Individual differences in response to psychological stress and chlordiazepoxide in adult mice: Relations with changes in early social milieu

GIAMPIETRO LOGGI, GIACOMO DELL'OMO and GIOVANNI LAVIOLA
Istituto Superiore di Sanità, Rome, Italy

Individual differences in the adult behavior of mice have been associated with variations in early social milieu, such as age of weaning and the sex of littermates. To better understand the roles that these two variables play in the organization of a behavioral profile, the long-term effects of their manipulation in CD-1 mice were assessed. On Postnatal Day (PND) 15, mouse litters were split in half. Each half litter contained 4 males or 4 females, or 2 of each sex. At the same time, one half litter was weaned (precocious weaning); the other half was left with the dam (regular weaning). At adulthood (PND 70), the animals were challenged with the benzodiazepine agonist chlordiazepoxide (CDP; at a 0-, 2.5-, or 5.0-mg/kg dose) and assessed in sequential order in an animal model for anxiety (black/white exploration test) and for pain reactivity (hot-plate test, set at 55°C ± 1°C) after a 2-min exposure to a threatening stimulus represented by cat urine. Balanced-sex reared males exhibited a shorter latency to leave the white area than did males raised unisexually, being apparently more prompt to react in a novel situation. No such difference appeared in the female group. In the hot-plate test, precocious weaning significantly reduced the latency to lick a forepaw, and prior exposure to cat's urine induced a clear-cut analgesia. The latter response was dose-dependently reduced by CDP administration in mice raised in a sex-balanced condition only. Baseline activity levels also differed as a consequence of gender and of rearing condition, and were slightly increased by CDP treatment. Overall, these results suggest that subtle variations of social environment early in development can exert long-term effects on both behavioral reactivity to environmental stimuli and on BDZ-induced behavioral changes.

Several studies on altricial rodents indicate that differences in early social environment are crucial for the organization of individual differences in adult behavior (e.g., Birke & Sadler, 1987; Denenberg & Morton, 1964; Moore & Power, 1992; Namikas & Wehmer, 1978; for a critical review of the literature, see Laviola & Loggi, 1992, and Terranova & Laviola, 1995). In this context, the gradual transition to nutritional and behavioral independence that takes place at weaning can well be seen as a focal point in development. Thus, experimental manipulation of age at weaning, a factor that varies considerably in natural conditions, has been shown to affect a wide range of facets of behavioral repertoire in both rats and mice (Smith, 1991; Terranova & Laviola, 1995). As outlined by Bateson, Mendl, and Feaver (1990), such behavioral changes are likely to represent a major contributor to the subsequent development of individual differences in behavior and to adult individual fitness. Our first aim in the present study was thus to investigate the outcomes of precocious weaning on the behavioral repertoire of adult male and female mice.

The inclusion in the present experimental design of a group of adult mice weaned precociously from the mother also enabled us to try to separate the causal factors (see below) accounting for another important phenomenon linked to the manipulation of early social milieu—the “sibling effect.” Each sibling in a family is part of the environment where other siblings develop, and if the phenotype (e.g., gender) of one sibling influences the behavior of another, this is a sibling effect (see Laviola & Alleva, 1995, for literature and discussion). Such an effect, which can also be obtained by early manipulation of litter gender composition, has been claimed to account for several individual differences in adult rat and mouse behaviors (Alleva, Caprioli, & Laviola, 1986; Laviola & Loggi, 1992; Musi, DeAcetis, & Alleva, 1993; Sharpe, 1975; also see Discussion).

As for the mechanisms that may be responsible for the differences related to the sex of littermates, one working hypothesis includes a joint action of at least two factors. First, both rat and mouse dams discriminate and interact differently with male and female pups, and such sex-biased
maternal behavior affects several aspects of the offspring's behavioral repertoire (Alleva, Caprioli, & Laviola, 1989; Moore & Morelli, 1979). A second factor, namely changes in the nature of social interactions among siblings (e.g., different levels of social grooming or playfulness) as a function of the sex of littermates may also play a prominent role (Laviola & Alleva, 1995; Terranova & Laviola, 1995).

