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**The Interaction of Sleep-Wake States
and Sucking Behavior in Young Rats**

by

Harry N. Shair

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

1986

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

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ABSTRACT

The Interaction of Sleep-Wake States and Sucking Behavior
in Young Rats

by

Harry N. Shair

Advisor: Dr. Myron Hofer

The fine structure of nursing behavior in mammals is largely unknown and the processes by which the motivational changes of deprivation affect this early feeding system are as yet poorly understood. This dissertation explores these two areas using novel methods devised for this purpose.

A series of three experiments were conducted examining the relationship between sleep-wake states and feeding behavior in two-week-old rats. Electrophysiological and behavioral measurements were performed for two hour periods on pups either interacting with their normal mother and littermates or while attached to the teat of their anesthetized dam. After the baseline period in each experiment, pups were separated from their mothers for 22 hours, then tested during a two hour reunion period under identical conditions.

Contrary to general belief, rat pups were found to be asleep much of the time while being nursed by their mothers. They fell asleep prior to receiving any milk. Another unexpected finding was that sucking by the pups, which has been shown to be necessary to elicit milk ejections (ME), occurred at appreciable levels while asleep. Pups were virtually always asleep at the onset of ME, were awakened briefly,

and returned rapidly to sleep.

Sucking was increased with deprivation, as expected, but the rates of sucking in each state did not increase. Instead, the proportion of time spent in states with high rates of sucking (awake and slow wave sleep) increased, while time spent in the state with lowest rates (paradoxical sleep) decreased. Even after deprivation, the pups were always asleep just prior to ME. They sucked more in response to milk upon reunion, but again mainly by shifting states.

This study provides one of the first demonstrations of an organized, essential behavior that is embedded in sleep, and suggests a novel role for altered sleep-wake state organization as a mediator of changes in motivated behavior in the infant rat.

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The phrase is trite, but the feeling is true: Words are not adequate to express my gratitude to Dr. Myron Hofer. The stimulating ideas, patient direction, professional and emotional support you have provided during the course of this study are a continuation of what has been going on since our association began. At the time of this writing, we have been working together for almost 18 years. In many ways, I have grown up in your laboratory and as your friend.

Many other people have helped with this research in one way or another. The first of these is Dr. Stephen Brake. Steve's study of the development of sucking behavior was one of the bases for this work. He also scored all the sucking data reported here. In addition, Steve, thank you for the hours of discussion. You will find many of your comments reprinted verbatim here.

I would like to thank the remaining members of my dissertation committee, Drs. Gerald Turkewitz, Gordon Barr and most recently Dennis Kelly. Their comments and criticism were both thoughtful and helpful. Together with Myron and Steve, they turned my dissertation defense into a lively discussion among colleagues.

The following technicians, graduate students, high school and college volunteers helped with the surgery, recordings and data analysis. Many also provided creative advice. Two of these people were associated with this project for long periods of time: Amy Fisher

and Shidan Tavana. In chronological order, the others included Lee Kupersmith, Dick Shindledecker, Charles Levine, Michael Macy, Jamie Panero, Alex Gottschalk, Gus Romano, Jenifer Leaf, Myra Joyce and Ann Zmitrovich. Thank you all.

Without Phyllis Blackman, I would never have graduated on time. Thank you for typing, editing and proofreading beyond the call of duty.

Susan Brunelli and I met and married in graduate school. Susan gave birth to our daughter Carolyn and her own dissertation as I was producing mine. Susan furnished the emotional support, intellectual exchange and the time necessary for me to graduate at all.

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INTRODUCTION

The format of the Introduction will be as follows:

1. Goals.
2. Selective Review of the Literature on Control of Food Intake in Pre-weanling Rats.
3. Studies of Sucking Behavior in Pre-weanling Rats.
4. Discussion of the Importance of Monitoring Sleep-Wake States in Studies of Young Organisms.
5. Evidence Linking Sleep-Wake States and Feeding.

Goals

Very little work has been done to investigate systematically the relationship of sleep-wake states and feeding behaviors during development. Each of these areas has been studied extensively, but separately. With a few important exceptions, most observations linking the two behaviors during ontogeny have been casual. Yet, there are reasons to believe that sleep-wake states and feeding behavior do interact in adult organisms and developmentally. This research is reviewed in the sections below.

One goal of this dissertation is to describe the relationship between sleep-wake states and nursing behavior in infant rats. A particular aspect of feeding behavior, sucking by the pups, is of primary interest, but other aspects are considered as well. To attain this goal, pups were studied with their normal mothers and litters while the pups' sleep-wake states and jaw muscle activity was monitored polygraphically. Pups of 12-14 days of age were chosen for investigation for two reasons. This is the earliest age at which slow

wave sleep is fully distinguishable (Jouvet-Mounier, Astic and LaCote, 1970). Also, at this age, a very streamlined head implant is possible (Hofer, 1976) which permits the test pups to compete with littermates during the normal nursing episodes.

A second goal is to begin to understand the controls for any of the relationships discovered. To this end, the mother-pup interactions were manipulated in several ways. Deprivation of the pups from the dam has known effects on both feeding behavior (e.g., Brake, Sager, Sullivan and Hofer, 1982; Hall and Rosenblatt, 1977; Lorenz, Ellis and Epstein, 1982; see below for more references) and sleep-wake states (Hofer, 1976; Hofer and Shair, 1982). Therefore, deprivation and reunion were used to perturb the normal relationships between states and feeding and thus highlight any interactions and control mechanisms. Another manipulation involved testing the pups while attached to the teats of their anesthetized mothers both before and after deprivation. In this preparation, the mother's behavior including milk delivery was removed as a variable for the study, allowing more precise focus on the pups themselves.

Selective Review of Literature on Control of Intake

One area that this dissertation was planned to help understand was how young rat pups are able to control their food intake during nursing. For some time there has been a controversy in the literature as to whether or not young rat pups can in fact control their intake while attached to the teat of a nursing mother. Many researchers believe that rat pups are passively attached to their mother's nipples and the mother controls how much milk they ingest. Even the name of

the milk delivery process, the milk ejection, suggests such a passive role for the pup: the mother ejects the milk into the passively receptive pup.

The basis for the controversy over rat pups' control of food intake are two series of deprivation experiments with seemingly different results. In both series of experiments, rat pups of various ages were separated from their dams and thus deprived of food and all other aspects of the mother-infant interaction including sucking. Upon reunion, most studies found that nondeprived and deprived pups younger than 10-11 days of age show no differences in latency to attach to the teats of their anesthetized dams or changes in the percent of time attached during the test session (Cramer, Blass and Hall, 1980; Hall and Rosenblatt, 1978; Brake, Sager, Sullivan and Hofer, 1982). One report has shown such a difference (Drewett, Statham and Wakerley, 1974). Even when returned to normal litters and mothers, deprived and well-fed pups remain attached equal amounts of time (Lorenz, Ellis and Epstein, 1982). Also, for pups less than approximately 15 days of age, deprivation level does not affect the amount of milk ingested by pups attached to the teat of an anesthetized dam and receiving pulses of milk through a posterior tongue cannula (Cramer and Blass, 1983a,b; Hall and Rosenblatt, 1977). In fact, very young pups remain attached to the nipple and draw in milk until extremely overengorged and often in respiratory difficulty (Cramer and Blass, 1983a,b; Hall and Rosenblatt, 1978).

In contrast to this startling result, when placed together with a normal unanesthetized dam, the deprived pups will ingest more than nondeprived pups (Friedman, 1975; Houpt and Epstein, 1973; Houpt and

Haupt, 1975). This increase in ingestion is true even though the attachment time to the teat is not increased (Lorenz, Ellis and Epstein, 1982). It is also true that very young rat pups will show the effects of deprivation on intake when tested in an independent (i.e., not attached to a teat) feeding experiment (Hall and Bryan, 1980). Thus, two lines of study with different experimental conditions, had seemingly contradictory results. It was these differences in experimental condition that provided one of the bases for thinking that the experimental results could be resolved.

The rats studied in this dissertation were two weeks old, approaching the age at which satiated pups will decrease intake even when receiving pulses of milk through a tongue cannula and attached to a teat. Since the controls of this behavior appear to change at this age, why not study younger rats? As mentioned above, two of the reasons had to do with the age at which slow wave sleep develops and at which a streamlined head implant was practical. However, the main reason is that we were studying the pups in the natural nursing situation. In the natural nursing situation, even very young pups regulate intake based on deprivation levels (Haupt and Epstein, 1973; Haupt and Haupt, 1975). There is no evidence that the controls of food intake during nursing change around this age. Thus, there is good reason to believe that our findings would generalize to younger pups as well.

Studies of Sucking Behavior

One measure missing from the above studies is the actual sucking behavior of the pups. Stephen Brake has demonstrated that sucking

levels do change in response to deprivation (Brake, Wolfson and Hofer, 1979). He measured sucking behavior by implanting the digastric muscle of the jaw with an electromyogram (EMG) electrode and recording EMG on a polygraph. Pups can be attached to a dam's teats for the same length of time yet be spending more or less time sucking. Different types of sucking are available to the pups as well. Brake has described three different kinds of sucking: rhythmic sucking, treadles, and bursts (see Methods for further description). These three sucking types are differentially influenced by deprivation and by the nutritive consequences of sucking (Brake, Wolfson and Hofer, 1979; Brake, Sager, Sullivan and Hofer, 1982). When tested on an anesthetized, nonnutritive dam, rhythmic sucking is increased by deprivation even if the pups are given a milk preload of 5% body weight prior to the test. Arrhythmic (i.e., bursts and treadles) sucking is not increased by deprivation in this test situation. However, if tested on an anesthetized mother, but under nutritive conditions, with milk available in a tongue cannula, giving the milk preload prevents the increase in sucking caused by the availability of milk. Thus, the level of the actual sucking behavior itself is influenced by both nutritive status (deprived or not deprived; preload or no preload), and by whether the sucking produces nutritive results or not.

Soon after Brake began his studies of sucking behavior, it was decided to attempt to study feeding in the natural nursing situation. If the changes in sucking behavior described by Brake are critical to how the pups control intake, similar changes should be seen during normal nursing bouts. At the same time, since the pups would be attached to a polygraph for the EMG measure, it was decided to monitor

the pups' sleep-wake state as well. There were two interrelated reasons for this decision. (1) Much work has been shown that monitoring sleep behavior is extremely important in assessing the capabilities of young organisms. (2) There is a great deal of evidence linking sleep-wake states and feeding behavior.

Importance of Monitoring Sleep-wake States

Before discussing the importance of monitoring states, it is critical to define what one is discussing.

"By state, one tries to describe constellations of certain functional patterns of physiological variables which may be relatively stable and which seem to repeat themselves. They are readily recognized as they occur again and again . . . The enigma of the state concept lies in its abstract character. Since such a concept stands or falls with the variables that are applied, as well as with their assessment, it is evident that only operational definitions are acceptable. This principle leads to the subjective and arbitrary nature of all rating scales for states, varying with the purposes for which they are designed (Precht, Akiyama, Zinkin and Kerr-Grant, 1968)."

Without attention to state, work with infants often produces confusing and/or contradictory results. Of course, if one's major emphasis is the study of sleep, this idea is crucial. Anders (1975) clearly makes this point.

"The attention paid to "state" as a necessary variable to control in neonatal investigations has enhanced the reliability and validity of all data that generated from newborn studies. An infant's responsivity varies according to its state . . . it is now considered essential and has become commonplace to standardize observations and compare responses only after controlling for the infant's state."

Not only sleep researchers feel that state behavior is a critical aspect of neonatal studies. Chase (1972) who studies the development of reflex activity and Junge (1979) from the area of cardiovascular development, also stress this idea. For example, in discussing early

work on fetal heart rate (F.H.R.) and movement pattern, Junge says, "unfortunately in these publications possible influences of spontaneous changes of central nervous coordination on F.H.R. and fetal motor activity have not been taken into account."

The most comprehensive discussion of this subject to my knowledge is the article by Anneliese Korner (1972) called "State as Variable, as Obstacle, and as Mediator of Stimulation in Infant Research". The title is practically self-explanatory. She proposes that there are three ways of viewing "state" while doing infant research. The idea of state as an obstacle is the most important one to this introduction. Dr. Korner describes how both false-positive and false-negative results can be generated if state behavior is left uncontrolled.

False-positive results are especially likely to occur in studies of individual differences. If behaviors occur with different frequencies in the different states, or have changes in thresholds of elicitation, then individual differences may be found simply due to the different states of the infant. Since in human infants, several behaviors including nonnutritive sucking have been shown to occur at different levels as state changes (Korner, 1972; Wolff, 1972), monitoring state behavior in any study which also measures sucking is especially critical.

False-negatives are most likely to be found in studies of the effects of experimental treatments or studies which explore what groups of infants may have in common. Korner (1972) states, "If, for example, in a conditioning study one does not strictly control for the infant state at the time of testing, the infant's response may be so variable that one may conclude wrongly that conditioning had little or no

effect." Martinius and Papousek (1970) were able to get habituation of an eye blink response in 5 day old infants while the infants were in quiet sleep, but not while they were in active sleep. Had state not been controlled, the results might have been quite confusing.

The idea that state acts as a mediator of stimulation may also be an important concept for this work. Korner (1972) presents an example in which the same stimulation of infants (e.g., placing them upright onto the caretaker's shoulder) may bring the infant into a state receptive for learning, depending on the state the infants were in prior to the stimulation. It is easy to imagine, that the response of infant rats to a milk ejection may differ depending upon the state of the pup prior to that milk ejection.

Evidence Linking Sleep-Wake States and Feeding

The interaction of sleep-wake states and feeding have been shown in many different species and at many different ages. Those interactions can be classed in four different types which will be discussed in turn below.

1. We have all had the sensation of feeling sleepy after a heavy meal. This is called post-prandial sleep. In fact, in adult rats entering sleep has been described as an important part of the satiety sequence (Young, Gibbs, Antin, Holt and Smith, 1974). Indeed, one of the major findings implicating cholecystokinin (CCK) as a satiety hormone is that fact that rats go to sleep after CCK injection (Mansbach and Lorenz, 1983). Post-prandial sleep has also been described in infant humans. Infants are more likely to be asleep following a feed than prior to that feed or than following a sham

feed (Emde and Metcalf, 1970; Harper, Hoppenbrouwers, Bennett, Hodgman, Sterman, and McGinty, 1977; Wolff, 1972). One way in which post-prandial sleep in infants can occur is described by Emde and Metcalf.

"A characteristic behavioral sequence is: sucking with eyes 'bright' and awake-type eye movements, sucking with eyes 'glassy' and without gross eye movements, sucking with a horizontal darting of the eyes, and, finally, continued nutritional sucking with horizontal and vertical eye movements indistinguishable from REMs as seen during drowsiness and sleep."

Thus again, in this situation, sleep could be considered part of the satiety sequence. In fact, this example suggests that the babies may enter a state which is at least similar to paradoxical sleep while continuing nutritional sucking.

Not only is feeding important, but the oral factors involved in feeding are also important in eliciting sleep. Wolff (1972) demonstrated that infants fed through a stomach tube are not as likely to fall asleep following a feed as infants fed by mouth. Feeding can affect other variables besides sleep-wake states either as correlative phenomena or perhaps due to the influence of the state. Heart rate also varies following a feed (Harper, et al, 1977).

2. Feeding behavior may help to regulate sleep-wake state patterning. This has been demonstrated by experiments involving changing meal size, composition, or timing, and observing changes in sleep-wake state behavior. The most dramatic change in sleep-wake state behavior is found following food deprivation. In general, the amount of sleep decreases severely following such deprivation (Danguir and Nicolaidis, 1979; Hockman, 1964; Jacobs and McGinty, 1971). The phase of sleep most depressed, the patterning change, and the amount of that depression all depend on the specifics of the experimental

preparation. In adult rats at least two studies have shown that increasing meal size or changing the timing of the meals can change sleep-wake state cycles (Danguir, Nicolaidis and Gerard, 1979; Mouret and Boblillier, 1971). Similar findings have been found in human infants. Gaensbauer and Emde (1973) showed that infants who are schedule-fed have a different sleep-wake response to feeding than infants who are demand-fed, even though the average time in between feeding is the same. Finally, the composition of the diet can influence the type of sleep in the period after feeding. Diets high in tryptophan will cause babies to enter quiet sleep and active sleep more quickly than diets high in valine (Yogman and Zeisel, 1983).

These influences of nutrient on sleep probably do not depend completely on gastric mechanisms. Intravenous infusions of nutrient also affect sleep-wake states. In lean, young adult rats given no other food, intravenous infusions of amino-acids increase paradoxical sleep. Neither glucose or lipid infusions have any significant affect, but a composite solution of all three nutrients increases both paradoxical and slow wave sleep (Dauguir and Nicolaidis, 1980). Both protein and glucose, given intravenously, increase slow wave sleep at the expense of paradoxical sleep in young women (Lacey, Stanley, Hartmann, Koval and Crisp, 1978).

3. Many studies in animals have demonstrated that there is a very quick acting link between nutrient and changes in electroencephalogram (EEG) patterns. The general finding is that infusion of nutrient causes the animal to have more slow wave EEGs. This has been found in response to milk infused into the mouth and gut in anesthetized animals (Sudakov, 1965); in response to nutrients

given via jugular cannulae in adult rats (Rosen, Davis and LaDove, 1971); and in infant cats either sucking at the teat of their mother or feeding from a bowl (Anokhin and Shuleikina, 1977). Intraduodenal infusions of fat in the adult cat seem to cause increases in paradoxical sleep (Rubenstein and Sonnenschein, 1971). This increase is blocked by atropine.

4. The reverse relationship between feeding and sleep has also been found. Siegel (1975) has shown that the amount of REM sleep in a 12 hour period was correlated to the amount of food intake in the next 12 hour period in adult cats. If there was increased REM during that time, there was a decreased food intake in the next 12 hours. It was not true that the total amount of sleep was predictive of changes in feeding behavior. Thus, one particular portion of sleep seems to be crucial for this prediction.

These known interactions between sleep-wake states and feeding behavior confirmed our desire to monitor sleep-wake states during natural nursing in the infant rat.

METHODS

The dissertation will consist of four separate experimental series; I will give the general methods first, and then the specific methods for each experiment.

General Methods

Subjects

Wistar derived rats (*Rattus norvegicus*; Marland Farms) were born and raised in our laboratories in Plexiglas terraria, 40 x 20 x 24 cm, under reverse 12-hour light:12-hour dark cycle. Cages were checked for births each morning and afternoon. The day of notation was labeled Day 0-1. Litters were culled to 8-9 pups within 2-3 days after birth. Food (Purina Rat Chow, #5001) and water were available ad libitum to the dams. Bedding material was pine woodchips. Temperature (25-27°C) and humidity (50%) were controlled in the laboratory building. In three of the four experiments, the litters were raised in an Animal Care room with a population of approximately 25 females near the time of parturition or with litters of under three weeks of age. No experimental procedures were carried out in this room.

Procedures

The procedures followed here have been previously reported in part (Hofer, 1976; Hofer and Shair, 1982; Shair, Brake and Hofer, 1984); however, they will be repeated and expanded here.

Litters were undisturbed until 11-12 days postnatally when one member, chosen at random¹, was implanted with chronic electrodes (see below). After recovery from anesthesia, the pup was intubated with 1.0 ml of bovine milk formula (2 parts Carnation evaporated milk: 1

part H₂O) and replaced with litter and dam. The intubation replaced nutrient which would have been received during the time of the operation and recovery thereafter. The litter was reduced in size from 9 to 6-7 pups. These procedures were necessary for the implanted pups to gain weight prior to the beginning of the experiment.

Two days later, at 1200 hours, in the mid-dark period of the light/dark cycle, the mother was removed from the cage, the infant was weighed and its rectal temperature taken. Only those pups which gained weight since the time of the electrode implantation were included in the experiment. The pup's electrodes were connected via leads to a Grass Model 7 polygraph and the pup returned to its home cage nest area. Two hours was chosen as the time of the recording session since it is sufficient to include a nursing bout as well as an inter-bout interval in most cases (Croskerry, Smith, Leon and Mitchell, 1976; Grotta and Ader, 1969; Hofer and Grabie, 1971). In addition, it was similar to our previous experiments (Hofer, 1976; Hofer and Shair, 1982). Plus, in a two hour period, the pup is unlikely to develop many separation effects since inter-bout intervals of that length are seen with litters of this age.

Depending upon the experiment, the pup was recorded while attached to the teat of its urethane-anesthetized dam or with its littermates and unanesthetized, freely moving dam. This recording session was labelled Baseline day. The polygraph was positioned near the home cage terrarium to facilitate concurrent recording of behavioral observations. Because recordings were made during the dark phase of the light cycle, the infant was observed under indirect and minimal light (equal to direct light from a 15 watt bulb). The paper speed on the polygraph was 10 mm per second. At the end of the Baseline

recording, the lead wires were removed from the pup and its rectal temperature and weight were taken again.

Note that any weight changes during the experiment may not accurately reflect intake or metabolic rate, since excretion was not controlled. Pups may have been stimulated to excrete during the session either by the dam or by the procedures themselves. We decided not to void the pups prior to the recording session for fear that their behavior or the mothers' reactions to them might have been changed.

The exact manner of housing overnight for the pup depended upon the experiment. However, all test pups were separated from their mothers, had at least two littermates as companions, were in a nest of home cage shavings, and had thermoregulated heat supplied (36.5 °C delivered to the bottom of the cage).

After 22 hours of separation, each test pup was recorded and observed during a two hour Reunion session. The procedures for the Reunion test were exactly the same as on Baseline day for that particular experiment.

Electrode Implantation and Physiological Recording

Ethyl ether anesthesia was used and the infant held in a modified stereotactic apparatus on a heating pad. Bipolar cortical electrodes (38 gauge braided steel wire, Cooner, Bioflex, wound at opposite sides of a 2 x .5 mm washer machined from Delrin plastic) were placed on the dura mater through a two mm burr hole drilled two mm lateral to the sagittal suture and midway between lambda and frontal sutures.

Electromyogram (EMG) electrodes were sewn onto the nuchal musculature.

The EMG electrodes were formed of the same braided wire with a drop of solder flattened on the end to be sown down to the muscle. The EMG

wire ran rostrally to an anchor post on the opposite side of the sagittal suture from the cortical electrodes. The electrodes were cemented in place (Cranioplastic, Plastic Products) and the skin sewn up to cover the entire head piece. The four strands of the insulated wire passed caudally for 4 cm from the head piece so they lay along the pup's back.

The digastric chin muscle was implanted with electrodes identical in design to the neck EMG muscles described above. The wires from the digastric EMG electrodes were led under the skin and out through a hole made in the skin of the back where they lay along the fur. This implantation procedure has been described previously (Brake, Wolfson and Hofer, 1979). Two 30 gauge silver wire loop electrodes were placed subcutaneously across the dorsal thorax (Hofer, 1970), from which was recorded respiratory rate by impedance pneumography.

All connections were made between implanted electrodes and recording wires by Amphenol Reliatac subminiature connectors. The implantation technique was designed to be maximally streamlined and flexible so as to interfere minimally with the pup's burrowing under the mother and competing with littermates during the nursing process. As noted above, after recovery from anesthesia, the implanted pup was fed 1 ml bovine milk by intubation and then placed with its littermates for one-half hour in the home cage before returning the mother to the cage. The incidence of the mother destroying such electrodes and/or the pup not gaining weight prior to Baseline day was about 15%.

Scoring of Sleep-Wake State and Sucking Behaviors

Three major states were defined by classical criteria (Anders, Emde and Parmalee, 1971; Gramsbergen, 1976; Jouvet-Mounier, Astic

and LaCote, 1970) from polygraph recordings of electroencephalogram (EEG), neck EMG, respiratory rate, and behavioral observation of activity. Illustrations of these are given in Fig. 1, 2 and 3 . Awake (A) equalled fast, low voltage EEG, high amplitude neck EMG, variable and fast respiration, and bouts of coordinated behavioral activity. Slow wave sleep (SWS) consisted of slow, low voltage EEG, moderately low EMG amplitude, very regular respirations and behavioral quiescence. Paradoxical sleep (PS) equalled fast, low voltage EEG, minimal neck EMG with occasional phasic twitches, irregular respiratory pattern, and behaviorally sudden movements superimposed on a loss of postural tone. Note that eye movements were not recorded because respiratory patterns gave a clearer distinction between the two major sleep states at this age. A pattern of phasic irregularity was seen during PS, whereas during SWS the respirations showed almost machinelike regularity. By these methods of dorsal subcutaneous electrodes and an impedance recording, body movements were also sensitively registered on the polygraph as baseline fluctuations. Fifteen-sec epochs were classified according to whatever state occupied a majority of the epoch. A rationale for these criteria for state scoring will be discussed later (see below).

Sucking was characterized according to three basic patterns of muscle activity previously described by Brake, Wolfson and Hofer (1979). The relationship between these patterns and actual suction on the teat was verified in that study. The three patterns consist of treadles, bursts, and rhythmic sucking (previously called rhythmic runs). Bursts (B) were characterized as discrete episodes of high frequency activity lasting at least two seconds. Treadles (T) consisted of discrete periods of activity with slow wave mixed with

Figure 1. Sample polygraph tracing of a nursing pup in the awake state. High neck electromyogram (EMG), activated electroencephalogram (EEG) and irregular respiration are seen. Note that the amount of slow waves are increasing towards the end of the tracing, as the pup begins to fall asleep. The digastric EMG shows treadles and rhythmic sucking. Thirty seconds (two scoring epochs) of recording are illustrated.

Top channel = EEG; channel 2 = timing (in seconds); channel 3 = digastric EMG; channel 4 = respiration (by impedance pneumography); channel 5 = neck muscle EMG.

RS = rhythmic sucking; T and TD = treadle; SCR = scratch. TD and SCR are behavioral observations written during the recording.

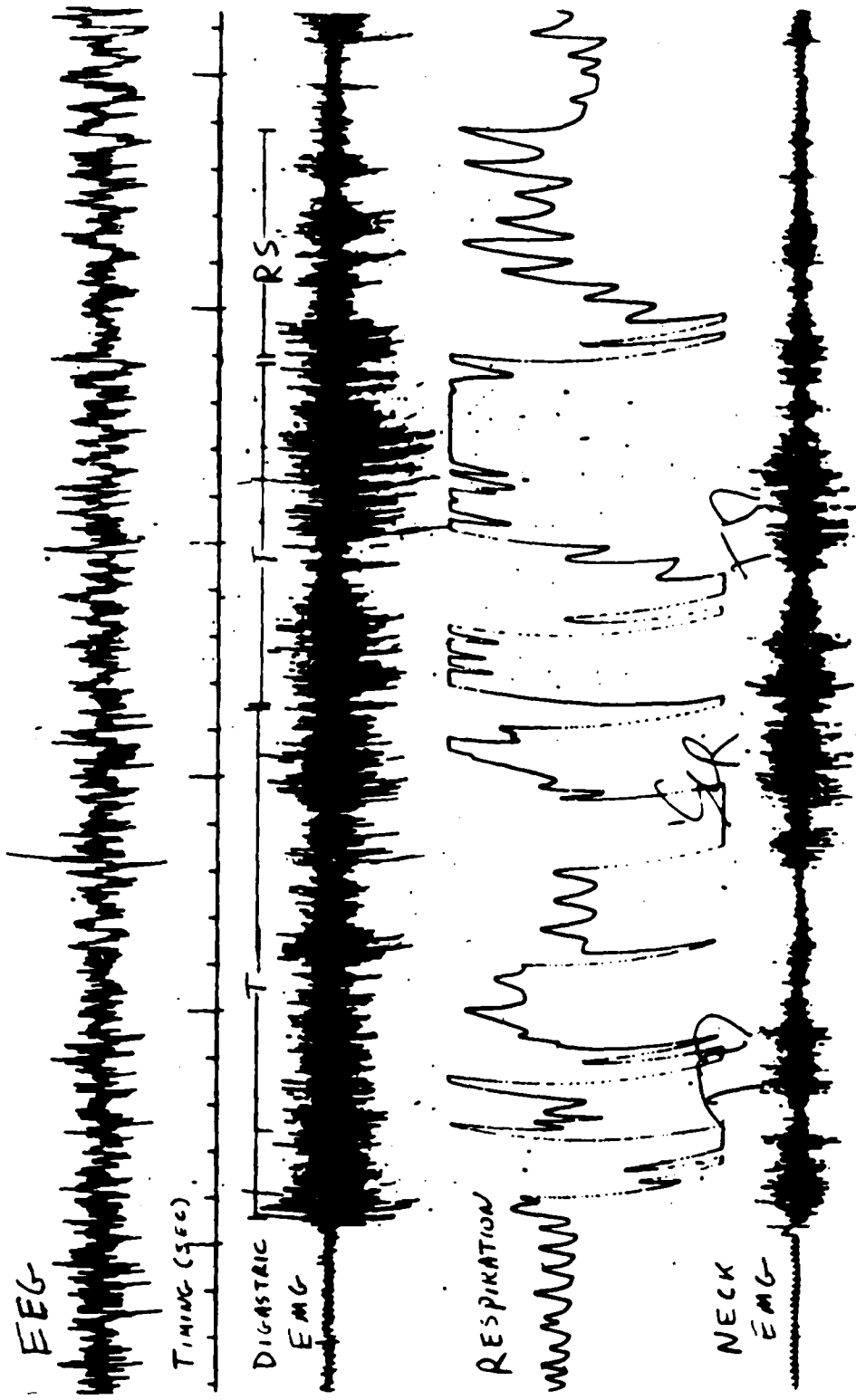
Figure 2. Sample polygraph tracing of a nursing pup in paradoxical sleep. Note the low amplitude neck EMG with evidence of phasic twitches, desynchronized low amplitude EEG and irregular respiration. The digastric EMG contains a burst (B).

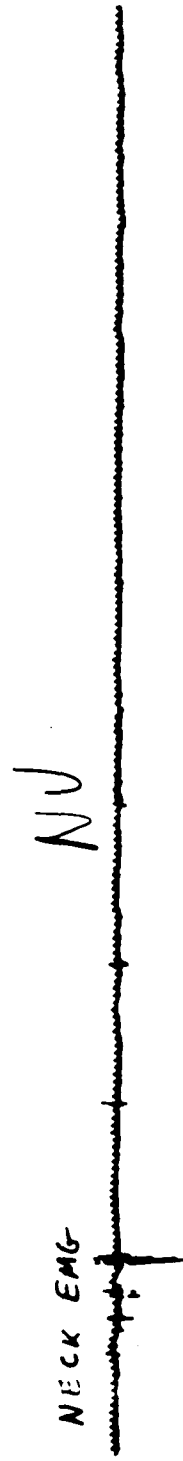
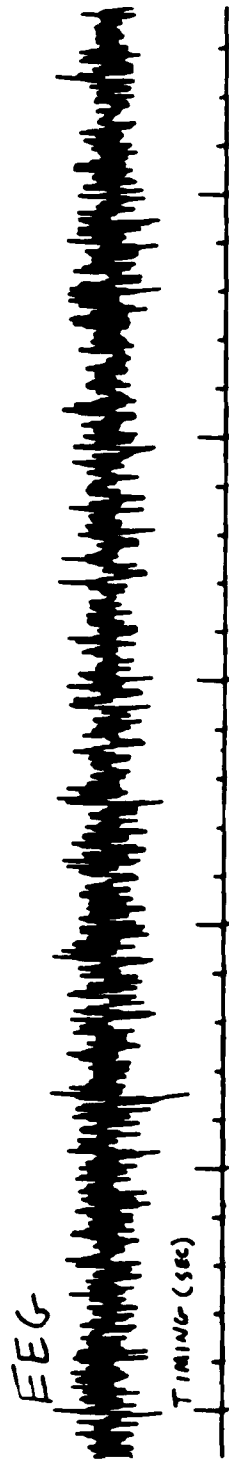
NV = no visible body movement to observer.

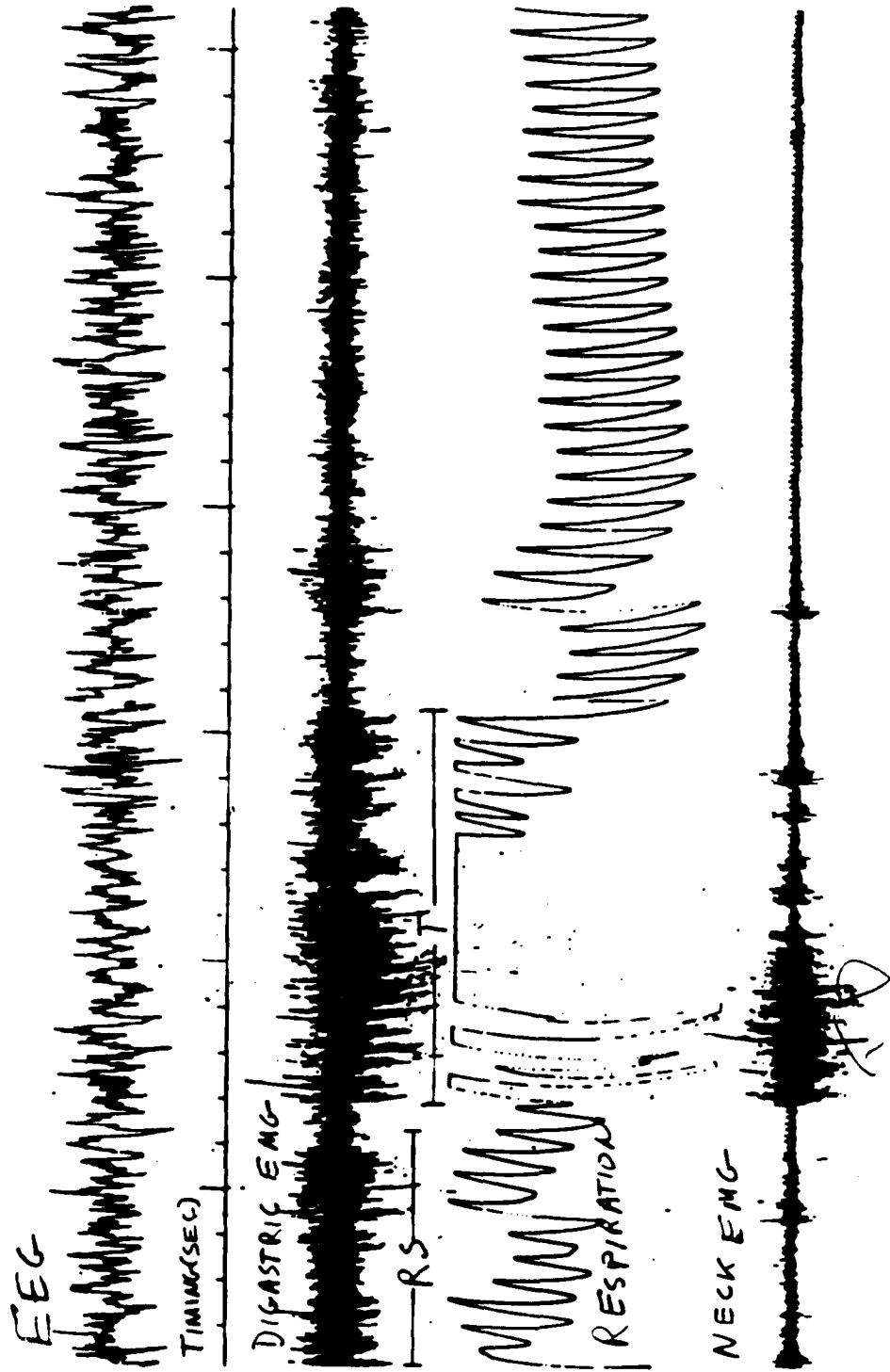
Channels and other abbreviations as in Fig. 1.

Figure 3. Sample polygraph tracing of a nursing pup in slow wave sleep. The low frequency, high amplitude EEG waves associated with tonic, moderate neck EMG tone, and regular respiration define slow wave sleep. Movement artifact during the treading prevents recording the respiration. The digastric EMG has examples of rhythmic sucking and treadles.

Channels and abbreviations as in Fig. 1.







fast wave lasting at least two seconds. The jaw muscle activity was accompanied by a characteristic movement of the fore limbs which supplied the name - treadles. Forelimb movement artifacts were shown to account for the slow wave activity (Brake, Wolfson and Hofer, 1979).

Bursts were unaccompanied by body movements. Bursts and treadles occurring sequentially had to be separated by at least two seconds to be counted as different episodes. Rhythmic sucking bouts (RS) were characterized as brief periods of regularly alternating increases and decreases in activity lasting at least five seconds, with no more than two seconds separating each activity peak. The intensity of jaw EMG between sucks was also rated as a measure of the tonic pressure seal developed by the pup. This intensity rank was rated for each epoch of 15 seconds on an arbitrary scale of one to four points.

The issue of experimenter bias must be addressed since I scored all sleep-wake state data and was not blind to the experimental condition of the record. There are three factors which lessened the likelihood of experimenter bias. First, the scoring criteria were objective. Inter-rater reliability in a previous experiment was 96.4% (Hofer, 1976). In this experiment, I checked to see if my own scoring criteria had drifted over time. My reliability separated by a two year period was 94.5% with approximately equal results for all three states. The second factor which worked to prevent bias was that I had no strong hypothesis as to the direction of any results. Finally, much of the experiment depends on the relationship between sucking behavior and sleep-wake states. Stephen Brake scored all the sucking data and was blind to experimental condition and to sleep-wake state.

Rationale for Sleep-Wake State Scoring Criteria

States are defined operationally by constellations of behaviors which do not always coalesce in exactly the same form (Prechtl, Akiyama, Zinkin and Kerr-Grant, 1968). Individuals may vary as to exactly how those behaviors coalesce. In one subject, respiratory pattern may not discriminate state changes well. In another, differences in EEG may not be as distinct. Thus, recognizing sleep-wake state behavior becomes a problem in pattern recognition. And, for each individual subject, the particular pattern recognition criteria must be reset slightly.

No single behavior, even EEG, is enough to define state, particularly when comparing different individuals. For example, in a study of adult rats using spectral analysis of the EEG, Young, Steinfeld and Khazan (1978) described most of the spectral power during PS as between 6-9 cycles per seconds (cps). Another study listed most power between 30-40 cps (Timo-Iaria, Negrão, Schmidek, Hoshino, Lobato de Menezes and Leme da Rocha, 1970). Similar discrepancies in results can be found for A and SWS. (The results for PS can probably be reconciled. In the first study, the EEG is dominated by theta waves, possibly due to electrode placement. The faster waves of PS are of low amplitude, probably superimposed on the theta, but with little spectral power).

