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Brain Serotonin Depletion and Nipple Attachment in Rat Pups

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LESHEM, M. AND M. KREIDER. *Brain serotonin depletion and nipple attachment in rat pups.* PHARMACOL BIOCHEM BEHAV 27(1) 7–14, 1987.—5,7-DHT (104 μg) was injected into the third brain ventricle of three-day-old rat pups 1 hr after desmethylimipramine pretreatment (20 mg/kg IP). Whole-brain serotonin was depleted by 50% , and there was some retardation of weight gain. Nipple attachment was observed in sucklings and in weaned pups. When 9 days old, attachment in 5-HT depleted pups was delayed and they were less active when undeprived, but not when 8 hr deprived. In comparison with pups raised in litters of 6 or 14, which served to control for reduced body weight, depleted pups' behavior was more similar to the heavier pups, suggesting that the behavioral differences were not due to retarded growth. After weaning at 21 days, depleted pups attached more up to 37 days of age, after which attachment was not reinstated by treatment with methysergide, suggesting that the waning of depletion induced attachment was not due to recovery of serotonergic function. Again, body weight controls showed that the effects were not due to retarded weight gain in the depleted pups. The results confirm the biphasic action of 5-HT antagonism on attachment, i.e., inhibition in the neonate and disinhibition in the weanling. The findings also show that central 5-HT systems are involved in the inhibition of attachment during weaning, but that this involvement is transient, and apparently not crucial.

Attachment 5,7-Dihydroxytryptamine Ontogeny Rat pups Serotonin

THERE is now a large and complex body of evidence implicating serotonergic systems in the control of nipple attachment in the rat pup. 5-Hydroxytryptamine (5-HT) antagonists inhibit attachment in the 3–8-day-old neonate [16,17], but in the older suckling 5-HT agonists inhibit attachment while antagonists promote it. Thus, the 5-HT agonist fenfluramine is anorexic in the 10-day-old suckling [11] before amphetamine achieves anorexic potency [11, 12, 15], and quipazine, another 5-HT agonist, also inhibits nipple attachment at 10 days and thereafter [14,19].

Even more persuasive are the findings that some 5-HT antagonists increase nipple attachment in weaning age pups and can induce it after weaning [14,19], and the remarkable demonstration that weanling pups will reverse their preference from food ingestion to nipple attachment when treated with the 5-HT blocker methysergide [18].

On the basis of these findings, two roles can be proposed for the developing brain 5-HT system in the weanling. Firstly, it may inhibit suckling to effect weaning [16, 18, 19]. Secondly, since all the above studies tested pups after acute drug administration, it is also possible that 5-HT acts to terminate discrete bouts of ingestion of suckled mother's milk, particularly since a role in promoting satiety has been proposed for brain 5-HT in the adult rat [3]. The two possible roles are not mutually exclusive.

One means of dissociating a short-term satiety effect from suppression of attachment behavior as such, is by examining the effects on preweanling pups of treatment with a serotonin specific, irreversible neurotoxin such as 5,7-dihydroxytryptamine (5,7-DHT). 5,7-DHT causes a long lasting depletion of brain 5-HT due to the destruction of serotonergic nerve terminals [2]. Thus, if the developing brain 5-HT system is indeed crucial to the promotion of weaning, its destruction should prolong nipple attachment beyond the age of natural weaning.

Two pharmacological issues arise in considering this evidence for a role of 5-HT in the weanling. The first is that of drug specificity. In the studies reviewed above, attachment was increased by peripheral injections of methysergide and metergoline, but not by cinanserin [14,19]. These drugs have relatively poor specificity as 5-HT blockers [8] so that the use of a more specific antagonist is necessary for confirmation of a 5-HT specific effect. The second consideration is whether evidence from peripheral 5-HT manipulations justifies the assumption of central 5-HT involvement, particularly since most 5-HT is associated with the gastro-intestinal tract, so that peripheral manipulations of 5-HT might well influence behaviors associated with ingestion *via* peripheral actions [19].

Both questions can be adressed by the use of central injections of 5,7-DHT, for its administration after desmethylimipramine pretreatment has been shown to be specifically toxic to 5-HT terminals [4,5], and injection of small amounts directly into the brain is unlikely to influence peripheral serotonergic neurons.

The present studies were designed to address these is-

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sues. Neonatal rats were treated intraventricularly with the serotonin specific neurotoxin 5,7-DHT and the influence of the treatment on the pups' attachment behavior both before and after weaning was evaluated.

EXPERIMENT 1: ATTACHMENT IN 5-HT DEPLETED SUCKLING RAT PUPS

METHOD

Subjects

Seven litters of Sprague-Dawley pups born in the lab were used in the experiment. Pups born before 5 p.m. were considered 0 days old. They were raised in litters with their dam, each litter in its own nesting bin. The animal room was maintained at 23°C and on a 14–10 hr light-dark cycle.

