernick and Alberts, 1983; Gubernick and Alberts, 1985). As part of the experimental protocol, we artificially emptied the pups' bladders prior to reunion in order to obtain more accurate weight information, and thereby may have eliminated an increase in AG licking which could have been elicited due to the increase in urine available post-separation. Also, it is hard to know at what stage the dams were at in recycling water and electrolytes due to the break in their lactation cycle, but perhaps because they had not nursed recently they were in balance, just as it can be assumed the nonseparated dams were. AG licking has been shown not be dependent solely upon the transfer of body fluids to the pups, as sensitized virgins were found AG lick but could be due also to the dam's salt appetite (Gubernick and Alberts, 1985). It was thought that perhaps the quality of the separated pups urine was altered due to the separation period, and this could have elicited more licking, however this was not seen. In fact, we found that separated and nonseparated pups did not elicit any difference in pup directed behaviors from their dams prior to the first nursing bout.

The disparate groups had more quiet epochs prior to the first nursing bout relative to the similar condition groups.

It is hard to know what to make of this, for if the dams and pups within these groups were asynchronous with each other due to their disparate conditions, one might have predicted them to be less quiet and/or more disrupted. It is possible that the dams and pups within these groups were not aroused by each other. Or, perhaps the groups were very aroused by each other but had mixed signals. In this latter case, the increase in quiet behavior would be the result of frequent unsatisfying attempts to interact. The dams and pups would come together, their behaviors would not mesh, and the dam would turn away from their attempt to nurse and become quiet, and these behaviors would cycle. If this is true, the analysis of transitions between dam behaviors prior to the first nursing bout will detect it.

Pup Behaviors:

Prior to the first nursing bout, the focal pup's behaviors were noted as being either 1) dam directed, which consists of changes of position directed towards the dam while in contact, nosing and probing the ventrum, mouthing and licking a teat, or treadling once attached, 2) other directed, which consists of changes of position directed away from the dam, and 3)quiet, which consists of periods of inactivity away from the dam, in contact with the dam, or attached. Analysis will be presented first on the overall behavioral category, then on each specific behavior within them.

As noted in the methods section, the use of the focal pup allowed the specific behavioral analysis to be done as described above, and this pup's behavior was taken to be representative of its littermates. In addition, measurements of contact with the dam and attachment time were taken of the litter as a whole.

Dam directed behavior:

There was no significant difference between the Nn and Ss group prior to the first nursing bout in the general category of dam directed behavior. Analysis across all four groups found no effects of separation on this measure for the dam or pup condition, or an interaction between the two.

When the dam directed behaviors were looked at individually, there were no significant differences between the Nn and Ss group in nosing and probing the ventrum, mouthing and licking the teat, or treadling once attached. Analysis across all four groups also found no effects of separation (see Figure 24).

There was however a difference in the rate of changing of position while in contact with the dam, as the pups of the Ss group changed position more than the pups of the Nn group ($t(10)=-2.196, p<.054$). Analysis across all groups revealed that increase in movement while in contact was due to separation changes occurring in the dam condition; the rates of changes of position in contact with the dam were significantly higher in pups with separated dams (Dam effect: $\underline{F}(1,20)=4.435, p<.049$), fitting a Model 1 of separation effects.

Other directed behavior:

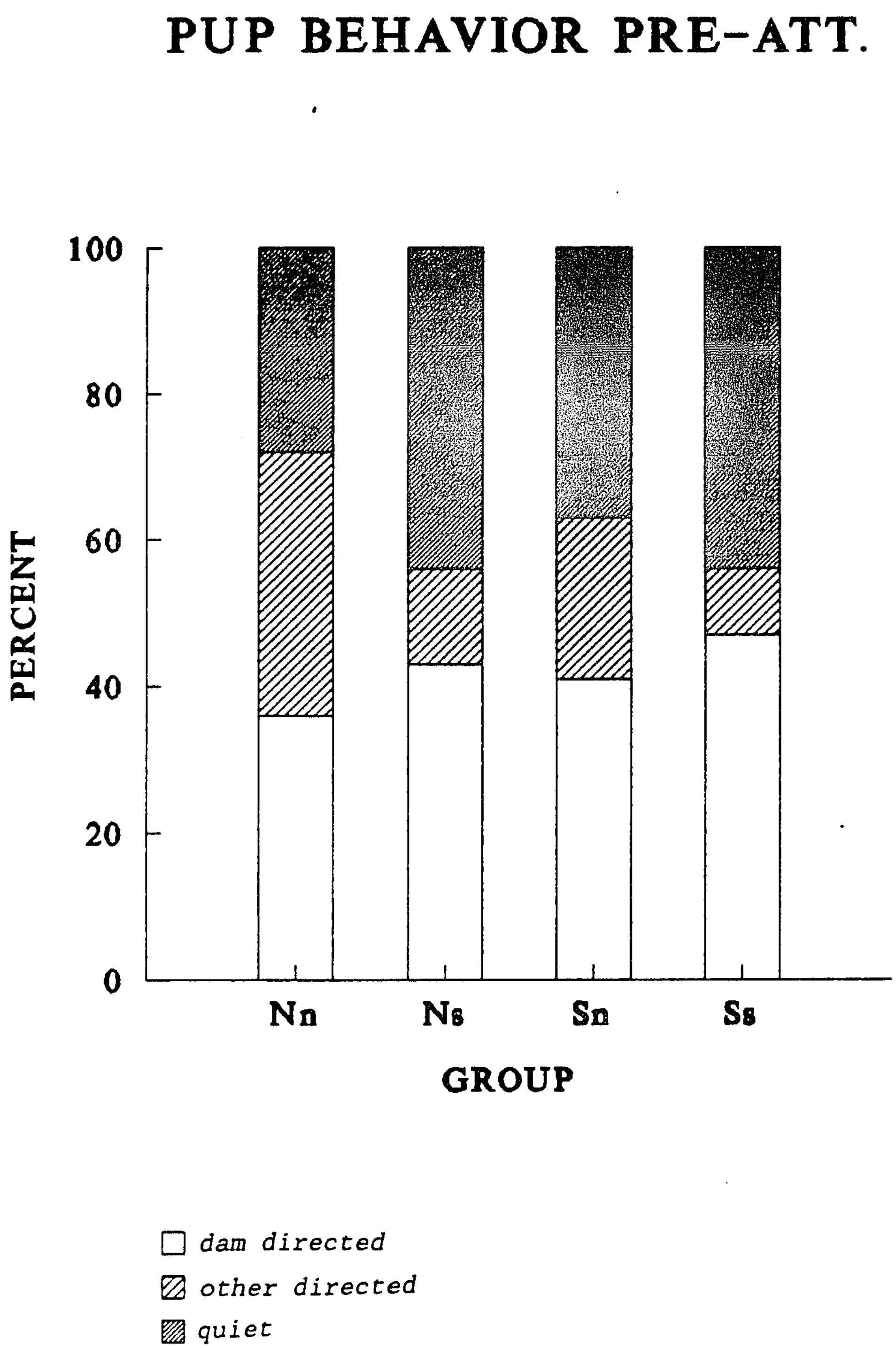
Pups of the Ss group were significantly less other directed than pups of the Nn group ($t(10)=3.368, p<.008$). As shown in Figure 24, this was due to alterations in the pup condition. Separated pups had lower rates of other directed behavior regardless of dam condition (Pup effect: $\underline{F}(1,20)=9.399$, $p<.007$).

Figure 24: The mean percent time the pups of the 6 litters of each group spent dam directed, other directed, or quiet prior to the first nursing bout.

There was no significant difference between the Nn and Ss group in time the pup spent dam directed prior to the first nursing bout. Analysis across all groups with the 2 way ANOVA also found no effects.

There was a significant difference between the Nn and Ss groups in time the pup spent other directed prior to the first nursing bout ($p<.008$). The 2 way ANOVA found a significant pup effect. These results fit a Model 2 of separation effects.

There was no significant difference between the Nn and Ss group in time the pup spent quiet prior to the first nursing bout. The 2 way ANOVA found no effects.


PUP BEHAVIOR PRE-ATT.
PERCENT
100
80
60
40
20
0
Nn
Ns
Sn
Ss
GROUP
dam directed
other directed
quiet

Quiet :

There was no significant differences between the Nn and Ss groups in their rate of quiet epochs. Analysis across all groups also found no effect of separation of the dam or pup condition, or an interaction between the two, in the rate of quiet epochs. This included rates of quiet epochs away from the dam, in contact with dam, while attached, or all combined (see Figure 24).

Contact and Attachment of Pups Prior to the First Nursing Bout:

Pups of the Ss group were in contact more than pups of the Nn group prior to the first nursing bout ($t(10)=-3.352, p<.016$). Analysis across all groups demonstrated that this was due to changes in the dam and pup conditions; separated pups tended to spend more time in contact with the dam (Pup trend: $\underline{F}(1,20)=3.925, p<.072$), and pups with separated dams tended to spend more time in contact (Dam trend: $\underline{F}(1,20)=3.423, p<.090$). As shown in Figure 25, these results are an example of Model 4 separation effects. Separation increased contact in both the dam and pup condition, and these effects were additive in the Ss group.

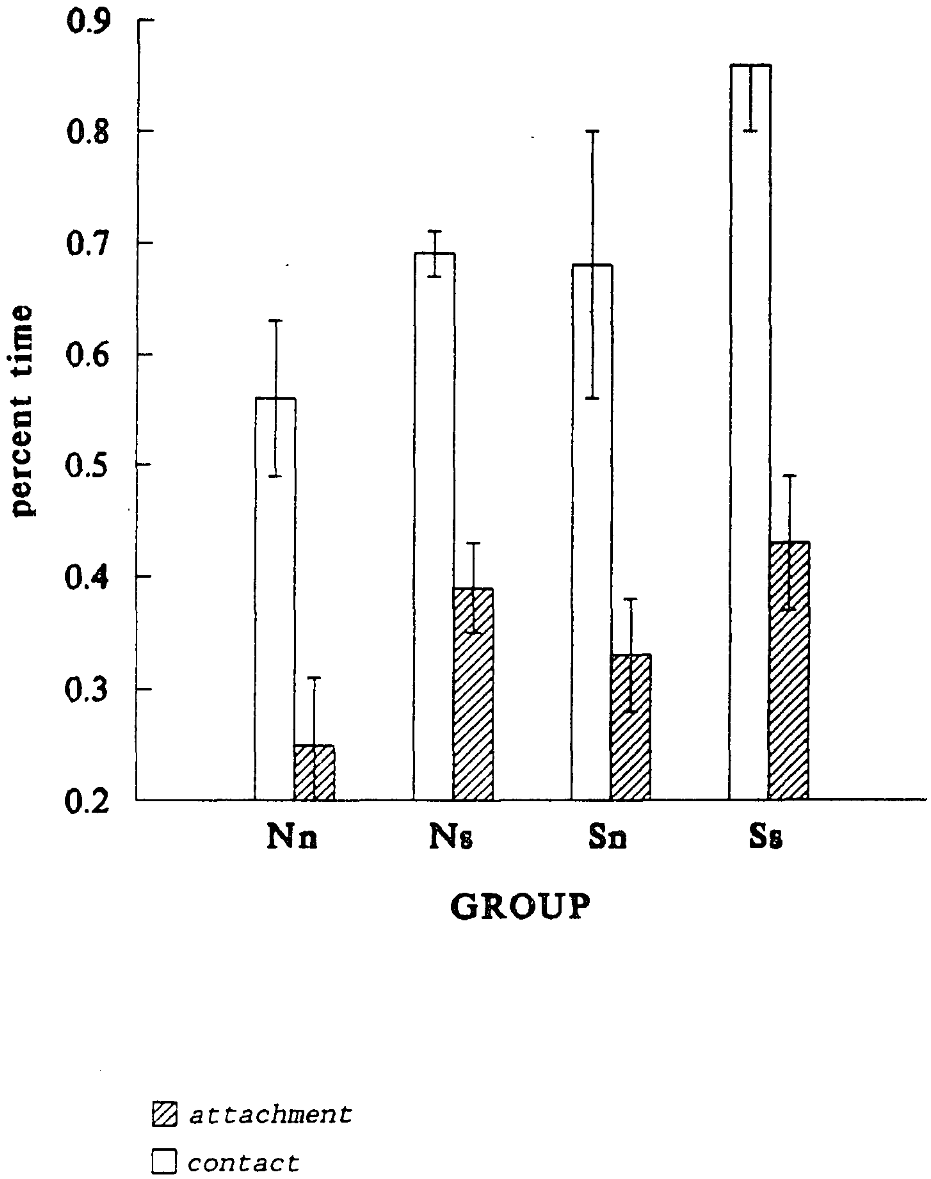
Pups of the Ss group were attached more than pups of the Nn group prior to the first nursing bout ($t(10)=-2.236, p<.050$). Analysis across all groups demonstrated that

Figure 25: The mean rate of contact and attachment by the litter of the 6 litters per group prior to the first nursing bout.

There was a significant difference between the Nn and Ss groups in rate of time in contact prior to the first nursing bout ($p<.016$). The 2 way ANOVA found a dam trend and a pup trend. These results best fit a Model 4 of separation effects.

There was a significant difference between the Nn and Ss groups in rate of time attached prior to the first nursing bout ($p<.050$). The 2 way ANOVA found a pup effect. These results best fit a Model 2 of separation effects.

CONTACT AND ATTACHMENT



this was due to separation changes in the pup condition; separated pups were attached more prior to the first nursing bout regardless of the dam condition (Pup effect: $F(1,20)=5.348, p<.033$). As shown in Figure 25, these results are an example of Model 2 separation effects, as separation increases attachment prior to the first nursing bout in the pup condition.

The total percent of time does not total 100 % in this figure because contact was not observed until the third trial of the experiment. Therefore percent of time attached is a mean of 6 litters per group, as opposed to the 4 litters for percent time in contact.

Summary and Discussion

In summary, the decrease in other directed behaviors and increase in contact and attachment by separated pups prior to the first nursing bout, support the hypothesis that separated pups were more directed toward nursing upon reunion. It was predicted that there would be an increase in behavioral activation of the separated pups toward the dam as they attempted to attach and consume more milk, and that this would contribute to the pups' increase in temperature and weight over time. The fact that separated pups were shown to be in contact with the dam more, and be less other directed during the time prior to the first nursing bout support this, although there were no changes in dam directed behavior.

The attachment of separated pups prior to the first nursing bout must have occurred sporadically, as there was no difference in latency to the first nursing bout. Perhaps this is a reflection of the dam not letting them attach, or a hyperarousal on the pups' part preventing them from attaching securely. It is possible that the dams were hyperaroused and their increase in body licking seen prior to the nursing bout prevented the pups from attaching for long. Or perhaps the increase in body licking increased the arousal of the pups so that they could not remain attached for long.

Transitions:

Even when groups had equal amounts of certain behaviors, these behaviors may have been organized differently over time. Similar behaviors could have occurred continuously in time or in brief epochs alternating with other behavior. The second mode would involve a greater number of transitions and might be representative of disruptive behavior.

The potential for a transition from one behavior to another occurred every time an observation was made, or every minute. Transitions were determined between the main categories of behaviors for both the dam and the pup. Transitions between dam or pup directed behavior, other directed behavior, and quiet behavior were totaled and then divided by the amount of time available for them to occur. Times of consistent behavior (quiet to quiet behavior, other directed to other directed behavior, and pup directed to pup directed behavior) were not counted as transitions, however, they did increase the time available for transitions to occur. Litter Q of the Sn group, which never attached, was included in the analysis and had a total of 120 minutes of preattachment behavior. Analysis was also done without litter Q to determine the effect of this increased variability on the ANOVA, and the results that differ without Q will be described.

Transitions between dam behaviors:

There was no difference between the Nn and Ss group in rate of transitions between dam behaviors. Analysis across all four groups, however, found that the Ns and Sn groups had significantly higher rates of transitions between behaviors than the Nn and Ss groups whether analysis included Q or not (Interaction effect: ($\underline{F}(1,20)=8.126, p<.011$; $\underline{F}(1,19)=7.563, p<.014$). As shown in Figure 26, these results are an example of Model 3 separation effects.

Transitions between pup behavior:

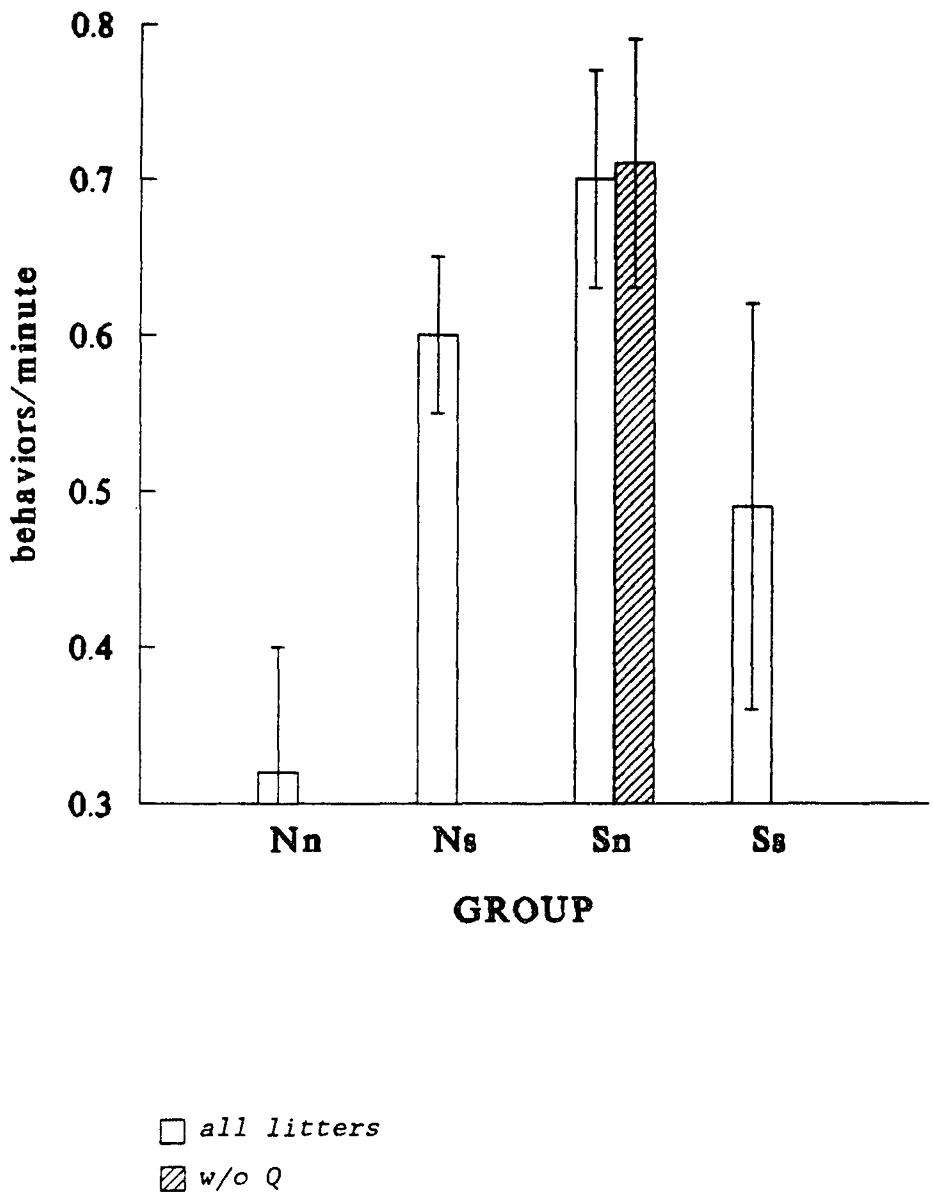
There was no difference between the Nn and Ss group in rate of transitions between pup behaviors. Analysis across all four groups also found no effect of the dam or pup condition, or an interaction between the two. Therefore, the rate of transitions between preattachment behaviors by pups was not significantly affected by separation.

<u>Figure 26</u>: **The mean rate of transitions between dam behaviors prior to the first nursing bout for the dams of the 6 litters per group, with and without litter Q of the Sn group.**

When all litters were included, there was no significant difference between the Nn and Ss group. The 2 way ANOVA found a significant interaction effect. These results best fit a Model 3 of separation effects.

When litter Q of the Sn group was excluded from analysis, the 2 way ANOVA found a significant separation effect. These results best fit a Model 3 of separation effects.

RATE OF TRANSITIONS BY DAM



Summary and Discussion:

There were more frequent transitions between dam behaviors prior to than during the first nursing bout in the disparate groups. Therefore, the increased quiet behavior seen in the Ns and Sn groups must be occurring in brief epochs alternating with pup directed and other directed behavior. (It is interesting to note that the rate of consistent quiet behavior (quiet to quiet epochs) was not lower in the disparate groups as there were no significant differences across the groups for this behavioral rate). This lack of continuity of a given behavior mode supports the hypothesis that the increase in quiet epochs seen in the disparate groups was a sign of disruption. The hypothesis proposed is that frequent, sporadic attempts at interacting with the pups were unsatisfying, so the dams became quiet, with later attempts made again and again with the same results until nursing began. It is thought that the interaction with the pups could be unsatisfying due to their disparate conditions.

BEHAVIORS DURING THE FIRST NURSING BOUT

Litter Q was omitted from all first nursing bout analysis because the litter was never observed to have had one.

All results reported are from analysis excluding the minutes in which definite MEs occurred. Analysis was also done in which both definite and possible MEs were excluded, and when this produced different results, these will be described.