Another, but equally difficult problem relates to the overlap in EEG frequencies in the different states. Waves of all frequencies are seen in all three states. Slow wave sleep is discriminated from A and PS, in part, by the proportion of slow waves seen. Thus, there can be much overlap in EEG patterns. In fact, the EEG patterns for PS and A are practically indistinguishable, both in adult and young rats

(Jouvet-Mounier, Astic and LaCote, 1970; Timo-Iaria, et al, 1970).

The work described in this dissertation differed from most of the studies in the literature in that the recordings were often done with the pup attached to its mother's teat. Did this procedure change sleep-wake state behavior such that the state ratings under different attachment conditions were incorrect? I suggest that the answer to this question is no, for four reasons.

1. Emde and Metcalf (1970), Jouvet-Mounier, Astic and LaCote (1970) and Ruckebusch (1972) have all monitored young mammals while nipple-attached and found no reference to any need to change state rating criteria.

2. The polygraph tracings and behavioral observations taken on pups when attached in the present studies appear the same as those recorded from unattached pups in our laboratory (Hofer, 1976; Hofer and Shair, 1982).

3. In the Normal Mother Experiment, pups were recorded both while attached and while unattached. Each individual could be compared in the two conditions. No changes in the behaviors which make up the criteria for state rating were observed. Respiration was regular, almost machine-like during SWS in both conditions. Irregularity in breathing of the same type was seen in PS. The EEG patterns looked similar, attached or unattached.

4. Some preliminary work using spectral analysis of the EEG confirms this last point. I have now recorded three pups, both while attached and while unattached. After scoring sleep-wake states by the normal criteria, the EEGs from epochs of each of the three states under both conditions were analyzed using a spectral analysis program (Williams and Gottman, 1982). This program counts the EEG waves and

sorts it according to frequencies. The pattern of EEG frequencies seen was not significantly influenced in any state by the attachment condition (unpublished data).

Data Analysis

Fifteen-sec epochs were scored by the above criteria and measures derived from duration and frequency of periods in each state, the percent of total recording time spent in each state, and the rate and pattern of transitions from one state to the next. We also scored the latency to sleep onset. The total amount of sucking for the three sucking types was measured for each animal. Finally, we also calculated for each animal the rate of sucking per unit of time spent in each sleep-wake state, a state-specific sucking rate.

In this experimental design, each infant served as its own control, with respect to changes in measures from Baseline to Reunion Day, to changes in measures within different states, and with respect to measures recorded when the pup was attached or not attached to its mother's teat. Dependent t-tests (dep t) and one- and two-way analyses of variance (ANOVA) with repeated measures took into account this strong experimental design. Comparisons across groups without repeated measures were done by independent t-test. The correlation of one variable to another was done using Pearson Product-Moment parametric correlations. Variation around all mean values are given by the standard error of the means (SEM), both in the text and the figures.

Note that the combination of the ANOVAs with repeated measures and the format of the Results section creates a problem in presentation. Baseline and Reunion data are discussed separately, then comparisons between the two days are drawn. The overall analyses are based on data

from both days. Rather than present the overall analyses twice, these larger ANOVAs are presented in the Appendix. Smaller ANOVAs and t-tests are used within the text to make specific comparisons. The use of these specific analyses where unplanned are validated by the overall ANOVAs. Dep t-test are used as post hoc tests since they are used for planned comparisons of similar data later. It seemed cumbersome to report different types of tests on figures which otherwise were very similar. Note that Newman-Keul's post hoc tests gave the same results as these "post hoc" dep t-tests in every case.

Following the Normal Mother Baseline experiment, most comparisons are planned, based on the results of that first experiment. For planned comparisons, dep t-tests are used and no justification by overall ANOVA is needed (Winer, 1974). Nevertheless, ANOVAs on all the data are given in the Appendix for researchers interested in testing other hypotheses than those presented here.

Specific Methods For Individual Experiments

Normal Mother and Litter Experiment

Six litters were born and raised near the polygraph machine in the testing room where they would be studied later. This procedure was followed to accustom the mothers to the presence of the investigators. On the day of electrode implantation, the usual procedures were followed except that the electrodes were made especially long (10 cm) to allow the pups more freedom of movement.

On Baseline day, 15 minutes prior to the test session, the dam was removed from the cage and the implanted pup was connected to leads buried under the nest shavings. After the dam was returned, care was taken to watch the dam closely during the first 15 minutes of the

recording session since she was likely to attack the electrodes during this time, if at all. It was sometimes necessary to distract her attention away from the electrodes by touching the top of the cage or occasionally by actually pushing her head away from the electrodes with a long probe. No heat was supplied to the bottom of the cage during the test sessions since the mother and litter were present to provide normal temperature controls to the test pup.

In this experiment the mother was free to come and go from the litter, thus the data were analyzed both while the pup was attached to the mother's teat and while the pup was unattached, as well as an overall analysis of sleep-wake behavior combining both conditions. Gaps in attachment of less than or equal to one minute were included as attachment time. During these short gaps the pups were frantically nosing their mother's ventrums, in the process of reattaching to the same or a new nipple. The short gaps usually occurred just following a milk ejection during which time a nipple switch is to be expected. The short gaps did not constitute a termination of the nursing bout itself.

Of course, sucking behaviors could only be analyzed when the pup was attached. It must be noted that it was impossible to see the test pup some of the time since the mother and littermates would block our view; thus the behavioral observations were occasionally incomplete.

As is known from the work of two groups of researchers, the mother rat must be in SWS for a milk ejection to occur (Lincoln, Hentzeen, Hin, Vunder Schoot, Clarke and Sumerlee, 1980; Voloschin and Tramezzani, 1979). She is generally in a relaxed posture with the pups quietly attached to her ventrum. The milk ejection activates both mother and pups. The mother wakes and stands on four splayed legs over her litter with her back arched (Drewett, Statham and Wakerley, 1974;

Lincoln, et al. 1980). The pups begin to suck vigorously and display a stereotypic "stretch" response, showing a rigid body extension with limbs pressed against the mother's ventrum (Drewett et al., 1974; Lincoln, Hill and Wakerley, 1973; Lincoln, et al. 1980).

We used these already known behaviors of mother and the entire litter to rate the occurrence of a milk ejection as have other workers in the field (e.g., Drewett and Trew, 1978; Voloschin and Tremezzani, 1979). A variety of measures have justified the use of these behaviors as a marker for milk ejection, including their temporal correlation with a rise in intramammary pressure, with the administration of oxytocin to the dam, etc. (see Drewett, 1983 for review). However, it has not been conclusively proven that in the natural nursing situation these behaviors occur only when the pups receive milk or that the pups never receive milk without their occurrence. Thus, following common practice in the field, when I discuss the pups' response to a milk ejection, I am referring to this defined "milk ejection response" behavior.

This problem is especially relevant since the sucking pattern was also used to define the milk ejection response. The EMG was rated independently by Brake in four categories ranging from Definite to Barely Possible as to whether a milk ejection had occurred. Since in the data analysis we describe sucking changes during milk ejection responses, it would be circular if we tried to imply that these behaviors were a necessary part of all milk delivery. The two rating systems overlapped very well for 28 cases and these were called milk ejection responses. In five cases, either the behavioral or EMG rating was so high that these instances were called milk ejection responses despite reservations of the second scoring system. There

were another 30 cases with low ratings as milk ejection responses by either system or more usually both. These examples were not included in the data analysis. Thus, we used a conservative approach to calling a particular behavior a milk ejection, deliberately accepting the chance of more "misses" than "false positives".

After the Baseline recording session, the test pup and two littermates were placed in a new cage overnight. As noted in the General Methods, home cage shavings and thermoregulated heat were supplied. Four pups were left with the mother in the home cage in order to reduce any changes in the mother's behavior which might be caused by the experimental procedure. Thus on Reunion Day, four of the seven pups had not been deprived while three had been. The observations of the mother-infant interaction of Reunion Day should be interpreted with the differential experience of the two groups of pups in mind.

Anesthetized Mother Experiment

In this experiment six litters were studied, both on Baseline and Reunion days. Each implanted pup was recorded for two hours without littermates in its home cage while attached to its urethane-anesthetized mother. The mothers were anesthetized at a dosage of 2-3 mg per kg. After Baseline recording, the pup was separated from the mother overnight with two littermates in the home cage. The mother was placed in another cage with three littermates and home cage shavings. The three pups with the mother often remained attached to nipples overnight despite the fact that milk ejections are unlikely to have occurred (Lincoln, Hill and Wakerley, 1973). Just prior to the Reunion test, the mother was placed on new bedding from

the home cage nest and two of the separated littermates were placed on the mother to draw out her teats, if necessary. They were then removed before recording from the experimental pup.

Cycled Diet Experiment

In this experiment, six litters were studied. The test procedure was almost the same as that in the Anesthetized Mother Experiment with the following exceptions. At the time of electrode implantation, each animal was provided with a gastric cannula made from 30 gauge polyethelene tubing (Clay-Adams, PE-10) by techniques modified from Messer, Thoman, Terrasa and Dallman (1969), and described in Hofer (1973). The tube passed through the ventral surface of the stomach, through a purse-string suture, through a 1.5 mm slit in the peritoneum, and curved laterally and dorsally in the region of the spine. At this point, it curved abruptly in a caudal direction after surfacing in the middle of the pup's back, and the external 3-5cm lay on the surface along the lower back extending to the tail. Sutures distal to beads in the wall of the polyethelene tubing at the stomach and abdominal walls, as well as a small patch of collodion at the exit point in the skin prevented the displacement of the cannula by traction. Bends in the polyethelene tubing were formed with heat prior to surgery to conform approximately to body contours. These cannulae remained patent to infusions for several days and were rarely destroyed by the mother.

After the Baseline recording, the pup's cannula was connected to tubing (Silastic 602-105) through which the pup was infused with an enriched formula diet. The major constituents were condensed bovine milk and corn oil (to raise fat content). The diet also had small amounts

of vitamins, minerals and amino acids and was diluted with water to mimic, as closely as possible, the composition of rat milk. A detailed comparison between rat milk, condensed bovine milk and this formula is available (Messer, Thoman, Terresa and Dallman, 1969). The diet was infused during the 22 hrs of separation on a schedule approximating that of the normal nursing cycle. A timer was used to activate a Harvard infusion pump for 15- or 20-min periods alternating with 40-min to 60-min periods of no infusion. The flow rate of nutrients were adjusted so that the pup received approximately the same amount of milk it would have received from its mother. The tubing was supported in such a way as to prevent tangling by the movement of the test pup or its two littermates. The last infusion occurred approximately 30-min before the Reunion test period.

¹ Sex of the test pup was not controlled in these experiments. In each series of experiments, there were a minimum of two pups of the less-well-represented sex. No sex differences had been noted in our previous work (Hofer, 1976; Hofer and Shair, 1982). However, with the small number of subjects in the present study, sex differences were very unlikely to be found. Whether any such differences exist in the behaviors described here remains to be tested.

NORMAL MOTHER AND LITTER EXPERIMENT BASELINE

The purpose of this section is to describe the behavior of two-week-old rat pups during the normal nursing cycle. For the reasons discussed in the Introduction, the emphasis will be on the relationship of sleep-wake states and sucking behavior in the nursing process. Two secondary goals will also be addressed. The first of these aims is to provide normative data on many aspects of the mother-infant interaction and compare these results with previous studies. The behavioral observations which were performed during the test sessions suggested the final goal. During a nursing bout, the pups alternated brief periods of activity -- treadles, changing nipples, burrowing -- with longer periods of quiet attachment. When not attached, the pups also alternated between short periods of activity and longer periods of quiet. Behavioral and physiologic recordings of nursing bouts and inter-bout intervals will be compared to see if this impression of similarity is sustained.

The data will be presented in the following fashion. First, there will be a detailed quantification of the behavioral observations of the six experimental litters. Included in this data will be such measures as the number and timing of nursing bouts and milk ejections, activity levels of the implanted pups, and weight and temperature changes.

Second, the polygraph results will be presented. Sleep-wake states and sucking patterns will be emphasized. Trends in the data over the time of the nursing episodes will be examined.

Third, the results will be analyzed for any trends over the time of the recording sessions.

Behavioral Observations

During the two hour test session, the implanted pup was able to compete fully with its littermates. As shown in Fig. 4, the test pup could burrow under its dam which was in good nursing posture. Impressionistic visual observations did not reveal any gross behavioral differences between the implanted pups and their littermates. For example, the test pups never missed a milk ejection received by their littermates. They were able to attach to their dams $79.4 \pm 3.9\%$ of the time that the adult was in the vicinity of the litter, demonstrating the pups' mobility. Also, as will be seen below, the sleep-wake patterns of the pups were quite comparable to the norms described for pups of this age recorded without their dams or littermates (Hofer, 1976; Jouvet-Mounier, Astic and LaCote, 1970). Thus, there seems to be little reason to believe that the following results were an artifact of the recording procedure.

Attachment Time

During the two hour test session, both the number and length of the nursing interactions between pups and dams varied among the six litters. This variability is displayed in Fig. 5. Note the irregular patterns of milk ejections as well. A few constants do appear to be true, however. No milk ejections occurred in any nursing bout of less than five minutes. Only rarely was there a milk ejection in the first several minutes after attachment.

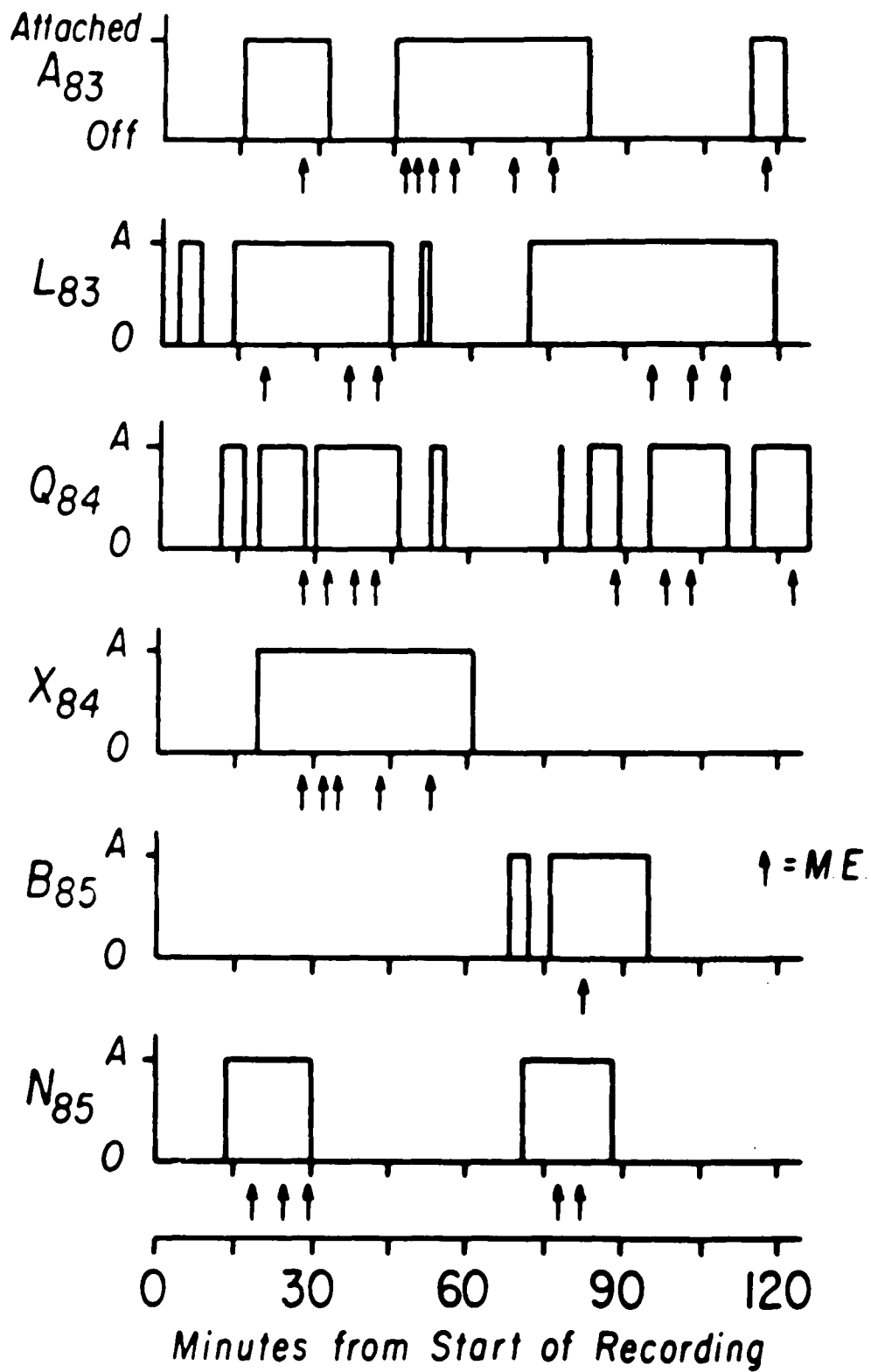
The data from this figure and its derived means (see Table A) are well within the range of results found by other investigators. Two-week-old rats are suckled approximately 40% of day (Grota and Ader,

Figure 4. An illustration of the mother and litter during the recording session. The mother is unanesthetized, nursing her litter in the normal fashion. The implanted pup is under the mother's ventrum, presumably attached to a teat.



Figure 5. Patterns of nursing bouts for all six litters during the two hour Baseline recordings.

This data is based on the times during which each implanted pup either was or was not attached to a teat. The results for any other individual in a litter may vary slightly. Gaps in attachment of less than or equal to one minute are not indicated. Milk ejections (ME) are designated by arrows.



1969; Leon, Adels and Coopersmith, 1985). In this study, nipple switches were seen following 22% of the milk ejections (data derived from Table A), as has been reported for pups of this age (Hall, Cramer and Blass, 1975). (Nipple switching is a behavior exhibited increasingly by older pups and may be an attempt to obtain more milk by emptying two nipples at each milk ejection.) The average length of the nursing bouts in this experiment was a little shorter than the 20 minutes found by others for pups of this age (Grota and Ader, 1969; Leon, Croskerry and Smith, 1978). However, these researchers monitored maternal nest time, not actual attachment time. As one can see in Fig. 5, there were nursing bouts interrupted by only a few minutes. In some instances, the dam did not leave the nest area during this time.

Milk Ejection Responses

There were a total of 33 milk ejection responses observed during the 4.92 hours of attachment of all implanted pups. The data in Table A concerning the defined milk ejection responses are comparable to other reports in the literature (Lincoln, Hill and Wakerley, 1973). These investigators found a very similar range in inter-ejection intervals from about two to over 20 minutes with a mean of 6.6 minutes. Thus, since the present recording procedure did not interrupt the normal pattern of milk letdown, the test procedure had to have allowed the mothers to fall asleep (Lincoln, Hentzeen, Hin, Vunder Schoot, Clarke and Summerlee, 1980; Voloschin and Tramezzani; 1979).

Weight and Temperature Data

Pups were within the normal range for weight and temperature at

Table A

Data from behavioral observations of mother-pup interactions on Baseline day.

	Time Att. (min)	% TRT Att.	Latency to 1st NB (min)	# of NB'S	Lengths of NB's (min)	# of nipple ^a switches	# of ME's	Latency to 1st ME ^b (min)	Time ^c betw. later ME's (min)
Mean	51	41	21	3.5	15.5	1.2	5.5	7.5	6.3
SEM	9.1	6.8	9.4	1.0	3.1	.8	1.5	1.5	.8
Range	23 to 84	19 to 65	3 to 67	1 to 8	.5 to 48	0 to 5	1 to 8	1.8 to 24	2.8 to 16

^a A nipple switch was defined as an event in which a pup detached from a nipple and reattached to the same or another nipple within one minute.

^b This figure is latency to the first ME of each NB, not the TRT.

^c Figured only for ME's within the same NB.

ME = milk ejection NB = nursing bout TRT = total recording time
Att. = attached

the start of the recording session (Table B; Hofer and Gräbe, 1971; Messer, Thoman, Terrasa and Dallman, 1969). Temperature was maintained at the end of the session. There was a small but significant increase in weight during the two hour period. If the pups had continued to gain weight at that rate for 24 hours, their percentage change in weight would have been $.8\% \times 12 = 9.6\%$. The normal gain in weight for this age pup is approximately 11% (Messer, Thoman, Terrasa and Dallman, 1969). Neither change nor percent change in weight was significantly correlated to the amount of time the pup was attached to the nipple, to the number of milk ejection responses, or to the number of nipple switches. Remember, as mentioned in the Methods section, the pups had not been voided prior to the start of the test procedure, thus variable amounts of elimination might have influenced the weight measures.

Activity Levels

Hofer and Gräbe (1971) reported that for the two-week-old pup, the distribution of active and inactive episodes did not depend on whether the pup was being nursed. Results in the present experiment were similar. As shown in Fig. 6 (and confirmed by ANOVA results in the figure legend), there was no influence of attachment condition on the duration of either active or inactive episodes.

Two ideas were suggested by these data. From the fact that the present results confirmed a previous study, there was more reason to believe that our recording procedure was not too disruptive. The larger implication concerned the controls for the patterning of the pup's behavior. Even though the presence or absence of the dam changed which specific behaviors the pup displayed, the timing of the emission

Table B

Body weight and rectal temperature.

	(-----Body Weight (gr.)-----)			(---Rectal Temp.(°C)---)	
	pretest	posttest	% change ^a	pretest	posttest ^b
Mean	27.6	27.9	0.8	35.9	36.3
SEM	1.0	1.0	.26	.16	.21
Range	24.5 to 31.6	24.5 to 31.8	0 to 1.6	35.4 to 36.4	35.8 to 36.9

^a The mean weight gain was statistically significant (dep t = 3.478, df=5, p<.05).

^b The mean temperature change was not statistically significant (dep t = 1.765, df=5).

Figure 6. Histogram analysis of active and inactive periods during nursing and when the mother was away, illustrating the relative frequency of occurrence of periods of different durations.

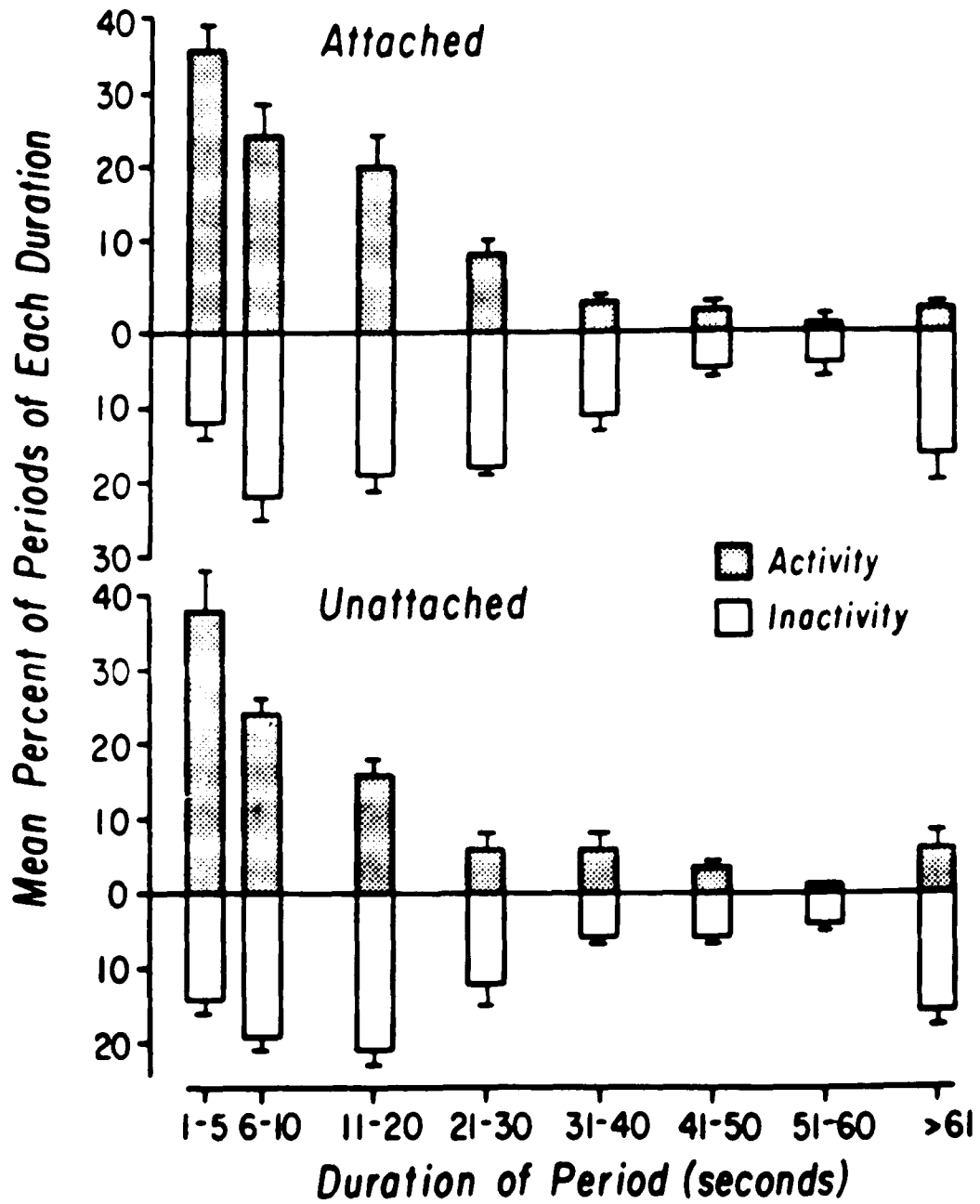
Two-way ANOVAs with repeated measures on factors A and B were run on both the periods of activity and the periods of inactivity. In neither case was there any effect of attachment condition on the relative frequencies of the episode durations. There was no interaction effect either.

Periods of activity: Factor A Attached vs. Unattached
Factor B Episode duration

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.361	5	.072	
A	.028	1	.028	.000
B	13521.4	7	1931.6	53.541
AB	112.4	7	16.1	.445
Resid	2705.8	75	36.08	

Periods of Inactivity: Factors A and B the same as above

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.058	5	.011	
A	.264	1	.264	.010
B	3076.7	7	439.5	17.249
AB	271.8	7	38.8	1.524
Resid	1911.1	75	25.5	



of behavior was quite similar. Several hypotheses might explain these data. A null hypothesis would be that either random chance or the crudeness of the recording measures prevented a distinction from being drawn. One alternative would be that there existed some internal pattern generator which caused the pup to be active within certain time intervals. Such a generator might be programmed by the pattern of behavior evoked during previous interactions with the mother, including nursing. Or, the putative generator might be relatively independent of environmental input. Clearly, other hypotheses are possible. For example, perhaps the environmental cues in the two attachment conditions were more similar than appearances suggested. The first step in answering the questions raised by these speculations was to continue the comparisons of behavior in the attached and unattached conditions. Such comparisons of sleep-wake states will be presented below.

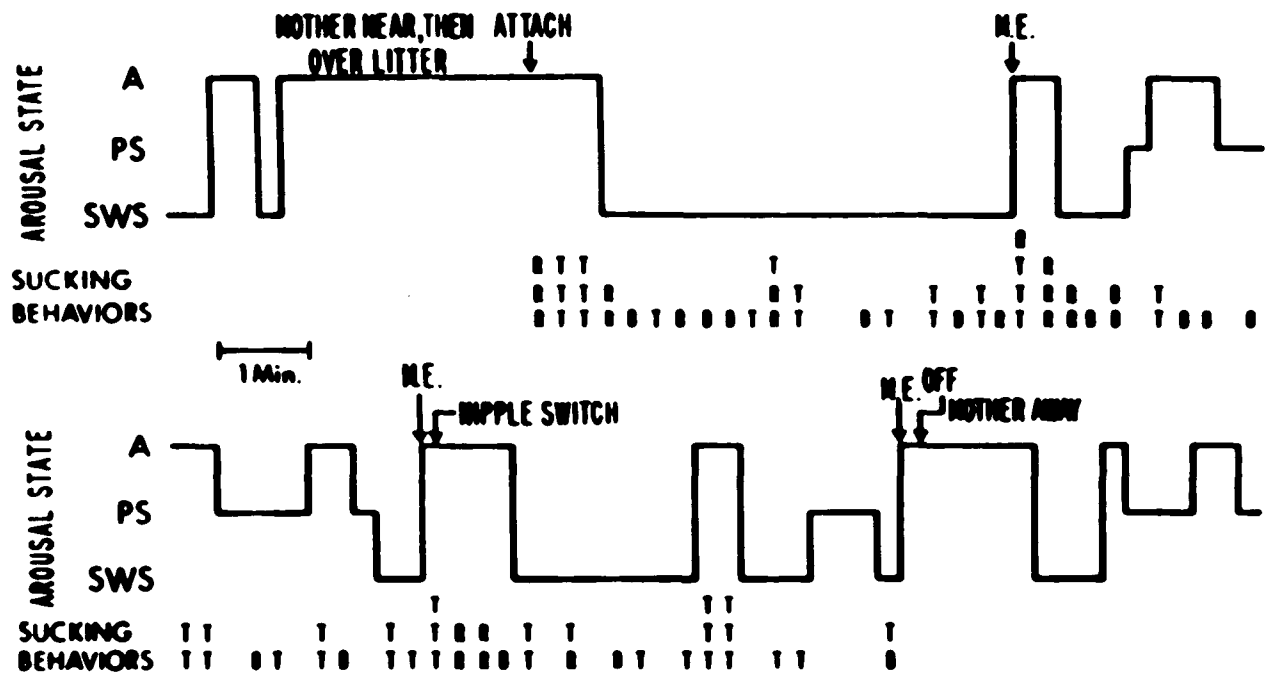
Electrophysiological Data

Rat pups were found to be asleep most of each nursing bout. A typical example (Fig. 7) shows how quickly one pup fell asleep after attaching. The pup alternated among all three sleep-wake states during the period it was attached to the mother's teat. Note that the pup was asleep at the start of each of the three milk ejections, then awakened only briefly during and after the milk ejection. The example also shows that sucking behaviors occurred in all three states. The rest of this section will characterize these observations in greater detail. First sleep patterns, then sucking data, and finally the periods around milk ejection will be analyzed.

Figure 7. Sleep-wake state data and sucking behavior during a typical nursing episode.

The height of the top line indicates which state the pup was in at any given time. After the pup was attached, the type and amount of sucking during each 15 second interval is shown by the letters below the sleep-wake state tracing. Thus three Rs indicate from 11 to 15 seconds of rhythmic sucking. Other behaviors of interest are noted above the state tracing.

A = awake; PS = paradoxical sleep; SWS = slow wave sleep; R = rhythmic sucking; B = bursts; T = treadles; ME = milk ejection.



NURSING EPISODE EXAMPLE: Normal Mother

Sleep-Wake States

As suggested by Fig. 7, rat pups were asleep a high percentage of the time during nursing bouts. Table C summarizes the findings. In fact, the sleep-wake state percentages were not significantly different whether the pup was or was not attached. Other results did show a difference between the two attachment conditions. The sleep latency immediately following nipple attachment was actually shorter than the sleep latency after the pups had come off the nipple. There is another interesting point having to do with sleep latencies. When attached, in most cases pups entered slow wave sleep. However, when the pups were not attached, they showed an equal likelihood of entering into slow wave or paradoxical sleep. The duration of episodes was shorter in all three states while pups were nursing than when not nursing. Those episodes also occurred at a higher frequency, which resulted in the equal percentage recording time noted above. The fewer number of state transitions per hour of the pups when not attached reflects the data on duration and frequency in a single figure. The more state transitions per hour of the attached animal was probably due to the fact that the pup was being disturbed from external sources -- the mother's movements and her milk ejections.

One can examine the pattern of duration of state episodes more closely in Fig. 8. From these histograms one can see that the mean attached vs. not-attached changes in durations from Table 5 were not evenly distributed. There were many more very short and few very long state episodes of awake and paradoxical sleep when attached. The intermediate episode durations were fairly comparable.

Table C

Measures of sleep and wakefulness of infant rats recorded with their normal mother and litter for two hours on Baseline day.

	Sleep Latency (min)	% Time in State ^a			-----State Episode Analysis-----						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Overall	2.3	31	39	30	1.02	1.82	1.33	18	13	14	46
Attached	.8	29	33	38	.68	1.22	1.18	26	17	20	61
Not Attached	2.4	33	43	25	1.34	2.66	1.46	16	10	10	35
Comparisons of Att. and Not Att.:											
dep t =	1.913	.637	1.347	1.866	2.479	3.104	1.344	5.097	3.008	5.513	7.377
df=5					+	*		**	*	**	***

^a % time in state is based on the total recording time for the overall condition, on the time being nursed in the Attached condition, and on the time with mother away in the Not Attached condition.

A = awake PS = paradoxical sleep SWS = slow wave sleep + = p<.10, ns
 * = p<.05 ** = p<.01 *** = p<.001

Figure 8. Histogram analysis of state episode durations during nursing and with the mother away on Baseline day, illustrating the relative frequencies of occurrence of periods of different durations.

A = awake; PS = paradoxical sleep; SWS = slow wave sleep; E = episode; = $p < .10$; * = $p < .05$; ** = $p < .01$.

Indications of statistical significance refer to comparisons within a state for episodes of the same duration between the Attached and Not Attached conditions. These indications are based on the results of t-tests whose use are validated by the findings of three two-way ANOVAs with repeated measures on A and B, one ANOVA for each state.

Awake: Factor A Attached vs. Not Attached
Factor B Episode Duration

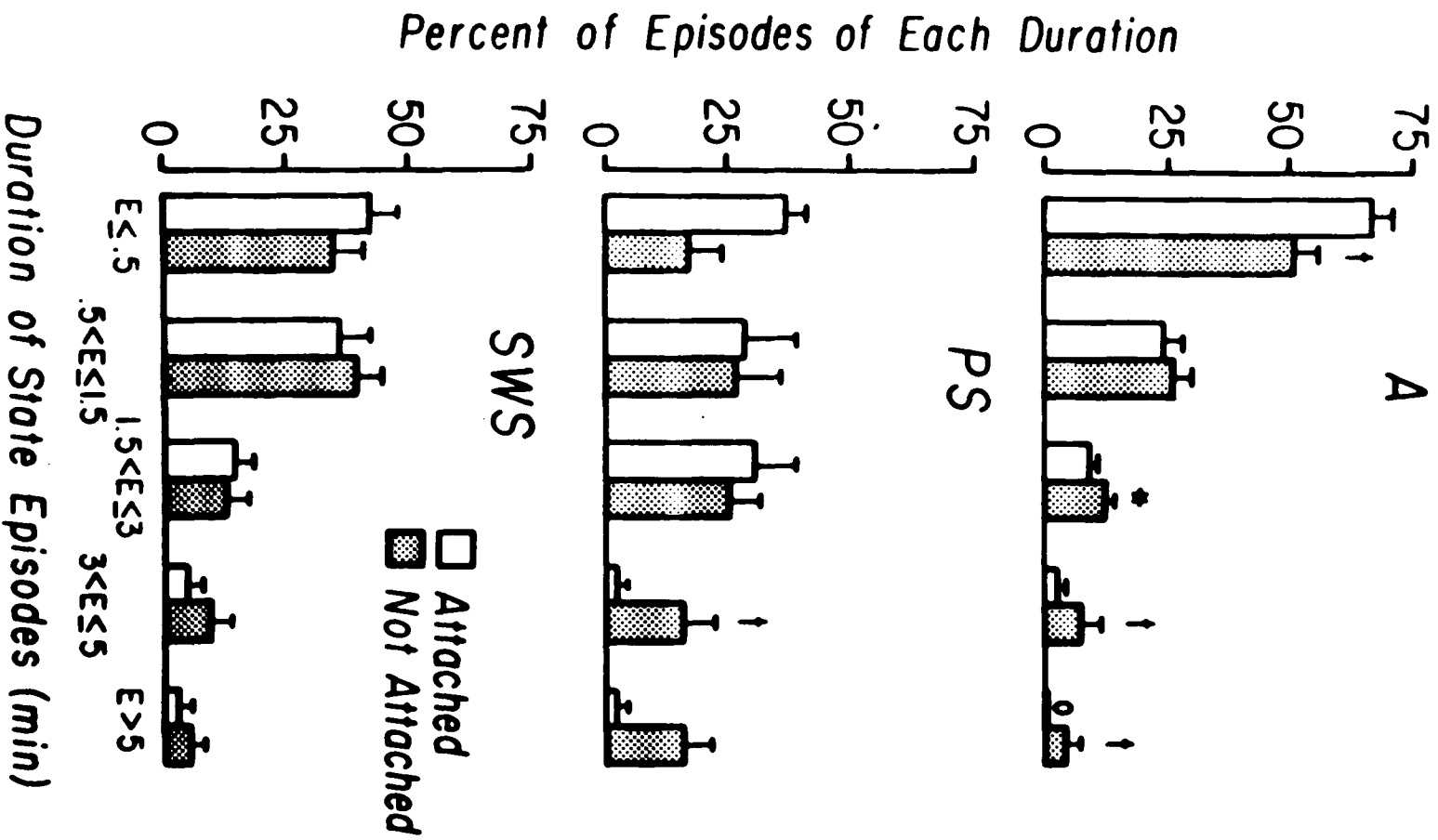
<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.500	5	.100	
A	.021	1	.021	.000
B	26553.4	4	6638.3	90.752**
AB	955.0	4	238.8	3.264*
Resid	3291.7	45	73.1	

PS: Factors A and B the same as above

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.213	5	.043	
A	.078	1	.078	.000
B	4992.0	4	1248.0	4.237**
AB	2490.9	4	622.7	2.114
Resid	13,254.8	45	294.6	

SWS Factors A and B the same as above

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.490	5	.098	
A	.141	1	.141	.001
B	13450.1	4	3362.5	24.466**
AB	281.6	4	70.4	.512
Resid	6184.7	45	137.4	



Another characteristic of state behavior is the probability of transition from one state to another. In this study, there were no significant changes in transitional probability depending upon whether the pups were being nursed or not (Fig. 9). Under both conditions, the most likely sequence of transitions was from awake to slow wave to paradoxical and back to awake. However, note that only two cases (awake to slow wave; paradoxical sleep to awake) were above 20% in probability. Thus, these pups did not have the adult pattern of more predictable transitions, something which has also been reported for other young mammals (Meier and Berger, 1965; Shimizu and Himwich, 1968).

Sucking Behavior

All six pups engaged in frequent sucking while they were asleep and attached to their mother's nipple. This finding has been noted above in the nursing example illustrated in Fig. 7. Table D summarizes the mean sucking times for the three sucking types: bursts, treadles and rhythmic sucking. Overall, almost 50% of all sucking occurred while the pups were asleep. The sucking pattern called "burst" was different from treadles and rhythmic sucking in that this was the only sucking pattern predominantly (71%) seen during sleep. The other two patterns were seen more in slow wave than paradoxical sleep, but still less than half as much as while awake.