5-HT Depletion

At 3 days of age pups were removed from the litter, marked by toe clipping, and injected IP with 20 mg/kg desmethylimipramine (DMI) (Desipramine HCl, Sigma) 1.0 ml/100 g b.wt., or saline vehicle. The pups were then returned to the litter, and removed again 60 min later for a pulse intracerebroventricular injection (pICV). The injection was aimed at the third ventricle as described elsewhere [13]. Briefly, a 23 g guide cannula is pushed through the cartilagenous skull 1.0 mm lateral to bregma, angled medially to a depth of 2.0 mm. A 30 g injection needle is then inserted to a depth of 4.0 mm below the surface of the skull. The procedure is rapid and benign, and requires no anesthesia. DMI treated pups were injected with 104 μg (salt weight) of 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT, Sigma) in 5 μl of 0.1% ascorbic acid solution prepared just prior to injection. Control pups were injected with the vehicle. The pups were returned to the dam immediately after the treatment.

Determination of Brain 5-HT Levels

At 7 weeks of age the rat pups were sacrificed by decapitation and the brains rapidly removed, weighed, and frozen on dry ice. For serotonin determinations each brain was homogenized in 5.0 ml 0.1 N perchloric acid on ice. The homogenates were centrifuged at 20,000 rpm at 4°C to pellet the precipitated protein. The supernatant was saved and stored at −20°C prior to HPLC separation and quantitation by electrochemical detection.

The HPLC chromatographic system consisted of a 5 μm Zorbax ODS column and a mobile phase of 0.15 M monochloroacetic acid and a 2 mM Na EDTA, pH 3.0, at a flow rate of 1.2 ml/min. The electrochemical detection system consisted of a BAS LC-4 amperometric detector and a glassy carbon electrode set at +0.72 V vs. an Ag/AgCl reference electrode. Current deflections (nA) in response to injections of 50 or 100 μl of perchloric acid extracts of brain tissue were recorded on an X-Y recorder. Standard amounts of serotonin ranging from 1 through 100 pmoles were analysed in an identical manner to the brain extracts. A standard curve relating peak height deflection vs. amount (pmoles) was generated using linear regression and this equation was used to quantitate serotonin in the brain samples. Serotonin concentrations are expressed as pmoles/ mg wet weight.

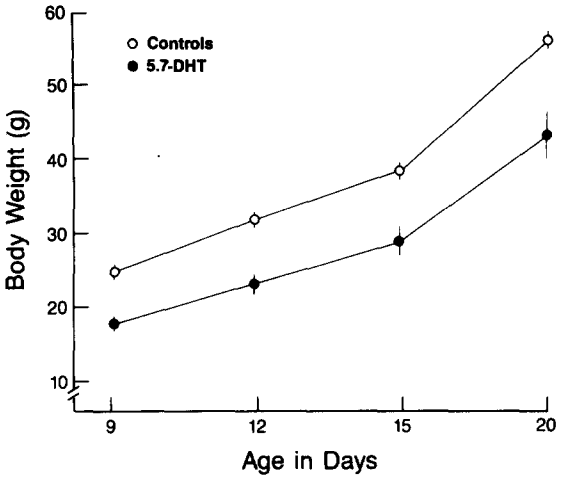

FIG. 1. Effect of 5,7-DHT treatment at 3 days of age on weight gain in sucklings.

Body Weight Controls

Our pilot studies had shown that 5,7-DHT treatment caused a loss of body weight, and in order to control for this, 5,7-DHT treatment was compared with control treatments both within litters (where body weights would unavoidably differ), and between litters (where we attempted to equalize body weights).

For the within litter comparisons, two litters of 10 pups each were treated with 5,7-DHT and vehicle (5 pups of each per litter). The between litter control was effected by treating two litters of 5 pups each with 5,7-DHT, and one litter of 12 pups with vehicle. Since litter size is inversely related to body weight [1] it was anticipated that these experimental and control pups would have comparable body weights during the subsequent tests.

Testing Procedure

The testing procedure followed that described by Hall *et al.* [6]. Briefly, anesthetized dams were placed on their backs along the side of a tilted transparent box (18×28×13 cm) under a lamp to provide warmth and illumination. The pups were placed on the ventrum of their dams and activity levels were scored during the first 30 sec on a 4 level scale: 0=little or no movement, 1=side to side head movement, 2=general locomotor movement, 3=vigorous locomotion. Side to side head movement (level 1) was not observed, but it was replaced in the assessment by slow locomotion with pauses. Latency to attach to a nipple was timed, and pups not attaching during the 5 min test were given a score of 300 sec. During the session, each pup was tested 3 times, but not consecutively.

Pups were tested repeatedly at 9, 12, 15 and 20 days of age. They were tested under 2 deprivation conditions (8 hr deprived and undeprived) at alternate ages, counterbalanced within litters.

