

Olfactory-Guided Orientation in Neonatal Rats Is Enhanced by a Conditioned Change in Behavioral State

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In Experiment 1, 3–6-day-old rats were simultaneously exposed to an unfamiliar odor which they will normally avoid (orange extract) and the odor of maternal saliva, or to either orange alone or saliva alone. One hour later, in a two-odor choice test, those pups which were simultaneously exposed to orange and saliva exhibited an enhanced orientation to the orange odor. In Experiment 2, orange odor was presented while pups were exposed to the odor of saliva, or while they received one of two types of tactile stimulation (stroking or tailpinching). Pups in control groups were first presented with saliva or tactile stimulation and then the orange odor. Pups that had received any of the three simultaneous exposure treatments subsequently exhibited an enhanced orientation toward the orange odor, but not the pups in the control groups. The results suggest that the tendency of neonates to avoid a novel odor can be reversed by pairing that odor with events that elicit significant increases in behavioral activity. The possibility that this phenomenon reflects a classical conditioning process is discussed.

Rat pups are attracted to odors emanating from the nest, particularly those associated with the dam or littermates (Alberts & May, 1984; Galef & Kaner, 1980; Leon, 1974). In contrast, they usually avoid novel odors. Under certain conditions, however, an unfamiliar odor can also acquire the ability to act as an attractant. For example, pups presented an unfamiliar and slightly aversive odor in conjunction with intraoral infusions of milk will subsequently orient to that odor (Brake, 1981; Johanson & Teicher, 1980; Johanson & Hall, 1982).

In very young pups, other procedures conducted in conjunction with presentation of a novel odor will alter the value of that odor. Pedersen, Williams, and Blass (1982) and

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Pedersen and Blass (1982) have shown that a previously unfamiliar odor can be used to promote nipple attachment in 1-day-old pups, provided the pups had previously been stroked with an artist's brush in its presence. This was a particularly striking demonstration because the naturally occurring odors which help the pup attach to the nipple (e.g., Hofer, Shair, & Singh, 1976; Teicher & Blass, 1976, 1977) had been removed by chemically washing the dam's ventrum. Only the previously unfamiliar odor could have guided the pup to the teat.

In each of these cases, it appears that the value of the unfamiliar odor had been enhanced through a conditioning process. In the first instance, milk served as the reinforcer. Milk delivery has been shown to support other forms of learning in young pups. For example, Johanson and Hall (1982) have shown that newborn pups will learn to manipulate a paddle in order to obtain a pulse of milk. In the second case, stroking of the pup's body seems to have served as the reinforcer (Pedersen & Blass, 1982; Pedersen et al, 1982). This manipulation was meant to approximate the way the dam licks and grooms the pups.

Are milk delivery and stroking two of the relatively few stimuli that serve as primary reinforcers in newborn rats, or is something more general about reward processes being revealed by these experiments? These are important questions because if milk delivery and stroking, two apparently different types of stimuli, are only two of a very broad class of stimuli that might serve as rewards in neonates, then the bases of reinforcement may be quite different in newborns than in adults, or even older pups.

Both milk delivery and stroking increase the level of activity displayed by neonatal rats in a very dramatic way. Infusions of milk delivered to deprived neonates elicits intense and prolonged motor behavior. This behavioral activation, first described by Hall (1979), seems restricted to young pups and consists of mouthing, probing the floor, rolling over, and vigorous limb movements. Stroking elicits similar motor activity in neonates, but not in older pups (Sullivan, Sager, & Brake, 1980).

In the current study, we examine the hypothesis that any procedure which elicits increased behavioral activity in 3-6-day-old pups is sufficient, when paired with an unfamiliar odor, to enhance the value of that odor. Underlying these investigations (see also Sullivan, Brake, Hofer, & Williams, subsequent article) is the possibility that behavioral activation may reflect a change in the neonate's state similar to that usually attributed to reward.

Experiment 1

In the studies described above, a tactile stimulus and a gustatory stimulus were both shown to elicit behavioral activity, and both seemed to serve as reinforcers. In this experiment, we examined whether an olfactory stimulus might act as a reinforcer in the same way. The odor of maternal saliva, collected from 9-day-post-parturient dams, served as this stimulus. Saliva was selected because it is a stimulus encountered repeatedly in the pup's natural environment (as dams frequently lick pups), and because it seems to elicit vigorous behavioral activity. The saliva of newly parturient females has been shown to initiate a sequence of activity which ultimately leads to nipple attachment and suckling (Teicher & Blass, 1977). However, we chose to use saliva from dams of slightly older post-partum age because it does not elicit nipple attachment in young pups (Blass & Teicher, 1980), even though it still elicits increased behavioral activity (Sullivan, Sager, & Brake, 1980). This is important because any enhancement of a novel odor's ability to act as an attractant after pairings with saliva cannot then be attributed to any obvious value saliva might have as a primary reinforcer. Instead, the enhanced value of the novel

odor might be attributed to a more general change in state elicited by the saliva, a property shared by stroking and the delivery of milk.