Dam Behaviors:

Pup directed behavior:

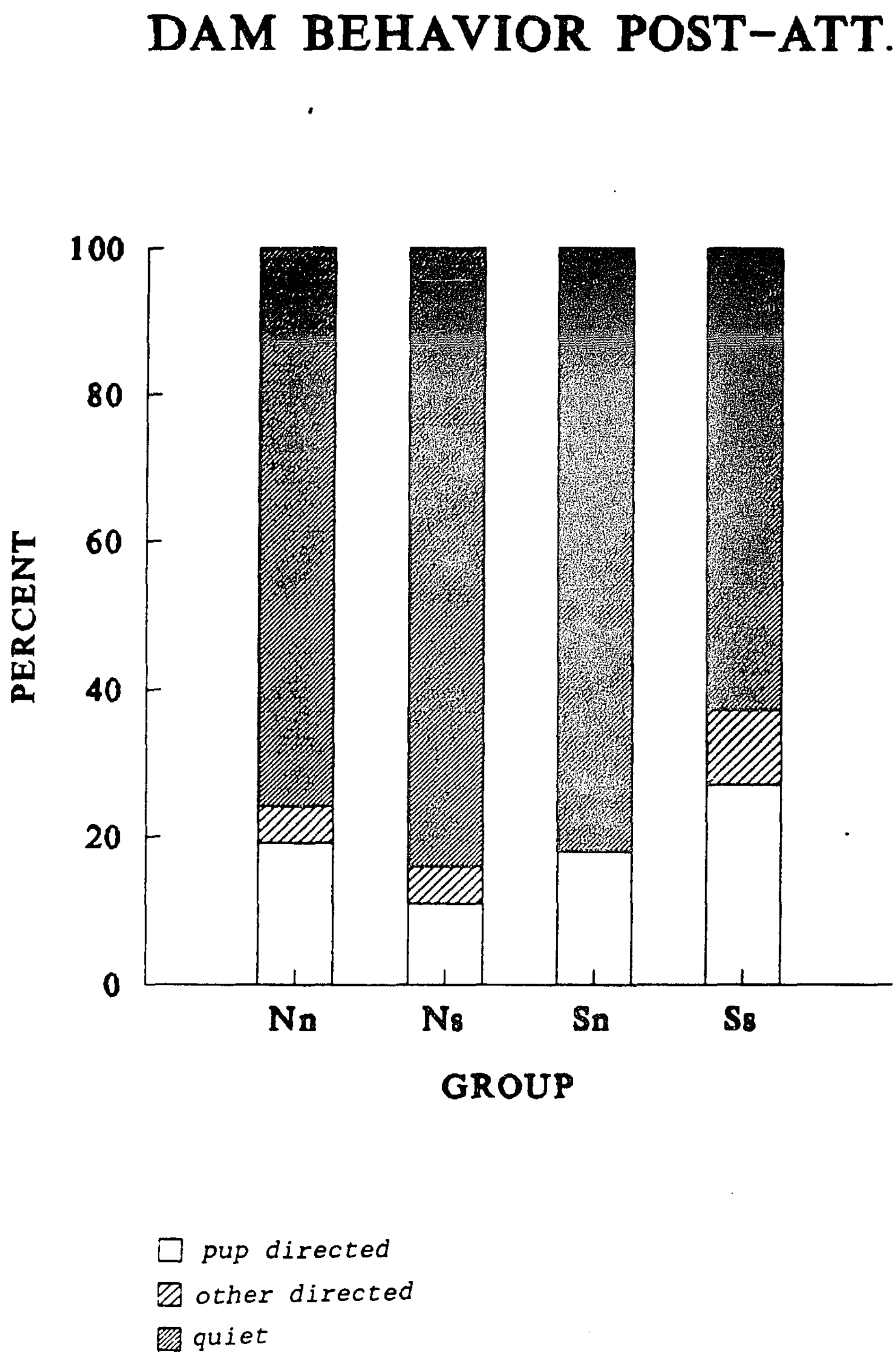
There was no significant difference between the dams of the Nn and Ss groups in their rate of pup directed behavior. Analysis across all four groups showed, however, that separated dams tended to have higher rates of pup directed behavior (Dam trend: $\underline{F}(1,19)=3.696, p<.071$) and the Nn and Ss groups had significantly higher rates of pup directed behavior than the disparate groups (Interaction effect: $\underline{F}(1,19)=5.430, p<.032$). As shown in Figure 27, the Ss group appears to have the highest rate of pup directed behavior, but post-hoc analysis found that it was only significantly higher than the Ns group, tended to be higher than the Sn group, and was not significantly different from the Nn group (post-hoc, Ss vs. Ns: $\underline{F}(1,19)= 9.495, p<.006$; Ss vs. Sn: $\underline{F}(1,19)=3.064, p<.097$). Because there is not one

Figure 27: **The mean percent time the dams of the 6 litters of Nn, Ns, and Ss groups and the 5 litters of the Sn group spent pup directed, other directed, or quiet during the first nursing bout.**

There was no significant difference between the Nn and Ss group in time the dam spent pup directed during the first nursing bout. Analysis across all groups with the 2 way ANOVA found an interaction effect and a dam trend. These results best fit a Model 3 of separation effects. (Note: without times of possible MEs, the 2 way ANOVA found only a dam trend. These results best fit a Model 1 of separation effects).

There was no significant difference between the Nn and Ss groups in time the dam spent other directed during the first nursing bout. The 2 way ANOVA found an interaction effect and a pup effect. These results fit a Model 6 of separation effects because the Ss group is significantly higher than the other groups.

There was a trend for the Nn and Ss group to be different in the time the dam spent quiet during the first nursing bout. The 2 way ANOVA found a significant interaction effect and a dam trend. These results fit a Model 6 of separation effects, because the Ss group is significantly lower than the other groups.


DAM BEHAVIOR POST-ATT.
100
80
60
40
20
0
PERCENT
Nn
Ns
Sn
Ss
GROUP
pup directed
other directed
quiet

unique interaction that is solely responsible for the significant effects found due to separation, these results do not fit the Model 6 of separation effects. Therefore, although a trend exists for the separated dams to have higher rates, these results best fit the Model 3 of separation effects which illustrates differences between similar-condition groups and the disparate groups.

When times of possible MEs were excluded from analysis in addition to the times of definite MEs, there was no significant difference between the Nn and Ss group in the rate of pup directed behavior. However analysis across all groups found that without the times of possible MEs, the interaction previously seen is lost and only a trend exists for separated dams to be more pup directed during the first nursing bout (Dam trend: $\underline{F}(1,19)=3.598, p<.074$), fitting a Model 1 of separation effects. This was a result of an increase in the mean rate of the Sn group from .18 to .20 pup directed behaviors per minute.

When the behaviors that make up the pup directed behavior were looked at individually, there were no significant differences found between the Nn and Ss groups in rate of AG licking or retrieval behaviors by the dam postattachment. However, the dams of the Nn group tended to body lick their pups more than the dams of the Ss group ($t(10)=2.036, p<.070$), a difference opposite that seen prior to the first nursing bout. Analysis across all groups found a significant pup and interaction effect due to the high rate of body licking in the Nn group (Pup effect: $\underline{F}(1,19)=5.993, p<.025$; Interaction effect: $\underline{F}(1,19)=6.688, p<.019$). As shown in Figure 28, these results fit a Model 6 of separation as the combination of a nonseparated dam with nonseparated pups increased the rate of body licking above all other groups (post-hoc, Nn vs. Ns: $\underline{F}(1,19)=13.305, p<.003$; Nn vs. Sn: $\underline{F}(1,19)=5.827, p<.027$; Nn vs. Ss: $\underline{F}(1,19)=5.913, p<.026$). It appears that the nonseparated dam licked the nonseparated pups more than the separated pups. The separated dams had a consistent rate of body licking regardless of what pup type they were with perhaps because they were less responsive to the pups, or responding to something else in the environment. It is interesting to note that the dams of the Ss group licked their pups' bodies more than the Nn group prior to the first nursing bout, and then less during the first nursing bout.

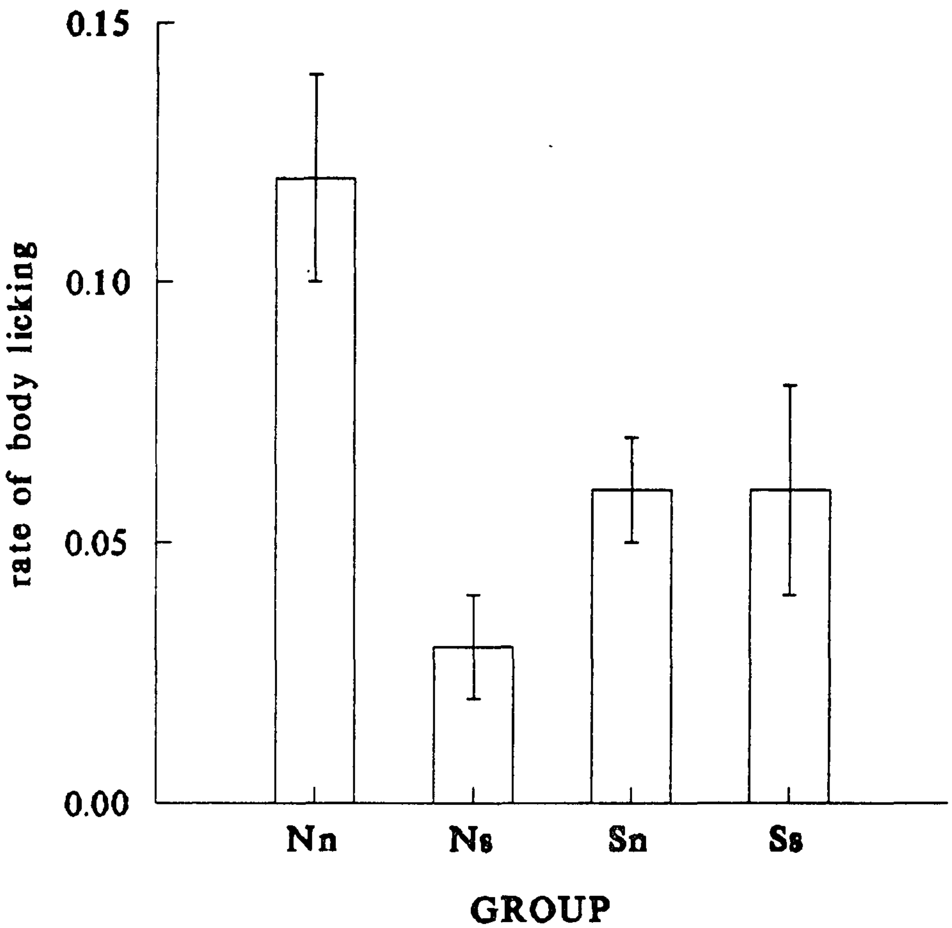
Other directed behavior:

There was no significant difference between the Nn and Ss groups in the dams rate of other directed behavior during the first nursing bout. Analysis across all four groups found that dams with separated pups were more other directed (Pup effect: $\underline{F}(1,19)=5.644, p<.029$), and the Ss group appeared to have higher rates of other directed behaviors than the other groups (Interaction effect:

Figure 28: The mean rate of body licking of pups by the dams during the first nursing bout of the 6 litters of the Nn, Ns, and Ss groups and the 5 litters of the Sn group.

There was a trend for the Nn and Ss groups to be different ($p<.070$). The 2 way ANOVA found a pup effect and an interaction effect. These results are a Model 6 of separation effects. The Nn group is significantly higher than the other groups.

BODY LICKING POST-ATT.



$\underline{F}(1,19)=6.036, p<.025$). As shown in Figure 27, these results apply best to the Model 6 of separation effects; the Ss group was significantly higher than the Sn group, and tended to be higher than the Ns and Nn groups (post-hoc, Ss vs. Sn: $\underline{F}(1,19)=11.147, p<.004$; Ss vs. Ns: $\underline{F}(1,19)=3.317, p<.085$; Ss vs. Nn: $\underline{F}(1,19)=3.107, p<.095$). The Ss group had the highest, and the Sn group the lowest rate of other directed behavior, while the Nn and Ns groups had equal rates, perhaps revealing that in this case the separated dam was more responsive to pups, and the nonseparated dam was more intrinsically controlled.

There were no significant differences between the Nn and Ss groups in rates of self-grooming, rearing, or changing of position. (Note: there were no occurrences of eating or drinking in any of the groups during the first nursing bout). Analysis across all groups found no dam or pup effects or an interaction between the two for rates of self-grooming or rearing. However, there was a trend for the dams with separated pups to have higher rates of changing of position, and there was a trend for an interaction to exist (Pup trend: $\underline{F}(1,19)=3.422, p<.081$; Interaction trend: $\underline{F}(1,19)=3.422, p<.081$). The Ss appeared to have the highest rate of this behavior, which would explain both of these effects, however the post-hoc analysis found that the rate of changing position of the Ss group was only significantly higher than the Sn group (post-hoc, Ss vs. Sn: $F(1,19)=6.533, p<.020$). When analysis was done excluding the times of possible MEs, the pup and interaction trends seen became significant (Pup effect: $F(1,19)=4.514, p<.048$; Interaction effect: $F(1,19)=4.279, p<.053$) and these effects were found to be contributed to mainly by a higher rate of position change in the Ss group (post-hoc, Ss vs. Sn: $F(1,19)=8.392, p<.010$; Ss vs. Ns (trend): $F(1,19)=3.891, p<.064$; Ss vs. Nn: $F(1,19)=4.053, p<.059$). The rate of changing position was therefore responsible for the differences seen in the overall rate of other directed behavior, including the increased rate of other directed behavior seen in the Ss group.

Quiet:

There was a trend for the dams of the Nn group to be more quiet than the dams of the Ss group during the first nursing bout ($t(10)=1.991, p<.075$). Analysis across all groups revealed that this was the result of a trend for nonseparated dams to be more quiet, and for the disparate groups to be more quiet (Dam trend: $F(1,19)=3.101, p<.095$; Interaction effect: $F(1,19)=10.202, p<.006$). These results are an example of Model 6 ANOVA results, as there the unique combination of the separated dam with separated pups (Ss) resulted in a decrease in amount of time spent quiet when compared to all other groups (post-hoc, Ss vs. Nn:

$F(1,19)=4.640, p<.045$; Ss vs. Ns: $F(1,19)=12.890, p<.003$; Ss vs. Sn: $F(1,19)=9.266, p<.008$) (see Figure 27). The rates of quiet behavior are confirmed by analysis excluding both possible and definite MEs.

Nursing behavior:

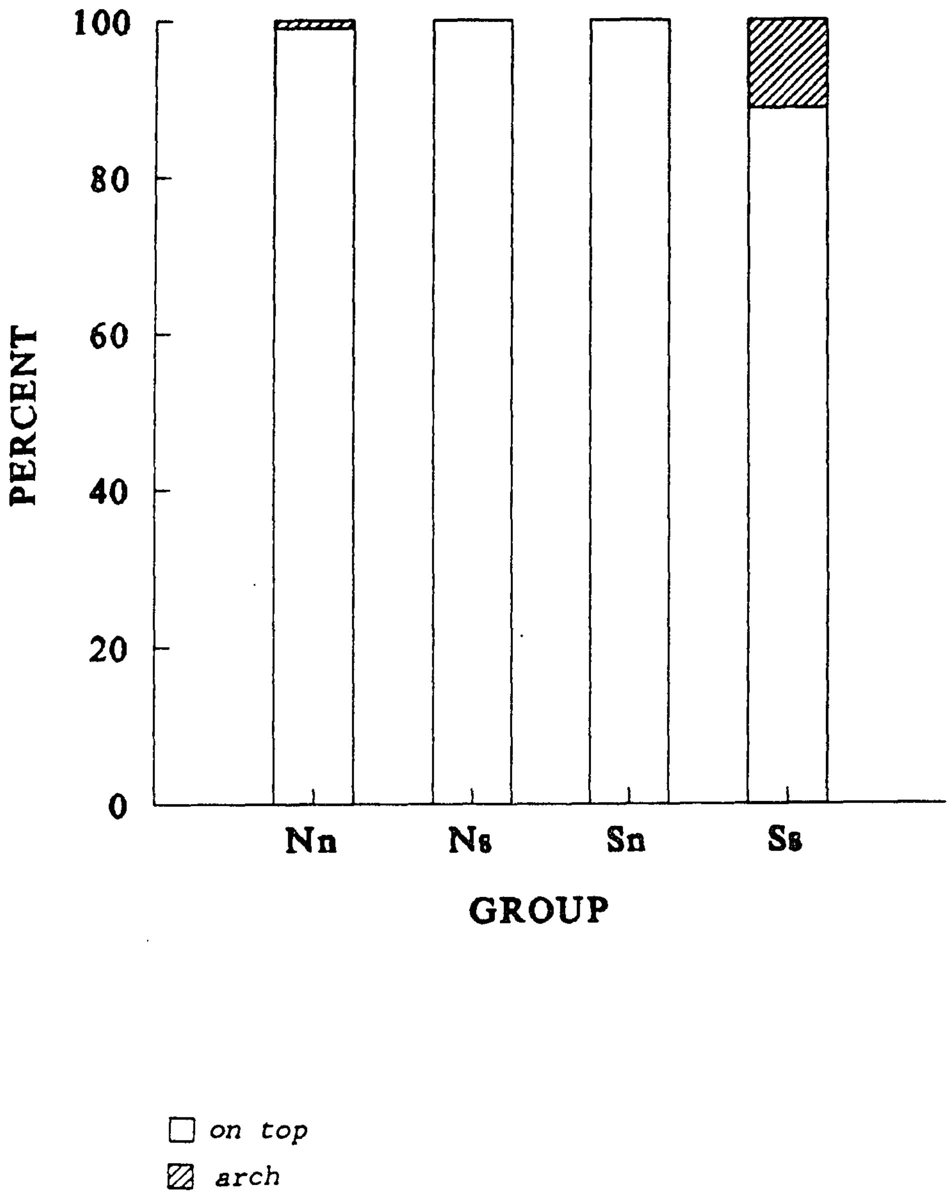
As shown in Figure 29, there was a trend for the Ss group to have higher rates of the arched nursing position and lower rates of the on top nursing position when compared to the Nn group (Arch: $t(10)=-1.903, p<.087$; On top: $t(10)=2.118, p<.061$). These results appear to be due to effects that fit the Model 6 ANOVA results; the Ss group spent more time arched and less time on top than all the other groups (On top, Pup effect: $F(1,19)=5.270, p<.034$; Interaction effect: $F(1,19)=5.542, p<.030$; post-hoc, Ss vs. Sn: $F(1,19)=10.324, p<.006$; Ss vs. Ns: $F(1,19)=8.106, p<.011$; Ss vs. Nn: $F(1,19)=7.862, p<.012$) (Arched, Dam trend: $F(1,19)=3.291, p<.086$; Pup trend: $F(1,19)=3.263, p<.088$; Interaction effect: $F(1,19)=4.785, p<.042$; post-hoc, Ss vs. Sn: $F(1,19)=7.614, p<.013$; Ss vs. Ns: $F(1,19)=8.407, p<.010$; Ss vs. Nn: $F(1,19)=6.882, p<.018$). Therefore, separation increased the rate of arched nursing while decreasing the rate of on top nursing, and this was particularly evident when a separated dam was reunited with separated pups.

<u>Figure 29</u>: **The mean percent time the dams of the 6 litters of Nn, Ns, and Ss groups and the 5 litters of the Sn group spent in the arched or on top nursing position during the first nursing bout when minutes affected by a definite ME were excluded from analysis.**

There was a trend for the Nn and Ss groups to be significantly different in percent time on top during the first nursing bout ($p<.061$). The 2 way ANOVA found an interaction effect and a pup effect. These results fit a Model 6 of separation effects, as the Ss group is significantly lower than the other groups.

There was a trend for the Nn and Ss groups to be significantly different in percent time arched during the first nursing bout ($p<.087$). The 2 way ANOVA found an interaction effect, a dam trend, and a pup trend. These results fit a Model 6 of separation effects, as the Ss group is significantly higher than the other groups.

NURSING POSITION W/O DEF. MEs



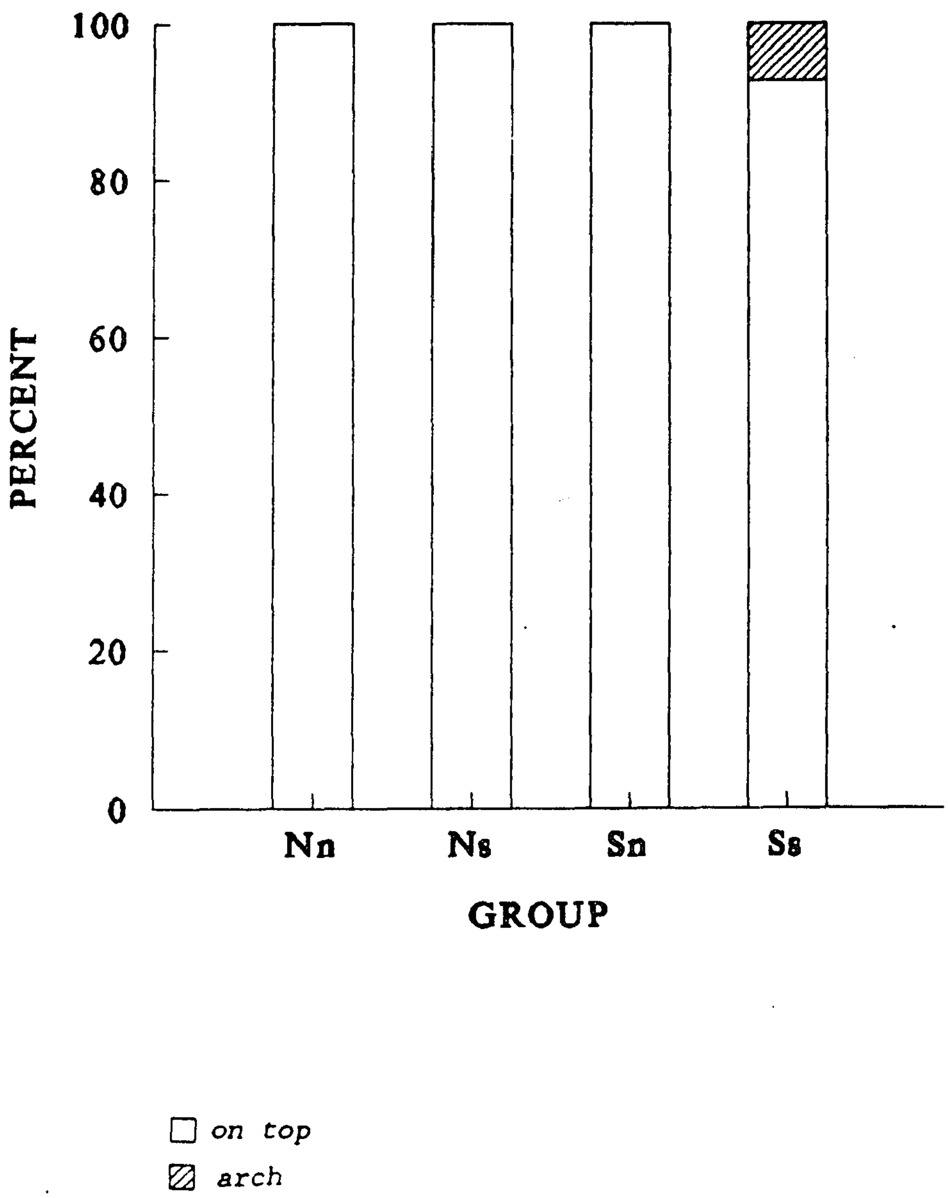
If the minutes associated with possible MEs were removed from the analysis the trends for differences between the Nn and Ss group disappeared (see Figure 30). Only the Ss group had periods of arched nursing not associated with either type of ME. Analysis across all groups for the arched nursing position found no effects of separation on the dam condition, pup condition, or an interaction between the two. Analysis across all groups for the on top nursing position found that only the interaction effect remained (Interaction effect: $\underline{F}(1,19)=4.433, p<.050$). These results fit a Model 3 of separation effects, depicting the disparate groups as having higher rates of on top nursing. However, it can be seen that it was the decrease in on top nursing in the Ss group (caused by occurrences in arched nursing) that was responsible for this effect, as all other dams were on top of their litters 100 % of the time possible when all MEs were excluded from analysis.

Figure 30: The mean percent time the dams of the 6 litters of Nn, Ns, and Ss groups and the 5 litters of the Sn group spent in the arched or on top nursing position during the first nursing bout when minutes affected by a definite ME or possible ME were excluded from analysis.

There was no significant difference between the Nn and Ss group in percent time on top during the first nursing bout. The 2 way ANOVA found an interaction effect. These results fit a Model 3 of separation effects.

There was no significant difference between the Nn and Ss group in percent time arched during the first nursing bout. The 2 way ANOVA found no effects.

NURSING POSITION W/O ANY MEs



Summary and Discussion

It was hypothesized that the separated dam might show signs of increased sensitivity to the pups' suckling, as her mammary glands were distended with milk, because it has been shown that in this state she has an increased sensitivity to oxytocin (DeNuccio and Grosvenor, 1971). This was proposed to be reflected in an increase in movement and delay in settling down to quiet nursing. Analysis did support this hypothesis as the Ss group was seen to change position more during nursing, and tended to be less quiet. It appears that the separated pups elicited higher rates of changing of position in the separated dam, perhaps due to the sensitivity of the dam, or perhaps because they were so activated to nurse the dams could not easily settle down. Prior to the first nursing bout separated pups were in contact and attached more, and less other directed. It remains to be seen if they continue to demonstrate this increase in behavior toward nursing during the first nursing bout, which would increase the dam's movement during nursing.

The increase seen in separated dams in arched nursing, a nursing position often associated with a let down of milk, supports the idea that separated dams were more sensitive to suckling and/or perhaps they were delivering milk between observed MEs. However, the Sn group had no occurrences of arched nursing when MEs were excluded from the analysis.