Pups to be deprived were removed from the litters at 8 a.m. and placed in bins with littermates for 8 hr before testing at 4 p.m. The bins contained bedding from the home cage, and were placed in a humidified incubator maintained at 33°C. Undeprived pups were placed in bins with their deprived littermates just prior to testing, when the dams were anesthetized. The dams were anesthetized with IP

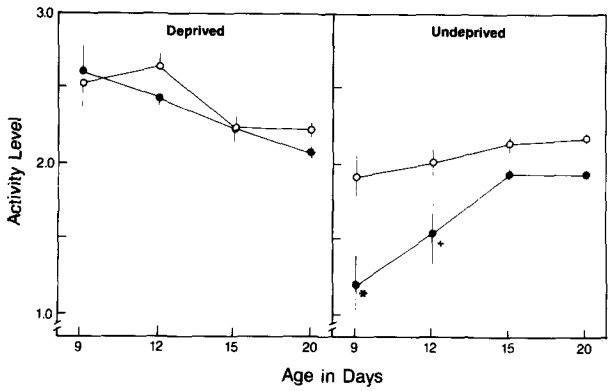

FIG. 2. Activity levels in suckling pups when 8 hr deprived and when undeprived. $+p<0.05$, $*p<0.01$, different from controls, Mann-Whitney U.

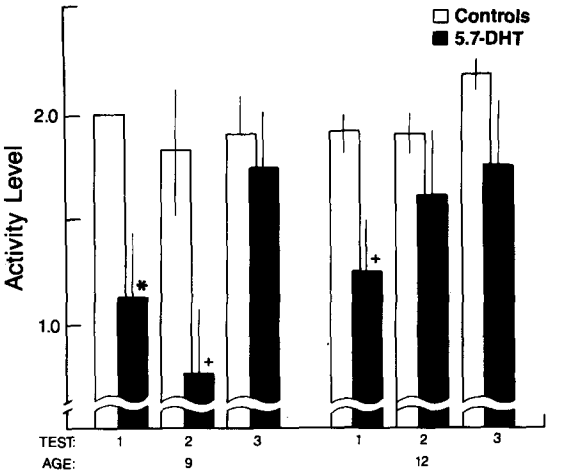

FIG. 3. The influence on activity of repeated tests at 9 and 12 days of age in undeprived sucklings $+p<0.03$, $*p<0.01$, different from controls.

ketamine (20 mg with 0.2 mg acepromazine in 0.2 ml per dam) which was sufficient to anesthetize them for precisely the duration of the test. If necessary, 0.05 ml ketamine was injected during the test to maintain anesthesia. At the end of the test, litters and dams were returned to the nesting bins.

The experimenter did not know what the treatment conditions of each pup were during the test.

Statistical Analysis

Activity was analyzed by appropriate non-parametric tests (see below). Attachment latencies were analyzed by means of *a priori* selected orthogonal comparisons using *t*-ratios [9].

RESULTS

Brain 5-HT

Four of the 20 5,7-DHT treated pups died before testing commenced, once in each litter. Tested litter sized were, therefore, of 4, 9, and 12 pups.

Whole-brain 5-HT levels were significantly reduced in 5,7-DHT treated pups (0.68±0.11 picomoles/mg wet weight±SE) compared to control levels (1.35±0.08) a 50% reduction ($p<0.001$). However, there was considerable variability across litters (range: 73–28% of control values). Three litters were analysed for levels in forebrain and hindbrain separately (posterior collicular transection), and forebrain depletion (0.24±0.02 vs. 1.47±0.10 pmoles/mg wet weight ±SE, $p<0.001$) was greater than hindbrain (0.81±0.25 vs. 1.41±0.13, $p<0.05$), respectively 16% and 58% of control values ($p<0.05$).

Brains of treated pups were lighter than control pups' brains, varying between 97% (NS) and 91% ($p<0.05$) of their respective littermate and body weight controls' brain weights.

Brain 5-HT levels at sacrifice (at 7 weeks) among the treated pups did not correlate significantly with the behavioral measures and since all treated pups had lower brain 5-HT than all their respective controls, they were all included in the analysis.

Body Weight

5,7-DHT treatment retarded growth during the first few days after treatment, and the weight differential, $F(1,144)=105.5$, $p<0.001$ established at this time between treated and control pups was subsequently maintained up to 20 days of age (Fig. 1). This weight difference was not ameliorated by raising 5,7-DHT treated pups in the smaller litters (4 pups/litter), which is otherwise known to increase body weight [1]. Since there was also no difference in the weights of the control pups raised in the different sized litters, subsequent analyses were performed on pooled data for 5,7-DHT treated pups and for controls.

Activity

Activity was increased by deprivation, although this effect was reduced with age. 5,7-DHT pups were as active as controls after 8 hr deprivation, but at 9 and 12 days they were less active when not deprived (Fig. 2).