If the pups' aversion to a novel odor can be reversed by pairing it with saliva, then pups simultaneously exposed to the two odors should subsequently orient to the novel odor, while pups exposed to only one of the two odors should not. The odor of orange extract was selected as the novel odor. Alcohol-based orange extract has a rather pungent odor which pups tend to avoid (Brake, 1981). To assess orientation, a two-odor choice test was employed.

Method

Subjects

The subjects were 50 rat pups, 3–6 days of age, selected from 13 litters of Marland Farm Wistar dams. Five males and five females were assigned to each treatment condition. Litters were born in the Animal Care facilities of Montefiore Medical Center. Dams and litters were housed in 40 × 40 × 24-cm Plexiglas tarraria, on pine shavings, in a temperature- (22°C) and humidity-controlled room. Dams were allowed free access to food and water, and were maintained on a 12:12-hr light-dark cycle, with light onset at 7:00 A.M.

Procedure

Collection of saliva. Maternal saliva was collected for use immediately prior to the start of treatment. A lactating dam (mean days postpartum = 9) was anesthetized (Urethane, 2 g/kg) and placed in a warm (37°C) plastic container for 1 hr. After approximately 30 min, saliva accumulated in the dam's mouth and was collected with a pipet for the next 30 min. Approximately 2 ml of saliva was collected, enough for treatment of 2 subjects.

Deprivation conditions. All pups were separated from their dams 18 hr prior to treatment and individually housed in plastic tubs in a warm (31°C), moist incubator (Forma Scientific Diurnal Growth Chamber). Pups were deprived because we believed from our own preliminary data (Sullivan et al., 1980) and the data of others (e.g., Hall, 1979), that this would increase the probability that treatment would elicit behavioral activation. None of the pups were debilitated in any obvious way following the deprivation period.

Treatment procedures. Treatment consisted of removing pups from the housing incubator and placing them in individual tubs, which were then placed into another warm (32°C), moist treatment incubator for 10 min. The orange odor was presented by lining the floor of each pup's tub with 3 ml of orange-scented pine shavings. The orange-scented shavings were prepared 10 min prior to treatment by spraying 1.25 cc of orange extract (Ann Page) onto 500 ml of pine shavings and shaking them thoroughly in a closed container. The odor of maternal saliva was presented on a cotton swab positioned within 5 cm of the pup's snout. The cotton swab was continually repositioned to accommodate the pup's movement. The cotton swab was resoaked (for 2–3 sec) every 2 min.

Pups were randomly assigned to one of five treatment conditions. Each lasted 10 min: (1) Orange & Saliva, simultaneous presentation of the orange odor and the odor of maternal saliva; (2) Orange & Water, simultaneous presentation of the orange odor and a cotton swab soaked in tap water (as a control for any odors which might be emitted by damp cotton); (3) Orange, presentation of the orange odor alone; (4) Saliva, presen-

Results and Discussion

Behavioral Activity

Presentation of maternal saliva, whether alone or in combination with orange odor, elicited significant increases in behavioral activity, as previously described in Sullivan et al. (1980). Independent assessment of activity in the two separate groups of pups showed that this phenomenon, obvious to the naked eye, was also statistically reliable. Pups which were presented maternal saliva received a mean activity score of 1.76 while those which were not presented saliva received a mean score of 0.33 (independent *t*-test, $p < .01$).

Two-Odor Choice Test

Pups simultaneously exposed to the orange odor and the odor of maternal saliva spent a greater amount of time over the orange odor than did pups in any of the other groups (Fig. 1; $F(4,45) = 6.66$, $p < .01$; a post-hoc Tukey test revealed that the mean of the Orange & Saliva group differed from the means of the other groups at the $p < .01$ level). In fact, only pups in the Orange & Saliva group seemed to prefer the odor of the orange-scented pine shavings to the more familiar odor of unscented pine shavings. Thus, the simultaneous exposure to orange odor and the odor of maternal saliva is sufficient to produce enhanced orientation toward the orange odor, but simple exposure to the orange odor is not.

These results suggest that the odor of maternal saliva may be added to the list of other stimuli which are perceived by 3–6-day-old pups as rewarding. It has now been shown that a neonate's preference for novel odors can be enhanced when those odors are paired with a gustatory stimulus (milk delivery), a tactile stimulus (stroking), and an olfactory stimulus (odor of maternal saliva). All of these stimuli elicit changes in the neonate's gross motor activity as well, but do not elicit activity changes in older animals. Thus, it appears that an increase of behavioral activity in response to sensory stimulation seems to be equivalent to positive reinforcement in neonates. In Experiment 2, we further test this possibility.