In this context, a series of previous studies by our group have shown long-term influences of early social events on the function of opioid as well as GABA/benzodiazepine (BDZ) receptor systems in the CNS (Alleva et al., 1986; Laviola & Loggi, 1992). With respect to the latter, the BDZ receptor matures early in ontogeny and thus may play an important physiological role in the mediation of affiliative bonds during the course of development (Cirulli, Santucci, Laviola, Alleva, & Levine, 1994; Insel, Hill, & Major, 1986; Laviola & Loggi, 1992; Laviola, Terranova, Sedowofia, Clayton, & Manning, 1994; Terranova, Alleva, & Laviola, 1994). Consistent with this, GABA/BDZ systems are widely expressed in limbic areas responsible for receiving and organizing incoming sensory information from which arise integrated social bonding behaviors and accompanying affective states (Izquierdo, Cunha, & Medina, 1990).

Overall, these data suggest that (1) brain GABA/BDZ systems modulate socially guided motivations and behaviors, (2) the social environment modulates the functional activity of the GABA/BDZ receptor complex in the CNS, and (3) alterations in an organism's social environment may be an important factor in the genesis of anxiety-related disorders (Cirulli et al., 1994; Drugan & Holmes, 1991; Insel et al., 1986; Insel, Scanlan, Champoux, & Suomi, 1988; Izquierdo et al., 1990; Laviola & Loggi, 1992). Thus, the second aim of this study was to test whether or not manipulation of the early social milieu that mice are raised in would in some way affect the development and subsequent functioning of this important CNS regulatory system (Denenberg & Morton, 1964; Hayashi & Kimura, 1978; Terranova, Laviola, & Alleva, 1993; Terranova et al., 1994; also see Discussion).

Several studies have also suggested that rodents have a stress response to the olfactory stimuli emitted by a predator that results in a variety of physiological effects. Experimental evidence has also demonstrated that endogenous pain-inhibiting systems involving CNS opioid and GABA/BDZ systems interact with the social environment and can be activated by predator-emitted stimuli (R. J. Blanchard, D. C. Blanchard, Weiss, & Meyer, 1990; Kavaliers, 1988). Rat and mouse responses to biological odors (cat urine or feces) have been amply described (Dell'Omo & Alleva, 1994; Dell'Omo, Fiore, & Alleva, 1994; fanselow & Sigmundi, 1986), and recently Kemble and Gibson (1992) demonstrated that cat odor can induce avoidance and analgesia in laboratory CD-1 mice.

In the following experiment, adult male and female mice that underwent an ontogenetic manipulation of social milieu were tested in sequence in an animal model of anxiety (black/white exploration test), and in a behavioral procedure commonly used to assess reactivity to noxious stimuli (hot-plate response). The animals were also challenged with a drug (chloridiazepoxide; CDP) known to act on the GABA/BDZ receptor complex—a neural substrate considered to be an important participant in an organism's response to environmental challenge (R. J. Blanchard et al., 1990; Cirulli et al., 1994; Drugan & Holmes, 1991; Laviola, DeAcetis, Bignami, & Alleva, 1991; Laviola & Loggi, 1992). In our case, this challenge was represented by a naturalistic, threatening predatory stimulus, cat urine. In fact, the application of a naturalistic approach allows for an analysis of the natural repertoire of an organism's responses which may be of great help in understanding the evolution of the acquired component of the endogenous pain-inhibiting system as well as the adaptive significance of other important physiological mechanisms (R. J. Blanchard et al., 1990; Dell'Omo & Alleva, 1994; Dell'Omo et al., 1994; fanselow & Sigmundi, 1986; Kavaliers, 1988; Kemble & Gibson, 1992; Zangrossi & File, 1992).

