Oxytocin Selectively Increases Holding and Licking of Neonates in Preweanling but Not Postweanling Juvenile Rats

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Preweanling rats exhibit components of maternal behavior (MB) after brief periods of contact with neonates; the latency of onset of MB rises considerably after weaning. Oxytocin (OXT) stimulates MB in adult rats. The effects of intracisternal (IC) administration of OXT (2 µg) on pup-directed and other behaviors in preweanling and postweanling juvenile rats were tested. Compared with saline and no treatment, OXT significantly increased active holding of pups in preweanling but not postweanling juvenile rats. No other components of adultlike MB were stimulated by OXT. OXT also decreased inactive touching of pups and robustly increased self-grooming in juveniles at all ages tested. It is concluded that OXT facilitation of active pup-holding and licking in preweanling rats may be an extension of OXT-induced self-grooming to pups and may also be related to OXT activation of MB in adult rats.

Naive adult rats initially avoid physical contact with neonates (Terkel & Rosenblatt, 1971) and display maternal behavior (MB) only if they are kept in close proximity to young pups for extended periods of time (Rosenblatt, 1967). Hormonal events late in gestation are essential for the rapid onset of postpartum MB (Numan, 1988; Rosenblatt, Mayer, & Siegel, 1985). In contrast, preweanling juvenile rats (females and males) do not avoid physical contact with neonates and, indeed, display some components of adult MB after relatively brief periods of exposure to young pups. Bridges, Zarrow, Goldman, and Denenberg (1974) observed a significantly shorter latency of onset of pup retrieval in 24-day-old rats compared with older juvenile rats. Mayer and Rosenblatt (1979) reported significantly more active pup-directed behavior (touching, sniffing, licking, and moving pups) in 20- and 22-day-old rats than in 26-day-old rats. Brunelli, Shindledecker, and Hofer (1985) found that during continuous exposure to young pups that were 18, 24, and 30 days old rats failed to display all components of MB in the persistent and integrated manner typical of postpartum rat mothers. However, many components of adultlike MB did emerge, with retrieval and licking occurring more often than crouching in a nursing posture or nest building. Twenty-four-day-old rats displayed retrieval more rapidly than older juvenile rats. The frequency of physical contact with pups declined with increasing age.

Weaning occurs gradually in rats between 20 and 30 days of age (Galef, 1981). Nursing drops to a very low frequency by 26 days of age. As weaning progresses, juvenile rats display increasing emotionality and defensive reactions in response to novel and aversive stimuli (Bronstein & Hirsch, 1976; Candland & Campbell, 1962; Livesey & Egger, 1970). It has been suggested that active avoidance of pups and the increasing latency of onset of MB that occurs with the completion of weaning are related to the emergence of neophobia (Mayer & Rosenblatt, 1979).

We and others (Fahrbach, Morrell, & Pfaff, 1984; Pedersen, Ascher, Monroe, & Prange, 1982; Pedersen & Prange, 1979; Wamboldt & Insel, 1987) observed that central administration of the neuropeptide oxytocin (OXT) induces rapid onset of MB in naive estrogen-treated female rats. In more recent studies, OXT has been reported to stimulate MB in mice (McCarty, Bare, & vom Saal, 1986) and sheep (Kennel, Keverne, & Baldwin, 1987). Central administration of OXT antiserum or an OXT antagonist blocked the onset of MB in ovarian steroid-treated nulliparous rats (Fahrbach, Morrell, & Pfaff, 1985; Pedersen, Caldwell, Johnson, Fort, & Prange, 1985) and postpartum rats (van Leengoed, Kerker, & Swanson, 1987) thereby suggesting that OXT endogenous to the brain plays a physiologically relevant role in the initiation of MB.
In this article, we report the effects of central administration of OXT on the display of pup-directed and other behaviors in preweanling (18, 20, 21, and 22 days of age) and postweanling (26, 30, and 34 days of age) rats.

Method and Materials

Subjects were 18-, 20-, 21-, 22-, 26-, 30-, or 34-day-old Sprague-Dawley (SD) rats that were bred and raised in our animal colony (University of North Carolina, Chapel Hill, NC) by mating adult SD rats purchased from Charles River Laboratories (Wilmington, MA). All dams were multiparous. At about 2 days of age (the morning after delivery was considered Day 0), litters were culled to 10 or 11 pups, with sex distribution as equal as possible in each litter. Litters were raised in 35 × 24 × 18 cm metal cages with metal floors that were covered with about 2 cm of pine shavings. Water and Purina Rat Chow were available ad libitum. The animals were main-

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in a 12:12-hr light-dark cycle, with lights on at 7:00 a.m. When cages were cleaned every 5–7 days, pups were handled as little as possible. At 25 days of age, subjects were separated from their mothers and housed in groups in a room that contained no young rats. The stimulus pups that were used in behavioral tests were 1–2-day-old offspring of SD mothers.