When the results of the individual tests within the sessions at each age were examined, it emerged that at 9 and 12 days the treated pups were least active in the first test and neared control activity levels in the subsequent tests (Fig. 3).

Latency to Attach

Latency to attach was decreased by deprivation, $F(1,36)=65.4$, $p<0.001$ largely due to differences at 20 days of age (Fig. 4). 5,7-DHT treatment increased attachment latencies at 9 days, again only in undeprived pups. There was an effect of test sequence within sessions, $F(1,64)=10.3$, $p<0.001$. At the first test at 9 days when undeprived, latencies to attach in the treated pups were 2.6 times as long as control latencies, but there was no significant difference at the third test (Fig. 5).

Behavioral Observation

Close observation of the pups failed to reveal any other overt differences due to the treatment, except that deprived 5,7-DHT pups appeared more vigorously active than their controls. This probably was not revealed by the activity

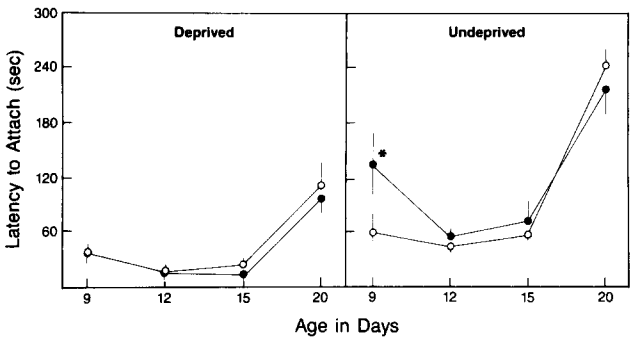

FIG. 4. Latencies to nipple attach (sec) during the 300 sec test. The sucklings were 8 hr deprived or undeprived. *$p<0.01$, different from controls.

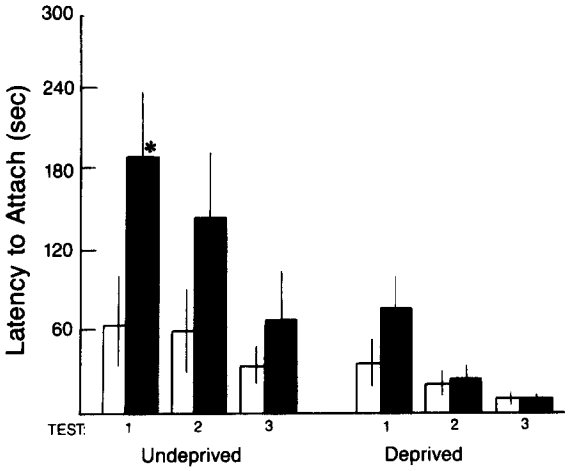

FIG. 5. The influence of repeated tests at 9 days of age on latencies to nipple attach. *$p<0.01$, different from controls.

measures because they distinguish pups primarily by the nature of their activity and less by its intensity.

Apart from the clear difference in body size between the 5,7-DHT and control pups, development appeared normal in both groups, although at 15 days of age, more of the controls seemed to have their eyes more open for more of the time during handling and testing.

DISCUSSION

Our depletions of whole brain 5-HT were milder than those reported by others [4,5]. However, this may be due to differences in injection site. Breese *et al.* [5] report severe depletion in brainstem (10% of control values) with their intracisternal injections, whereas with our third ventricular injections, hindbrain 5-HT was 58% of control levels. On the other hand, our depletion to 16% of control values of forebrain 5-HT is comparable to the values attained by these authors.

These regional differences in depletion may account for our lower mortality rate (20% vs. 70%, [4]), and possibly also for our failure to observe the hyperactivity they reported, although this may also be due to other methodological differences, such as in activity measurement.

The behavioral results show that undeprived 5-HT depleted pups are less active than controls at 9 and 12 days of age, and take longer to attach to the nipples of their anesthetized dams at 9 days of age. The finding that activity was reduced in undeprived 9 and 12-day-old pups is at variance with the report that 5,7-DHT treatment [4,5] and metergoline [16] increase locomotor activity in suckling pups. There are significant differences in the methods employed, but it would seem that the activity changes we have noted are more closely related to attachment insofar as they covary with latency to attach and its responsiveness to age and deprivation status.

Taken together our findings suggest that the undeprived, depleted pups are less keen to attach. However, this conclusion must be tempered by the issue of the degree to which these pups are behaviorally competent 6 days after neurotoxin treatment. The data suggest that they are, because when they were deprived they were as competent as controls, and when undeprived, observations showed that treated pups were active when not attached. They wandered about the dam's ventrum and rooted at nipples without attaching, even when replaced on the nipple. This is reminiscent of the behavioral effects of metergoline in 7–8-day-old pups reported by Ristine and Spear [16]. Moreover, in the condition showing the most robust effect on activity and latency (9 days undeprived) and when retested within the same experimental session, their performance was similar to that of controls by the third test repetition. It is possible that the progressive deprivation during the 90 min experimental session may have been of itself sufficient to reduce attachment latencies in both treated and control pups, again indicating that the depleted pups can respond adequately to mild deprivations, i.e., considerably shorter than the 8 hr deprivation period we employed.