However, since the proportions of time spent in the three sleep-wake states were different, a rate of sucking in each state gives a better measure of the relationship between sucking pattern and sleep-wake states. In Fig. 10, one can see that all four aspects of sucking

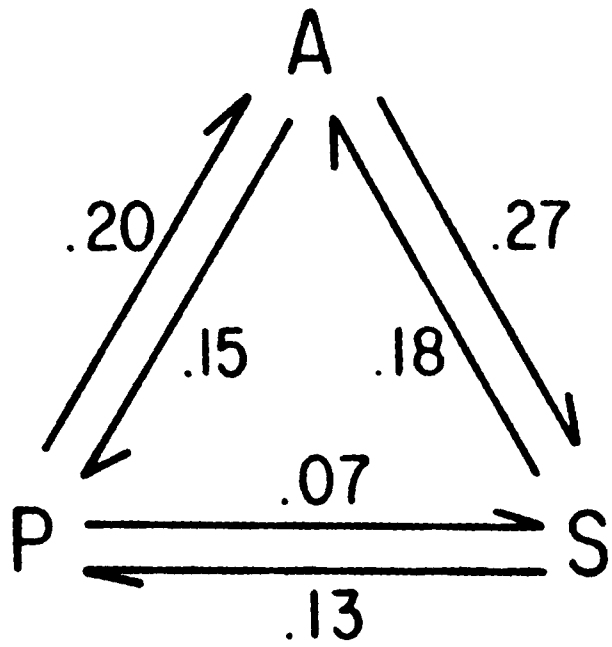
Figure 9. The probabilities of transition among the three sleep-wake states during nursing and while the mother was away on Baseline day.

There were no significant differences in transitional probabilities between the two attachment conditions, based on the lack of interaction effect in the ANOVA below.

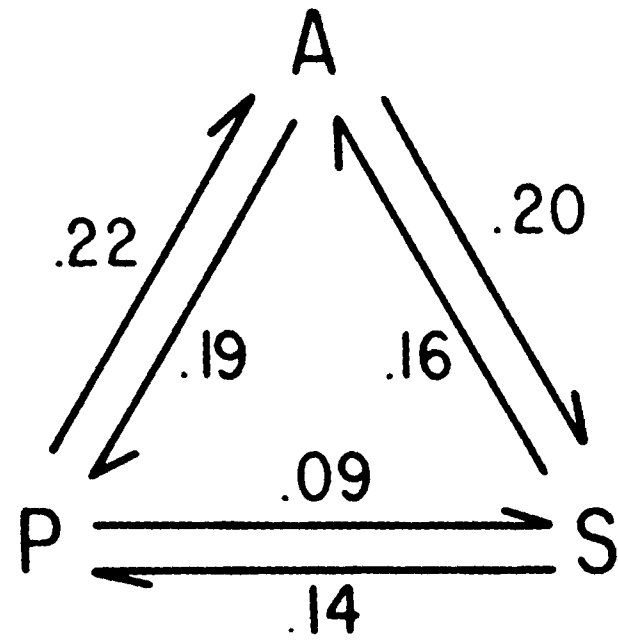
A = awake; P = paradoxical sleep; SWS = slow wave sleep.
 ** = $p < .01$

Factor A Attached vs. Not Attached
 Factor B Direction of State Transition Probability

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.000	5	.000	
A	.000	1	.000	.000
B	.182	5	.036	3.699**
AB	.024	5	.000	.496
Resid	.542	55	.009	



Attached



Not Attached

Table D

Mean and SEM sucking times^a in each sleep-wake state of infant rats being nursed by their mother on Baseline day.

State	RS	B	T
A	44 ± 11	19 ± 5	70 ± 15
PS	2 ± 1	17 ± 5	17 ± 6
SWS	19 ± 4	34 ± 9	30 ± 6

SEM = standard error of the mean A = awake
 PS = paradoxical sleep SWS = slow wave sleep B = bursts
 RS = rhythmic sucking T = treadles

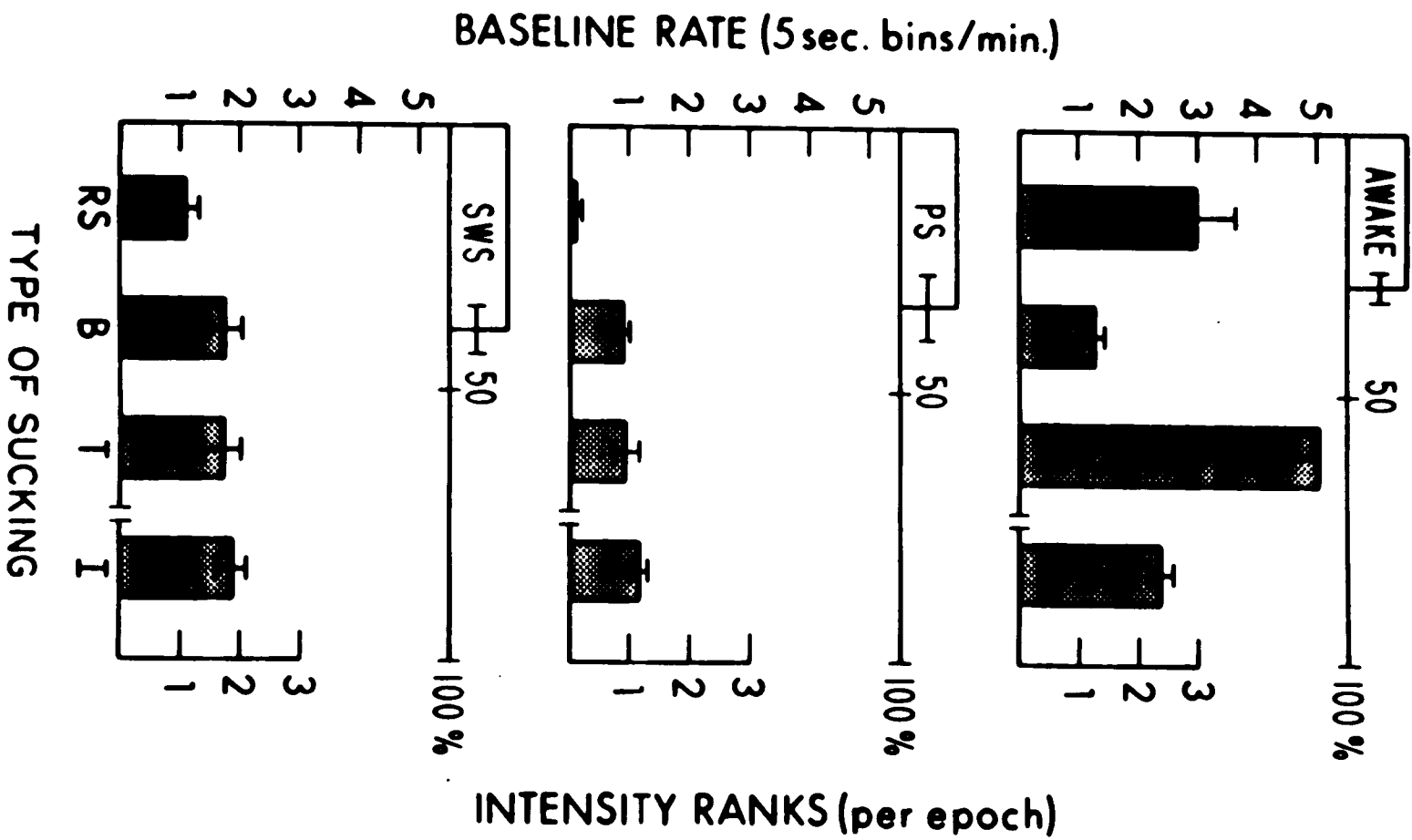
^a For each of the three sucking types, the time spent sucking was calculated as the number of five second bins in which a suck of that type occurred.

Figure 10. The rate of sucking behaviors and the percentage of time in each sleep-wake state during nursing on Baseline day.

The horizontal bars represent the mean percentage of recording time while attached to a teat spent in each sleep-wake state. The height of the three left-hand vertical bars gives the mean rate of sucking per minute for each kind of sucking. The bar to the right measures the amplitude of the digastric EMG recording when no sucking is occurring (an index of the muscle tone involved in maintaining a seal on the nipple).

EMG = electromyogram; PS = paradoxical sleep; SWS = slow wave sleep; RS = rhythmic sucking; B = bursts; T = treads; I = EMG intensity rank.

Each sucking type differs in its rate among the three states at the $p < .05$ level or better except for the comparison of treads between PS and SWS (dep $t = 2.581$, $df = 5$, $p < .10$). All significance findings are based on dep t -tests whose use are validated by the results of overall ANOVAs on both Baseline and Reunion data (see Appendix).



behavior occurred at appreciable levels during the two states of sleep as well as during the awake state with the single exception of rhythmic sucking, which was almost never observed in paradoxical sleep. Bursts actually occurred at a higher rate in slow wave sleep than while awake. The rate of each type of sucking differed significantly between the sleep-wake states in most cases, as is shown in the figure legend.

Milk Ejection Responses

The period around the milk ejection responses was analyzed closely in terms of sleep-wake states and sucking patterns. As mentioned above, there were 33 milk ejections observed. In Fig. 11, one can see that in all cases observed the pups were asleep prior to milk ejection, were awakened by the milk ejection, and in 50% of the cases were asleep again within 15 seconds. By 30 seconds the percentage asleep had risen to 74%.

The sucking levels on either side of the milk ejection responses are displayed in Fig. 12. Treadles and rhythmic sucking both rose during the minute post-milk ejection. Treadles rose to nearly five times normal rates immediately after milk ejection. Considering the entire minute post-milk ejection, the overall rate was doubled (see Table E). Thus, treadles and rhythmic sucking are implicated as nutritive sucking types for the rat pup. Bursts appeared to be unchanged by the milk ejection.

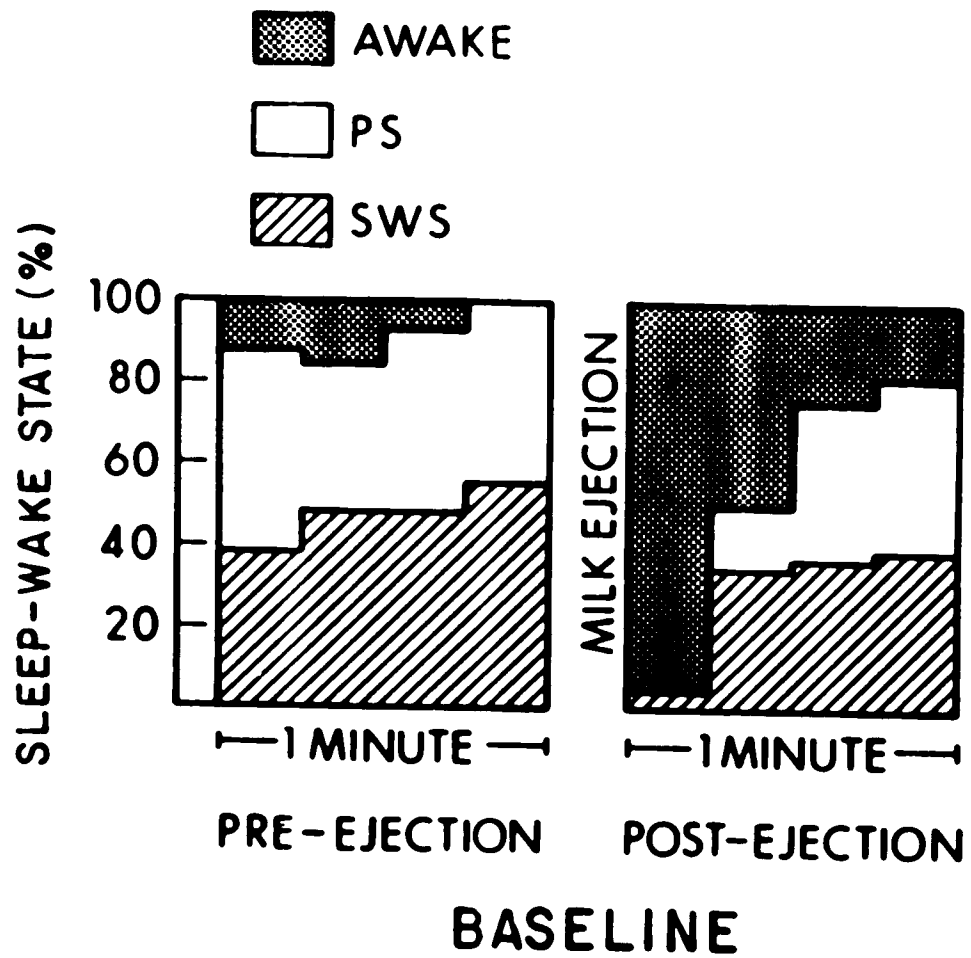
When the definition of the milk ejection response is expanded to include even those 30 behavioral events rated "Barely Possible" milk ejections (see Methods section), the above described sleep-wake and sucking patterns were not altered dramatically. The pups were still

Figure 11. The percentage of pups in each sleep-wake state during the minute before and the minute after milk ejection responses in 28 of 33 cases observed.

In five cases the pups detached for more than 15 seconds and were omitted from this analysis.

Figure 12. Sucking levels for the minute before, during and two minutes after milk ejection response (ME).

Mean values based on the 38 MEs in which the pups did not detach for more than 15 seconds.



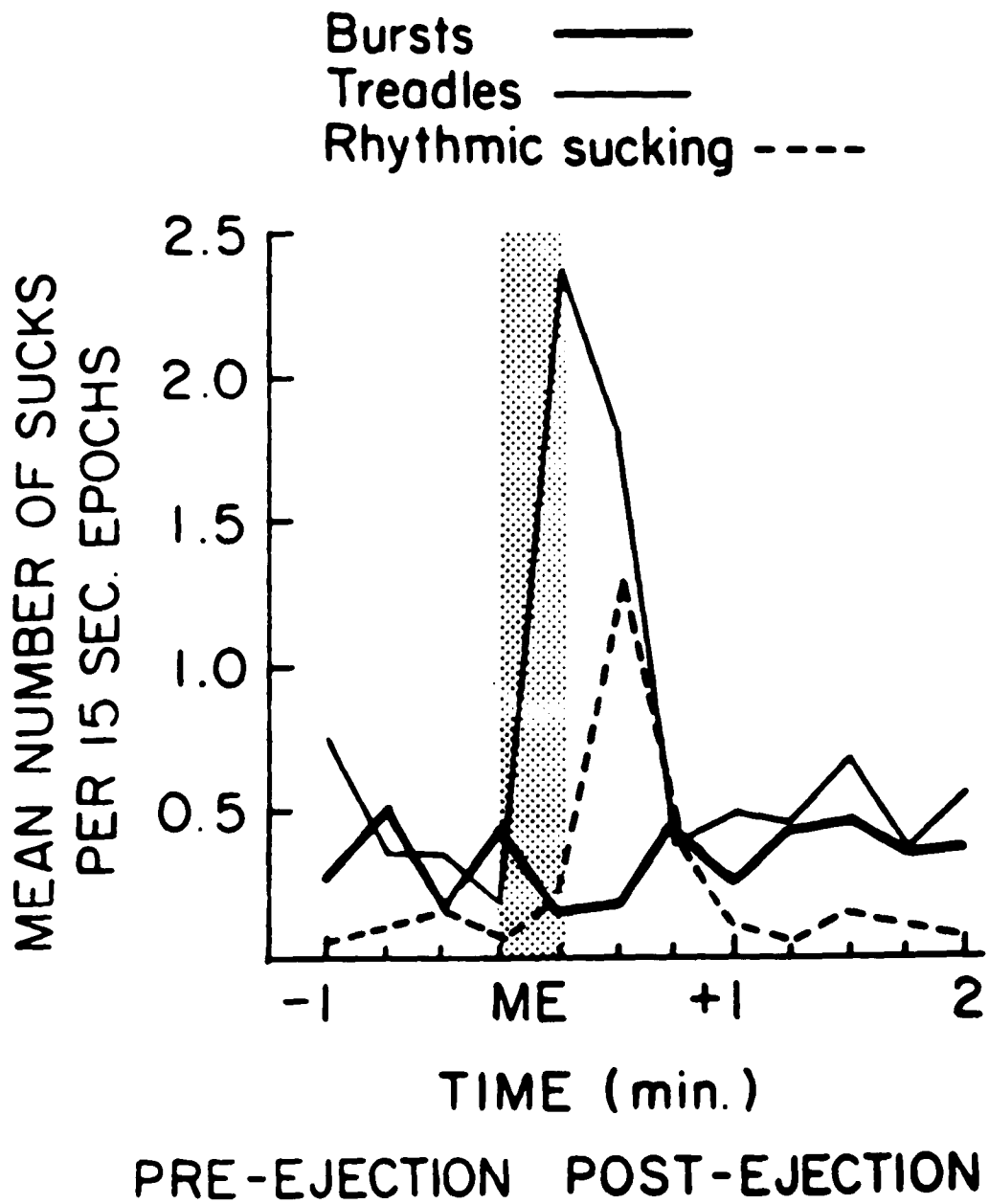


Table E

Comparison of mean (and SEM) sucking rates in the two minutes immediately post milk ejection to the overall sucking rates during attachment on Baseline day.

	-----Mean Sucking Rates ^a -----			(---Dep T-Tests, df=5---)	
	1st min post ME	2nd min post ME	Overall	1st min vs. overall	2nd min vs. overall
RS	2.05±.35	.37±.20	1.22±.16	2.180 +	5.751 **
B	1.09±.26	1.63±.48	1.38±.14	1.641	.642
T	4.95±.45	2.13±.47	2.37±.37	7.766 ***	1.582

SEM = standard error of the mean ME = milk ejection
 B = bursts RS = rhythmic sucking T = treadles
 + = p<.10, ns ** = p<.01 *** = p<.001

^a For each of the three sucking types, the rate of sucking was calculated as the number of five second bins per minute in which a suck of that type occurred.

asleep prior to virtually every milk ejection. They returned to sleep even more quickly thereafter. Sucking behavior changed in that neither rhythmic sucking nor treads rose quite as high in the period during and just after the suspected ejection.

What happened to the sucking rates in each of the three states in this period of milk ejection response (as originally defined)? As shown in Table F, only the rise in treads during the awake state was statistically significantly different from its overall awake treadle rate. Note that these data must be considered tentative, since there were a limited number of epochs available for comparison. The problem was especially acute for paradoxical sleep, since two pups did not enter paradoxical sleep in the minute following their milk ejections. However, these data suggested that it would be worth examining how fixed the rate of sucking is in each sleep-wake state. That point will be addressed further in the studies to follow.

Trends Over Time Within the Nursing Bout

As discussed in the Introduction, there is known to be a relationship between feeding behavior and sleep-wake cycles (e.g., Danguir and Nicolaidis, 1979; Siegel, 1975; Yogman and Zeisel, 1983). One of the simplest ways to think about this relationship is that young mammals may be awake when hungry and asleep when full, a formulation included as part of what Kleitman (1963) calls "wakefulness of necessity." The illustrated nursing bout (Fig. 7) already suggests that such a simple explanation cannot be true in all cases, since the pup fell asleep before receiving any milk. Another way to examine the hypothesis is to compare the pups' behavior in the first half of the

Table F

Comparison of mean sucking rates within each sleep-wake state from the first minute post milk ejection to the overall sucking rates in that sleep-wake state.

	(-----A-----)			(-----PS-----)			(-----SWS-----)		
	RS/M	B/M	T/M	RS/M	B/M	T/M	RS/M	B/M	T/M
Min post ME	2.45	.67	7.26	0	.93	2.32	2.05	1.14	2.55
Overall	2.99	1.30	5.08	.10	.89	.94	1.11	1.77	1.74
dep t = df=5 ^a	1.127	2.467 +	3.537 *	.000	.008	2.940 +	.921	.460	1.201

^aFor PS, df=3 since only four pups entered PS in the 1st. min post ME.

A = awake PS = paradoxical sleep SWS = slow wave sleep
 RS = rhythmic sucking B = bursts T = treadles ME = milk ejection M = minute
 + = p<.10, ns * = p<.05

nursing bouts to their behavior in the second half of the bout. In Table G, one can see that neither the sleep-wake state percentages nor the time spent sucking showed any significant change between the two halves of the nursing bouts. These data do not fit the predictions that the pups would spend more time asleep after receiving milk.

Did the behavior associated with milk ejections change over the time of the nursing bouts? For those 14 nursing \geq five min, there were an average of $1.1 \pm .32$ milk ejections in the first half and $1.3 \pm .16$ in the second half of the nursing bout (dep $t = .763$, $df = 13$, n.s.). The sleep-wake state percentages in the minute pre- and post-milk ejections did not differ when measured in the first half vs. second half of the nursing bout. Neither did the sucking rates show any change over time, as can be seen in Table H.

Trends Over Time During The Recording Session

In the sections above, a great deal of data have been presented on the mother-pup interaction. Much of that data confirmed earlier work by others (Grota and Ader, 1969; Hofer and Grabie, 1971; Leon, Adels and Coopersmith, 1985). As a final check to see if the recording procedure interfered with the behavior of the mother or pups, we examined the first vs. second half of the recording session. If these data were strongly influenced by the attachment and continued presence of the leads, one would expect to see changes in behavior over time as the mother and pups slowly adapted. As shown in Table I, there was no significant change either in percent time attached or number of milk ejections. From Fig. 5, one can see that the change in behavior across litters was much greater than any change over time.

Table G

Trends over time within the nursing bout:
Comparison of state percentages and sucking behavior from
the first to second half of the bout on Baseline day.^a

	(-Sleep-Wake State %'s-)			(-Time Sucking ^b -)		
	A	PS	SWS	RS	B	T
1st Half	35 ^c	23	42	16.4	14.7	23.7
2nd Half	32	32	36	10.2	13.5	24.0
dep t = df=13	.978 ^d	1.700	1.188	1.610	.641	.125

^a Data taken only from those 14 nursing bouts \geq 5 min.

^b For each of the three sucking types, the time spent sucking was calculated as the number of 5 sec bins in which a suck occurred divided by the attached time of that half.

^c Note that these figures were calculated as means per nursing bout (i.e., divided by 14, not 6). Therefore these data will not match the means given in Tables 1 and 2.

^d All t's not statistically significant.

A = awake PS = paradoxical sleep SWS = slow wave sleep
B = bursts RS = rhythmic sucking T = treadles

Table H

Trends over time within the nursing bout:
 Comparison of the rates of sucking in the minutes around
 milk ejection in the first vs. second halves of the nursing bout.

	1st min. prior to ME			1st min. post ME		
	RS	B	T	RS	B	T
1st Half	.5	1.8	1.4	2.1	.8	5.3
2nd Half	.3	1.1	1.7	2.3	1.2	4.7
dep t = df=5	.753 ^a	1.698	.558	.172	.945	.537

B = bursts RS = rhythmic sucking T = treadles
 ME = milk ejection

^a All t's are non-significant.

Table I

Trends over time during the recording session:
Comparison of behavior from the first to second half of
the Baseline session.

	% TRT Attached ^a	# of ME's
	Mean + SEM	Mean + SEM
1st Half	44 ± 10.6	3.3 ± .76
2nd Half	41 ± 10.7	2.2 ± .60
dep t = df=5	.200 ns	1.337 ns

^aData taken only from those 14 attached episodes ≥ 5min.

ME = milk ejection ns = not statistically significant
TRT = total recording time

These first vs. second half comparisons are admittedly crude measures. Many of the finer-grained comparisons of trends over time would be possible. In fact, too many are possible. Without specific hypotheses to guide us in which comparisons to make, we decided to be satisfied with these imperfect analyses. They are at least sufficient to test the simple questions proposed in these two subsections.

The Problem of "Indeterminate State"

As was discussed in the Introduction and Methods, states are defined based on a constellation of behaviors. Individuals may vary from each other or within themselves over time in the preciseness of the coherence of the parameters used to define states. Thus, certain epochs may be hard to characterize as one state or another. This problem is often especially acute in young organisms as all the parameters which constitute a state may not be fully developed yet (Anders, 1975; Petre-Quadens, 1974; Wolff, 1972). On Baseline day, 9% of the recording session were these difficult-to-score epochs which have been labelled indeterminate state (IDS). Each of these difficult epochs was placed in the state to which in my best estimation it belonged. However, these epochs were tracked for further analysis. Sixty-four percent of IDS were epochs which appeared to be a cross between awake-drowsy and slow wave sleep with movement. This state has been labelled intermediate state (ITS). ITS occurred an average of $6 \pm 1.3\%$ of the Baseline recording. The range of ITS among the animals went from 0 to 10%. Of these epochs, 69% were scored as slow wave sleep; 31% as awake.

To test whether possible mislabelling of these epochs might have

Table J

Influence of intermediate state scoring on sleep-wake state percentages.

	Mean % of Recording Time		
	A	PS	SWS
Overall: original scoring	31	39	30
Overall: ITS scored as A	34	39	27
Attached: ITS scored as A	38	33	29
Not Attached: ITS scored as A	34	43	24
Comparison of Att. vs. Not Att. dep t, df=5	.894,ns	1.493,ns	.890,ns

A = awake PS = paradoxical sleep SWS = slow wave sleep
ITS = intermediate state ns = not statistically significant

influenced the results in the sections above, the data in Table J were calculated. As can be noted, there was only small change in sleep-wake percentages caused by this recalculation. In fact, the similarities between the attached and not-attached conditions in sleep-wake percentages were even more striking when figured in this manner (compare with Table C).

If all ITS epochs in the minute following milk ejections were scored as awake, the speed at which pups fell asleep was still dramatic. In 42% of the cases the pups were asleep by 15 seconds. The figure climbed to 69% by 30 seconds. Thus we calculated that the problem caused by the difficult-to-score epochs did not significantly influence the general pattern of our results.

Discussion

The results of this experiment demonstrate that there are striking similarities in the behavior of the two-week-old rat while attached and not attached to its dam's teat. Activity levels do not distinguish between the two conditions, confirming Hofer and Grabie (1971). Most importantly, the basic sleep-wake pattern continues for the most part independently of attachment condition. For example, there is no change in the percent of time in each sleep-wake state. The rat pup alternates in a fairly rapid manner among the three basic arousal states, spending approximately two-thirds of the time asleep under both conditions. What does alter is the duration and frequency of the state episodes. While attached, the episodes tend to be shorter and more frequent, possibly due to the mother's behavioral stimulations.

Nothing in these results conclusively confirms or denies the

speculation that the pattern of activity is controlled by some internal generator mechanism, relatively independent of attachment condition. If such a mechanism exists, it may also explain the similarity of sleep-wake states under the two conditions. In fact, the sleep-wake state behavior may be part of such a control mechanism, or at least on the pathway of control.

The results also reveal that during nursing bouts almost half of all sucking behavior occurs while the infant rat is asleep. Characteristic rates of each sucking type are exhibited in the different states. These rates tend to be highest while the pup is awake, intermediate in slow wave sleep, and lowest in paradoxical sleep. However, one sucking pattern, the burst, has its highest rates in slow wave sleep.

The proportions of sleep-wake state percentages and the rates of sucking do not differ between the first and the second halves of the nursing bouts. Thus, in the normal suckling situation in the two-week-old rat, increasing sleepiness and decreased levels of sucking are not found to occur as the nursing bout progresses. In this species, in which milk letdown occurs periodically, sleep-wake state levels do not provide a simple explanation for the termination of feeding. Indeed, termination of nursing bouts has been found to be triggered by increases in the mother's ventral temperature rather than other feeding-related events (Woodside, Pelchat and Leon, 1980).

After the pup originally attaches to the teat, then only in the periods of the milk ejection responses can we predict what the pup's state will be. Before each milk ejection the animal is always asleep; after each milk ejection the pup awakens briefly. Rhythmic sucking

and treadles are both increased during milk ejection responses and are therefore candidates as ingestive sucking types.

NORMAL MOTHER AND LITTER REUNION

The Baseline experiment reported above raised some unexpected issues. If satiety is not simply linked with sleepiness, what is the relationship between feeding and sleep-wake states? The pups' sleep-wake patterns were similar whether attached or not-attached to their mother's teat. Is this similarity evidence that the sleep-wake states are generated internally by the pups and not easily influenced by the environment? How is the sucking behavior which occurs during sleep controlled? What factors influence that behavior?

Deprivation experiments have often been used to perturb normal behaviors and thus highlight any relationships among them. One such deprivation -- separation from the mother -- has known effects on both sleep-wake states and sucking behaviors, albeit studied separately and under different experimental conditions (Hofer, 1976; Hofer and Shair, 1982; Brake, Sager, Sullivan and Hofer, 1982). The goal for this experiment was to see how deprivation affected the mother-infant interaction and especially the relationship of sleep-wake states and sucking behaviors.

The data will be presented in a similar format as in the Baseline results section. First, as a part of behavioral observations, data will be presented on the number and timing of nursing bouts and milk ejection responses, the number of nipple switches, and weight and temperature changes.

Second, the polygraph data will be analyzed, especially the relationship of sleep-wake states and sucking behaviors. The period of milk ejection responses will be scrutinized.

Trends in the data over the time of the nursing bout will be examined in conjunction with trends over time in the recording session. This analysis is especially important since reunion effects are to be expected.

Reunion to Baseline day comparisons will be made throughout the data presentation as needed. These comparisons will be used to emphasize any effects of deprivation.

Behavioral Observations

Upon Reunion, the pups still showed the alternation of active and quiet periods which was observed on Baseline day, both when attached and unattached. However, implanted pups and their separated littermates appeared more active than their nonseparated littermates. The separated pups were more vigorous in attempting to attach to the dams, occasionally going as far as leaving the litter pile and chasing the dam around the cage. Remember that since only three pups from each litter were separated overnight, the following data does not represent the normal response of mother and litter to deprivation, but comparisons can be drawn between the test pup's own responses on Baseline and Reunion days.

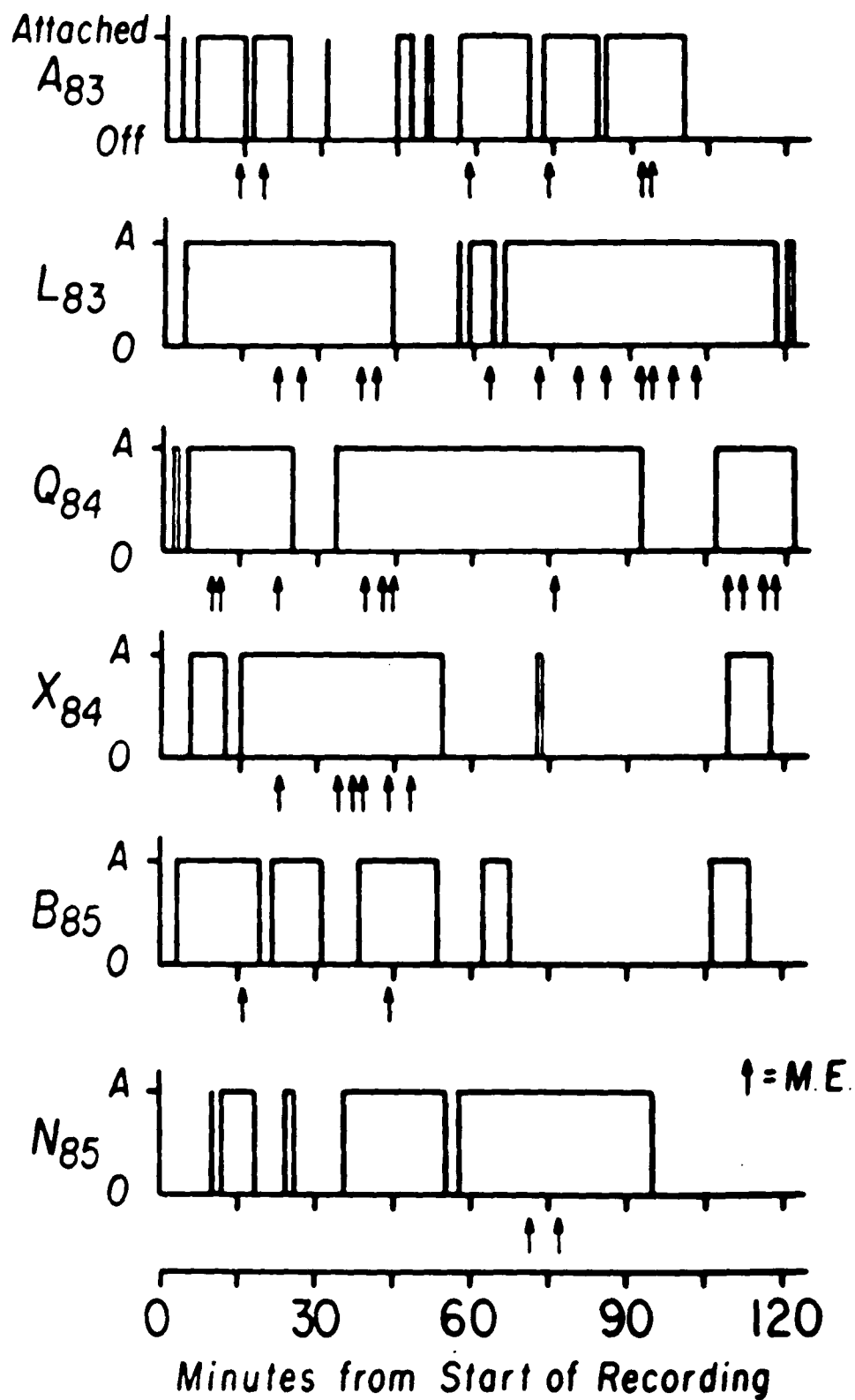
Attachment and Milk Ejection Response Data

As can be seen in Fig. 13, there was a great deal of variability in both attachment and milk ejection response patterns among the six litters. There was a significant increase in attachment time over the Baseline day (see Table K), but this did not translate into an increased number of milk ejections as defined by the criteria in the

Figure 13. Patterns of nursing bouts for all six litters during the two hour Reunion recordings.

This data is based on the times during which each implanted pup either was or was not attached to a teat. The results for any other individual in a litter may vary slightly. Gaps in attachment of less than or equal to one minute are not indicated. Milk ejections (ME) are designated by arrows.

Compare to Fig. 5 for the similar data from Baseline day.



Methods section. Four pups had more milk ejections on Reunion day; two pups had fewer. Neither the latency to the first milk letdown of a nursing bout nor the time between subsequent letdowns was significantly decreased on Reunion day.

The milk ejection response data was also examined for each pup in relation to the total recording time, i.e. independent of the variable timings of the nursing bouts. Thus, a mean figure was derived from each pup and that was used to get an overall mean. This method of analyzing the data allowed us to see if the pups were getting milk ejections sooner or more frequently on Reunion day. The mean latency to the first milk ejection of the recording for each pup on Baseline day was 34 ± 7.9 minutes. On Reunion day it was 26 ± 9.1 minutes (dep t = .538, df=5, ns). In the Baseline to Reunion latency change, four animals decreased latency to the first milk ejection on Reunion day, one increased latency, the last showed little change. When figured independent of breaks in attachment, the mean latency between subsequent milk ejections per pup was as follows: Baseline mean = 13.3 ± 2.0 min.; Reunion mean = 8.9 ± 2.0 min. (dep t = 1.664, df=4¹, ns).

The mean latency to the first milk ejection of each nursing bout of each pup was as follows: Baseline means² = 8.1 ± 1.5 min.; Reunion mean = 8.2 ± 1.6 min. (dep t = .079, df=5, ns). Thus, in this experiment in which only one half the litter had been separated, the deprived pups were not able to elicit significantly more or more frequent milk ejections from their dams. These results suggest, among other things, that any changes in the test pup's behavior and

Table K

Data from behavioral observations of mother-pup interactions on Reunion day.

	Time Att. (min)	% TRT Att.	Latency to 1st NB (min)	# of NB'S	Lengths of NB's (min)	# of nipple ^a switches	# of ME's	Latency to 1st ME ^b (min)	Time ^c betw. later ME's (min)
Mean	70	58	3.2	5.3	13.6	2.5	6.5	6.8	5.8
SEM	7.8	5.6	.5	.9	2.7	1.2	1.8	1.2	1.2
Range	52 to 94	43 to 77	1.0 to 4.8	4 to 9	.5 to 52	0 to 7	2 to 12	1.5 to 18	1.5 to 30

Comparison of means to Baseline data.

dep t =	3.071	3.229	1.913	1.642	.863	1.164	.745	.222	.334 ^d
df=5	*	*							

^a A nipple switch was defined as an event in which a pup detached from a nipple and reattached to the same or another nipple within one minute.

^b This figure is latency to the first ME of each NB, not the TRT.

^c Figured only for ME's within the same NB.

^d ind t-test, df=41, ns

ME = milk ejection NB = nursing bout TRT = total recording time
Att. = attached

electrophysiological data from Baseline to Reunion were not in response to major shifts in the dams' behavior patterns.

Weight and Temperature Data

During the 22 hours of deprivation, the test pups lost an average of $2.0 \pm .28$ gr., which was a significant decrease in weight (see Table L). The mean change in body weight during the Reunion session was not statistically significant, even though the average increase was greater than the change on Baseline day. Two pups actually lost weight during the Reunion test session. These results are similar to findings of Stephen Brake in which deprived pups had an increased variability in weight gain post separation. Some pups gained much more than prior to deprivation, some pups "seemed to fall apart" (personal communication). As on Baseline day, neither weight changes nor percent change were correlated significantly to attachment time, to the number of milk ejections or to the number of nipple switches.

The pups' average temperature was $35.2 \pm .4^{\circ}$ C prior to the recording. The temperature had not changed significantly due to deprivation, nor did it change significantly during the recording session.

Electrophysiological Data

Sleep-Wake States

Even after 24 hours of separation, when pups were returned to their mothers and litters, they were asleep a great deal of the time both attached and unattached. The percentage of time spent in each of the sleep-wake states did change from Baseline day, however. One of

Table L

Body weight and rectal temperature.

	(-----Body Weight (gr.)-----)			(---Rectal Temp.(C)---)	
	pretest	posttest	% change ^a	pretest	posttest ^b
Mean	25.8	26.9	4.3	35.2	36.3
SEM	1.2	1.2	2.4	.4	.4
Range	22.4 to 30.0	22.4 to 30.2	-2.6 to +11.6	33.6 to 36.2	35.6 to 36.6
Comparison of means: Baseline and Reunion					
dep t =	7.129 ^c	-----	1.521	2.130 ^c	-----
df=5	***			+	

^a The mean weight gain was not statistically significant (dep t = 1.829, df=5).

