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Neonatal Administration of Oxytocin Increases Novelty-Induced Grooming in the Adult Rat

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NOONAN, L. R., G. CONTINELLA AND C. A. PEDERSEN. *Neonatal administration of oxytocin increases novelty-induced grooming in the adult rat.* PHARMACOL BIOCHEM BEHAV **33**(3) 555–558, 1989.—Three-day-old Sprague-Dawley rat pups were intracisternally infused with a single dose of oxytocin (1 μg/2 μl) or saline, or were untreated. As adults, these animals were observed for novelty-induced grooming, analgesia measured by the hot-plate test, and behavior in the open field. Oxytocin treatment during infancy resulted in an elevation of novelty-induced grooming when compared to saline and untreated animals. There were no significant oxytocin treatment effects on analgesia response or open-field behaviors. Oxytocin given early in life may have permanent effects on certain behavioral responses to stress.

Oxytocin Grooming Ontogeny Analgesia Open-field behavior Neurohypophyseal peptide

THE period around birth in the rodent is a time of rapid neural development and increased sensitivity to exogenously administered steroid and peptide hormones. Administration during infancy of corticosterone (3, 11, 19, 20), ACTH (2,23), CRF (24–26), opiates (13, 18, 24, 26), TRH (6,22) and vasopressin (7,14) have been shown to have long-term effects on behavior and physiology. In general, administration of a specific peptide during infancy alters, in the adult animal, either spontaneous behavior known to be influenced by the endogenous peptide or responses to readministration of the peptide (24). For instance, treatment with β-endorphin during infancy results in elevated pain thresholds during adulthood, and alters the socio-sexual behavior produced by β-endorphin administration in adult rats (13,18).

Oxytocin is known to influence a number of behavioral events in the adult rodent including novelty-induced grooming (8,12), nociceptive threshold (1) and locomotor behaviors (4). Grooming, analgesic responses and locomotor behaviors are observed in the rat soon after birth and probably undergo neural organization in the neonatal period. During this period of rapid brain growth, application of peptides may have organizational effects on behavior. In this experiment we examine the spontaneous grooming, analgesic and locomotor responses of adult rats that had been treated with oxytocin during the sensitive neonatal period.

METHOD

Six litters of Sprague-Dawley-derived rat pups at 3–4 days of age were treated with a single infusion of either oxytocin (OXT), saline (SAL), or were untreated (UNT). These animals were returned to their mother until weaning and then group-housed by sex for the remainder of the experiment. At approximately four months of age, these animals were observed for 1) novelty-induced grooming, 2) hot-plate analgesia and, 3) behavior in the open field.

Neonatal Treatment

Untreated pups remained undisturbed, except for weekly cage maintenance, until weaning on day 24. For each litter receiving treatment, half of the animals were intracisternally infused with 1 μg/2 μl OXT, and half received 2 μl of normal saline. Infusions were delivered at a rate of 2 μl over 34 seconds. Following treatment the pups were returned to the home cages such that a litter contained pups of a single treatment. The total number of subjects for each treatment group was: OXT n = 26; SAL n = 25; UNT n = 33. There were approximately equal numbers of male and female rats in each treatment group.

Adult Testing

Each animal was observed during three types of tests by an experimenter who was unaware of the treatment condition. For novelty-induced grooming, the animals were placed individually in small plastic cages with wire tops (19 × 30 × 13 cm), housed

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within dimly-lit, sound-attenuating chambers. Four animals were observed simultaneously during the 15-minute observation period. At the end of each 20-second interval the following behaviors were noted if they had been observed during that interval: forelimb or hindlimb licking, face washing, scratching with the hindpaw, body, tail or genital grooming. A score for total grooming was arrived at by noting the number of 20-second intervals in which any type of grooming occurred (9). The observation cages were cleaned following each session.

For observations in the open field, individual subjects were placed in a dimly-lit open field ($66.5 \times 66.5 \times 48.5$ cm) divided into 16 squares of equal size. Each rat was observed continuously for 5 minutes. Matrix crossings, defined as the rat's head and forepaws crossing from one square into another, were recorded. In addition, number of supported and unsupported rears, cumulative time spent in grooming, and number of fecal boli produced were noted. The open field was cleaned after each rat.

Nociception was tested by the hot-plate analgesia test. The rat was grasped at the base of the tail and placed on a hot plate (52 ± 1°C). The latency for the rat to lick a paw was recorded, up to a maximum of 10 seconds. (All of the rats paw-licked in less than 10 seconds.) The rat was removed from the hot plate immediately after the first paw lick. Escape from the hot plate was prevented by plastic walls enclosing the hot plate, and a removable top that was closed immediately after placing the rat on the plate. The temperature of the hot plate was checked and the plate was cleaned after each rat. There was no injury to the animals' feet or tails from the hot plate.

