

Olfactory Determinants of Rat Pup Locomotion

James V. Gallivan

Thesis presented to
the Faculty of Graduate Studies
of Carleton University
in partial fulfillment of the requirements
for the degree of Master of Arts in Psychology

Theses
M.A.
1980
G34

For Valerie

Abstract

Home cage odors, of which maternal droppings' odor comprises a central element, influenced locomotion in the day 15 laboratory rat even in the presence of its mother. In contrast, weanling age pups were mostly indifferent to these odors. In environments devoid of home cage odors, the day 15 pup locomoted little. However, when home cage bedding odor, or maternal fecal odor was presented to the isolated pup, or when the pup was placed on thoroughly disturbed home cage bedding, high rates of locomotion ensued. Clean bedding material elicited intermediate locomotor activity. These differences between groups exposed to various odor and bedding conditions were time-dependent -- strongest initially and decaying thereafter. Whereas initial contact with disturbed home cage bedding produced high velocity locomotion, isolation in the undisturbed home cage energized a strikingly different behavioral profile, with locomotion rare and vigorous digging predominating. These findings were interpreted under the rubric of strategies for dealing with threat given the pup's vulnerability, use of olfactory information and separation from the nest.

Acknowledgements

Gratitude is extended to Bruce Pappas for his advice, support and patience throughout this endeavor. Appreciation is also extended to Gerry Owen for his time and labour, with which he was most generous, and to Jose Bell for his able technical assistance.

Table of Contents

| | |
|--|-----|
| Title | i |
| Abstract | iii |
| Acknowledgements | iv |
| Table of Contents | v |
| Figure Captions | vi |
| Introduction | 1 |
| Experiment 1 | 5 |
| Experiment 2 | 11 |
| Experiment 3 | 14 |
| Experiment 4 | 17 |
| Experiment 5 | 18 |
| Experiment 6 | 23 |
| General Discussion | 29 |
| Reference Note | 32 |
| References | 33 |
| | |
| Appendix 1: Diagram of odor presentation chamber | 37 |
| Appendix 2: Effects of ventilation and lighting on bedding- elicited locomotion in the day 15 rat pup | 38 |
| Appendix 3: Effect of amphetamine on bedding-elicited locomotion in the day 15 rat pup | 40 |
| Appendix 4: Analysis of variance tables | 44 |

Figure Captions

- Figure 1. Mean crossings per minute for the first 10 min of exposure to maternal odor or unscented room air at 15 or 26 days of age.
- Figure 2. Mean (+S.E.) crossings per hour for the first and sixth hours during continuous exposure to maternal odor or unscented room air at 15 or 26 days of age.
- Figure 3. Mean crossings per minute for the first 10 min of exposure to home cage bedding or no bedding at 15 or 26 days of age.
- Figure 4. Mean crossings per 5-min interval for the first hour of exposure to home cage bedding or no bedding at 15 or 26 days of age.
- Figure 5. Mean (+S.E.) crossings per hour for the first and sixth hours during continuous exposure to home cage bedding or no bedding at 15 or 26 days of age.
- Figure 6. Mean crossings per hour for six hours of continuous exposure to home cage bedding, clean bedding, or no bedding.
- Figure 7. Mean crossings per hour for six hours of continuous exposure to home cage bedding or no bedding in Wistars or Sprague Dawleys.
- Figure 8. Plot of mean crossings per hour versus percent time not huddling with a sibling, two anesthetized siblings, the dam or the anesthetized dam. The lines are linear regression estimates of mean crossings as a function of percent non-huddling time in home cage bedding or no bedding.

- Figure 9. Mean crossings per 5-min interval over a one-hour exposure period to home cage, clean, or no bedding in the presence of a non-anesthetized sibling. Scores are means for each sibling pair.
- Figure 10. Behaviors of day 15 rat pups in disturbed or undisturbed home cage bedding or near disturbed or undisturbed home cage bedding but in clean bedding. Illustrated are mean (+S.E.) percent time digging (10a), mean (+S.E.) cage crossings (10b) and mean (+S.E.) percent side preference (10c) as a function of these odor/bedding conditions over the test hour.
- Figure 11. Mean crossings per 5-min interval over the first half hour of exposure on day 15 to home cage bedding or no bedding under white lighting or with fans turned off.
- Figure 12. Mean (+S.E.) crossings per half-hour over a one-hour exposure period in disturbed home cage bedding or no bedding when injected 5 min before testing with d-amphetamine or saline.
- Figure 13. Mean (+S.E.) crossings per half-hour over a one-hour exposure period in disturbed home cage bedding or no bedding when injected 30 min before testing with d-amphetamine or saline.
- Figure 14. Mean (+S.E.) crossings per half-hour over a one-hour post-injection period. Pups were injected with d-amphetamine or saline 30 min after initial exposure to disturbed home cage bedding or no bedding.

Introduction

During the weeks after birth, the rat pup progresses rapidly towards independence of maternal care and control, a progression highly related to the development of locomotor skills. Through locomotion, the pup gains some ability to select its environment, and additionally, to travel out of the relative security of the nest and into environments of high risk. Outside the nest, the pup is threatened by cold, starvation and predation, while inside it receives temporary sanctuary from these perils. Eventually, however, synchronous changes in the pup and its mother force the pup out of the nest permanently. At this point, insufficient extra-nest experience may leave the pup at a survival disadvantage¹.

To best serve the pup's dual needs of risk minimization and preparation for autonomy, the pup is immersed in a program of gradually increasing exposure to the extra-nest environment. As the pup's motoric competence, thermoregulatory independence of the huddle, sensory capabilities and experience increase, it can sustain longer and further separations from the nest. However, before the pup can remain in and explore the environment outside the nest, maternal protectiveness must be relaxed. In young pups, less than two weeks of age, the dam aggressively controls litter dispersion by retrieving pups that wander outside the nest (Rosenblatt & Lehrman, 1963). This method of litter dispersion control

1. No distinction between the wild and laboratory rat pup is undertaken here, although, of course, pup exploration and litter dispersion are invariably constrained in the laboratory.

places an increasing tax on maternal energy with each increment in pup mobility. To conserve maternal energy, each pup must become increasingly responsible for its own separation from the nest; to enable control over this separation, the pup utilizes olfactory cues emanating from the nest area (Altman, Sudashan, Das, McCormick & Barnes, 1971; Cornwell-Jones & Sobrian, 1977; Gregory & Pfaff, 1971; Scerzenie & Hsiao, 1977; Sobrian & Cornwell-Jones, 1977). Thus each pup comes to exercise continuous control over its exposure to the risky benefits of extra-nest experience.

Research on odor-mediated separation control has focussed on the reactive aspects of this control system, typically by experimentally inducing a separation between the rat pup (Altman et al., 1971; Cornwell-Jones & Sobrian, 1977; Gregory & Pfaff, 1971; Holinka & Carlson, 1976; Leon, 1974; Leon & Behse, 1977; Leon & Moltz, 1971, 1972; Sobrian & Cornwell-Jones, 1977), hamster pup (Devor & Schneider, 1974; Gregory & Bishop, 1975) or kitten (Freeman & Rosenblatt, 1978a, 1978b) and its mother or nest. The vectored approach response that results has two components, direction and velocity, which with appropriate techniques can be studied separately. To date, most research effort has concentrated on the directional aspects of this odor-guided behavior - a rewarding strategy that has dramatically enhanced our perception of the importance of odor cues to the infants of these species.

During the first two weeks of life, isolated rat pups orient themselves towards odor from their home cage; this rudimentary approach response has been noted as early as 2-3 days postpartum (Bolles & Woods, 1964; Cornwell-Jones & Sobrian, 1977; Scerzenie & Hsiao, 1977). Towards

the end of the second week the dam begins to emit large quantities of caecotrophe, a volatile substance secreted with the feces and a powerful marker of the nest site (Leon, 1974). Separated pups reliably home towards this odor until the fourth week, when coincident with weaning, the pups begin to emit caecotrophe in their own droppings and their strong attraction to the nest site dissolves (Leon & Behse, 1977).

Thus for the first month postpartum, the nest defines the meeting-place for mother and young. To ensure successful reunions, the spatial distribution of pups around the nest is controlled, primarily by maternal efforts at first, then increasingly by the pup itself. By the third week postpartum, maternal involvement is limited to the production and selective deposition of volatile marker. In contrast, the pup assumes a more active role, routinely using nest intensity cues to monitor and control its separation from the nest, and analogously, its exposure to risk, and employing this same information to provide guidance for rapid homing when threatened.

To the young pup, whose only experience has been in the nest and its immediate environs, experimentally induced separation from the nest constitutes an exceptional threat. When threatened by isolation in an unfamiliar environment, the pup will attempt to search out and move towards security -- which at two weeks of age is defined by the nest and the mother but is also typified by familiar odor environments to which the pup has simply had exposure unaccompanied by harm (Leon, Galef & Behse, 1977). To accomplish this odor-guided transition from high to low threat, the pup needs an olfactory gradient of sufficient steepness and a physical

environment that allows unimpeded locomotion. Unfortunately, most studies of pup locomotor activity have been performed using inescapable test environments of unspecified olfactory dimensions. Not surprisingly then, consensus has been rare.

More recent investigations of pup locomotion have attempted to specify the odor environment at testing (Campbell & Raskin, 1978; Randall & Campbell, 1976). They reported that pups placed in the home cage or with the mother are relatively quiescent compared to agemates separated from both of these stimuli. Interpreted within the context of homing behavior, these findings suggest that contact with the goal object (the nest or the mother) elicits a re-orientation in pup behavior from approach to contact maintenance, and consequently, locomotor activity levels (which reflect approach velocity) drop from high to near zero. Thus a critical dimension in determining pup activity may be the presence/absence of separation from an attractive odor source -- when in contact with an attractive odor source, low activity ensues; otherwise activity is high and is characterized by attempts to approach the odor source by moving up the odor gradient. The following series of experiments examines the effects of nest-relevant odors on pup locomotor activity levels during nest-seeking and approach.

EXPERIMENT 1

Campbell and Raskin (1978) concluded on the basis of their experiments that day 15 rat pups respond to an unfamiliar environment with high locomotor activity levels and display low activity in a familiar environment. No differential response to environment familiarity was observed on day 25. The present experiment, using presence/absence of maternal odor as the familiarity modifier, was designed to reconfirm the conclusion of Campbell and Raskin.

Method

Animals

Wistar pups were born in 2 L of hardwood chips in standard 23x46x15-cm polypropylene cages from mothers bred in our colony or purchased pregnant from Canadian Breeding Farms, St. Constant, Quebec. Day of birth is called day 1. Litters were culled to nine by day 3. Bedding remained unchanged until after testing on day 15 when 1.5 L of soiled bedding were removed and replaced with clean bedding. Litters were housed under 12L:12D h reversed light schedule (light on at 20:00) and tested during the dark portion of their cycle.

Apparatus

Locomotor activity was measured in four 40x40x16-cm plywood boxes with tops of clear plexiglass. Each box (diagram in Appendix 1) comprised a separate odor environment. A fan-powered supply and exhaust system moved

air at 3.5 L/min upwards in the box floors and out of the test room through exhaust lines connected to the plexiglass tops. Removable mylar cylinders (20 cm diam) contained the main air flow to the central portion of the box. Lines on the box floor divided the floor into 5 equal areas: the central area inside the cylinder, and the remains of 4 squares missing their central corners. Four cut-aways (3.2X3.8 cm) at the cylinder bottom permitted free pup movement in and out of the central area and rapid equilibration of odor throughout the box. The air delivery line to each box was interrupted by an air-tight plastic reservoir into which could be placed a small petri dish filled with odorant material. Test room lighting was a single 100-W red bulb.

Procedure

Pups (n=12/cell) were exposed for 6 h to one of two odor conditions (maternal fecal odor or no odor) on day 15 (range 15-16) or day 25 (range 26-27). On test day the dam was removed from the home cage and isolated in a hanging wire cage with ad lib food and water. Feces were collected and stored in covered petri dishes until at least 1 g had been collected. Owing to large differences in the rates of defecation, the collection procedure took considerably longer with the day 25 (up to 16 h) than the day 15 (less than 8 h) mothers. When sufficient fecal material was collected, but not before 8 h maternal separation, the deposit was divided and placed in the air delivery reservoirs of two boxes dedicated to the maternal odor condition. Four littermates were removed from the home cage and transported to the test room in a holding cage. Pups were placed in

the boxes outside the central cylinders and the supply/exhaust fans turned on. The first and sixth hours of locomotor activity were recorded using a video camera mounted over the boxes and videorecording equipment in a separate room. After testing, the apparatus was thoroughly washed with non-odorized detergent solution.

Tapes were scored for two dependent measures, time spent inside the central cylinder and number of line crossings, including movements into and out of the central area.

Results

When placed in a test box, pups of all ages and treatments soon investigated the central cylinder interior (mean entrance latency 2.1 ± 0.3 min). However, analysis of variance (ANOVA), with factors ODOR, AGE and repeated measure factor HRS, revealed that exposure to maternal odor or room air did not differentially affect the amount of time spent within the cylinder at either age. Although the central area constituted 1/5 of the total box floor area, all groups spent less than 1/5 of any hour inside the cylinder indicating a general preference for the box periphery. This preference was stronger in day 26 pups than in their juniors ($F(1,44)=4.05, p=.05$).

Figure 1 displays locomotor activity of day 15 and 26 pups in the two odor conditions during the initial 10 min of odor exposure. A repeated measures ANOVA with TIME (1-min intervals) as the repeated measures variable revealed a significant ODOR X AGE X TIME interaction ($F(9,396)=2.85, p=.003$). There was no significant effect of odor

Figure 1. Mean crossings per minute for the first 10 min of exposure to maternal odor or unscented room air at 15 or 26 days of age.