**METHOD**

**Animals, Breeding, and Rearing Conditions**

Mice of an outbred Swiss CD-1 strain, purchased from Charles River Italia (Calco, Italy), were used. Upon arrival, they were housed in standard conditions (temperature 21±1°C, relative humidity 60±10°C) in a reversed 12:12-h white-light:red-light cycle (red lights on at 0930 h). Males and nulliparous females were housed separately in groups of 8 in 42×27×15 cm Plexiglas boxes with sawdust as bedding and a metal top. Pellet food (Enriched Standard Diet purchased from Piccioni, Brescia, Italy) and water were continuously available.

After 2 weeks, breeding pairs were formed and housed in 33×18×14 cm boxes. The females were inspected daily at 1000 h for the presence of vaginal plug (Pregnancy Day 0) and for delivery (Postnatal Day 1; PND 1). The stud was removed 10 days after the plug was found. At birth, the litters were reduced to 8 pups—4 males and 4 females. On PND 15, each litter was divided in half; one half was randomly assigned to the precocious weaning (PW) condition, in which the mother was removed on PND 15; the other half was assigned to the regular weaning (RW) condition, in which they were kept with the mother and transferred to a new cage. At the same time, the sexes were segregated (Laviola & Loggi, 1992; Terranova & Laviola, 1995), with each half litter being randomly assigned to one of three different family units: to one with all male pups, to one with 2 male and 2 female pups, or to one with all female pups. The result was the formation of the following four experimen tal groups: MM and MF males, FM and FF females in each of the two weaning conditions.

This age period was chosen because PNDs 14–25 constitute a critical period in development during which future patterns of social response in mice are determined (Denenberg & Morton, 1964; Hayashi & Kimura, 1978). Milk cream in a dish and food pellets were put on the floor of the cage on PNDs 14–16 in order to limit the potentially stressful experience related to the sudden removal of the dam and the consequent abrupt suppression of food intake. The first appearance of weaning behavior and eating of solid food are normally observed around PNDs 16–17 (for the literature, see Terranova & Laviola, 1995). Moreover, it has been reported that weaned mice can subsist on solid food as soon as their eyes open (around PND 15), and a series of previous studies from our group (Terranova et al., 1994; Terranova & Laviola, 1995) have shown that there are no significant long-term effects of precocious weaning on body weight.
On PND 25, the mice were rehoused, according to sex, in 42 × 27 × 15 cm Plexiglas boxes (4 individuals per cage). The cohorts of 4 individuals per cage were derived from different litters, with only 1 animal per litter being assigned to the new group. Each single treatment group was represented by 12 animals, with only 1 animal per original litter contributing to each mean. The animals were classified as adults when they reached PND 70±1. All mice were used only once.

Drug Treatment
An aqueous suspension of chlordiazepoxide hydrochloride (CDP) obtained from AGRAR, Rome, was prepared fresh daily by mixing the compound in the vehicle [VEH, saline (NaCl 0.9%) containing two drops of Tween 80 per 10 ml of solution]. To maintain an even suspension, it was stirred at low speed until the time of injection. The animals in each litter were weighed and assigned randomly to one of the following treatments given i.p. in a volume of 0.01 ml/g body weight: CDP at 0, 2.5, or 5.0 mg/kg. The range of doses and the time interval between injection and testing were selected on the basis of findings from previous work by our group (Laviola & Loggi, 1992). The experimental design incorporated temporal counterbalancing in order to equilibrate the different test times of various groups.

Activity/Exploration in a Black/White Apparatus
Tests were performed in an experimental room isolated from the animal colony and maintained under similar light:dark cycle and humidity and temperature conditions. The exploration assessment was conducted in a white/black test box (described by Costall, Jones, Kelly, Naylor, & Tomkins, 1989), which consisted of a rectangular Plexiglas chamber divided into two compartments (16 × 15 × 30 cm each) by a wall with a 4 × 7 cm opening; one compartment had white and brightly illuminated walls and floor (60-W white bulb suspended 100 cm from the ceiling above the apparatus); the other was black, dark, and covered on the top. Passages from one side to the other were registered by photocells, while activity scores (body displacement) were obtained by passive infrared sensors placed on the ceiling of each side of the apparatus, which relayed signals to an automated digital counter (ELMA System, Rome). The animals were transported in their home cages to the experimental room for a 1-h habituation period prior to the start of testing, after which they were administered injections. Thirty minutes after the injection, the mice were individually transferred from their home cages into the white side of the apparatus. Latency to leave the white side, activity measurements, and total time spent in each of the two compartments were collected on line during the test session.