Approximately 24 hr before behavioral observations, each subject was placed in a clean Plexiglas cage (34 × 24 × 15 cm) with water, a rat chow pellet, and a 1-cm layer of pine shavings for 3.5 hr and then returned to its dam. A Plexiglas plate with four air holes covered each cage. The observation room was illuminated with one 15-W light bulb. Observation cages were arranged in groups of four within 2 × 2 × 2 ft (0.6 × 0.6 × 0.6 m) observation cubicles; each cubicle was illuminated with one 7.5-W incandescent light bulb.

The next day, each subject was weighed, returned to the observation room, and placed in the same-size Plexiglas cage that contained food, water, pine shavings, and eight 3.6-cm² pieces of paper towel for 1 hr. Some subjects were then removed to the injection area, anesthetized with ether, and given an intracisternal injection that contained 2 μg OXT in 10 μl normal saline or saline vehicle alone (SAL). Pilot studies in which 1–4 μg OXT was tested revealed that 2 μg was well tolerated and produced vigorous self-grooming at all of the ages that we wanted to investigate. Injection solutions were coded so that the person who performed the injections was unaware of the different treatments. Other subjects were briefly removed from their cages but given no anesthesia or injection and designated as no treatment (NTX). OXT and SAL were administered intracisternally through a 30-gauge needle with a depth guide. Injection into the cisterna magna was over 90% accurate as determined by inspection of methylene blue-injected subjects. Food and water were removed from cages before the return of subjects.

Fifteen minutes after being returned to its cage, each subject received 3 stimulus pups (chosen randomly without regard to sex), each of which was placed approximately 15 cm apart at the apexes of an equilateral triangle centered in the middle of the cage. Behavioral recording began 10 s later. Each rat was observed in turn for a 1-min period. The observer recorded the juvenile rat behavior every 5 s. After the first sweep of 1-min observation periods of all subjects, sweeps were repeated at 10-min intervals for a total of 12 min periods (one hundred forty-four 5-s observations) for each subject over a 2-hr period. All observations were made by the same observer, who was unaware of the treatments given.

Fifteen behaviors were recorded. They were grouped into three categories: active pup-directed, inactive pup-directed, and other. Active pup-directed behaviors included (a) active hold with lick: holding both forepaws on a pup or pups with the juvenile or pup(s) or both in motion while the juvenile licks pup(s); (b) active hold: same as (a) but no licking of pup(s); (c) lick: placing tongue against pup(s) at least once; (d) sniff: placing snout and vibrissae against or very near pup(s); (e) crouch: taking a crouching position over pup(s); (f) moving pup: pushing or carrying pup(s) at least 2 cm; (g) paper over pup: moving paper to partially or completely cover pup(s).

Inactive pup-directed behaviors included (a) inactive hold: forepaws on pup(s), no movement by juvenile or pup(s); (b) inactive over pup: sitting or lying with some of its body (excluding the tail or forepaws) on pup(s); (c) inactive touch: sitting or lying with some of its body (excluding the tail) in contact with but not on pup(s).

Other behaviors included (a) self-groom: self-licking, washing, or scratching; (b) moving paper: lifting, holding, shifting, or chewing of paper; (c) pica: chewing wood shavings or droppings; (d) explore: locomoting, air sniffing, or rummaging through shavings; (e) inactive alone: sitting or lying with no pup contact. Because no nest building was observed in our preliminary studies, this behavior category was not included.

Only one behavior was scored during each 5-s interval. A behavior was scored only if it occurred for at least 1 s during the interval. If two or more behaviors occurred, then the hierarchy of scoring priority was as follows: crouch, moving pup, active hold and lick, active hold, paper over pup, self-groom, lick, sniff, moving paper, pica, explore, inactive hold, inactive over pup, inactive touch, and inactive alone. Behavioral data from juveniles that chewed or killed pups (this rarely occurred) were not included in the analysis.

A one-way analysis of variance was used to determine whether there were any main effects of treatment, age, sex, or weight on behavioral measures. When appropriate, t tests were used for post hoc comparisons between treatments within each age group. Tukey's studentized range tests were used for post hoc comparisons of each treatment condition between ages. Paired t tests were used to compare behavioral measures in Hr 1 and Hr 2.