^b The mean temperature change was not statistically significant (dep t = 2.559, df=5, p<.10).

^c Thus, the pups started the Reunion session lighter than on Baseline day (mean wt. change = -2.0 gr), but the loss in rectal temperature did not quite reach significance.

+ = p<.10, ns *** = p<.001

these changes, the decreased time spent in paradoxical sleep (see Table M) confirmed similar findings on pups recorded in isolation described by Hofer (1976) for Carworth Wistar rats and by Hofer and Shair (1982) for Marland Farms Wistar rats.

In the present study the amount of time awake also increased significantly on Reunion day, while the amount of time spent in slow wave sleep did not change. There was no difference in the number of state transitions per hour, probably due to the behavioral changes which will be discussed later.

Few Baseline to Reunion correlations of sleep parameters reached significance. However, the animals that showed the least paradoxical sleep on Baseline day showed the most on Reunion day, suggesting a possible rebound effect ($r = -.78$, $df=4$, $p<.05$). There was a strong positive correlation of mean duration of episodes in slow wave sleep between Baseline and Reunion days, possibly indicating the resistance of slow wave sleep to change ($r = .93$, $df=4$, $p<.01$).

Upon Reunion, the patterning of sleep-wake states of the pups depended upon whether or not they were attached to a teat. Attachment condition influenced the percent of time spent in each state, as well as the episode mean duration and frequency per hour in many cases (Table M). These differences may possibly be explained by the interaction of the environmental stimuli of reunion and the internal motivational state of the deprived pups. For example, when not attached, the test pups were often observed chasing their dams around the cage, nosing frantically at her ventrum, attempting to attach. It was this type of behavior which led to being awake 77% of the time with an average episode duration of 3.3 minutes. In Fig. 14, one can see

Table M

Measures of sleep and wakefulness of infant rats recorded with their normal mother and litter for two hours on Reunion day.

	Sleep Latency (min)	% Time in State ^a			(-----State Episode Analysis-----)						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Overall	6.5 ^h	57	9	33	1.92	1.02	1.35	19	5	16	40
dep t = 1.787 df=5		4.372**	7.057***	.834	3.976*	3.451*	.234	1.337	3.924*	1.234	1.812
Attached	1.6	48	4	48	1.24	.60	1.46	24	4	21	48
Not Attached	4.4	77	13	11	3.27	1.62	.79	17	6	9	30
dep t = 2.191 ^c df=5		2.674*	2.091+	4.884***	2.132+	3.030 ^d +	3.296 ^e *	1.777	.552	2.350+	1.455

^a % time in state is based on the total recording time for the overall condition, on the time being nursed in the Attached condition, and on the time with mother away in the Not Attached condition.

^b Comparison of Baseline to Reunion

^c Comparison of Attached and Not Attached

^d df = 4 ^e df = 2

A = awake PS = paradoxical sleep SWS = slow wave sleep + = p<.10, ns
* = p<.05 ** = p<.01 *** = p<.001

that the longer mean duration was especially related to a large percent of very long (\geq five min) episodes.

Of the small amount of paradoxical sleep during the Reunion test, less than one quarter of that occurred while attached (Table M). Also, while attached, any episodes which did occur were of very short duration (Fig. 14), usually terminated by awakening (Fig. 15). When not attached, there were no more frequent episodes of paradoxical sleep, but the mean duration was longer. I believe that the low amount of paradoxical sleep while attached was related to the pattern of sucking behavior elicited by Reunion. This point will be addressed further after the sucking data has been presented. With the demands of the sucking behavior released while not attached, the higher percentage of paradoxical sleep may have been a kind of "rebound" phenomenon.

The percent of slow wave sleep while not attached was significantly lower than when being nursed (Table M), possibly because of how active the not-attached pups were. The high percentage while attached could have been another example of "rebound". Another possible explanation is that some element of the Reunion nursing situation elicited slow wave sleep in the pup. Figure 14 demonstrates that the lower mean duration and frequency per hour of episodes when not being nursed was due to a general shift in the percent of episodes to be shorter, rather than a heavy weighting in episode lengths at one end of the scale or the other, as was the case with awake and paradoxical sleep.

In the transitional probability of sleep-wake state changes data (Fig. 15), there is further evidence of the different demands on the pups in the attached and not-attached conditions. When attached, pups

Figure 14. Histogram analysis of state episode durations during nursing and with the mother away on Reunion Day, illustrating the relative frequencies of occurrence of periods of different durations.

A = awake; PS = paradoxical sleep; SWS = slow wave sleep; E = episode; = $p < .10$; * = $p < .05$; ** = $p < .01$.

Indications of statistical significance refer to comparisons within a state for episodes of the same duration between the Attached and Not Attached conditions. These indications are based on the results of t-tests whose use are validated by the findings of three two-way ANOVAs with repeated measures on A and B, one ANOVA for each state.

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
Awake: Factor A Attached vs. Not Attached Factor B Episode Duration				
SWG	.215	5	.043	
A	0	1	0	0
B	6426.2	4	1606.5	11.638**
AB	3887.8	4	972.0	7.041**
Resid	6211.8	45	138.0	
PS: Factors A and B as above				
SWG	.074	2	.037	
A	.028	1	.028	.000
B	6847.2	4	1711.8	12.670**
AB	8950.5	4	2237.6	16.562**
Resid	2431.9	18	135.1	
SWS: Factors A and B as above				
SWG	.123	4	.031	
A	.496	1	.496	.002
B	13028.6	4	3257.1	13.966**
AB	1463.4	4	365.8	1.569
Resid	8395.9	36	233.2	

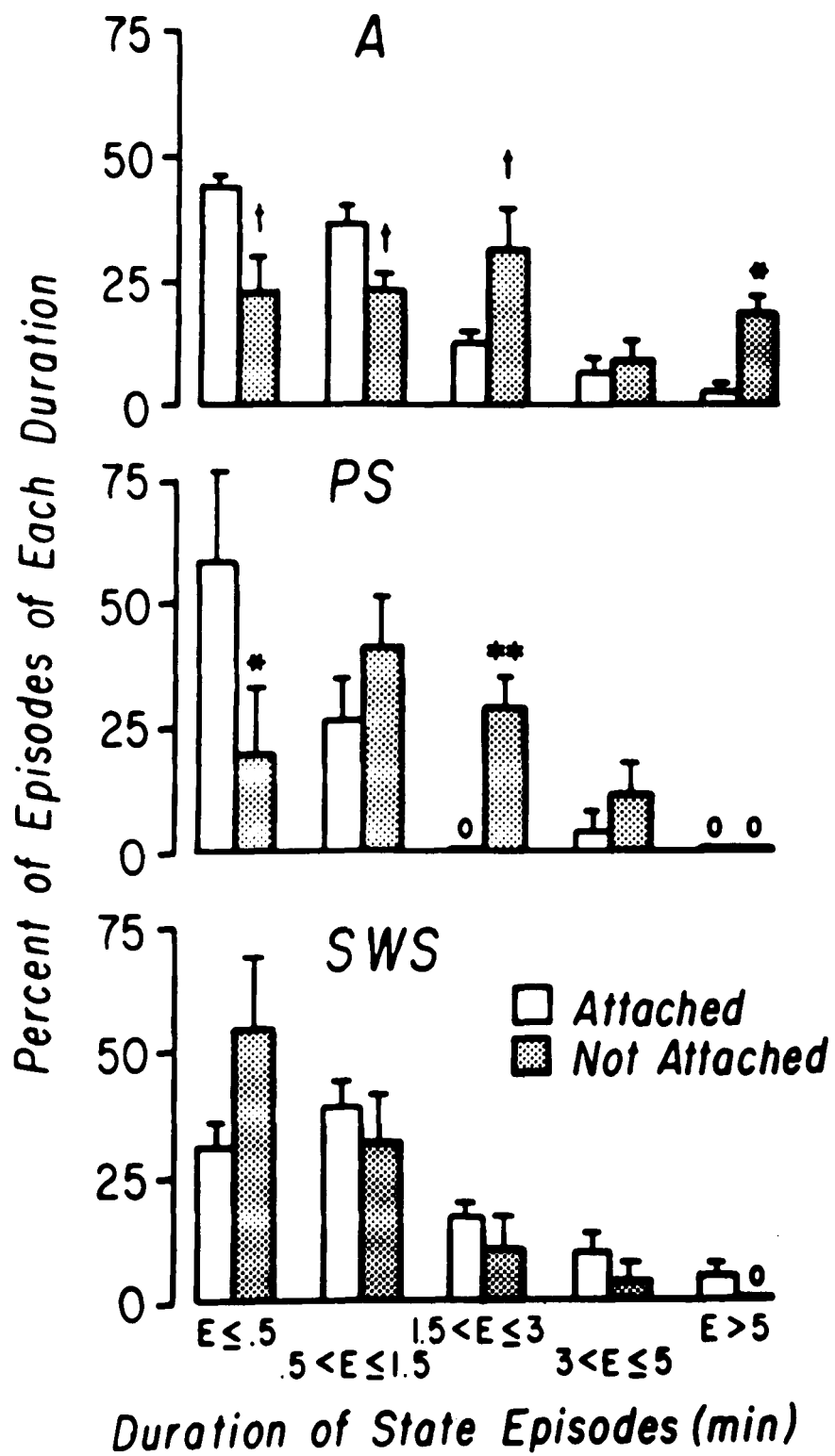


Figure 15. The probabilities of transition among the three sleep-wake states during nursing and while the mother was away on Reunion day.

An overall ANOVA on both Attached and Not Attached transitional probabilities revealed both main and interaction effects (see below). These results allowed the use of dep t-tests for planned, individual comparisons.

Unlike Baseline day (see Fig. 9), there were significant differences in transitional probabilities between the two attachment conditions:

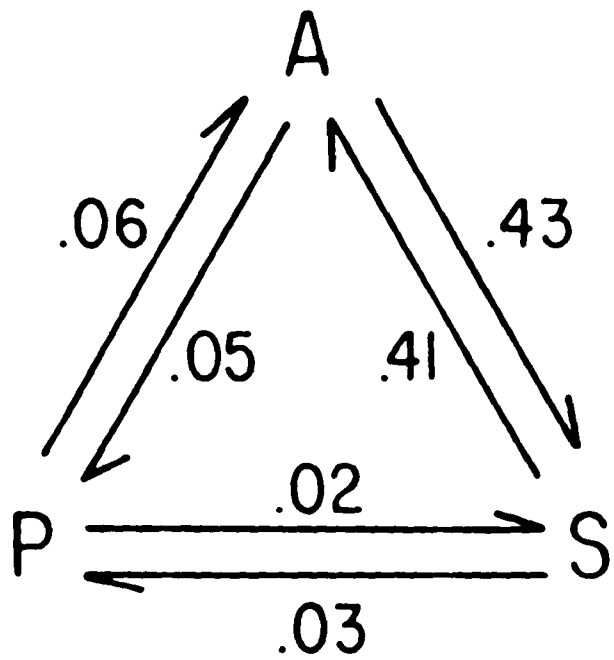
S to A: $t=3.443$, $p<.02$
 P to A: $t=3.607$, $p<.05$
 A to S: $t=3.717$, $p<.02$
 A to P: $t=2.835$, $p<.05$
 $df=5$ in all cases

Individual comparisons of Reunion to Baseline data were performed only for the Attached condition. The behavioral observations revealed such gross differences in actions of the pups when not attached that comparisons seemed pointless. Even when attached, however, there were significant differences between Reunion and Baseline in the likelihood of all state transitions at the $p<.05$ level or better (use of dep t-tests justified by ANOVA results in Appendix)

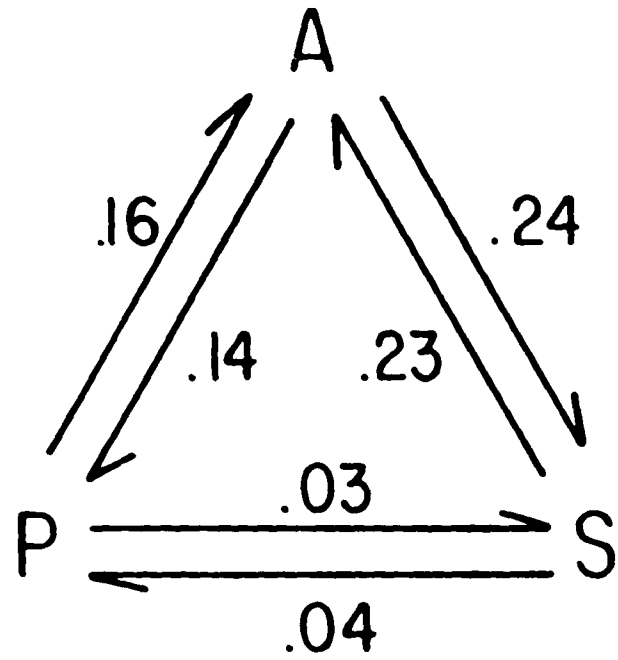
A = awake; P = paradoxical sleep; SWS = slow wave sleep;
 df = degree of freedom; $p<.01$.

Reunion data: Factor A Attached vs. Not Attached
 Factor B Direction of State Transition Probability

<u>Source</u>	<u>SS</u>	<u>DF</u>	<u>MS</u>	<u>F ratio</u>
SWG	.069	5	.014	
A	.014	1	.014	2.073
B	1.186	5	.237	35.326**
AB	.257	5	.051	7.658**
Resid	.369	55	.006	



Attached



Not Attached

were three times more likely to shift from paradoxical sleep to awake, than to shift from paradoxical sleep to slow wave sleep. This finding occurred despite the fact that awake and slow wave sleep percentages of total attached time were equal. When not attached, pups in paradoxical sleep were more likely to shift to slow wave than would have been predicted by random chance based on the sleep-wake state percentages. In neither attachment condition was the most common adult mammal pattern seen, that of awake to slow wave to paradoxical to awake.

Comparison of these data with the similar data from the Baseline session demonstrates the greater influence of attachment condition on sleep-wake behavior after separation and Reunion. Especially note that percent of time in each state differed with attachment condition after Reunion and did not on Baseline Day. Thus, attachment condition was a more salient stimulus on Reunion day than during the Baseline session.

The above data might lead one to believe that 22-hour deprived pups were simply hyperaroused, probably because of hunger. And, it is true that the pups were awake more after deprivation, even while being nursed (Table N). However, note that the increased time awake was due to a selective reduction in paradoxical sleep. Slow wave sleep was once again resistant to change. Neither percent time in slow wave sleep nor duration or frequency of episodes differed significantly between Baseline and Reunion recordings. Also, sleep latency was not significantly increased on Reunion day. It is therefore hard to think of these deprived pups as just hyperaroused by hunger, since they all still fell asleep prior to receiving any milk. The significant decrease in state transitions per hour after separation might be considered as evidence against hyperarousal as well, but the very low

Table N

Comparison of sleep-wake state measures during attachment: Baseline to Reunion.

	Sleep Latency (min)	% TAT in State			(-----State Episode Analysis-----)						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Baseline	.8	29	33	38	.68	1.22	1.18	26	17	20	61
Reunion	1.6	48	4	48	1.24	.60	1.46	24	4	21	48
dep t_a df=5	1.612	2.667*	4.228**	1.705	2.698*	1.581	1.859	1.309	6.852**	.329	3.620*

^adf=3, since two reunion pups had no PS attached

A = awake PS = paradoxical sleep SWS = slow wave sleep
TAT = total attached time * = p<.05 ** = p<.01

amount of paradoxical sleep explains that finding. Shifting between two states in a limited amount of time leads to fewer transitions than changing among three.

The increased percent time awake on Reunion day was made up from longer, but no more frequent episodes (Table N). Many of these longer episodes awake occurred following milk ejections and will be discussed below.

Sucking Behavior

Upon Reunion after deprivation, two week old rat pups increase their intake from the dam or in an artificial feeding test with continuous milk flow (Hall and Rosenblatt, 1978; Houpt and Epstein, 1973; Lorenz, Ellis and Epstein, 1982). Privation not only influences attachment time, beginning at this age (Table K, also Cramer, Blass and Hall, 1980; Dollinger, Holloway and Denenberg, 1978; Hall and Rosenblatt, 1977), but the time spent sucking as well (Brake, Wolfson and Hofer, 1979; Brake, Sager, Sullivan and Hofer, 1982). As shown in Fig. 16, our data confirmed this previous work. Total of treads, rhythmic sucking and the nipple attachment intensity all increased from Baseline to Reunion day.

However, examination of the sucking rate per unit time in each state demonstrated a pattern very similar to that of Baseline day. High rates for sucking were seen while awake, low rates in paradoxical sleep and intermediate levels in slow wave sleep (Fig. 17). Bursts were again the exception, being highest in slow wave sleep. In fact, the sucking rate per unit time in each state seemed relatively resistant to change. There were no significant increases in rates per

Figure 16. The average time spent in each sucking type while attached to an unanesthetized dam during Baseline and then Reunion from 22 hours separation.

Time of sucking is represented as the number of five-second periods in which a suck of a given type occurred.

RS = rhythmic sucking; B = bursts; T = treadles;
I = EMG intensity rank; * = $p < .05$; ** = $p < .01$.

Statistical indications compare Baseline and Reunion results for a given sucking type and are based on dep t-tests, $df=5$ for each.

RS: dep t = 2.571, $p = .05$
B: dep t = 1.840, ns
T: dep t = 17.740, $p < .001$
I: dep t = 2.597, $p < .05$

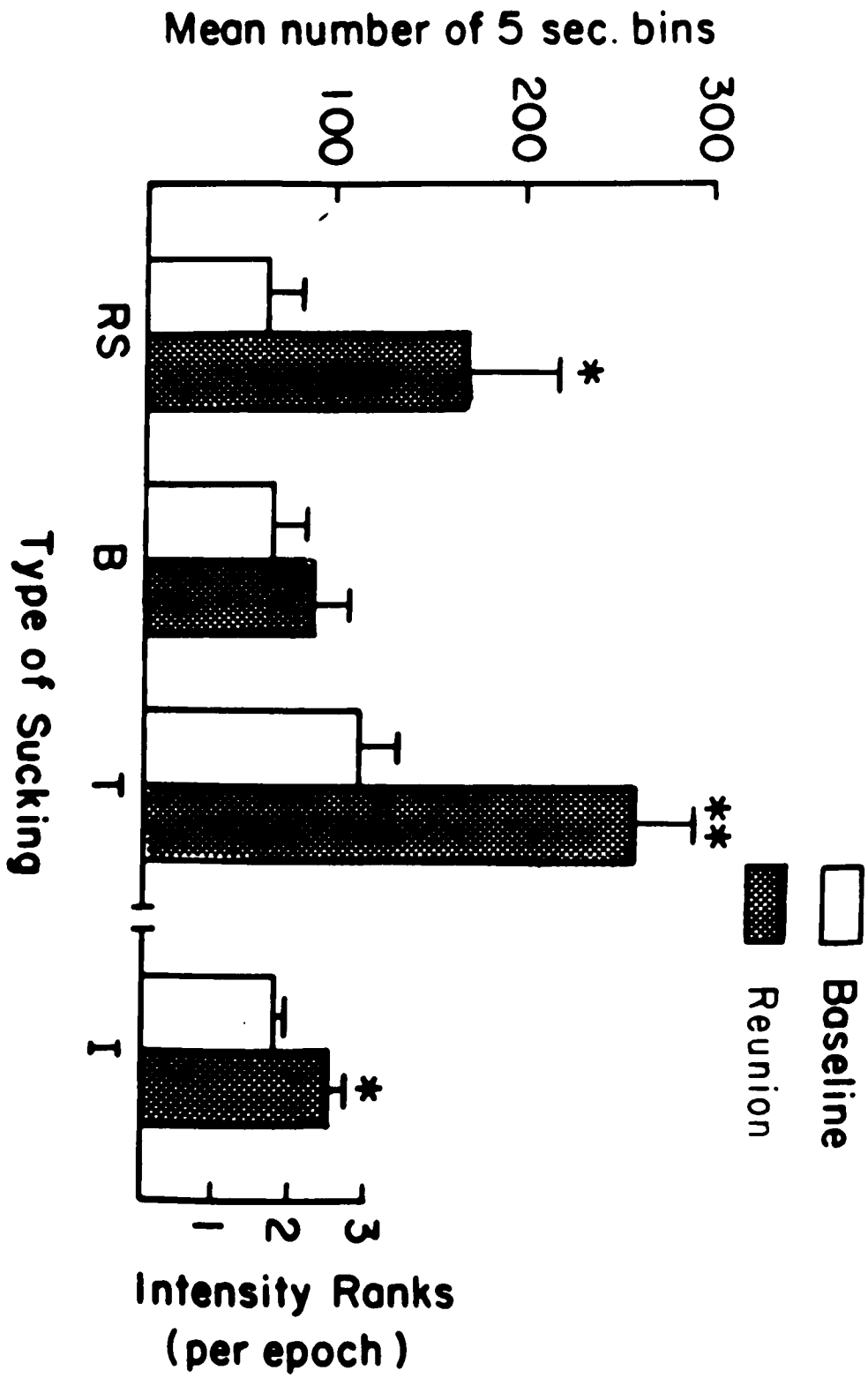


Figure 17. The rate of sucking behaviors and the percentage of time in each sleep-wake state during nursing after Reunion from 22 hours separation.

The horizontal bars represent the mean percentage of recording time while attached to a teat spent in each sleep-wake state. The height of the three left-hand vertical bars gives the mean rate of sucking per minute for each kind of sucking. The bar to the right measures the amplitude of the digastric EMG recording when no sucking is occurring (an index of the muscle tone involved in maintaining a seal on the nipple).

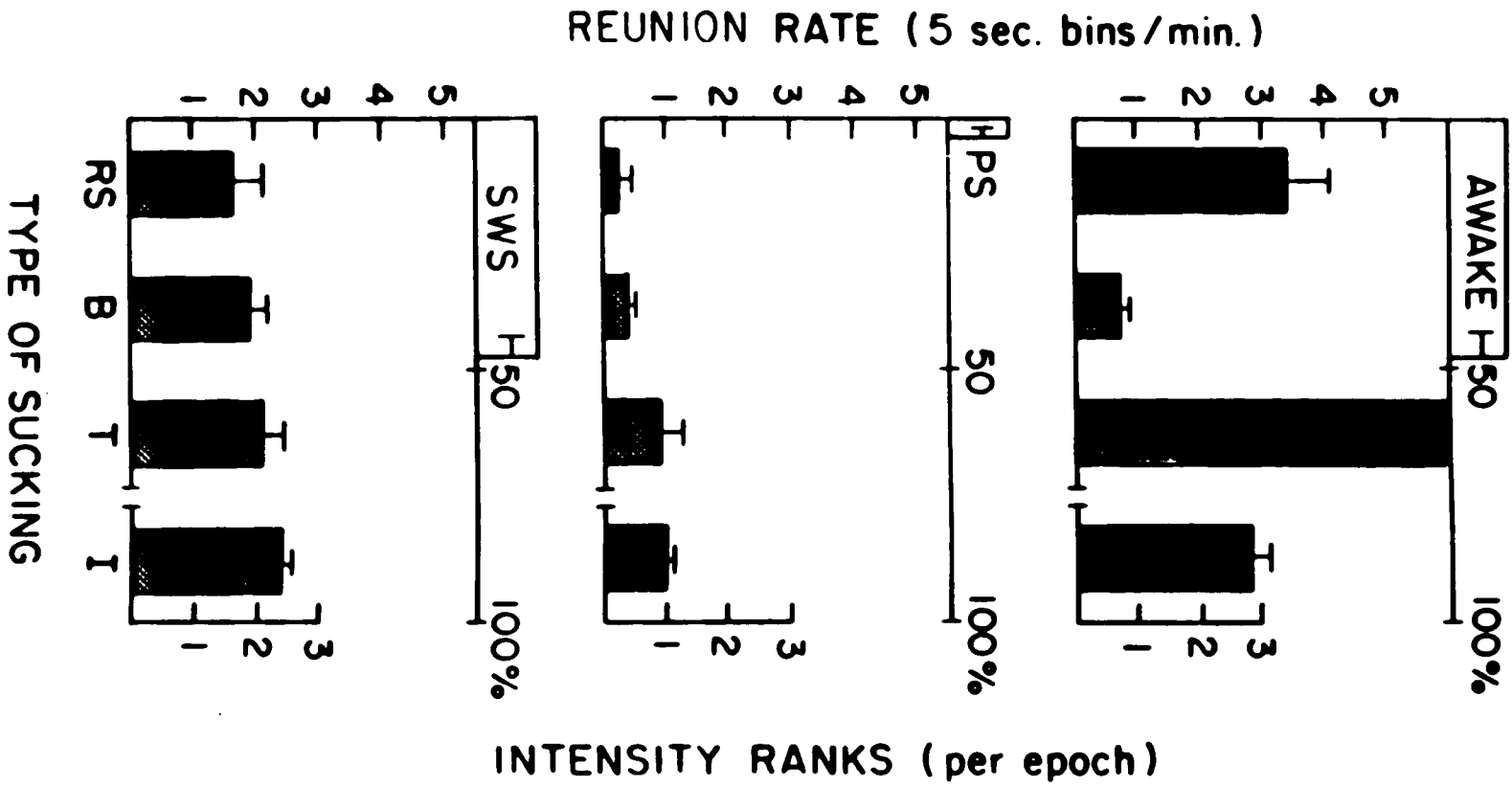
EMG = electromyogram; PS = paradoxical sleep; SWS = slow wave sleep; RS = rhythmic sucking; B = bursts; T = treadles; I = EMG intensity rank.

Each sucking type differs significantly in its rate among the three states at the $p < .05$ level or better except for RS: A to PS, dep $t = 2.963$, $df=3$, $p=.058$ and RS: SWS to PS, dep $t=1.860$, $df=3$, ns.

Comparisons of Reunion and Baseline rates within a state reveal only two significant changes (compare graph with Fig. 10). The rates of bursts during both awake and PS are decreased on Reunion day.

B/awake: dep $t=9.790$, $df=5$, $p<.001$
 B/PS: dep $t=3.502$, $df=3$, $p<.05$

All significance findings are based on dep t-tests whose use are validated by the experimental results of overall ANOVAs on both Baseline and Reunion data (see Appendix).



state from Baseline to Reunion test. Thus, the increase in total amounts of sucking were not simply due to a generalized increase in sucking in all three states. (See figure legend for statistics.)

These sucking rates measures were not absolutely fixed, however. The rate of bursts decreased significantly after deprivation both in awake and in paradoxical sleep. Also, as shown in Table O, individual animals could either increase or decrease sucking rates within each state. I am not trying to say that sucking rates per unit time within each state never increase. Note that such an increase did occur for treadles while awake in five of the six pups. However, such an increase was not necessarily a part of the response to deprivation. One pup had a decreased rate and for several the magnitude of the increase was quite small.

Milk Ejection Responses

The periods surrounding milk ejections were defined and examined closely as was done on Baseline day. There were 39 observed milk letdowns. In 34 of these the pups did not detach from the teat for more than 15 seconds. These 34 were used for analysis. As on Baseline day, pups were almost always asleep just prior to letdown, as shown in Fig. 18. Deprivation and Reunion did shift the ratio of sleep states dramatically. In only 3% of the cases were the pups in paradoxical sleep; in 94% they were in slow wave sleep. After milk ejection the pups returned to slow wave sleep, but more slowly than on Baseline day. By one minute, pups were asleep in 50% of the cases.

As shown in Fig. 19 and in Table P, the rate of treadles rose precipitously after milk ejection, returning to normal by the end of

Table 0

Direction^a of sucking rate change from Baseline to Reunion tests.

	(-----A-----)			(-----PS-----)			(-----SWS-----)		
	RS/M	B/M	T/M	RS/M	B/M	T/M	RS/M	B/M	T/M
B85	+	-	-	b	b	b	+	-	+
X84	-	-	+	b	b	b	+	-	-
Q84	+	-	+	+	-	0	+	-	+
N85	-	-	+	-	-	-	-	+	-
A83	-	-	+	-	-	+	-	+	+
L83	+	-	+	-	-	+	+	+	+

^a Remember that this chart gives no indication of the magnitude of the change.

^b No PS while attached during the Reunion test.

+ = positive change in rate - = negative change in rate
 0 = no change in rate A = awake PS = paradoxical sleep
 SWS = slow wave sleep RS = rhythmic sucking B = bursts
 T = treadles M = minute

Figure 18. The percentage of pups in each sleep-wake state on Reunion day during the minute before and the minute after milk ejection response in 34 of 39 cases observed.

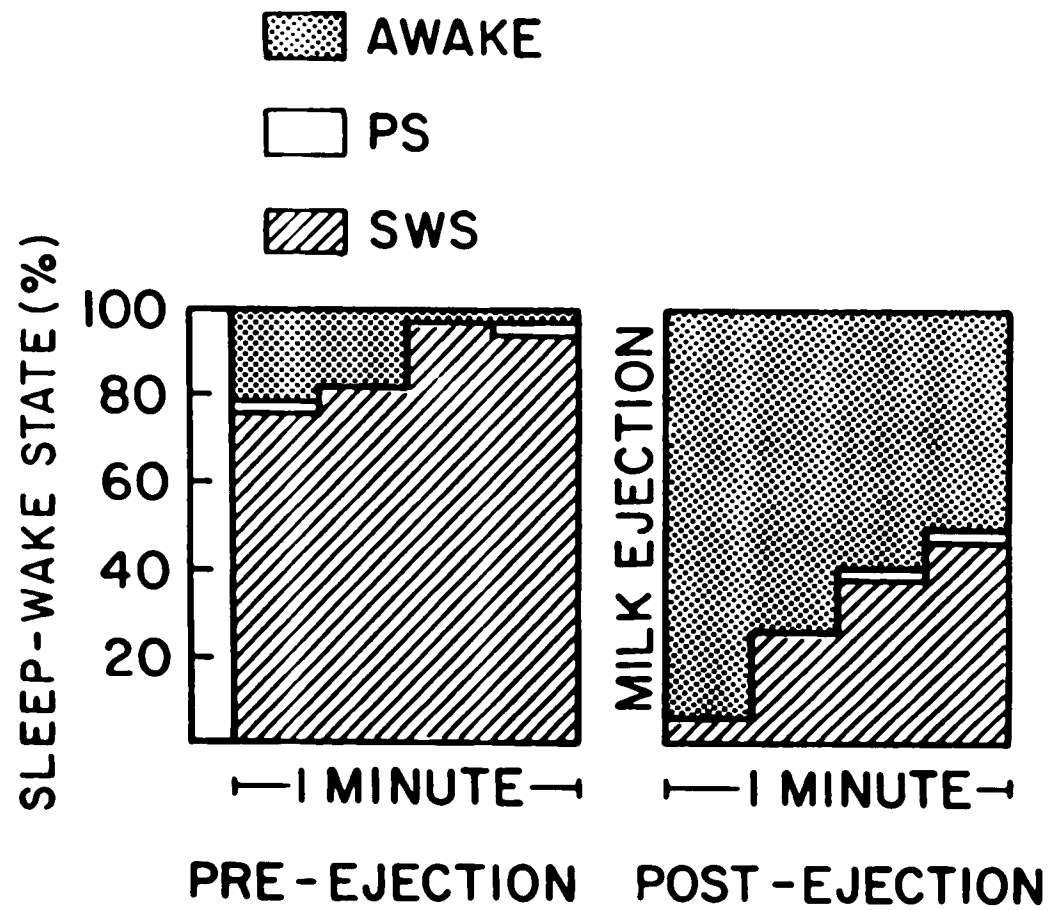
In five cases the pups detached for more than 15 seconds and were omitted from this analysis.

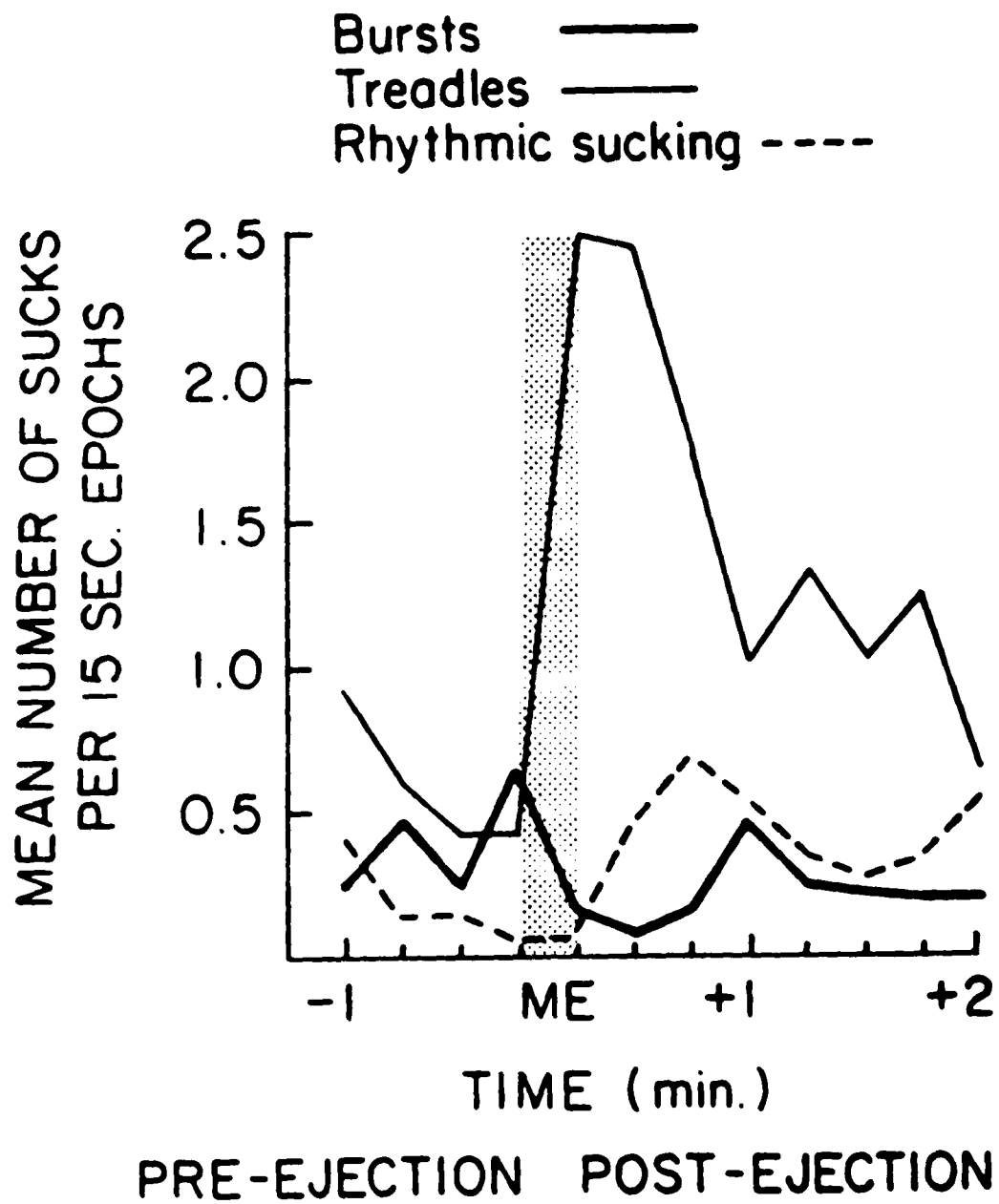
Compare to Baseline results in Fig. 11.

Figure 19. Sucking levels on Reunion day for the minute before, during and two minutes after milk ejection response (ME).

Mean values based on the 34 MEs in which the pups did not detach for more than 15 seconds.

Compare to Baseline results in Fig. 12.





the second minute. Although rhythmic sucking was not increased in comparison with its overall rate, it did show an increase when the minute after milk ejection was contrasted with the preceding minute (dep $t = 3.595$, $df=5$, $p<.05$). Why didn't rhythmic sucking increase compared to its overall rate? It may have been due to the high rate of rhythmic sucking observed during the times of attachment and detachment, as well as during shifts of maternal position. Another explanation may be that the large body movements of treading emitted by these pups may have prevented the EMG leads from picking up the underlying rhythmic sucking.

Recent unpublished work by Brake suggests that treading and rhythmic sucking may actually be the same sucking type. The EMG activity of the limb movements of treading may be obscuring the underlying rhythmic sucking pattern on the polygraph tracing. Brake now employs a new technique involving a tongue cannula and pressure transducer to record sucking (Brake, Tavana and Myers, 1986). The output of this technique is not influenced by feeding movements. With this technique rhythmic sucking appears to be underlying most, if not all, treading during the milk ejection response. However, this hypothesis of sameness of sucking types has not yet been proven. Further experiments directed specifically to that point are needed. Also, even if sucking patterns are the same, the presence or absence of treading motions may be an important difference in the pup's response to milk ejection. Kneading the ventrum may cause more milk to be released or allow the pup to suck better by positioning its body more advantageously. Thus the distinction between rhythmic sucking and

Table P

Comparison of mean (and SEM) sucking rates in the two minutes immediately post milk ejection to the overall sucking rates during attachment on Reunion day.

	(-----Mean Sucking Rates ^a -----)			(---Dep T-Tests, df=5---)	
	1st min post ME	2nd min post ME	Overall	1st min vs. overall	2nd min vs. overall
RS	1.69±.53	1.37±.62	2.46±.54	1.673	3.254 *
B	.88±.40	.86±.24	1.29±.21	1.168	1.315
T	7.36±.79	4.12±1.26	3.81±.35	4.456 **	.235

SEM = standard error of the mean ME = milk ejection
 B = bursts RS = rhythmic sucking T = treadsles
 * = p<.05 ** = p<.01

^a For each of the three sucking types, the rate of sucking was calculated as the number of five second bins per minute in which a suck of that type occurred.

rhythmic sucking with treads may still be an important one to be made.

The rate of treading while awake and while in slow wave sleep also increased significantly in this minute after milk ejection (Table Q). The significant decrease in rates of bursts and rhythmic sucking while awake were probably due to the amount of time taken up by treading. These data again implicate treading as the nutritive sucking pattern and demonstrate that the sucking rate per unit time in a state can change in response to environmental stimuli.

Trends Over Time

Unlike Baseline day, the recording session did not occur against the background of normal interaction between the pups and their dams. On Reunion day, the pups were abruptly presented with their mothers from whom they had been separated for 22 hours. With this in mind, it was especially important to look for changes over the course of the recording session as well as during the nursing bouts. For example, one might expect to see pups awake more at the start of the nursing bouts or recording session and asleep more after having received several milk ejections or spent time sucking. As shown in Table R, there was such a decrease in the percent time awake while attached in the second half of the recording session. Note however, that neither the percent time attached nor the number of milk ejections differed between the two halves. Thus, pups were still actively seeking the mother, attaching and sucking during the second half. The pups also showed a decrease in the rate of treads from the high rates during the first half of the session (Table R). When considered in

Table Q

Comparison of the mean sucking rates in each sleep-wake state^a in the first minute post milk ejection to the overall sucking rates in that sleep-wake state during the Reunion test.