Therefore it might not be a sign of milk delivery between ejections because pups of the Sn group also gained more weight over the 2 hour period in comparison to pups with a nonseparated dam. Also, it is possibly not a sign of increased sensitivity because the Sn group was not observed to arch in times not associated with MEs, unless the nonseparated pups were not as activated as the separated pups with a separated dam, and therefore did not test the dam's sensitivity.

It is interesting to note that the dams of the Ss group licked their pups' bodies more than the Nn group prior to the first nursing bout, and then less during the first nursing bout. Also, nonseparated dams had the highest rate of body licking nonseparated pups (the Nn group) but had the lowest rate when with separated pups (the Ns group), while the separated dams had a lower rate of body licking than the Nn group but was equivalent with both separated and nonseparated pups. Perhaps prior to the first nursing bout, the separated dam licks her pups more to stimulate them to nurse sooner, and once attached they do not need to be stimulated to maintain the nursing posture because the increased supply of milk maintains their suckling behavior. It has been shown that separated pups maintain an increased level of sucking if milk is present (Brake and Hofer, 1980; Brake, Wolfson, and Hofer, 1979). The nonseparated dam however does need to lick her nonseparated pups more to stimulate them nurse and suckle during the first bout, but does not need to stimulate the separated pups as their deprivation has increased their sucking rate and amplitude.

Pup Behaviors:

As in the pup behaviors prior to the first nursing bout, the rates of dam directed, other directed, and quiet were rates of the focal pup's behaviors for each litter, within each group. Rates of contact with the dam and attachment were variables defined by the behavior of the litter as a whole.

Dam directed behavior:

As shown in Figure 31, the pups of the Ss group showed significantly more dam directed behaviors than those of the Nn group ($t(10)=-4.741, p<.002$). Analysis across all groups demonstrated that this was a result of increases of dam directed behavior by pups with separated dams, and an increase of dam directed behavior by separated pups (Dam effect: $\underline{F}(1,19)=6.429, p<.021$; Pup effect: $\underline{F}(1,19)=12.661, p<.003$). These results are an example of Model 4, as the increases seen in dam directed behavior in conditions with a separated dam or separated pup are combined in the Ss condition, thus the separated effects are additive.

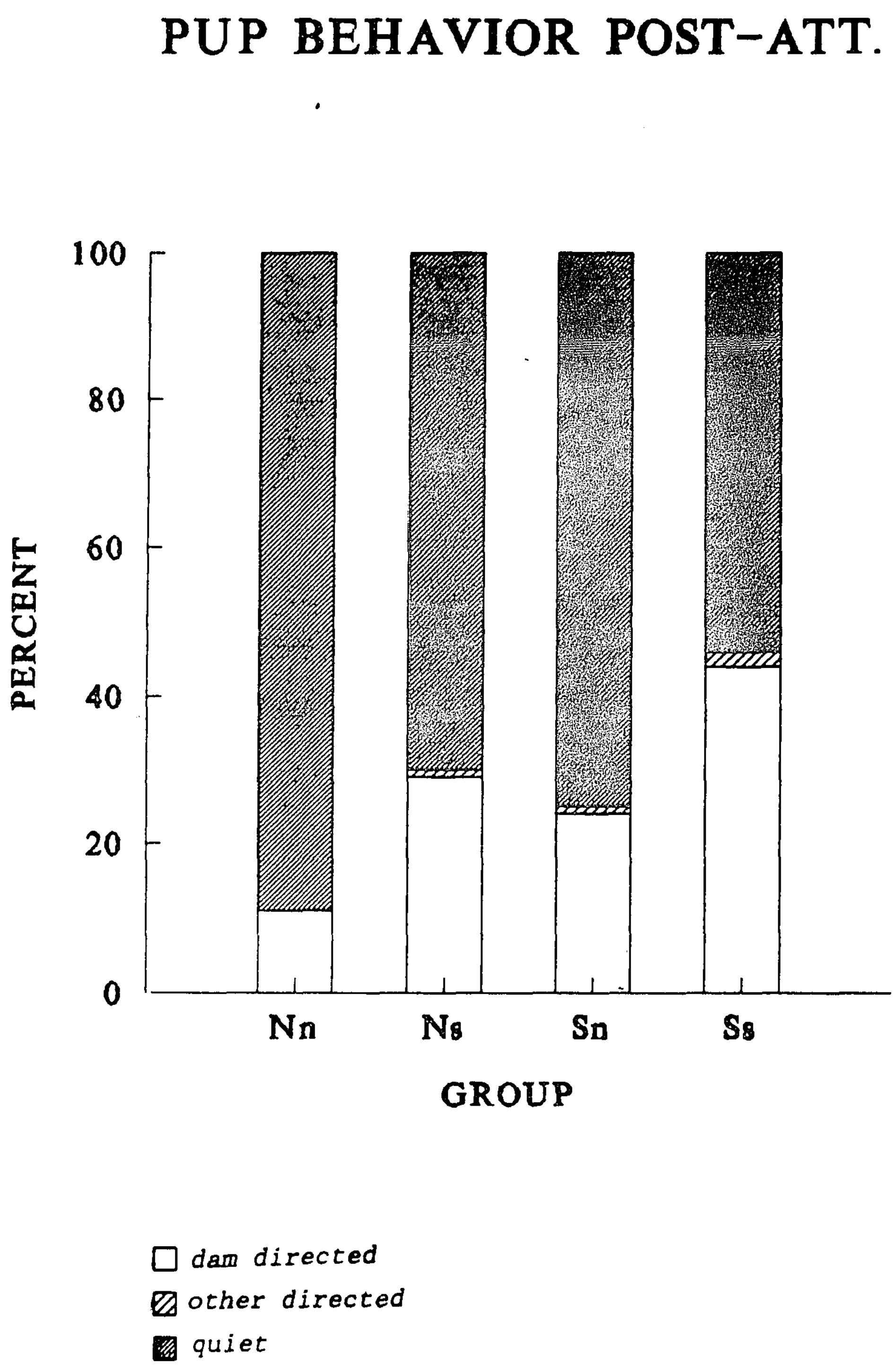
The pups of the Ss group had a higher rate of nosing and probing the dam's ventrum than the pups of the Nn group ($t(10)=-2.557, p<.030$). Analysis across all groups revealed that this was a result of a trend for separated pups to have higher rates than nonseparated pups (Pup trend: $\underline{F}(1,19)=3.143, p<.093$). This is an example of a Model 2, as the separation effects seen are due to changes in the pup condition. When possible MEs were excluded from the analysis, the separation effects seen are due to changes in both the dam and pup condition as in Model 4; pups with separated dams tend to have higher rates of nosing and probing, and separated pups tend to have higher rates of

Figure 31: The mean percent time the pups of the 6 litters of Nn, Ns, and Ss groups and the 5 litters of the Sn group spent dam directed, other directed, or quiet during the first nursing bout.

There was a significant difference between the Nn and Ss group in rate of dam directed behaviors by the pups during the first nursing bout ($p<.002$). Analysis across all groups with the 2 way ANOVA found a dam effect and a pup effect. These results best fit a Model 4 of separation effects.

There was no significant difference between the Nn and Ss groups rate of other directed behaviors by the pups during the first nursing bout. The 2 way ANOVA found no effects.

There was a significant difference between the Nn and Ss groups in time the pup spent quiet during the first nursing bout ($p<.001$). The 2 way ANOVA found a dam effect and a pup effect. These results fit a Model 4 of separation effects.


PUP BEHAVIOR POST-ATT.
100
80
60
40
20
0
PERCENT
Nn
Ns
Sn
Ss
GROUP
dam directed
other directed
quiet

nosing and probing (Dam trend: $\underline{F}(1,19)=3.088, p<.096$; Pup trend: $\underline{F}(1,19)=3.079, p<.096$).

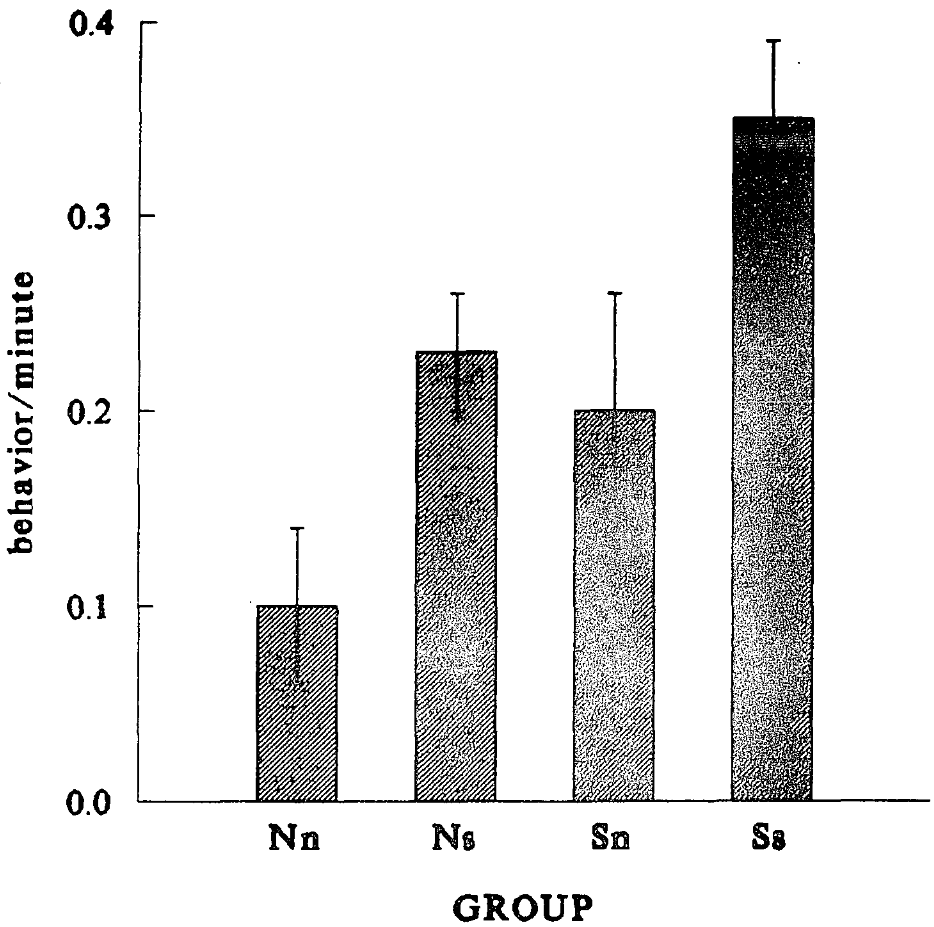
There was no difference between the pups of the Ss and Nn groups in the rate of mouthing and licking the dam's teat. However, analysis across all groups found a trend for separated pups to mouth and lick the dam's teats more than nonseparated pups (Pup trend: $\underline{F}(1,19)=3.143, p<.093$). This effect fits a Model 2, as the changes seen due to separation were contributed by changes in the pup condition. These changes were slight, as there was only a trend for separated pups to mouth and lick the teats more, and this trend was lost when analysis was done excluding possible MEs.

There were no significant differences between the pups of the Ss or Nn group in rate of changing of position once in contact. Analysis across all groups found no separation effects on the dam or pup condition, or an interaction between the two. This dam directed behavior might be considered less relevant for behavioral analysis once the pups are attached, however separated pups were shown to have episodes during the first nursing bout in which partial nursing occurred for 3 minutes or less during which time these behaviors could have occurred, yet separated pups were not different in position change in contact.

As shown in Figure 32, pups of the Ss group had a higher rate of treadling than the pups of the Nn group ($t(10)=-4.386, p<.002$). Analysis across all groups

Figure 32: The rate of treadling during the first nursing bout of the pups of the 6 litters of Nn, Ns, and Ss groups and the 5 litters of the Sn group. There was a significant difference between the Nn and Ss group ($p<.002$). The 2 way ANOVA found a dam effect and a pup effect. These results fit a Mode 4 of separation effects.

TREADLING BY PUPS



determined that this effect was due to an increase in treadling of pups with separated dams, and an increase in treadling by separated pups (Dam effect: $F(1,19)=6.069, p<.024$; Pup effect: $F(1,19)=10.570, p<.005$). These results best fit Model 4 representing the additive model of separation effects, with the Ss group having the highest rate. These results were present when the times of possible MEs were excluded so differences in treadling must be present during minutes without any MEs. The treadling behavior just described appears to be the main contributor to the overall increase in dam directed behavior seen in pups with separated dams and in separated pups.

To determine the effect of treadling rate on the weight gain of the pups, correlations of the treadling rate and the weight gain over two hours were done within groups and across groups. Although there were no significant correlations within any of the groups, a positive relationship was found when all the groups were combined (Pearson $r=.427(24), p<.05$).

Other directed behavior:

There was no significant difference between the Ss group and the Nn group in the rate of other directed behavior by the pup. Analysis across all groups also found no effects of separation. As shown in Figure 31, the rates of other directed behavior were very low or zero for all groups. Higher rates of this behavior would have been unusual as this behavior consists of pup movement away from the dam which is not common during a nursing bout.

Quiet:

The pups of the Ss group had lower rates of quiet epochs than the pups of the Nn group ($t(10)=5.770, p<.001$). Analysis across all groups determined that pups with separated dams were less quiet, and separated pups were less quiet (Dam effect: $\underline{F}(1,19)=7.732, p<.013$; Pup effect, $\underline{F}(1,19)=14.553, p<.002$). These results fit a Model 4 of separation effects as the decreases seen in time spent quiet by separated dams and separated pups are additive, with the Ss group having the lowest rate of quiet time (see Figure 31). These results were consistent with the results obtained when analysis is done without the time of possible MEs.

Summary and Discussion

It was hypothesized that separated pups were sucking harder to stimulate milk release, or treadling more to actually mechanically induce contractions of the mammary glands and thereby release milk. Brake, Shair, and Hofer (1988) stated that separated pups stay awake longer following MEs, perhaps contributing to their increased intake. In support of this hypothesis, separated pups were found to be less quiet and more dam directed, treadling more once they were attached. Separated pups also had higher rates of nosing and probing the dam's ventrum, and mouthing and licking the dam's teats, due to the fact that only separated pups had disruption periods, or times of unattachment during the first nursing bout when these behaviors could occur.

The increase in treadling by separated pups was found to be expressed most when pups were with a separated dam. Perhaps these pups treadled more because they were activated by the increased milk supply available, or were inducing the mechanical contractions described previously, which can release milk in a distended mammary gland. As noted before, pups with separated dams were probably ingesting milk during the times possible MEs were observed, which would contribute to their greater weight gain. But pups with a separated dam may be also be ingesting milk when no MEs were observed, as reflected by their decreased quiet behavior, and increase of treadling during periods without definite or possible MEs. Treadling was found to be positively correlated with weight gain when analyzed across all groups, the lack of significance between treadling and weight gain within the Ss group was probably due to the small n available (n=6).

CONCLUSION

The Ss group was different from the Nn group in many ways. Briefly, they were disrupted and highly aroused, gluttonous, and heat producing. The interaction that occurred upon reunion flowed as follows. Upon reunion, the pups were cool, due to the combination of their deprived state and lack of thermogenesis during the manipulation immediately prior to the reunion. The dams appeared very interested in stimulating the pups to nurse as they were less other directed, and licked the pups' bodies more prior to the first nursing bout. During this time, the pups also appeared very interested in nursing, as they moved toward the dam's ventrum, increased their contact with the dam, and were even attached although not in consecutive minutes. Although both the dam and pups appeared to have an increased motivation to nurse, there was no alteration of the latency to the first nursing bout. The pups were thought to be hyperaroused, because the separated dam was not preventing them from attaching but rather was stimulating them to do so. Of course, she might have been hypersensitive to the suckling attempts, and was not comfortable when they frantically attached. Once attached, during the first nursing bout, there were periods of disruptions suggesting that the hyperarousal of the pups and hypersensitivity of the dam continued. Further support for this is that the pups treadled more and were less quiet, and the dams were seen to change position more and be less quiet. There was no difference in latency to the first let down of milk, if latency was measured to the first definite or possible ME. The possible MEs were believed to be an actual let down of milk, as supported by the positive correlation between possible MEs and weight gain, and more possible MEs were released during the first nursing bout and over the 2 hour period contributing to the pups' increased weight gain. It has been shown that deprived pups increase the rate and amplitude of their sucking. The separated pups were perhaps doing this, stimulating more oxytocin release to which the separated dam was more sensitive, thereby increasing the rate of milk release. The pups were also treadling more possibly inducing contractions of the mammary gland mechanically, which has shown to be quite possible in dams with distended, full mammary glands. The contribution of treadling to milk release is also supported by a positive correlation between rate of treadling and weight gain. The bout did not last longer than the nonseparated bouts, nor were there more bouts in the two hour period, nor an increase in nursing time. Therefore, it is through these possible MEs and perhaps milk let down between observed ejection responses that contribute to the pups' increase in weight over time. More milk was supplied at first, which then decreased in amount over the two hour period, and the ME rate of the dam does decrease from hour 1 to hour 2.

After the reunion, in addition to the pups' increased weight gain, they also were much warmer as a result of their contact with the dam as well as their consumption of warm milk. Over the next 22 hours, these pups lose weight due to the separated dams' decreased milk supply.

Analysis comparing all four groups, the Nn, Ns, Sn, and Ss, found that some of the changes seen due to separation were clearly due to changes in the dam condition or pup condition. The groups were not just unique interactions. For example, separated dams delivered more possible MEs during the first nursing bout, and over the two hour period regardless of what pup types they were with. This increase in possible MEs in the separated dam condition was thought to be due to her increased milk supply, as they were probably an actual let down of milk. This increase in milk supply increased weight gain of the pups with separated dams during the first two hours of reunion, but once depleted the pups lost weight over the next 22 hours. These weight changes were clearly a result of separation changes in the dam's condition, as separation affected her milk supply.

An example of a separation effect due to an alteration of the pups' behavior is the occurrence of disruption periods in during the first nursing bout, as they occurred in interactions with separated pups regardless of what dam type was present. Also during the first bout, separated pups treadled more and were less quiet. Therefore, these aroused behaviors of the separated pups during the first nursing bout were the result of the separation experience on the pups, as they were seen in interaction with both the separated and nonseparated dam.

The interaction of the Ss group, in contrast to the Nn group, was a mixture of expected and unexpected results. It was surprising that the separated pups did not nurse more over the two hour reunion period as a result of their cooler temperatures. This was predicted due to the study by Jans and Leon (1983) which demonstrated that contact time between a dam and her pups increased when pups were cool, consistent with the Leon hypothesis that nesting time is regulated by maternal temperature and it would take cooler pups longer to create the increase in dam temperature which would curtail the nursing time. Therefore, our results suggest that under these experimental conditions, something other than maternal temperature is regulating nest time. We found that the Ns, Sn and Ss groups nursed more than the Nn group (Model 6, Nn spent the least time nursing during the two hour period). Stern and Johnson (1990) have recently proposed that ventral somatosensory stimulation of the dam by the pups' nosing and probing and attaching, is critical to maintain the dam's nursing posture. Our results are consistent with this theory, as separated pups were shown to be very active during the first nursing bout, treadling and nosing and probing, and perhaps were able to maintain the dams' nursing position in the Ns and Ss groups longer than that seen in the Nn group. The increased nursing time in the Sn group relative to the Nn group, is perhaps a result of the separated dam being more sensitive to the pup stimulation, as these dams were found to change positions more and spend less time quiet during the first bout.

It has been demonstrated that upon reunion, separated pups engage in more frequent and vigorous sucking (Brake and Hofer, 1980; Brake, Wolfson, and Hofer, 1979). Also, deprived pups have been shown to have altered sleep- wake patterns which result in an increase in time spent in states with higher sucking rates (awake and slow wave sleep) (Shair, 1986). Due to these changes in sucking and sleep-wake states, we hypothesized that separated pups would consume more milk regardless of what dams they were with. We found no difference between pup conditions in weight change during the two hour period, probably due to the masking of any differences by the increase in milk supplied by the separated dam. This is consistent with Friedman (1975) who has shown that the build up of milk in the separated dam's mammary glands increases the intake of pups upon reunion regardless of their deprivation experience. However, over the next 22 hours of reunion, separated pups did gain more weight than nonseparated pups when with a nonseparated dam, and lose less weight when with a separated dam. Although we didn't measure sucking, we found that separated pups were active during nursing, treadling, nosing and probing, and creating disruptions. Perhaps these changes in behavior were a reflection of separated pups sucking harder and longer, and staying awake longer, thereby increasing their milk consumption.

It was surprising that the Ss group did not attach faster, have a shorter latency to the first ME, or shorter IMEIs. It has been shown that deprivation of pups decreases their latency to attach to an anesthetized dam (Drewett, Statham, and Wakerley, 1974; Hall, Cramer, and Blass, 1977). Also, Lincoln, Hill, and Wakerley (1973) found a decrease in latency to the first ME, and a decreased IMEI in litters that had previously been separated for 18 hours. However, we found none of these effects. It is possible that the increased motivation of the separated dams and pups to nurse was productive in that the interaction was highly intense, but was counterproductive in producing the quiet nursing interaction needed for milk transfer. Lincoln, Hentzen, Hin, van der Schoot, Clarke, and Summerlee (1980) found that the dam needs to be in slow wave sleep before the milk ejection reflex can occur. Similarly, Shair (1986) has found that pups also need to be asleep prior to the milk ejection. Therefore, the most efficient reunion of the Ss group in this experiment would have consisted of an increased arousal toward attachment, and then once attached, an immediate quieting down of both the dam and pups so that milk transfer could occur. Perhaps the hyperarousal of both the dams and pups prevented the expected decrease in ME latency and IMEI from occurring, and perhaps even prevented a decrease in attachment latency from occurring because their behaviors were too agitated.

Normally in the rat, the transfer of milk is restricted to periods of active milk ejections stimulated by oxytocin release, and the young display the characteristic stretch response while the dam arches. However, what we saw was the occurrence of possible MEs, during which either the dam did not fully arch or the pups fully stretch, but which was most likely a let down of milk. Perhaps these possible MEs that occurred in the separated dam condition were periods of milk let down when the dam was not in slow-wave sleep. The presence of these possible MEs was unusual but consistent in the separated dam condition, and it appears that these MEs contributed to the separated dams' increased delivery of milk to her pups. Another unexpected finding was that the condition of pups was able to affect the ME pattern. The Ss group had the highest rate of MEs during the first hour, contributed to not only by the dam condition, but the pups' condition as well.

When looking at all of the effects of separation on the dam-pup interaction in terms of them being an evolutionary advantage or disadvantage, the results appear ambiguous. Some alterations appeared to have an evolutionary advantage, creating a more efficient reunion. Changes that support this include the increased consumption of milk by the pups over a time period equivalent to that seen in the nonseparated interaction. This result was possibly contributed to by the increase in possible MEs seen over the 2 hour period, and during the first nursing bout. Changes in both the dam and pup condition contributed to the increased number of possible MEs that occurred, and the additive nature of these changes could be considered support for changes beneficial to a reunion. Also, the behaviors that demonstrated an increase in motivation to nurse were changes that may have contributed to the increased milk consumption by pups. These include the dams' increased rate of body licking and the pups' increased time spent in contact with the dam prior to attachment, and an increase in arched nursing and pup treadling once attached. On the other hand, these changes may be seen as hyperarousal of the dams and pups and preventative of an increase in efficiency during the interaction, as there was no decrease in latency to attachment, to the first ME, or IMEI. These changes therefore are paradoxical.