Results

Active Pup-Directed Behaviors

Figure 1 summarizes total active holding of pups (active hold or hold with lick) and total licking of pups (lick alone or hold with lick) that occurred in the 1st hr of the observation period (Sweeps 2–6) for each treatment group at each age tested. Compared with SAL and NTX, OXT increased total active holding of pups in rats 18, 20, 21, 22, and 30 days of age but not in rats 26 or 34 days of age. Total licking of pups was significantly increased after OXT compared with SAL and NTX in rats 20 and 21 days of age; compared with SAL, OXT significantly increased total licking in 30-day-old rats. There were no differences in total active holding or total licking between rats that received SAL and NTX. There were no differences between treatment groups in the amount of other active pup-directed behaviors (sniff, crouch, moving pup, and paper over pup) that were displayed. These behaviors were either not exhibited or were exhibited at very low rates.

Active hold alone was significantly increased by OXT compared with SAL and NTX at ages 18, 20, 21, 22, and 30 days (p < .05). Hold with lick was greater after OXT than after other treatments at ages 20 and 21 days (p < .05). Lick alone was not increased by OXT at any age. Thus, the primary effect of OXT on active pup-directed behavior was to increase total active holding of pups by stimulating active hold alone and hold with lick. Total licking was elevated in OXT-
Figure 1. Mean percentage (±SE) of the total number of 5-s observations made on each animal during Observation Sweeps 2–6 (n = 60) during which active holding of pups (hold with lick or hold alone) or licking of pups (hold with lick or lick alone) occurred in each treatment group (O = oxytocin, S = normal saline, N = no treatment) of juvenile rats tested at 18, 20, 21, 22, 26, 30, or 34 days of age. (n indicates the number of animals in each group defined by age and treatment. *p < .05; **p < .001 [O vs. S; t test after analysis of variance]. *p < .05; **p < .001 (0 vs. N; t test after analysis of variance).)

Figure 2 summarizes the time course of total active holding during the 2-hr observation period for each treatment group at each age tested. OXT significantly (p < .01) increased total active hold in the 1st hr versus the 2nd hr of observation (Sweeps 2–6 vs. Sweeps 8–12) at ages 18, 20, 21, and 22 days but not at ages 26, 30, or 34 days. Rats that received NTX displayed significantly less (p < .05) total active holding in the 1st hr than in the 2nd hr of observation at ages 20 and 22 days. SAL-treated rats displayed no differences in total active holding between the 1st and 2nd hr of observation at any age.

OXT stimulated significantly (p < .05) more total active holding in the 1st hr of observations at ages 18, 20, 21, and 22 days than at ages 26, 30, and 34 days. In the 2nd hr of

Figure 2 (opposite). Mean percentage of the total number of 5-s observations made on each animal in each 20-min interval of the 2-hr observation period (n = 24) during which active holding of pups, inactive touching of pups, or self-grooming occurred in each treatment group of juvenile rats tested at 18, 20, 21, 22, 26, 30, or 34 days of age.
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ACTIVE HOLD
Hour 1 Hour 2

INACTIVE TOUCH
Hour 1 Hour 2

SELF-GROOM
Hour 1 Hour 2

Percent of Total Observation

Time (Intervals of 20 minutes)

- OXYTOCIN
- SALINE
- No Treatment

18 Days
20 Days
21 Days
22 Days
26 Days
30 Days
34 Days
observation, OXT-treated rats displayed significantly more total active holding at age 18 days than at ages 21, 26, 30, and 34 days and more at age 22 days than at age 34 days. In rats that received NTX, total active holding in the 1st hr of observation was greater at age 18 days than at 20, 22, 30, and 34 days of age; in the 2nd hr of observation, total active holding was significantly greater at ages 18, 20, and 21 days than at ages 26, 30, and 34 days and greater at age 22 days than at age 34 days. In rats treated with SAL, total active holding in the 1st hr of observation was greater at age 22 days than at ages 21, 30, and 34 days; in the 2nd hr of observation, total active holding was significantly greater at ages 18, 20, and 22 days than at ages 30 and 34 days.

**Inactive Pup-Directed Behavior**

As is summarized in Figure 3, OXT compared with SAL and NTX significantly decreased inactive touching of pups in the 1st hr of behavioral observations at all ages except 30 days, in which OXT produced significantly less inactive touch than SAL but not less than NTX. Comparisons of amounts of inactive touch between rats given SAL and NTX were significant only at age 30 days. Other inactive pup-directed behaviors (inactive hold and inactive over pups) were displayed at low rates, and there were no differences between treatment groups.