	(-----A-----)			(-----SWS-----)		
	RS	B	T	RS	B	T
Min post ME	1.58	.30	8.92	1.87	1.44	3.89
Overall	3.43	.72	5.95	1.46	1.42	1.87
dep t = df = b	2.794 *	3.181 *	3.001 *	.349	.051	2.727 ^c +

B = bursts RS = rhythmic sucking T = treadles
ME = milk ejection + = p<.10, ns * = p<.05

^a Too few examples of paradoxical sleep in the minute post ME for any meaningful analysis of sucking levels.

^b df=5 while awake; df=4 in slow wave sleep since one pup had no SWS in the minute post ME.

^c p = .053

Table R

Trends over time during the recording session:
 Comparison of attached pups from the first to second half of the Reunion session.^a

	%TRT Attached	# of ME's	Sleep-Wake State %'s			Sucking Rates ^b		
			A	PS	SWS	RS	B	T
1st Half								
Mean	61	3.5	53	.5	46	2.7	1.4	4.4
2nd Half								
Mean	54	3.0	40	7.2	53	1.8	1.3	3.1
dep t = df=5	.457	.355	3.448 *	1.834	1.333	1.223	.084	4.460 **

^a Data taken only from those 20 nursing bouts (i.e., attached episodes) \geq 5 min.

^b As a reminder, sucking rates were figured as the number of 5 sec bins/min in which sucks of that particular type occurred. The rates in this table were calculated independently of sleep-wake state.

A = awake PS = paradoxical sleep SWS = slow wave sleep B = bursts
 RS = rhythmic sucking T = treadles ME = milk ejection TRT = total
 recording time * = p<.05 ** = p<.01

conjunction with the decreased awake time in the second half shown above, it suggests that the treadle rate decrease was due to the lower level of awake time, not necessarily a change in the treadle rate per unit time in each state.

This idea was tested by comparing the treading rate while awake in first vs. second half of the total recording time and finding no significant change in rate (dep $t = 1.928$, $df=5$, ns). In fact, there were no significant changes in rate for any sucking type within any state from the first to second half of the recording session.

If attachment to teat, feedback from sucking or milk ingestion during Reunion acted quickly to reverse the effects of deprivation on the pups, then one might predict a difference in sleep-wake state percentages from the first to second half of each nursing bout. On the contrary, the sleep-wake state percentage change approached statistical significance only for paradoxical sleep (Table S). Even in this case, an increase from one to five percent may not have much biological significance; most nursing bouts had no paradoxical sleep in either half.

The amount of rhythmic sucking decreased dramatically over the time of each nursing bout (Table S). This datum supports the impression gained during the recording sessions that rhythmic sucking is used to attach to the teat, as well as for ingestion. If the number of milk ejections had been concentrated in either half of the nursing bout, sleep-wake percentages and sucking behavior might have been influenced. In fact, the mean number of milk ejections were the same in each half (first half: 1.3 ± 2.9 ; second half: $1.3 \pm .32$). Fig. 13 provides a visual impression of these data. Thus the increased

Table S

Trends over time within the nursing bout:^a
 Comparison of state percentages and sucking behavior from
 the first to second half of the bout on Reunion day.

	(-Sleep-Wake State %'s-)			(----Time Sucking ^b ----)		
	A	PS	SWS	RS	B	T
1st Half	53	1	46	34.0	10.9	39.7
2nd Half	46	5	49	15.0	16.4	38.6
dep t = df=19	.927	2.061 ^c +	.451	3.898 ***	1.970 +	.348

^a Data taken only from those 20 nursing bouts \geq 5 min.

^b For each of the three sucking types, the time spent sucking was calculated as the number of 5 sec bins in which a suck occurred divided by the attached time of that half.

^c p = .051

A = awake PS = paradoxical sleep SWS = slow wave sleep
 B = bursts RS = rhythmic sucking T = treadles
 + = p<.10, ns *** = p<.001

rhythmic sucking in the first half of nursing bouts noted above was not due to the number of milk ejections. Finally, the sucking rates in the minute just before and the minute following milk ejection were examined for the first and second halves of the nursing bouts (Table T). As can be seen, there were no significant changes in rates of sucking behaviors in the periods around milk ejections from the first to the second halves of the nursing bouts. It may be that these data are too complex to analyze in this way, but at least we can reject the simplest hypothesis that the pups would suck more vigorously early in the nursing bout due to deprivation effects.

Intermediate State

What has been called "intermediate state" was described in the Baseline section. During Reunion, intermediate state accounted for an average of $10 \pm 1\%$ of the recording session. This amount was a significant increase over Baseline day ($t = 2.538$, $df=5$, $p < .05$). Of intermediate state epochs, 74% were scored as slow wave sleep, 26% were scored as awake. If all such epochs were scored as awake, the overall sleep-wake percentages would become: awake = $64 \pm 3.7\%$; paradoxical sleep = $9 \pm 1.9\%$; slow wave sleep = $27 \pm 2.7\%$. If the data in the sections above were analyzed with these changes in scoring, the major Baseline to Reunion day shifts would still have the same form; i.e., an increase in time awake, less time in paradoxical sleep and no significant change in slow wave sleep.

Table T

Trends over time within the nursing bout:
 Comparison of the rates of sucking in the minutes around
 milk ejection in the first vs. second halves of the nursing
 bout.

	1st min. prior to ME			1st min. post ME		
	RS	B	T	RS	B	T
1st Half	.6	1.2	1.6	1.4	.5	7.1
2nd Half	.8	1.2	2.4	2.8	1.1	7.6
dep t = df=4 ^a	.386 ^b	.000	.625	1.737	.964	.563

B = bursts RS = rhythmic sucking T = treadles ME = milk
 ejection

^a df=4 because pup N85 had no ME's in the first half of any
 nursing bout, except those after which it detached for
 more than 15 seconds.

^b All t's are non-significant.

Discussion

What has been learned from the Reunion procedure? First, pups are not simply hyperaroused by the deprivation experience. The pups still fall asleep quickly after attachment to the nipple and before receiving any milk. They spend over half the time asleep while being nursed. There are changes in state behavior, however. The pups do spend more time awake and for longer durations. The amount of paradoxical sleep is reduced confirming earlier work (Hofer, 1976; Hofer and Shair, 1982). Another major difference between Baseline and Reunion days is that after deprivation sleep-wake patterns are dramatically changed by attachment condition. When not attached, the pups are awake almost continually, often actively chasing their mother around the cage. When attached, slow wave sleep time equals time spent awake and there is very little paradoxical sleep.³

The amount of time spent sucking is increased by deprivation, confirming in this naturalistic procedure the work using an anesthetized dam (Brake, Wolfson and Hofer, 1979). The most intriguing finding about sucking behavior is that the characteristic rates of sucking within each state are not significantly increased by deprivation. One strategy by which pups could increase total time spent sucking would be to raise sucking rates within one or all of the three states. This strategy was not employed. Instead, pups shift sleep-wake states to those states with higher sucking rates (awake and slow wave sleep) and decrease that state with the lowest rates (paradoxical sleep). Sucking rates per unit time in each state are not totally fixed, however. There are transient increases in the minute

just after milk letdown. Also, the rate of bursts while awake and in paradoxical sleep is decreased by deprivation and reunion.

These results demonstrate that deprivation and reunion influence both sleep-wake states and sucking behavior in an interactive way. The data does not imply that one change is primary and the other secondary. One might imagine that changes in sucking behavior cause the state variations. For example, the increased levels of treads and rhythmic sucking just following milk ejections may cause the pups to stay awake longer. However, an alternative explanation is that milk ejections are more stimulating to deprived pups, causing them to remain awake longer, with the consequent higher levels of sucking. Finally, milk itself may stimulate both the sleep-wake and sucking changes independently of one another. The small percentage of paradoxical sleep seen while attached post-deprivation is a similar case. Those few episodes which did occur often ended with some sucking activity and awakening. Perhaps it was the sucking which woke the pups. If this idea is true, however, it is hard to explain why pups recorded in isolation also have decreased amounts of paradoxical sleep post-deprivation (Hofer, 1976; Hofer and Shair, 1982).

What effects does the increase in sucking time have on milk intake? First, in this atypical situation with only three deprived pups in each litter, the pups do not elicit more milk ejections or shorter latencies between the ejections. However, the pups do stay awake longer during the milk ejection responses. They may use this period to ingest more food. In fact, the average weight gain was higher on Reunion day, although not statistically significantly (due to increased variability of intake on this second day).

During Reunion after deprivation one expects to find recovery from the deprivation effects. This recovery can occur gradually or rapidly. It may or may not be complete. In the two-hour Reunion period, pups did not spend less time attached or receive fewer milk ejections as time passed. Thus, the pups were still showing the effects of the deprivation at the end of the test session. There was however, less time awake while attached in the second half of the recording session. This finding may show the beginning of a recovery process. Something about Reunion must have been influencing the state change. Milk intake and sucking stimulation are two obvious possibilities.

¹ The degrees of freedom equal four since one pup (B84) had only one milk ejection on Baseline day, thus no time between milk ejections.

² As noted above, only bouts greater or equal to five minutes in length were considered, except that on Reunion day one bout of 4.5 minutes had a milk ejection. These data differ from the results in Table K since bouts without any letdowns were included. In any bout meeting criterion length without a milk ejection, the latency was considered to be total bout length.

³ The changes in sleep-wake state percentages cannot be explained by maturational factors. If rats of these ages remain with their dams between the two test sessions, no differences in sleep-wake state behavior are observed (Hofer, 1976). Nor is it likely that deprivation causes alterations in the polygraph records such that the scoring criteria need to be reexamined. In the study cited above, the individual polygraph channels were scored separately and then recombined to get state percentages. There was no increase in the percent of time spent in some kind of transitional sleep after deprivation. However, it is not possible to eliminate totally the idea that the polygraph tracings may be altered so much by deprivation that one major state is defined as another (e.g., the EMG amplitude during paradoxical sleep increases so much that many epochs are scored as awake). Such speculation is unanswerable. States are defined by the assemblage of their component parts; there are no independent markers for states.

ANESTHETIZED MOTHER BASELINE

In this series of studies, the goal was to examine the possible interaction between sleep-wake states and sucking behaviors which had been suggested by the Normal Mother Experiment. In order to get a clear picture of the pup's behavior, a simplified recording situation was required. One possibility immediately suggested itself, that of recording each pup while attached to its anesthetized dam with no littermates. This preparation would minimize the external stimuli which influenced the pup's behavior. There would be no milk ejections, no body movements of the mother or littermates, and no coming and going by the dam. Also, with this preparation, each pup would be exposed to the same conditions during the test session. The similarity of experimental stimuli should facilitate comparisons among the individual pups.

Clearly this test situation was an artificial one, with some drawbacks. The lack of external stimulation might in essence be a stimulation in itself. A pup may have learned to associate its mother with periodic milk ejections, with changes in position, with being licked and manipulated. However, the similarities between the anesthetized dam and normal dam are also quite striking. During a nursing bout, the normal dam sleeps throughout much of the time between milk ejections. That time period is irregular and can be as much as 20-25 minutes apart (Lincoln, Hill and Wakerley, 1973). Thus, there were grounds to believe that the pup's behavior exhibited with an anesthetized dam should be comparable with normal behavior.

There are three goals in this section. The first is to look at the levels of sleep-wake and sucking behaviors in the Anesthetized

vs. Normal Mother preparations. This examination will not only help verify the generality of the Anesthetized Mother findings, but also aid in understanding the effect on the pups of the mother's behavior including milk ejections. Secondly, any interactions of sleep-wake state and sucking behavior will be analyzed. Are state episode length and sucking rates related? Do states and sucking behaviors change together over the time of the recording session? Do other variables like body weight and temperature influence the sleep-wake and sucking interactions? Finally, these results will provide the Baseline day for a deprivation and Reunion experiment which was carried out to investigate further the stability of sucking rate per unit time within each state. The data will be presented in a similar order to that of the previous experiments.

Behavioral Observations

When tested with anesthetized mothers, the pups' behaviors were much simplified. Each of the six pups attached rapidly to its dam's teat and remained attached virtually the entire recording session: mean attachment percent = $98 \pm .3\%$ of the total recording time. While attached, the pups showed some similar patterns of behavior to those seen in the normal nursing situation. Pups on the anesthetized dam alternated brief periods of activity -- changing postures, treadles -- with longer periods of quiet attachment. Four of the six pups rarely switched nipples, but one pup nipple-switched seven times: mean = 2.2 ± 1.0 nipple switches. Of course, in this test situation, nipple switching could not be a response to milk ejections. Correlations between the number of nipple switches and other pup parameters will be

reported throughout the section in order to help understand the reasons for this difference in behavior among the pups.

Weight and Temperature Data

As can be seen in Table U, the pups were a healthy weight and temperature at the start of the recording sessions, well within the normal range for this age (Hofer and Grable, 1971; Messer, Thoman, Terrasa and Dallman, 1969). All pups lost weight during the recording session, as we expected since no milk was available. There was no significant difference in body weight or rectal temperature between Anesthetized and Normal Mother pups (ind. $t = -1.132$ and 1.332 respectively, $df = 10$).

Neither pretest body weight, % change in weight, nor rectal temperature was significantly correlated with the number of nipple switches. Using the first two variables as extremely tentative markers for hunger and high metabolic rate, the results ruled against a simple link between any of the three parameters and nipple switching behavior.

Electrophysiological Data

Sleep-Wake States

During the two hour recording session, the test pups were asleep over 84% of the time (see Table V). Pups always went into slow wave sleep first during the recording session, thus the sleep onset data given in the table is also slow wave sleep onset.

As can be seen in Fig. 20, the pups had no long episodes of awake when tested in isolation with their anesthetized dam. Paradoxical

Table U

Body weight and rectal temperature.

	(-----Body Weight (gr.)-----)			(---Rectal Temp.(°C)---)	
	pretest	posttest	% change ^a	pretest	posttest ^b
Mean	29.2	29.0	-.9	35.6	35.7
SEM	1.0	1.0	.1	.16	.19
Range	26.4 to 33.0	26.2 to 32.9	-.3 to -1.1	35.0 to 36.0	35.2 to 36.4

^aThe mean weight gain was statistically significant (dep t = 7.319, df=5, p<.001).

^bThe mean temperature change was not statistically significant (dep t = .664, df=5).

Table V

Measures of sleep and wakefulness of infant rats recorded while attached to their anesthetized dams for two hours on Baseline day.

	Sleep Latency (min)	% TRT in State			(-----State Episode Analysis-----)						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Mean	1.7	12	57	30	.56	2.05	1.95	14	18	10	41
SEM	.65	1.9	1.0	1.5	.04	.18	.16	2.2	1.3	.7	3.2

A = awake PS = paradoxical sleep SWS = slow wave sleep
 TRT = total recording time SEM = standard error of the mean

sleep episode durations were distributed fairly evenly in length, while slow wave sleep episodes tended to have either short or long duration, something which is not apparent from the mean durations given in Table V.

Transitional probabilities are given in Fig. 21. It is worth noting that the pups were more likely to go from awake to paradoxical than from awake to slow wave despite the fact that sleep onset after initial attachment was always into slow wave sleep. Part of this was no doubt due to the much larger percent of paradoxical than slow wave during the recording session. Still, as in the Normal Mother Experiment, one sees that at this age the awake to slow wave to paradoxical and back to awake sequence is not fixed. Note, however, that transitions from awake to slow wave to paradoxical were much more common than the reverse.

Correlations were run to measure the influence of various pup parameters on the percent of time in each state and on the number of state transitions per hour, a measure of sleep fragmentation. Neither body weight, rectal temperature nor number of nipple switches showed any significant relationship with the above measures.

In comparisons of state variables between the Anesthetized and Normal Mother Experiments, only the time pups were being nursed during the Normal Mother recordings were considered. This procedure was an attempt to minimize the differences in recording situations between the two experiments. As shown in Table W, pups studied with their mothers anesthetized were awake less and had fewer state transitions per hour. These data provide the first evidence that the pups may be less activated on the anesthetized dam, probably due to lack of stimulation

Figure 20. Histogram analysis of state episode durations while attached to the teat of an anesthetized dam, illustrating the relative frequencies of occurrence of periods of different durations.

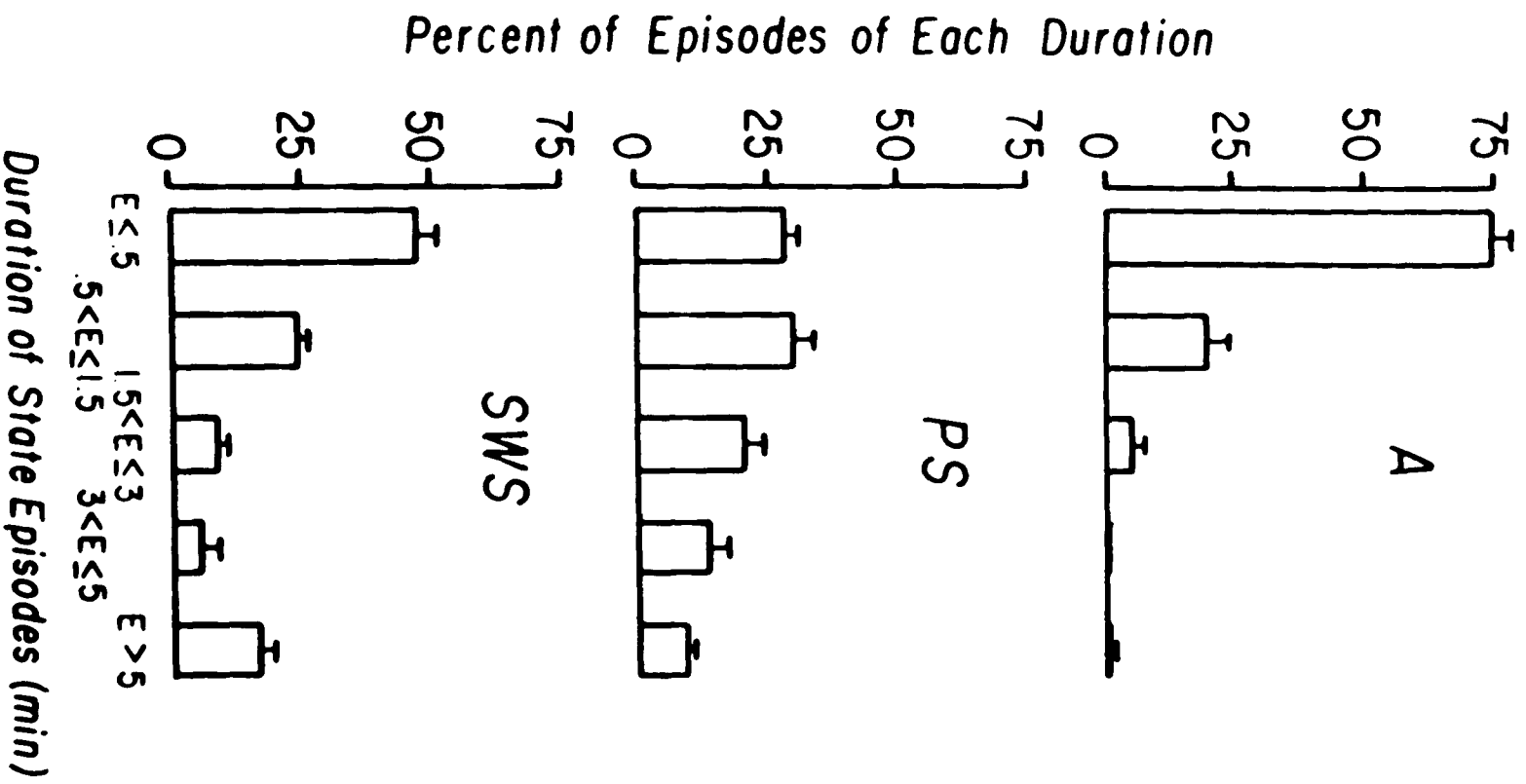
A = awake; PS = paradoxical sleep; SWS = slow wave sleep;
E = episode.

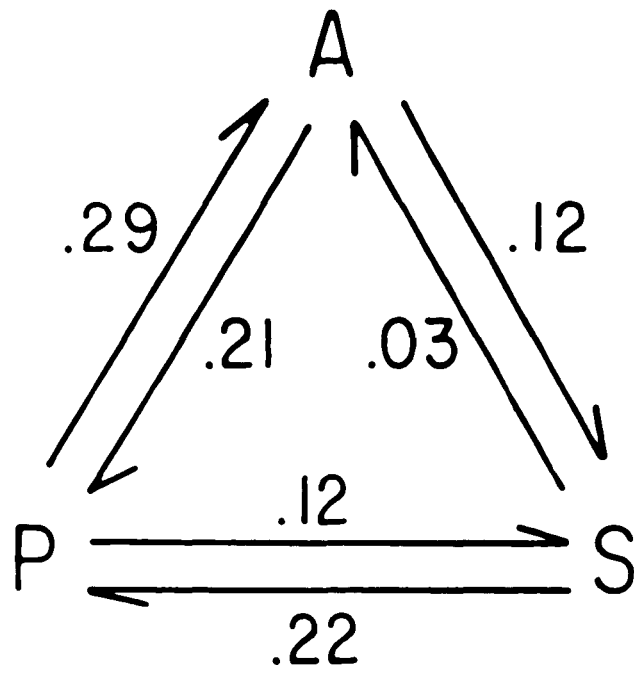
Compare to Fig. 8 Attached for Normal Mother results.

Figure 21. The probabilities of transition among the three sleep-wake states while attached to the teat of an anesthetized dam.

A = awake; P = paradoxical sleep; S = slow wave sleep.

Compare to Fig. 9 Attached for Normal Mother results.





Baseline

Table W

Comparison of sleep-wake data means of pups attached to an anesthetized dam and pups attached to a normal dam.

	sleep latency (min.)	percent time in state			state trans. per hour
		A	PS	SWS	
AM pups	1.7	12	57	30	41
NM pups (while attached)	.8	29	33	38	61
ind t = df=10	1.362	4.725 ***	4.079 **	1.511	5.553 ***

A = awake PS = paradoxical sleep SWS = slow wave sleep
 AM = anesthetized mother NM = normal mother
 ** = p<.01 *** = p<.001

in the nest. Note, however, that the percent of time in slow wave sleep was not different in the two conditions. The lack of arousal with the mother anesthetized can also be seen by comparing Fig. 20 with Fig. 8 (Normal Mother Baseline). Looking at the percent of state episodes of each duration, with the dam anesthetized, there were increases in the percent of long episodes of sleep which reached statistical significance for the longest duration episodes of slow wave sleep (ind. $t = 3.693$, $p < .01$; see Appendix for ANOVA).

The probabilities of state transition showed a great difference between the two test settings. Only the probability of transition from awake to paradoxical was not significantly changed (compare Fig. 21 and Fig. 9 from Normal Mother Baseline). With the dam anesthetized, the transitions from slow wave to awake and vice versa were decreased (both $p < .01$). Thus, the most likely transition from awake was to paradoxical in this experiment but was to slow wave in the previous one. All other transitional probabilities were increased on the anesthetized dam at the $p < .05$ level, except for paradoxical to slow wave which only showed a trend toward increase ($p < .10$; see Appendix for ANOVA).

Sucking Behavior

Sucking behaviors were seen in all three sleep-wake states. As shown in Table X, approximately 66% of all sucking took place during sleep. As in the Normal Mother Experiment, bursts were predominately seen during sleep. The total time spent in treadles and rhythmic sucking were highest when the pups were awake, even though awake time was so low (Fig. 22). However, the rate of sucking per unit time in each state gives a truer picture of the pups' behavior since the time

Table X

Mean and SEM sucking times^a in each sleep-wake state of infant rats attached to their anesthetized dam on Baseline day.

State	RS	B	T
A	7 + 3	26 + 8	74 + 12
PS	0 + 0	65 + 10	55 + 10
SWS	1 + 1	55 + 6	33 + 9

SEM = standard error of the mean A = awake
 PS = paradoxical sleep SWS = slow wave sleep B = bursts
 RS = rhythmic sucking T = treadles

^a For each of the three sucking types, the time spent sucking was calculated as the number of five second bins in which a suck of that type occurred.

Figure 22. The rate of sucking behaviors and the percentage of time in each sleep-wake state while attached to the teat of an anesthetized dam.

The horizontal bars represent the mean percentage of recording time while attached to a teat spent in each sleep-wake state. The height of the three left-hand vertical bars gives the mean rate of sucking per minute for each kind of sucking. The bar to the right measures the amplitude of the digastric EMG recording when no sucking is occurring (an index of the muscle tone involved in maintaining a seal on the nipple).

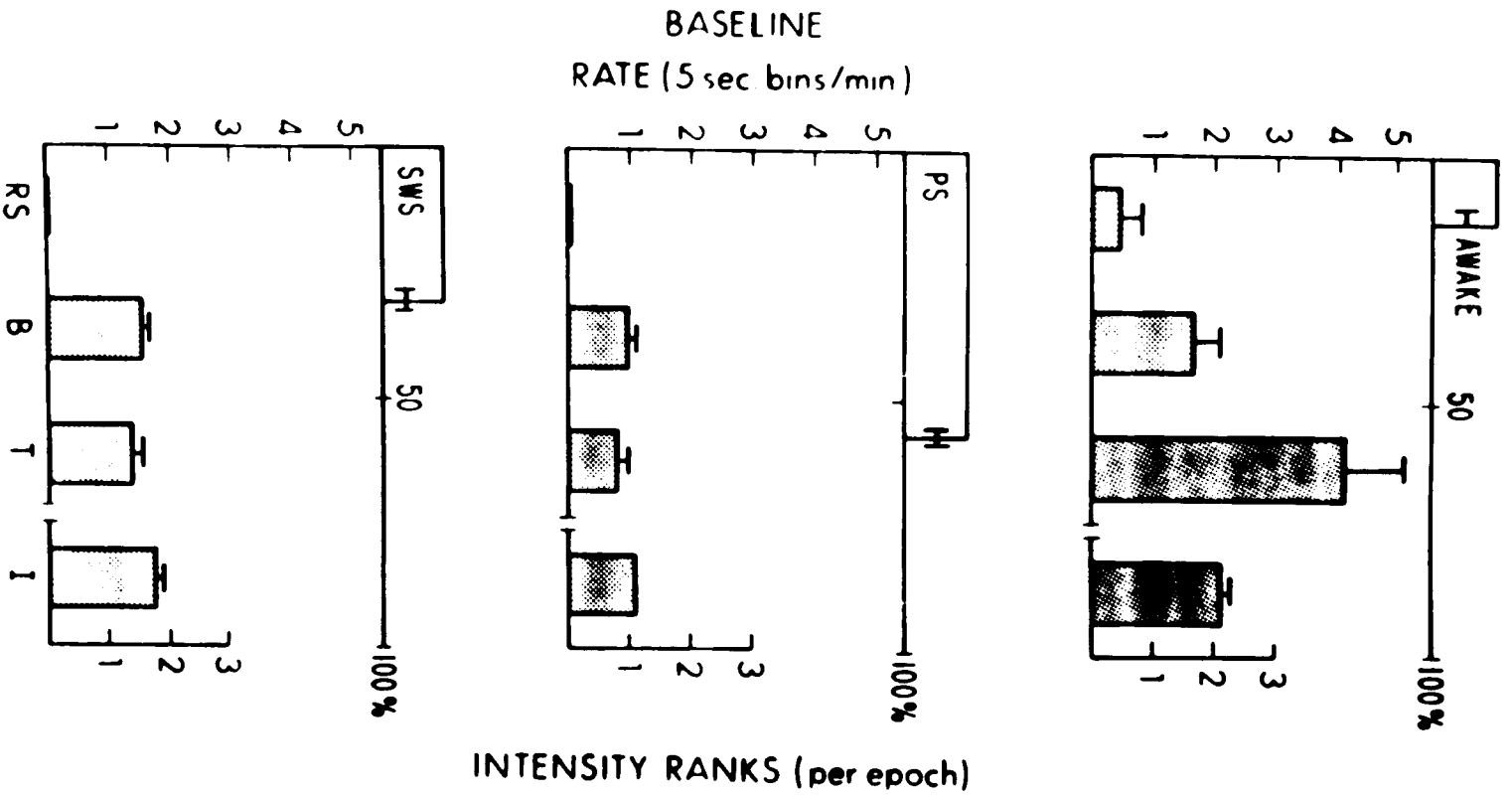
EGM = electromyogram; PS = paradoxical sleep; SWS = slow wave sleep; RS = rhythmic sucking; B = bursts; T = treads; I = EMG intensity rank.

Only the rate of treading and the EMG intensity rank differed significantly when compared between sleep-wake states.

T: A>PS, dep $t=3.755$, $p<.05$
 A>SWS, dep $t=3.624$, $p<.05$
 I: A>PS, dep $t=7.279$, $p<.01$

All significance findings are based on dep t-tests, $d=5$ for each, whose use are validated by the experimental hypotheses based on the results of the Normal Mother Experiment. Overall ANOVAs on both Baseline and Reunion data are given in the Appendix.

Comparisons to the sucking rates of the Normal Mother Experiment are given in Table Y.



spent in each sleep-wake state varied. Fig. 22 demonstrates that the patterns seen with the anesthetized dam were similar to those seen in the previous experiment: sucking rates were generally highest while awake, lowest in paradoxical sleep, and intermediate in slow wave. When tested on the anesthetized mother, however, the burst rate in the awake state was as high as the rate in slow wave sleep (see figure legend and Appendix for statistics).

Neither the number of nipple switches, pretest body weight nor change in body weight during the test session correlated significantly with any measure of time spent sucking. Pre-test rectal temperature, however, was positively correlated with one type of sucking: the total time spent treadling ($r = .83$, $df = 4$, $p < .05$). The direction of temperature change during the recording period did not influence sucking totals, since no correlations of sucking and change in body temperature reached significance.

As we noted above, temperature did not correlate with sleep-wake state percentages. Thus, its relationship with treadling could not be explained by a shift in states to those states with high rates of treadling. For this reason, the relationship of rectal temperature to the rate of sucking by states was analyzed. There were no significant correlations between body temperature and treadle rate within any state. However, in both slow wave and paradoxical sleep, the correlations were positive and quite high, approaching significance ($r = .70$ and $.74$ respectively).

Two correlations between sucking rates by state and body temperature actually reached significance. While the pups were awake, both the rate of rhythmic sucking and of bursts had a negative

relationship with temperature ($r = -.87$ and $-.88$ respectively, $df = 4$ and $p < .05$ for both). Thus, in some cases, the rate of sucking within a state can be modified, possibly while asleep and certainly while awake.

As noted above, the pattern of the rates of sucking within each state looked similar in both the Anesthetized and Normal Mother Experiments. Were there any differences in the actual rate levels themselves? As shown in Table Y, for bursts there was no significant change in rate by state in either awake, paradoxical or slow wave sleep. For treads, only the slow wave sleep data approached significance, with a possible decrease in rate with mother anesthetized. For rhythmic sucking, there was a very different state of affairs. There was a significant increase in the rate of rhythmic sucking in all three states, when the dam was awake and giving milk ejections. These results bolster the idea that rhythmic sucking and possibly treads may be the sucking patterns of milk ingestion and nipple attachment.

Correlations were performed to determine if there was a relationship between the length of state episodes and the rate of sucking within the episodes. No significant correlations were found for any state. The relationship between the average length of awake episodes and the rates of both rhythmic sucking and bursts while awake almost reached significance ($r = -.763$ and $-.755$ respectively, $df = 4$ and $p < .10$ for both). Thus, the rates of sucking within each state were not affected by episode duration in general, but this result must be examined again after deprivation.

Table Y

Comparison of mean sucking rates within each sleep-wake state from the Baseline sessions of the Normal and Anesthetized Mother Experiments.

	(-----A-----)			(-----PS-----)			(-----SWS-----)		
	RS/M	B/M	T/M	RS/M	B/M	T/M	RS/M	B/M	T/M
AM pups	.5	1.7	4.2	0	1.0	.8	0	1.6	.9
NM pups	3.0	1.3	5.1	.1	.9	.9	1.1	1.8	1.7
ind t = df=10	3.532 **	1.008	.768	2.591 *	.375	.411	4.331 **	.716	2.127 +

AM = Anesthetized Mother NM = Normal Mother A = awake PS = paradoxical sleep
 SWS = slow wave sleep RS = rhythmic sucking B = bursts T = treadles
 M = minute + = p<.10, ns * = p<.05 ** = p<.01

Trends Over Time

Examining the experimental results for any changes over time serves two purposes. One, variations in state and sucking patterns may interact in ways useful in helping spot any relations between them. Secondly, if the pups were reacting to the artificial recording situation, their behavior might show changes over time.

The data was analyzed for the first and second hours separately, as shown in Table Z. As can be seen, neither sleep-wake percents nor sucking time changed significantly. However, the rise in paradoxical sleep almost reached significance. Since paradoxical sleep was the state with the lowest rates of sucking, one might suspect that the rates within one or more states may have increased in order for sucking time to remain constant.

This suspicion proved false (see Table AA). There were no significant rises in sucking rates. In fact, there was a decrease in the rate of treads during slow wave sleep. As has been stated before, the sucking rates by state appeared to be relatively stable, but not totally fixed.

Discussion

The anesthetized mother preparation served the purpose for which it was designed. It was a much less "active" situation than in the normal litter.¹ The test pups remained attached to the teats for almost the entire two hour recording session. They spent less time awake, had fewer state transitions, and the duration of episodes in paradoxical and slow wave sleep were longer than in the normal litter situation. Yet, much of the sleep-wake behavior was similar to the previous

Table Z

Trends over time during the recording session:
 Comparison of mean state percentages and sucking behaviors
 from the first to second half of the Baseline test.

	(-Sleep-Wake State %'s-)			(-Time Sucking ^a -)		
	A	PS	SWS	RS	B	T
1st Half	13	54	32	4	84	90
2nd Half	12	60	28	4	64	79
dep. t = df=5	.403	2.008 +	.877	.193	1.749	1.556

^a For each of the three sucking types, the time spent sucking was calculated as the number of 5 sec bins in which a suck of that type occurred.

A = awake PS = paradoxical sleep SWS = slow wave sleep
 B = bursts RS = rhythmic sucking T = treadles
 + = p<.10, ns

Table AA

Trends over time during the recording session: Comparison of the rates of sucking within each sleep-wake state from the first to second half of the Baseline session.

	(-----A-----)				(-----PS-----)				(-----SWS-----)			
	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep
1st Half	.7	1.5	5.7	2.1	0	1.0	.8	1.3	0	2.0	1.2	1.7
2nd Half	.5	1.8	5.4	2.3	0	.9	.8	1.2	0	1.1	.7	1.8
dep t = df=5	.537	.713	.314	.734	.000	1.450	.415	1.369	1.581	1.977	2.948	.192
Pearson r =	.68	.69	.70	.45	--- ^a	.95 **	.87 *	.28	--- ^a	-.22	.76 +	.31

A = awake PS = paradoxical sleep SWS = slow wave sleep B = bursts RS = rhythmic sucking
 T = treadles I = EMG intensity rank ep = epoch m = minute + = p<.10,ns * = p<.05
 ** = p<.01

^a No comparison possible due to lack of RS.

experiment. Sleep onset latency did not differ nor did the percent of time in slow wave sleep. Also, there were few changes in state behavior over time. Thus, factors like hunger or the artificiality of the test situation were unlikely to have been a major factor.

The pattern of sucking in the three sleep-wake states was quite similar between the two experiments. Pups sucked at the highest rate while awake, intermediate in slow wave sleep, and lowest in paradoxical sleep. In this experiment, however, the rate of bursts while awake was as high as the burst rate during slow wave sleep. Almost no rhythmic sucking was seen in any state with the mother anesthetized. This difference between the two experiments begins to confirm the link between rhythmic sucking and milk ingestion/nipple attachment.

The relationship between sucking and sleep-wake behaviors appears to be relatively stable. The rate of sucking within each state is unaffected by state episode duration. There is not much change in either state or sucking behaviors over the time of the recording session. However, body temperature was correlated with certain sucking rates while the pups were awake and possible while in slow wave sleep as well. There were no such correlations between temperature and state measures. Thus, at least one pup parameter influenced sucking without affecting state.

Finally, this experiment provided the Baseline data for the next deprivation and Reunion Study. The issues raised here and in the Normal Mother sections will be examined further in the next chapter. Special interest will be paid to the stability of the sucking rates per unit time within each state, since so few manipulations have changed those rates.

¹ The presence or absence of littermates during the recording session is unlikely to have been a major factor in the results described. Hofer (1976) found that the sleep-wake patterns of pups of this age recorded in isolation were very similar to those recorded in the presence of littermates. This similarity was true both before and after maternal deprivation. On Baseline day, the pups recorded with littermates did spend more time in paradoxical sleep and less time awake than infants recorded singly. However, the presence of littermates brings the sleep-wake state percentages into line with the findings of the Anesthetized Mother experiment. It is not likely that the presence of littermates in this situation would have caused the Normal Mother and Anesthetized Mother Baseline experiments to differ further.

ANESTHETIZED MOTHER REUNION

Twenty-two hours after the Baseline recording period, the pups were returned to their anesthetized dams for a two-hour Reunion session. Based on the results of Stephen Brake (Brake, Wolfson and Hofer, 1979; Brake, Sager, Sullivan and Hofer, 1982) and the Normal Mother Experiment described above, we predicted that deprivation would lead to increase time spent sucking during Reunion. Similarly, we predicted that sleep-wake state behavior would change as well. We hoped that the changes in the two systems under study would permit close examination of any interactions between them. Specifically, what would happen to the rates of sucking within each state? Would the stability of rate by state be maintained in this experiment without the influence of the mother's behavior and milk ejections? Also, would amount of weight loss or, especially, change in body temperature affect states or sucking patterns? Would pups show individual responses to deprivation that were hidden in the previous experiment by the variability introduced by differences in maternal behavior? Perhaps those individual responses might highlight any relationships between sleep-wake states and sucking behavior.

The data will be presented in the same format as the previous sections: first, behavioral observations of the pup; then electrophysiological data including sleep-wake states and sucking behavior; and finally any trends in behavior over the time of the test session. Some figures will repeat data from the Anesthetized Mother Baseline section in order to facilitate comparisons.