Other changes support the alternative view that the changes that were occurring due to separation were changes for adaptation to separation, or to independence. In the dams' case, this includes the ceasing of milk production after the pups' suckling stimulus is removed. This is reflected in the pup weight loss seen between hours 2 and 24 when with a separated dam. Theoretically, the sooner lactation is stopped when a dam becomes separated from her pups, the sooner she can become receptive and reproduce again, thereby investing her time and energy into creating a new litter rather than maintaining maternal behavior to help the separated pups if they are ever reunited.

Another possibility is that changes seen due to separation in the pups' case were just symptoms of the lack of interaction, and a were representative of a gradual fading of control mechanisms, until death. In other words, perhaps these changes had no function at all. At this age, there is little chance of the pups surviving a permanent separation. At this age they would need a constant supply of heat, in addition to a supply of milk or food they could consume. This is quite unlikely as their consumption of solid food is minimal to none and they are not quite old enough to forage for it. Nor can they protect themselves against predators. They are completely dependent on the dam for warmth, food and protection, and perhaps if their dam disappears, they just slowly die.

Although the results appear ambiguous when examined for changes that were advantageous for reunion or not, it was true that the stress of separation altered the mother-infant interaction. Perhaps what we are seeing, rather than a completely effecient reunion, or a disrupted reunion, is a combination of the two due to the fact that not all measures were actively selected for during separation in preparation for reunion, and the withdrawal of regulators of the mother-infant interaction also increases the individual variability in reponse to situations.

EXPERIMENT 2

INTRODUCTION

The goals of Experiment 1 were to characterize the mother-infant interaction upon reunion after a period of separation, and to determine the relative contributions of the dam and the pups to the changes seen. The latter goal is quite difficult, as the contributions of the dams and pups within an interaction are complex due to the interactive or interresponsive quality of the dam-pup relationship. For example, if a dam was observed to be eating, not nursing, it could be inferred that the dam was hungry enough to prioritize her eating over the feeding of the pups. However, perhaps the pups had not attached to her ventrum when made available and thereby signaled the dam that they were already satiated giving her the freedom to leave the nest and perform other behaviors. In the latter example, the pups were contributing greatly to the direction of the dam's behavior.

Experiment 2 studied the contributions of the dam to the alterations seen in the mother-infant interaction upon reunion after a period of separation while the pups were anesthetized thereby eliminating their behavior, and hopefully some of the complexity of the interaction. This experiment asks 4 main questions listed below.

1) What dam behaviors are dependent on an interaction with active pups and therefore will not occur at all in this

experiment? For example, the differences between separated and nonseparated dams in their pups' 2 hour weight gain or delivery of possible MEs will not be seen.

2) What changes in dam behavior due to separation are dependent on the active characteristics of pups? Differences between separated and nonseparated dams for these behaviors would therefore not be seen in this experiment. For example, perhaps there will be no increase body licking by separated dams, as see prior to the first nursing bout in Experiment 1. If body licking was functioning to stimulate the pups to nurse, it is possible that the lack of response by the anesthetized pups would decrease or eliminate this behavior. Or perhaps both the separated and nonseparated dam with anesthetized pups will increase their rate of body licking of the pups in an attempt to stimulate them, and therefore this behavior would not differentiate between dam conditions.

3) What changes in interaction due to separation are mediated by the passive characteristics of pups? In this experiment pup temperature, smell, urine composition or taste may vary due to separation and elicit different behaviors from the dam. For example, if as in Experiment 1, the separated pups are cooler upon reunion perhaps they would be seen to elicit an increase in contact time. In Experiment 1, cooler pups did not attach faster, have a longer first nursing bout, or nurse more over the entire two

hour period. However, perhaps if the pups were anesthetized the measure of contact would be increased because the interactive behaviors associated with the transfer of milk have been eliminated.

4) What behaviors occur that are unique to the interaction of dams with passive pups? For example, dams might be seen to cannibalize the unresponsive pups. This behavior is known to occur when a pup is the runt of the litter, sick, or dead, and perhaps the passivity of the entire litter would elicit it.

The procedure of anesthetizing a partner of the mother-infant interaction to tease apart the behaviors of the dams and pups has been used with success. Multiple studies have measured a particular pup behavior on an anesthetized dam. For example, latency to attach and milk intake after deprivation have been measured on anesthetized dams (Cramer and Blass, 1983; Hall, Cramer, and Blass, 1975; 1977). By eliminating dam behavior or completely controlling for it the accuracy of measurements of the dependent variables was increased.

Studies of dams' behaviors with anesthetized pups have been less frequent but have shown that information can be obtained, as described below. In a series of experiments Stern and Johnson (1990) have suggested the importance of somatosensory stimulation by the pups in the maintenance of nursing behavior. They found that dams were shown never to crouch actively over their pups if the pups were anesthetized, or pups whose mouths had been sewn shut to prevent active rooting and suckling. Brewster and Leon (1980) demonstrated that between postnatal day 0 and 14, if the nest was disturbed, the transport of pups to a new nest location was not affected by anesthetizing the pups, concluding that active pup behavior was not necessary for the dams' retrieval response. Other studies have shown that retrieval patterns vary depending upon pup activity. Stern (1985) and Beach and Jaynes (1956a) determined that fewer dead pups than live pups were retrieved during the first ten minutes of reunion, but Stern and Johnson (1990) determined that the total retrieval of dead litters was shown to occur faster than that of live litters once the first pup had been retrieved. Therefore pup activity decreased the latency to the initiation of the retrieval process, but might actually have prolonged the completion of the retrieval of the entire litter to a new nest site. These studies have demonstrated what role active pup behavior has in maintaining or eliciting a particular maternal behavior.

METHODS

Experiment 1 was replicated except that the pups were anesthetized. The four conditions were:

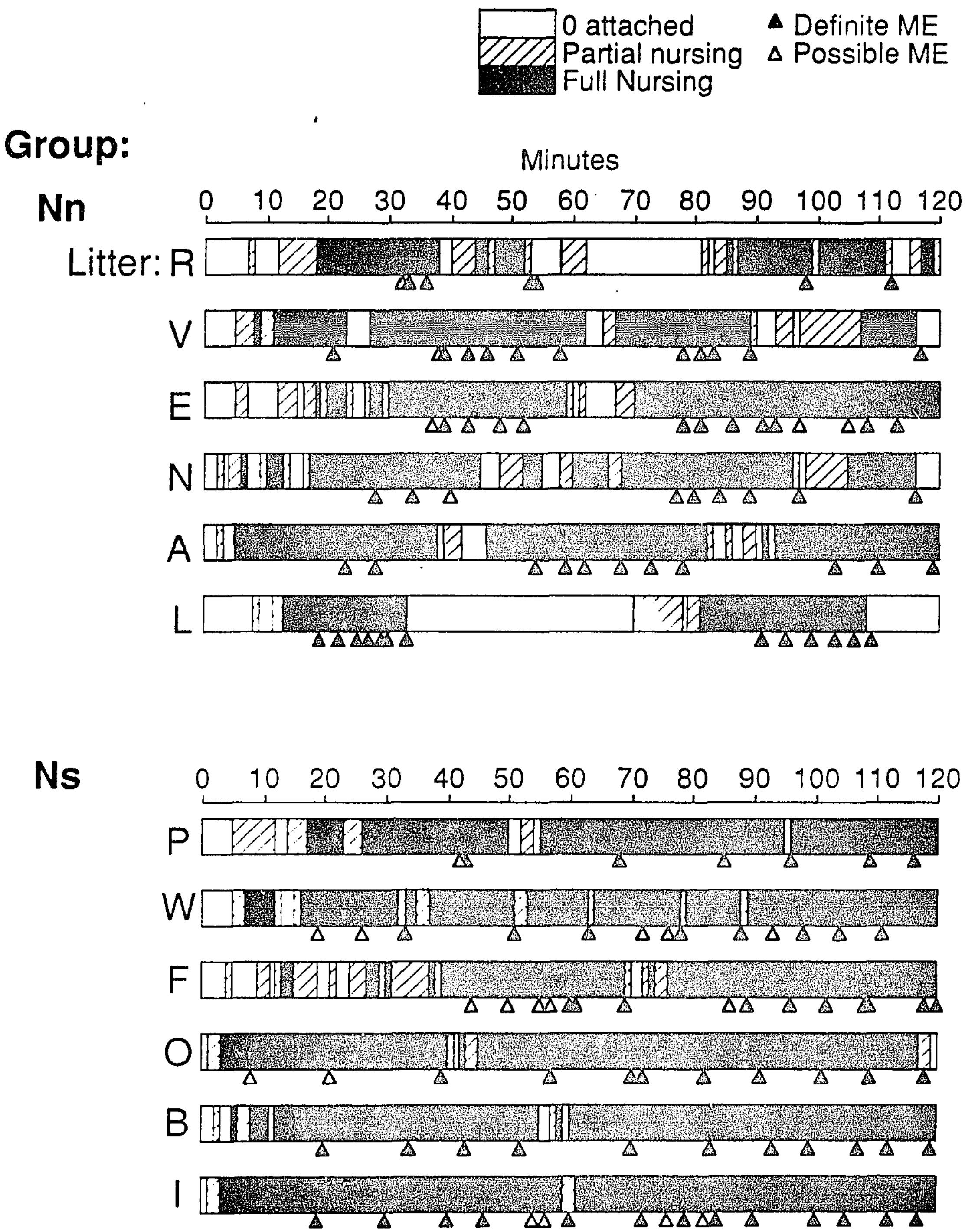
1. A nonseparated dam with nonseparated, anesthetized pups, Nna
2. A nonseparated dam with separated, anesthetized pups, Nsa
3. A separated dam with nonseparated, anesthetized pups, Sna
4. A separated dam with separated, anesthetized pups, Ssa

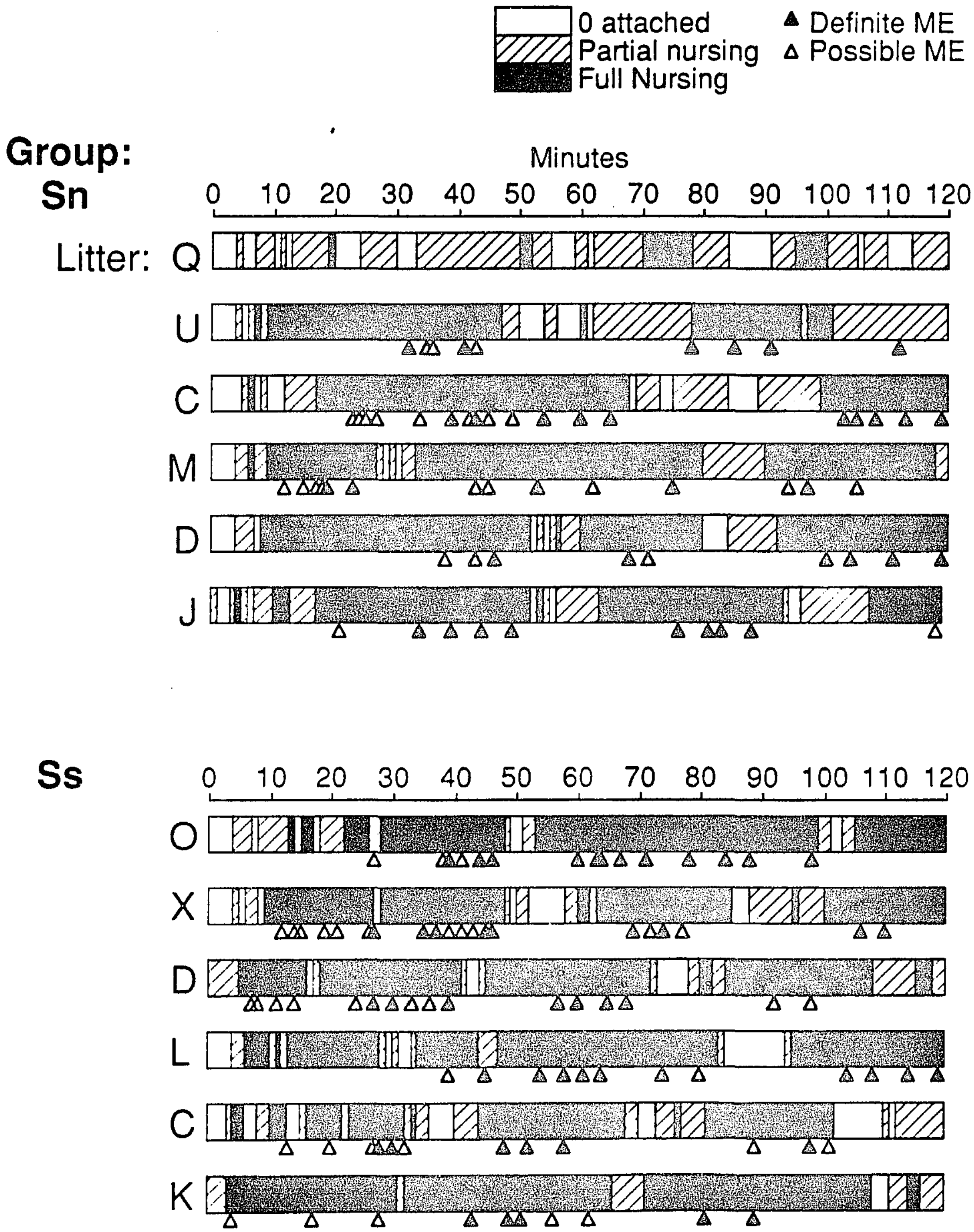
After the stimulating, weighing and marking of each pup at the end of the treatment period, each was given .01 mg/kg of urethane subcutaneously. Pups were then placed in a pile in their home cage, axillary temperatures of two pups taken, and the dam returned for the reunion period. Observations of the dams behaviors were made for two hours. In addition to the previously listed behavioral observations, the dam was also noted as being pup directed if she was observed to nose and probe the pups, or lick and probe the pups. These behaviors seemed to be elicited by the anesthetized pups perhaps because they were unresponsive. If the dam began cannibalizing the pups, she was immediately removed from her pups, and observations of that condition were stopped. At the end of the two hour period, the axillary temperatures of the pups were taken and the test was terminated. The four conditions listed above were tested simultaneously in six trials, creating a total of six litters per condition.

ANALYSIS

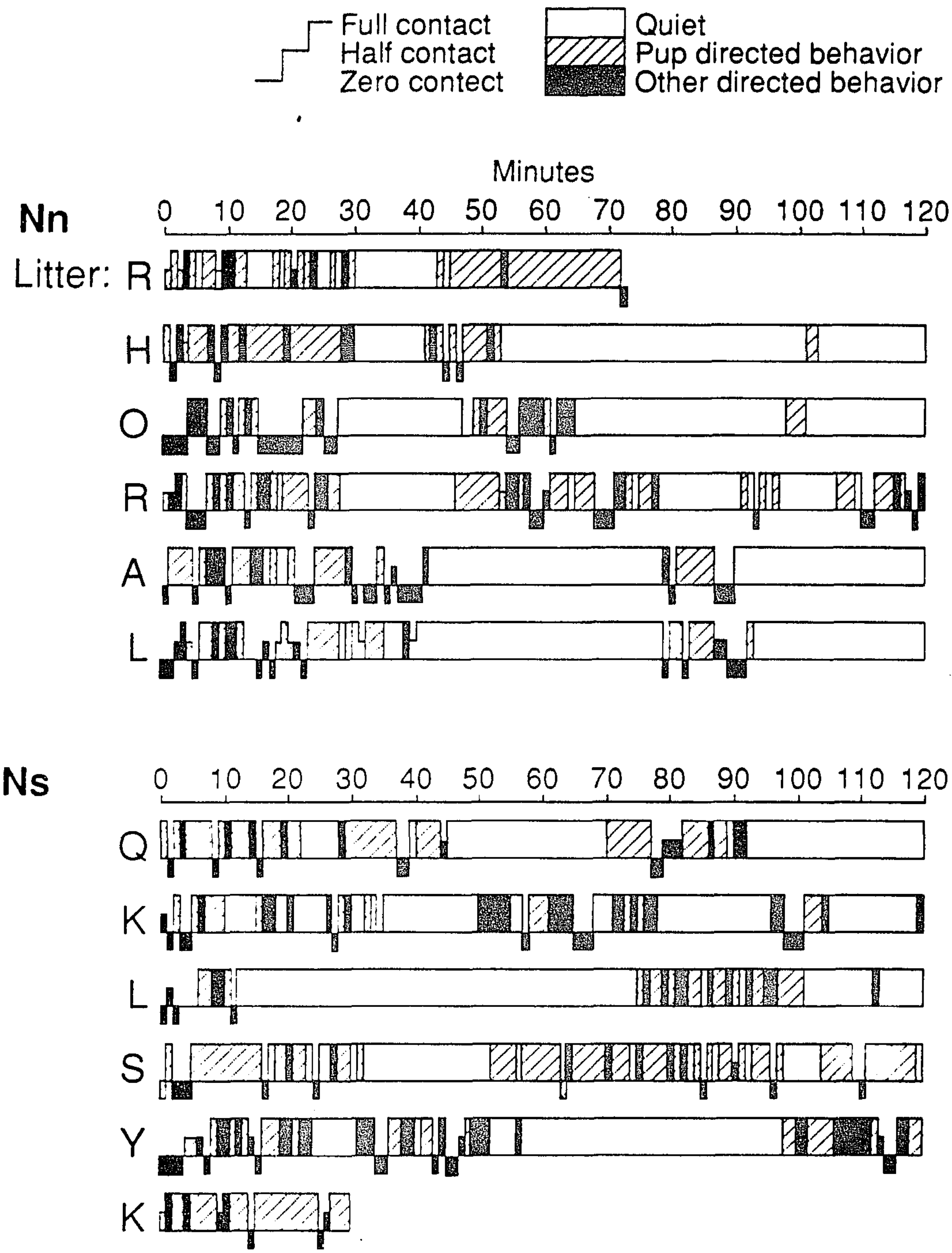
Analysis was done for behaviors totaled over 2 time periods: the first 10 minutes of reunion, and the total two hour observation period. The first 10 minutes of the reunion were analyzed to examine the time when the dams were first exposed to the pups' sleep-like condition. Ten minutes was long enough to include a measure of full contact for all of the litters, but not so long that a stable behavioral pattern was observed. During the first 10 minutes one might expect that the dams would attempt to stimulate the pups and try to initiate nursing, however after the 10 minute point, the dams might have lost their expectancy to nurse and be settling down into behavior directed elsewhere. We expected that the first ten minutes of this experiment would include such behaviors because it is comparable to the period prior to the first nursing bout in Experiment 1. Only 1 litter in Experiment 1 had begun their first bout prior to the tenth minute of reunion. Figures 33 and 34 are histograms of Experiment 1 and 2 consecutively. From them one can see the complexity of data we are attempting to analyze. These figures will be referred to again later.

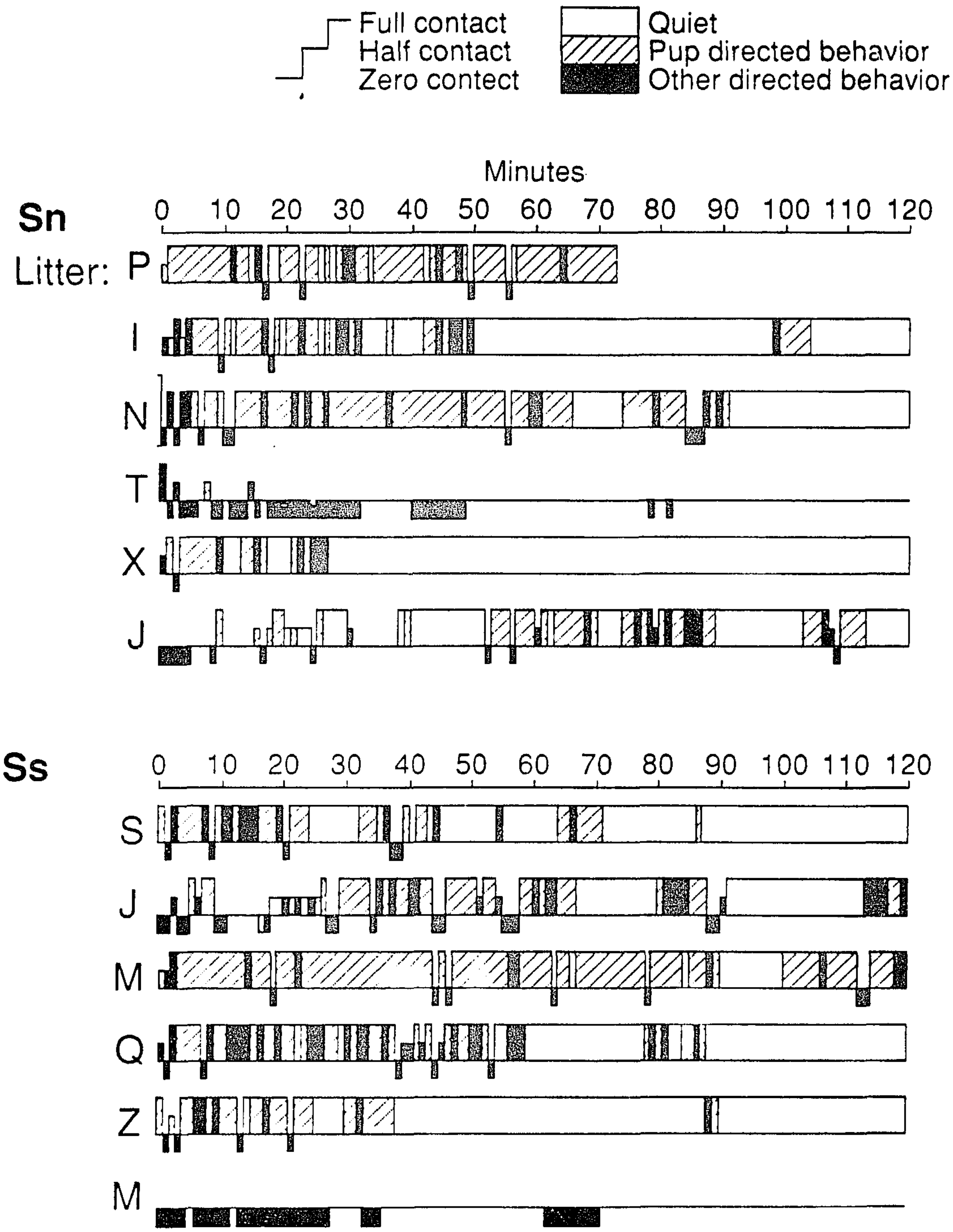
Figure 33: Histograms of the 6 litters per group in Experiment 1 over the 2 hour reunion period, done minute by minute. Attachment levels (none, less than half the litter, or more than half the litter) and time of definite and possible MEs are represented.


0 attached
Partial nursing
Full Nursing
Definite ME
Possible ME
Group:
Minutes
Nn
0 10 20 30 40 50 60 70 80 90 100 110 120
Litter: R
V
E
N
A
L
Ns
0 10 20 30 40 50 60 70 80 90 100 110 120
P
W
F
O
B
I


0 attached
Partial nursing
Full Nursing
Definite ME
Possible ME
Group:
Sn
Minutes
0 10 20 30 40 50 60 70 80 90 100 110 120
Litter: Q
U
C
M
D
J
Ss
0 10 20 30 40 50 60 70 80 90 100 110 120
O
X
D
L
C
K

<u>Figure 34</u>: Histograms of the 6 litters per group in Experiment 2 over the entire 2 hour reunion period, done minute by minute. Contact levels (none, less than half the litter, or more than half the litter) are represented, in addition to the category of the dam behaviors (pup directed, other directed, or quiet).