The lack of effect of litter size on the body weights of the treated pups was unexpected. Whether raised in litters of 4 or 9 (with 5 control siblings), the treated pups had similar body weights. At face value, this would suggest that availability of dam's milk was not a limiting factor in these pups' growth rates.

However, our failure to experimentally separate between body weight reduction and 5-HT depletion leaves the possibilty that the behavioral effects are due to the reduced body weight of the treated pups. This issue is clearly critical in determining whether reduced body weight is consequent on reduced suckling by 5-HT depleted pups, or conversely, whether the alterations in behavior can be ascribed to some consequence or correlate of reduced growth. The following experiment addressed this issue by evaluating activity and latency to attach in pups of different body weights that were not treated with 5,7-DHT.

EXPERIMENT 2

METHOD

Six litters participated in this experiment. Growth rate was manipulated by culling 4 litters to 6 pups each, and raising 2 litters of 14 pups. All other procedures employed were the same as for experiment 1.

RESULTS

Body weights of the pups in the litters of two sizes diverged significantly, $F(1,95)=595$, $p<0.001$, and the weights of the lighter pups (raised in litters of 14) and the heavier pups (raised in litters of 6) were similar to the respective

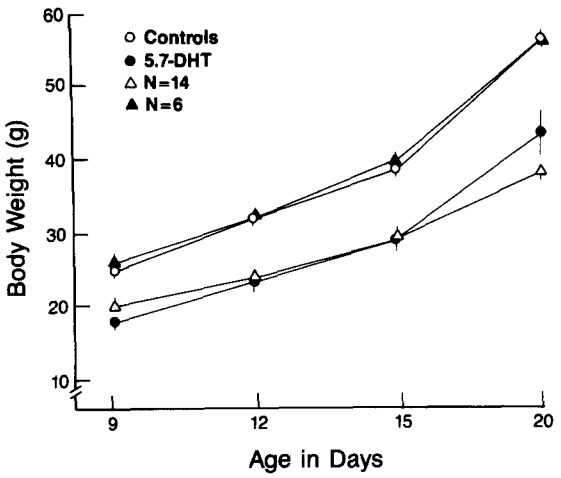

FIG. 6. Growth curves of 5,7-DHT treated sucklings and their littermate controls raised in litters of 9 compared to uninjected pups raised in litters of 14 pups (N=14) or 6 pups (N=6).

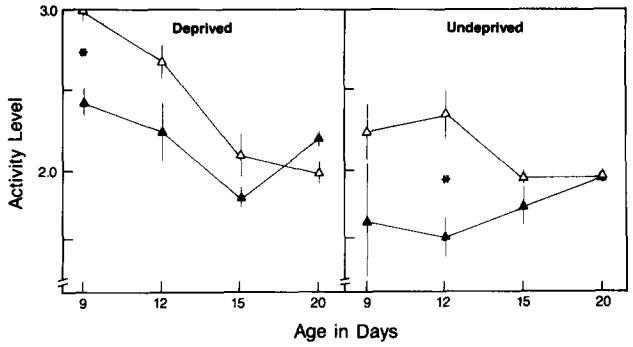

FIG. 7. Activity levels in sucklings raised in litters of 6 or 14 pups when 8 hr deprived or undeprived. *$p<0.01$, for the difference between the two litter sizes.

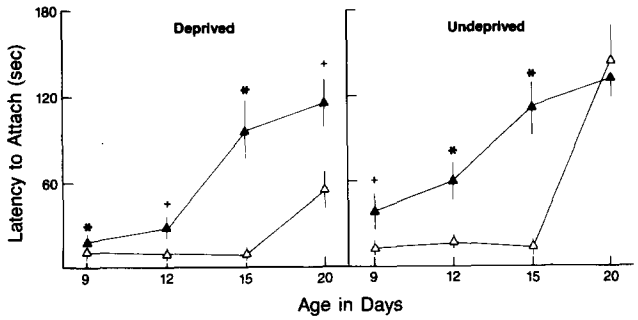

FIG. 8. Attachment latencies in sec during the 300 sec tests for deprived and undeprived sucklings raised in litters of 6 or 14 pups. +$p<0.05$, *$p<0.01$, for the difference between the two litter sizes.

body weights of the treated and control pups of Experiment 1 (Fig 6).