The order in which the three tests were presented was systematically varied. The animals in each treatment group were randomly assigned to three subgroups, with approximately equal numbers of males and females in each subgroup. We considered the experience of being placed into the open field or cage used for grooming observations to be a similar type of novelty stressor. We hypothesized that the hot-plate analgesia test might be more stressful and the most likely to cause carry-over effects on other observations. Because the limited number of animals did not allow us to test the animals in the possible six presentation orders, we chose three in which the hot-plate analgesia test was given first, second or third in order. Thus, the three test orders were 1) analgesia, grooming, open field (AGO), 2) open field, analgesia, grooming (OAG), 3) grooming, open field, analgesia (GOA). The order of presentation was used as a factor, along with treatment and sex, in the multiple regression analysis by cell means parametrization performed on the data.

RESULTS

Grooming

There were significant effects of treatment on total grooming (see Fig. 1A). OXT treatment during infancy increased the incidence of adult spontaneous grooming when compared to SAL, $F(1,54) = 5.4$, $p = 0.02$, and UNT, $F(1,54) = 6.7$, $p = 0.01$, adults. SAL animals did not differ from UNT for total grooming.

Body grooming occurred most frequently of the individual grooming behaviors. OXT treatment significantly increased this type of grooming when compared to UNT, $F(1,54) = 5.0$, $p = 0.03$, and approached a significant increase when compared with SAL animals, $F(1,54) = 3.8$, $p = 0.058$. SAL and UNT rats did not differ in the amount of body grooming exhibited. There were not significant treatment effects for forelimb or hindlimb licking or face grooming.

The effects of peptide on grooming did not vary between sexes. However, there were main effects of order of presentation of the three tests on grooming behavior (see Fig. 1B). The order of

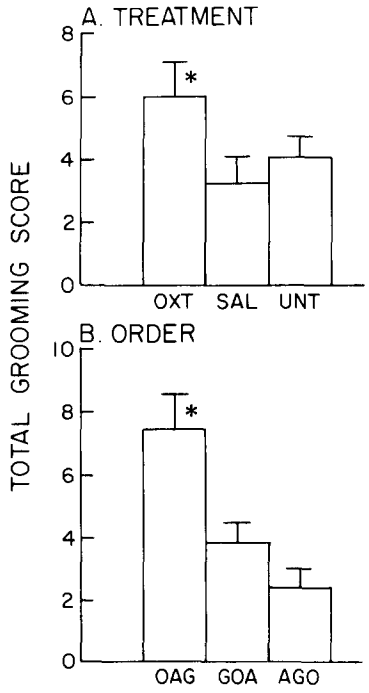

FIG. 1. (A) A single intracisternal infusion of OXT to the neonate increased novelty-induced grooming when the rats were tested four months later. (B) There were order effects of test presentation order on grooming which did not interact with the OXT treatment effects. When the open-field (○) and hot-plate analgesia (A) testing was done prior to grooming (G) observations, novelty-induced grooming was significantly increased. *Significantly different from SAL and UNT, $p<0.05$, for A, and significantly different from GOA and AGO, $p<0.05$, for B.

presentation variable did not interact with the treatment or sex variable. Comparisons between the presentation orders indicated that the novelty-induced grooming of the OAG group was greater than GOA and AGO group for total groom [OAG vs. AGO: $F(1,54) = 17.5$, $p = 0.0001$; OAG vs. GOA: $F(1,54) = 9.1$, $p = 0.004$], forelimb lick [OAG vs. AGO: $F(1,54) = 13.9$, $p = 0.0001$; OAG vs. GOA: $F(1,54) = 4.8$, $p = 0.04$] and body groom [OAG vs. AGO: $F(1,54) = 22.6$, $p = 0.0001$; OAG vs. GOA: $F(1,54) = 8.9$, $p = 0.004$]. Additionally, OAG grooming was greater than AGO grooming for face washing, $F(1,54) = 8.1$, $p = 0.007$. AGO and GOA groups did not differ for any of the grooming measures. It is difficult to assess the reason for this effect. The presentation of both the open-field and analgesia testing conditions prior to the grooming observations (OAG) elevated grooming above levels observed when the animals are naive (GOA) or when only analgesia testing is experienced prior to the grooming observations (AGO). Thus, it appears that the number of mildly stressful experiences prior to testing may be an important factor determining the amount of novelty-induced grooming exhibited.

Analgesia or open-field testing experience did not influence the increase in novelty-induced grooming produced by neonatal OXT treatment, as evidenced by the lack of an interaction between treatment and order of test presentation variables. An examination of total grooming behavior of only animals which were experimentally-naive at the time of observation indicated that grooming of neonatally-treated OXT animals was greater than either SAL or UNT animals (t-tests, $p<0.05$).