Behavioral Observations

As on the Baseline day, the test pups quickly grasped their mothers' teats. The pups stayed attached for $97 \pm .9\%$ of the test period. During the session, the pups appeared more active than on the previous day. For example, four of the six pups had many more nipple switches upon Reunion, although the increased number did not quite reach statistical significance. The mean number of nipple switches equalled 6.3 ± 1.1 (dep. $t = 2.076$, $df = 5$, $p < .1$, ns). Also, the electrophysiological data which will be described below provided more evidence of increased activity on the second day. Despite the increased level of activity, all of test pups were quietly attached for some period of time. For five of the six pups, there were long episodes of quiet attachment. One pup (E79) was aberrant in behavior. Although attaching to a nipple quickly and remaining attached for 98% of the time, this pup was active for much of the session, especially during the first hour.

Weight and Temperature Data

At the start of the Reunion session, the pups were both lighter and slightly cooler than on Baseline day (see Table BB). The weight loss was, of course, expected. The temperature loss occurred despite the warming of the pups by placing their cages on heating pads as described in the Methods section. Note, however, that these lower temperatures are within the normal range for pups in this series. For example, one of the pups in the Normal Mother Experiment had a temperature of 33.9° after deprivation. Intriguingly pup E79 had both the lowest weight (24.4 gr) and temperature (33.4°) at the start of

Table BB

Body weight and rectal temperature.

	(-----Body Weight (gr.)-----)			(---Rectal Temp.(°C)---)	
	pretest	posttest	% change ^a	pretest	posttest ^b
Mean	26.9	26.5	-1.5	34.7	34.6
SEM	1.0	1.0	.3	.30	.29
Range	24.4 to 31.1	23.7 to 30.7	-.8 to -3.0	33.4 to 35.6	33.7 to 35.5
Comparison of means: Baseline and Reunion					
dep t =	9.268 ^c	----	1.476	2.821 ^c	----
df=5	***			*	

^a The mean weight loss was statistically significant (dep t = 4.824, df=5, p<.01).

^b The mean temperature change was not statistically significant (dep t = .642, df=5).

^c Thus, the pups started the Reunion session both lighter and cooler than on Baseline day.

* = p<.05 *** = p<.001

Reunion. Its percent change in body weight was mid-range however (-8.7%). Thus, after deprivation, the range of weight and temperatures was wide, yet within the normal range, allowing examination for any effects on the other behavioral and physiological parameters. Body weight was correlated with rectal temperature ($r = .76$, $n = 6$) which does not quite reach significance ($p < .10$). However, the scatter plot of body weight and rectal temperature data (see Fig. 23) suggest that in midrange of temperature and body weight, the correlation was not significant, but at the extremes the correlation might be valid.

Neither rectal temperature ($r = .54$) nor percent change in body weight ($r = .33$) was significantly correlated to the number of nipple switches that pups showed. However, the correlation of body weight itself to number of nipple switches ($r = .78$, $p < .10$) was high and approached significance. In the scatter plot of this data (Fig. 24) there is nothing to suggest that any extreme points were having too great an influence. If nipple switching is a technique used to elicit more milk from the mother, one might expect the pups with the lowest body weights to show more nipple switches. Of course, a caveat applies. The pups may have those low body weight because they are less interested in food or are less mature. Thus, no firm conclusion on the purpose of nipple switching can be drawn.

Electrophysiological Data

Sleep-Wake Stages

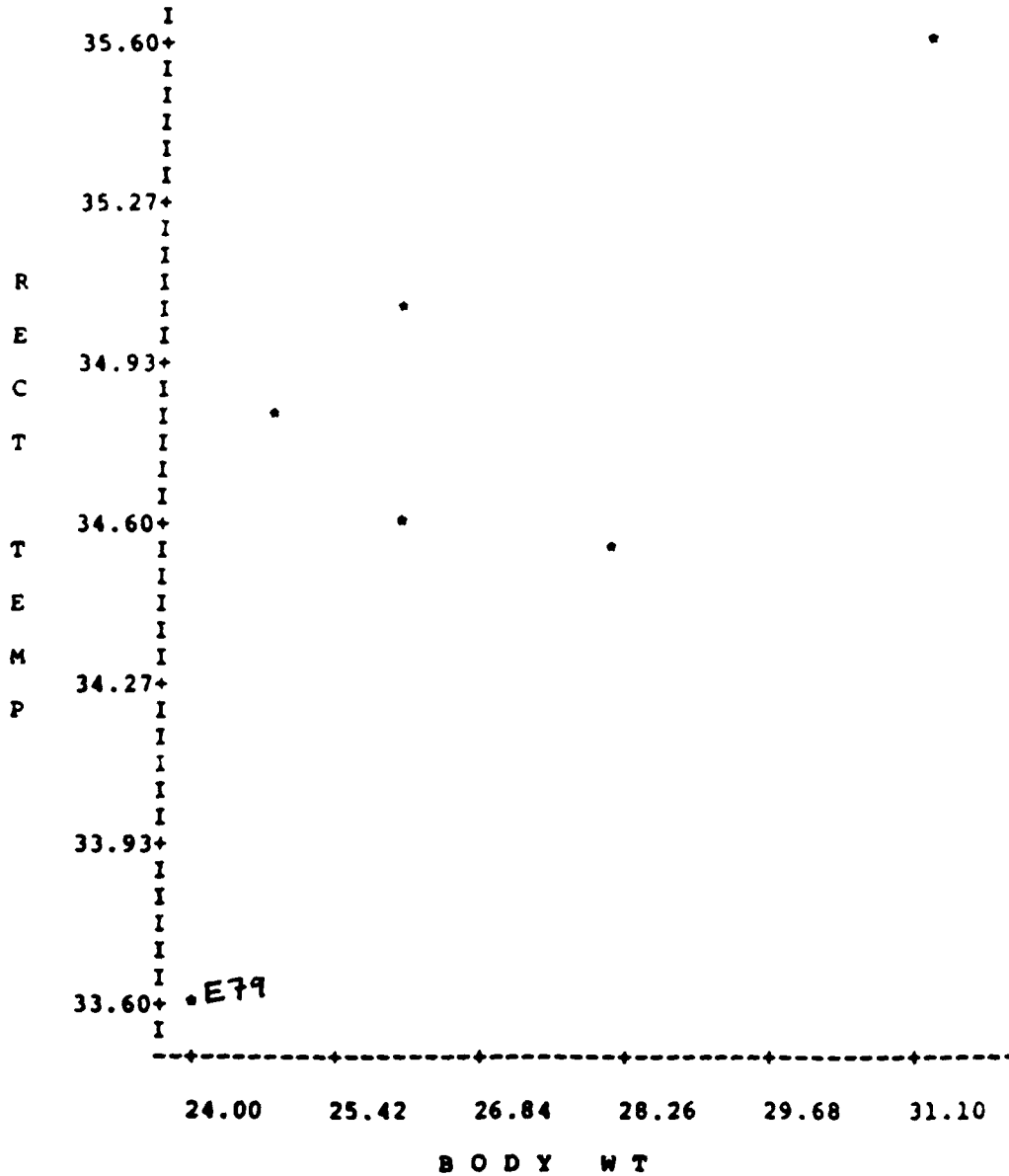
Even after 22 hour separations, the pups were asleep an average of 64% of the time. As shown in Table CC, this percentage was a decrease from Day 1. The decrease was due to a large loss in paradoxical sleep

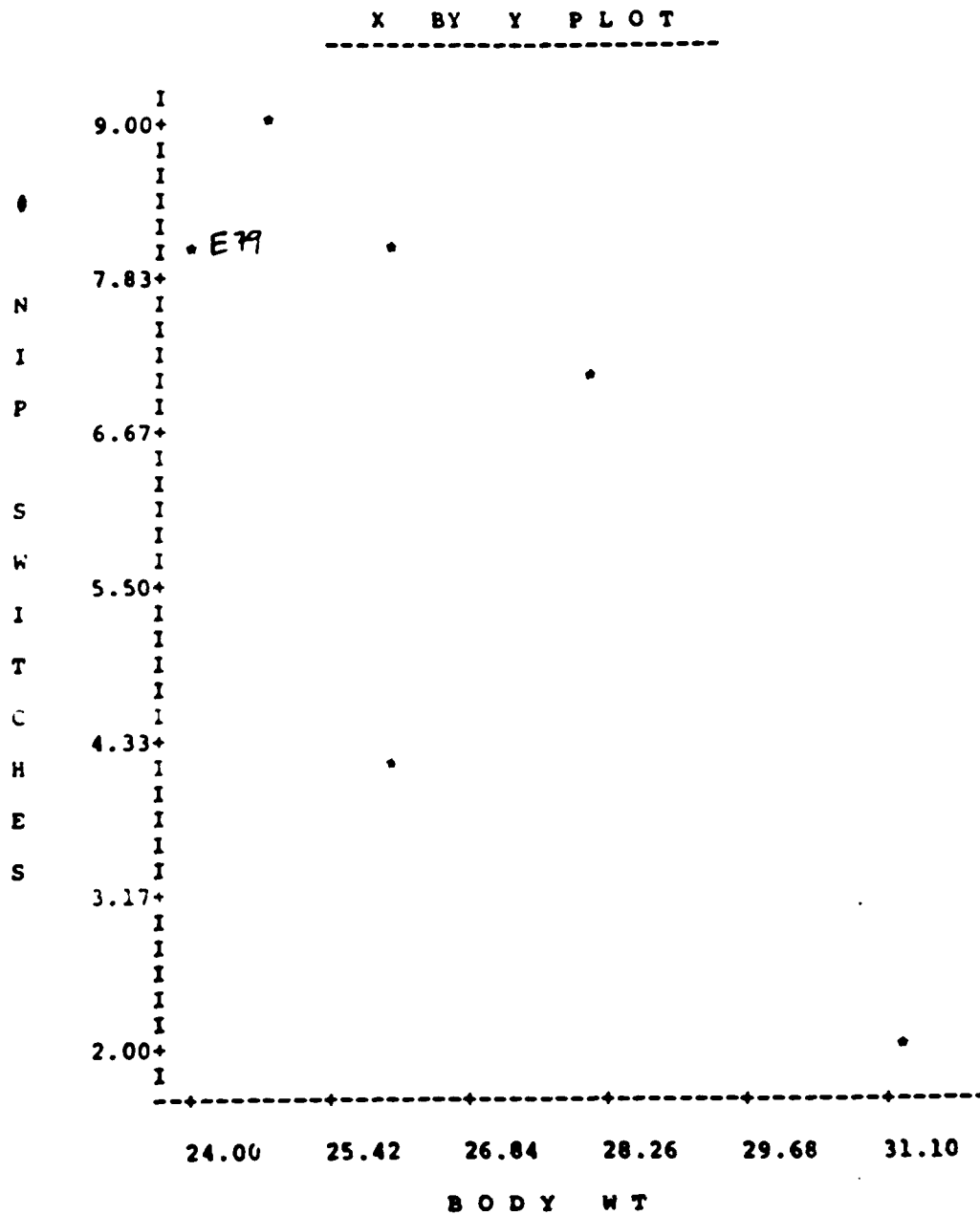
Legend 23. Scatter plot of the pretest body weights (gr) and rectal temperatures ($^{\circ}$ C) for the six test pups on Reunion day.

The value for subject E79 is labelled on this and all other scatter plots since the pup's values lie in the extremes on so many variables.

Figure 24. Scatter plot of the pretest body weights (gr) and the number of nipple switches emitted by the six pups during the Reunion session.

X BY Y P L O T





time, while slow wave time tended to increase. Thus, the alterations in sleep-wake state percentages were in similar directions in both the Normal and Anesthetized Mother Experiments.

Note that in Table CC the information for the Reunion day is compared to the data for Baseline day, both with and without pup E79. This procedure was followed since E79 was so aberrant in terms of percent time spent in each state (awake = 82%, PS = 10%, SWS = 9%). Despite the results above, I have no reason to think there was anything wrong with E79. I believe it was merely on one end of the spectrum of responses to separation and reunion. Note that in the Normal Mother Experiment, there was increased variability in the weight gain data on Reunion compared to Baseline day. In the present experiment, without differences in the mothers' behavior as a variable, the pups had more variability in their state data after deprivation. Also, Brake has recently found increased variability in responses of young rats to deprivation on measures of sucking behavior and weight gain as well (unpublished data). The varied responses have predictive value for long-term growth after reunion with the dam. Thus, deprivation may cause the pups to separate into two (or more?) groups. For this reason, the data to follow will be presented both with and without pup E79 where important to do so.

As with the Baseline day, the relationships of sleep-wake state percentages to body weight, percent change in weight, number of nipples switches and rectal temperature were examined. After separation, some correlations appeared suggestive. The percent of paradoxical sleep was positively correlated to rectal temperature ($r = .91$, $df = 4$, $p < .05$) and negatively correlated to the number of nipple switches ($r = -.82$,

Table CC

Measures of sleep and wakefulness of infant rats recorded while attached to their anesthetized dams for two hours on Reunion day.

	Sleep Latency (min)	% TRT in State			(-----State Episode Analysis-----)						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Mean	13.6	36	20	44	1.31	.72	1.60	21	14	17	52
SEM	10.1	9.4	3.4	7.7	.61	.03	.23	2.4	2.0	2.6	5.6
Comparisons of Reunion to Baseline:											
dep t =	1.098	2.708	8.570	2.020	1.226	6.012	1.886	2.020	1.706	2.978	1.546
df=5		*	***	+		**		+		*	
Comparisons as above w/o pup E79:											
dep t =	.770	4.510	7.768	5.332	1.318	7.823	2.444	3.861	1.754	4.733	6.288
df=4		*	**	**		**	+	*		**	**

A = awake PS = paradoxical sleep SWS = slow wave sleep
 TRT = total recording time + = p<.10, ns * = p<.05 ** = p<.01 *** = p<.001

$p < .05$). The correlations of percent of paradoxical sleep to body weight ($r = .80$, $p < .1$) and percent time awake to rectal temperature ($r = .80$, $p < .1$) did not quite reach statistical significance. No other correlations were even close to significance. An examination of the scatter plots of the above data (Fig. 25, 26, 27) suggests that the relationships of body weight, rectal temperature and number of nipple switches to percent recording time in paradoxical sleep are not obviously due to the effect of any extreme points. The relationship between percent time awake and rectal temperature is less clear (Fig. 28). The aberrant point of E79 may be having too much influence.

The pattern of duration of state episodes is given in Fig. 29B. Both awake and paradoxical sleep showed a high proportion of short episodes. However, the pups did have some long episodes awake as well, which accounted for the relatively high mean duration in Table CC. Slow wave sleep episodes were more evenly spread in length, but still with a preponderance of short episodes. Comparing Baseline and Reunion data demonstrated that statistically significant shifts occurred in the pattern of episodes durations while asleep. The most dramatic change was seen in paradoxical sleep. On the second day long episodes were virtually eliminated. There was also a significant decrease in the number of long episodes of slow wave sleep. (See the figure legend and Appendix for the statistical results.)

On the second day of recording, the most common transitional probability of state change (Fig. 30B) was from awake to slow wave sleep. The pups were more likely to return to awake from slow wave sleep than go into paradoxical sleep. Remember that paradoxical sleep

Figure 25. Scatter plot of the pretest rectal temperatures ($^{\circ}\text{C}$) and the percent of the total recording time spent in paradoxical sleep by the six test pups on Reunion day.

Figure 26. Scatter plot of the pretest body weights (gr) and the percent of total recording time spent in paradoxical sleep by the six pups during the Reunion session.

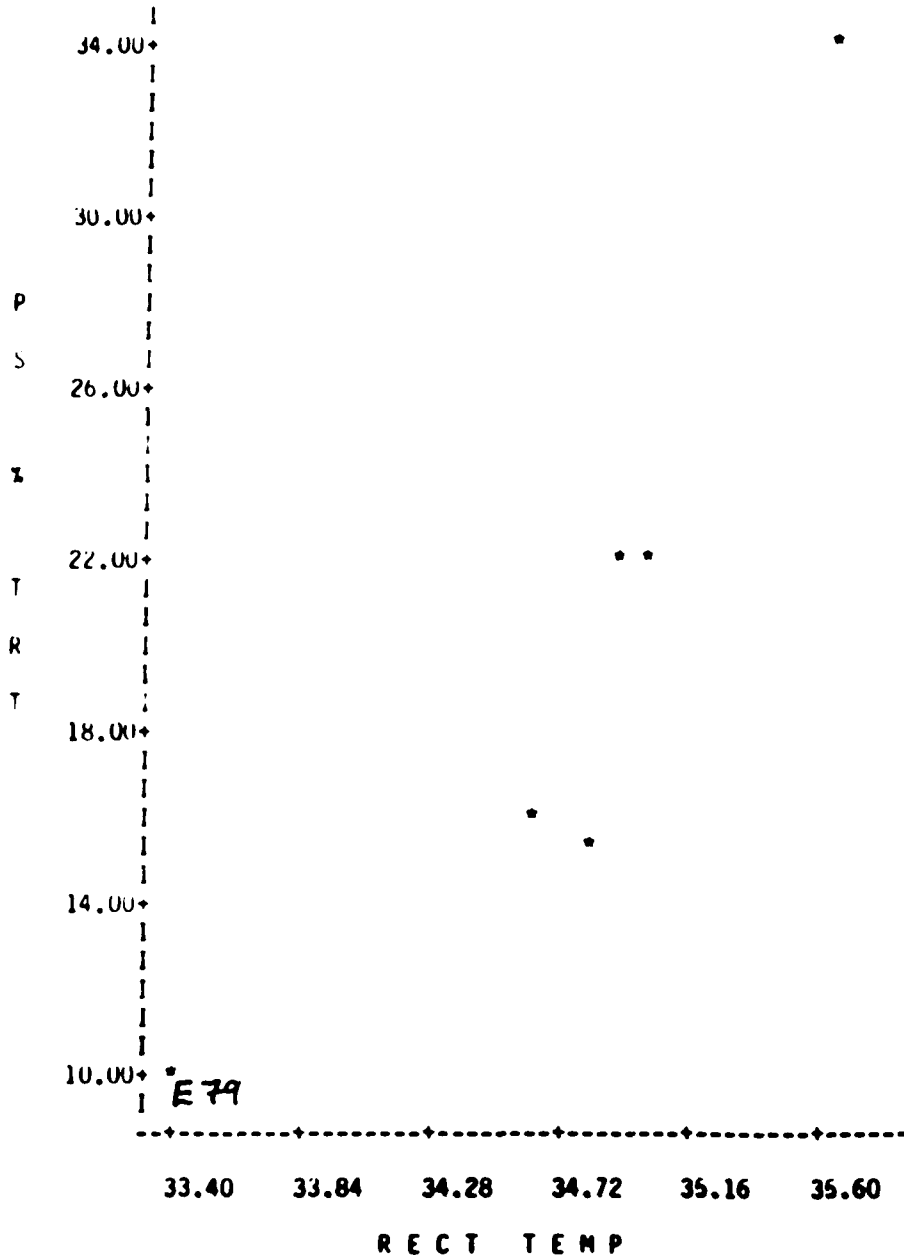
Note that the point labelled A is actually two overlapping values.

Figure 27. Scatter plot of the number of nipple switches and the percent of total recording time spent in paradoxical sleep by the six pups during the Reunion session.

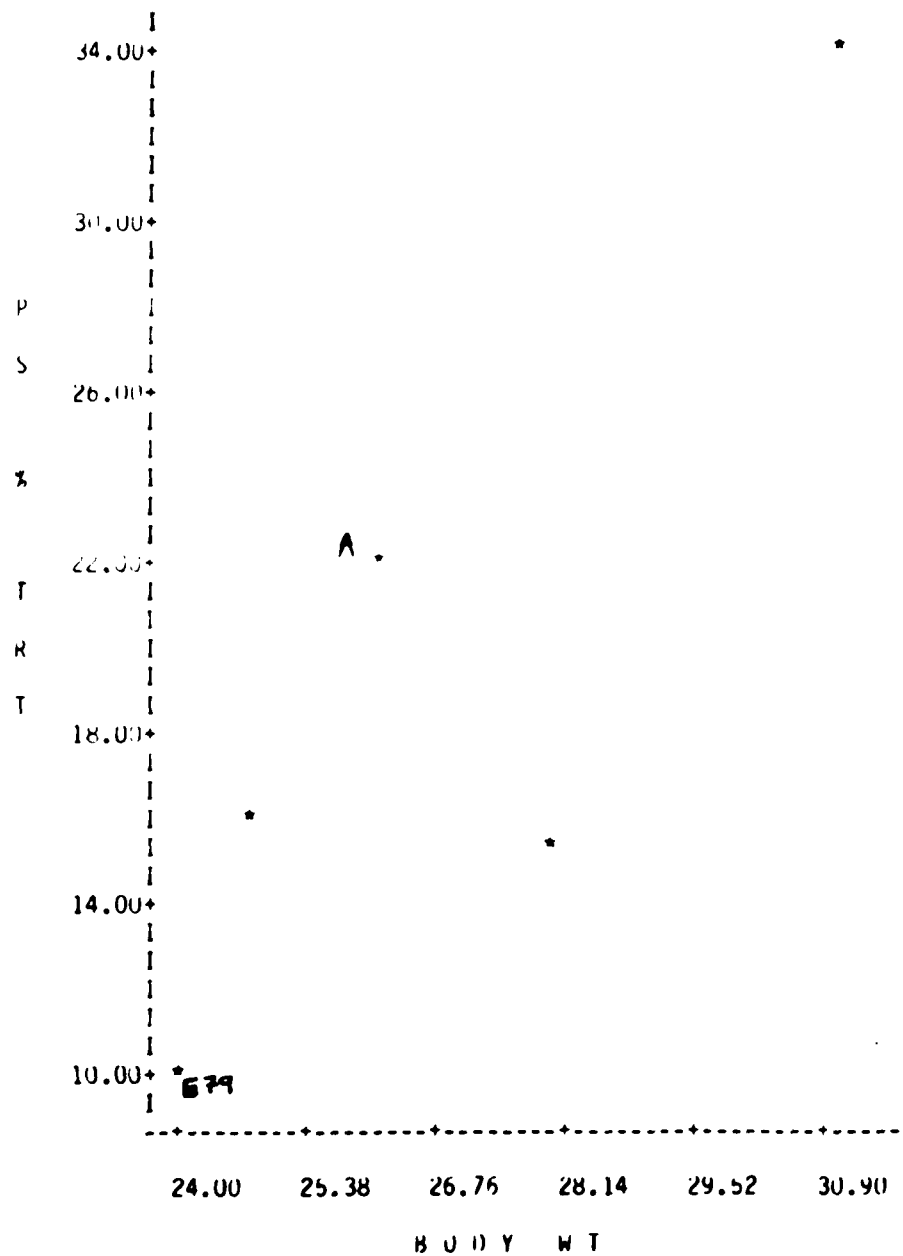
Figure 28. Scatter plot of the pretest rectal temperatures ($^{\circ}\text{C}$) and the percent of total recording time spent awake by the six pups during the Reunion session.

Note the extreme value of pup E79 which acts to inflate the results of the correlation given in the text.

X BY Y P L O T



X BY Y PLOT



X BY Y PLOT

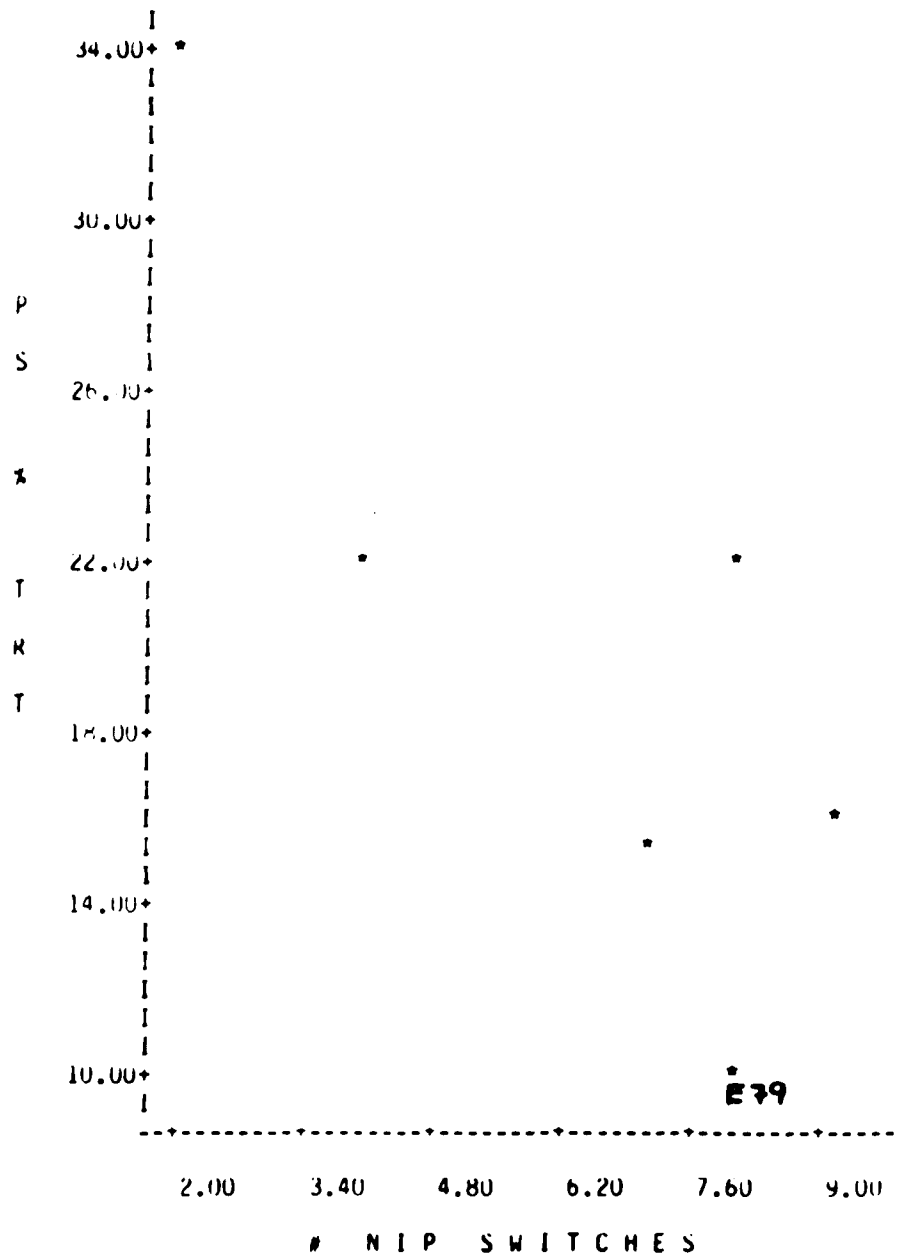


Figure 29. Histogram analysis of state episode durations while attached to the teat of an anesthetized dam, illustrating the relative frequencies of occurrence of periods of different durations.

A. Baseline B. Reunion

A = awake; PS = paradoxical sleep; SWS = slow wave sleep;
E = episode; = $p < .10$; * = $p < .05$; ** = $p < .01$.

Indications of statistical significance refer to comparisons between Baseline and Reunion sessions for episodes of the same duration within the same state. These indications are based on the results of dep t-tests ($df=5$ for each). Overall ANOVA results are given in the Appendix.

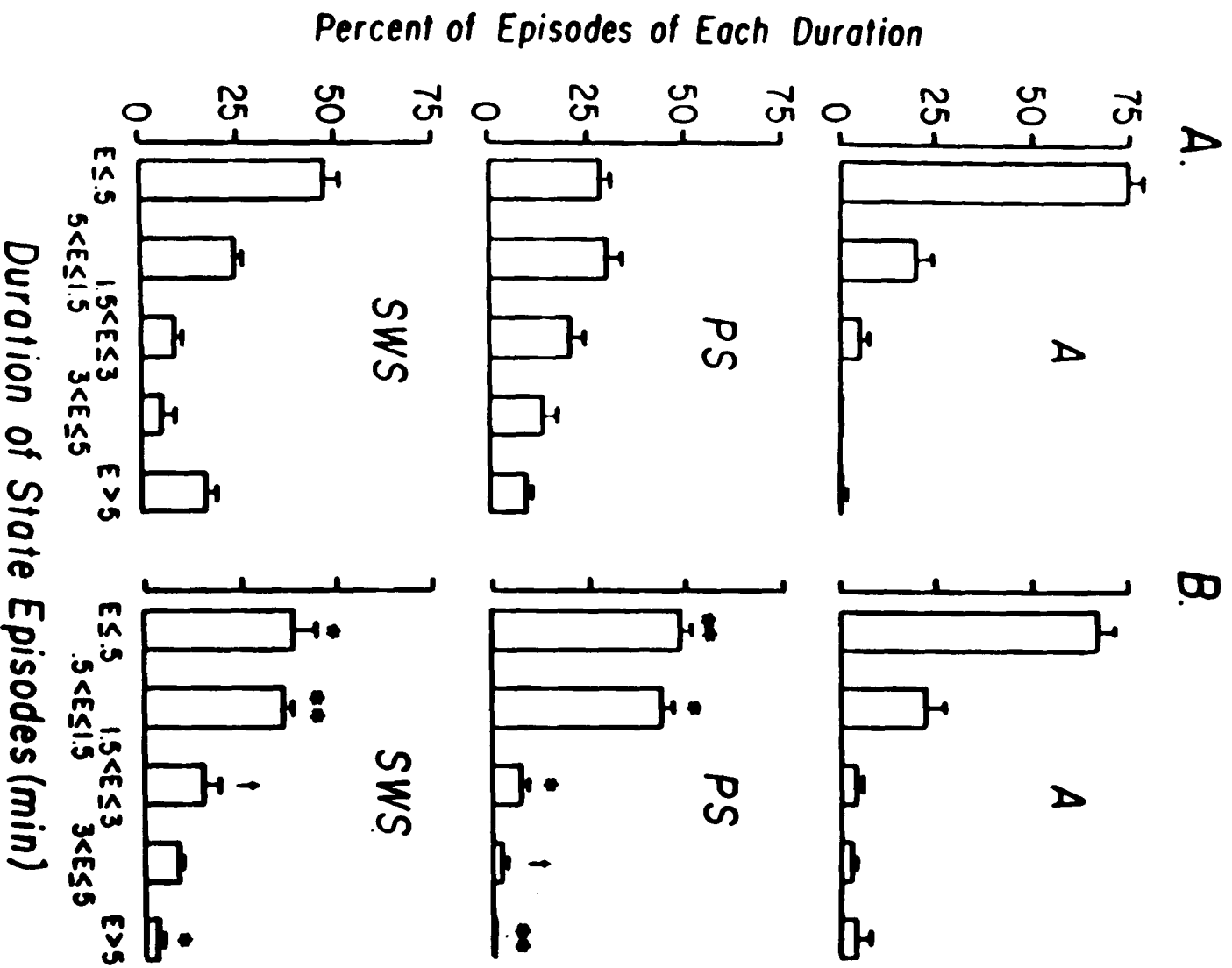


Figure 30. The probabilities of transition among the three sleep-wake states while attached to the teat of an anesthetized dam.

A. Baseline B. Reunion

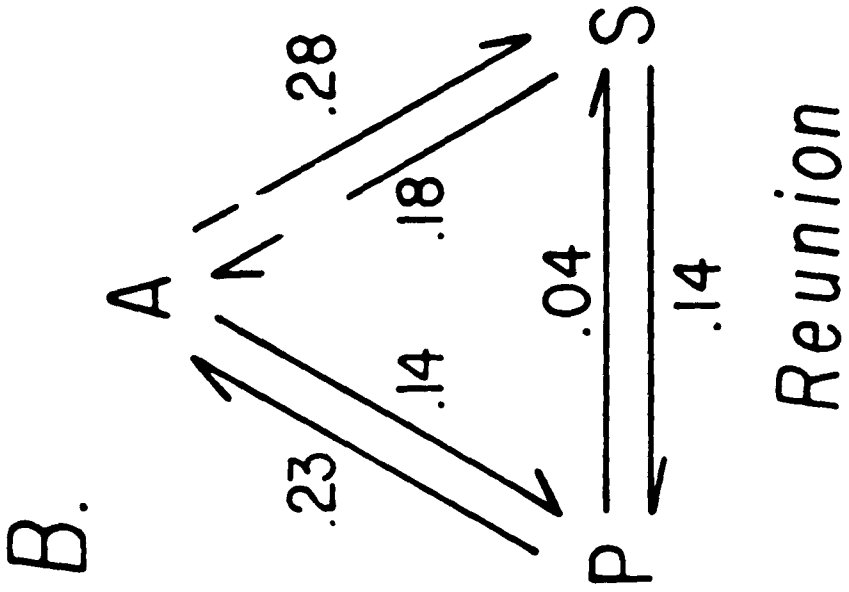
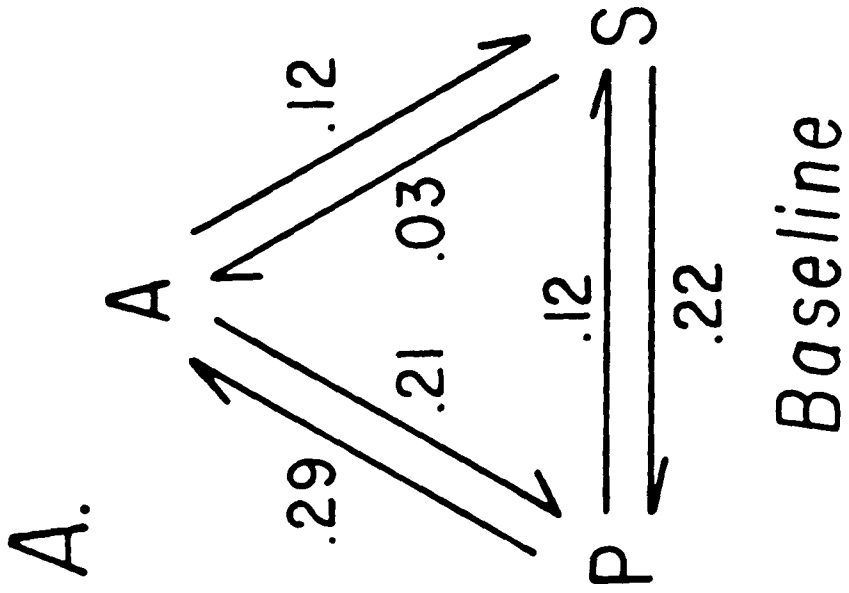
Planned, individual comparisons were by dep t-tests, df=5 for each.

R>B: S--A, dep t = 6.505, p<.01
A--S, dep t = 5.746, p<.01

B>R: S--P, dep t = 3.636, p<.05
P--S, dep t = 3.793, p<.05
P--A, dep t = 2.803, p<.05
A--P, dep t = 2.802, p<.05

The overall ANOVA on both Baseline and Reunion transitional probabilities also revealed both main and interaction effects (see Appendix).

A = awake; P = paradoxical sleep; S = slow wave sleep.



occupied only 20% of the total recording time. Once in paradoxical, the pups awoke in 85% of the cases. All the transitional probabilities differed significantly from their counterparts on the first day at the $p < .05$ level or better (see figure legend and Appendix for detailed data). The major difference was in the high percentage of awake to slow wave sleep (and vice versa) interactions on Day 2, instead of awake to paradoxical sleep which was seen on Day 1. Also note the low number of paradoxical to slow wave sleep interchanges on Day 2.

Sucking Behavior

As shown in Fig. 31, the amount of sucking time upon Reunion increased after the 22 hour separation, as was predicted by the work of Stephen Brake (Brake, Sager, Sullivan and Hofer, 1982) and the results of the Normal Mother Experiment. There was no significant change in bursts, but the other three sucking measures increased dramatically. The increase in rhythmic sucking was found statistically significantly only by non-parametric tests (see figure legend) due to the extreme variability in the rhythmic sucking which was seen on Day 2. This pattern of change caused by deprivation closely matched the results of the Normal Mother procedure (compare with Fig. 16).

When the sucking data was analyzed in terms of the rate of sucking per unit time in each state (see Fig. 32B, and figure legend and Appendix for statistics), the pattern which emerged was generally comparable to the results of the previous experiments. Sucking rates were highest while awake, but appreciable levels of sucking were emitted while asleep, especially in slow wave sleep. The rate of

Figure 31. The average time spent in each sucking type while attached to the teat of an anesthetized dam during Baseline and then Reunion from 22 hours separation.

Time of sucking is represented as the number of five second periods in which a suck of a given type occurred.

Statistical indications compare Baseline and Reunion results for a given sucking type, and are based on dep t-tests, df=5 for each.

RS = rhythmic sucking; B = bursts; T = treadles;
I = EMG intensity rank; * = $p < .05$.

SUCKING BEHAVIORS

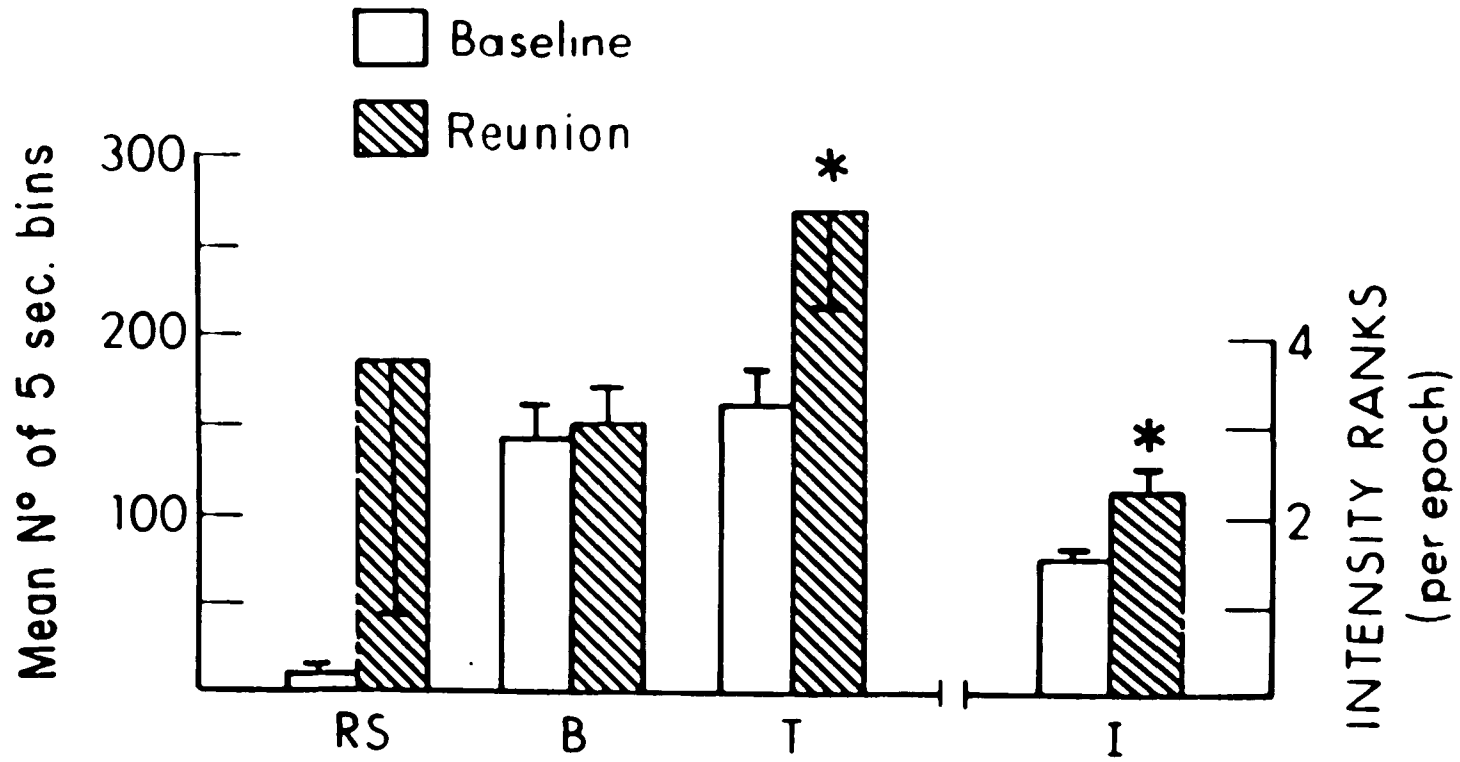


Figure 32. The rate of sucking behaviors and the percentage of time in each sleep-wake state while attached to the teat of an anesthetized dam.