Activity

Activity levels were increased by deprivation at 9 and 12 days of age (Mann-Whitney U, p's<0.03), and the increase was similar for both groups of pups. However, the lighter pups were more active than the heavier ones in both deprivation conditions at these ages (Mann-Whitney U, $p<0.001$). There were no significant effects of litter size or deprivation at 15 and 20 days of age (Fig. 7).

Attachment Latencies

The lighter pups attached more rapidly, $F(1,87)=43.2$, $p<0.001$. Attachment latencies were shortened by deprivation, $F(1,87)=12.3$, $p<0.001$ but mainly in the heavier pups. The lighter pups were unaffected by deprivation (except at 20 days) because they attached immediately under both deprivation conditions (Fig. 8).

Behavioral Observations

The lighter pups, up to 15 days of age and irrespective of deprivation status, behaved much like deprived heavier pups at 9 days of age. They were vigorously active, and the activity was orientated to nipple attachment. As the pups grew older, there was an age related decrease in hyperactivity, which probably reflects the progressive reduction of the stress of 8 hr deprivation as pups increase their body weight with age. This typified both the depleted pups of Experiment 1 and their controls, and the heavier pups in this experiment. However, this reduction in activity was conspicuously delayed in the lighter pups. When returned to the nesting bins after the experiments, these lighter pups were observed to scramble to attach to the nipples of their recovering dam, in contrast to the exploration, play, and grooming which characterised the behavior of all the other pups in both experiments.

At 15 days-of-age, much like the treated pups in Experiment 1, the eyes of the lighter pups seemed to be less consistently open than those of the heavier pups.

DISCUSSION

The results clearly demonstrate that reduced body weight *per se* cannot account for the behavioral results of 5-HT depletion obtained in Experiment 1. When undeprived, 9-day-old depleted pups were less active and took longer to attach than their heavier controls. In sharp contrast, the weight matched lighter pups in Experiment 2 showed increased activity and reduced attachment latencies. Indeed, up to 12 days of age, the behavior of the 5-HT depleted pups was more similar to that of the heavy pups raised in litters of 6 (cf. Figs. 2 and 7, 4 and 8).

Consideration of the experimental findings and behavioral observations of Experiment 2 makes it fairly obvious that the lighter pups were hungry, and that their lower body weights are evidence of a lower nutritional status than that of the heavier pups from the small litters. This clearly distinguishes these pups from the weight-matched 5-HT depleted pups. The depleted pups did not behave as if they were hungry. In fact, their behavior was the opposite of that of the hungry

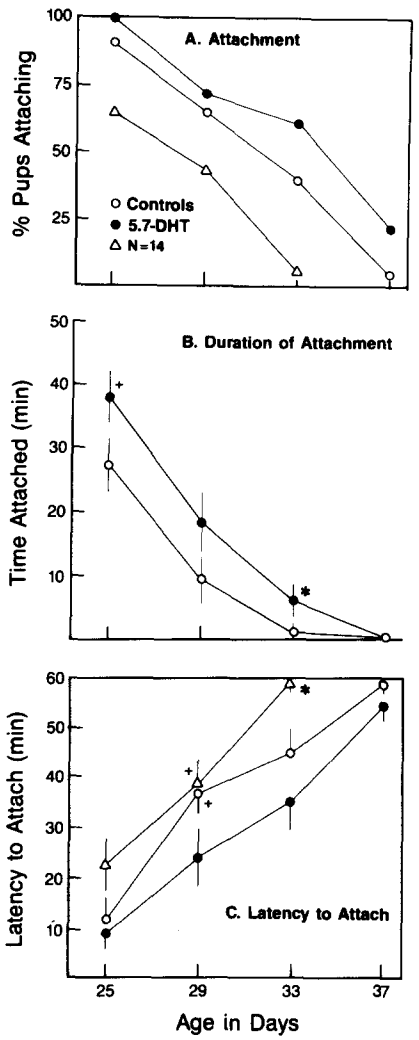

FIG. 9. Effects of 5,7-DHT treatment in weaned pups. (A) Percent pups attaching. (B) Duration of attachment (min) during the 60 min test. (C) Latency to attach (min). Data for uninjected pups raised in litters of 13–14 pups is included in A and C for comparison. For clarity, data for litters of 6 pups is not included. $+p<0.05$, $*p<0.01$, different from 5,7-DHT treated pups.

pups, suggesting the possibility that the 9-day-old 5-HT depleted pups were sated when removed from their dam. In turn, this would indicate that their reduced body weight is consequent upon a reduced level of milk intake.

EXPERIMENT 3: NIPPLE ATTACHMENT IN 5-HT DEPLETED WEANED PUPS

This experiment was designed to investigate the effects of 5-HT depletion on attachment in recently weaned pups and to compare it with the effects of acute 5-HT blockers administered peripherally as reported by others [14,19].