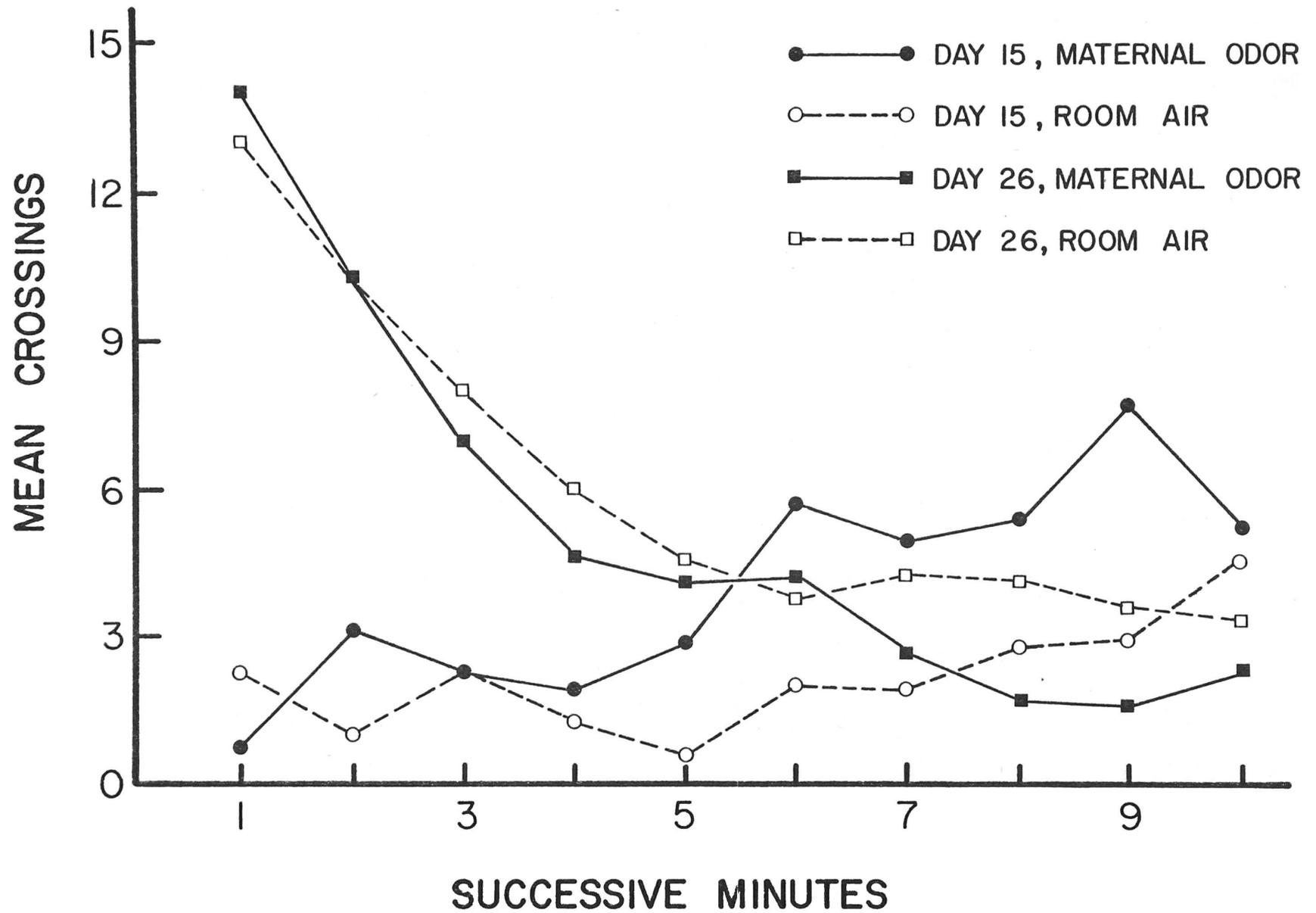
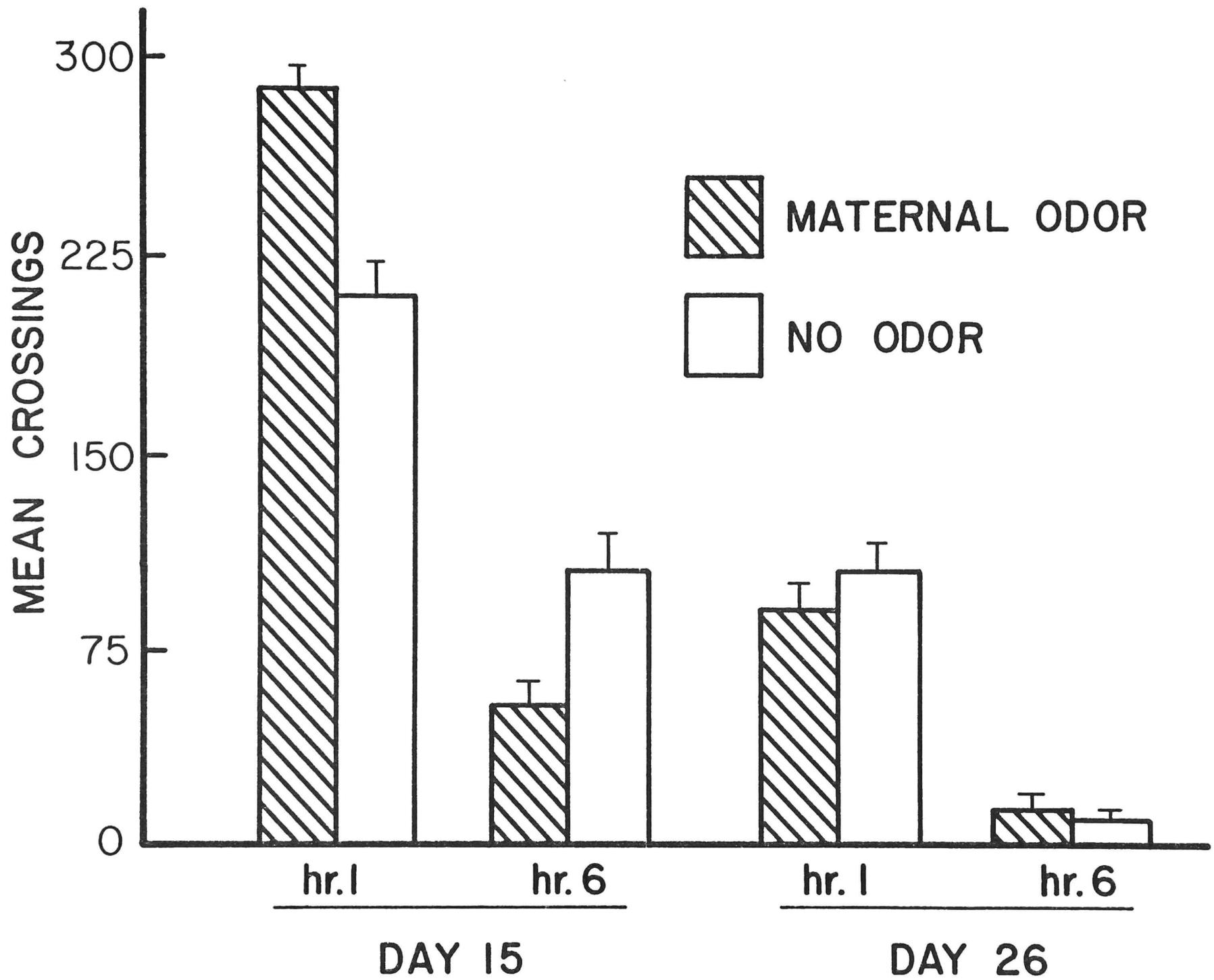


Figure 2. Mean (+S.E.) crossings per hour for the first and sixth hours during continuous exposure to maternal odor or unscented room air at 15 or 26 days of age.



condition on day 26 during this period. On day 15, however, Scheffe post hoc comparisons revealed that in the second half of this 10-min period pup activity was significantly higher in the maternal odor group compared to no odor controls ($p < .01$).

Tapes of hours 1 and 6 were scored for locomotor activity in 5-min segments also (factor TIME). ANOVA with repeated measures on HRS and TIME revealed a very strong AGE X HRS X TIME interaction ($F(11,484)=20.78$, $p < .001$) arising from two distinct age-dependent reactions to the box environment. Day 26 pups continued the precipitous drop in locomotor activity seen in Figure 1, leveling off to baseline at about 15 min. Day 15 pups displayed a modest increase in activity throughout the initial hour. Within hour 6, neither age group displayed TIME-related alterations in activity. From hours 1 to 6, however, day 15 pups displayed a large decrease in activity, whereas day 26 pups displayed no change (Figure 2, $F(1,44)=7.76$, $p < .01$).

Odor effects were revealed in this analysis by a significant ODOR X AGE X HRS interaction ($F(1,44)=4.68$, $p < .05$). Scheffe comparisons between maternal odor exposed day 15 pups and their no odor controls revealed significantly higher hour 1 activity in the pups exposed to maternal odor ($p < .05$, Figure 2).

Discussion

In apparent contradiction to Campbell and Raskin (1978), day 15 pups in this experiment responded to a familiar environment with high activity. However, the basis of this contradiction depends on the rather fragile assumption that simple addition of maternal odor to an otherwise unfamiliar test environment renders this environment familiar. More likely, and in accordance with Leon's many reports (Leon, 1974; Leon & Behse, 1977; Leon & Moltz, 1971, 1972), maternal odor acts to energize pup locomotion and direct this locomotion towards the odor source. In this view, the novel test environment remains aversive and threatening and elicits odor-guided escape (Leon, 1978).

With regard to the direction-guiding effects of maternal odor, the limitations imposed by the apparatus of this experiment must be considered. The test environment was specialized to permit selective odor presentation and observation of odor effects on locomotor activity over a sustained period. To achieve these design goals, pup contact with the odor source was denied and direction-guiding odor cues were minimized -- in essence, frustrating approach and contact while facilitating extended observation of pup search locomotion.

Nonetheless, some spatially-discrete odor cues may have existed in this environment, enabling some approach behavior. It was reasoned that maternal odor fan-forced through the test chamber may have defined attractive air currents, inducing the pup to search in their vicinity. However, pups exposed to maternal odor blown through the central cylinder spent no more time occupying this area than their no odor controls,

suggesting that pup search behavior was randomly directed and that the procedure used here did not produce an uneven distribution of odor.

Over the first hour, maternally deprived day 15 pups displayed a mild increase in locomotor activity which was enhanced by maternal odor presence but was at dramatic variance with the rapid, adult-typical habituation pattern observed in 26-day-old pups. This rapid habituation in day 26 pups has been noted by others (Bronstein & Dworkin, 1974; Bronstein, Neiman, Wolkoff & Levine, 1974; Melberg, Ahlenius, Engel & Lundborg, 1976); however, the day 15 pattern reported here has not been reported elsewhere. Typically, studies of day 15 locomotion have yielded little interstudy consistency suggesting that this age is characterized by a marked sensitivity to procedural variation.

A noteworthy procedural variation used in this experiment is the minimum 8 h maternal deprivation to which all pups were subjected. This appears to have had no effect on the activity of the largely self-sufficient day 26 pups. However, Hofer (1975) has reported that 8 h of maternal deprivation significantly increases day 14 baseline activity suggesting that similar baseline alterations occurred on day 15 in this experiment. The next experiment, similar in design, avoids pretest maternal deprivation by testing pups in a more easily obtainable odor environment.

EXPERIMENT 2

In experiment 1, maternal fecal odor elicited high day 15 activity, which was interpreted as vigorous search behavior in an approach-restricting environment. According to the model espoused here, pup separation from an attractive odor source in a threatening environment elicits a vigorous attempt by the pup to extract itself from its present environment and move towards greater security. For the young pup, the locus of maximum security is that site emitting the attractive odor, usually the nest or mother. Movement towards this locus is characterized by high velocity; its successful attainment is marked by relative quiescence. It follows, then, that by placing the pup in direct contact with an attractive odor source, thus bypassing the approach process, immediate low activity should be observed. Supporting this hypothesis, Randall and Campbell (1976) and Hofer (1975, 1976) have observed that the presence of the mother subdues the young. Campbell and Raskin have reported similar low activity in the presence of soiled home cage bedding, both when left in the home cage (1978, experiment 1) and when transferred to a clean cage (1978, experiment 2). As an attractive odor source, home cage bedding offers considerable practical advantages: it is easily collected, it is abundant and it is divisible, permitting simultaneous testing of more than one animal. Thus home cage bedding, of which maternal droppings are a natural component, was selected as the attractive odor source in this experiment. Of major interest was whether contact with this familiar material would elicit a settling reaction in the day 15 pup.

Method

Animals

Source of animals and housing were as reported in experiment 1.

Apparatus

The boxes used in experiment 1 were modified slightly for this experiment. The mylar cylinders were removed as was a section of the air delivery system containing the reservoir for odorant material.

Procedure

Pups (n=12/cell) were exposed for 6 h to one of two bedding conditions (home cage bedding or no bedding) on day 15 or day 26. On test day the dam and litter were removed from the home cage and the bedding material mixed. A 1-L sample was removed and divided between the two boxes dedicated to this odor condition. Littermates transported to the test room in a holding cage were randomly assigned to a box. After testing the boxes were thoroughly washed.

Pup behavior was videotaped over hours 1 and 6. Locomotor activity was scored as crossings of box floor lines or the lines redrawn on a video monitor screen.

Results

Figure 3 illustrates the effects on locomotor activity of bedding conditions (BED) and age at testing (AGE) during the initial 10 min of

Figure 3. Mean crossings per minute for the first 10 min of exposure to home cage bedding or no bedding at 15 or 26 days of age.

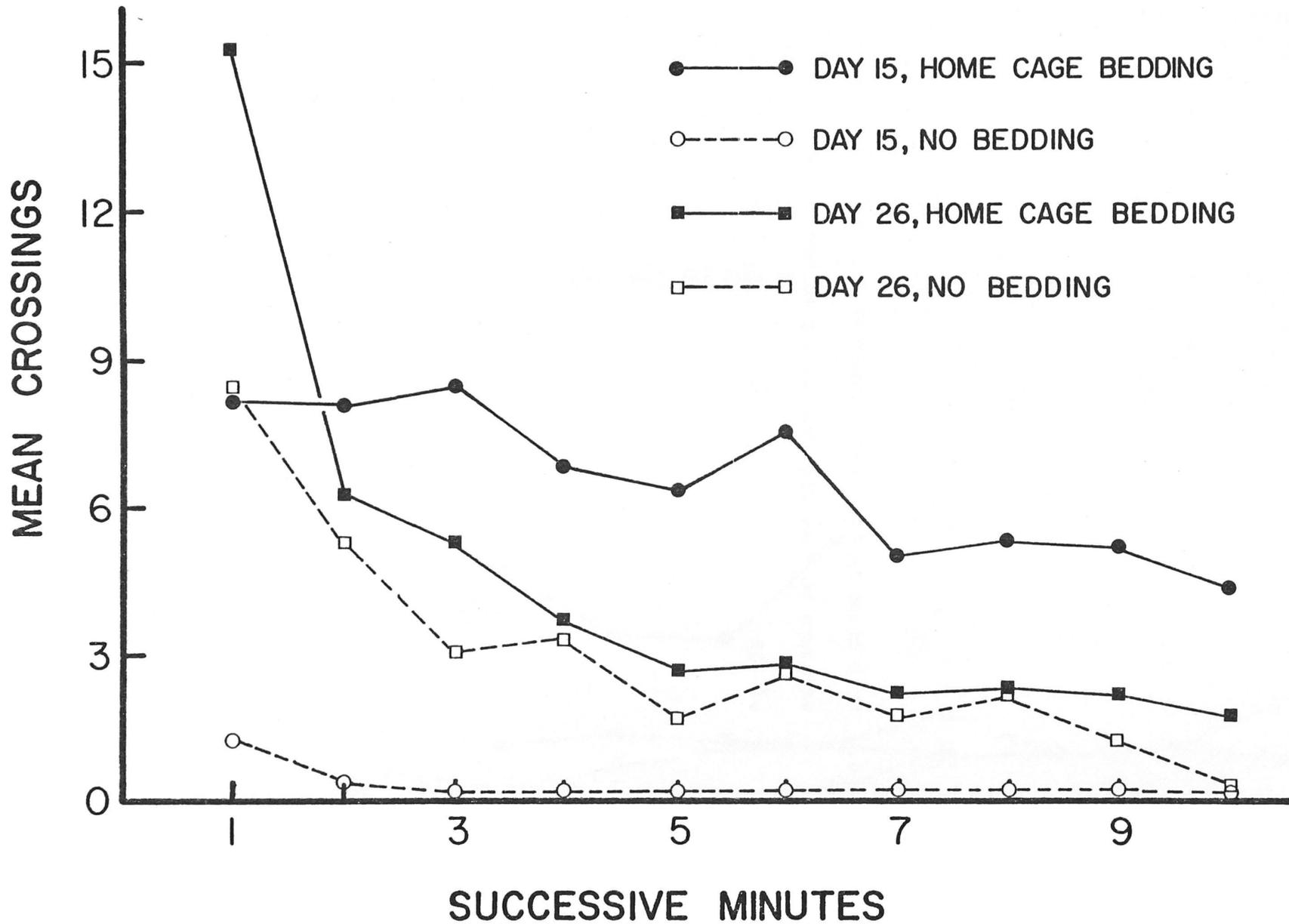


Figure 4. Mean crossings per 5-min interval for the first hour of exposure to home cage bedding or no bedding at 15 or 26 days of age.

