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Infant Rat Hyperactivity Elicited by Home Cage Bedding Is Unaffected by Neonatal Telencephalic Dopamine or Norepinephrine Depletion

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PAPPAS, B. A., G. VICKERS, M. BUXTON AND W. PUSZTAY. *Infant rat hyperactivity elicited by home cage bedding is unaffected by neonatal telencephalic dopamine or norepinephrine depletion.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 151–154, 1982.—Newborn rats received either stereotaxically guided bilateral injections of 6-hydroxydopamine (6-OHDA) in the neostriatum so as to deplete dopamine (DA) there, or subcutaneous 6-OHDA to deplete forebrain norepinephrine (NE). Both the DA and NE depleted rats as well as their respective control rats were significantly more active at 15–16 days of age when tested in a novel environment containing soiled bedding from their home cages, than when tested in the presence of clean bedding material. Furthermore, under both the home cage and clean bedding conditions the DA depleted rats were more active at this age than their controls. Thus while transiently elevated locomotor activity is one consequence of neonatal, neostriatal DA depletion, inattention to olfactory stimuli (which occurs after adult neostriatal DA depletion) is not another. The NE depleted rats in both home cage and clean bedding test conditions showed activity levels equivalent to that of their control groups. Furthermore, the NE depletion did not affect hyperactivity elicited by artifically (peppermint) scented bedding like that in which the rats had been reared. Thus, contrary to expectations based on the reported reduction of preference for conspecific odor after neonatal and adult forebrain NE depletion, these data show that the locomotor activating effects of neither conspecific nor artificial odors associated with the nest odors are attenuated by neonatal NE depletion. The mortality rates among NE depleted rats raised in the peppermint scented shavings was unusually high and overall, these rats were less active than similarly raised controls.

Young rat Hyperactivity Home Bedding Catecholamines

WHEN infant rats of different ages are tested for spontaneous locomotor activity in a novel environment, their levels increase to a peak between the second and third week of life and then decline [2]. The early work of Campbell and associates indicated that the increase and later decrease in activity in the rat was due to the maturation of noradrenergic excitatory followed by cholinergic inhibitory circuits respectively [2,4]. The distinctive maturational link of activity, coupled with this plausible neurochemical explanation encouraged investigators to use this phenomenon to assess the consequences for neurobehavioral ontogeny of diverse prenatal treatments, such as stress [12] or drug administration [13].

We have found that when activity is recorded for the first hour of exposure to a novel environment, the presence of home cage bedding (HCB) induces hyperactivity in the 15 but not the 9 or 26 day old rat (Gallivan and Pappas, unpublished observations). Furthermore, this effect may depend upon the presence of maternal excretion odor in HCB, since this odor alone elicited hyperactivity. These results contrast with those of Campbell and Raskin [1] who had earlier reported that the extent of activity over a six-hour test period in the 15 day old rat was inversely related to the similarity of the test environment to the home cage. There is no obvious reason for the difference between our results and those of Campbell and Raskin.

In the following experiment we determined whether neonatal depletion of either neostriatal dopamine (DA) or norepinephrine (NE) altered the activity elevation by home cage bedding. In the first instance, neonatal forebrain DA depletion causes transient hyperactivity this effect occurring around the age at which HCB modulates activity [9, 11, 15]. Since DA depletion in the adult rat induces generalized sensory neglect including impaired orientation to olfactory stimuli [7], it follows that neonatal DA depletion may be expected to attenuate the modulation of activity by home cage bedding.

Second, we determined whether neonatal forebrain NE depletion would reduce the activational effect of HCB which contains familiar biological, odorous material such as maternal and offspring excrement but not to bedding artifically scented to resemble the home cage's artificial scent. It has

been shown that such NE depletion reduces the preference of rat pups for home nest (conspecific) odors but not for simple botanical odors associated with the home nest [5,14]. Research with adult rats sustaining bilateral lesion of the dorsal tegmental NE bundle has shown that, unlike intact rats [3], they also fail to prefer conspecific (maternity bedding) odors over botanical odors.

METHOD

Animals

Wistar rats borne by mothers obtained 14 days pregnant from Canadian Breeding Farms, St. Constant, Quebec were raised in standard 23×46×15 cm polypropylene maternity cages under reversed light schedule (lights off at 8 hr, on at 20 hr). Each cage contained either 2 litres of hardwood chips ("Beta Chips", Northeastern Product Corp., Warrensburg, NY) in the DA depletion experiment or 4 litres of pine shavings in the NE depletion experiment. Litters were crossfostered on the day of birth and culled to 10 pups.