Full contact
Half contact
Zero contect
Quiet
Pup directed behavior
Other directed behavior
Minutes
Nn
0 10 20 30 40 50 60 70 80 90 100 110 120
Litter: R
H
O
R
A
L
Ns
0 10 20 30 40 50 60 70 80 90 100 110 120
Q
K
L
S
Y
K


Full contact
Half contact
Zero contect
Quiet
Pup directed behavior
Other directed behavior
Minutes
Sn
0 10 20 30 40 50 60 70 80 90 100 110 120
Litter: P
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Ss
0 10 20 30 40 50 60 70 80 90 100 110 120
S
J
M
Q
Z
M

For analysis of the first 10 minutes, behaviors were not made into rates for comparisons because the time period of 10 minutes was constant for all conditions. To determine if there was a difference between dam behavior when reunited with active pups (Exp. 1) and dam behavior when reunited with anesthetized pups (Exp. 2), a 2 way ANOVA (Experiment=2, Group=4) was done on each measure. A difference between the four groups would be detected by a group effect, and a difference between any individual groups in the 2 different experiments would be picked up by an interaction effect. However, these latter effects will not be focused on. This analysis was done to detect a difference between Experiment 1 and Experiment 2 for a particular behavioral measure during the first 10 minutes of reunion, to answer the question whether changes in dam behavior due to separation are dependent on active characteristics of pups.

To determine if there was a difference between separated and nonseparated dams when reunited with anesthetized pups, behavioral measures were analyzed as in Experiment 1: an independent t-test determined if there was a significant difference between the Nna and Ssa groups, and a 2 way ANOVA (Dam X Pup) across all four groups attempted to determine if the difference that exists was a result of changes in the dam condition, the passive qualities of the pup condition, or an interaction between the two. Results could illuminate effects of separation due solely to changes in the dam condition (Model 1), due solely to changes in the passive qualities of the pups which elicit different behaviors from the dams (Model 2), an interaction between these two effects (Model 3), or various combinations of these effects (Models 4, 5, 6, and 7) (see Figure 1).

To determine if there was a difference in the first 10 minutes between separated and nonseparated dams with active pups a 2 way ANOVA (Dam X Pup) was done across all groups. By comparing the results of the 2 way ANOVAs of Experiment 1 to Experiment 2, we can determine if changes due to separation in dam behavior with passive pups were the same changes seen with active pups.

The second period of time analyzed was the entire two hour observation period, to determine if the dams demonstrated a stable pattern of behavior over time, and what that behavioral pattern was. Rates of behaviors (behaviors/time available) were analyzed over the two hour period because the time available for behaviors was not equal among litters due to the occurrence of cannibalization, at which point the observations were stopped and the interaction terminated.

Analysis done over the entire 2 hour period of reunion with anesthetized pups cannot be directly compared to the analysis done in Experiment 1. The analysis of the reunion with active pups in Experiment 1 was based on attachment, looking at the time prior to solid attachment which was the beginning of the first nursing bout and then during the first nursing bout. Over the entire 2 hour period, analysis of Experiment 1 focused on the time the pups spent attached and the number and rate of MEs to determine if these measures could have contributed to the weight changes seen. This approach cannot apply to the reunion with anesthetized pups.

Analysis over the entire 2 hour period of reunion in this experiment attempted to determine if the effects of separation on the dam's behaviors as they were elicited only by the passive qualities of the pups, and if the passive characteristics of the pups elicit different dam behaviors. Behavioral measures were again analyzed as in Experiment 1: an independent t-test determined if there was a significant difference between the Nna and Ssa groups, and a 2 way ANOVA (dam=2, pup=2) across all four groups attempted to determine if the difference that exists was a result of changes in the dam condition, the passive qualities of the pup condition, or an interaction between the two.

Also, as in Experiment 1 ANCOVAs were run to determine if differences between groups for certain measures still existed when the means were adjusted by the pups' axillary temperatures, and if there was a relationship within groups between the pup temperatures and the behavior.

RESULTS

Cannibalization

Cannibalization occurred in 3 out of the 24 litters. It did not occur consistently in the same condition, as it was present in one litter each of the Nna, Nsa, and Sna group. None occurred in the Ssa group. Two of the occurrences of cannibalization did occur on the same day (Nna and Sna) at approximately the same time, around the 73rd minute of reunion. Perhaps this was the result of an additional disturbance being present on that day in the laboratory or a cross disturbance between dams. The other occurrence of cannibalization, in the Nsa group, occurred on a different day approximately 30 minutes after reunion began.

All analyses were done with and without these litters to determine their effect on the overall results.

Pup Axillary Temperature

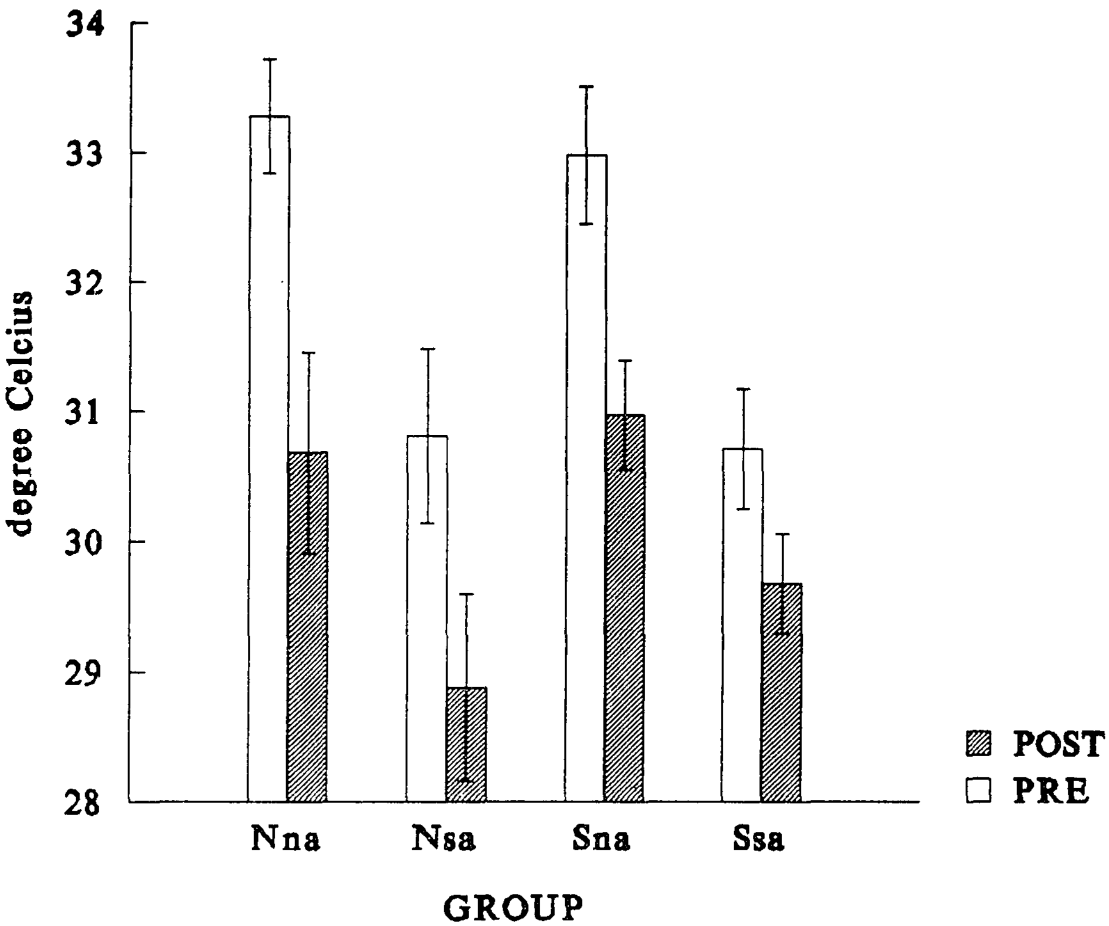
As shown in Figure 35, pups of the Ssa group were significantly cooler than pups of the Nna group prior to reunion ($t(10)=4.037, p<.003$). Analysis across all groups revealed that both groups of separated pups were cooler prior to reunion (Pup effect: $\underline{F}(1,20)=19.710, p<.001$), even though they were kept on a heating pad during the separation period as in Experiment 1. After 2 hours of reunion, pups of the Ssa group were no longer significantly cooler than

Figure 35: The mean axillary temperatures of anesthetized pups for 6 litters per group prior to reunion and after 2 hours of reunion.

There was a significant difference between the Nna and Ssa groups prior to reunion ($p<.004$) . The 2 way ANOVA found a pup effect. These results represent a Model 2 of separation effects.

There was no significant difference between the Nna and Ssa groups after reunion. The 2 way ANOVA found a pup effect. These results again represent a Model 2 of separation effects.

PUP TEMPERATURE



pups of the Nna group ($t(8)=1.168, p<.28$), but again, analysis across all groups revealed that separated pups were still cooler (Pup effect: $\underline{F}(1,20)=6.945, p<.020$). Both of the pre and post temperatures fit the Model 2 of ANOVA results depicting the differences seen due to separation as the result of changes within the pup condition.

Over the 2 hour period of reunion, all anesthetized pups in all groups lost temperature. There was no significant difference between the Nna and Ssa groups in the decrease in temperature over 2 hours, nor was there any effect of separation on pup temperature due to changes in the dam condition, passive pup condition, or an interaction between the two.

Summary and Discussion

As in Experiment 1, separated pups were cooler prior to reunion even though they were kept in a cage with shavings and a nest area on a heating pad. As stated before, this was probably due to the lack of periodic ingestion of warm milk during the separation period which has been shown to help pups maintain their normal body temperature (Leon, 1979), as well as the pups inability to respond to the cold challenge of being without their dam's warm milk and ventrum (Stone et al. 1976). Unlike Experiment 1 in which pups of all groups showed an increase in temperature over the 2 hour reunion period, all anesthetized pups showed a decrease. There was no difference in the amount of temperature decrease between groups, resulting in the separated pups again being cooler than nonseparated pups.

The difference between separated and nonseparated pups temperature represents a major difference in the passive cues of the pups, and therefore might be responsible for eliciting a difference in behavior between the separated and nonseparated dams. The lack of difference between groups in the decrease in temperature over the 2 hours could possibly reflect a lack of difference in most pup-directed maternal behaviors that would warm or even maintain the pups' temperature. Or, perhaps anesthetized pups were not easily warmed due to their continued lack of ingestion of warm milk, and maternal behaviors had little or no effect on them. In the experimental description that follows, we will try to distinguish between these possibilities.

<u>The Initial Ten Minutes of Reunion</u>

Contact

As shown in Table 2, the amount of time the dams were in contact with the anesthetized pups overall and in the individual groups was not different from the amount of time dams spent in contact with active pups during the first 10 minutes of reunion in Experiment 1. This is shown by the lack of Experimental effect found in the 2 way ANOVA

TABLE 2

The First 10 Minutes of Reunion of Experiment 1 & 2

The mean and SEM of measures

Measure	Exp.	Nn(a)	Ns(a)	Sn(a)	Ss(a)	2 W-ANOVAs Betw. Exps. -Exp. -Group	Within Exps -Dam -Pup
Contact	1	5.34(.97)	6.75(1.65)	6.50(.84)	8.13(.97)	No effects	NS
	2	6.50(.66)	6.33(.84)	5.92(1.45)	6.00(1.38)		NS
On Top	1	2.00(1.03)	5.00(1.32)	4.50(.67)	5.00(1.13)	Exp.effect	NS
	2	1.50(.76)	1.67(.80)	1.50(1.15)	2.17(.98)		NS
Pup Dir.	1	1.33(.49)	2.67(.84)	4.00(.52)	3.67(.67)	Exp.effect	SD>ND
	2	4.17(.70)	5.17(.91)	4.83(1.45)	4.67(1.23)		NS
-Nosing +	1	0	0	0	0		
Probing	2	2.67(.42)	2.33(.67)	1.67(.56)	3.00(.78)		NS
-Retrieve	1	.50(.34)	.67(.49)	.50(.22)	.67(.33)	No effects	NS
	2	.17(.17)	.83(.54)	.50(.34)	.17(.17)		NS
-AG Lick	1	.67(.33)	1.67(.40)	1.83(.40)	1.17(.31)	Exp.effect	NS
	2	.50(.22)	1.17(.31)	.17(.17)	.50(.22)	Interaction	sp>np ND>SD
-Body	1	.17(.17)	.83(.40)	1.67(.42)	1.83(.31)	Gr. effect	SD>ND
Lick	2	.83(.17)	1.00(.45)	2.50(1.20)	1.00(.45)		NS

Table 2 cont.

Measure	Exp.	Nn(a)	Ns(a)	Sn(a)	Ss(a)	2 W-ANOVAs Betw. Exps. -Exp. -Group	Within Exps -Dam -Pup
Other Dir.	1	7.67(1.02)	3.67(1.20)	4.50(.22)	3.33(.88)	Gr. effect	NS
	2	5.50(.92)	4.33(.67)	4.50(1.25)	5.17(1.11)		NS
-Self Gr.	1	.17(.17)	.17(.17)	.33(.21)	.17(.17)	Exp.effect	NS
	2	.33(.21)	.83(.40)	.33(.33)	1.17(.45)		NS
-Rear	1	2.83(1.01)	2.50(1.15)	1.83(.48)	1.83(.60)	No effects	NS
	2	3.83(.75)	2.17(.54)	2.67(.67)	2.67(.72)		NS
-Change in Position	1	4.83(.75)	1.00(.45)	2.33(.56)	1.50(.50)	Exp.effect	Nn grt
	2	1.33(.42)	1.33(.42)	1.50(.62)	1.33(.49)	Gr. effect Interaction	NS
Quiet	1	1.00(.82)	3.67(1.20)	1.50(.50)	2.83(.48)	Exp.effect	sp>np
	2	.33(.33)	.50(.50)	.67(.49)	.17(.17)	Interaction	NS

(Experiment X Group). There also was no group or interaction effect.

An independent t-test found no significant difference between the Nna and Ssa groups in amount of time the dams were in contact with the anesthetized pups during the first 10 minutes of reunion. Analysis across all four groups (2 way ANOVA, Dam X Pup) also found no effects. The 2 way ANOVA of Experiment 1 also found no effects. Therefore, separation did not affect the dams contact time with pups during the first ten minutes of reunion whether the pups were active or passive.

There were also no differences between the Nna and Ssa groups, nor were differences seen in analysis across all groups for the latency to the first time contact with the pups was observed. (Note: because observations were made using the snapshot technique rather than continuously, this measure is not necessarily an accurate measure of the very first time the dams were in contact with the pups, however this limitation was equal for all groups). The means of the latency to the first contact observed ranged from 2.8 (SEM=.6) minutes in the Nna group to 22 (19.55) minutes in the Ssa group. This long latency to contact in the Ssa group was the result of one litter, labeled M, in which contact was never observed (see Figure 34). Analysis was done excluding litter M of the Ssa group to determine if its contribution of variability was masking differences between the groups, and this also revealed no significant differences between conditions in latency to contact. Without M, the mean latency of the Ssa groups latency to contact to 2.8 (.92) minutes.

An ANCOVA found no difference in latency to contact between groups when the pups' pre-reunion axillary temperatures were taken into account, nor was there a relationship found within groups between latency to contact and pup temperature. Actually, the ANCOVAs done other measures of Experiment 2 did not contribute any new information and therefore will not be described.

Another way to determine if the anesthetized pups' temperatures were affecting time in contact with the dam during the first ten minutes of reunion was to do a correlation between these measures across all groups. Although the difference between the conditions of the groups were not taken into account, the lack of a significant correlation between pre-reunion axillary temperature and time in contact is consistent with the above nonsignificant ANCOVA result.

On Top Position

Technically, the on top position displayed by dams when with anesthetized pups was not the on top nursing posture seen in Experiment 1 because the pups did not attach, however the dams were seen to lay over their pups who were most often in a pile. Analysis comparing experimental conditions and groups found that dams with anesthetized pups were on top of their litters significantly less than dams with active pups (Exp. effect: $\underline{F}(1,40)=11.648, p<.002$) (see Table 2). This was probably due to the lack of pup stimulation in maintaining it as suggested by the work of Stern and Johnson (1990). There were no effects of group, nor an interaction between experiment and group.

Comparing the anesthetized pup groups of Experiment 2, there was no difference between the Nna and Ssa groups, nor were there any differences across all four groups in the time spent nursing on top. Therefore separated and nonseparated dams spent similar amounts of time on top nursing, and there was no difference in the time elicited by the passive qualities of separated and nonseparated anesthetized pups.

Analysis between the active pup groups of Experiment 1 also found no dam or pup effects. In conjunction with the analysis of Experiment 2 previously described, this means that there were no differences due to separation in dam behavior in time spent in the on top position when with anesthetized or active pups. However, as was shown in the comparison between experiments, dams with active pups were on top more.

Pup Directed Behavior

Dams with anesthetized pups spent significantly more time pup directed than dams with active pups during the first 10 minutes of reunion (Exp. effect: $\underline{F}(1,40)=7.789, p<.009$) (see Table 2).

Analysis of Experiment 1 found that separated dams were more pup directed toward the active pups during the first 10 minutes of reunion (Dam effect: $\underline{F}(1,20)=8.067, p<.011$). This effect was not seen in Experiment 1; there was no difference between the Nna and Ssa groups, and no effects found in the analysis across all groups. Therefore active pups were necessary to elicit the increase in pup directed behaviors by separated dam. However, pup directed behaviors were greater in all groups with anesthetized pups during the first 10 minutes of reunion.

The specific pup directed behavior that contributed to the increase in the general class of pup directed behaviors in Experiment 2, was the occurrence of nosing and probing the pups. This behavior occurred frequently in interactions with anesthetized pups, and was not observed in interactions with active pups. Dams with anesthetized pups were observed to actively probe or push the pups with her snout, often licking them simultaneously. Possibly this was how the dam recognized the unusual lack of response by the pups and was attempting to stimulate them.

When other individual pup directed behaviors were looked at, there were no differences between dams with active or anesthetized pups in time spent retrieving. Nor were there any differences between experiments in time spent body licking. (Note: there was a significant group effect however this will not be focused on because all group and interaction effects seen from this analysis are contributed to by the effects seen in the analysis within experiments which will be described in detail). In Experiment 1, separated dams spent more time body licking (Experiment 1, dam effect: $\underline{F}(1,20)=13.554, p<.002$) (see Table 2). This effect of dam condition was not seen in Experiment 2. Therefore, the dam behavior of body licking was contributing to the separation effect seen in Experiment 1 between dams with active pups which was not seen in Experiment 2 by dams with anesthetized pups.

The last pup directed behavior, AG licking, was found to be significantly higher in interactions with active pups (Exp. effect: $\underline{F}(1,40)=8.333, p<.007$). This was thought to be due to the loss of reflexive urination in anesthetized pups. A small pilot in the lab confirmed this; anesthetized pups, unlike active pups, cannot be stimulated to urinate and defecate by actively stroking their AG area.

Analysis across groups with active pups in Experiment 1 found no effects of separation on this dam behavior. Nor were there changes in the amount of AG licking prior to or during the nursing bout as reported previously. Perhaps this was because the behaviors associated with nursing (attachment, suckling, treadling, consuming) interfered with the possible differences.

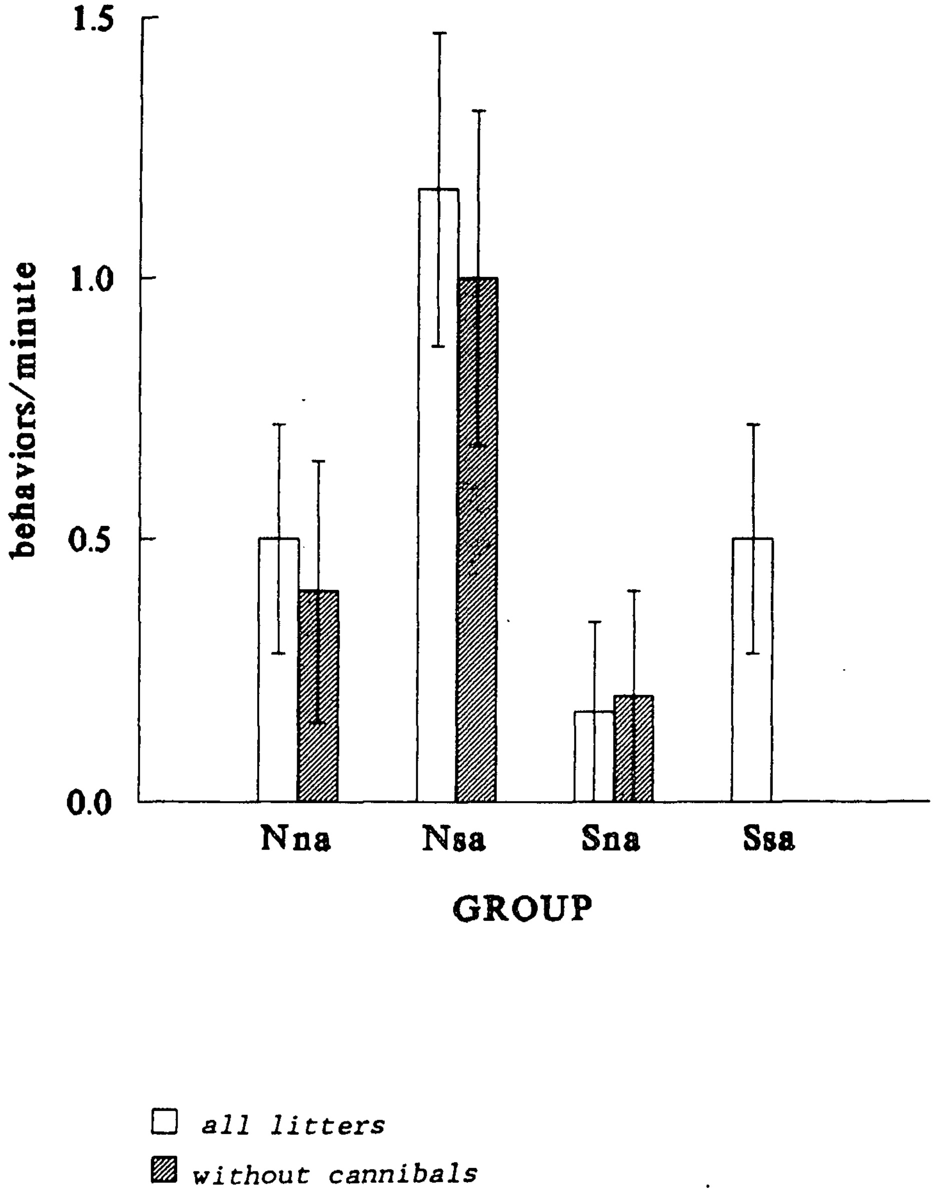
Analysis across groups with anesthetized pups in Experiment 2 found that separated dams AG licked anesthetized pups less, and separated pups were licked more (Dam effect: $\underline{F}(1,20)=4.500, p<.048$; Pup effect: $\underline{F}(1,20)=4.500, p<.048$) (see Figure 36). These results fit the Model 5 of separation effects, as there was a decrease of this behavior in groups with separated dams and increase in groups with separated anesthetized pups. When combined in the Ssa group, these effects cancelled each other out and no difference was seen when compared to the Nna group.

When the analysis of AG licking excluded the three litters in which cannibalization occurred, the results were different. Again there was no difference between the Nna and Ssa groups, but now analysis across all groups found only a trend for separated pups to be AG licked more than nonseparated pups (Pup trend: $\underline{F}(1,17)=3.266, p<.089$). As shown in Figure 35, the cannibals in the Nna and Nsa groups had elevated levels of AG licking and when excluded, the dam effect disappeared, but the remaining dams still tended to maintain the same distinction between separated and nonseparated pups in amount of AG licking during the first 10 minutes of reunion.