Exposure to Predatory Stimulus
Immediately after the end of the exploration test, the mice were placed, for 2 min, in a cage identical to their home cages, which was placed into a metal box (60 × 60 × 50 cm). One half of the vehicle-injected (CDP-0) animals (between-odor controls) were tested in an empty box, while the other half (within-odor controls) as well as animals from both CDP groups were exposed to fresh cat urine contained in 100 cc of scented sawdust collected from the cage of an adult male domestic cat. This schedule of exposure was selected on the basis of the research of D. C. Blanchard, R. J. Blanchard, Tom, and Rodgers (1990), Dell’Omo et al. (1994), Fanselow and Sigmundi (1986), Kemble and Gibson (1992), and Zangrossi and File (1992).

Nociceptive Assessment
Immediately after the exposure to cat urine, pain reactivity and analgesia, using a hot-plate apparatus (Socrel Hot-Plate Model DS37: Ugo Basile, Italy) were measured as latencies of forepaw licking. The apparatus temperature was set at 55±0.5°C, and cutoff was set at 60 sec.

Data Analysis
The data were analyzed by mixed-model analyses of variance (ANOVA) for all variables—namely, the litter random variable (Abbey & Howard, 1973; Chiarotti, Alleva, & Bignami, 1987) nested under the prepubertal sexual segregation (PSS), time of weaning, sex, and treatment administered before test. Moreover, for the analysis of pain sensitivity, the ANOVA also included the exposure to cat urine (Od). Post hoc comparisons within logical sets of means were performed using the Tukey’s HSD test, whose use is permissible or even recommended in the absence of significant main or interaction effects in the ANOVA in order to minimize both Type I and Type II errors (Wilcox, 1987, pp. 187–189).

RESULTS

Body Weight Assessment
As usual, males were heavier than females [$F(1,1) = 122.07, p < .001$], while no significant carry-over effects of weaning conditions were evident. Consistent with previous reports, no changes in body weight were related to differences in litter gender composition (Alleva et al., 1986; Laviola & Loggi, 1992; Terranova & Laviola, 1995).

Mouse Behavior in the Black/White Apparatus
The assessment of latency to leave the white area failed to show simple effects of weaning, sex, or PSS variables, but did yield a main effect of treatment [$F(2,112) = 6.24, p < .01$]. That is, mice treated with CDP-2.5 moved to the black side of the apparatus earlier than did control animals and the other CDP group (11 ± 1.2 sec vs. 14 ± 0.9 or 16 ± 2.1 sec—means ± SE). Although a sex × PSS interaction (Figure 1) just missed significance [$F(1,56) = 2.76, p = .07$], post hoc tests suggested that males reared unisexually entered the black area later than did males from mixed-sex litters ($p < .05$); no such difference appeared within the female group.

Effects on Locomotion and Exploratory Behavior
Since the scores obtained for the black compartment are complementary to those obtained in the white one, for the sake of brevity, only the latter scores are presented.

![Figure 1. Mean latency (in seconds ± SEM) to leave the bright white area in a black/white exploration test of adult male and female mice reared in unisex or mixed-sex litters ($n = 48$). *$p < .05$ within the male group.](image-url)
As expected, mice preferred to spend more time in the black compartment than in the white one, and this time also varied as a joint function of weaning and treatment \([F(2,112) = 7.04, p < .01]\). In fact, as shown in Table 1, mice weaned precociously spent less time in this compartment after a 2.5 CDP dose than did both controls and the other CDP group ( \(p < .05\) or less, respectively). CDP had no effects in the regularly weaned mice. Moreover, the sex of the subjects and the PSS condition seemed to affect the time spent in the white compartment, as suggested by a weaning \(\times\) sex \(\times\) PSS \(\times\) treatment interaction, just missing significance in the ANOVA \([F(2,112) = 2.79, p = .06]\).