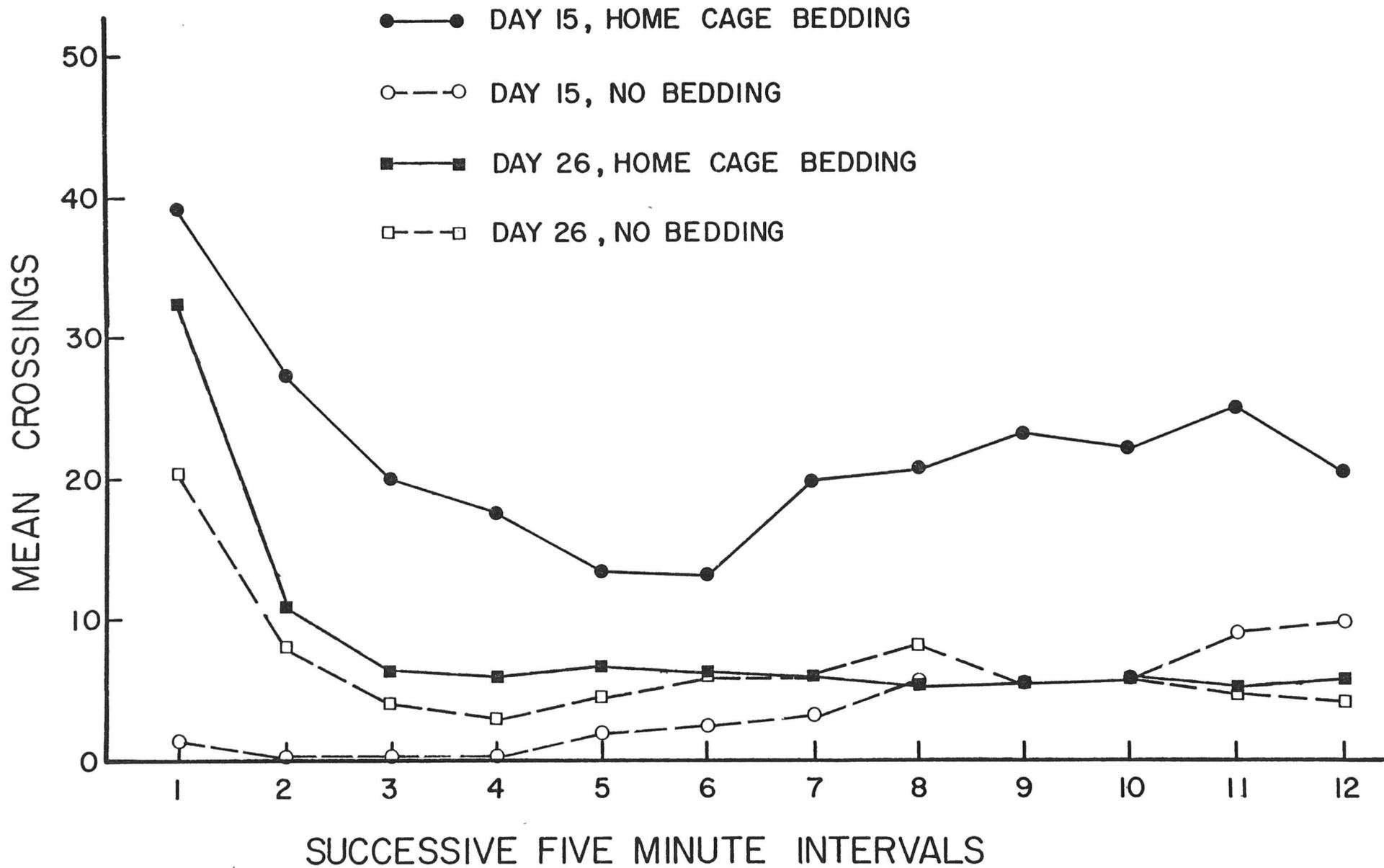
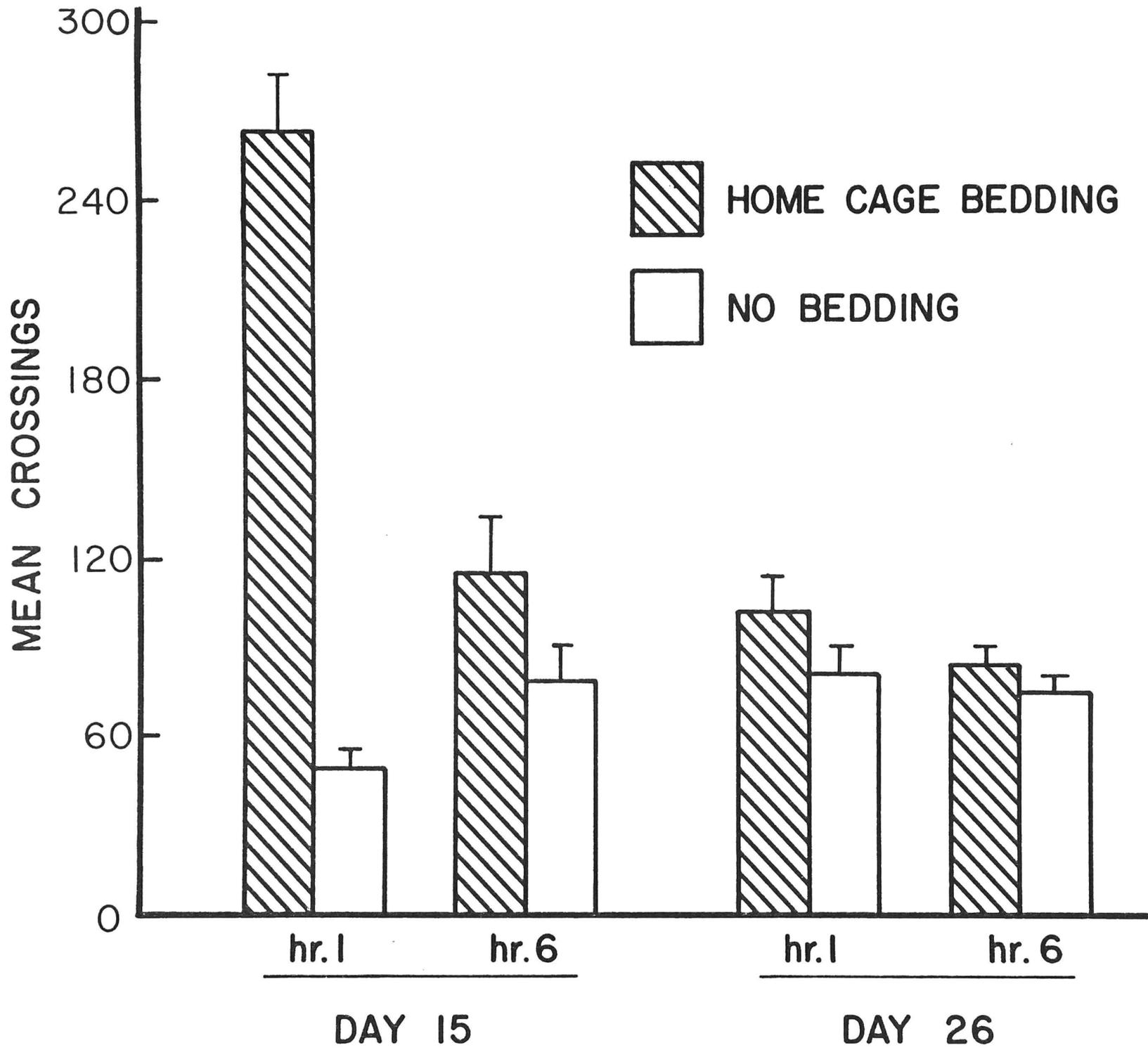


Figure 5. Mean (+S.E.) crossings per hour for the first and sixth hours during continuous exposure to home cage bedding or no bedding at 15 or 26 days of age.



odor exposure (TIME). Repeated measures ANOVA revealed a significant BED X AGE X TIME interaction ($F(9,396)=2.71, p=.005$) which testified to the different time-related patterns of activity on days 15 and 26 as well as the large differences in response to bedding conditions at the two ages. Home cage bedding produced significantly higher activity than no bedding in day 26 pups during the first minute only ($p<.05$). Over a 1-h time period the bedding effect disappeared entirely on day 26 but persisted on day 15. As Figure 4 illustrates, however, in both age groups the bedding effect was strongest at the beginning of exposure.

Figure 5 summarizes locomotor activity during hours 1 and 6 of the four AGE X HRS combinations. Repeated measures ANOVA with HRS and TIME (5-min intervals) as the repeated measures variables revealed a highly significant BED effect ($F(1,44)=14.39, p<.001$). A BED X AGE X HRS interaction ($F(1,44)=5.81, p=.02$) was clarified by the significant difference between the effects of BED conditions on day 15 at hour 1 ($p<.001$). During this hour, pups in the home cage bedding condition were more active than those in the no bedding condition.

Discussion

The major finding in this experiment is the 5-fold difference in bedding-elicited activity observed on day 15 in the first hour of exposure. As in experiment 1, day 15 pups exposed to nest-relevant stimuli exhibited higher activity than non-exposed age-mates, but the magnitude of the difference was much greater in the present experiment. Comparison of control groups from experiments 1 and 2 suggests that the

lesser difference found in experiment 1 is due mainly to an elevated activity baseline resulting from pretest maternal deprivation (Hofer, 1975).

The effect of home cage bedding on day 15 activity reported in this experiment does not support the hypothesis that pups will settle upon contact with nest material. Perplexingly, the low activity in no bedding exposed pups and high activity in home cage bedding exposed pups reported here for hour 1 of a 6-h exposure period is completely opposite to the mean activity levels Campbell and Raskin reported for an exposure period of identical length.

These conflicting findings can be reconciled only if significant procedural differences are uncovered or if large alterations in bedding elicited activity occur between hours 1 and 6.

Since the day 25 data of this experiment closely replicated the findings of experiment 1 for this age, succeeding experiments concentrated on better describing the more intriguing day 15 phenomenon. Of interest also was the extent to which the conflict between this and the findings of Campbell and Raskin could be resolved. Before engaging in large scale investigations, however, I wished to gain some insight into the robustness of the day 15 bedding response (pilot studies in Appendix 2).

EXPERIMENT 3

The purpose of this experiment was to determine whether the different time-sampling procedures employed by myself and Campbell and Raskin might

account for the apparent incongruity of results. Campbell and Raskin sampled locomotor activity continuously over a 6-h period but reported only the total score (their experiment 1). In their experiment 2, the results were broken down into 2-h totals but the effects of their bedding conditions were confounded with the effects of lighting changes. Thus no clear picture emerges of the bedding response time course. In order to remain consistent with the results of my experiment 2 and yet support Campbell and Raskin, day 15 pup activity must undergo dramatic temporary alterations between hours 1 and 6. The following experiment was designed to uncover these alterations, if they existed.

Method

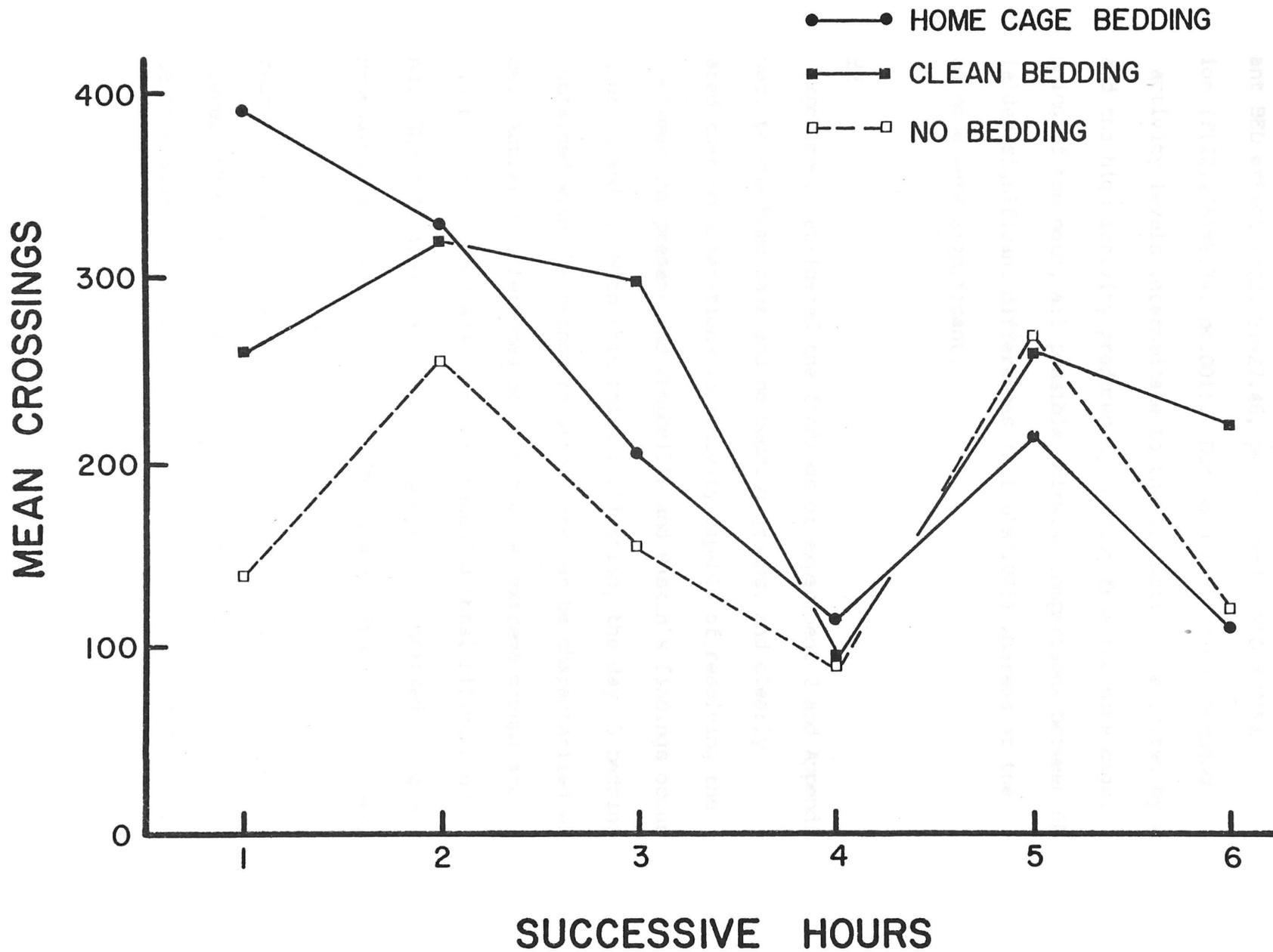
Procedure

Animals and apparatus were as in experiment 2. Day 15 (range 15-16) Wistar pups were exposed for 6 h to one of three bedding conditions (home cage bedding, clean bedding or no bedding). Time lapse videorecording was used to record the entire 6 h period and reduce scoring time on tape playback. Locomotor activity (line-crossing) was scored as in experiment 2.

Results

The data were collected into six 1-h blocks (HRS) for each of the three bedding conditions (BED). Although HRS exerted a significant effect over the 5 h test duration ($F(5,105)=5.86, p<.001$), BED-elicited differences for the 6 h period were nonsignificant (Figure 6). Reanalysis

Figure 6. Mean crossings per hour for six hours of continuous exposure to home cage bedding, clean bedding, or no bedding.



of first hour scores in 5-min blocks (TIME), however, yielded a highly significant BED effect ($F(2,25)=27.46, p<.001$) and a BED X TIME interaction ($F(22,275)=8.74, p<.001$). During hour 1, clean bedding produced activity levels intermediate to the low activity elicited by no bedding and the high activity produced by bedding from the home cage. At the beginning of the hour, all possible pairwise comparisons between BED groups yielded significant differences (all $p's<.001$) whereas at the hour's end none were significant.

Discussion

This experiment replicated the findings of experiment 2 and Appendix 2 with respect to the home cage and no bedding groups, and clearly demonstrated that no alterations in activity capable of resolving the conflict between the present and Campbell's and Raskin's findings occur between hours 1 and 6. With this third replication, the day 15 bedding response obtained with the method reported here can be characterized with confidence. Activity differences between the two extreme groups are greatest initially and decrease over time becoming nonsignificant by hour's end. These findings have been independently confirmed using a vibration-sensing technique for activity measurement (Pusztay & Pappas, Note 1).

Interestingly, pups exposed to clean shavings displayed intermediate activity levels throughout hour 1. While this finding is of practical importance in evaluating day 15 behavior in a host of test conditions, no explanatory framework is offered here.

EXPERIMENT 4

The purpose of this experiment was to explore interstrain differences in the bedding response, again with the goal of uncovering a variable that would resolve the differences between the present and published results. Campbell and Raskin used pups of the Sprague-Dawley strain. I used Wistars in my experiments.

Method

Animals

Pups were offspring of Wistar and Sprague-Dawley females purchased pregnant from Canadian Breeding Farms, St. Constant, Quebec. Twelve Wistar (n=6/cell) and 24 Sprague-Dawley (n=12/cell) pups were used.

Procedure

Animal housing and apparatus were as in experiment 2. Day 15 (range 15-16) pups of two strains (Wistar or Sprague-Dawley) were exposed for 6 h to one of two bedding conditions (home cage bedding or no bedding). Locomotor activity was scored from time lapse recordings.

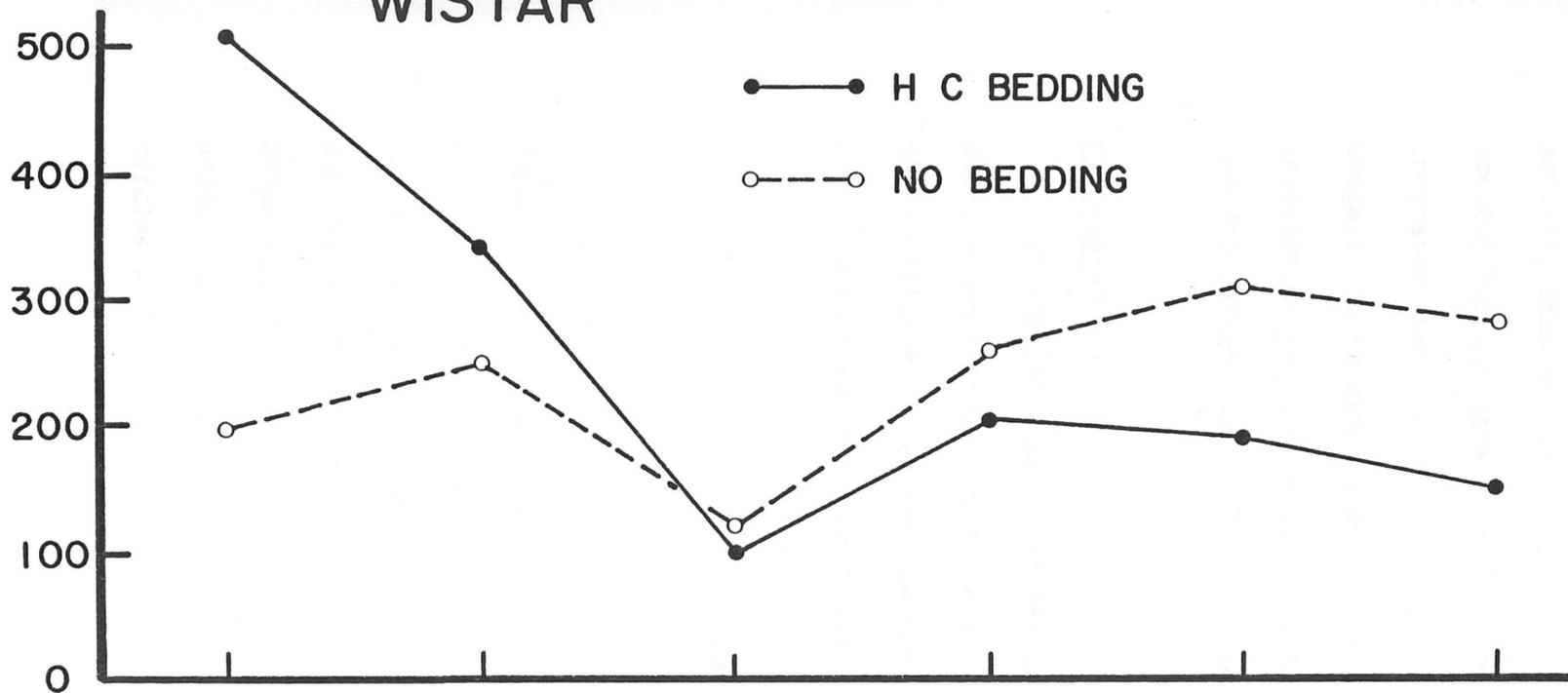
Results

Both Sprague-Dawley and Wistar pups initially responded with high activity in the presence of home cage bedding and low activity in the absence of bedding (Figure 7). However, in both strains this

Figure 7. Mean crossings per hour for six hours of continuous exposure to home cage bedding or no bedding in Wistars or Sprague Dawleys.