Figure 36: The mean rate of AG licking by the dams of the 6 litters per group during the first 10 minutes of reunion with anesthetized pups. There was no significant difference between the Nna and Ssa groups in rate of AG licking by the dam during the first 10 minutes of reunion. The 2 way ANOVA found a dam effect and a pup effect. These results fit a Model 5 of separation effects.

When litters in which cannibalization occurred were excluded from the analysis, again there was no significant difference between the Nna and Ssa groups in mean rate of AG licking by the dams during the first 10 minutes of reunion. However, the 2 way ANOVA now found only a pup trend. These results best fit a Model 2 of separation effects.

AG LICKING. 1ST 10 MINUTES



Other Directed Behavior

There was no significant difference between dams with active pups of Experiment 1 and dams with anesthetized pups of Experiment 2 in the amount of time spent doing other directed behaviors (see Table 2). Analysis across all four groups in Experiment 1 found no effects of the dam or pup condition, nor did analysis across all four groups in Experiment 2. Therefore, there was no difference in amount of other directed behavior by dams with active or anesthetized pups, and separation had no affect on the dams' other directed behavior when with either active or anesthetized pups, and the differences in passive qualities of the pups did not contribute differently to the dams' other directed behaviors. In looking at the rate of changing position, there was a significant experimental effect, group effect, and interaction effect (Exp. effect: $\underline{F}(1,40)=7.530, p<.010$; Group effect: $\underline{F}(3,40)=5.024, p<.006$; Interaction effect: $\underline{F}(3,40)=5.056, p<.006$). These effects were the result of the increased rate of this behavior in the Nn group of Experiment 1 (Exp. 1: Dam trend: $\underline{F}(1,20)=3.025, p<.098$; Pup effect: $\underline{F}(1,20)=16.471, p<.002$; Interaction effect: $\underline{F}(1,20)=6.807, p<.018$; Post hocs: Nn vs. Ns: $\underline{F}(1,20)=22.227, p<.001$; Nn vs. Sn: $\underline{F}(1,20)=9.454$, $p<.007$; Nn vs. Ss: $\underline{F}(1,20)=16.807, p<.002$). This increase in other directed behavior was seen for the Nn group when analyzed during the period prior to the first nursing bout.

It is interesting that the Nna group does not show these elevated rates. Perhaps the anesthetized pups elicit pup directed behavior that detracts from the changing of position or cage exploration seen when the pups were active in this group. Or perhaps pups of the Nn group do not elicit pup directed behaviors.

There were no differences between experiments or within experiments in time the dam spent rearing during the first 10 minutes of reunion. But dams with anesthetized pups displayed an increased amount of self grooming during the first 10 minutes of reunion (Exp. effect: $\underline{F}(1,40)=5.000, p<.032$). Dams with anesthetized pups perhaps increased their self grooming in an attempt to arouse the pups to attach; by licking her ventrum, saliva coats the nipples and normally helps the pups to locate and attach to them. (Note: in this context, the act of self grooming could also be considered a pup directed behavior). Or perhaps, this was a displacement behavior, as such behaviors are known to occur in ambiguous situations when it is not clear what other behaviors should be indulged in.

There was no significant difference between the Nna and Ssa groups in the amount of time the dam spent self grooming, nor were there differences found in the dam or pup condition in analysis across all groups with anesthetized pups. Also, no effects were found across groups in Experiment 1 with active pups either. Therefore, there was no difference between separated or nonseparated dams when with anesthetized pups, or with active pups, however all dams self groomed more with anesthetized pups.

Quiet

During the first 10 minutes of reunion, dams with anesthetized pups had less quiet periods than dams with active pups, as seen when comparing Experiment 1 to Experiment 2 (Exp. effect: $\underline{F}(1,20)=20.000, p<.001$) (see Table 2). This is consistent with their increase in pup directed behavior during this time. These results are of interest because the interaction could have gone either way. The first 10 minutes of reunion with anesthetized pups could have shown an increase in time spent quiet by the dams as they recognized the lack of responsiveness of the pups, or they could have been more pup directed behavior and less quiet in an attempt to stimulate the passive pups. The latter hypothesis has been supported as dams with anesthetized pups were more quiet than dams with active pups (Experiment 1).

Analysis across all four groups in Experiment 1 with active pups indicates that dams with separated pups were quieter than dams with nonseparated pups during the first 10 minutes of reunion (Pup effect: $\underline{F}(1,20)=7.784, p<.012$).

In this experiment, there was no significant difference between the Nna and Ssa groups in time spent quiet during the first 10 minutes of reunion. Analysis across all groups also found no differences between dam or pup condition, or an interaction between the two. In fact, there were only five litters out of all 24 that had at least one minute of quiet during the first 10 minutes. The latency to the first quiet minute ranged from 17 to 25 minutes for all four groups, and was not different between them.

Separation therefore did not affect the amount of time that dams spent quiet when reunited with anesthetized pups. However, dams with anesthetized pups were significantly more quiet than dams with active pups during the first 10 minutes of reunion. And dams with separated active pups were more quiet than dams with nonseparated active pups during the first 10 minutes of reunion.

Summary and Discussion:

In summary, dams with anesthetized pups, when compared to dams with active pups, probed their pups more, self groomed more, and spent less time quiet. This is consistent with the hypothesis that dams with anesthetized pups will attempt to stimulate the pups to nurse as well as make their ventrum more desirable, and due to their passivity, had to increase this stimulation. It was also possible that the dams would neglect the pups after an initial investigation, but this was not seen.

Dams with anesthetized pups also spent less time on top. This is consistent with the literature demonstrating that without pups attaching, treadling, and sucking, the dams do not become immobile and crouch over their pups (Stern and Johnson, 1990). These differences were seen between the experiments although there were no differences in amount of pup contact.

There were some effects of separation during the first 10 minutes that were only seen when dams were interacting with active pups in Experiment 1. Separated dams were more pup directed and spent more time licking the pups' bodies. Dams with separated pups were less quiet. And the Nn group spent the most time changing position during the first 10 minutes of reunion. These behaviors which differentiated the groups of this experiment were dependent upon the presence of active pup behavior, and disappear when pups were anesthetized. Separated dams have may spent more time licking their pups' bodies due to their increased motivation to nurse, and these active pups are responsive and therefore are attempting to attach. Dams with separated pups might be more quiet because the separated pups are activated to attach and need no stimulation. And perhaps pups of the Nn group do not elicit pup directed behavior, and the pups of the Nna group do.

There was only one effect of separation seen when comparing interactions of dams with anesthetized pups during the first 10 minutes of reunion. Nonseparated dams were seen to AG lick their pups more, and separated pups were AG licked more, but these differences were contributed to by the elevated rate of AG licking in the litters in which cannibalization occurred with nonseparated dams. Without cannibals, there was only a trend for separated pups to be AG licked more. One might have expected to see separated pups AG licked more if they had not been stimulated to urinate and defecate prior to their anesthetization and reunion. But they had been, and this procedure of stimulating the pups appeared to control for the AG licking in Experiment 1, as there were no differences between dam or pup conditions during the first 10 minutes of reunion or the time prior to the first nursing bout. Perhaps there was another passive quality of the separated anesthetized pups that elicited this behavior. Since separation involves a decrease in fluid intake, the urine of separated pups was more concentrated. However, a pilot study demonstrated that anesthetized pups do not reflexively urinate when stimulated: pups which had been separated from their dam for approximately 6 hours were anesthetized by a procedure identical with that of the present experiment then stimulated with a soft tissue at their AG area. Two pups who were anesthetized for a two hour period after the six hour separation period from their dam were stimulated and urination and defecation were not observed. Three active, unanesthetized pups were stimulated successfully for a brief period to confirm presence of urine and then anesthetized. None of these pups reflexively urinated or defecated when stimulated to do so. Therefore dams were not consuming urine when observed to be AG licking the anesthetized pups. Why then did the anesthetized separated pups elicit an increased rate of AG licking? It is not necessarily an effect of non-reward or the dam licking more because no urine was elicited, because the nonseparated anesthetized pups were not licked more. Perhaps traces of the urine were still found on the pups from the stimulation done to them prior to anesthesia and reunion, and the difference in concentration or odor due to separation has marked them uniquely.

Behaviors that appear unique to interactions of dams with anesthetized pups include cannibalization, nosing and probing the pups, and a decrease in pup temperature over time. These behaviors were not seen in interactions with active pups in Experiment 1, and therefore must be due to the passive characteristics of the pups.

The First Two Hours of Reunion

Contact

There was no significant difference between the Nna and Ssa groups in the percent of time the dams' were in contact with their pups. Analysis across all four groups also found no significant differences, the rate of time in contact ranging from 74 to 88%. When percent of time in contact was divided into percent of full contact (contact with more than half the litter), partial contact (contact with less than half the litter), or no contact, there were again no differences between the Nna and Ssa groups, nor in analysis across all groups. Although no differences between groups existed, there were two litters with separated dams which appeared aberrant because contact was minimal to none. The dam of litter T of the Sn group spent only 4 minutes in contact with the pups over the two hour period, and the dam of litter M of the Ss group was never observed to be in contact with her litter (see Figure 34).

The time the dam spent in contact with active pups in Experiment 1 was affected by the attachment of the pups, and other variables involved in the transfer of milk. However, a comparison between the two experiments might illuminate how much these variables of active nursing affect contact time. Percent of time in contact ranged from 82 to 95% of the two hour period of interactions with active pups. There was no significant difference within Experiment 1 between dam or pup conditions in time spent in contact, and analysis between experiments revealed that dams spend equivalent amounts of time in contact with active and anesthetized pups. This is surprising as an increase in contact time in Experiment 1 might have been predicted due to the study by Stern and Johnson demonstrating the importance of the active pup stimulation in maintaining the dam's nursing position by rooting and probing and attaching to the dam's ventrum.

Jans and Leon (1983) demonstrated that the initial pup temperature of the pups had no effect on the dam's latency to enter the nest but increased the contact time with the dam if pups were cool. This was not supported in Experiment 1 with active pups. As mentioned earlier, the all ANCOVAs of behavioral measures in Experiment 2 with anesthetized pups demonstrated that there were no differences in the analyses when the group means were adjusted by the pups' axillary temperature prior to reunion and no differences were found within groups between pup temperature and a specific measure. This was true of the contact measure over the two hour period with anesthetized pups. A correlation across all groups also found no relationship percent of time the dams were in contact with the pups and the pups' axillary temperature prior to reunion or the decrease in temperature over the 2 hour period. Therefore Experiment 2 also is not consistent with Leon hypothesis that contact time is regulated by maternal homeostasis. Something other than active pup stimulation and or the passive characteristic of pup temperature must be affecting contact time.

Transitions Between Contact Levels

There was no significant difference between the Nna and

Ssa groups in number of transitions seen between the different levels of contact mentioned above. However, as shown in Figure 37, analysis across all groups revealed that nonseparated dams had more transitions between contact levels (Dam effect: $\underline{F}(1,20)=4.441, p<.049$). Analysis without the cannibalized litters again found no difference between the Nna and Ssa groups, but a significant dam effect across all four groups (Dam effect: $\underline{F}(1,17)=4.301, p<.055$). These results fit a Model 1 of separation effects, that is separation alters the mother-infant interaction due to changes in the dam condition.

On Top Position

There was no significant difference found between the Nna and Ssa group in percent of time the dam spent lying on top of her pups. Nor was there a difference found when analysis was done across all groups. The percent of time spent in this position ranged from 57% to 77% over the 2 hour period. When the group means of the rate of on top position were adjusted for by pup temperature, there were still no differences found between groups.

Pup Directed Behavior

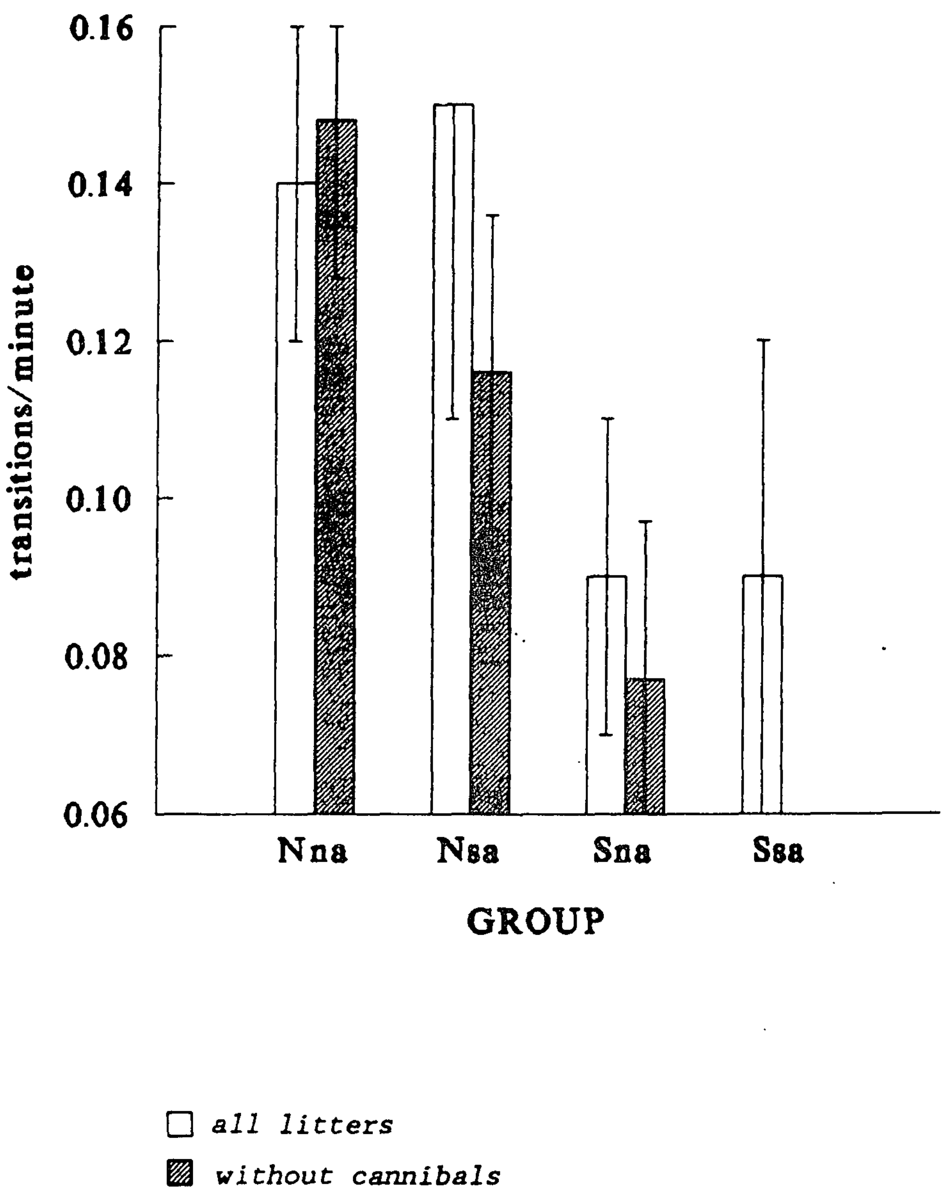
There was no significant difference found between the Nna and Ssa groups in percent of time the dam was pup directed. Analysis across all groups also revealed that

<u>Figure 37</u>: The mean rate of transitions between contact levels (zero, less than half the litter, more than half the litter) over the 2 hour period of reunion with anesthetized pups.

There was no significant difference between the Nna and Ssa groups in rate of transitions between contact levels. The 2 way ANOVA across all groups found a dam effect. These results best fit a Model 1 of separation effects.

When litters in which cannibalization occurred were excluded from the analysis, again there was no significant difference between the Nna and Ssa groups in rate of transitions between contact levels. The 2 way ANOVA across all groups found a dam effect. These results still fit a Model 1 of separation effects.

TRANSITIONS: CONTACT LEVELS



there were no effects of separation on this behavioral measure. The means of the four groups ranged from 27% to 35% of the time over the 2 hour period. Looking at the individual pup directed behaviors, there were no differences seen in rate of AG licking, body licking, licking and probing, nosing and probing, total probing, or in the retrieval of pups, whether analysis was done comparing the Nna and Ssa groups, or was done across all four groups.

Retrieval of Pups

The above mentioned rate of retrieval represents how many times the dams were observed to be retrieving the pups divided by the time available. This retrieval measure is a general category of dam behavior is which the dam is transporting the pups around the cage. She could be relocating the nest, or pup carrying due to nest disturbance. It is not the retrieval response elicited by pups who stray from the nest, as obviously the anesthetized pups cannot. From the data available, we attempted to get the latency to the first time a pup was retrieved, and the time it took from the beginning of the first retrieval of a pup to when all 9 pups were relocated. Unfortunately, this information was not noted for all litters. However data each group is represented in the observation of each measure. Because of the small subject pool available, the data for groups Nna and Nsa were combined, and the data for groups Sna and Ssa were combined. Therefore comparisons were done between nonseparated and separated dams for the latency to the first retrieval and the total time it took to retrieve the entire litter. These informal observations of retrieval measures also found no differences between separated and nonseparated dams. Although the data available were accurate, the amount of data were limited decreasing the possibility of detecting significant differences.

Other Directed Behavior

Separation did not affect the dams other directed behaviors when analysis compared the Nna and Ssa groups, or was done across all four groups. There were no significant differences in rate of overall other directed behaviors, or individual rates of selfgrooming, rearing, changing of position, or eating and drinking. The percent of time the dams were other directed over the 2 hour period ranges from 17% to 22%.

Quiet

There was no significant difference between the Nna and Ssa groups in rate of time spent quiet. Analysis across all groups also found no differences. The percent of time spent quiet ranged from 43% to 53% across all groups for the 2 hour period.

Transitions Between Behaviors

There was no significant difference between the Nna and Ssa group in the number of transitions seen between pup directed behavior, other directed behavior, and time spent quiet. Nor were there differences found when analysis was done across all four groups. This was also true when the litters in which cannibalization occurred were excluded from the analysis.

Summary and Discussion

There were some behaviors that were dependent upon active pup behaviors, and just did not occur in this experiment with anesthetized pups. These include attachment or nursing, MEs, weight changes, and the arched nursing position. Because the pups are anesthetized they do not attach, elicit MEs, or gain weight. And as mentioned earlier, without the active probing of the dam's ventrum by pups attempting to attach or treadling once attached, the arched nursing position is not elicited (Stern and Johnson, 1990).

There were some changes due to separation that were dependent upon the active characteristics of pups. When placed with active pups in Experiment 1, separated dams were shown to be less other directed and have greater rates of contact with pups prior to the first nursing bout. However, when dams were reunited with anesthetized pups in Experiment

2, the experience of separation did not affect the rate of pup directed behavior, other directed behavior, or time spent quiet. Nor did it affect the transitions between them. Thus, these behaviors of the dams seen in the first experiment described above were dependent upon interacting with active pups.

Also in Experiment 1, all pups showed an increase in body temperature over 2 hours, but in this experiment a decrease in body temperature was seen in all anesthetized pups. All groups had high rates of dam- pup contact and of the dam being on top of the pups, however these behaviors were not successful in warming up the pups even though they were equivalent to those seen in Experiment 1. One might think that the cold challenge of being without their dam's ventrum had ended since contact time was unchanged, but perhaps because the anesthesia had turned off thermogenesis the heat transfer from the dam was not sufficient without active nursing. As mentioned previously, the periodic ingestion of warm milk has been shown to help pups maintain their normal body temperature (Leon, 1979).

From the Jans and Leon (1983) study demonstrating that contact time was increased when initial temperatures of pups were cooler, one might have expected the cooler separated pups' to increase the time of contact. However, the pups' axillary temperature prior to reunion did not affect the dams' on top, or contact behavior.

Stern and Johnson (1990) found that chilled pups (2 days old, 9.5 to 22.7° C) were not sniffed more quickly, but were sniffed more often. Chilled pups also elicited more nest building and burrowing by the dam. Although the reduction of shavings prevents normal nest building and burrowing behavior, there were no effects of pup temperature on the dams' pup directed, other directed, or quiet behavior.

The one difference between separated and nonseparated dams with anesthetized pups over the two hour period was the decrease in transitions between contact levels by separated dams. Perhaps nonseparated dams were more disturbed by the lack of movement in the pups as the dams had recently experienced active pups. Also, this effect is probably due in part to the two litters in the separated dam condition who were observed to have minimal to no contact over the 2 hour period, thereby decreasing the separated dams' rate of transitions between contact levels. Nonseparated dams were also shown to AG lick their pups more during the first 10 minutes of reunion, but this effect was mainly contributed to by the litters in which cannibalization occurred. Separated pups were shown to be AG licked more than nonseparated pups during the first 10 minutes of reunion, with or without the cannibalized litters. This was perhaps due to separation changes in the urine present on the pups' bodies, which would disappear over the entire 2 hour period.

Dams with anesthetized pups AG licked their pups less, probably because anesthetized pups do not reflexively urinate.

Over the two hour period, there were no changes in dam behavior mediated by the passive characteristics of pups. Therefore, the results seen in Experiment 1 are not due to the smell, size, or temperature of the pups.

The anesthetized pups did elicit unique behaviors that were not seen in interactions with active pups. These behaviors include cannibalization of the pups, as well as nosing and probing the anesthetized pups frequently. Also, the anesthetized pups lost temperature over the two hour period, something that also did not occur in Experiment 1. These unique behaviors were elicited by or the result of the passiveness of the pups.

The two litters in which contact was limited are of interest because they seem most disturbed by the experience of separation and the anesthetized conditions of the pups. As seen in litter Q of the Sn group in Experiment 1, perhaps separation effects some dams more than others, and could potentially result in a complete disruption nursing behavior.

CONCLUSION

It was interesting that not all dam behaviors were eliminated by the passivity of the pups. Of course those behaviors associated with nursing and weight gain were, but rather than maintain contact and attempt to stimulate the pups, it seemed just as likely that the dams would investigate the pups briefly and then either neglect them or cannibalize them. The rate of cannibalization was actually quite low considering the stress of separation for some dams and the complete lack of response in the pups. Also, in addition to being passive, the separated pups were quite cold. But perhaps the dams were able to detect breathing, and therefore delayed cannibalizing.

It was also interesting that no consistent effects of separation on dam behavior were seen when with either active or anesthetized pups. Therefore, the changes in the passive characteristics of the pups, including their size, temperature, and possibly smell, were not responsible for altering the dam's behavior in Experiment 1. It was the active behavior of the pups that, for example, increased body licking of pups and decreased other directed behavior prior to the first nursing bout in separated dams.

EXPERIMENT 3

INTRODUCTION

The dams of the Nn control group of Experiment 1 were given cross fostered pups during the 18 hour treatment period, and were reunited thereafter with their own pups which had meanwhile been nursed by another mother. The purpose of this experiment was to determine if these pup exchanges altered the mother-infant interaction of our control group. The following study compared the mother-infant interaction of litters which were treated like the control group in Experiment 1, Nn(x), to litters which were with their natural partners throughout the procedure and test, but handled equivalently. This latter group will now be called Nn. If the results of this experiment find that the Nn(x) group is not different from the Nn group, than the effects of separation seen in Experiment 1 can be generalized to a more natural dam-pup interaction. However, if differences are found between the Nn(x) and Nn group, the meaning of the results of Experiment 1 become limited. The differences between the Nn(x) and Ss group of Experiment 1 would then be specific to interactions of dams and pups who have recently experienced cross fostering.

Little is known about the effects of cross fostering, and no extensive behavioral study has been done comparing dams interacting with their natural offspring to dams interacting with cross fostered pups. This study was not a direct comparison between the two, but rather looked at the effects of a recent experience of cross fostering on the mother-infant interaction upon reunion.