Figure 2 summarizes the time course of inactive touch during the 2-hr behavioral observation period for each treatment group at each age tested. OXT-treated rats displayed significantly less (p < .01 for all comparisons) inactive touch in the 1st hr than in the 2nd hr of behavioral observations at all ages. Except for the NTX group at age 26 days, in which inactive touch was significantly less (p < .05) in the 1st hr, there were no differences in inactive touch between the 1st and 2nd hr of the behavioral observation period in SAL or NTX groups at any age.

In rats that received OXT, inactive touch in the 1st hr of observation was significantly greater at age 22 days than at all other ages; in the 2nd hr of observation, inactive touch was significantly less at age 26 days than at ages 20, 21, and 22 days. In rats that received NTX, inactive touch in the 1st hr of observation was significantly less at age 26 days than at ages 18 and 22 days. In rats that received SAL, inactive touch in the 1st hr of observation was significantly greater at age 20 days than at age 26 days; in the 2nd hr of observation, there were no differences between age groups.

**Behaviors Not Directed Toward Pups**

As is summarized in Figure 4, OXT compared with SAL and NTX significantly increased self-grooming in the 1st hr of observation at all ages. There were no differences between treatment groups in the amount of other behaviors not directed toward pups (moving paper, pica, explore, and inactive alone).

Figure 2 summarizes the time course of self-grooming over the 2-hr observation period for each treatment group at each age tested. OXT-treated rats displayed significantly more (p < .01 for all comparisons) self-grooming in the 1st hr than in the 2nd hr of the observation period at all ages. SAL-treated rats displayed significantly less (p < .05) self-grooming in the 1st hr than in the 2nd hr of the observation period at ages 18 and 22 days. Rats that received NTX displayed...
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Figure 4. Mean percentage (±SE) of the total number of 5-s observations made on each animal during Observation Sweeps 2–6 (n = 60) during which self-grooming occurred in each treatment group (oxytocin [O], saline [S], or no treatment [N]) of juvenile rats tested at 18, 20, 21, 22, 26, 30, or 34 days of age. (The numbers of animals in each group defined by age and treatment are the same as in Figure 1. **p < .001 [O vs. S; t test after analysis of variance]. ++p < .001 [O vs. N; t test after analysis of variance].)

no significant difference in self-grooming between the 1st and 2nd hr of the observation period at any age.

Some comparisons of the amount of self-grooming between age groups within each treatment group in each hour of the observation period were significant. In rats that received OXT, self-grooming in the 1st hr was less at age 22 days than at age 26 days; in the 2nd hr self-grooming was significantly greater at age 30 days than at all other ages. In rats that received NTX, self-grooming in the 1st hr was less at ages 18, 20, and 34 days than at age 30 days; in the 2nd hr, there were no differences between ages. In rats that received SAL, self-grooming in the 1st hr was less at age 21 days than at ages 30 and 34 days; in the 2nd hr, there were no differences between ages.

There were no effects of sex or body weight on behavioral outcomes.

Discussion

Central administration of OXT in juvenile rats stimulated significant increases in active pup holding and licking at some ages (18, 20, 21, 22, and 30 days) but not at other ages (26 and 34 days). OXT was much less effective in older rats (26, 30, and 34 days of age) that were further along in weaning. In contrast, central OXT administration potently increased self-grooming and decreased inactive touching of neonates at every age tested. Thus, only the effects of OXT on active pup holding and licking were related to age and weaning status.

Central infusion of OXT markedly increases self-grooming behavior in adult female rats (Drago, Pedersen, Caldwell, & Prange, 1986). Clearly preweanling and postweanling juvenile rats are highly sensitive to this behavioral effect of OXT. Might OXT stimulation of active pup holding and licking be related to OXT activation of self-grooming? The time course of OXT induction of self-grooming parallels the time course of OXT facilitation of active holding and licking of pups (see Figure 2). Self-grooming induced by OXT includes forepaw grasping and licking of various body surface areas. These behaviors may “spill over” from OXT-treated preweanling rats thereby resulting in increased holding and licking of nearby pups.