A. Baseline B. Reunion

The horizontal bars represent the mean percentage of recording time while attached to a teat spent in each sleep-wake state. The height of the three left-hand vertical bars gives the mean rate of sucking per minute for each kind of sucking. The bar to the right measures the amplitude of the digastric EMG recording when no sucking is occurring (an index of the muscle tone involved in maintaining a seal on the nipple).

EMG = electromyogram; PS = paradoxical sleep; SWS = slow wave sleep; RS = rhythmic sucking; B = bursts; T = treads; I = EMG intensity rank.

During the Reunion test the following sucking types differed significantly when compared between sleep-wake states:

B: A>P?, dep t = 2.209, p<.10, ns

S>P, dep t = 4.570, p<.01

T: A>P, dep t = 5.321, p<.01

A>S, dep t = 5.273, p<.01

RS: All pups had higher rates of RS when awake than in either SWS or PS. Dep t-tests did not show significance due to the large variability in the awake data and the number of zeros when asleep. According to the non-parametric sign test, both A>PS and A>SWS are significant at p=.016.

I: A>P, dep t = 4.897, p<.01

A>S, dep t = 3.053, p<.05

S>P, dep t = 6.558, p<.01

Comparisons of Reunion and Baseline rates within a state revealed no significant effects of day of testing on sucking rates. The EMG intensity rank was higher on Reunion day, however.

I/awake; dep t = 2.744, p<.05

I/PS: dep t = 2.433, p<.10, ns

I/SWS: dep t = 4.300, p<.01

All significance findings, except where noted, are based on planned dep t-tests, df=5 for each, whose use are validated by the experimental hypotheses based on the Normal Mother Experiment. The results of overall ANOVAs on both Baseline and Reunion data are given in the Appendix.

bursts was again highest in slow wave sleep, although not significantly higher than its rate while awake.

Comparing the rates by state on Baseline and Reunion days, rates of sucking within states were not increased. The only significant change was a decrease in the number of bursts per minute during paradoxical sleep (see figure legend for statistics). Once again, the resistance to change of sucking rate within each state was demonstrated. Yet, the pups were attached more tightly to the teat after deprivation, as shown by the significant increases in Intensity Rank/Epoch.

If sucking rates in each state were truly fixed for each individual animal, one would expect high positive correlations between the rates by state on Baseline and Reunion days. As shown in Table DD, while such correlations were positive in general none of the rate by the state comparisons and only one of the EMG intensity measures reached statistical significance. Removing E79 from the comparisons caused the correlations to become more positive in all cases except one. Clearly, sucking rates within each state were not absolutely fixed for each individual. Some pups increased rates, some pups decreased. Yet, the positive correlations do suggest that some individual stability was present from Baseline to Reunion. Other variables including changes of weight and body temperature may have influenced the sucking rate measures differentially in each pup. A further study with more animals would be needed to discover if the positive correlations truly reflect an underlying individual stability in sucking rates within states.

As on Baseline day, the relationships among various pup parameters and sucking measures were examined (Table EE). As one can see, there

Table DD

Baseline to Reunion correlations of sucking rate measures.

	(-----A-----)				(-----PS-----)				(-----SWS-----)			
	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep
Pearson												
r =	.27	.64	.13	.56	---	.46	-.25	-.09	.56	.39	.75	.81
n=6											+	*
Pearson (as above w/o E79)												
r =	.78	.92	.48	.75	---	.59	-.21	-.06	1.00 ^b	.13	.77	.89
n=5		*							**?			*

A = awake PS = paradoxical sleep SWS = slow wave sleep B = bursts RS = rhythmic sucking
 T = treads I = EMG intensity rank ep = epoch m = minute + = p<.10,ns * = p<.05
 ** = p<.01

^a No comparison possible due to lack of RS.

^b Perfect correlation is based on the following data:

pup	Q75	B76	I74	B79	I79
Base.	0	0	.1	0	0
Reun.	0	0	.1	0	0

Thus, while the relationship is statistically significant, it is unlikely to have biological relevance.

was a significant relationship between the Intensity Ranking of EMG amplitude and both pretest body weight and temperature. Those pups which were the smallest and coolest clung most firmly to their dam's teats. Even when the data point for E79 was removed, the same high negative correlations were found.

Time spent in rhythmic sucking was negatively correlated to two measures as well, but those correlations were not at all high without E79. The scatter plot for the relationship between time of rhythmic sucking and percent change in weight during the test is given to demonstrate the extent to which an outlying point can influence a correlation (Fig. 33). Without E79, the correlation changes from $-.90$ to $.60$.

Unlike on Baseline day, there was no positive correlation between body weight and the time spent treading, even when the data for E79 was not included. If the Baseline relationship was not due to chance, then some aspect(s) of the deprivation and reunion experience must have overridden or changed that relationship. One can speculate as to what caused the change, but without any firm support. There is no logical reason to assume that body weight has exactly the same significance to the pups before and after deprivation. Perhaps, after deprivation all the pups were hungry, whereas before deprivation only the lighter pups were. Note the high negative correlation between treading and percent change in body weight during deprivation. Although not quite statistically significant, this datum suggests that after deprivation it was those pups which lost the most weight that treadled most. Perhaps this new factor altered the direct correlation between weight

Table EE

Pearson Product-Moment Correlations: Measures of pups' weights, temperatures and nipple switching behaviors correlated with levels of sucking.

	(------Body Weight-----)			(--Rectal Temp.--)		# of nipple switches
	pretest wt.	%change from Base.	%change during Reun.	pretest temp.	change during Reun.	
Time spent in RS	-.54	.19	-.90* .06 ^a	-.90* -.44 ^a	.49	.37
Time spent in B	.36	.06	.31	.64	.15	-.28
Time spent in T	-.43 -.55 ^a	-.74+ -.73 ^a	.08	-.06	-.35	-.03
EMG Intensity Rank	-.82* -.79 ^a	-.46	-.60	-.94** -.89* ^a	.12	.62

B = bursts RS = rhythmic sucking T = treads + = p<.10,ns
* = p<.05 ** = p<.01

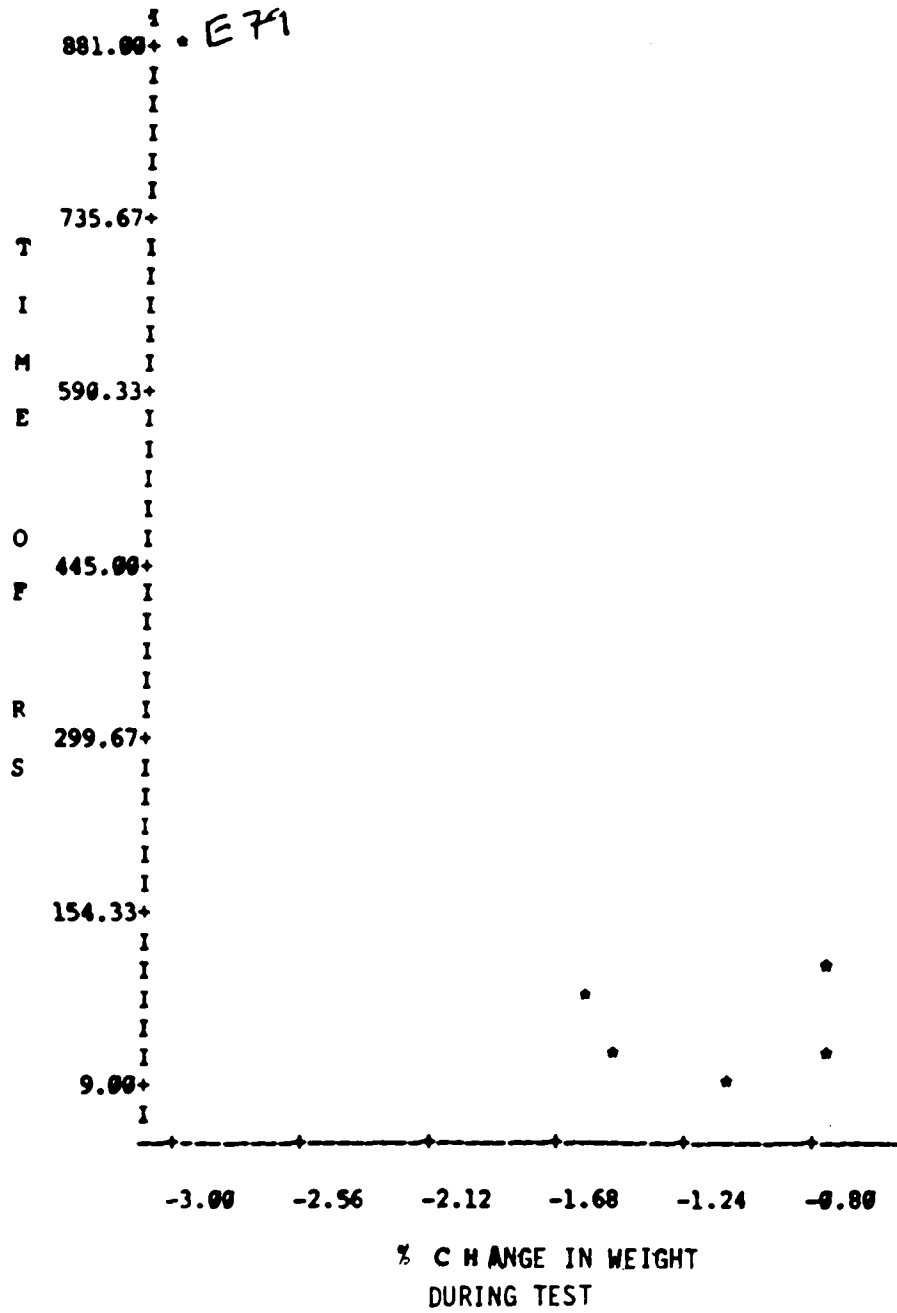
^a Correlation run again without data from pup E79.

Figure 33. Scatter plot of the percent change in weight during the two hours of the Reunion test to the total time spent rhythmic sucking by the six test pups.

Time of sucking is figured as the number of five second bins in which RS occurred.

Note the extreme value of pup E79, which acts to inflate the results of the correlation given in the text.

X BY Y PLOT



and treading. As mentioned above, such reasoning is quite speculative.

Before deprivation, changes in rectal temperature were related to changes in certain sucking rates while awake and possibly while asleep. What happened to those relationships after deprivation? Again, bursts per minute in awake animals was correlated significantly to body temperature ($r = .86$, $df = 4$, $p < .05$). However, the direction of the correlation altered. It had been negative, but was positive on Day 2. I do not have any good speculations as to the cause of this difference. The Reunion correlation between rhythmic sucking while awake and body temperature was similar to the Baseline one, except that after deprivation the correlation did not quite reach statistical significance ($r = -.74$, $df = 4$, $p < .10$). No correlations between temperature or body weight and any sucking rate during sleep came close to significance. Thus, after deprivation as before, sucking rates while awake were affected by body temperature.

As you recall, during the Baseline recording, there were high negative correlations between the average length of awake episodes and the rates of both rhythmic sucking and bursts while awake which almost reached statistical significance. Deprivation and Reunion altered those relationships. The awake episode duration was definitely not significantly correlated to burst rate ($r = -.51$) and was positively correlated to the number of rhythmic sucks per minute ($r = .96$, $df = 4$, $p < .01$). However, this positive correlation was due to the extreme point of E79, since without E79 'r' equalled .57. Thus, deprivation did not confirm the tentative relationships seen on Day 1. Of course, as noted above, there is no a priori reason to assume that Baseline and

Reunion relationships must be the same. No sleep state durations were significantly correlated to sucking rates within that state. A possible relationship was suggested by the correlations between treads per minute and episodes duration in paradoxical sleep, both with and without E79 ($r = -.78$, $df = 4$, $p < .10$ and $r = -.82$, $df = 3$, $p < .10$ respectively). Therefore, the question of the relationship between state episode duration and any sucking within that episode must still remain open.

Trends Over Time

The data was analyzed in the first hour vs. the second hour of recording to see if there were any changes during the recording session. As shown in Table FF, the high sucking time upon Reunion occurred both in the first and second hours of the recording session. However, the pups did tend to shift the proportions of sleep states as the recording progressed with very little paradoxical sleep early, but appreciable amounts later in the recording where it replaced both slow wave sleep and awake time. This rise in paradoxical sleep percentage over time on Reunion day was also seen while attached in the Normal Mother Experiment (Table R), although in that case statistical significance was not attained. The fall in amount of time spent awake did not reach statistical significance, unlike the previous study.

Sucking rate changes in treads per minute in both paradoxical sleep and slow wave sleep again showed that sucking rates by state were not totally fixed (see Table GG). Note that this change in treads/minute in slow wave sleep was also seen on Baseline day (Table

Table FF

Trends over time during the recording session:
Comparison of mean state percentages and sucking behaviors
from the first to second half of the Reunion test.

	(-Sleep-Wake State %'s-)			(-Time Sucking ^a -)		
	A	PS	SWS	RS	B	T
1st Half	41	9	50	156	88	148
2nd Half	32	30	38	56	62	142
dep t =	1.512	9.561 ^b	2.022 ^b	1.700	1.122	.609
df =		***	+			

^a For each of the three sucking types, the time spent sucking was calculated as the number of 5 sec bins in which a suck of that type occurred.

^b The decrease in SWS was statistically significant when pup E79 was excluded from the analysis (dep t = 15.438, df=4, p<.001). The change in PS remained significant at the same probability level. Pup E79 had neither SWS nor PS during the first hour.

A = awake PS = paradoxical sleep SWS = slow wave sleep
B = bursts RS = rhythmic sucking T = treadles
+ = p<.10, ns *** = p<.001

Table GG

Trends over time during the recording session: Comparison of the rates of sucking within each sleep-wake state from the first to second half of the Reunion session.

	(-----A-----)				(-----PS-----)				(-----SWS-----)			
	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep	RS/m	B/m	T/m	I/ep
1st Half	2.9	1.5	5.2	2.7	0	.7	.3	1.7	.4	1.9	1.4	2.4
2nd Half	1.9	1.3	5.4	2.6	0	.4	.6	1.6	0	1.6	.7	2.2
dep t = df=5 or 4	^b 1.334	.450	.427	1.341	--- ^a	1.883	2.982 [*]	1.725	.999	1.677	3.344 [*]	1.206
Pearson r =	.94 ^{**}	.70	.96 ^{**}	.92 ⁺	--- ^a	.89 [*]	.76	.85 [*]	--- ^a	.60	.94 ^{**}	.60

A = awake PS = paradoxical sleep SWS = slow wave sleep B = bursts RS = rhythmic sucking
 T = treadles I = EMG intensity rank ep = epoch m = minute + = p<.10,ns * = p<.05
 ** = p<.01

^a No comparison possible due to lack of RS.

^b df=4 for PS and SWS measures, since pup E/9 was awake the entire first hour.

AA). However, most of the sucking types showed no alteration in rate by state. Not only that, there were many highly significant positive correlations in rate by state measures between the first and second hours. Thus, within the two-hour recording period, there was great stability of the rates of sucking within each state for the individuals, as well as for the group as a whole. It may need a massive experience like deprivation to affect that stability.

Discussion

In this experiment, the response of the test pups to deprivation and Reunion was very similar to what had been seen in the Normal Mother Experiment. Sleep-wake state percentages altered so that the pups spent more time awake and less in paradoxical sleep. The pups also had higher levels of sucking behavior. Yet again, there were no increase in the sucking rates within each state. The only significant change in rate was a decrease in bursts per minute during paradoxical sleep, a decrease found in the previous experiment as well. After deprivation, the rat pup has a very different state pattern. The picture is of a pup awake for much more of the time and for longer episodes. Sleep-wake cycles are fragmented. The pup is rarely in paradoxical sleep and wakes rapidly from that state. As the recording session progresses, more paradoxical sleep is seen. Perhaps something about the Reunion experience, even with an anesthetized dam, enables the pup to begin a recovery process. One explanatory hypothesis suggested by the work of Szymuziak and Satinoff (1981) is that the pup gains heat and enters the thermoneutral zone, known to have an influence on the percent of paradoxical sleep. A positive correlation

was found between body temperature and the percent of time in paradoxical sleep. However, the pups in this study did not gain temperature over the recording period. Some other factor must explain the increase in paradoxical sleep time.

Other findings suggest that individual animals may be affected differentially by deprivation. The amount of variability in state behavior increased dramatically from Day 1 to Day 2. The variability in sucking time increased as well. There were positive correlations between sucking rates within states from Baseline to Reunion day but few reached statistical significance. Of course, these changes may be clues to shifts in some underlying mechanisms. For example, pups with the lowest body weights and temperatures have the least amount of paradoxical sleep. They also tend to nipple shift more. Temperature influences the rate of sucking behavior as well, but only when the pup is awake.

A final point about sucking rate by state: in both the Normal and Anesthetized Mother Experiments, the characteristic sucking rates within each state appeared stable. Yet, these rates were not totally fixed. There were a few differences from Baseline to Reunion in each experiment. There were a few differences from Baseline to Baseline between the two experiments. There were a few differences between the first and second hours of the Anesthetized Mother Experiment. Temperature can influence sucking rate while awake. Milk ejections bring a transient increase in treads while awake. However, it is now clear that the general response to deprivation and reunion is not one of increasing sucking rates within the various states. The preponderance of the results suggest that pups increase sucking time

after deprivation by increasing the percentage of time spent in states with the higher rates of sucking, that is awake and slow wave sleep. The final experiment will attempt to investigate how tightly coupled are the sucking rates and the sleep-wake states.

CYCLED DIET EXPERIMENT

The purpose of this experiment was to study the connection between particular sleep-wake states and the characteristic sucking rates within those states. The experiments reported above demonstrated that state and sucking rates remained relatively firmly linked. Yet, a certain lability was seen. How great was that lability? Could rate and state be disassociated?

A possible way to disassociate the two variables was suggested by previous research. Hofer and Shair (1982) demonstrated that normal sleep-wake patterns can be maintained, even after 22 hours of maternal separation. The method involved infusion of a nutrient formula comparable to rat milk on a periodic schedule similar in timing to the dam's pattern of nursing. A different series of experiments showed that prefeeding does not influence the amounts of non-nutritive sucking of maternally-deprived pups (Brake, Sager, Sullivan and Hofer, 1982). Thus, in theory, providing such periodic nutrient in the time between Baseline and Reunion should affect sleep-wake states but not sucking amounts. The pups should have a Baseline-type sleep-wake pattern and Reunion-type sucking time, forcing the states and rates to disassociate.

Certain caveats had to be considered. The two background studies just described had protocols different from one another and from the present series of experiments. In the sleep-wake work, pups were tested without their mothers in the cages (Hofer and Shair, 1982), thus no attachment was possible. Brake and colleagues (1982) gave their rats a single stomach-load of 5% body weight rather than infusing formula periodically throughout the deprivation period. These caveats

suggested that all of the following possible outcomes must be considered:

- 1). Normal sleep-wake patterns and increased sucking time; therefore, increased sucking rates within some or all of the states;
 - 2). Reunion-like sleep-wake patterns and increased sucking time; therefore, no change in sucking rates by states;
 - 3). Normalized sleep-wake patterns and no increased sucking time; therefore, no change in sucking rates by state;
 - 4). Some interaction of sleep-wake patterning and sucking level change; therefore, no specific prediction of rate by state results.
- With those outcomes in mind, the experiment was performed as described in the Methods section.

The data will be presented in two sections. First, to examine whether the gastric cannula implantation influenced the test pups' behavior, comparisons of the Baseline days of the Cycled Diet and Anesthetized Mother Experiments will be carried out. The comparisons will include behavioral observations, sleep-wake state patterns and sucking rates within each state.

Second, the influence of the diet infusions on the Reunion to Baseline comparisons within this experiment will be examined. Only sleep-wake patterns, total sucking time and sucking rates within states will be discussed, since these are the key behaviors which address the possible results given above.

BASELINE COMPARISONS: CYCLED DIET TO ANESTHETIZED MOTHER EXPERIMENTS

In the previous experiments, the pups showed good recovery from the electrode implantation. Was the addition of a gastric cannula

operation enough to hinder complete recovery?

The behavioral observations suggested that the gastric cannulae implanted pups behaved normally. The cannulae did not inhibit their movements. They did not direct any appreciable attention to the cannulae, either scratching or chewing at them. During the Baseline recording, the Cycled Diet (CD) pups attached to the dams' teat almost immediately and remained attached an average of 96 and 1.2% of the session. This result did not differ significantly from the attachment percentage of the Anesthetized Mother (AM) pups (ind t = 1.601, df=10). The number of nipple switches on Baseline day did not distinguish between the two groups either: CD pups mean = $1.7 \pm .8$; AM pups mean = 2.2 ± 1.0 (ind t = .707, df = 10). However, between the day of surgery and the Baseline recording day, the CD animals gained only an average of $8.5 \pm 1.8\%$ body weight. The AM pups in the same length of time gained $17.0 \pm 1.8\%$ body weight (ind t = 2.755, df = 10, $p < .05$).

Still, the CD pups did not appear stunted; while their pretest weight of 28.0 ± 1.0 gr was slightly lower than that of the AM pups (29.2 ± 1.0 gr), this difference was not significant (ind t = .717, df = 10). Thus, the gastric cannula did appear to exert some effect on recovery from surgery, yet the overall behavior of these pups appeared normal.

Next, the CD and AM pups were compared on sleep-wake state variables as shown in Table HH. As can be seen, the average amount of time spent awake did not differ between the two groups, but the CD pups spent less time in slow wave and tended to spend more time in paradoxical sleep than the pups in the previous experiment. The change in paradoxical sleep percentage was so small that neither the duration

Table HH

Measures of sleep and wakefulness of infant rats previously implanted with gastric cannulae recorded while attached to their anesthetized dams for two hours on Reunion day.

	Sleep Latency (min)	% TRT in State			(-----State Episode Analysis-----)						State trans. per hour
		A	PS	SWS	Mean Duration (min)			Frequency/hour			
		A	PS	SWS	A	PS	SWS	A	PS	SWS	
Mean	.5	15	62	24	.60	2.16	2.21	18	18	7	42
SEM	.16	1.6	2.1	2.2	.05	.20	.39	1.5	1.3	1.0	3.2
AM Mean	1.7	12	57	30	.56	2.05	1.95	14	18	10	41
Comparisons of CD and AM means:											
ind t =	1.730	.442	1.924	2.425	.638	.438	.678	1.591	0	2.516	.297
df=10			+	*						*	

AM = Anesthetized Mother Experiment CD = Cycled Diet Experiment A = awake
 PS = paradoxical sleep SWS = slow wave sleep TRT = total recording time
 + = p<.10, ns * = p<.05

nor frequency per hour of the episode showed a significant difference between the two groups. The lower slow wave sleep time was due to fewer number of episodes per hour. The mean duration of the episodes was not significantly different between the two groups.

The comparison of sucking rates by state showed more differences between the pups on Baseline day in the two experiments. The rate of bursts and treads in both paradoxical sleep and slow wave sleep was much lower in the CD Experiment Baseline (see Table II). While awake, CD pups had a higher rate of rhythmic sucking. There were no other significant changes in sucking rates by state between the two experiments.

Whether or not the above changes were a result of the abdominal surgery is uncertain. Another possibility is that the rat's strain and/or rearing conditions may have shifted slightly in the five years between the two experiments. Thus the pups on Baseline day were not identical in the Anesthetized Mother and Cycle Diet experiment. Yet, if we look at the overall picture, the two groups look similar. Sleep-wake state percentages are not far different. The pattern of sucking rates by state was also similar in the two groups -- highest while awake, intermediate in slow wave sleep, lowest in paradoxical. In the CD pups, the distinction between slow wave and paradoxical sleep was not as great as with the AM experiment, mainly due to the low levels of sucking in slow wave sleep. With the above differences in mind, we proceeded to separate the CD pups for 22 hours while providing food on a periodic schedule. No comparison was run to the Normal Mother data due to the problem of having attached and unattached portions of the recording time.

Table II

Comparison of mean sucking rates within each sleep-wake state from the Baseline sessions of the Cycled Diet and Anesthetized Mother Experiments.

	(-----A-----)			(-----PS-----)			(-----SWS-----)		
	RS/M	B/M	T/M	RS/M	B/M	T/M	RS/M	B/M	T/M
CD pups	2.1	1.2	2.8	.1	.4	.3	.1	.7	.2
AM pups	.5	1.7	4.2	0	1.0	.8	0	1.6	.9
ind t = df=10	4.419 **	.973	1.403	1.672	3.072 *	3.258 **	.914	3.680 **	3.316 **

AM = Anesthetized Mother CD = Cycled Diet A = awake PS = paradoxical sleep
 SWS = slow wave sleep RS = rhythmic sucking B = bursts T = treadles
 M = minute * = p<.05 ** = p<.01

REUNION TO BASELINE COMPARISONS: CYCLED DIET EXPERIMENT

First, the diet infusion did cause the pups to gain weight during the maternal deprivation period. The pups gained a significant amount of weight, an average of $13 \pm .9\%$ of body weight (dep t = 17.300, df = 7, $p < .001$). They received an average of $6.9 \pm .2$ cc of diet. As predicted by the work Stephen Brake (Brake, Sager, Sullivan and Hofer, 1982), food intake did not prevent the increase in total sucking time caused by maternal deprivation (see Fig. 34). The amount of treading doubled upon Reunion, while rhythmic sucking almost doubled. Again, bursts did not change significantly (see figure legend for statistics and Appendix for ANOVA). Thus, possible result 3 was disproved. Time spent sucking was increased.

An examination of the sleep-wake state percentage bars in Fig. 35A and B demonstrates that there was a change in sleep-wake state per cent from Baseline to Reunion day. Percent total recording time of awake and slow wave sleep were significantly increased, paradoxical sleep was decreased (see figure legend for statistics and Appendix for ANOVA). Thus the cycled diet infusion did not normalize the sleep-wake state percents when the pups were tested attached to an anesthetized mother.

This result was not predicted from the previous work (Hofer and Shair, 1982), where the pup was tested without the mother. Since sleep-wake states were not regularized, possible result 1 cannot be true. There was no generalized increase in sucking rates by state that occurred independently of sleep-wake state patterning.

Why was sleep-wake state behavior not normalized? If these pups had been studied without their dams, such normalization would have resulted. One must assume that the testing situation, probably some

Figure 34. The average time spent in each sucking type while attached to the teat of an anesthetized dam during Baseline and then Reunion from 22 hours separation.

Pups fed intragastrically during the period of separation.

Time of sucking is represented as the number of five second periods in which a suck of a given type occurred.

Statistical indications compare Baseline and Reunion results for a given sucking type and are based on dep t-tests.

RS = rhythmic sucking; B = bursts; T = treadles;
I = EMG intensity rank; = $p < .10$; * = $p < .05$; ** = $p < .01$.

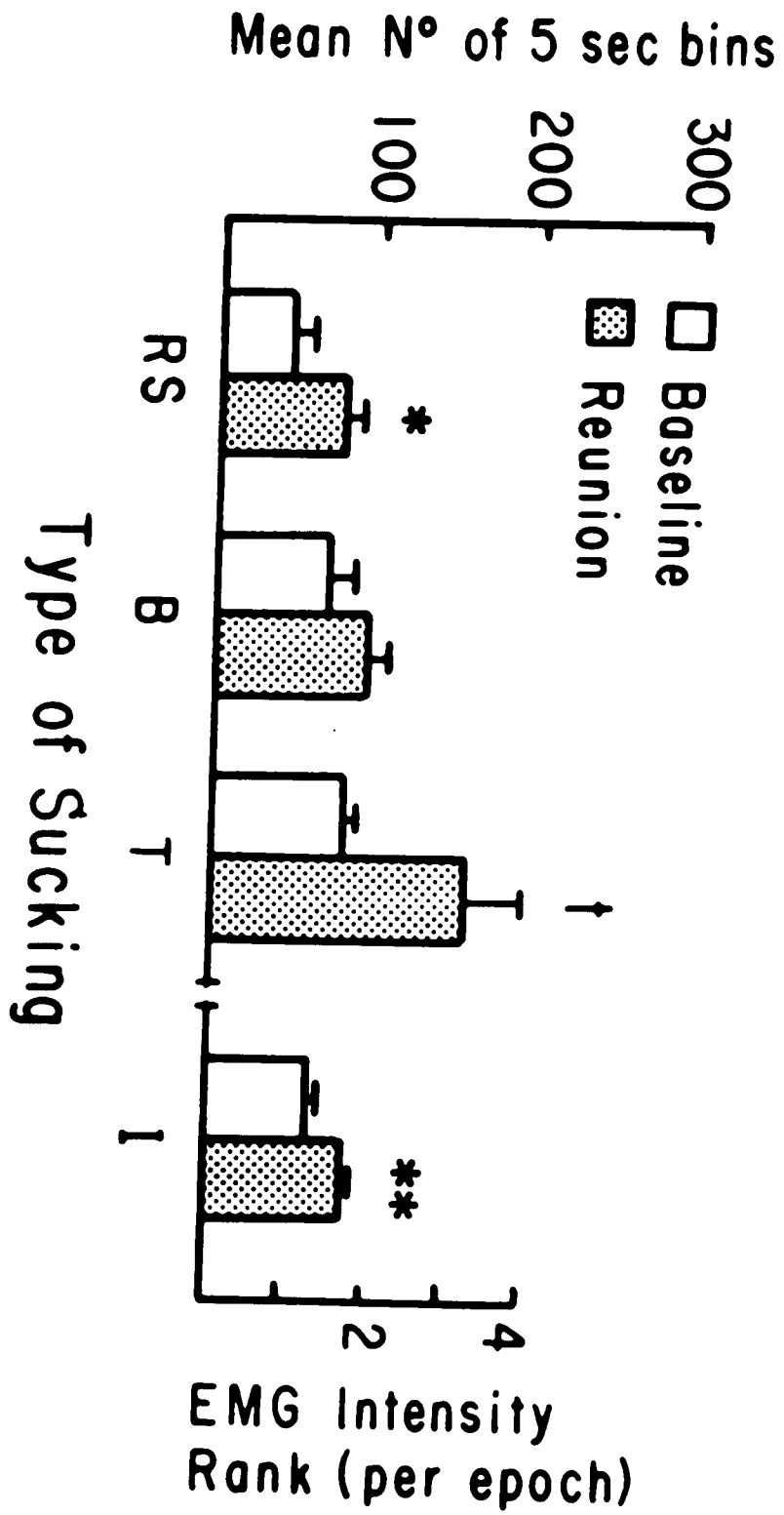


Figure 35. The rate of sucking behaviors and the percentage of time in each sleep-wake state while attached to the teat of an anesthetized dam.

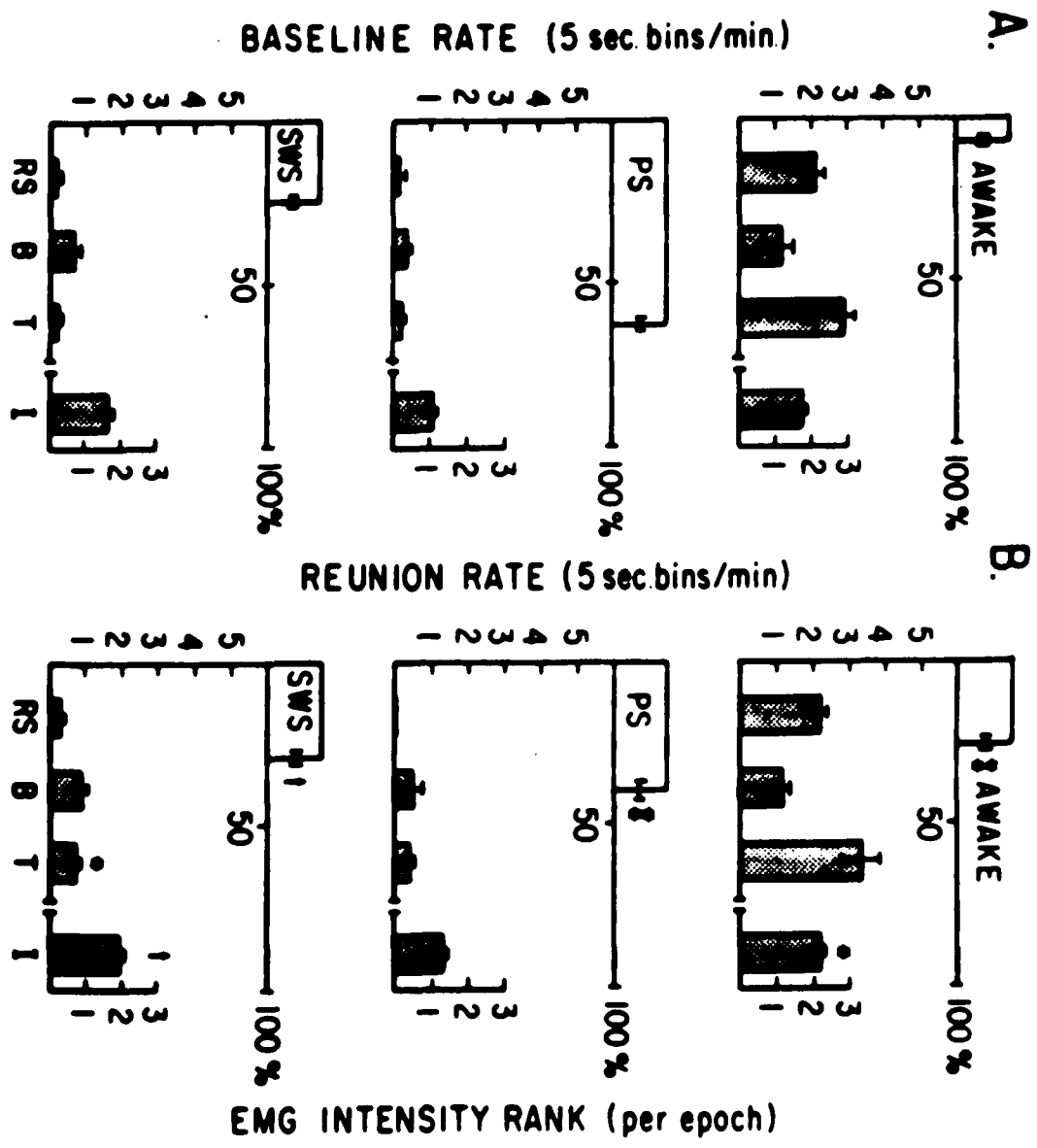
A. Baseline B. Reunion

Pups fed intragastrically during the period of separation.

The horizontal bars represent the mean percentage of total recording time spent in each sleep-wake state. The height of the three left-hand vertical bars gives the mean rate of sucking per minute for each kind of sucking. The bar to the right measures the amplitude of the digastric EMG recording when no sucking is occurring (an index of the muscle tone involved in maintaining a seal on the nipple).

EMG = electromyogram; PS = paradoxical sleep; SWS = slow wave sleep; RS = rhythmic sucking; B = bursts; T = treadles; I = EMG intensity rank; = $p < .10$; * = $p < .05$; ** = $p < .01$.

Significance indications on the graph are comparisons of the Reunion bar to its equivalent Baseline bar. These comparisons include % total recording time of a given state, sucking rates and EMG intensity within a given state. All significance findings are based on dep t-tests whose use are validated by the experimental hypotheses based on the results of the previous experiments. Overall ANOVAs are given in the Appendix.



aspect of attachment to the anesthetized dam, prevented the cycled diet from having its predicted effect. Given the series of experiment described in this work, it is tempting to assume that the lack of state normalization was related to the sucking behavior. Perhaps feedback from the sucking activity kept the pups awake more. Alternatively, one can hypothesize some central mechanism which drove both state and sucking behavior. Or, this hypothetical mechanism could have driven sleep-wake patterns which were in turn permissive for sucking rates within each state. Any of these speculations appear possible. The hypothesized central mechanism might simply be excitement. Upon Reunion, the pup's dam is arousing, whereas on Baseline day she may have a pacifying effect. Only further experiments designed specifically to address these speculations can differentiate among them.

What happened to the sucking rates within each state? The results were a bit equivocal in terms of the predicted possible results. As shown in Fig. 35A and B, the sucking rate by state did not change significantly between Baseline and Reunion days in either awake or paradoxical sleep. However, in slow wave sleep there may have been an increase in the rate of treads, based on the results of a dep t-test ($t = 3.310$, $df = p < .05$). If true, this increase in rate was the first statistically significant increase in sucking rate within a state for any comparison from Reunion to Baseline recordings in any of the three experiments. Why it should occur in this experiment, in which the pups were fed during separation, is unclear. One possibility is that the increase was due to random effects. Why might that possibility be correct? An overall ANOVA on treads/minute showed state effects, but no deprivation or interaction effects (see Appendix). Note that the

results of the t-tests and ANOVAs for all other variables (RS, B and I) were concordant. However, this experiment was specifically designed to test effects of deprivation on the rate of sucking within a state. The hypothesis demanded specific comparisons of the individual cells from Baseline to Reunion (Winer, 1971, p. 384). One other explanation for the results is that the treadle rate was abnormally low on Baseline day (see Table II) possibly due to some effect of gastric cannula implantation. The measure of EMG intensity also showed a significant increase in all three states, as had occurred in the Anesthetized Mother Experiment for slow wave sleep and awake.