A number of studies comparing dams with their natural or fostered pups have measured retrieval behavior, which has often been used as an index of maternal behavior. An early study by Wiesner and Sheard (1933) found no evidence of discriminatory retrieval of pups when dams were offered either a natural or foster pup, dropped one at a time down a delivery chute. A later study supported this result, finding that dams showed no preference when offered both a natural or foster pup in a t-maze retrieval test (Misanin, Zawacki, and Krieger, 1977). However, Beach and Jaynes (1956a, 1956b) found that when offered both simultaneously in a pile together, the dam retrieved her natural young prior to the alien young. Also, although there was no difference in latency to the retrieval of the first pup of a natural or fostered litter, the total retrieval time of all the pups was significantly slower for the foster litters. This suggested that dams, when given a direct choice, will care for their natural young, and might even be less directed when caring for a litter of foster pups. But, in complete contrast to this, Misanin et al. (1977) found that although the latency to pick up either the natural or fostered pup was not different, the fostered pup was then returned to the nest more quickly than the natural pup. They then concluded that fostering might lead toward more maternal attention than that provided by the pups' natural dam. The conflicting results of the above studies are possibly due to the various methods by which retrieval behavior were measured. Also, they provide reason to be cautious in defining maternal behavior by retrieval behavior alone.

One might predict that the dam's experience of having fostered another litter for an 18 hour period and the pups' experience of being cross fostered would affect the pups' weight gain upon reunion. However, Reddy, Donker, and Lennerud (1964) found that dams with cross fostered pups did not differ significantly from dams with their natural pups in milk transfer after separation periods ranging from 3 to 12 hours on postnatal days 11, 13 or 15. Pup weight gain after 1 hour was the dependent variable. This would mean that not only do dams nurse fostered and natural pups equally, but pups that were cross fostered were not deprived during the cross fostering period, which could have altered their behavior upon reunion. The results of this study may be affected by the increase in milk build up that occurs in a separated dam, as this has been shown to increase pups' intake regardless of their deprivation condition (Friedman, 1975) and therefore may mask any possible differences between the fostered and natural pups. This study does not involve separated dams, and will therefore not run into this problem.

Based on the limited literature available, it was predicted that few if any differences will exist between the Nn(x) and Nn group.

METHODS

The conditions of Experiment 3 were:

1. A nonseparated dam with nonseparated pups, cross fostered during the treatment period (Nn(x))
2. A nonseparated dam with nonseparated pups, not cross fostered during the treatment period (Nn)

The pups of the Nn condition were treated identically to those of the Nn(x) condition, which is described in Experiment 1, except that they were placed back with their original dam for the 18 hour treatment period. This experiment was terminated at the end of the two hour observation period, after post-reunion weights and temperatures of the pups were taken. A total number of 8 litters per condition were observed over 6 trials.

ANALYSIS

Independent t-tests were utilized to compare the Nn(x) and Nn conditions. Analysis was done on the full two hour period, and the dams' and pups' behavior prior to first nursing bout and during the first nursing bout.

To determine if the behavioral measures of this experiment replicated the measures of Experiment 1, independent t-tests compared the Nn(x) group of this experiment to the Nn(x) group of Experiment 1.

RESULTS

Weight

Cross fostering did not affect the weight gain of the pups. The Nn group gained a mean of .55 (SEM=.15) grams over 2 hours, and 2.30 (.14) over 24. The Nn(x) group gained a mean of .56 (.08) grams over 2 hours, and 2.45 (.26) over 24 (see Table 2).

It is important to note that after the first two hours of reunion before the 24 hour weights, these litters were handled more than in Experiment 1 as weights were also taken at hours 0, 2, 6, 8, and 22. This was due to the procedure of Experiment 4 that was run simultaneously using the Nn(x) as its control litter. However, there were no significant differences between the Nn and Nn(x) groups in weight gained over the first 6, 8, or 22 hours of reunion.

Temperature

Cross fostering did not affect the temperature of the pups. There were no significant differences between the Nn and Nn(x) groups in the pup axillary temperatures taken prior to reunion, immediately after 2 hours of reunion, or in the change of temperature over the two hour period. The temperatures prior to reunion averaged 35.3 to 35.5° C for the Nn and Nn(x) groups respectively, and 35.4°C for both groups after reunion (see Table 3). Because no differences existed, ANCOVAs to determine the effect of pup temperature on certain variables was not necessary.

Attachment, MEs, and Nursing Time

The First Nursing Bout

1. Latency to Attach

There were no significant differences between Nn and Nn(x) groups in their latency to attach, whether it was defined as the first minute of full nursing, the first minute of three consecutive minutes of full nursing, or the first minute of 10 consecutive minutes of full nursing which is the defined beginning of the first nursing bout (see Table 3).

There was one litter in the Nn group, litter J, which was never observed to have more than half the litter attached. Analysis was done on all measures with and without litter J to determine its effect on the statistical results. For all the following analyses this outlier litter will be included, and results that differ without it will be noted. This litter will be discussed in detail later.

TABLE 3
The Control Litters of
of Experiment 3 & 1

The mean and SEM of measures

Experiment:	3 Nn	3 Nn w/o J	3 Nn(x)	1 Nn(x)
Measure				
Weight (g)				
2 hours	.55(.05)		.56(.03)	.66(.08)
24 hours	2.30(.06)		2.45(.09)	2.72(.24)
Temp (C)				
pre reunion	35.3(.2)		35.54(.27)	34.98(.34)
post reunion	35.4(.18)		35.41(.10)	35.65(.45)
Latency to Att.				
init. att.	27.13(13.37)	13.86(1.88)	14.25(2.40)	12.33(2.39)
stable att.	29.75(13.40)	16.86(4.21)	18.38(4.86)	13.83(2.24)
solid att.	31.00(13.18)	18.29(4.00)	18.38(4.86)	16.67(3.44)
Bout length	30.71(3.14)	NA	32.13(3.58)	23.67(3.15)
MEs in 1st bout				
definite	3.57(.78)	NA	5.00(.91)	3.00(.89)
possible	.57(.43)	NA	.75(.25)	.50(.22)
total	4.14(.86)	NA	5.75(.80)	3.50(.88)
Lat to 1st ME				
from sol.att.	11.58(.79)	NA	10.81(1.15)	10.33(1.82)
from reunion	33.07(4.55)	NA	28.19(4.67)	27.50(3.31)
2 Hour Data				
Time nursing				
full	64.38(9.79)	73.57(3.87)	66.88(9.26)	73.50(8.04)
partial	18.75(3.06)	21.43(1.72)	22.50(4.27)	15.67(3.48)
total	83.13(12.48)	95.00(4.42)	89.38(6.16)	89.17(6.08)
MEs				
definite	8.38(1.28)	9.57(.53)	9.38(1.27)	10.17(1.08)
possible	1.38(.82)	1.57(.92)	.88(.35)	1.00(.45)
total	9.75(1.61)	11.14(.94)	10.25(1.36)	11.17(1.11)
ME rate	.13(.02)	.15(.01)	.16(.02)	.15(.03)

TABLE 3 cont.

Measure	Experiment: 3 Nn	3 Nn w/o J	3 Nn(x)	1 Nn(x)
Behaviors Prior to 1st Bout				
Dam Behavior				
Pup Dir.	.33(.06)	.38(.05)	.34(.05)	.25(.07)
Other Dir.	.57(.03)	.59(.03)	.61(.05)	.68(.08)
Quiet	.10(.07)	.04(.04)	.05(.04)	.07(.03)
Transitions	.36(.06)	.40(.05)	.35(.02)	.32(.08)
Pup Behavior				
Dam Dir.	.40(.07)	.46(.05)	.34(.08)	.36(.07)
Other Dir.	.25(.06)	.28(.06)	.28(.07)	.36(.06)
Quiet	.34(.10)	.26(.07)	.37(.11)	.28(.11)
Transitions	.50(.08)	.56(.06)	.49(.08)	.51(.08)
During the 1st Bout				
Dam Behavior				
Pup Dir.	.17(.04)	NA	.18(.05)	.19(.02)
Other Dir.	.05(.03)	NA	.06(.02)	.05(.03)
Quiet	.78(.06)	NA	.75(.07)	.75(.03)
Pup Behavior				
Dam Dir.	.22(.03)	NA	.22(.03)	.11(.04)
Other Dir.	.01(.01)	NA	0(0)	0(0)
Quiet	.78(.03)	NA	.78(.03)	.89(.04)

2. Length of the First Bout

Cross fostering did not affect the length of the first nursing bout (see Table 3).

3. Disruptions During the First Nursing Bout

There was only one occurrence of disruption between both groups. It occurred in the Nn group, and lasted for 3 minutes. All other litters of each group did not have any disruptions during their first nursing bout, supporting results found in Experiment 1 demonstrating that litters with separated pups showed more fluctuations in attachment during nursing.

4. Milk Ejections During the First Nursing Bout

There were no significant differences between the Nn and Nn(x) groups in their latency to the first ME, whether measured from time of reunion or time of attachment (see Table 3).

Cross fostering did not affect the number of definite MEs, possible MEs, or total MEs during the first nursing bout, and both dams had more definite than possible MEs (see Table 3).

The First Two Hours of Reunion

1. Time Spent Nursing

Cross fostering did not effect the time spent nursing. There were no significant differences between the Nn and Nn(x) groups in time spent full nursing, partial nursing, or the combination of them both (see Table 3).

2. Number of Milk Ejections Over Two Hours

There were no differences found between the cross fostered and just handled litters in number of definite MEs, possible MEs, or total MEs over the two hour period (see Table 3).

3. Rate of MEs

The rate of MEs over the two hour periods were similar in the cross fostered and the just handled litters (see Table 3).

<u>Behaviors</u>

Behaviors Prior to the First Nursing Bout

1. Dam Behaviors

There were no effects of cross fostering on pup directed behavior, other directed behavior, or time spent quiet prior to the first nursing bout. There were also no differences between the dams of cross fostered and just handled litters in the rate of transitions between these behaviors (see Table 3).

2. Pup Behaviors

There were no effects of cross fostering on dam directed behavior, other directed behavior, or time spent quiet prior to the first nursing bout. There were also no differences between the pups of the cross fostered and just handles litters in the rate of transitions between these behaviors (see Table 3).

Behaviors During the First Nursing Bout

1. Dam Behaviors

There were no effects of cross fostering on pup directed behavior, other directed behavior, or time spent quiet during the first nursing bout. There were also no differences between dams of cross fostered and just handled litters in their rate of on top and arched nursing (see Table 3).

2. Pup Behaviors

There were no effects of cross fostering on pup directed behavior, other directed behavior, or time spent quiet during the first nursing bout (see Table 3).

<u>The Nn group outlier, Litter J</u>

There was one litter in the Nn group, litter J, which was never observed to have had more than half the litter attached. It therefore never had a nursing bout, and also was never observed to have had a ME during the entire 2 hour period. Unlike a previously described outlier litter of Experiment 1 which was never observed to have had a ME yet gained weight over the 2 hour period, all pups of litter J were shown to lose weight over the first 2 hours of reunion. The mean pup weight decreased from 25.01g to 24.88g. In addition, the pups were shown to lose temperature, going from a mean of 35.4 C to 34.5 C. The disruption of litter J appears not to have lasted during the next 22 hours of reunion, as during this time all pups gained weight.

During the two hour period of reunion, the dam spent most her time quiet or other directed. In fact, a behavior directed toward the pups was only observed once. The pups were mainly quiet, with little other directed behavior and no dam directed behavior. Basically, the dam was quiet on one side of the cage, while her pups were quiet in a pile on the other side. Their rate of transitions between behaviors was the lowest for this litter, therefore the scenario described was quite constant.

The outlier litter present in Experiment 1, during which the dams were reunited with active pups, was litter

(Q) of the Sn group, and was never observed to have a nursing bout or any MEs during the 2 hour period. Also, there were two litters in Experiment 2, during which dams were reunited with anesthetized pups, in which contact was minimal to none. These litters were in the Sna and Ssa groups. It therefore might appear that the experience of separation by the dam was somehow involved in the lack of interactive behavior upon reunion. However, litter J of this experiment was of the Nn group and was therefore manipulated the least. Perhaps the Nn group had been nursing just prior to the observed reunion time and was therefore not interested yet. Or perhaps, the manipulations affect some dams more than others regardless of their or their pups condition. These disrupted interactions may be representative of the far end of the normal curve of the specific group population.

Comparison of Nn(x) groups between Experiment 1 & 3

The following analysis was done to determine if the above behavioral measures found for the Nn(x) group of this experiment replicated those found in Experiment 1. The measures are shown in Table 3. In summary, there were few differences between the Nn(x) groups of Experiment 1 and Experiment 3. There was a trend for the Nn(x) group of Experiment 3 to have more total MEs during the first nursing bout ($t(12)=1.879, p<.086$) possibly due to the greater time available for them to occur as the Nn(x) group of Experiment 1 had a shorter mean length of the first nursing bout, although this was not significant. This trend for having more MEs during the first bout was not reflected in a difference in weight gain, and in fact when the entire 2 hour period was looked at there was no difference in the number of MEs, time spent nursing, or the ME rate.

There were no differences in behavioral rates of the dams or pups prior to the first nursing bout. However during the first bout, pups of the Nn(x) group of Experiment 1 were less dam directed and more quiet than the pups of the Nn(x) group of Experiment 3 (Dam directed: $t(12)=2.102, p<.058$; Quiet behavior: $t(12)=-2.160, p<.053$). Perhaps the dams of Experiment 3 were more active, and the pups were less quiet and treadled more in an attempt to stay attached. However, there were no differences in dam behavior during this time. Pups of the Nn(x) group in Experiment 3 tended to elicit more MEs during the first bout, and although not significant, appeared to have a longer nursing bout. Perhaps these factors resulted in an increase of dam directed behavior, but this is also not likely, as the behavioral comparisons were based on rates taking into account the time available for the behaviors to occur, and all times associated with MEs were excluded from analysis. Perhaps the increase in dam directed behavior seen in the pups of the Nn(x) group of Experiment 3 was a result of possible MEs, which appear to be greater in number in Experiment 3 than Experiment 1, although not significantly. However, analysis excluding the times of both definite and possible MEs also found the pups of Experiment 3 to be more dam directed and less quiet (Dam directed: $t(12)=2.479, p<.030$; Quiet behavior: $t(12)=-2.540, p<.027$). Perhaps pup behavior is in general more variable than dam behavior during the first nursing bout.

DISCUSSION

No significant differences were found between the Nn and Nn(x) groups for the major variables of the nursing interaction, or in analysis of the dam and pup behaviors within it. Therefore cross fostering did not significantly affect the variables being measured, and the Nn(x) group in Experiment 1 can be generalized to a mother-infant interaction in which cross fostering did not occur.

The differences found between the Nn(x) groups of Experiment 1 and 3 were minimal. With the exception of pup behavior during the first nursing bout, the behavior of these control groups was replicated during these different experiments.

EXPERIMENT 4

INTRODUCTION

In Experiment 1, the ability of the separated pups to increase their own intake was not detected until after the first 2 hours of reunion. During the first 2 hours of reunion there was no significant difference in weight gain between the pup conditions, but over the next 22 hours separated pups gained more weight than nonseparated pups when with a nonseparated dam, and lost less weight when with a separated dam. Experiment 4 was designed to reexamine the behaviors of separated pups using extended observation periods during the first 24 hours of reunion in an attempt to determine how these pups increased their milk intake compared to nonseparated pups when with a nonseparated dam. An Nn group and Ns group were observed during hours 0-2, 6-8, and 22-24. Weights and temperatures of pups were taken before and after each observation period.

Although in Experiment 1, the separated pups did not gain more than nonseparated pups over the first two hours of reunion, there were some behaviors that might have contributed to the separated pups' increase in weight seen over 24 hours if these behaviors continued over time. Separated pups were less other directed prior to attachment, and treadled more and were less quiet during the first nursing bout, possibly reflecting their increased activation to nurse. Also, over the full 2 hour period, separated pups spent more time full nursing, and less time partial nursing perhaps reflecting that all pups within the litter were motivated to nurse.

Behaviors that might have hindered the separated pups' weight gain during the first 2 hours of reunion in Experiment 1 also existed, but perhaps over time they would be observed to decrease in rate or disappear thereby contributing to the later increase in weight seen by separated pups. These behaviors occurred in groups with separated pups (Ns and Ss) during the first nursing bout and included an increase in disruption periods and an increase in other directed behavior by the dams. Therefore separated pups were seen to interrupt the interaction, but perhaps over time the dam and pup interaction settled down, and by doing so allowed the separated pups to increase their weight gain.

METHODS

In addition to the observations made during the first two hours of reunion, as in Experiment 1, observations were made during hours 6 to 8, and hours 22 to 24. All observation periods were during the daily light cycle.

The conditions present for Experiment 4 were:

1) A nonseparated dam with nonseparated pups (Nn(x))

2) A nonseparated dam with separated pups (Ns)

Another litter was needed during the treatment period to supply the foster pups and dam necessary, and as shown in Figure 2, a Sn litter was created by this need but was not observed.

The experimental procedure was identical to that of Experiment 1. After weights were taken at hour 2, the pups were reunited again with their dam. Before and after the 6-8 and 22-24 observation periods, all pups were weighed and axillary temperatures were taken of 2 pups. Also, prior to these observation periods the markings on the pups were made darker. However, pups were not stimulated to urinate and defecate as was done prior to the first observation period, as all had been with dams between these observation times.

The reduction of the shavings to allow visibility of the litter was performed as in Experiment 1, however, after hour 2 and hour 8, a large handful of shavings was placed back in the home cages of the litters so there was bedding material available between the observation periods. This amount was removed prior to the 6 to 8 and 22 to 24 hour observation periods.

Experiment 4 was run simultaneously with Experiment 3, the Nn(x) condition being represented in both. In Experiment 4, the total number of litters per condition observed over 6 trials were: Nn(x)(n=8) and Ns(n=6).

ANALYSIS

Independent t-tests were utilized to compare the Nn and Ns groups. Analysis of weight changes were determined from measures taken at hour 0, 2, 6, 8, 22, and 24. Selective behaviors were analyzed during hours 0 to 2.

RESULTS

Weight

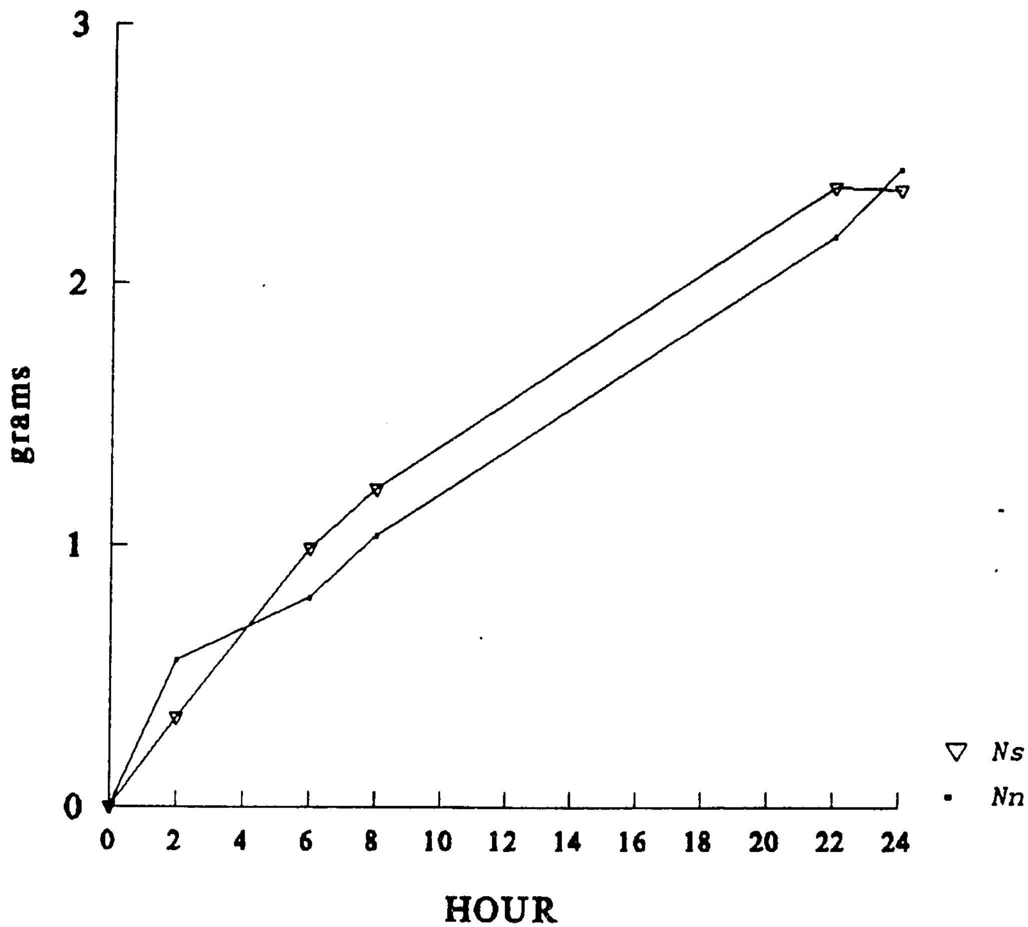
As shown in Figure 38, separated pups did not gain more weight than nonseparated pups over 24 hours. Therefore, the effect we were attempting to study, which was present in Experiment 1, was not replicated. There was no significant difference between the Nn and Ns group in change of weight from hours 0-2, 0-6, 0-8, 0-22, or 0-24.

In one interval between observation periods, between hour 2 to 6, separated pups gained significantly more than nonseparated pups ($t(12)=-2.475, p<.030$).

During the last observation period, between hours 22 and 24, separated pups were shown to maintain their weight rather than gain as a result of one litter whose pups gained a mean of only .16 grams. The other five litters of the Ns group had an average weight gain of 2.80 grams. When the litter with the minimal weight gain was excluded from analysis, there was still no difference seen between the Nn and Ns group in weight gain between hours 22 and 24.

Figure 38: The mean weight gain of the 8 litters of the Nn(x) group and the 6 litters of the Ns group before and after each observation period. These include hours 0 to 2, 6 to 8, and 22 to 24.

WEIGHT GAIN OVER 24 HOURS



Summary and Discussion

Although, in Experiment 1, there was no difference in weight gain between separated and nonseparated pups during hours 0-2, we had believed that in this experiment the later observation periods would demonstrate how separated pups were gaining more than nonseparated pups over 24 hours. The most likely reason the 24 hour weight effect of Experiment 1 was not replicated was due to the increased handling and manipulations of the litters before and after the additional two observation periods. Separated pups were seen to gain more during hours 2 to 6, perhaps due to the lack of handling or observing, however, they did not gain more during hours 8 to 22, an even longer time of uninterrupted nursing.

Although the weight effect seen in Experiment 1 was significant (See Graph 6: Pup effect of all four groups, hours 0-24: $\underline{F}(1,20)=10.28, p<.005$; post-hoc, Nn vs. Ns: $\underline{F}(1,20)=4.540, p<.047$) perhaps it was not a robust effect that is easily replicable, yet this of course, would need to be tested using a procedure identical to that used in Experiment 1.

As described before, in Experiment 1, there was no difference between pup conditions in weight gain between hours 0 and 2 (see Figure 4, Nn and Ns groups). Sometime over the next 22 hours the separated pups started to gain more than the nonseparated pups (see Figure 5, Nn and Ns groups). As shown in Figure 38, the weight changes up to hour 6 replicate those seen in Experiment 1. There was no difference between the Nn and Ns groups at hour 2 or 6, but the mean weight gain of the separated pups is greater than that of the nonseparated pups between hours 2 and 6. Perhaps observation periods during this time, without the handling and weighing disruptions, should be looked at more carefully to detect behavioral differences between the pup conditions, as it appears the differences in milk intake might be occurring at this time.