METHOD

Four litters participated in this study. Two of the litters were those used in Experiment 1 for the within-litter comparisons. The others were similarly treated, i.e., half the pups in each litter were treated with DMI and 5,7-DHT as described

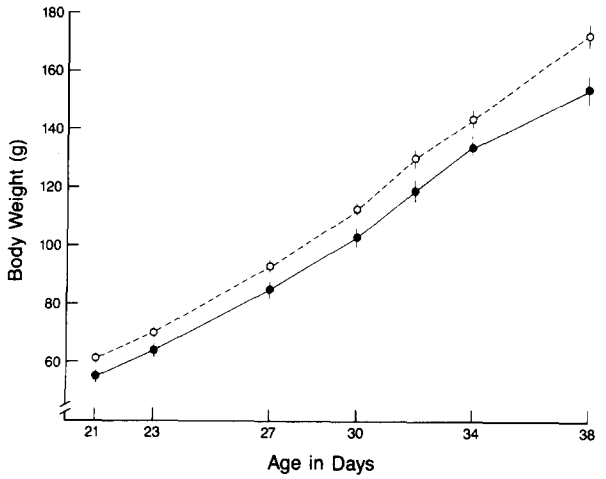

FIG. 10. Post-weaning growth curves for 5,7-DHT treated pups and their littermate controls.

above. The pups used in Experiment 2 as body weight controls (6 litters) were also examined for attachment after weaning. The pups tested were: 18 depleted pups, 20 littermate controls and 27 and 23 body weight controls raised in litters of 13–14 and 5–6 pups respectively.

Procedure

Pups were weaned at 21 days of age by removing the dam from the nesting bin. Nipple attachment was examined at 25, 29, 33, and 37 days of age. Tests of attachment were conducted after the pups had been deprived of food and water overnight for 17 hr. For the test, the pups were placed in small, transparent bins (18×28×13 cm) in groups of 2–4 with an anesthetized (Ketamine, 10 mg/0.1 ml/100 g body weight) dam placed on her side. Foster dams from litters less than 15 days of age were used. Latency to nipple attachment, and frequency and duration of episodes of attachment were recorded during 1 hr at 1 min intervals.

The data were analyzed by a split-plot analysis of variance with repeated measures for age. The data of the depleted pups and their littermate controls were analyzed separately from those of the body weight controls from litters of different sizes.

RESULTS

Effects of 5,7-DHT

Whole brain 5-HT levels were reduced by 5,7-DHT to 58% of control levels (0.89±0.11 pmoles/mg wet weight±SE, vs. 1.54±0.09, $p<0.001$). Depletion across litters varied from 73 to 33% of littermate control values.

Frequency of attachment among the treated pups was greater, i.e., more of them attached ($p<0.001$, Fig. 9A). Total duration of time attached fell with age, but 5,7-DHT pups remained attached longer than their littermate controls, $F(1,36)=4.4$, $p<0.05$, Fig. 9B). Latency to first nipple attachment mirrored these trends, increasing with age and being briefer in depleted pups at 29 and 33 days, $F(1,36)=4.2$, $p<0.05$, Fig. 9C.

Body weight of the treated pups was lower than that of their littermate controls ($p<0.01$, Fig 10). Body weight at 30 days among the 5,7-DHT pups correlated with brain 5-HT

levels at sacrifice ($r=0.74$, $p<0.001$). Although none of the behavioral measures achieved significant correlations with brain 5-HT levels, when the 10 pups with lowest 5-HT levels were analysed separately, the behavioral effects were more prominent.

Body Weight Controls

Body weights of the pups raised in large (14 pups) and small litters (6 pups) differed ($p<0.001$). As in Experiment 2, the body weights of pups raised in litters of 14 were similar to those of the depleted pups, and the body weights of pups raised in litters of 6 were similar to the depleted pups' littermate controls. There was no significant difference in duration of attachment, $F(1,30)=0.01$, or latency to attach, $F(1,30)=2.2$, $p>0.1$ between light and heavy pups. Direct comparison of the lighter pups and the depleted pups (of similar body weights) revealed that relatively more depleted pups attached ($p<0.001$, Fig. 9A). Depleted pups attached for longer on the average at 25, 29 and 33 days, but this did not attain significance. 5,7-DHT pups attached with significantly shorter latencies, $F(1,43)=15.5$, $p<0.001$, Fig 9C.

DISCUSSION

These results demonstrate that 5-HT depleted pups attach with shorter latencies and for longer periods for up to 37 days of age, i.e., 16 days after they were weaned, than their littermate controls. Moreover, control pups attached for a shorter post-weaning period. Interestingly, the pups from the different sized litters attached far less, irrespective of their body weight, and it could be, therefore, that the depleted pups influenced their control siblings' attachment behavior.

The matched body weight controls show that increased attachment in depleted pups is not a simple consequence of reduced body weight *per se*, for body weight did not influence attachment. Moreover, in direct comparison, more of the depleted pups attached, and they did so more rapidly than controls of equivalent body weight.