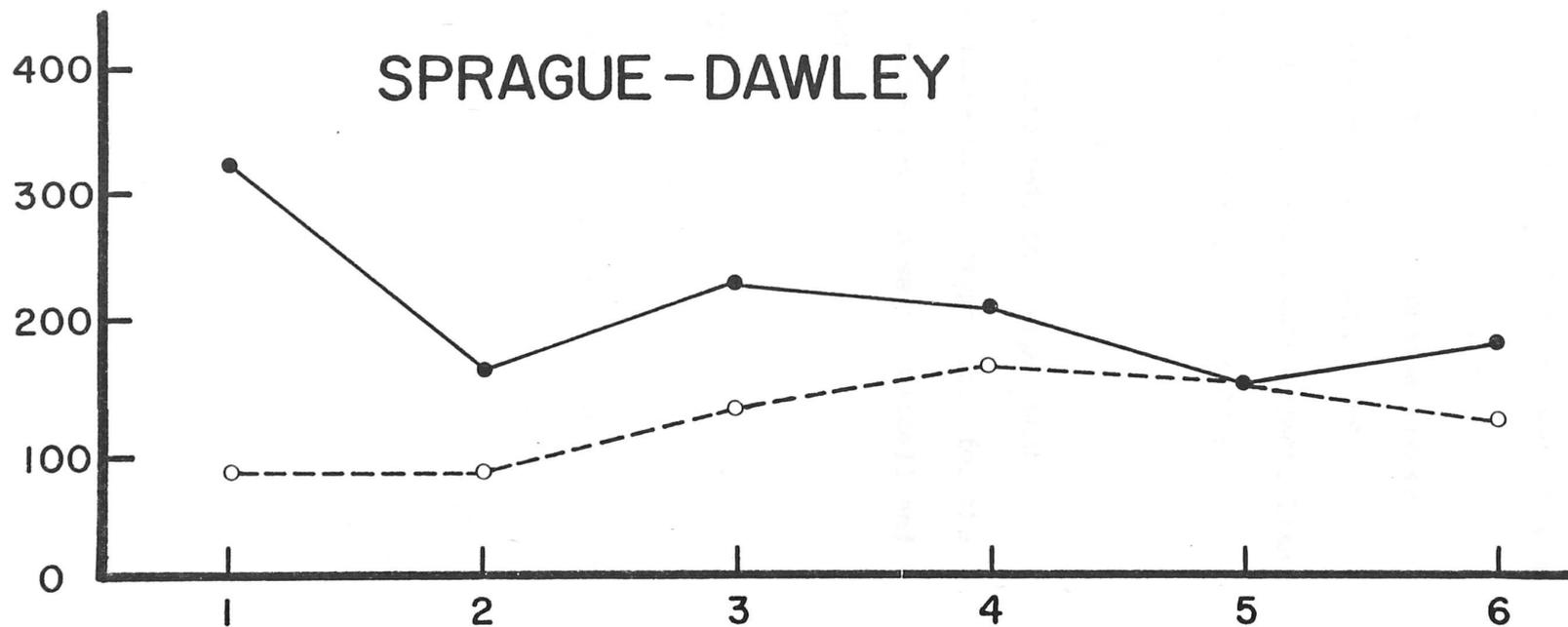
WISTAR

MEAN CROSSINGS



SPRAGUE - DAWLEY

MEAN CROSSINGS



SUCCESSIVE HOURS

bedding-elicited activity difference decayed over time (BED X HRS $F(5,160)=7.54, p<.001$). Home cage bedding produced reliably higher activity than no bedding in the first hour ($p<.001$) and in the second hour ($p<.05$) but not afterwards. A STRAIN effect ($F(1,32)=4.33, p<.05$) indicated that generally Wistars were more active than Sprague-Dawleys. Comparisons of Wistars with Sprague-Dawleys within a STRAIN X HRS interaction ($F(5,160)=3.93, p=.002$) revealed that the STRAIN effect, like the BED effect, was significant only during the first 2 h ($p<.001$).

Discussion

Although interstrain differences were detected, this experiment clearly demonstrates that the strain variable is not responsible for the major differences between the present findings and those of Campbell and Raskin with respect to pup bedding response.

EXPERIMENT 5

The conspicuously high activity I have repeatedly observed when day 15 pups are exposed to home cage bedding in a unfamiliar environment is notably higher than the activity of these same pups in the home cage with their siblings and mother. Gross observation of home cage bedding-exposed pups suggests that they do not spend a disproportionate amount of time inspecting corners or the test box periphery. Instead, the pups rush around the bedding-covered floor in discontinuous and often reversing circles with gradually dissipating velocity. Is this search behavior? If

so, perhaps more complete mimicry of the familiar home environment is required before pup quiescence can be elicited. To test this notion, conspecifics were added to the test environments with the intent of assaying how this familiarity increment would change the pup bedding response.

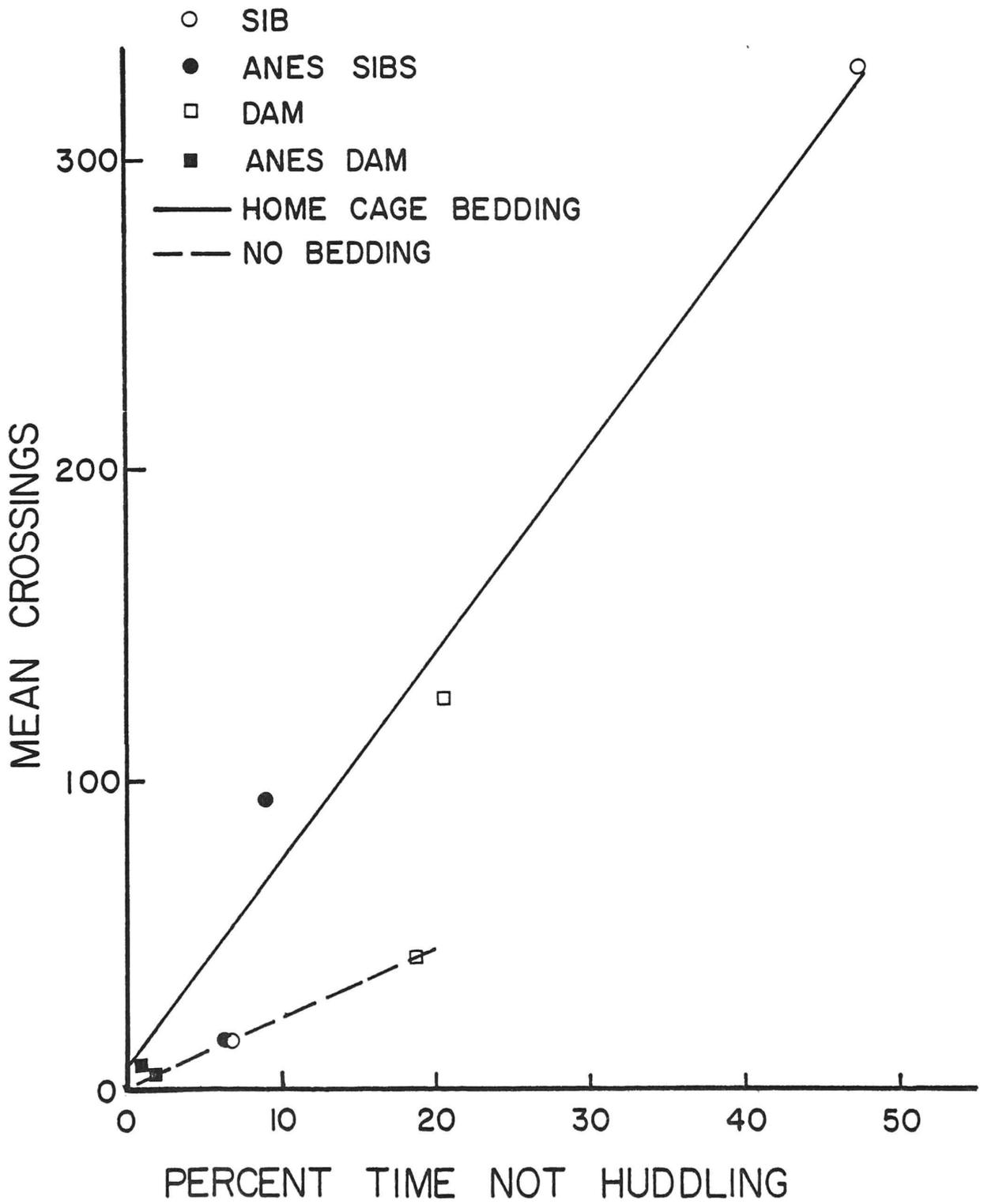
A complication arises in that conspecific presence in the test environment introduces opportunity for huddling, generally incompatible with locomotion. In this experiment it was necessary to dissociate low activity caused by huddling from a changed reaction to home cage bedding. To this end, the following methodological notes are in order. Both locomotor activity and huddling time were measured to form estimates of pup velocity in each bedding condition. 'Time spent not huddling' was selected as the denominator since virtually all locomotion occurred during the non-huddling episodes. To gain generality for these velocity estimates, huddling time was varied by using conspecific targets of differing attractiveness and contact difficulty.

Method

Procedure

Animal housing and apparatus were as in experiment 2. Day 15 (range 15-16) Wistar sibling pairs (n=8 pairs/cell) were exposed for 1 h to one of three bedding conditions (home cage bedding, clean bedding or no bedding). Other pups from the same litters were assigned to one of two bedding conditions (home cage bedding or no bedding) modified by the presence of two anesthetized sibs, the anesthetized dam or the

Figure 8. Plot of mean crossings per hour versus percent time not huddling with a sibling, two anesthetized siblings, the dam or the anesthetized dam. The lines are linear regression estimates of mean crossings as a function of percent non-huddling time in home cage bedding or no bedding.



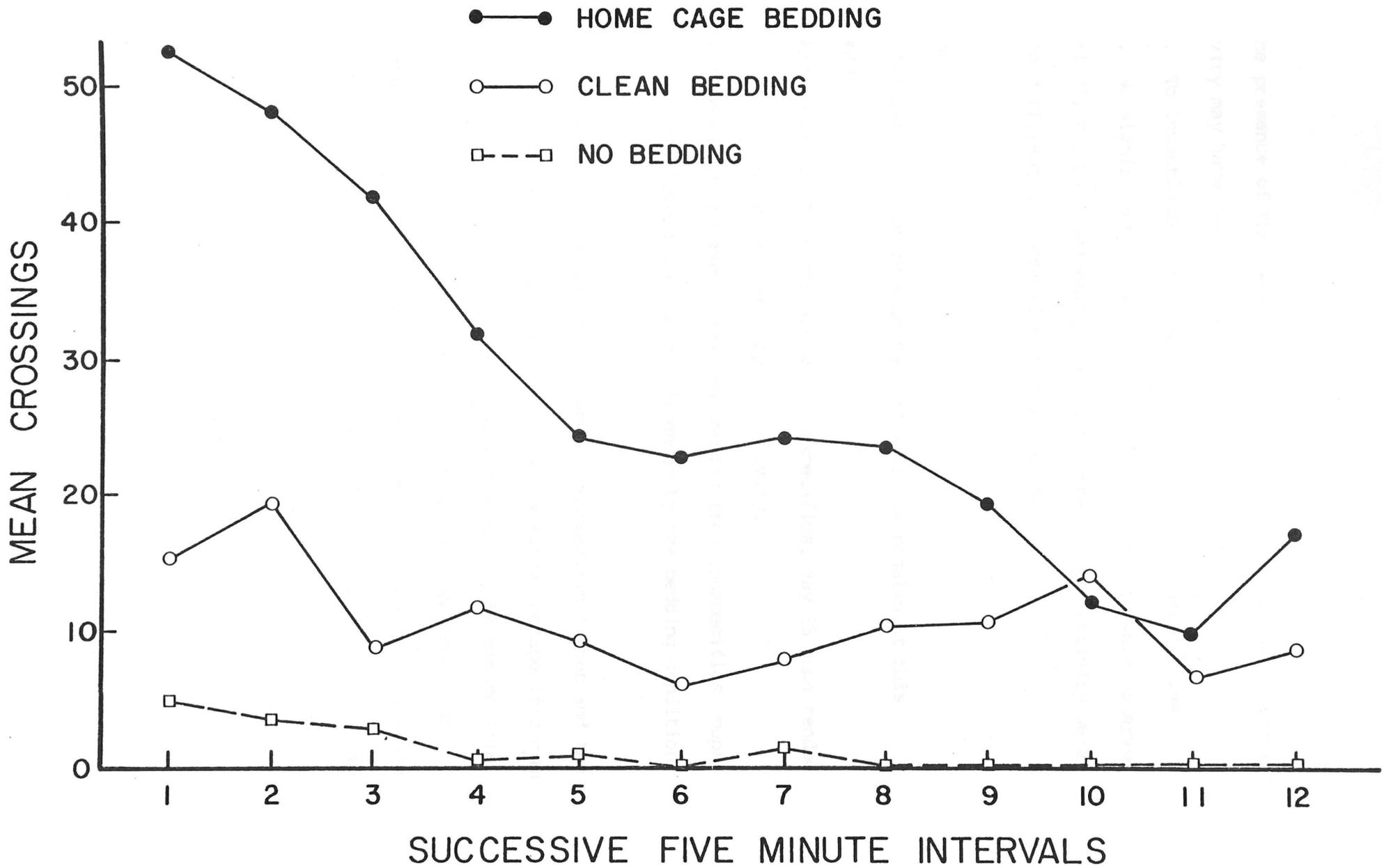
non-anesthetized dam (n=8 pups/cell). Before a session began, the anesthetized (Equithesin, 2.3 mL/kg i.p.) animal(s) were positioned midway along a randomly selected wall. The test pup was placed along the opposite wall. Non-anesthetized dams, however, were introduced at the same time as their day 15 pup. The dam was used in non-anesthetized condition on day 15 and used anesthetized on day 16. Two dependent measures were extracted from tapes of the sessions, lines crossed and time spent not in contact with conspecifics. The relationship between these two dependent measures was examined through regression analysis.

Results

Figure 8 displays the major findings of this experiment. Mean line crossings over the test hour are plotted against mean percent time spent not huddling for the four conspecific target groups within each of the home cage bedding and no bedding groups. Separate regression lines for each bedding group fit the data significantly better than a single regression line ($F(1,47)=37.1, p<.001$), indicating that per unit time not huddling, pups exposed to home cage bedding displayed significantly more locomotion than pups exposed to no bedding.

With the exception of the non-anesthetized sibling, all conspecific targets elicited intense huddling that was unaffected by bedding conditions ($F(3,47)=50.0, p<.001$). The time course of sibling pair activity was highly similar to that noted in previous experiments using single pups (Figure 9). For comparability with other experiments, the sibling pair activity scores were calculated as means of the two individual scores.

Figure 9. Mean crossings per 5-min interval over a one-hour exposure period to home cage, clean, or no bedding in the presence of a non-anesthetized sibling. Scores are means for each sibling pair.



In the presence of the awake mother, bedding-associated differences in pup activity may have been due, in part, to differential maternal activity. To investigate this possibility, maternal locomotion was measured. No significant differences in maternal activity were observed ($F(1,14)=1.21, p>.05$), suggesting that differences in pup activity were not due to differential locomotion by their mothers.