A gender \(\times\) PSS interaction for general activity \([F(1,56) = 3.70, p < .05]\) was also evident. Specifically, MF males (73.5 ± 7.1) were significantly more active than MM males (62.1 ± 3.8; \(p < .05\)). By contrast, an opposite trend (59.1 ± 4.5 for FM and 68.9±3.9 for FF) was evident in females ( \(p < .05\)).

For general activity, there was a main effect of treatment \([F(2,112) = 7.08, p < .01]\) and a weaning \(\times\) treatment interaction \([F(2,112) = 4.91, p < .01]\). In particular, inspection of data and multiple comparisons revealed that this effect was limited to the regularly weaned group, CDP-2.5 mice being more active than both controls and CDP-5 animals and than similarly treated subjects weaned precociously ( \(p < .05\); see Table 1). A far more complex profile was also suggested by the finding of a higher level interaction—weaning \(\times\) sex \(\times\) PSS \(\times\) treatment \([F(2,112) = 3.18, p < .05]\). In particular, MF males weaned regularly were more active with CDP-2.5 than were similarly treated MM males ( \(p < .05\)). No other significant differences emerged.

**Effects on Nociception**

Latency to forepaw licking in response to the thermal stimulation was in general greater in males than in females \([sex, F(1,56) = 5.50, p < .05]\). Moreover, time of weaning also affected this measure \([weaning \times sex, F(1,56) = 6.35, p < .01]\), with males weaned regularly showing a higher latency than those weaned precociously and than both groups of females.

The above variables also interacted with PSS \([F(3,168) = 3.11, p < .05]\). In fact, the group of sex-balanced and regularly weaned mice (see Figure 2) exhibited a significant analgesia following the exposure to the cat odor (CDP-O/ no-Od vs. CDP-0/Od, \(p < .01\)). In contrast, animals weaned precociously failed to show such a response, as confirmed.
by the finding of a significant difference between this group and the corresponding group of regular weaning, mixed-sex, and CDP-0/Od animals ($p < .01$). In the group weaned regularly, CDP administration strongly reduced the stress-induced analgesia, particularly at the 5-mg/kg dose ($p < .05$), that was otherwise observed in CDP-0/Od animals. This effect was limited to the regularly weaned and mixed-sex animals [$\text{weaning} \times \text{PSS} \times \text{treatment}$, $F(3,240) = 258$, $p < .05$]. It should be noted that the same dosages of CDP were reported to significantly decrease the threshold of the pain response (Laviola & Loggi, 1992). Specifically, this effect has been observed in sex-balanced adult mice without prior exposure to cat urine. As in the present report, CDP administration was ineffective in sexually segregated subjects.

**DISCUSSION**

The results clearly show that:

1. Adult male mice sexually segregated during prepuberty were less fearful and showed a longer latency to leave a bright white area than did males reared in a balanced-gender condition. No such difference was evident in the group of females.

2. Adult mice of both sexes raised in a balanced-gender condition showed a prominent analgesia in response to a potentially threatening stimulus such as cat urine. As expected, prior CDP administration dose-dependently reduced this response. Interestingly, no response changes, neither the simple response to the odor nor the CDP effects, were found in the sexually segregated animals.

3. Precocious weaning (a) abated the cat-odor-induced analgesia, and (b) strongly reduced the PSS-related differences in behavior, which otherwise appeared clear-cut in animals weaned regularly.

The present results confirm and extend previous observations concerning the influence of early social environment on both the behavioral repertoire of adult animals and the functioning of the GABA/BDZ system in the CNS (see the introduction to this paper). They also suggest that manipulation of social milieu during prepuberty has a long-term influence on systems that serve exploratory pattern and pain sensitivity by modulating both the process of coping with a threatening, natural stimulus such as a predatory odor and its possible results as exemplified by the response to painful stimulation.