Thus, in general, possible result 2 was supported, but result 4 was not totally eliminated. Rat pups can change sucking rates within states on occasion. Even increases in rate by state are possible. However, this technique is not the way that pups increase sucking upon reunion after deprivation. Rat pups increase sucking times by switching states. Paradoxical sleep, the state with the lowest sucking rates, decreases in time. Awake and slow wave sleep, with their higher rates, both increase.

GENERAL DISCUSSION

The format of the general discussion will be as follows:

1. Summary of Major Findings
2. The Relationship of the Rate of Sucking and State:
Possible Implications for Motivational Processes
3. How Pups Regulate Feeding in Response to Deprivation Level
4. Possible Functions of Sucking During Sleep
5. The Effect of Feeding on State Behavior
6. Interaction of Sleep States With Other Behavioral
Processes: Implications and Speculations

1. Summary of Major Findings

One of the principal findings of this study is that two week old rat pups are asleep much of the time while being nursed by their mothers. Tested with normal, unanesthetized dams, the percent of time in each sleep-wake state is equal in the attached and unattached conditions. Rat pups are asleep an even larger percent of the time while attached to the teats of anesthetized mothers.

Deprivation causes alterations of sleep-wake state behavior in pups tested during reunion with their dam. The percent of paradoxical sleep is reduced, while awake and possibly slow wave sleep time are increased, as is also true for pups tested without their mothers present (Hofer, 1976). Even after periodic gastric infusion of nutrient during separation, a procedure which normalizes sleep-wake behavior in young rats tested without their dams (Hofer and Shair, 1982), pups exhibit "deprivation-type" states when tested during reunion. However, despite the increase in awake time, deprived pups

still fall asleep soon after attaching to teats, before receiving any milk, and are asleep for a large proportion of the reunion test. Thus, any arousal caused by the deprivation experience is not a simple phenomenon which causes the pups to remain awake continually.

A second major finding is that pups suck while attached to the teat both while awake and during the two states of sleep. Even when tested with the normal mother, almost 50% of all sucking occurs while the pups are asleep. Characteristic rates of sucking are seen in each sleep-wake state. The pattern of those rates were similar on the baseline day in all three experiments. In general, sucking rates are highest while awake, intermediate during slow wave sleep, and lowest in paradoxical sleep. However, the sucking type called 'bursts' actually has its highest rate during slow wave sleep. Deprivation increases the amount of sucking upon reunion, as has been previously reported (Brake, Wolfson and Hofer, 1979; Brake, Sager, Sullivan and Hofer, 1982). Yet the pattern of sucking rates within each state is similar to pre-separation. This similarity leads to the third major finding of this dissertation.

The rates at which young rats suck within each state are stable across many of the experimental conditions. In general, deprivation does not change the rates of sucking within each state. In general, the young pups do not shift sucking rate by state during the time of the recording session. Yet, these sucking rates within each state are not fixed, not totally unchangeable. For example, the treadle rate while awake goes up during the milk ejection response, although no other rate within a state shows a similar increase. Another set of findings from the Anesthetized Mother Experiment illustrates this

point as well. Over a short period of time (within one day's recording session), not only does the group show no changes in sucking rates within states, but the individual pups' sucking rates are also stable. This individual stability was demonstrated by the very significant positive correlations for sucking rates by state for the members of the group, from the first to second hour of the test session. However, either the experience of deprivation and/or the time between the two recording periods decreases that individual stability (few correlations across the two days reached significance). Therefore, although as a group there were very few changes in sucking rates within a given state, the rates of any individual may have increased or decreased.

Sleep-wake state behavior has a very characteristic pattern during the defined milk ejection responses. In fact, the only times one can accurately predict what states pups will be in are during the nipple attachment process and in the period around the milk ejections. Two week old rat pups are always asleep just before milk ejection. If state behavior in the pups were randomly related to milk ejections from the dam, one would expect the pups to be asleep prior to only 70% of the milk ejections. Possible explanations for this relationship will be discussed below. The young pups are aroused briefly by the milk ejection and return quickly to sleep afterwards. Thus, it is possible that milk or some other aspect of the milk ejection experience has stimulatory properties. Even after deprivation, the rat pups show the same pattern. In this case, however, the sleep prior to milk ejection is almost exclusively slow wave sleep. Also, the pups stay awake longer during the milk ejection response. Possible reasons for this longer period awake will be considered below.

The sucking behavior also shows characteristic changes during milk ejection responses. Treadles and rhythmic sucking are implicated as nutritive sucking types in that they both increase. And, those increases are longer lasting after deprivation, especially for treadles.

2. Relationship of Sucking Levels and State: Possible Implications for Motivational Processes

As has been shown in this dissertation, the rate for each type of sucking within each of the three sleep-wake states is a stable phenomenon. This idea does not imply that sucks are emitted at precise intervals within each state, under the control of some central pattern generator. Rather, the implication is that the relative frequency at which sucks of a certain type are distributed is related to the state of the animal. That frequency would be highest in the awake state for treadling, but highest in slow wave sleep for bursts.

Changes in state have been linked to changes in the frequency of many behavioral and physiological events. This concept is not new with this dissertation. The behaviors used to define the states themselves certainly fit this category. For example, phasic twitches, one of the defining characteristics of paradoxical sleep, are also seen during other states as well, but at a much lower frequency (Petre-Quadens, 1974). Other kinds of behavior also have state-linked frequencies. In infant humans, rhythmic mouthing is highest during slow wave sleep; reflex smiles are highest during paradoxical sleep (Korner, 1969). The regulation of the cardiovascular system changes during the different states (Combs, 1982). In fact, the amount of

heart rate variability, which is largest during paradoxical sleep, has been used as a way of monitoring behavioral states in fetuses (Junge, 1979). Michael Chase (1972) has demonstrated that amplitude of the reflex responses of the jaw muscles of the cat is modulated differently, depending upon state. The masseter muscle EMG is greatest while awake, intermediate in quiet sleep, and lowest in active sleep. The digastric reflex response has its peak amplitude during quiet sleep, smaller while awake, and smallest in active sleep.

The rate of sucking itself has previously been linked to the behavioral state of the organism. In a study of the effect of food satiation and auditory stimulation on the nonnutritive sucking of human infants, Kaye (1966) states, "It would appear that within the current testing procedures the sucking rates are 'locked' by the general level of arousal, the latter being determined in part by pretest ingestional factors. Only stimuli altering the level of arousal will change the sucking rate." Wolff (1972) makes similar points based on his study of healthy and neurologically impaired infants. He even suggests that the basic sucking frequency "is controlled by central oscillatory mechanisms which can operate independent of peripheral input." He goes on to say, "Nowhere was it assumed, however, that such hypothetical oscillators would actually function independent of environmental or peripheral influences in the intact organism." Of course, there are many differences between these two studies and this dissertation. Different species were used. Drs. Wolff and Kaye are only considering nonnutritive sucking in the results discussed above. By changes in arousal level, Dr. Kaye means arousal level while awake (although Dr. Wolff is referring to sleep-

wake states). Despite these differences, their observations agree very well with the results of this dissertation. In the rat, levels of sucking also seem set by behavioral state. But, as shown by the higher rate of rhythmic sucking in all three sleep-wake states in the Normal Mother Experiment than in the Anesthetized Mother Experiment, environmental and/or peripheral influences can alter those levels.

This dissertation extends the findings of Drs. Wolff and Kaye by showing the stability of the sucking rates within each state in response to deprivation. This finding of stability may have implications for understanding how changes in motivation affect an organism's behavior.

Any discussion of motivation has certain circularity built into it. We define motivational levels in terms of the response of an organism. We assume that rat pups, upon reunion from deprivation, have a high motivation to get milk and to suck. The evidence for this assumption is the increased levels of sucking (Brake, Wolfson and Hofer, 1979) and of intake (e.g., Houpt and Houpt, 1975). The rat pups could respond to deprivation by showing increased levels of sucking in all states, or even just while awake. In fact, evidence for central coding of motivational state in response to hunger has been found during both paradoxical and slow wave sleep (Jacobs, Harper and McGinty, 1970). Yet, the pups described in this dissertation suck at the same rate within each state in response to a deprivation stress. The way their behavior does change with deprivation is to snift sleep-wake states. Paradoxical sleep is reduced while awake and slow wave sleep are increased. This shift is away from the state with the lowest rates of sucking.

Which comes first, the state change or the sucking change? My dissertation supplies no clear answer to that question. Yet, state changes in rats tested without mothers are almost identical to my results with rats tested attached to their mother's teat (Hofer, 1976; Hofer and Shair, 1982). It is tempting to think that the state change is the primary response to deprivation and that that change sets the level of sucking.

Dr. Wolff might agree with that speculation. He states (1971), "Hunger satiation has remarkably little influence on the temporal organization of nonnutritive sucking; the changes of sucking rate observed in the course of gastrostomy feeding were probably due to changes in state rather than the direct effects of gastric loading."

3. How Pups Regulate Feeding Based on Deprivation Levels

As discussed in the Introduction, there has been a controversy in the literature about deprivation effects on feeding in young rats. Pups prior to approximately two weeks of age when attached to teat of an anesthetized dam but given milk through a tongue cannula will continue to take in milk until seriously overengorged, even to the point of respiratory distress (e.g., Hall and Rosenblatt, 1978). And, stomach loading has been reported to affect neither attachment nor sucking levels on nonnutritive nipples (e.g., Brake, Sager, Sullivan and Hofer, 1982). Yet, rats of the same age tested while unattached do regulate intake (Hall and Bryan, 1980). Also, rats of these young ages will increase intake over nondeprived pups when returned to normal litter nursing situations (e.g., Houpt and Houpt, 1975).

The more recent work of Brake and his colleagues and the results in this thesis suggest a resolution to this controversy. In the natural nursing situation, there is a limited amount of milk available and it comes only periodically. Based on the defined milk ejection responses, deprived pups suck longer and more vigorously following milk pulses (Brake, Sullivan, Sager and Hofer, 1982; Normal Mother Experiment: Baseline and Reunion). Deprived pups are also awake longer following milk ejection and then are more likely to enter slow wave sleep rather than paradoxical -- awake and slow wave being the states with higher rates of sucking. Thus, in the normal situation, deprived pups may extract more milk from each milk ejection. This possibility is now being tested directly.

In the tongue cannula preparation, the pup is not only being required to extract milk, but also it is required to demonstrate satiation by detaching from the nipple. This is a very different requirement than merely being asked to extract milk. Young rats infrequently leave the teat, even when not receiving any milk reinforcement. In the natural nursing situation, the dam terminates the nursing bouts, in a process controlled by thermoregulation (Leon, Adels and Coopersmith, 1985). Pups younger than approximately 14 days will remain attached to an anesthetized dam for six hours and even longer (Brake and Hofer, 1980; Drewett, 1983). Prolonged attachment will reduce the amount of sucking of pups tested in a nonnutritive preparation (Brake, Sager, Sullivan and Hofer, 1982). Thus, sucking appears to be under the same kinds of motivational controls as ingestion.

It is the interaction of the two motivational systems which explains the disparate results in the feeding literature. Young pups implanted with tongue cannulae and attached to an anesthetized dam over-ingest because they are motivated to suck and cannot avoid the concurrent intake. In fact, the rate at which these food-satiated pups suck and ingest is less than the rate of deprived pups (Brake, Sager, Sullivan and Hofer, 1982). Their decreased motivation to ingest depresses the rate of sucking.

Another distinction between the different types of feeding studies while attached to the teat of anesthetized dams must be made. In some, the milk is given to the pups in a continuous fashion. In others, the milk delivery is pulsatile, more accurately mimicking the natural situation. We are aware that milk has stimulative properties. It wakes the pups up as demonstrated in this thesis. It also increases sucking levels, wiping out the distinction between deprived and nondeprived pups (Brake, Sager, Sullivan and Hofer, 1982). In some unpublished work, it has been demonstrated that pups will sleep through very small and/or slow infusions of milk through a tongue cannula, but will wake up to larger or more rapid infusions (Shair, Gottschalk, Brake and Hofer, 1983). In response to large pulses through a tongue cannula, the pups are probably awakened for long periods of time due to the stimulation of the milk. Thus, a ceiling effect may be involved. The pups may all be sucking at a maximum rate, and therefore unable to show a distinction between deprived and nondeprived animals.

There are more speculations which can be made about the possible effects of deprivation levels on aspects of the feeding interaction. It is known that sucking stimulation by the pups is necessary to

induce milk letdown by the dam (Drewett and Trew, 1978). It is possible that deprived pups, with their increased amount of time spent sucking, may stimulate larger and/or more frequent milk ejections. This idea has not been tested as yet. In this thesis only three pups of the litter were deprived and they were not able to elicit more frequent milk ejections. If the entire litter were deprived, the results might have been different.

Another intriguing possibility, based on as yet unpublished work by Brake, is that everything stated for deprived vs. nondeprived pups may also be true for competent and incompetent feeders. It will be interesting to monitor sleep-wake state behavior in pups who are small for gestational age vs. those who are large for gestational age. It is conceivable that smaller pups may not stay awake as long after a milk ejection. Thus, their sucking rates might be lower and intake less. At least, this idea is testable.

4. Possible Functions of Sucking During Sleep

Why do rat pups suck during the two sleep states? One idea is based on the work showing that sucking is necessary to induce milk ejection (Drewett and Trew, (1978). The mother rat must be asleep for milk ejections to occur (Lincoln, Hentzen, Hin, Vunder Schoot, Clarke and Summerlee, 1980; Voloschin and Tramezzani, 1979). Perhaps the optimal stimulus for the mother rat is quiet (i.e., sleeping) pups who are sucking. The sleeping pups will not disturb their mother and thus allow her to go to sleep as well. It is possible to imagine that for this reason sucking evolved during sleep. Such speculation provides an 'ultimate' reason for the finding that rat pups were always asleep

prior to the milk ejection response on both baseline and reunion days. How this ultimate function was translated into the physiology and behavior of the individual pup is of course unknown. One explanation is based on statistical probabilities and the idea of the optimal stimulus described above. When the entire litter is asleep, the dam is most likely to be able to enter slow wave sleep herself. Therefore, milk ejections should be most probable at these times.

Sucking during sleep might also be explained as an aid to digestion. Sucking stimulates saliva production. It has also been suggested that sucking may increase gastric motility. It would be interesting to test the digestive capabilities of animals given equal amounts of food intragastrically. If half the pups were allowed to suck normally and half prevented from sucking, would the food be digested better/more quickly by those pups allowed to suck?

A final theoretical idea is that sucking during sleep may allow pups to extract every drop of milk available from the mother's teat. Even though milk ejections are periodic, small amounts of milk may be available between milk ejections. Whether milk is available in these intervals has yet to be tested directly. However, an anesthetized mother who has been deprived of her pups for one day prior to anesthesia has milk in her teats that can be withdrawn prior to any oxytocin injection (Lincoln, Hill and Wakerley, 1973). Thus it is possible that milk does build up in the teats in the normal situation. If such milk is available, the pilot work mentioned above demonstrates that 14 day old rats can ingest small quantities of milk while asleep (Shair, Gottschalk, Brake and Hofer, 1983). The pups can swallow milk in either paradoxical or slow wave sleep. However, the

amount that can be ingested while remaining asleep is approximately twice as large in slow wave than in paradoxical sleep. This fits very well with the reduction in paradoxical sleep seen after deprivation. Whether or not the two findings are actually related more than coincidentally is yet unknown.

5. The Effect of Feeding on State Behavior

As discussed in the Introduction, many studies have shown that state regulation and feeding behavior are linked. This is true at many different ages, in many different species, and studied with many different preparations. One typical finding is that the amount of paradoxical sleep is decreased in food-deprived animals (Danguir and Nicolaidis, 1979). Similar results were found in this dissertation in the Normal Mother and Anesthetized Mother Experiments. Yet, food deprivation is not the only cause for this reduction in paradoxical sleep. Giving pups nutrient on a periodic schedule during deprivation causes pups to gain weight at normal levels and when tested without the mother present, normalizes sleep-wake state behavior (Hofer and Shair, 1982). However, if the pups are tested attached to an anesthetized dam, delivery of food during the deprivation period has no effect on state patterns (Cycled Diet Experiment). This finding leads one to postulate the greater stimulative properties of the dam to a sucking-deprived pup. In a future experiment, I plan to give pups sucking experience during deprivation either with or without cycled diet, then test while attached to an anesthetized mother.

Short-term linkages between feeding and sleep have also been reported. In fact, falling asleep after feeding has been described as

part of the satiety sequence in rats (e.g., Mansbach and Lorenz, 1983). Also, infusion of nutrient into the gut causes almost immediate changes in the EEG (e.g., Sudakov, 1965). In the Normal Mother Experiment as well, a linkage between feeding and sleep was also found. The pups were always asleep prior to milk ejection, even after the deprivation experience. They were awakened by milk ejections and quickly fell back to sleep. Perhaps the milk ejection and/or the nursing bouts serve as zeitgebers for the setting of the sleep-wake cycles. Milk in the gut may cause animals to go to sleep. This could either be an immediate mechanism of taste or some sensation in the gut causing over a neural pathway to the brain. Also, there could be some longer latency effect of food on the stomach and digestion which influences the sleep-wake state cycles.

Another possibility is that rather than milk causing sleep, milk may wake the pup up out of sleep due to its stimulative properties. The milk may keep the pup awake as long as it is flowing. This awake time may reset the sleep-wake cycles. This idea is often not considered by researchers in the field. For example, Wolff (1972) describes a study of the effects of feeding on state in normal infants vs. infants fed through gastrostomy. Infants fed in a normal fashion are more frequently asleep 30 min after the feeding than infants fed through a stomach tube. Dr. Wolff takes this as evidence that the sucking as well as feeding has an influence on sleep-wake states. However, he does not comment on the fact that half the gastrostomy infants are asleep during the feeding whereas all of the normal infants are awake. Thus, it seems likely that the normal operation of

the sleep-wake cycle would cause fewer gastrostomy infants to be asleep in the period following the feeding.

It is even possible that the sleep-wake and feeding interaction is learned or partly learned during development. In the young rat, each milk ejection appears associated with a very specific change in sleep-wake states. The temporal pairing of milk in the mouth and/or gut with the rapid transition into sleep may provide the substrate for Pavlovian conditioning. It is also possible that instrumental processes may be involved. Perhaps the more rapidly the pups return to sleep, the quicker the dam can reenter slow wave sleep and provide another milk ejection. Thus, the pups rapid return to sleep is a conditioned anticipatory response. In older animals in which feeding takes place independently, the response of postprandial sleep may have been learned in part by this earlier association of falling asleep upon the teat. It is possible to test these speculations by raising pups fed on different schedules using the pup-in-cup technique.

6. Interaction of Sleep States with Other Behavioral Processes:

Implications and Speculations

Learning has been shown to occur during feeding in the young rat (Brake, 1981; Martin and Alberts, 1979). Yet, in this dissertation it has been shown that pups are awake only briefly following a milk ejection. How can learning occur in this very brief time? There are several possibilities. One is that some learning experiments are not naturalistic. The pup may be kept awake longer by a longer infusion of milk. In those studies in which learning has been shown to occur in the natural situation, the possibility exists that milk is so

stimulating that it causes attention to all other stimuli in the environment, even during that brief time. The work of Regina Sullivan (Sullivan, Hofer and Brake, in press; Sullivan, Brake, Hofer and Williams, in press) has shown that stimulation can be important in promoting learning in the young rat.

Attachment between the young rat pup and the dam is assumed to develop at least in part during the nursing period. Again, as in the paragraph above, how can such attachment occur in the brief period of awake time? One possibility is that the stimulative properties discussed above may be important in the attachment phenomenon as well. Also, one must remember that milk ejection experience and awakening thereafter is repeated over and over again as the pup grows. This repetition allows more time for attachment to develop.

In adults, many physiological processes are linked to sleep-wake states in a circadian or ultradian manner. Among those are hormone secretion (Åkerstedt, 1984), cardiovascular regulation (Combs, 1982), and thermoregulation (Obál, 1984). Often these processes are not linked very early in development. Perhaps the milk ejection and/or nursing bouts are zeitgebers for these processes as well. This could be through either immediate or through longer, i.e. digestive, pathways. As an example of this, a recent finding from our lab is that blood pressure shows an abrupt transient increase to milk ejection (Shair, Brake, Hofer and Myers, 1986). This blood pressure response always accompanies milk ejection and no similar transient blood pressure changes are seen at any other point in the normal behavior of the young pup. Thus the milk ejection has been shown to be linked to both sleep-wake state changes and cardiovascular changes. This linkage

may provide the substrate by which sleep-wake state and cardiovascular systems become linked together during development. Further experiments are needed to test this somewhat speculative idea.

What is the relationship between the phenomenon described in the young rat to similar processes in humans and other species? One similarity is that many species have sucking behavior while asleep (Emde and Metcalf, 1970; Jouvet-Mounier, Astic and LaCote, 1970). However, it remains to be tested systematically as to whether such sucking will occur in and around a nutritive feeding situation. Secondly, as discussed above, many species including humans fall asleep soon after feeding. One difference is that humans and many other species get milk in a more continuous flow. During a 10 minute feed, breast-fed human infants take in almost 90% of all the milk within four minutes. The last half of the feed is almost nonnutritive (Lucas, Lucas and Baum, 1979). This difference in milk flow may have important effects on the response of the organism to the feeding situation. Perhaps human infants are kept awake longer by the stimulative properties of milk. They then may sleep longer afterwards. It is even possible that the length of the sleep-wake cycle may in part be set by the feeding experience. A more continuous flow may be too great to allow any sleep during the feeding situation, at least in the beginning of the nursing bout. In goats (Ruckebush, 1972) the young are awake while receiving milk. To the best of my knowledge, sleep-wake states have not been studied systematically in any other species during the actual intake of food. I would like to

do a study with human infants studying sleep-wake states during feeding. There are anecdotal reports of infants asleep at their mother's breasts.

APPENDIX

Overall ANOVAs which are presented in this section would have fit awkwardly in any other chapter. These ANOVAs justify many of the dependent and independent t-tests used for comparisons of smaller portions of the data, as reported in the text, figures and tables.

As an example, in the discussion of sleep latencies in the Normal Mother Experiment (Tables C and M), comparisons could be made between the latencies Attached and Not-Attached on both Baseline and Reunion days, as well as between the two days. For this one variable, there might be a total of six comparisons by dep t-tests within and between the two tables.

Clearly some justification is necessary to allow the performance of so many tests. That reason is supplied by a two-way ANOVA with repeated measures on factors A and B, where A is Day of Test and B is Attachment Condition. If both main effects are statistically significant and/or the interaction is significant, then comparisons of the individual cells of the matrix are justified.

As you will see below, in this example, both main effects reach significance. Thus all six comparisons are statistically justified. However, as you will notice by looking at Tables C, M and N, only three individual comparisons were actually carried out. These three address specific points raised in the text. The other contrasts were not relevant to this study, even though statistically allowable.

The example above illustrates standard statistical procedure. In a few cases, I use the statistics in a less traditional manner. These cases can be grouped into two classes. The descriptions and rationales are given below.

1. In some tables, I report non-significant dep t-test results whose use is not justified by the results of the overall ANOVA. If the ANOVA results are not significant, then carrying out the t-tests is not justified. These t-tests are presented merely for the ease of the reader, to make it clear that the differences are not significant.

2. In some two-way ANOVAs with a single main effect the only significant result, I nevertheless make comparisons of the individual cells. An example is the comparisons of rhythmic sucking(RS) rates from the Normal Mother Experiment (Fig.'s 10 and 17). There was a significant State effect on rate of RS, but no effect of Day of Test or interaction.

In this case, the tests seemed to me to be based on common sense. The ANOVA had already indicated that the sucking rates differed based on state and there was no interaction with test session. Thus, any dep t-tests comparisons between states on a single day were more likely to be insignificant due to smaller n's than a similar comparison using data from both days. Therefore, I felt I could have reasonable confidence in any significant results obtained, even though the comparisons were post hoc.

After the Normal Mother Experiment had been analysed, specific

hypotheses were generated and tested in the later experiments. The t-tests were planned comparisons. No overall ANOVA is needed to justify this kind of test (Winer, 1971, p. 384). The ANOVAs were performed to see if any unexpected results were significant and to provide information for researchers interested in testing hypotheses other than those presented here.

TWO-WAY ANOVAS WITH REPEATED MEASURES ON A and B

Normal Mother Experiment

Tables C,M+N: Factor A Day of Test
Factor B Attachment Condition

	SOURCE	SS	DF	MS	F RATIO
Sleep Latency	SWG	3.780	5	.756	
	A	11.718	1	11.718	4.962*
	B	28.798	1	28.798	12.195**
	AB	2.059	1	2.059	.872
	Resid	35.422	15	2.361	
	AxSWG	9.180	5	1.836	
	BxSWG	2.224	5	.445	
	ABxSWG	24.018	5	4.804	
% Time Awake	SWG	477.359	5	95.472	
	A	5828.19	1	5828.19	31.791**
	B	1568.22	1	1568.22	8.554**
	AB	988.137	1	988.137	5.390*
	Resid	2749.95	15	183.33	
	AxSWG	576.313	5	115.263	
	BxSWG	993.273	5	198.655	
	ABxSWG	1180.36	5	236.072	
% Time in PS	SWG	149.226	5	29.845	
	A	5192.05	1	5192.05	44.598**
	B	513.382	1	513.382	4.010+
	AB	2.038	1	2.038	.018
	Resid	1746.28	15	116.419	
	AxSWG	689.200	5	137.840	

	AxSWG	25.224	5	5.045	
	BxSWG	147.228	5	29.446	
	ABxSWG	115.228	5	23.038	
PS #Epi/hr	SWG	62.833	5	12.567	
	A	468.165	1	468.165	21.597**
	B	37.499	1	37.499	1.730
	AB	104.170	1	104.170	4.805*
	Resid	325.166	15	21.678	
	AxSWG	114.835	5	22.967	
	BxSWG	173.501	5	34.700	
	ABxSWG	36.830	5	7.366	
SWS #Epi/hr	SWG	441.215	5	88.243	
	A	.379	1	.379	.011
	B	672.049	1	672.243	19.990**
	AB	12.036	1	12.036	.358
	Resid	504.285	15	33.619	
	AxSWG	71.871	5	14.374	
	BxSWG	251.203	5	50.241	
	ABxSWG	181.211	5	36.242	
State Trans/hr	SWG	931.820	5	186.364	
	A	486.180	1	486.180	2.928
	B	2730.64	1	2730.64	17.077**
	AB	112.703	1	112.703	.705
	Resid	2398.52	15	159.901	
	AxSWG	167.844	5	33.569	
	BxSWG	1627.400	5	325.480	
	ABxSWG	603.277	5	120.655	

	BxSWG	494.869	5	98.974	
	ABxSWG	562.213	5	112.443	
% Time in SWS	SWG	589.484	5	117.897	
	A	20.166	1	20.166	.163
	B	3800.15	1	3800.15	30.770**
	AB	888.182	1	888.182	7.192*
	Resid	1852.53	15	123.502	
	AxSWG	247.840	5	49.568	
	BxSWG	1068.86	5	213.771	
	ABxSWG	535.830	5	107.166	
A Mean Dura.	SWG	4.847	5	.969	
	A	9.338	1	9.338	7.001*
	B	10.787	1	10.787	8.088*
	AB	2.835	1	2.836	2.126
	Resid	20.006	15	1.334	
	AxSWG	5.388	5	1.078	
	BxSWG	7.821	5	1.564	
	ABxSWG	6.780	5	1.359	
PS Mean Dura.	SWG	.244	2	.122	
	A	1.519	1	1.519	4.182+
	B	9.991	1	9.991	27.502**
	AB	.612	1	.612	1.684
	Resid	2.180	6	.363	
	AxSWG	.685	2	.343	
	BxSWG	.567	2	.283	
	ABxSWG	.928	2	.464	
SWS Mean Dura.	SWG	2.805	4	.701	
	A	.065	1	.064	1.074
	B	.318	1	.318	5.248*
	AB	.977	1	.977	16.146**
	Resid	.726	12	.061	
	AxSWG	.093	4	.023	
	BxSWG	.758	4	.190	
	ABxSWG	-.125	4	-.031	
A #Epi/hr	SWG	126.862	5	25.372	
	A	.029	1	.029	.001
	B	425.028	1	425.028	22.164**
	AB	22.053	1	22.053	1.150
	Resid	287.643	15	19.176	

TWO-WAY ANOVAS WITH REPEATED MEASURES ON A and B

Normal Mother Experiment

Fig.'s 10 + 17: Factor A Day of Test
Factor B Sleep-Wake State

	SOURCE	SS	DF	MS	F RATIO	
RS/min.	SWG	4.855	3	1.618		
	A	.041	1	.041	.043	
	B	41.202	2	20.601	21.518**	
	AB	.021	2	.010	.011	
	Resid	14.361	15	.957		
	AxSWG	4.722	3	1.574		
	BxSWG	6.584	6	1.097		
	ABxSWG	3.056	6	.509		
	B/min.	SWG	.431	3	.144	
		A	.226	1	.226	1.918
B		8.490	2	4.245	35.993**	
AB		2.016	2	1.008	8.549**	
Resid		1.769	15	.118		
AxSWG		.260	3	.087		
BxSWG		.604	6	.101		
ABxSWG		.905	6	.151		
T/min.		SWG	17.245	3	5.748	
		A	3.581	1	3.581	6.993*
	B	83.784	2	41.892	81.821**	
	AB	3.453	2	1.727	3.372+	
	Resid	7.680	15	.512		
	AxSWG	1.264	3	.421		
	BxSWG	5.740	6	.957		
	ABxSWG	.677	6	.113		
	EMG I/epoch	SWG	1.291	3	.430	
		A	.034	1	.034	.183
B		8.941	2	4.470	24.241**	
AB		.257	2	.129	.698	
Resid		2.766	15	.184		
AxSWG		1.571	3	.524		
BxSWG		.242	6	.040		
ABxSWG		.953	6	.159		

Tables B + L: Factor A Day of Test
Factor B Pre- vs. Post-Test

Weight	SWG	143.490	5	28.698		
	A	10.277	1	10.277	22.271**	
	B	2.611	1	2.611	5.659*	
	AB	1.064	1	1.064	2.307	
	Resid	6.922	15	.461		
	AxSWG	1.582	5	.316		
	BxSWG	3.020	5	.604		
	ABxSWG	2.320	5	.464		
	Temperature	SWG	3.469	5	.6937	
		A	.748	1	.748	3.090+
B		3.238	1	3.238	13.378**	
AB		.660	1	.661	2.727	
Resid		3.631	15	.242		
AxSWG		.244	5	.049		
BxSWG		1.260	5	.252		
ABxSWG		2.127	5	.425		

Fig.'s 9 + 15: Factor A Day of Test
Factor B Direction of State Transition

Trans. Prob.	SWG	.000	5	.000	
	A	.000	1	.000	.000
	B	.969	5	.194	42.383**
	AB	.362	5	.072	15.827**
	Resid	.251	55	.004	
	AxSWG	.000	5	.000	
	BxSWG	.136	25	.005	
	ABxSWG	.115	25	.005	

TWO-WAY ANOVAS WITH REPEATED MEASURES ON B

Anesthetized Mother Experiment
Comparison with Normal Mother BaselineFig.'s 8 + 20: Factor A Experiment
Factor B Episode Duration

	SOURCE	SS	DF	MS	F RATIO
Awake	Between Subj				
	A	0	1	0	0
	SWG	.318	10	.032	
	Within Subj				
	B	42571.7	4	10642.9	175.34**
	AB	279.845	4	69.961	1.153
	BxSWG	2427.96	40	60.699	
PS	Between Subj				
	A	.009	1	.009	.155
	SWG	.566	10	.057	
	Within Subj				
	B	7697.71	4	1924.43	9.687**
	AB	1108.09	4	277.022	1.394
	BxSWG	7946.62	40	198.665	
SWS	Between Subj				
	A	0	1	0	0
	SWG	1.557	10	.156	
	Within Subj				
	B	13491.1	4	3372.78	35.258**
	AB	1150.83	4	287.706	3.008*
	BxSWG	3826.46	40	95.661	

Fig.'s 9 + 21: Factor A Experiment
Factor B Direction of State Transition

Trans. Prob.	Between Subj				
	A	.000	1	.000	1.776
	SWG	.000	10	.000	
	Within Subj				
	B	.195	5	.039	6.888**
	AB	.210	5	.042	7.445**
	BxSWG	.282	50	.000	

TWO-WAY ANOVAS WITH REPEATED MEASURES ON A and B

Anesthetized Mother Experiment

Tables U + BB: Factor A Day of Test
Factor B Pre- vs. Post-Test

	SOURCE	SS	DF	MS	F RATIO	
Weight	SWG	122.605	5	24.521		
	A	35.779	1	35.779	286.533**	
	B	.572	1	.572	4.583*	
	AB	.018	1	.018	.141	
	Resid	1.873	15	.125		
	AxSWG	1.764	5	.353		
	BxSWG	.035	5	.007		
	ABxSWG	.074	5	.015		
	Temperature	SWG	2.986	5	.597	
		A	6.389	1	6.389	24.619**
B		-.023	1	-.023	-.090	
AB		.105	1	.105	.406	
Resid		3.892	15	.260		
AxSWG		2.891	5	.578		
BxSWG		.428	5	.086		
ABxSWG		.574	5	.115		

Fig. 29: Factor A Day of Test
Factor B Episode Duration

Awake	SWG	4.293	5	.859		
	A	.021	1	.021	.000	
	B	41878.0	4	10469.5	165.338**	
	AB	309.197	4	77.299	1.221	
	Resid	2849.48	45	63.322		
	AxSWG	4.273	5	.855		
	BxSWG	1663.22	20	83.161		
	ABxSWG	1181.99	20	59.099		
	PS	SWG	.578	5	.116	
		A	.164	1	.164	.003
B		12460.3	4	3115.07	64.461**	
AB		2691.22	4	672.806	13.922**	

	Resid	2174.63	45	48.325	
	AxSWG	.135	5	.027	
	BxSWG	854.492	20	42.725	
	ABxSWG	1320.0	20	66.0	
SWS	SWG	38.818	5	7.764	
	A	.078	1	.078	.001
	B	11460.5	4	2865.12	46.397**
	AB	1327.76	4	331.939	5.376**
	Resid	2778.87	45	61.753	
	AxSWG	5.119	5	7.764	
	BxSWG	1973.69	20	98.685	
	ABxSWG	800.053	20	40.003	

Fig. 30: Factor A Day of Test
Factor B Direction of State Transition

Trans. Prob.	SWG	.000	5	.000	
	A	.000	1	.000	.000
	B	.270	5	.054	11.324**
	AB	.211	5	.042	8.845**
	Resid	.262	55	.005	
	AxSWG	.000	5	.000	
	BxSWG	.211	25	.008	
	ABxSWG	.052	25	.002	

Fig.'s 22 + 32: Factor A Day of Test
Factor B Sleep-Wake State

RS/min.	SWG	11.379	5	2.276	
	A	4.134	1	4.134	2.226
	B	16.921	2	8.460	4.555*
	AB	7.671	2	3.835	2.065
	Resid	46.434	25	1.857	
	AxSWG	9.312	5	1.862	
	BxSWG	20.440	10	2.044	
	ABxSWG	16.683	10	1.668	
B/min.	SWG	1.809	5	.362	
	A	.234	1	.234	.506
	B	6.812	2	3.406	7.379**
	AB	1.054	2	.527	1.142
	Resid	11.539	25	.462	

	AxSWG	1.567	5	.313	
	BxSWG	8.422	10	.842	
	ABxSWG	1.553	10	.155	
T/min.	SWG	14.842	5	2.969	
	A	1.068	1	1.068	.704
	B	124.141	2	62.070	40.914**
	AB	3.270	2	1.635	1.078
	Resid	37.928	25	1.517	
	AxSWG	5.859	5	1.172	
	BxSWG	16.006	10	1.601	
	ABxSWG	16.063	10	1.606	
EMG I/epoch	SWG	2.646	5	.529	
	A	1.734	1	1.734	24.045**
	B	6.792	2	3.396	47.100**
	AB	.074	2	.037	.513
	Resid	1.802	25	.072	
	AxSWG	.348	5	.070	
	BxSWG	.805	10	.081	
	ABxSWG	.649	10	.065	
%Time in State	SWG	.594	5	.119	
	A	.125	1	.125	.001
	B	1435.75	2	717.877	3.495+
	AB	6418.68	2	3209.34	15.624**
	Resid	5135.44	25	205.418	
	AxSWG	.535	5	.107	
	BxSWG	2993.22	10	299.322	
	ABxSWG	2141.68	10	214.168	

TWO-WAY ANOVAS WITH REPEATED MEASURES ON A and B

Cycled Diet Experiment

Fig. 35: Factor A Day of Test
Factor B Sleep-Wake State

	SOURCE	SS	DF	MS	F RATIO	
RS/min.	SWG	1.122	5	.224		
	A	.004	1	.004	.042	
	B	34.357	2	17.179	163.641**	
	AB	.054	2	.027	.257	
	Resid	2.624	25	.105		
	AxSWG	.409	5	.082		
	BxSWG	1.343	10	.134		
	ABxSWG	.873	10	.087		
	B/min.	SWG	2.893	5	.579	
		A	.111	1	.111	.695
B		3.332	2	1.666	10.420**	
AB		.107	2	.054	.335	
Resid		3.997	25	.160		
AxSWG		1.096	5	.219		
BxSWG		1.755	10	.176		
ABxSWG		1.146	10	.115		
T/min.		SWG	2.913	5	.583	
		A	1.068	1	1.068	3.639+
	B	58.862	2	29.431	100.287**	
	AB	.271	2	.135	.461	
	Resid	7.337	25	.263		
	AxSWG	1.802	5	.360		
	BxSWG	4.105	10	.410		
	ABxSWG	1.429	10	.143		
	EMG I/epoch	SWG	.834	5	.167	
		A	.734	1	.734	20.714**
B		4.413	2	2.207	62.284**	
AB		.026	2	.013	.341	
Resid		.886	25	.035		
AxSWG		.410	5	.082		
BxSWG		.234	10	.023		
ABxSWG		.241	10	.024		

%Time in State	SWG	.492	5	.098	
	A	.020	1	.020	.000
	B	6425.74	2	3212.87	58.629**
	AB	1768.07	2	884.037	16.132**
	Resid	1370.00	25	54.800	
	AxSWG	.480	5	.096	
	BxSWG	812.594	10	81.259	
	ABxSWG	556.922	10	55.692	

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