Discussion

Two statements can be made on the basis of the results of this experiment:

1) When close to or in contact with conspecifics, day 15 pups tended to huddle, resulting in zero or very low activity.

2) When separated by even a few centimetres from conspecifics, pups displayed activity levels that were influenced by the bedding condition used.

Thus, depending on the separation between conspecific target and the pup, pup activity was predominantly controlled by either conspecific cues or bedding cues. Outside of some critical range around the conspecific target, pup activity passed under bedding control, a phenomenon most clearly observed in the home cage bedding environment, where pup average velocity abruptly shifted from zero during huddling to a level three times that found in the no bedding environment. These bedding-controlled activity responses (i.e., Figure 9) were not remarkably different from the bedding responses observed in previous experiments.

On the basis of this failure to obtain home cage-typical quiescence through mimicry, it must be assumed that at least one cue salient to the pup was omitted. Furthermore, an essential property of this missing stimulus element was that its absence induced high rates of locomotion in the pup. A review of pup responses and the stimulus environment to which the pup was exposed suggests a promising candidate.

The pup responded to conspecific presence and home cage bedding in alternation. The former stimulus comprised a well-defined locus and elicited huddling, a contact maintenance behavior characterized by low activity. The latter stimulus defined a homogenous layer, thoroughly mixed and uniformly distributed over the test chamber floor. When in contact with bedding, the pup continued to move, presumably search-motivated. However, it was not searching for the conspecific. Engaged in a locomotor bout, the typical pup was oblivious to conspecific presence, racing by and brushing against the conspecific without pause. The bedding itself appeared to consume the pup's total attention. Assuming the pup was searching for a particular locus, which in the home cage might normally elicit approach and settling, then the mixing procedure had assured that this attractive locus was now evenly distributed over the box floor. Might not such a confusing stimulus presentation elicit apparently aimless searching? The next experiment assessed the possible influence of odor structure within the home cage as a determinant of pup settling.

EXPERIMENT 6

In most laboratory environments, rats are raised on standardized bedding materials which are often granular in texture. I used hardwood chips. This material is poorly suited for nest construction since it cannot be interwoven. As a result, a distinct home cage nest area is often not discernible to the experimenter.

Despite this poor visual definition, the nest area may be well defined along olfactory or other modalities salient to the rat pup. To test this notion, I compared pup behavior in undisturbed and disturbed home cage bedding. My primary interest was whether pups would preferentially settle in the undisturbed home cage.

While the disturbed nest hypothesis might shed light on how exposure to home cage bedding can produce both high and low activity, it offers nothing towards resolution of the conflicting results reported for no bedding environments. However, from experiment 1 it can be surmised that the odor environment exerts a powerful influence over locomotor activity. To obtain replicable locomotor activity measurements, the odor environment must be precisely regulated. In experiment 1, high activity resulted when the isolated pup was exposed to maternal odor; presumably, home cage odor would elicit the same response. Campbell and Raskin tested pups exposed to various bedding conditions at the same time and in the same test room. No special precautions to prevent odor diffusion were reported. Therefore this experiment had a second purpose, to determine whether presence/absence of home cage odor was an important mediator of pup locomotor activity.

Method

Animals

Wistar pups were raised on hardwood chips in 23x46x15-cm polypropylene cages. Source and housing of Wistar pups were as in previous experiments.

Procedure

Eight pups from each of nine litters were randomly assigned to various bedding and odor conditions; four siblings were tested on day 15 and four on day 16. Two rooms were used to ensure adequate isolation of home cage odors as required (see Table 1).

One room held three polypropylene cages containing no bedding, home cage bedding, and clean bedding, respectively. The three cages were placed beside each other with the cage containing home cage bedding in the middle. Wire tops prevented pup escape and permitted free diffusion of odor into the cages. The second room contained a single cage that was isolated from bedding, conspecifics, and home cage odors. Both rooms were illuminated by a 100-W red bulb and were serviced with videorecording equipment.

The procedure for presenting home cage bedding merits special attention. On day 15 the undisturbed home cage itself was used. On day 16, home cage bedding was removed from the home cage, thoroughly mixed and then transferred to a clean cage.

Prior to testing, pups were transferred to a holding cage and transported to the test rooms. One pup was placed in each of the four

Table 1

Test Cage and Room Assignment Across Test Days

| Group | Room 1 | Room 2 |
|--------|----------------------------------|------------|
| Day 15 | | |
| Cage 1 | No bedding | No bedding |
| Cage 2 | Clean bedding | - |
| Cage 3 | Undisturbed home cage bedding | - |
| Day 16 | | |
| Cage 1 | No bedding | No bedding |
| Cage 2 | Clean bedding | - |
| Cage 3 | Disturbed home cage bedding | - |

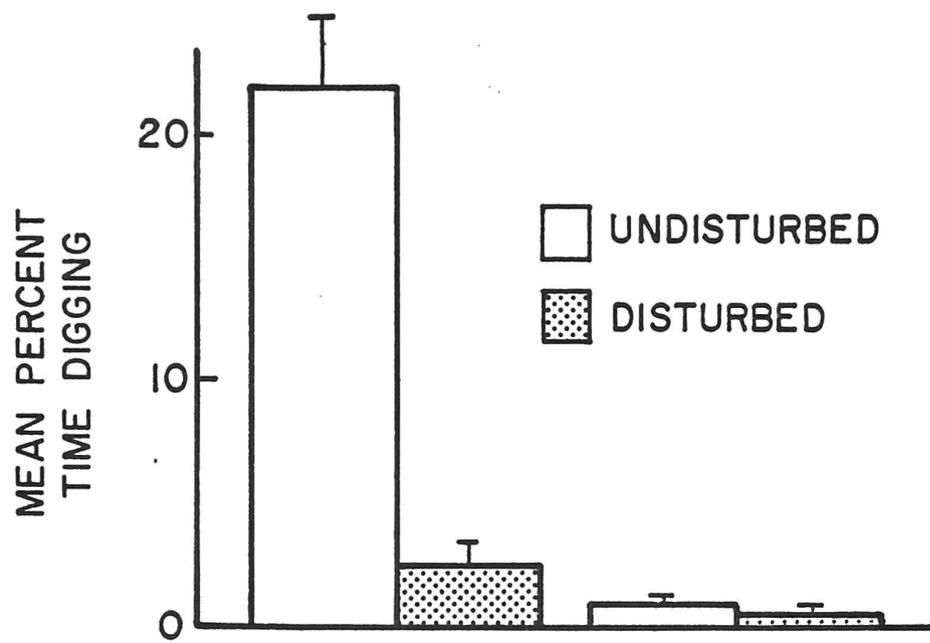
test cages and videorecorded for 1 h. Individual pups in all eight groups were scored for the number of cage crossings across an imaginary line bisecting the cage long axis. Pups in the clean and home cage bedding groups were scored for two additional dependent measures, percent time spent in the preferred half of the cage and percent time spent digging. Pups tested on day 15 were killed after testing.

Results

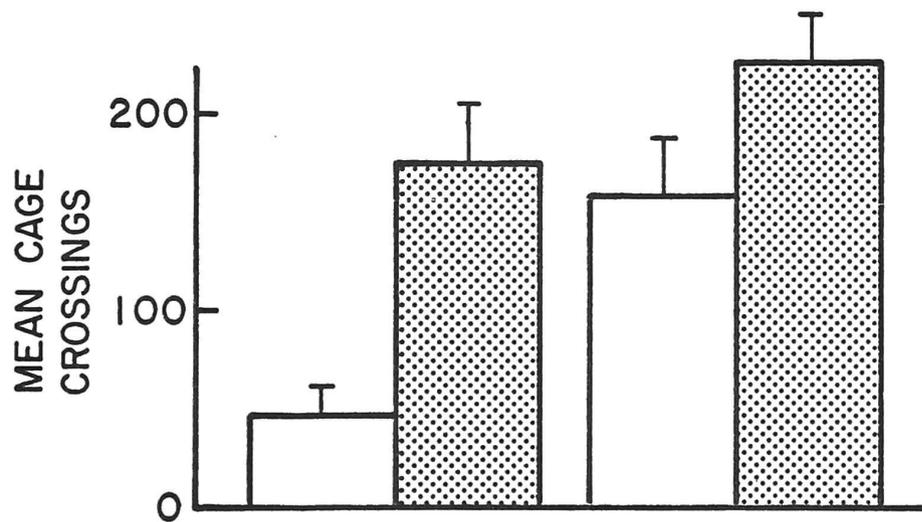
Home cage and clean bedding groups. Figure 10 displays the hour total cage crossings, percent digging time, and percent side preference for the home cage and clean bedding-exposed groups. The most striking result is the difference in digging time between the group exposed to undisturbed home cage bedding and the other three groups (Figure 10a). This behavior involved vigorous rooting through the bedding material with the snout and the scattering of bedding material with swimming-like motions of the fore and hind limbs. Digging always preceded settling and the pup always settled in a dug site. Two of the nine undisturbed home cages contained well-defined cleared areas surrounded by a collar of maternal droppings. In these two home cages, digging and settling occurred exclusively at this "nest". After the first half-hour, digging behavior was rarely observed in any group, although before that time industrious digging was a clear marker for the undisturbed home cage bedding group. As a reflection of this, during the initial half-hour there was no overlap between the lowest digging score in the undisturbed home cage bedding group and the highest score for any other group.

Pups in the undisturbed home cage spent a median of 40% (range 15% to 60%) of their time in the first half-hour digging at one or two sites. Over the hour they locomoted little compared to littermates in the disturbed home cage and clean bedding groups (Figure 10b). Analysis of variance of total cage crossings for the two bedding groups (factor BED) and the two amounts of disturbance of home cage bedding (factor DISTURB) revealed a significant BED effect ($F(1,32)=9.6$, $p<.005$) and DISTURB effect

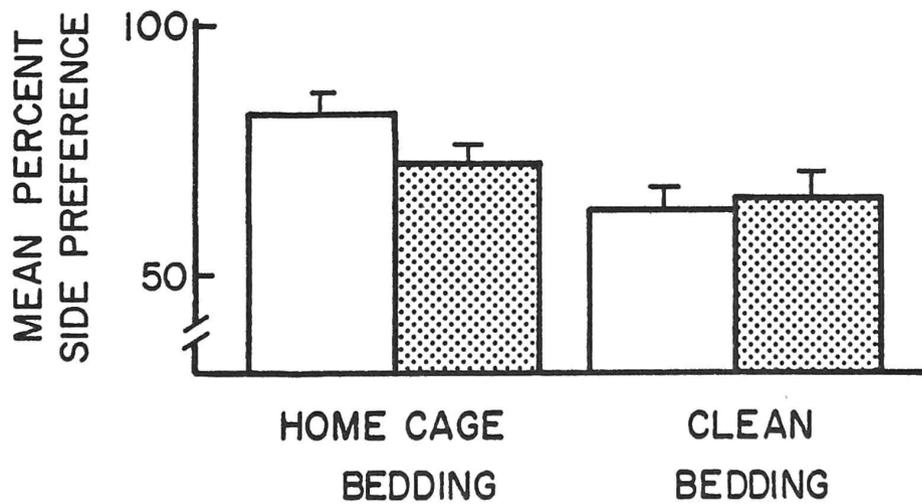
Figure 10. Behaviors of day 15 rat pups in disturbed or undisturbed home cage bedding or near disturbed or undisturbed home cage bedding but in clean bedding. Illustrated are mean (+S.E.) percent time digging (10a), mean (+S.E.) cage crossings (10b) and mean (+S.E.) percent side preference (10c) as a function of these odor/bedding conditions over the test hour.



a



b



c

($F(1,32)=12.3$, $p<.001$), suggesting that clean bedding with home cage odor induces higher locomotor activity than exposure to home cage bedding alone and supporting the hypothesis that disturbed home cage bedding elicits more pup locomotion than undisturbed.

Figure 10c shows the mean percent time pups in the four BED X DISTURB combinations spent on the preferred half of the cage. For this dependent measure the method of cage bisection was defined as for locomotion. The preferred side was defined as that side of the cage in which the pup spent the majority of the test hour. ANOVA revealed a significant BED effect ($F(1,32)=17.1$, $p<.001$), indicating that pups exposed to soiled home cage bedding were more likely to develop a side preference than those exposed to clean bedding. Within the home cage bedding groups, digging behavior was positively correlated with side preference ($r=.69$, $F(1,16)$, $p<.005$) and side preference behavior was negatively correlated with locomotor activity levels ($r=-.71$, $F(1,16)=16.3$, $p<.001$), confirming the impression given by the group means in Figures 10a, b, and c.

No bedding groups. Table 1 shows that there were four no bedding groups. The no bedding groups within Room 1 were not significantly different from each other in cage crossing scores ($F(1,16)=0.16$, $p>.05$). Thus no bedding groups were collapsed across test days within each room and a one-way ANOVA performed using ODOR (home cage odor present or absent) as the independent variable. Over the test hour, no bedding-exposed pups in Room 1 (home cage odor present) showed 136.0 ± 14.1 (mean \pm S.E.) cage crossings and pups in Room 2 (home cage odor

absent) showed 13.6 ± 5.6 cage crossings. ANOVA confirmed that this difference was highly significant ($F(1,34)=65.6, p<.001$).

Discussion

The results of this experiment offer compelling evidence of the influence of social odors on the rat pup and provide for reconciliation of my findings with those of Campbell and Raskin (1978). My findings demonstrate that control of the pup's odor environment during testing is essential. Barring this, variability between and within studies will result, ranging in magnitude from the small and innocuous, to the diametrically opposite results found by Campbell and Raskin and by me.

I suggest that the high activity reported by Campbell and Raskin for their no bedding condition was the product of freely diffusing home cage odors and the low activity reported for their home cage condition was due to pups settling in the nest. Despite the use here of granular bedding material which seemingly would have little value in nest-building, especially with the constant trampling unavoidable in the restricted living conditions of a typical maternity cage, 15-16-day-old pups could discriminate disturbed from undisturbed home cage bedding. Visually, little or no organization was discernible in the bedding of most home cages, suggesting that the major sensory cues were olfactory in nature.

This experiment reports a new observation, a digging/settling sequence, which occurred almost exclusively in the undisturbed home cage. While preliminary evidence suggests that this sequence takes place at the nest site, the significance of this behavior remains unknown.

Speculatively, it could indicate attempted self-concealment in the relative safety of the nest, digging for concealed siblings, digging for positive identifiers of the nest, or thermal conservative behavior. If, as the results of this study appear to indicate, an intact nest structure is the cue that triggers digging behavior, the vigor and persistence with which pups engage in digging may provide its own shut-off mechanism. By the end of a half-hour, little semblance of any structure in the bedding can remain.

Unlike digging and locomotion, side preference behavior did not reliably differentiate pups in disturbed and undisturbed home cage environments. In part, this attests to the fact that many pups dug in both ends of the undisturbed home cage, suggesting that the dam had recently relocated the nest site. In any case, the major differences in digging and locomotion observed between disturbed and undisturbed home cage bedding provides ample testimony that the home cage is not a homogenous entity to the rat pup.

General Discussion

The principle emerging from these experiments is that the presence/absence of nest-relevant odors constitutes a powerful variable affecting rat pup behavior. Much of the available literature on preweanling pup activity does not give sufficient information about the extent of odor control during testing. Consequently, the results are difficult to interpret. In contrast, future research with the immature rat should move towards better definition of the odor environment during

testing, driven by a growing body of data that already suggests a strong role of the odor environment in task performance and conditioning (Infurna, Steinert & Spear, 1979; Smith & Spear, 1978) and interactions with drug effects (Appendix 3; Campbell & Randall, 1977).

One unexpected finding in these studies was the existence of a behaviorally significant structure in the home cage. Although poorly defined visually, I equated this structure to the nest. Pups responded to the nest in a peculiar fashion, digging persistently in one or at most two locations for the first half-hour. Consequently, little ambulatory activity was observed. When home cage bedding was thoroughly mixed, distributing fragments of the nest structure over the test chamber floor, the pups engaged in a flurry of energetic activity that had a similar half-hour time course but a different constitution. In this test condition, digging was rare and locomotor activity predominated. Thus while presence/absence of nest-relevant odors is a powerful energizing variable, it is also clear that dramatic differences in the behaviors observed depend on the method of presentation of these odors. This serves to illustrate that the olfactory world of the preweanling rat pup is organized.

Analysis of this organization suggests that day 15 locomotor activity is a trivariate function of odor intensity and direction cues and time in isolation. When the familiar odors of its mother or the nest are undetectable to the pup, the pup tends to remain immobile for roughly a half-hour. After this time, or earlier if the pup has been subjected to prior maternal deprivation, the pup exhibits more movement (experiment 1;

Hofer, 1975). However, when familiar odors are superthreshold and directional cues are present, the pup displays reliable approach behavior (Leon, 1974; Leon and Moltz, 1971, 1972). Given the same odor presence but minus the directional cues, the pup displays high locomotion, interpreted as randomly vectored search behavior which, futile, habituates over time. Finally, when the nest or mother is reached, the pup maintains regular contact, displaying little locomotion.