The results also show that at 37 days of age 5-HT depleted pups do not continue to attach. If the supposition that 5-HT activation is necessary for inhibition of attachment is valid, then it could be that these depleted pups have recovered 5-HT activity, possibly due to the emergence of supersensitivity. We tested this possiblity in the following experiment by administering methysergide, which has been shown to induce attachment in weanlings [14,19].

EXPERIMENT 4

METHOD

Two litters of depleted pups and their littermate controls which were tested in the previous experiment were injected SC with methysergide bimaleate (1 litter with 10 mg/kg, the other with 20 mg/kg) and tested for attachment as in Experiment 3. They were tested at 39 days of age.

RESULTS

Twenty mg/kg methysergide sedated the pups, and the test was therefore extended in duration for 1 hour after they were fully recovered. None of the pups attached at either dose although there were brief episodes of treadling, nibbling of the nipples and other protuberances of the dam's anatomy such as snout, toes, eyes and tail. There was no difference in the incidence of these behaviors between depleted and control pups. It thus seems that 5-HT blockade in these pups did not disinhibit attachment, i.e,. that recovered 5-HT systems were not inhibiting attachment in the depleted pups.

GENERAL DISCUSSION

Our findings have clarified a number of issues relevant to understanding the growing body of information about the role of serotonergic systems in the ontogeny of nipple attachment behavior. By using 5,7-DHT we have confirmed the involvement of 5-HT systems in the behaviors previously obtained with methysergide and metergoline [14, 16–19] which are relatively non-specific 5-HT blockers [8].

Firstly, we have shown that in the preweanling 5-HT depletion reduces nipple attachment in the sated 9-day-old pup, and this is consonant with previous findings [16,17] indicating that the acute receptor blockers methysergide and metergoline reduce attachment in the 3–8-day-old neonate, but not at 10 days or later.

Secondly, in the weaned pup, we have shown that 5-HT depletion disinhibits nipple attachment in deprived pups for up to 16 days after imposed weaning, in line with the effects of the acute blockers [14,19].

We have therefore demonstrated, in the same pups, the biphasic effects of 5-HT antagonism (namely inhibition of attachment in the neonate and disinhibition in the weanling), previously reported in separate experiments. These results bolster the confidence with which we can ascribe these behaviors to a specific serotonergic antagonism.

In addition, we have shown that the effects in the weanling are mediated by the brain 5-HT system, thereby ruling out the possible peripheral effects that complicate the use of the acute blockers.

Finally, as previously argued [19] we have shown that an intact brain 5-HT system is not crucial for weaning to occur. All the growing pups in our experiments ceased to nipple-attach after 33 days, despite whole-brain 5-HT levels as low as 14% of control values. This was probably not due to recovery of 5-HT function because of supersensitivity of undamaged neurons, since methysergide failed to reinstate attachment in these pups.

At face value the curtailment of weight gain evident in the neonatally depleted pups appears contradictory in the light of their subsequent increased attachment. However, our findings and those of others [5, 7, 17] suggest that suckling may be reduced in the preweanling by 5-HT depletion or antagonists. In the weanling, insofar as increased attachment suggests increased suckling in the depleted pups, this increase could be expected to occur only after 20 days of age (Experiment 1 and [14] and [19]), i.e., when our pups were weaned and thereby prevented from regaining weight by any putative increase in suckling. It is also the case that curtailment of weight gain early in suckling establishes a long-term weight deficit as shown by our control litters of 6 and 14 pups and by others [1], so that if suckling is indeed reduced by 5-HT depletion in the neonate, the weight differential established at that age might be expected to persist. It should also be recognized that damage to the brain's 5-HT system by neonatal neurotoxin treatment is bound to have consequences which might also influence growth and development beyond the domain of suckling and feeding behavior, such as by pertubation of endocrine function in general, and growth hormone in particular [10].

For these reasons too, it is difficult to rule out the possibility that 5-HT mediates post ingestive (by suckling)

satiety, since reduced growth might mask the increase in weight resulting from increased intake due to chronic suppression of satiety. Nevertheless, parsimony does not favor the argument that depleted pups are suckling more yet weigh less, and our finding that light pups raised in large litters and which are presumably hungry, do not attach more after weaning, further weakens the putative link between nipple attachment and appetitive state. It thus appears unlikely that 5-HT mediates satiety during suckling.

In conclusion, while our results lend weight to the specific effect of 5-HT antagonism in the disinhibition of attachment, the role of endogenous 5-HT in weaning remains elusive. Our findings and those of others, show that the role of 5-HT in mediating the inhibition of attachment is facilitative rather than obligatory, and it is transient. Is this disinhibition of nipple attachment merely a neurological curio, or has it broader behavioral significance somewhere in the gamut of transitions that occur during weaning, from the ingestive to the affective?

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