Neostriatal DA Lesion Procedure

Within 12 hr of birth and again 24 hr later, the pups were injected with desmethylimipramine (25 mg/kg in 0.05 ml saline) and 45 min later were anesthetized by immersion in crushed ice for 4 min. Using a wax mold to immobilize the head, a 30 gauge needle was stereotaxically lowered to deliver either 25 ug 6-hydroxydopamine hydrobromide (6-OHDA, Sigma) dissolved in 1 μl of vehicle (saline plus ascorbic acid, 0.2 mg/ml) or vehicle only to the neostriatum. The needle tip was placed 1.5 mm. anterior, 1.5 mm. lateral and 3.0 mm. ventral to bregma. The fluid was delivered by microsyringe pump to either the left or right caudate on the first day and the contralateral side on the second.

On each of days 9 and 10, 15 and 16 or 25 and 26, four pups from two 6-OHDA and two vehicle injected litters were separated from their mother during the mid-hours of the dark portion of their light cycle and placed individually in clean maternity cages containing either 0.75 litres of bedding from their home cage or 0.75 litres of clean bedding. Activity was monitored by closed circuit television and recorded for 45 min with a videotape recorder. The tapes were subsequently scored for activity by counting the number of crossings across the transverse line bisecting the cage into its two ends.

Forebrain NE Lesion Procedure

Forebrain NE depletions were effected by injecting the rats subcutaneously within 12 hr of birth and again 24 hr later with 100 mg/kg 6-OHDA in 0.05 ml saline plus ascorbic acid (0.1 mg/ml). This treatment has been shown to produce a pattern of reduced forebrain NE much like that observed after 6-OHDA lesion of the dorsal tegmental NE bundle in the adult [8]. The rats were the offspring of 12 Wistar dams who were randomly crossfostered within 12 hr of birth with the restriction that each dam received 5 males and 5 females.

Six vehicle and six 6-OHDA litters were raised in bedding which was scented with peppermint. (Pure Mint and Peppermint Extract, Club House Foods Ltd., London). For these rats, four litres of shavings were sprinkled with 7 ml of the peppermint extract and then thoroughly mixed. This bedding was changed daily. An additional six vehicle and six 6-OHDA litters were raised in four litres of unscented pine shavings which were left unchanged until test day. To equate for handling effects, however, these rats were removed from the maternity cage daily in a simulated change of bedding. The pine (control) and peppermint scent raised rats were housed in separate vivarium rooms.

Locomotor activity of these rats was measured for 45 min on days 15 and 16 only. Each rat was isolated in a novel maternity cage. Some of the subjects raised under normal (unscented) bedding were tested in cages containing 2 litres of clean pine shavings—the other subjects were tested in cages containing 2 litres of mixed bedding from their home cage. Of the subjects raised on peppermint-scented bedding, some were tested in cages containing 2 litres of clean bedding while others were tested in cages containing 2 litres of otherwise clean bedding that was newly scented with peppermint as described earlier.

Tests involving only clean, unscented shavings were conducted in a room about 10 m from that used for tests with soiled home cage or peppermint-scented bedding. For the latter, one hour of room air exchange was allowed between soiled and scented bedding tests. Different maternity cages were also used for soiled and scented bedding tests.

Both test rooms were equipped with a video camera and the cages remotely recorded on videotapes. Activity was later scored as the total crossings of longitudinal and transverse cage bisectors drawn on the monitor screen so as to divide each cage into quadrants. We adopted this change in activity scoring procedure because the first (DA depletion) experiment indicated about as many tranverse as longitudinal cage crossings by our rats. Therefore the additional quantification afforded by totaling transverse and longitudinal crossings could have been more sensitive to the effects of the neonatal treatments than longitudinal crossings alone.

Assays

Spectrophotofluorometric assays of regional brain NE and DA were performed as described previously [8]. Samples of eight DA depleted and control rats were sacrificed at 30 days of age while samples of eight NE depleted and control rats who had completed activity testing were killed at 15 days. These rats were littermates of those undergoing behavioral testing. Brain tissue was stored in liquid nitrogen for about two weeks prior to assay.

RESULTS

Neostriatal DA Lesions and Activity

As shown in Fig. 1, and in confirmation of other results [9], both the vehicle and the 6-OHDA treated rats were markedly more active at 15 days than at either 9 or 25 days. Analysis of variance confirmed this effects of age on activity, $F(2,84)=19.03$, $p<0.001$. This age related variation in activity occurred for both the home cage bedding and clean bedding test conditions. The 6-OHDA treated rats were, as expected, markedly more active only at 15–16 days than their DMI-only control group ($p<0.001$, Sheffe test). This elevated activity was evident both under HCB and clean bedding test conditions. As Fig. 1 shows, the difference between the lesioned and vehicle controls was in fact greater under HCB test conditions than under the clean bedding condition. The activating effects of HCB, $F(1,84)=19.03$, $p<0.001$, were evident for both vehicle and 6-OHDA treated rats only on days 15–16.