The above analysis can be recast in terms of threat to the pup and the optimal strategy for removing this threat in environments of differing information content. When isolated from the nest and mother with no cues to guide its return, the pup is lost. Since random movement by the pup is sub-optimal, its observed low activity may facilitate maternal retrieval or at least not further deteriorate the situation. In this predicament, pup vocalizations might be expected (Allin & Banks, 1972; Bell, 1974; Bell, Nitschke, Bell & Zachman, 1974; Conely & Bell, 1978; Smotherman, Bell, Starzec, Elias & Zachman, 1974). Only when no maternal response is forthcoming might it benefit the pup to begin searching. At lesser separations from the home environment, the pup can use odor intensity as an indicator of proximity, bypassing the need for better directional cues. On this olfactory tether (Devor and Schneider, 1974), the pup is not lost. It is in control of its separation from the nest and when threatened, can briskly close the distance between itself and the nest. This model proffers what is in effect a simple notion, that is, that the pup reacts to its separation from the nest in a manner consistent with the goal of maximizing its chances for survival.

Reference Note

1. Puszta, W. & Pappas, B. A. Unpublished observations, March, 1979.

References

- Allin, J. T. & Banks, E. M. Effects of temperature on ultrasound production by infant albino rats. Developmental Psychobiology, 1972, 4, 149-156.
- Altman, J., Sudarshan, K., Das, G. D., McCormick, N., & Barnes, D. The influences of nutrition on neural and behavioral development. III. Development of some motor, particularly locomotor, patterns during infancy. Developmental Psychobiology, 1971, 4, 97-114.
- Bell, R. W. Ultrasounds in small rodents: Arousal-produced and arousal-producing. Developmental Psychobiology, 1974, 7, 39-42.
- Bell, R. W., Nitschke, W., Bell, N. & Zachman, T. Early experience, ultrasonic vocalizations, and maternal responsiveness in rats. Developmental Psychobiology, 1974, 7, 235-242.
- Bolles, R. C. & Woods, P. J. The ontogeny of behavior in the albino rat. Animal Behavior, 1964, 12, 427-441.
- Bronstein, P. M. & Dworkin, T. Replication: The persistent locomotion of immature rats. Bulletin of the Psychonomic Society, 1974, 4, 124-126.
- Bronstein, P. M., Neiman, H., Wolkoff, F. D. & Levine, M. J. The development of habituation in the rat. Animal Learning and Behavior, 1974, 2, 92-96.
- Campbell, B. A. & Randall, P. K. Paradoxical effects of amphetamine on preweanling and postweanling rats. Science, 1977, 195, 888-890.
- Campbell, B. A. & Raskin, L. A. Ontogeny of behavioral arousal: The role of environmental stimuli. Journal of Comparative and Physiological Psychology, 1978, 92, 170-184.

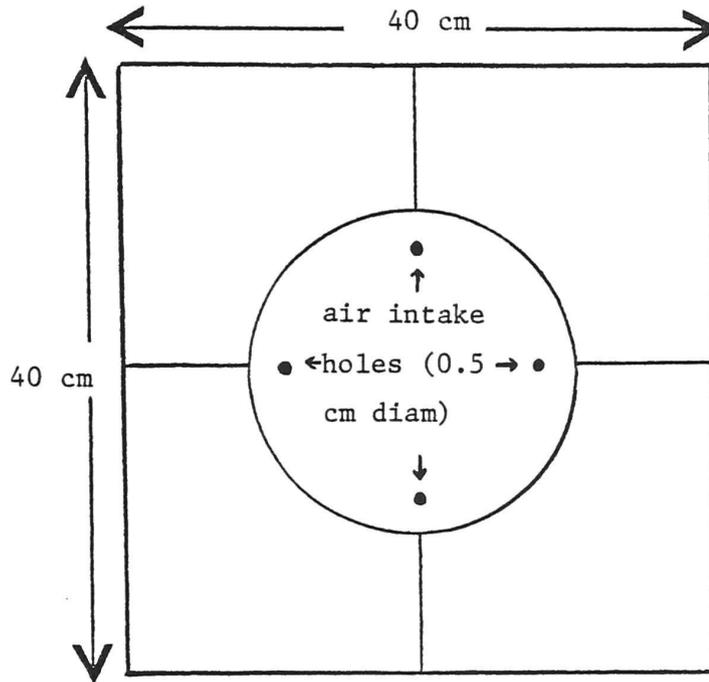
- Conely, L. & Bell, R. W. Neonatal ultrasounds elicited by odor cues. Developmental Psychobiology, 1978, 11, 193-197.
- Cornwell-Jones, C. & Sobrian, S. K. Development of odor-guided behavior in Wistar and Sprague Dawley rat pups. Physiology and Behavior, 1977, 19, 685-688.
- Devor, M. & Schneider, G. E. Attraction to home-cage odor in hamster pups: Specificity and changes with age. Behavioral Biology, 1974, 10, 211-221.
- Freeman, N. C. G. & Rosenblatt, J. S. The interrelationship between thermal and olfactory stimulation in the development of home orientation in newborn kittens. Developmental Psychobiology, 1978a, 11, 437-457.
- Freeman, N. C. G. & Rosenblatt, J. S. Specificity of litter odors in the control of home orientation among kittens. Developmental Psychobiology, 1978b, 11, 458-471.
- Gregory, E. & Bishop, A. Development of olfactory-guided behavior in the golden hamster. Physiology and Behavior, 1975, 15, 373-376.
- Gregory, E. H. & Pfaff, D. W. Development of olfactory-guided behavior in infant rats. Physiology and Behavior, 1971, 6, 573-576.
- Hofer, M. A. Studies on how early maternal separation produces behavioral change in young rats. Psychosomatic Medicine, 1975, 37, 245-264.
- Hofer, M. A. Olfactory denervation: Its biological and behavioral effects in infant rats. Journal of Comparative and Physiological Psychology, 1976, 90, 829-838.

- Holinka, C. & Carlson, A. D. Pup attraction to lactating Sprague Dawley rats. Behavioral Biology, 1976, 16, 489-505.
- Infurna, R. N., Steinert, P. A. & Spear, N. E. Ontogenetic changes in the modulation of taste aversion learning by home environmental cues in rats. Journal of Comparative and Physiological Psychology, 1979, 93, 1097-1108.
- Leon, M. Maternal Pheromone. Physiology and Behavior, 1974, 13, 441-453.
- Leon, M. Filial responsiveness to olfactory cues in the laboratory rat. In: Advances in the Study of Behavior, 8, J. S. Rosenblatt, R. A. Hinde, C. Beer and M.-C. Busnel eds. Academic Press, New York, 1978, 117-155.
- Leon, M. & Behse, J. H. Dissolution of the pheromonal bond: Waning of approach response by weanling rats. Physiology and Behavior, 1977, 18, 393-397.
- Leon, M., Galef, B. G. & Behse, J. H. Establishment of pheromonal bonds and diet choice in young rats by odor pre-exposure. Physiology and Behavior, 1977, 18, 387-391.
- Leon, M. & Moltz, H. Maternal Pheromone: Discrimination by pre-weanling albino rats. Physiology and Behavior, 1971, 7, 265-267.
- Leon, M. & Moltz, H. The development of the pheromonal bond in the albino rat. Physiology and Behavior, 1972, 8, 683-686.
- Melberg, P.-E., Ahlenius, S., Engel, J. & Lundborg, P. Ontogenetic development of locomotor activity and rate of tyrosine hydroxylation. Psychopharmacology, 1976, 49, 119-123.

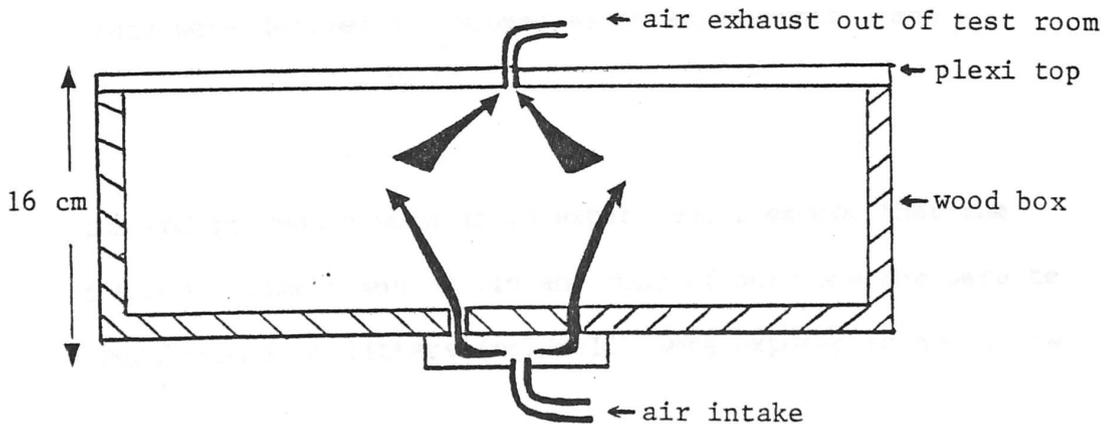
- Randall, P. K. & Campbell, B. A. Ontogeny of behavioral arousal in rats: Effect of maternal and sibling presence. Journal of Comparative and Physiological Psychology, 1976, 90, 455-459.
- Rosenblatt, J. S. & Lehrman, D. S. Maternal behavior of the laboratory rat. In: Maternal Behavior of Mammals, H. L. Reingold ed. Wiley, New York, 1963, 122-168.
- Sczerzenie, V. & Hsiao, S. Development of locomotion toward home nesting material in neonatal rats. Developmental Psychobiology, 1977, 10, 315-321.
- Smith, G. J. & Spear, N. E. Effects of the home environment on withholding behaviors and conditioning in infant and neonatal rats. Science, 1978, 202, 327-329.
- Smotherman, W. P., Bell, R. W., Starzec, J., Elias, J. W. & Zachman, T. A. Maternal responses to infant vocalizations and olfactory cues in rats and mice. Behavioral Biology, 1974, 12, 55-66.
- Sobrian, S. K. & Cornwell-Jones, C. Neonatal 6-hydroxydopamine alters olfactory development. Behavioral Biology, 1977, 21, 329-340.

APPENDIX 1: DIAGRAM OF AN ODOR PRESENTATION CHAMBER

floor view



side view



APPENDIX 2: EFFECTS OF VENTILATION AND LIGHTING ON BEDDING-ELICITED
ACTIVITY IN THE DAY 15 RAT PUP

In these pilot studies I tested the hypothesis that the day 15 bedding response is actually mediated by non-bedding variables. First, I examined the proposal that home cage bedding-elicited high activity is essentially an irritation artifact. This view holds that delicate pup membranes are irritated by air-borne bedding particles disturbed by the ventilation system. The simplest way to test this hypothesis was to measure pup activity in the presence of home cage bedding with the ventilation system shut off.

Second, I wanted to know whether lighting condition was an important determinant of pup bedding response. Randall and Campbell (1976) noted some sensitivity of day 15 pup activity to lighting conditions and Campbell and Raskin used different lighting conditions than were employed here.

Method

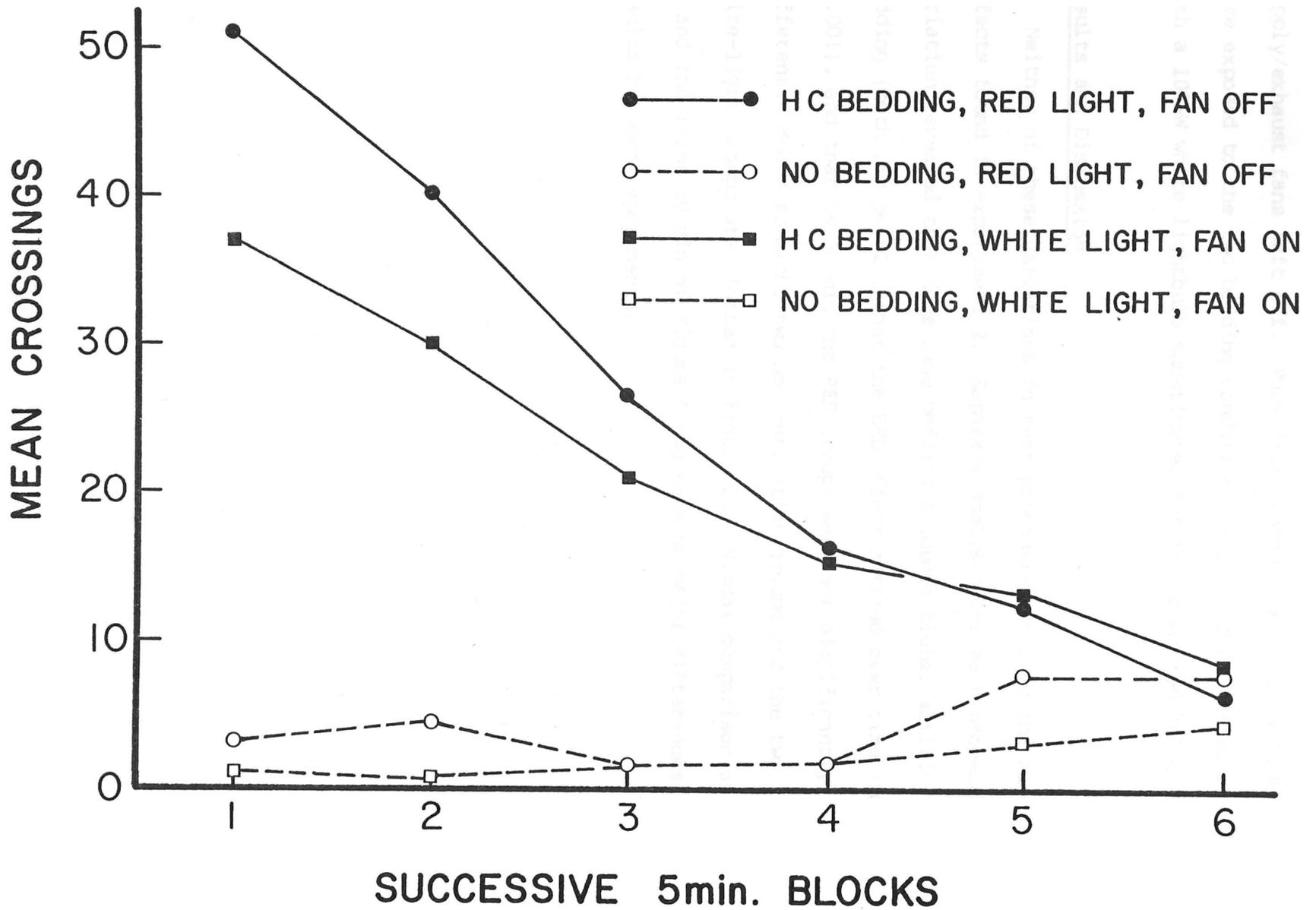
Animals

Wistar rats were derived and housed as in experiments 1 and 2.

Procedure

Apparatus and procedure were as in experiment 2 except that the duration of the experiment was 30 min and pups of only one age were tested (day 15). Pups from four litters (n=7/cell) were exposed to one of two

Figure 11. Mean crossings per 5-min interval over the first half hour of exposure on day 15 to home cage bedding or no bedding under white lighting or with fans turned off.



bedding conditions (home cage bedding or no bedding) with the supply/exhaust fans left off. Pups from another two litters (n=4/cell) were exposed to the two bedding conditions with the fans turned on but with a 100-W white lightbulb substituted for the usual red bulb.

Results and Discussion

Neither of these variations in test procedure reversed the bedding effects found in experiment 2. Separate analyses for each procedural variation revealed that home cage bedding produced higher activity than no bedding (both F's $p < .02$), that the BED effect altered over time (both F's $p < .001$), and that by 30 min the BED groups were not significantly different. Means from the two no-ventilation groups and the two white-light groups are plotted in Figure 11. Visual comparison of Figure 11 and the first 30 min of Figure 4 suggests no major differences in results between experiments.

APPENDIX 3: EFFECT OF AMPHETAMINE ON BEDDING-ELICITED ACTIVITY IN THE
DAY 15 RAT PUP

Amphetamine has been reported to increase approach behavior strength in day 15 rat pups (Campbell & Randall, 1977). In this regard, amphetamine might potentiate the vigorous search behavior characterized by high activity that is observed when day 15 pups are exposed to disturbed home cage bedding. If true, the degree of potentiation might also depend on the post-injection time at which initial exposure commenced (reflecting drug absorption levels) and the extent of exposure to disturbed home cage bedding prior to injection (reflecting ongoing search response strength). The following experiments were designed to weigh the possible influence of amphetamine as a co-determinant of locomotor stimulation in disturbed home cage bedding.