Experiment 2

In this experiment, 3–6-day-old pups were simultaneously presented with orange odor and one of three activating stimuli. One was the odor of maternal saliva and two were tactile stimuli (produced by stroking the pup's body or pinching its tail). Like the odor of maternal saliva, both tailpinching (Szechman & Hall, 1980) and stroking (Pedersen et al., 1982) produce robust behavioral activity in neonates. We also included groups that received the activating stimuli immediately before the presentation of the orange odor (a backward pairing) in order to provide a more stringent test of whether the phenomenon we observed in Experiment 1 was the result of a Pavlovian learning process.

Method

Subjects

The subjects were 56 rat pups, 3–6 days of age, selected from 14 litters of Marland Farm Wistar dams. Four males and three females were assigned to each treatment condition. Animals were born and raised as described in Experiment 1.

Procedure

In this experiment, the odor of maternal saliva was presented as homogenized salivary gland tissue taken from a donor dam (mean days postpartum = 12) immediately prior to treatment. The gland was excised from the dam, mashed and mixed with 1 cc of distilled water, and magnetically spun (Corning PC-351) for 5 min. Each extract preparation was used for 1–2 pups.

Deprivation conditions were identical to those described in Experiment 1. Following deprivation, pups were randomly assigned to one of these treatment conditions: (1) Orange & Saliva, simultaneous presentation of the orange odor and the odor of maternal saliva for 10 min; (2) Saliva/Orange, presentation of the odor of maternal saliva for 10 min followed immediately by a 10-min presentation of the orange odor; (3) Orange & Stroking, presentation of the orange odor for 10 min accompanied by repeated stroking of the pup's back, legs and belly with a sable-hair brush; (4) Stroking/Orange, 10 min of stroking followed by presentation of the orange odor for 10 min; (5) Orange & Tailpinch, presentation of the orange odor for 10 min with a tailpinch occurring every 15 sec; (6) Tailpinch/Orange, 10 min of tailpinching followed by a 10-min presentation of the orange odor; and (7) Naive, pups were placed in the tub for 10 min but received no treatment. All pups were tested 1 hr after treatment, as described in Experiment 1.

Each of these procedures elicited increases in body movements of various kinds. As in Experiment 1, we quantified this activity more exactly by assessing the behavior of five separate groups of pups ($n = 5$) according to the rating scale. The first group received presentation of maternal saliva for 10 min, as described above, the second group received stroking, the third received tailpinches, the fourth received presentation of the orange odor alone, and the fifth was untreated.

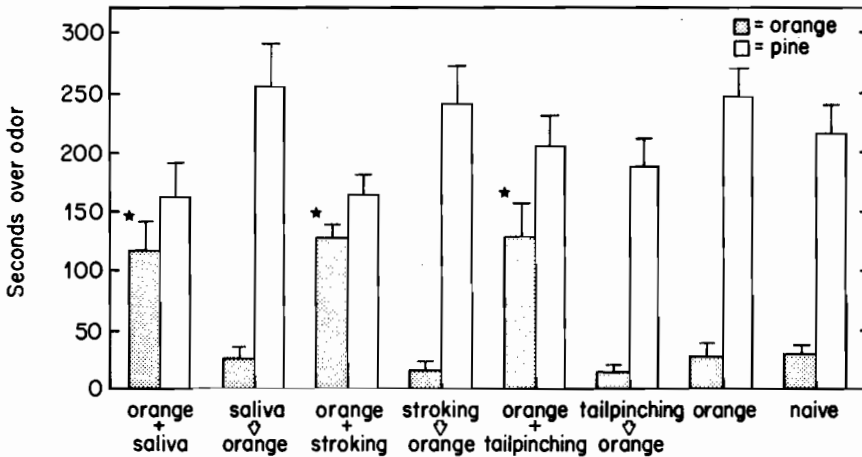


Fig. 2 Mean number of seconds spent over orange-scented shavings and unscented pine shavings. Pups were simultaneously exposed to orange odor and either saliva (orange + saliva), stroking (orange + stroking), or tailpinching (orange + tailpinching), or were exposed to orange odor immediately after being exposed to either saliva (saliva > orange), stroking (stroking > orange), or tailpinching (tailpinching > orange). Naive pups received no treatment. The stars indicate that pups in these groups spent more time over the orange-scented shavings than pups in the other groups. Bars indicate standard error of the means.

Results and Discussion

Independent Assessment of Behavioral Activity

Of the separate groups of pups assessed for behavioral activity, those which received tailpinch and stroking were significantly more active during treatment than those which received presentations of maternal saliva, $F(4,24) = 39.69$, $p < .001$; Table 1. Pups which received presentations of maternal saliva were, in turn, more active than pups which received presentations of the orange odor and pups which were untreated. Pups which received orange odor were no more active than pups which were untreated. Thus, each of our treatments (presentation of maternal saliva, tailpinching and stroking) resulted in significant elevations of behavioral activity.