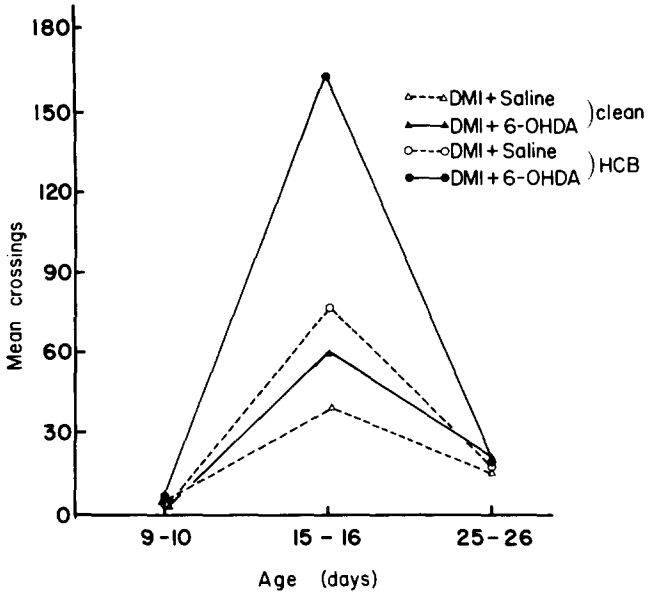

FIG. 1. Mean spontaneous locomotor activity (cage crossings) in infant rats depleted of neostriatal dopamine (DMI+6-OHDA) or in controls (DMI+saline). Half of these rats at these different ages were tested in cages containing home cage bedding (HCB) or clean bedding (clean) material. Each mean is based on the score of 8 rats.

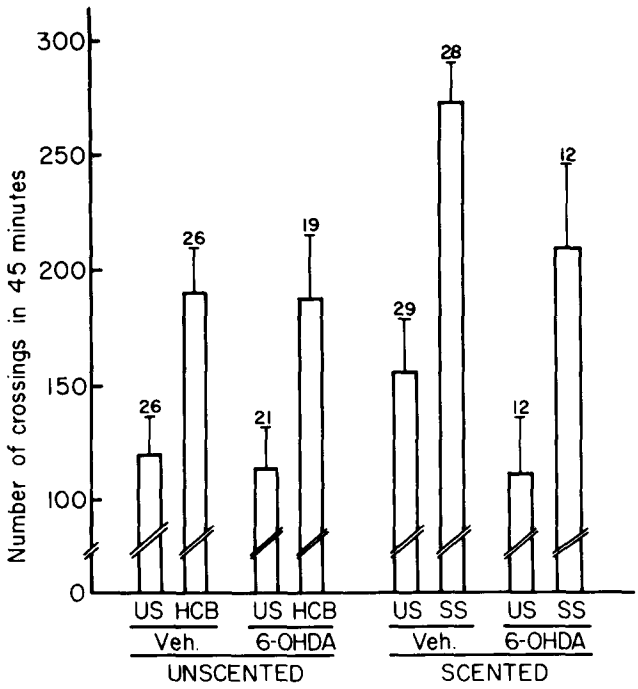

FIG. 2. Mean (±S.E.M.) spontaneous locomotor activity for rats raised either in unscented or artifically scented shavings. Vehicle (Veh) or systemic 6-hydroxydopamine (6-OHDA) treated animals were tested either in clean, unscented shavings (US) or in shavings resembling the home cage (i.e. normal, soiled bedding, HCB), or artificially scented bedding, SS, in the case of rats raised in such beddings. Sample n's are indicated on the figure.

NE Depletions and Activity

Figure 2 indicates that both the vehicle-control and systemic 6-OHDA groups who were raised in unscented shavings showed significantly higher levels of activity when tested with home cage bedding than with clean bedding, $F(1,60)=10.13, p<0.01$). Neither was there any effect of systemic 6-OHDA on activity nor was there an interaction between this treatment and test condition on activity.

Consistent with the data from the normally reared rats tested in home cage bedding, both the control and 6-OHDA groups who were raised in scented shavings were significantly more active when tested in scented shavings, $F(1,105)=22.8, p<0.001$. While again there was no interaction between the neonatal treatment and test condition, the scent-raised, vehicle animals were significantly more active overall than similarly-raised 6-OHDA treated rats, $F(1,105)=9.15, p<0.01$.