Because the weight effect was not found, the additional observation periods will not be described. However, it seemed useful to analyze hours 0 to 2, during which time the procedure was identical to that used in Experiment 1, to determine what other effects were replicated. These results will be described briefly. Although there were not four groups to compare as in Experiment 1 which contributed to the detection of the separation effects seen in the dam and pup condition, we can still look for the behavioral differences that were seen between separated and nonseparated pups as described in the introduction.

Pup Axillary Temperature

The separated pups of the Ns group were significantly cooler than the nonseparated pups of the Nn group prior to, and after 2 hours of reunion (hour 0: $t(12)=4.358, p<.002$);

hour 2: t(12)=4.942,p<.001). Also, the pups of the Ns group tended to increase their temperature more over the first 2 hours of reunion (t(11)=-2.082,p<.062). Since they had a lower starting point they were more likely to warm up more over time. There were no significant differences in pup temperature between the Nn and Ns groups at hour 6, 8, 22, or 24.

In Experiment 1, separated pups were cooler prior to and after 2 hours of reunion, therefore the above results replicate previous findings.

Attachment, MEs, and Nursing Time

As in Experiment 1, there were no differences between the Nn and Ns groups in latency to initial attachment, stable attachment, or solid attachment, the length of the first nursing bout, or the latency to the first ME. Also in Experiment 1, separated pups had disruption periods during the first nursing bout: the Ns group had significantly more disruptions than the Nn group during the first nursing bout (t(11)=-3.186,p<.010). In fact, the Nn group did not have any periods during the first nursing bout when the pups were not fully attached, while 3 out of 5 litters in the Ns group did.

The Nn group had more definite MEs during the first nursing bout (t(11)=2.313,p<.042). This could be due to the two litters of the Ns group which were never observed to have MEs during the first bout. These litters did have MEs during the 2 hour period, and did gain weight over this time, and there were no differences between the groups in number of definite, possible or total MEs over the two hour period, nor time spent nursing or ME rate. The separated pups of this experiment were not found to do more full nursing and less partial nursing over the 2 hour period, as in Experiment 1. The lack of effect for this measure may be due to not having the contributions of the Ss group.

Behaviors Prior to the First Nursing Bout

Dam Behaviors

The dams of the Nn group tended to have a higher rate of other directed behaviors than the dams of the Ns group ($t(12)=1.947, p<.076$). This is consistent with Experiment 1, as the Nn group of Experiment 1 had a significantly higher rate of other directed behavior prior to the first nursing bout than all other groups, contributed to mainly by an increased rate of changing position.

One might have predicted that the Ns group would be more quiet than the Nn group prior to the first nursing bout, as the disparate groups of Experiment 1 were shown to be (Interaction effect: $F(1,20)=5.197$, $p<.035$), however this was not seen perhaps because the Sn group was not represented in this experiment.

In Experiment 1 the dams of the disparate groups had higher rates of transitions between behaviors prior to the first nursing bout. This difference was seen again, as the Ns group had higher rates than the Nn group ($t(12)=-2.498, p<.024$).

Pup Behaviors

In Experiment 1, separated pups had a lower rate of other directed behavior prior to the first nursing bout. This effect was replicated in this experiment, as pups of the Ns group tended to be less other directed than pups of the Nn group ($t(12)=1.967, p<.074$).

No effect of separation was seen in preattachment rate of dam directed behavior, quiet epochs, or transitions between pup behavioral categories in this experiment, or in Experiment 1.

Behaviors During the First Nursing Bout

Dam Behaviors

In Experiment 1, the disparate groups were less pup directed and more quiet postattachment, however, in this experiment the Ns group was no different than the Nn group in rate of either behavior.

Dams with separated pups were found to be more other directed during the first nursing bout, and that was confirmed in this experiment. The separated pups appear to stimulate the dam while attached to be more other directed, and change position more often for example, as the dams attempt to settle down and nurse. Or perhaps the pups, due to their deprivation, were nursing longer than the dams were interested.

In Experiment 1, separation mainly affected the rate of on top and arched nursing positions in the Ss group, increasing the rate of arched nursing and decreasing the rate of on top nursing. Therefore it was not unexpected that there were no difference in rate of nursing positions between the Nn and Ns groups.

Pup Behaviors

There were rates of 0 for all but one litter of the Ns group for other directed behavior. This was not unexpected, as it means that pups were rarely seen to move away from the dam while nursing. Similar results were seen in Experiment 1.

In Experiment 1, separated pups were found to be more dam directed than nonseparated pups having higher rates of treadling, nosing and probing the dam's ventrum, and mouthing and licking the dam's teat. They were also less quiet postattachment. These rates were confirmed in this experiment; separated pups were more dam directed and less quiet, perhaps attempting to consume more milk.

CONCLUSION

The question still remains how separated pups gained more weight than nonseparated pups over time in Experiment 1. However, we now think that perhaps the weight effect occurs early on in the reunion. Because separated pups gained more than nonseparated pups during hours 2 to 6 in this experiment, perhaps it is this time period which should be looked at more closely in the future for behavioral differences. Of course, the fact that the separated pups did not gain more during other time periods in this experiment could simply be due to the increased handling of the litters.

Analysis of behaviors demonstrated that the Nn and Ns groups did not differ much, but when they did, they did in the same way as in Experiment 1. The fact that all expected changes in measures seen in Experiment 1 were not detected, such as an increase in partial nursing by separated pups, or an increase in quiet behavior by the disparate group Ns during the first nursing bout, was not surprising as there were only 2 groups to compare which decreased the opportunity of changes to be contributed to.

OVERALL CONCLUSION

The goals of this dissertation were to characterize the mother-infant interaction upon reunion after an 18 hour separation period and to determine the relative contributions of the dam and pups to the interaction seen during the first two hours. The following conclusion will discuss the results in terms of these goals, and then attempt to place these results in a theoretical framework.

I. Characterization of the Mother-Infant Interaction

Upon reunion, the separated pups were cool, due to the combination of their deprived state and lack of thermogenesis. The separated dam appeared very interested in stimulating the pups to nurse as she was less other directed than the nonseparated dam, and licked the pups' bodies more prior to the first nursing bout. During this time, the pups also appeared very interested in nursing, as they increased their contact time with the dam and were attached for brief periods of time. Although both the dam and pups appeared to have an increased motivation to nurse there was no alteration of the latency to the first nursing bout. One interpretation of this paradox was that the separated pups were hyperaroused and the separated dam was hypersensitive to the suckling attempts.

Once attached, during the first nursing bout, there were periods of disruptions, or nonconsecutive minutes during which the pups were off the nipples, suggesting that the possible hyperarousal of the pups and hypersensitivity of the dam continued. Further support for the presence of hyperarousal and hypersensitivity is that the pups treadled more and were less quiet, and the dams were seen to change position more and be less quiet.

There was no difference in latency to the first let down of milk, total number of milk ejections, or in the inter-milk ejection-interval, yet the pups with the separated dam gained more weight. This paradox could possibly be explained by a novel variation of the milk ejection response which was frequently observed in which the dam did not fully arch, or the pups did not fully stretch, called the "possible milk ejection". I believe that these particular milk ejections contributed to the increased weight gain. In addition, the pups' increased rate of treadling may have induced contractions of the mammary gland mechanically without eliciting any arch or stretch responses. Thus, the increased weight gain of the pups with the separated dam may have been the result of variations not typically seen in the way in which milk was let down.

The first nursing bout of the separated litters did not last longer than the nonseparated litters' bouts, nor were there more bouts in the two hour period, nor an increase in nursing time. Considering the increased weight gain seen, the lack of difference in time spent nursing further supports the idea that variations in the milk let down pattern were the contributing factors, as possible MEs appear to be the main alteration in the interaction upon reunion after separation. The lack of difference in time spent nursing was surprising, not only because the pups with separated dams gain more weight, but they also showed a greater increase in body temperature over the two hour period. The lack of increase in time spent nursing seen in the separated litter is not consistent with the Leon hypothesis of the thermal regulation of nursing time, which would have predicted that the cooler, separated pups would be nursed longer. Rather the control of nursing time appears to be consistent with the Stern hypothesis of ventral stimulation by the pups maintaining the dam's nursing position. A possible explanation for the increased temperature of the separated pups could be increased milk consumption, as well as the fact that they started out cooler.

It was predicted that separated pups could influence the rate of MEs by increasing the rate and amplitude of their sucking as well as the time they spent in sleep/wake states associated with increased sucking rates, however this was not supported. The ME rate of the dam decreased from hour 1 to hour 2 as a result of a decrease in the number of possible MEs. Rather than due to alterations in the separated pups' suckling, this decrease in rate of ME was believed to be a result of a depleted supply of milk available from the separated dam. Although the dam and pup remained together, over the next 22 hours, the pups lost weight, supporting the hypothesis that the separated dam's milk supply was limited.

II. The Relative Contributions of the Dam and Pups

Some effects seen were due to changes in the separated dam as they occurred equally in the groups with separated dams regardless of what pup type they were with (the Ss and Sn groups). These changes include the increased number of possible MEs and the increased weight gain of pups over the first two hours of reunion, and weight loss over the next 22. Some effects seen were due to changes in the separated pups as they occurred equally in the Ns and Ss groups. These changes include the increased number of disruption periods during the first nursing bout, and the increased rates of treadling. An interaction between the dam and pup condition was evident in the number of nursing bouts seen over the two hour period, as the disparate groups had less nursing bouts than the Nn and Ss groups.

It was also found that the active participation of the pups was necessary to elicit separation-induced changes in the dam's behavior, for they did not occur in reunions with anesthetized pups. In addition, the experience of cross fostering itself did not significantly affect the major variables of the nursing interaction upon reunion. Therefore the control, the Nn group, is generalizable to a more natural mother-infant interaction. Finally, weight gain of the separated pups over 24 hours was not increased if the procedure was altered to obtain more frequent weight measures. It was therefore believed that the weight gain effect was a sensitive effect, as it was eliminated by 3 additional brief moments of separation and handling. This implies that the separated pups, although gaining weight in the first experiment, were not really fully recovered from their separation experience as they were affected by increased disruptions.

III. Theoretical Framework

What was seen in the separated litter upon reunion was an interaction that was in some ways more efficient, in some ways disrupted, and in many surprising ways not different from the nonseparated litter. As described in the Introduction, we would like to consider three time dependent processes as a framework for understanding the separation changes seen: attachment, nursing and suckling motivation, and multiple regulators.

A. Attachment

Attachment, by some definitions requires a relationship that is specific in focus (Gubernick, 1981). Neither the dams nor pups displayed specificity in nursing or suckling behavior, as demonstrated in Experiment 3: the cross-fostered litter did not differ from the non-cross-fostered litter for all behavioral measures observed. Therefore this restrictive definition of attachment is not useful in describing the observations done on mother-infant interactions in the rat.

The attachment theory presented by Bowlby may be more useful. The initial stage he describes in the human infant in response to separation is the "protest" response and is characterized by an intensification of motivation toward the mother. Consistent with this "protest" stage, an intensification of some attachment measures was seen upon reunion in the mother-infant interaction in the rat as the pups increased the time in contact with and attached to the dam immediately upon reunion. Although this appears consistent with Bowlby's "protest" stage, it is unusual that the pups would still be in this initial stage of protest after an 18 hour separation period. One would think that the following stages of "despair" or "detachment" might be present after such a prolonged separation period; it was hypothesized that if a behavior such as detachment occurred, the rat pups would be observed to initially ignore their dam upon reunion, and the recovery to an apparently normal interaction would take some time. However, "detachment" was not observed as the pups were more dam directed upon reunion, and there was no delay in recovery of normal functioning of the interaction as there was no difference in latency to attach, latency to the first ME, or IMEI. Therefore, nothing comparable to the despair and detachment responses of human infants to separation were seen in the rat. Also, if the rat pup appears to still be in the "protest" stage, we could ask why the pups are still not actively protesting, as they do immediately upon separation, with an increased rate of ultrasonic distress calls and locomotion. In summary, it appears that in the rat pup the evidence does not support `detachment' and while it provides some incomplete support of the `protest' stage, it is not in the classic use of the attachment theory as presented by Bowlby.

Another aspect of Bowlby's theory is that despite the wide variations that exist in mother-infant relationships, such as in age, experience, or environment, the response observed was remarkably uniform. Evidence does not support a unitary response to separation and reunion as both efficient and disrupted behaviors were present in the interaction upon reunion. Perhaps the motivational framework will be more in line with the results obtained including the lack of a unitary response to the separation.

B. Nursing and Suckling Motivation

As described in the Introduction, a motivated behavior increases in intensity after deprivation, and therefore would result in an enhancement of the motivated behavior upon reunion after separation. In the pup, the motivation to suckle is vital as it is the means by which pups consume essential nutrients. Suckling by the pup has been shown to be a result of the motivational system to feed, which is affected by gastrointestinal fill, as well as the motivation to suck, which is affected by perioral stimulation (for review see Brake, Shair, and Hofer, 1988). It was hypothesized that the pups' motivation to feed upon reunion would increase in strength after separation. This was supported by literature demonstrating a decreased latency to attach in separated pups at 11 to 13 days of age (Drewett, Statham and Wakerley, 1974; Hall, Cramer, and Blass, 1977) and an increase the time and force of their sucking (Brake, Shair, and Hofer, 1988; Shair, 1986), thereby possibly increasing their milk intake.

In the studies presented here sucking was not directly measured, however latency to attach was observed as well as latency to the first ME and IMEI, which can possibly be seen as indirect measures of suckling frequency and/or strength. Also, pup weight gain was used as a measure of milk intake. We observed that behaviorally the pups had increased contact with the dam and increased dam directed behavior, however there was no decrease in latency to attach. There was also no decrease in latency to the first ME, or decreased IMEI. Therefore there is no evidence to support the notion that the pups' motivation to feed or to suck was increased by separation, as they did not attach faster, nor was there evidence to suggest they increased the time and force of their sucking. This lack of evidence of increased pup motivation is supported by the lack of increased weight gain in the separated pup condition.

It appears that there is little or no evidence to support an increase in the pups' motivation to suckle, however the increase in the pups contact with the dam as well as in dam directed behavior prior to attachment must be mentioned. As described in the section characterizing the mother-infant interaction, the possibility of the pups being hyperaroused, and the dam being hypersensitive was presented. Perhaps the pups' motivation to feed was increased in strength, however the result was disruptive in that an efficient attachment could not be made due to extreme excitability which was compounded by the dam's sensitivity to the pups' attempts. Therefore, if the pups' motivation to feed did indeed increase in strength it was not completely beneficial in that the efficiency of the reunion was not increased.

The motivational drive of the dam to nurse is not as clearly understood. Briefly, as described in the Introduction, the literature available on the elicitation of maternal behavior, or what motivates the dam to nurse, includes three descriptions of factors which contribute to some aspect of maternal behavior. Rosenblatt and colleagues have described the existence of a nonhormonal regulation of maternal behavior after parturition, which entails pup stimulation or feeding and suckling behaviors which are necessary to maintain her attentiveness to pups (Rosenblatt, Siegel, and Mayer, 1979). Leon's thermoregulatory control of the dam's nesting behavior suggests that the return of the dams to their pups between nursing bouts, and the amount of time the dam spends nursing has to do with the maintenance of maternal thermal homeostasis (Leon, 1979). And Stern and colleagues have shown that ventral somatosensory stimulation elicits nursing behavior in the dam, and thereby contributes to the motivation of the dam.

In the studies presented here, the dam was observed to be less other directed prior to attachment and licked the pups' bodies more, perhaps attempting to stimulate them to nurse. Her motivation toward the pups might also be an attempt to experience the stimuli that have been lacking, such as the pups' smell and tactile stimulation, to reestablish the interaction effectively. This latter possibility is consistent with the literature describing the maintenance of maternal behavior by pup stimulation rather than hormones after the time of parturition (Rosenblatt et al., 1979).

The maternal thermoregulatory control of nesting bout time was not supported. Theoretically, pups that were cooler upon reunion should have been nursed longer as it would have taken the dam longer to reach the critical rise in temperature shown to terminate nursing bouts. This was not the case as there was no difference in nursing bout length or total time spent nursing between the separated and nonseparated litters. Therefore these results were not consistent with the Leon hypothesis of maternal thermal homeostasis, although it is possible that the reestablishment of the thermoregulatory control of nursing bout rhythms would eventually occur. It would be interesting in future experiments to monitor the dam's temperature during the separation period as well as during the interaction upon reunion to more accurately assess the effect of separation on the thermoregulatory control of nesting time.

The motivation of the dam to nurse appeared consistent with the theory proposed by Stern and Johnson (1990) in which the ventral somatosensory stimulation of the dam by the pups' nosing, probing, and attaching is critical to maintain the dam's nursing posture. The increased activity of the pups to nurse, and the dam's receptivity to this stimulation appeared to be more important in determining the dam's motivation to nurse rather than her thermal homeostasis. However, there is no evidence to support the notion that the separated dam's motivation to nurse, or her sensitivity to the ventral somatosensory stimulation was increased as there was no increase in total time spent nursing over the two hour period. What was observed were periods of disruption during the nursing bouts of the separated dams, when the pups were briefly unattached. Perhaps this represented a hypersensitivity on the dam's part to the ventral somatosensory stimulation provided by the pups, and therefore the separated dam, although motivated to nurse more, did not.

In summary, suckling and nursing motivation were somewhat useful concepts to help understand the separation effects seen, however, they too were limited. The only evidence to support an increase in pups' motivation to suckle was an increase in time in contact with the dam and an increase in dam directed behavior. There was no change in latency to attach, latency to the first ME, IMEI, or time spent nursing. The only evidence to support a possible increase in the separated dams' motivation to nurse was the decrease seen in other directed behaviors and the licking of the pups' bodies more prior to attachment. So, although the dam did appear to seek out and be receptive to pup stimulation, there was no increase seen in time spent nursing. There wasn't even a thermoregulatory response to the cooler, separated pups which would have resulted in an increased time spent nursing according to Leon. A possibility which exists is that there was an increase in both the pups' motivation to suckle and the dam's motivation to nurse, however it was not helpful in creating an efficient interaction upon reunion as they had become hyperaroused and hypersensitive. Therefore what resulted was an effective reunion but the hyperaroused state of the pups and the hypersensitive state of the dam prevented an efficient reunion from occurring by being disruptive.

C. Multiple Regulators

The concept of multiple regulators describes the existence of overlapping and integrated controls for certain processes within the mother-infant interaction. Upon separation, or the withdrawal of specific regulatory actions provided to the pups by the dam, a shift in functions of pups' physiological systems are seen. Many of these separation effects plateau after 18 hours of separation, at which point, the pup appears to set in motion self-correcting or adaptive processes which operate to reverse the biological responses to separation. What is the adaptive value of the presence of these multiple regulators? Do they allow the pup, upon their removal, to be more adaptive to the independent state as the control of the pups' physiological systems becomes more intrinsic? Or, perhaps they create changes which prepare the pup for an intense reunion, compensating for the separation period.

Of course, the interaction is bidirectional, and the pups' stimulation regulates the dam's behavioral and physiological state, and upon their removal she also experiences a withdrawal. Therefore, the separation period is also affecting the dam, possibly either preparing her for permanent separation or eventual reunion.

Let us explore the possibility that the removal of the multiple regulators prepares the pup for independence from the dams, possibly through conservation of energy. If this indeed had occurred during the 18 hour separation period, one would expect that upon reunion, the pups would be unable to reconnect with the dam. Overall there appears to be little evidence to support this idea since the nonseparated and separated litters both had successful reunions. The pups suckled, the dams nursed, nursing time was consistent across groups, and all pups gained weight. This evidence also does not support the idea that the dam had been adjusting to the separated state at the cost of being unable to reunite.

Perhaps the removal of the multiple regulators prepares the pups for an interaction upon reunion that has increased in intensity, as the pups become more wakeful and active despite the metabolic preparation for conservation that occurred. However, there is little evidence to support this theory either, as there was no decrease in latency to attach in the separated litter, nor a decrease in latency to the first ME, or increase in total number of MEs, or even an increase in time spent nursing. Therefore, neither the pups nor the dam were more intense upon reunion in their ability to interact and exchange nutrients after separation.

Another possible outcome is that the initial stages of reunion would appear disrupted, as the dams and pups become reexposed to these systems being regulated externally within the mother-infant interaction, and then a gradual recovery of the multiple regulators' function occurs and the interaction readjusts. However, it does not appear that this occurred either. As described in the previous paragraph, not only was there no increase in the efficiency of the interaction, there was no initial disruption as the separated litter did not take longer than the nonseparated litter to attach and settle down into the first nursing bout.

Our results show that recovery upon reunion is not a unified process; the interaction upon reunion contained both efficient and disrupted behaviors as a result of separation effects. In other words, the mother-infant interaction upon reunion was not a unitary phenomenon, with all the behaviors holding together and the relationship being renewed as a whole. Rather the result could be interpreted as the interaction of many processes at different stages of adaptation to the separated state being reexposed to the various aspects of the mother-infant relationship that were present prior to separation. The assumption is that the processes in the pup that are at an early stage of adaptation to separation are more easily resumed upon reunion, and those that are in more advanced stages of adaptation to separation appear disrupted upon reunion.

By determining which behaviors were easily renewed upon reunion, and which behaviors appeared disrupted, we can see that many different processes exist within the relationship that are possibly under the control of multiple regulators working through various sensory pathways. A behavior that not only appears to resume easily but increases in efficiency as well is milk delivery and consumption by separated litters. The pups consume more milk over a time period equivalent to that seen in the nonseparated interaction. Behaviors that may have contributed to this weight gain include the increased motivation of the dams and pups to nurse (increased contact and nosing and probing of the dams' ventrum by the pups, and licking of the pups by the dam), an increased number of possible MEs, and increased rate of treadling by the pups. Other behaviors that appear do not help the efficiency of the reunion include the hyperarousal of the pups and hypersensitivity of the dam possibly preventing a decrease in latency to attach, latency to the first ME, or a decrease in the IMEI. And finally, other behaviors were clearly disruptive, such as the disruption in the nursing bouts and weight loss in pups over

24 hours possibly due to a decreased milk supply.

The presence of both adapted and disrupted behavior in the interaction upon reunion is consistent with the concept that there are so many variables at work in the mother-infant relationship that an observed behavior is the result of multiple regulators, and each system would have a different functional curve of adjustment to separation and effect upon reunion. The presence of these multiple regulators is an advantage in situations of environmental stress. The variability in the stress response provides the flexibility to adapt to multiple situations, including a separation period. Selection pressure, rather than narrowing the pups' optimal environmental choice, has provided the animals with the ability to survive in a wide range of environments and withstand severe levels of stress.

Due to the separation effects, whether they are considered adaptive to independence or to an efficient recovery of the interaction upon reunion, or both (or neither), the pup and dam have been changed by the experience, thereby becoming new or different pups and a new or different dam. The divergent changes that occurred in both the dam and pups during separation can be seen as creating a qualitatively new relationship upon reunion with novel features. The presence of the novel milk ejection reflex, or possible MEs, in the separated dam condition, and the presence of disruption periods, or periods of unattachment during nursing in the separated pup condition represent these novel features which were not found in the nonseparated group. Also, the increased rate of position change by the dam, and rate of treadling by the pup are different rates of activities that occur post-separation that did not occur in the control group. It is interesting that although these novel behaviors or rates of behaviors were present, there was no effect of separation on the length of nursing bouts, total time spent nursing, latency to attach, latency to the first ME or IMEI. Perhaps the separated litter are achieving these same critical aspects of the mother-infant interaction through different behavioral approaches and thus the nonseparated and separated groups appear surprisingly similar. Therefore, the separation might appear to have little or no effect on the major variables of the interaction when actually the achievement of these aspects of the interaction is the result of great flexibility and adaptability on the part of the dam and pups within the mother-infant relationship.

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