Two-odor Choice Test

Pups simultaneously exposed to the orange odor and any of the three activating stimuli (saliva, stroking or tailpinching) spent a greater amount of time over the orange-scented shavings than did all other pups (Fig. 2; $F(7,48) = 11.19$, $P < .01$; post-hoc Tukey test revealed that the means of these three groups were different from the means of the other groups at the $p < .05$ level). In fact, pups in each of these three groups oriented to the pungent orange-scented shavings as much as to the more familiar pine shavings, while pups in the remaining groups clearly avoided the orange-scented shavings.

The data also show that only simultaneous presentations of the activating stimuli and the orange odor enhanced subsequent orientation. Neither exposure to the orange odor alone, nor exposure to the orange odor immediately following exposure to the activating stimulus, affected subsequent orientation. These results suggest that enhanced orientation is due to an associative learning process (although a nonassociative interpretation cannot be excluded; see below).

The results also suggest that the acquisition of a new association about an unfamiliar odor, and the enhanced orientation towards that odor which results, is not mediated by any specific sensory stimulus but rather by some property common to many. This shared property seems to be reflected by the increased behavioral activity which these stimuli elicit.

General Discussion

The results indicate that unfamiliar odors presented to neonatal pups can take on new meaning when paired with a variety of stimulus events. A property common to each of these events is that they elicit increases in behavioral activity. Previous research suggests that such activity may be a *necessary* component of associative learning in

TABLE 1. *Effects of Various Stimuli on Behavioral Activity.*

	Stroking	Tailpinch	Maternal Saliva	Orange Odor	Naive
Mean	2.47	2.30	1.52	0.39	0.27
S.E.	0.05	0.06	0.33	0.08	0.11

Means represent summed scores of general activity as assessed by the rating scale (see text). S.E. indicates standard error of the mean.

neonates. For example, neonates will not learn an enhanced orientation to a novel odor previously paired with milk, unless the initial presentations of milk elicited increased behavioral activity (Hall, 1982; Johanson & Hall, 1982; Johanson, Polefrone & Hall, 1984). The current research adds to these results by showing that increases in behavioral activity may be *sufficient* to support the acquisition of new associations.

This might happen in one of several ways; we suggest two. First, the enhanced orientation to the unfamiliar odor displayed by pups simultaneously exposed to that odor and an activating stimulus might be due to an associative learning process. This would mean that each of the activating stimuli resulted in similar changes in the pup's behavioral state, as if each were rewarding. It also suggests that increases in activity may be a way to identify stimuli which can be expected to function as rewards for neonates. In this connection, it is interesting to note that many of these stimuli (stroking, tailpinch, maternal saliva) do not appear to elicit exaggerated behavioral activity in older animals nor, to our knowledge, to support associative learning in older animals. It would seem that sensory stimulation capable of eliciting marked increases in behavioral activity in young pups somehow engage an undifferentiated reward system peculiar to pups of this age (Hall, 1982; Sullivan & Hall, 1985).

It is also possible, however, that pups in the simultaneous pairing groups may have exhibited enhanced orientation as the result of differential attention and habituation processes. Presentation of the activating stimulus may have alerted these pups to the presence of the orange odor so that they habituated to the odor's aversive qualities more completely than pups in the other groups. If so, pups in the simultaneous pairing groups could be expected to avoid the odor less than the other pups during the choice test. This notion gains some support from the fact that pups spend nearly 80% of the time asleep (Gramsbergen, Schwartze, & Precht, 1970; Hofer, 1975), and so might not fully attend to unfamiliar odors unless awakened by an activating stimulus. Milk delivery, for example, reliably awakens sleeping pups (Shair, Brake, & Hofer, 1984). This explanation is made less likely, however, by the fact that pups which were awakened by stimulation immediately prior to presentation of the unfamiliar orange odor (the backward pairings groups) displayed no evidence of reduced aversion to orange in the choice test.

Regardless of the mechanisms which may be involved in mediating the effects we observed, the data have important implications for what may occur to pups during the first few days of life. Most strikingly, they suggest that a wide variety of olfactory, tactile and gustatory stimuli provided by the dam (licking or trampling the pups, the presence of maternal odors, milk delivery) may serve to impart important meaning to previously novel cues in the environment. For example, home-orientation may be guided, in part, by odors which have gained their meaning by being conditioned to behavioral activation elicited by the dam early in development. Similarly, the pup's approach to the teat may be guided by novel odors which have acquired value as attractants. If so, it would seem that sensory stimulation, and the resulting change in behavioral state it elicits, may contribute significantly to the development of olfactory-based social behaviors.

Notes

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