Method

General Procedure

Different injection schedules (5 or 30 min before or 30 min after bedding exposure) identified three separate experiments. Except as noted below, all methodological details were as described as in experiment 2. Day 15-16 Wistar pups (minimum 6 pups/cell) were injected with saline or d-amphetamine sulphate (1 mg/kg, i.p.) and exposed to one of two bedding conditions (disturbed home cage bedding or no bedding) for 1 h in the odor-tight boxes previously described. Those pups that were injected prior to bedding exposure were retained together in a holding cage lined

Figure 12. Mean (+S.E.) crossings per half-hour over a one-hour exposure period in disturbed home cage bedding or no bedding when injected 5 min before testing with d-amphetamine or saline.

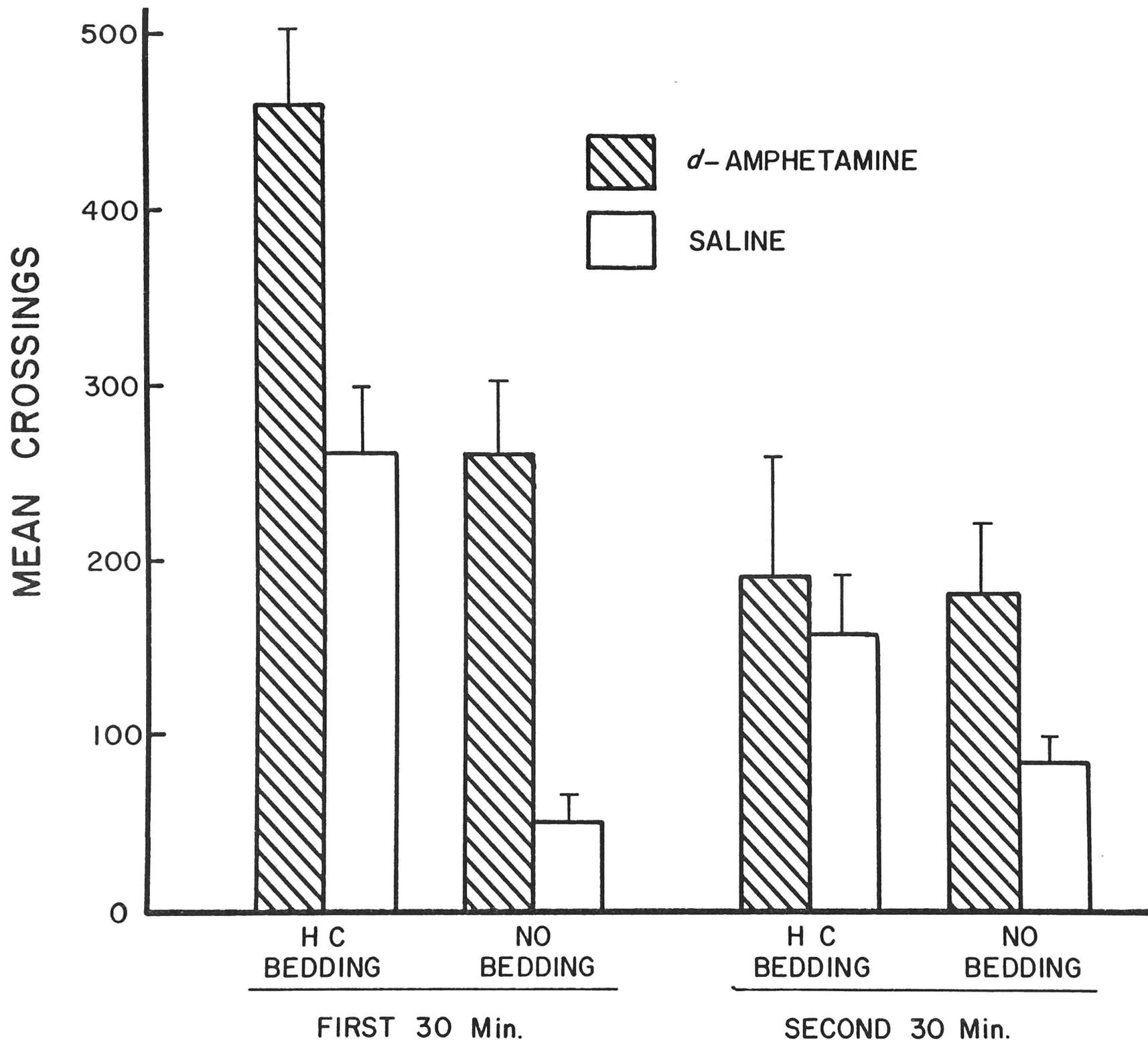
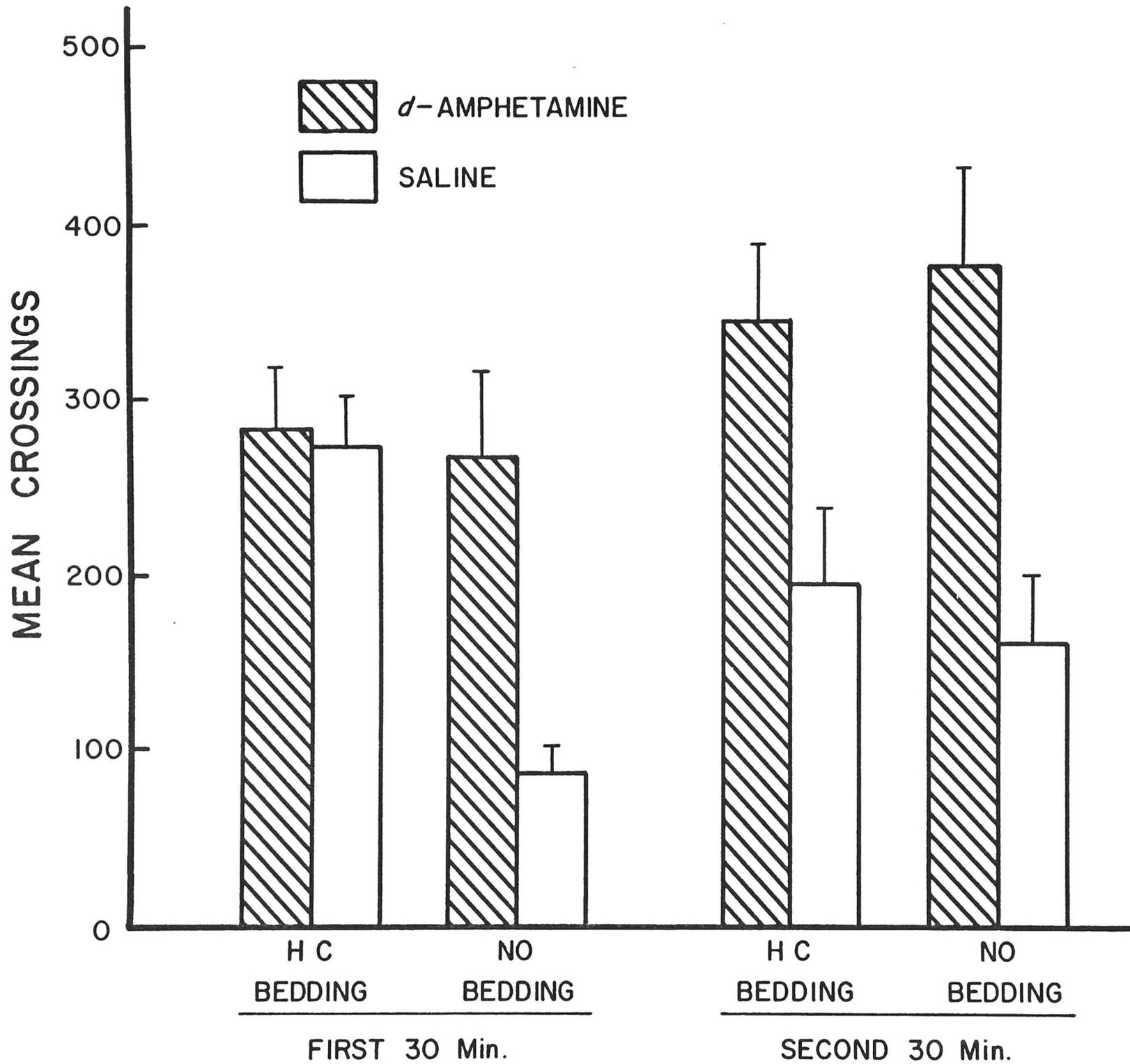


Figure 13. Mean (+S.E.) crossings per half-hour over a one-hour exposure period in disturbed home cage bedding or no bedding when injected 30 min before testing with d-amphetamine or saline.



with clean bedding until testing. Those pups that were injected after 30 min of bedding exposure were removed from the test box, promptly injected and then returned. Locomotor activity was scored from time-lapse videorecordings.

EXPERIMENT 7

Results

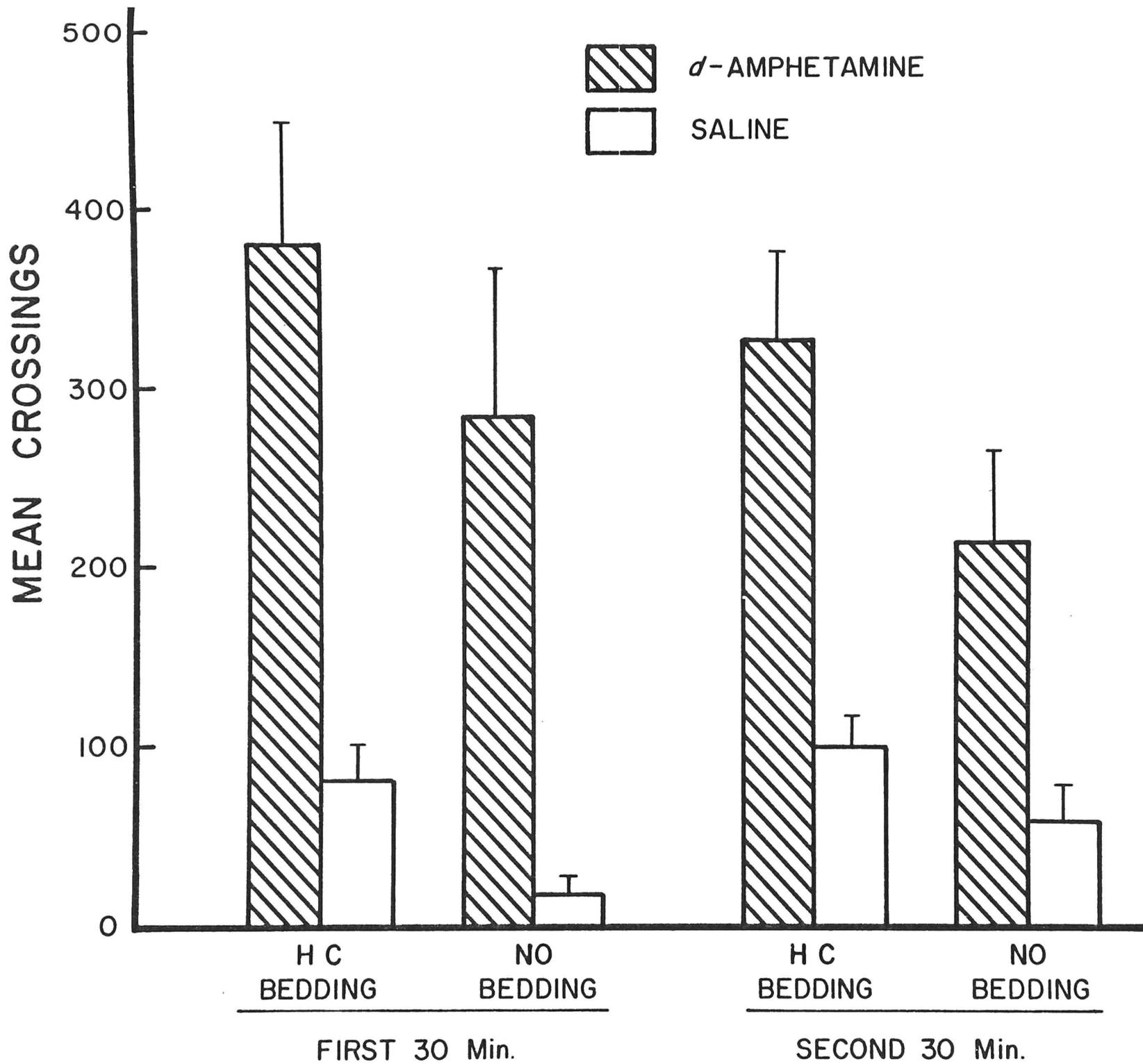
ANOVA with between groups variables BED and DRUG and repeated measures variable TIME (twelve 5-min intervals) was performed for each experiment. These time course data are compressed into half-hour blocks in Figures 12, 13 and 14. Figure 12 illustrates the locomotor activity of pups injected with d-amphetamine or saline 5 min before bedding exposure. Significant interactions of DRUG X TIME ($F(11,353)=7.91, p<.001$) and BED X TIME ($F(11,352)=12.53, p<.001$) justify Scheffe post hoc comparisons. During the initial 5-min interval amphetamine produced higher activity than saline ($p<.01$) and disturbed home cage bedding produced higher activity than no bedding ($p<.001$). After 30 min, both the DRUG and the BED effects had dissipated and were non-significant.

EXPERIMENT 8

Results

Figure 13 shows the activity of pups injected 30 min prior to bedding exposure. Again the DRUG X TIME ($F(11,352)=2.84, p=.001$) and the BED X TIME ($F(11,352)=7.81, p<.001$) interactions were significant. During the first 5 min, disturbed home cage bedding produced reliably higher activity

Figure 14. Mean (+S.E.) crossings per half-hour over a one-hour post-injection period. Pups were injected with d-amphetamine or saline 30 min after initial exposure to disturbed home cage bedding or no bedding.



than no bedding ($p=.001$). At the same time, comparisons between DRUG levels did not reveal a significant difference. Over the course of the test hour, the initially strong BED effect became weak and non-significant. Conversely, the initially weak DRUG effect became sufficiently pronounced so that that during the last 15 min amphetamine-treated pups were reliably more active than those treated with saline ($p<.05$).

EXPERIMENT 9

Results

Figure 14 displays the locomotor activity of pups who were injected with saline or amphetamine after most of the activity differences due to bedding conditions had dissipated (i.e., after 30 min of bedding exposure). Over the following hour, activity differences attributable to the BED variable were small and non-significant. However, the DRUG effect was clearly significant ($F(1,28)=20.02$, $p<.001$) and unchanging.

General Discussion

These experiments illustrate an interaction between four variables: duration of exposure to bedding condition, type of bedding condition, stimulant drug injection, and injection protocol. While this interaction is complex, two points are noteworthy. First, in a no-bedding environment, amphetamine elicited generally higher activity than saline. Second, in disturbed home cage bedding, the magnitude of the drug effect was highly dependent upon the injection protocol used and the

post-injection time at which behavior was observed. For example, in experiment 7, amphetamine elicited a significant increase in activity over saline controls in disturbed home cage bedding during the first half-hour but not in the second (Figure 12). In experiment 8, the opposite pattern is evident (Figure 13). In the final experiment, amphetamine potentiated activity throughout (Figure 14). These experiments do not isolate one particular variable to explain the findings, although they do suggest that prior bedding exposure (i.e., clean bedding in experiment 8, home cage bedding in experiment 9) is involved.

The finding here that disturbed home cage bedding and amphetamine interactively determine preweanling locomotor activity levels has particular relevance for stimulant drug research. Drug effects are usually assessed in an environment without bedding; drug effects so obtained using the preweanling are apparently not generalizable to other bedding conditions at this age. The inclusion of soiled home cage bedding (or perhaps even its odor alone) in a test environment can dramatically affect pup activity levels -- so much so that the expected drug effects may be completely masked, depending upon the length of the test period (i.e., Figure 13). Future investigations should aim at more fully characterizing these intriguing interactions between attractive odors, pup behavior and drug effects.