Central administration of OXT in adult female rats stimulates the rapid onset of the full spectrum of maternal behaviors (Fahrbach et al., 1984; Pedersen et al., 1982; Pedersen & Prange, 1979; Wamboldt & Insel, 1987). In contrast, OXT increases only a limited subset of these behavioral components (i.e., holding and licking of newborns) in preweanling juvenile rats. Further investigation will be necessary to determine whether OXT administration shortens the latency of onset of a broader spectrum of maternal behaviors in preweanling rats when observations are extended longer than 2 hr. The time course of OXT effects also differs between juvenile and adult rats. OXT-induced maternal behavior in adults persists indefinitely, whereas OXT-induced holding and licking of pups in juvenile rats lasts for less than 1 hr. Despite these considerable differences, OXT facilitation of pup-directed behavior in juvenile and adult rats may be related. Other neuropeptides, such as ACTH and CRF, stimulate considerable self-grooming in preweanling juvenile rats but fail to increase holding and licking of pups (unpublished observations). Thus, OXT specifically extends the focus of oral and forepaw behavior from self to newborn conspecifics. This unique effect may be the common mechanism underlying OXT induction of pup-directed behavior in juvenile and adult rats.

Why does OXT stimulation of active pup-directed holding and licking diminish in rats that are close to completing or have completed weaning? Other researchers have observed
that emotionality and defensive reactions in response to novel and other aversive stimuli increase across the weaning period in rats (Bronstein & Hirsch, 1976; Candland & Campbell, 1962; Livesey & Egger, 1970). Mayer and Rosenblatt (1979) have argued that the marked increase in latency of onset of maternal behavior that occurs at the completion of weaning may be related to fear of novelty (neophobia), which emerges at that point in development. Our observations, which were conducted over a 2-hr period during which neonates were introduced to juvenile rats for the first time, may shed some additional light on the development of behavioral responses to novelty across the weaning period. In our untreated control groups, active pup holding and licking in the 1st hr after introduction of pups was elevated at 18 days of age compared with 20 and 22 days of age. Active pup holding and licking rose significantly in untreated 20- and 22-day-old rats in the 2nd hr of contact with pups. Inactive touching of pups, however, was elevated equally in the 1st and 2nd hr at all preweaning ages. These findings suggest that the novelty of pups or the testing situation may begin to inhibit the onset of active pup holding and licking after 18 days of age but has no effect on the high rate of inactive touching of pups in preweanlings. At 26 and 30 days of age, however, inactive touching of pups declined significantly in untreated rats thereby suggesting that active avoidance of neonates had begun. This parallels the observations of Mayer and Rosenblatt (1979) and Brunelli et al. (1985) that physical contact with neonates declines in juvenile rats as they grow older and wean. Avoidance of physical contact with pups may mark the onset of neophobia. Loss of sensitivity to OXT induction of active pup holding and licking may be related to the increasing avoidance of neonates that develops with the completion of weaning.

But what is the neural basis underlying changes in OXT behavioral effects at different ages? Recent autoradiographic studies have demonstrated that the distribution of OXT binding in the rat brain undergoes considerable reorganization between the first 2 weeks of life and adulthood (Shapiro & Insel, 1989; Tribollet, Charpak, Schmidt, Dubois-Dauphin, & Dreifuss, 1989). The changeover from the infant to the adult pattern occurs in two discrete stages. The first transition occurs during the preweaning period (between 16 and 22 days of age) and involves a large decrease or disappearance of OXT binding in numerous brain sites (cingulate cortex, nucleus accumbens, caudate-putamen, anterior and paraventricular thalamic nuclei, mammillary complex, dorsal subiculum, and lateral portion of the bed nucleus of the stria terminalis) and the appearance of OXT binding in the ventral subiculum. After 35 days of age, and coinciding with the onset of puberty, OXT binding also appears in the hypothalamic ventromedial nucleus, the olfactory tubercle, and some cell groups in the ventral pallidum.

These dramatic shifts in the distribution of OXT binding may underlie the changes in behavioral effects of OXT that are observed at different stages of development. The first shift in OXT binding, which occurs during the preweaning period, coincides with a transition in the focus of OXT-induced grooming from self and neonates to self alone. It is also of interest that behavioral responses to novelty change during and after the first shift in the distribution of OXT binding. Perhaps alterations in the neural pathways affected by OXT contribute, in part, to the appearance of neophobia. The second shift in OXT binding pattern, which occurs during puberty, may be necessary for OXT to induce the full spectrum of maternal behaviors as well as other "adultlike" behaviors such as mating (Argiolas, Collu, Geesa, Melis, & Serra, 1988; Arletti, Bazzani, Castelli, & Bertolini, 1985; Arletti & Bertolini, 1985; Caldwell, Pedersen, & Prange, 1986).

References


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Received July 18, 1989
Revision received December 14, 1990
Accepted December 17, 1990

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