Analgesia

In general, latency to paw lick was longer in the UNT/AGO

TABLE 1

UNTREATED, EXPERIMENTALLY-NAIVE RATS EXHIBIT LONGER LATENCIES IN THE HOT-PLATE TEST FOR ANALGESIA

	Treatment		
Order	OXT	SAL	UNT
AGO	5.46	4.77	8.26*
	±0.47	±0.39	±1.32
GOA	5.03	5.11	5.51
	±0.22	±0.41	±0.41
OAG	5.16	6.24	5.14
	±0.65	±0.84	±0.28

*Significantly different from all other groups except SAL/OAG, $p<0.05$. Values are means ± S.E.M. A = analgesia, G = grooming, O = open field.

animals than most other treatment/order groups (see Table 1). The UNT/AGO animals most closely represent a naive or unstressed control group at the time of analgesia testing since this test was administered first in the 3-test order. Moreover, these rats were not injected or manipulated by the experimenter during infancy. Consequently, the significantly shorter paw-lick latencies (Tukey tests; $p \leq 0.05$) in all other treatment/order groups except SAL/OAG might be viewed as a hypoalgesia resulting from injection or manipulation during infancy (OXT/AGO, SAL/AGO), testing in adulthood prior to the analgesia trial (UNT/GOA, UNT/OAG), or both (OXT/GOA, OXT/OAG, SAL/GOA).

For the AGO animals, both OXT and SAL rats exhibited shorter paw-lick latencies than UNT, but did not differ from each other. Consequently, one must view treatment effects on hot-plate analgesia to be a result of infant manipulation and not specific to OXT administration per se.

Open Field

There were no treatment, order or sex effects on number of matrix crossings or rears in the open field. There were effects of sex on the cumulative time spent grooming in the open field, $F(1,54) = 5.6$, $p = 0.02$, and the number of fecal boli produced, $F(1,54) = 11.9$, $p = 0.001$. Female rats spent about twice as much time grooming in the open field as did males. Male rats produced more fecal boli than females (see Table 2).

TABLE 2

SEX DIFFERENCES IN OPEN-FIELD BEHAVIOR

	Sex	
	Male	Female
Grooming (sec/300)	5.12	10.63*
	±1.74	±1.46
Fecal Boli	3.53*	1.89
	±0.35	±0.31

*$p<0.05$ for males vs. females. Values are means ± S.E.M.

DISCUSSION

In summary, treatment with a single infusion of OXT during infancy appears to cause permanent changes in behavior. This effect appears to be somewhat specific to increasing novelty-induced grooming in adulthood, as there were no indications of specific OXT treatment effects on hot-plate analgesia or various behaviors exhibited in the open field.

The behaviors we chose to measure were some of those for which there is evidence for change following acute treatment of the neonatal or adult rat. Injections of OXT acutely increases novelty-induced grooming (8,12), defecation and locomotion in the open field (4), and induces analgesia (1) in adult rodents. In 5-day-old neonates, infusions of OXT produce grooming and locomotor movements 5 minutes after treatment (15). The acute effect of OXT on nociceptive responses in neonates has not been investigated to our knowledge.

The results of this study suggest that, of the behaviors observed, only grooming behavior is sensitive to reorganization by OXT administration at 3–4 postnatal days. This is the age when grooming is first observed in the rat (17). Changes in grooming behavior frequency, complexity, coordination and integration of component behaviors continue until approximately 30 days of age (10,17). During the first postnatal week, grooming becomes associated with environmental perturbations. As with the adult rat, removal of the pup from the familiar environmental (mother, nest) to an unfamiliar one (empty cage) elicits a short grooming bout (17). These rapid maturational changes in grooming may reflect a plastic neural substrate which is sensitive to administered OXT.

There were significant OXT treatment effects on grooming when the animals were observed in a small cage that restricted locomotion but not when they were observed in an open field. This result is similar to what has been reported for adult animals treated with OXT immediately prior to grooming observations (i.e., animals that were not treated as neonates). Oxytocin increased grooming when the animal is placed in a small cage (8), but not when placed in an open field (4). In the present study, animals placed in the open field groomed very little (37 out of 300 seconds was the highest individual score). These animals spent most of the test time engaged in exploratory behaviors such as locomotion and rearing, which potentially obscured facilitating effects of neonatal OXT treatment on grooming. In addition, the difference in scoring of the behavior in the two tests (time sampling vs. cumulative time spent grooming) may have led to the difference in results. These results, plus the indication of experiential (order) effects, indicate that grooming behavior is sensitive to change in environmental conditions. A defined environment appears to be particularly important when subtle changes in behavior, such as those observed after neonatal OXT treatment, are investigated.

Alterations in adult behavior or physiology following neonatal treatment with peptide hormones have been associated with changes in peptide receptors (5, 6, 18, 24–26). For instance, neonatal β-endorphin treatment which subsequently alters pain threshold also reduces opiate receptor number (24–26). While there has been little investigation on the maturing OXT receptor, a receptor which binds OXT appears to exist during early postnatal life. Oxytocin potently displaces arginine vasopressin from binding sites in neonatal cingulate gyrus and septum (16), suggesting that nonapeptide receptors in the neonatal brain are relatively undifferentiated. In contrast, the adult brain contains distinct nonapeptide receptor populations that selectively bind either OXT or vasopressin. OXT treatment in neonates may influence subsequent differentiation of nonapeptide receptors.

This study adds further support to the growing body of evidence that neonatal peptide administration can have permanent effects on behavior. The results confirm the importance of OXT in the expression of grooming behavior, and suggest that this peptide may exert organizational effects on the development of stress responses.

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