APPENDIX 4: ANALYSIS OF VARIANCE TABLES

Experiment 1

Dependent Variable: Time in central cylinder
 Independent Variables: 1. ODOR (maternal odor or no odor)
 2. AGE (15 or 26 days of age)
 3. HRS (hours 1 or 6 of odor exposure)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|----------|--------|----|------------|--------|------|------|
| OCON | | 1 | 11294. | 11294. | .86 | .358 |
| AGE | | 1 | 52907. | 52907. | 4.05 | .050 |
| OCON AGE | | 1 | 35967. | 35967. | 2.75 | .104 |
| | error | 44 | .57519E 06 | 13073. | | |
| | HRS | 1 | 19992. | 19992. | 1.69 | .201 |
| OCON | HRS | 1 | 46195. | 46195. | 3.90 | .055 |
| AGE | HRS | 1 | 8487.8 | 8487.8 | .72 | .402 |
| OCON AGE | HRS | 1 | 14373. | 14373. | 1.21 | .277 |
| | error | 44 | .52163E 06 | 11855. | | |

Experiment 1

Dependent Variable: Lines crossed per minute
Independent Variables: 1. ODOR (Maternal odor or no odor)
2. AGE (15 or 26 days of age)
3. TIME (successive minutes)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|----------|-----|--------|--------|-------|--------|
| OCON | 1 | 27.075 | 27.075 | .34 | .563 |
| AGE | 1 | 832.14 | 832.15 | 10.42 | .002 |
| OCON AGE | 1 | 205.39 | 205.39 | 2.57 | .116 |
| error | 44 | 3514.4 | 79.872 | | |
| OCON | 9 | 898.16 | 99.795 | 13.27 | P<.001 |
| TIME | 9 | 81.800 | 9.0889 | 1.21 | .288 |
| OCON AGE | 9 | 2469.6 | 274.41 | 36.48 | P<.001 |
| TIME | 9 | 192.65 | 21.405 | 2.85 | .003 |
| OCON AGE | 9 | 192.65 | 21.405 | 2.85 | .003 |
| error | 396 | 2979.1 | 7.5231 | | |
| total | 479 | 11200. | | | |

Experiment 1

Dependent Variable: Lines crossed
 Independent Variables: 1. ODOR (maternal odor or no odor)
 2. AGE (15 or 26 days of age)
 3. HOURS (hours 1 or 6 of odor exposure)
 4. TIME (successive 5-minute intervals)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|--------------|----|--------|--------|-------|--------|
| OCON | 1 | 118.84 | 118.84 | .07 | .786 |
| AGE | 1 | 24237. | 24237. | 15.16 | P<.001 |
| OCON AGE | 1 | 106.34 | 106.34 | .07 | .798 |
| error | 44 | 70358. | 1599.0 | | |
| HRS | 1 | 31878. | 31878. | 61.32 | P<.001 |
| OCON HRS | 1 | 1755.3 | 1755.3 | 3.38 | .073 |
| AGE HRS | 1 | 4035.0 | 4035.0 | 7.76 | .008 |
| OCON AGE HRS | 1 | 2432.5 | 2432.5 | 4.68 | .036 |
| error | 44 | 22874. | 519.87 | | |

Experiment 2

Dependent Variable: Lines crossed per minute
Independent Variables: 1. BED (home cage or no bedding)
2. AGE (15 or 26 days of age)
3. TIME (successive minutes)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|----------|-----|--------|--------|-------|----------|
| BED | | 1 | 1829.1 | 1829.1 | 36.94 | P < .001 |
| | AGE | 1 | 13.002 | 13.002 | .26 | .611 |
| BED | AGE | 1 | 722.75 | 722.75 | 14.60 | P < .001 |
| | error | 44 | 2178.8 | 49.519 | | |
| | TIME | 9 | 1651.9 | 183.54 | 37.02 | P < .001 |
| BED | TIME | 9 | 193.29 | 21.476 | 4.33 | P < .001 |
| | AGE TIME | 9 | 676.06 | 75.118 | 15.15 | P < .001 |
| BED | AGE TIME | 9 | 120.90 | 13.433 | 2.71 | .005 |
| | error | 396 | 1963.6 | 4.9585 | | |
| | total | 479 | 9349.4 | | | |

Experiment 2

Dependent Variable: Lines crossed
 Independent Variables: 1. BED (home cage or no bedding)
 2. AGE (15 or 26 days of age)
 3. HOURS (hours 1 or 6 of bedding exposure)
 4. TIME (successive 5-minute intervals)

Analysis of Variance

| Source | | df | SS | MS | F | SIG |
|---------|----------|------|------------|--------|-------|----------|
| BED | | 1 | 9741.8 | 9741.8 | 14.39 | P < .001 |
| AGE | | 1 | 3220.0 | 3220.0 | 4.75 | .035 |
| BED AGE | | 1 | 6197.6 | 6197.6 | 9.15 | .004 |
| | error | 44 | 29797. | 677.21 | | |
| | HRS | 1 | 2556.1 | 2556.1 | 4.46 | .040 |
| BED | HRS | 1 | 4425.8 | 4425.8 | 7.72 | .008 |
| AGE | HRS | 1 | 1077.3 | 1077.3 | 1.88 | .177 |
| BED AGE | HRS | 1 | 3334.7 | 3334.7 | 5.81 | .020 |
| | error | 44 | 25237. | 573.56 | | |
| | TIME | 11 | 6318.4 | 574.40 | 8.94 | P < .001 |
| BED | TIME | 11 | 1550.2 | 140.93 | 2.19 | .014 |
| AGE | TIME | 11 | 1292.9 | 117.54 | 1.83 | .047 |
| BED AGE | TIME | 11 | 396.42 | 36.039 | .56 | .861 |
| | error | 484 | 31112. | 64.280 | | |
| | HRS TIME | 11 | 4340.6 | 394.60 | 5.34 | P < .001 |
| BED | HRS TIME | 11 | 2778.6 | 252.60 | 3.42 | P < .001 |
| AGE | HRS TIME | 11 | 2642.4 | 240.21 | 3.25 | P < .001 |
| BED AGE | HRS TIME | 11 | 732.88 | 66.626 | .90 | .538 |
| | error | 484 | 35747. | 73.857 | | |
| total | | 1151 | .17250E 06 | | | |

Experiment 3

Dependent Variable: Lines crossed
Independent Variables: 1. BED (home cage, clean or no bedding)
2. HOURS (successive hours in a 6-hour exposure period)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|--------|-----|------------|------------|------|----------|
| BED | | 2 | .13164E 06 | 65820. | | |
| | error | 21 | .90648E 06 | 43166. | 1.52 | .241 |
| | HRS | 5 | .69032E 06 | .13806E 06 | 5.86 | P < .001 |
| BED | HRS | 10 | .28585E 06 | 28585. | 1.21 | .291 |
| | error | 105 | .24743E 07 | 23565. | | |

Experiment 3

Dependent Variable: Lines crossed
Independent Variables: 1. BED (home cage, clean or no bedding)
2. TIME (successive 5-minute intervals
in first hour of exposure)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|--------|-----|--------|--------|------|--------|
| BED | | 2 | 26384. | 13192. | 5.93 | .008 |
| | error | 25 | 55614. | 2224.5 | | |
| | TIME | 11 | 6190.1 | 562.73 | 6.31 | P<.001 |
| BED | TIME | 22 | 17148. | 779.46 | 8.74 | P<.001 |
| | error | 275 | 24533. | 89.209 | | |

Experiment 4

Dependent Variable: Lines crossed
 Independent Variables: 1. BED (home cage or no bedding)
 2. STRAIN (Sprague Dawley or Wistar)
 3. HOURS (successive hours in a 6-hour exposure period)

Analysis of Variance

| Source | | df | SS | MS | F | SIG |
|--------|----------|-----|------------|------------|------|----------|
| BED | | 1 | .12094E 06 | .12094E 06 | 1.82 | .187 |
| | STRN | 1 | .28778E 06 | .28778E 06 | 4.33 | .045 |
| BED | STRN | 1 | 74471. | 74471. | 1.12 | .297 |
| | error | 32 | .21246E 07 | 66394. | | |
| | HRS | 5 | .31042E 06 | 62083. | 3.88 | .002 |
| BED | HRS | 5 | .60304E 06 | .12061E 06 | 7.54 | P < .001 |
| | STRN HRS | 5 | .31416E 06 | 62832. | 3.93 | .002 |
| BED | STRN HRS | 5 | 86542. | 17308. | 1.08 | .372 |
| | error | 160 | .25586E 07 | 15991. | | |

Experiment 5

Dependent Variable: Lines crossed
Variables in Regression: 1. HTIME (percent time huddling)
2. BED (home cage or no bedding)
3. TARGET (sibling, 2 anesthetized siblings,
dam or anesthetized dam)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|------------------|----|-----------|-----------|-------|----------|
| HTIME | 1 | 862,022.7 | 862,022.7 | 640.6 | P < .001 |
| BED | 1 | 221,315.2 | 221,315.2 | 164.5 | P < .001 |
| TARGET | 3 | 232,626.3 | 77,542.1 | 57.6 | P < .001 |
| HTIME BED | 1 | 49,779.5 | 49,779.5 | 37.1 | P < .001 |
| HTIME TARGET | 3 | 23,978.6 | 7,992.9 | 5.94 | P < .01 |
| BED TARGET | 3 | 220,005.6 | 73,335.2 | 54.5 | P < .001 |
| HTIME BED TARGET | 3 | 12,675.6 | 4,225.2 | 3.1 | P < .05 |
| error | 47 | 63,242.3 | 1345.6 | | |

Experiment 5

Dependent Variable: Percent huddle time
Variables in regression: 1. ACTIVITY (total lines crossed)
2. BED (home cage or no bedding)
3. TARGET (sibling, dam, 2 anesthetized siblings or anesthetized dam)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|---------------------|----|-------------|-------------|-------|---------|
| ACTIVITY | 1 | 2,075,206.3 | 2,075,206.3 | 453.6 | P <.001 |
| BED | 1 | 305,973.0 | 305,973.0 | 66.9 | P <.001 |
| TARGET | 3 | 911,220.2 | 307,740.1 | 66.4 | P <.001 |
| ACTIVITY BED | 1 | 45,818.0 | 45,818.0 | 10.0 | P <.005 |
| ACTIVITY TARGET | 3 | 32,906.1 | 10,968.7 | 2.4 | P >.05 |
| BED TARGET | 3 | 686,425.8 | 288,806.6 | 50.0 | P <.001 |
| ACTIVITY BED TARGET | 3 | 19,585.1 | 6,528.4 | 1.4 | P >.05 |
| error | 47 | 215,001.6 | 4,574.5 | | |

Experiment 5

Dependent Variable: Lines crossed (Mean of scores from 2 siblings)
Independent Variables: 1. BED (home cage or no bedding)
2. TIME (successive 5-minute intervals)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|--------|-----|------------|--------|-------|----------|
| BED | | 2 | .13335E 06 | 66677. | | |
| | error | 21 | .13549E 06 | 6451.7 | 10.33 | P < .001 |
| | TIME | 11 | 37611. | 3419.1 | 8.27 | P < .001 |
| BED | TIME | 22 | 34890. | 1585.9 | 3.83 | P < .001 |
| | error | 231 | 95547. | 413.62 | | |
| | total | 287 | .43689E 06 | | | |

Experiment 6

Dependent Variable: Percent digging time
Independent Variables: 1. BED (home cage or clean bedding)
2. DISTURB (disturbed or undisturbed home cage bedding)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|----------|----|--------|--------|-------|----------|
| BED | 1 | 7641.7 | 7641.7 | 59.83 | P < .001 |
| DIST | 1 | 5662.6 | 5662.6 | 44.33 | P < .001 |
| BED DIST | 1 | 5196.0 | 5196.0 | 40.68 | P < .001 |
| error | 32 | 4087.1 | 127.72 | | |

Experiment 6

Dependent Variables: Cage crossings
Independent Variables: 1. BED (home cage or clean bedding)
2. DISTURB (disturbed or undisturbed
home cage bedding)

***Analysis of Variance*

| Source | df | SS | MS | F | SIG |
|----------|----|--------|--------|-------|------|
| BED | 1 | 16965. | 16965. | 9.60 | .004 |
| DIST | 1 | 21732. | 21732. | 12.30 | .001 |
| BED DIST | 1 | 2216.9 | 2216.9 | 1.25 | .271 |
| error | 32 | 56549. | 1767.2 | | |

Experiment 6

Dependent Variable: Percent time spent on preferred side of cage
Independent Variables: 1. BED (home cage or clean bedding)
2. DISTURB (disturbed or undisturbed home cage bedding)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|----------|----|--------|--------|-------|---------|
| BED | 1 | 7714.7 | 7714.7 | 17.05 | P <.001 |
| DIST | 1 | 1344.4 | 1344.4 | 2.97 | .094 |
| BED DIST | 1 | 1764.0 | 1764.0 | 3.90 | .057 |
| error | 32 | 14481. | 452.54 | | |

Experiment 6

Dependent Variable: Cage crossings
Independent Variables: 1. ODOR (home cage odors or no odors)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|--------|----|---------|--------|-------|----------|
| ODOR | 1 | 33917. | 33917. | 65.56 | P < .001 |
| error | 34 | 17644.2 | 518.99 | | |

Appendix 2 (Pilot Studies)

Dependent Variable: Lines crossed
Independent Variables: 1. BED (home cage or no bedding - fans off, red lighting)
2. TIME (successive 5-minute intervals)

Analysis of Variance

| Source | df | SS | MS | F | SIG |
|--------|------|--------|--------|--------|----------|
| BED | 1 | 9812.5 | 9812.5 | 41.99 | P < .001 |
| error | 12 | 2804.3 | 233.69 | | |
| | TIME | 5 | 4546.5 | 909.29 | P < .001 |
| BED | TIME | 5 | 5761.5 | 1152.3 | P < .001 |
| error | | 60 | 3228.7 | 53.811 | |

Appendix 2 (Pilot Studies)

Dependent Variable: Lines crossed
Independent Variables: 1. BED (home cage or no bedding - fans on, white lighting)
2. TIME (successive 5-minute intervals)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|--------|----|--------|--------|-------|----------|
| BED | | 1 | 4181.3 | 4181.3 | 12.80 | .012 |
| | error | 6 | 1959.3 | 326.54 | | |
| | TIME | 5 | 1046.0 | 209.20 | 7.53 | P < .001 |
| BED | TIME | 5 | 1429.4 | 285.88 | 10.29 | P < .001 |
| | error | 30 | 833.25 | 27.775 | | |
| | total | 47 | 9449.3 | | | |

Experiment 7

Dependent Variable: Lines crossed
 Independent Variables: 1. BED (disturbed home cage or no bedding)
 2. DRUG (saline or d-amphetamine)
 3. TIME (successive 5-minute intervals)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-----|--------|------|------------|--------|-------|----------|
| BED | | 1 | 45444. | 45444. | 11.45 | .002 |
| | DRUG | 1 | 54617. | 54617. | 13.76 | P < .001 |
| BED | DRUG | 1 | 1137.5 | 1137.5 | .29 | .596 |
| | error | 32 | .12699E 06 | 3968.3 | | |
| | TIME | 11 | 71234. | 6475.8 | 23.37 | P < .001 |
| BED | TIME | 11 | 38197. | 3472.5 | 12.53 | P < .001 |
| | DRUG | TIME | 24122. | 2192.9 | 7.91 | P < .001 |
| BED | DRUG | TIME | 1683.5 | 153.05 | .55 | .867 |
| | error | 352 | 97554. | 277.14 | | |

Experiment 8

Dependent Variable: Lines crossed
Independent Variables: 1. BED (disturbed home cage or no bedding)
2. DRUG (saline or d-amphetamine)
3. TIME (successive 5-minute intervals)

Analysis of Variance

| | Source | df | SS | MS | F | SIG |
|-------|--------|------|------------|--------|-------|----------|
| BED | | 1 | 8400.5 | 8400.5 | 2.31 | .138 |
| BED | DRUG | 1 | 58265. | 58265. | 16.05 | P < .001 |
| BED | DRUG | 1 | 10102. | 10102. | 2.78 | .105 |
| | error | 32 | .11614E 06 | 3629.3 | | |
| | TIME | 11 | 16290. | 1480.9 | 4.92 | P < .001 |
| BED | TIME | 11 | 25876. | 2352.3 | 7.81 | P < .001 |
| BED | DRUG | TIME | 9394.8 | 854.07 | 2.84 | .001 |
| BED | DRUG | TIME | 4584.3 | 416.75 | 1.38 | .178 |
| | error | 352 | .10599E 06 | 301.11 | | |
| total | | 431 | .35504E 06 | | | |

Experiment 9

Dependent Variable: Lines crossed
 Independent Variables: 1. BED (disturbed home cage or no bedding)
 2. DRUG (saline or d-amphetamine)
 3. TIME (successive 5-minute intervals)

Analysis of Variance

| Source | | df | SS | MS | F | SIG |
|--------|-----------|-----|------------|------------|-------|----------|
| BED | | 1 | 15226. | 15226. | 2.13 | .156 |
| BED | DRUG | 1 | .14307E 06 | .14307E 06 | 20.02 | P < .001 |
| BED | DRUG | 1 | 1750.1 | 1750.1 | .24 | .625 |
| | error | 28 | .20013E 06 | 7147.7 | | |
| | TIME | 11 | 10017. | 910.68 | 1.99 | .029 |
| BED | TIME | 11 | 1100.2 | 100.02 | .22 | .996 |
| BED | DRUG TIME | 11 | 7431.6 | 675.60 | 1.48 | .138 |
| BED | DRUG TIME | 11 | 2488.9 | 226.27 | .50 | .906 |
| | error | 308 | .14065E 06 | 456.64 | | |