Assays

The neostriatal injections of 6-OHDA significantly ($p<0.01$, t-test) reduced neostriatal DA to 63% of DMI control value (2.56 ± 0.12 μg/gm tissue) and also lowered ($p<0.01$, t-test) the combined pyriform cortex and olfactory tubercle DA to 75% of control (1.22 ± 0.02 μg/gm). Neither DA nor NE levels were altered in the remaining tissue samples (brain stem plus diencephalon, remaining cortex or spinal cord).

Systemic 6-OHDA significantly ($p<0.01$, t-tests) reduced NE levels in the olfactory bulbs (21.4% of vehicle control level of $0.16\pm$ μg/gm tissue), in the pyriform cortex plus olfactory tubercle (44% of control level of 0.42 ± 0.03 μg/gm) and in the remainder of the telencephalon (62.3% of 0.10 ± 0.01 μg/gm). Conversely, NE was significantly ($p<0.05$, t-test) elevated in the pons plus medulla (125% of control level of 0.58 ± 0.05 μg/gm). There were no changes in DA in any area.

Treatment Mortalities

There were no mortalities in any of the groups involved in the first (DA depletion) experiment. In the second (NE depletion) procedure, of those rats raised in unscented bedding, eight of 60 vehicle rats and 20 of 60 systemic 6-OHDA rats died before test day. These rates are unusually high for our laboratory. More striking, however, while there were only three deaths among the 60 vehicle rats raised in peppermint-scented shavings, 36 of the 60 peppermint-raised, 6-OHDA-treated rats died. Thus, systemic 6-OHDA increased the mortality rate and this rate was further exaggerated by rearing the animals in the non-biological scent. While the surviving 6-OHDA treated rats appeared slightly developmentally delayed, there was however, no obvious increase in this effect due to peppermint rearing.

DISCUSSION

As has been observed after intracisternal [11] and intraventricular [9] 6-OHDA in the infant rat, bilateral neostriatal

injections were found here to transiently elevate activity, this elevation manifested around 15–16 days. This behavioral effect occurred despite very modest (36% and 25%, respectively) depletions of DA in the neostriatum and adjacent olfactory tubercle-pyriform cortex. As it was for normal rats, activity in these already hyperactive rats was further elevated by testing them in the presence of home cage bedding. Thus, neostriatal DA depletion did not eliminate the activational influence of the bedding which is most likely due to its olfactory qualities. Although neostriatal DA depletion attenuates attention to olfactory stimuli in the adult rat [6,7], this attenuation was evident after far greater DA depletion than was achieved here with our neonatal 6-OHDA procedures. It could well be that more severe damage to the DA innervation of the neostriatum would eliminate the activational influence of HCB in weanling rats.

It may be more than coincidential that the activational effect of both home cage bedding and DA depletion was evident at 15–16 but not at 9–10 or 25–26 days. Perhaps the presence of home cage bedding in the test arena elicited a reduction of neostriatal DA function? Alternatively it could be argued that the elevated activity in the neostriatal 6-OHDA lesioned rats reflects a transient hyperfunction of this system caused by a modestly reduced presynaptic DA terminal input acting upon neostriatal cells made supersensitive through denervation. Thus the effect of home cage bedding might be to increase the functional activation of this system. Future research might concentrate on discerning the correct one of these two alternatives.

In general confirmation of earlier results [10], normally reared, systemic 6-OHDA and therefore forebrain NE depleted rats, showed levels of activity equivalent to controls when tested under conditions of home cage or clean bedding. Furthermore, when reared with either normal or artifically scented HCB, both vehicle and systemic 6-OHDA treated rats demonstrated the activating effects of this bedding at 15–16 days. In the artificial scent conditions, however, the overall activity levels of the NE depleted rats were lower. Thus, contrary to expectations based on the reduction of preference for conspecific odor after neonatal and adult forebrain NE depletion, [3, 5, 14] our data show that the activational effects of neither conspecific odors nor artificial odors associated with the home cage are attenuated by neonatal NE depletion. It is possible, of course, that this activational influence of HCB persists while preference for it is lost after this lesion.

One striking but unexpected feature of our data was the very high mortality rate (60%) among NE-depleted rats raised in artifically scented bedding. This was not observed for similarly raised control rats or neostriatal-DA depleted rats raised in normal bedding. If NE depletion indeed reduces responsivity to biologically important odors (such as nipple odors which encourage nipple attachment [16]) then a potent but biologically irrelevant nest odor may disrupt life-sustaining, odor-guided behaviors in these rats. Future research should determine whether this high mortality in artifically scented maternity cages is peculiar to the NE depleted rat and as well should more systematically assess what aspects of pup development and/or which feature(s) of mother-infant interactions may be disrupted by this treatment.

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