Behavioral alterations in response to challenge at the adult stage with BDZ agents have been shown in mice (Laviola & Loggi, 1992) and primates (Insel et al., 1988). These effects have been interpreted in terms of long-term influences of early life events on the function of the GABA/BDZ receptor system. The BDZ receptor matures early in ontogeny and may have an important physiological role in the mediation of affiliative bonds early in development either between the dam and her offspring (Cirulli et al., 1994; Insel et al., 1986, 1988; Laviola, Chiarotti, & Alleva, 1992; Laviola & Loggi, 1992) or among pups of different sex in the litter (Laviola et al., 1994; Terranova et al., 1994; Vandenberg, 1989). As outlined in the introduction, variations of postnatal maternal behavior depending on litter gender composition have also been considered as a major factor in the genesis of this phenomenon (Alleva et al., 1989; Moore & Morelli, 1979). The inclusion in the design of the present study of a group of adult animals removed precociously from the mother (PW group) made it possible to separate the effects of the two main confounding variables, namely the nature of dam–offspring and sibling–sibling relationships. We found a clear indication of the importance of a modulatory role for the mother early in development for the production of PSS-related differences in adult behavior. In fact, early removal of the dam reduced the long-term behavioral differences attributable to litter gender composition.

These data also demonstrate that in normally weaned subjects, CDP administration at nonsedating doses counteract a stress-induced analgesia, whereas sedating doses are known to be associated with analgesic effects (D. C. Blanchard et al., 1990; Drugan & Holmes, 1991; Kavaliers, 1988). This is consistent with previous demonstrations of significant scent-induced, BDZ-sensitive analgesia in laboratory and wild mice (R. J. Blanchard et al., 1990; Kavaliers, 1988; Kemble & Gibson, 1992). On the other hand, animals weaned precociously did not show any response change to CDP. This is not surprising, since the drug action is strictly dependent on the baseline level. In this case, only an elevated baseline response level was able to reveal the effects of the drug, and precociously weaned mice failed to show any increase in baseline threshold for pain response.

Behavioral changes related to age at weaning, a factor that varies considerably in natural conditions, are likely to represent a major contributor to the subsequent development of individual differences in behavior and to adult individual fitness (see Bateson et al., 1990). In this context, Bekoff (1988) outlined the importance of motor training and physical fitness for the development of intraspecific and intralitter variability. We have previously reported an altered expression of both locomotor and social behavioral patterns during development in mice weaned precociously that could well be put into this perspective (Terranova & Laviola, 1995). Inevitably, any experimental procedure involving the separation of mother from offspring is open to a variety of interpretations. The precociously weaned pups experienced a loss of maternal contact and care as well as a sudden change in diet earlier in development than did animals weaned regularly (Smith, 1991). Intraspecific variation in the time of weaning is evidently one source of behavioral variation between individuals and may continue to influence the subsequent course of development.

Since natural mouse litters are characterized by large differences in offspring sex ratio (Krackov, 1992), the same conceptual framework can be used to understand the results of our second manipulation of the family unit. We have previously reported that mice experiencing ongoing unisexual rearing were more involved in exploratory–escape activities and less in affiliative and huddling-like behavior than were pups reared in sex-balanced litters.
(Terranova & Laviola, 1995). It seems reasonable that individual pups experiencing a less varied social environment (i.e., the PSS condition) may show an impoverishment of the social behavioral repertoire. However, although functional explanations have been suggested for the effects of unisexual rearing on some physiological parameters (e.g., delayed onset of puberty in female mice; Drickamer, 1992; Vandenberg, 1989), it is not yet clear what kind of short- and/or long-term benefits may derive from the complex pattern of PSS-related behavioral changes that we observed.

As for the possible mechanisms underlying the above PSS effects, it should be noted that unisexual rearing causes changes, supposedly adaptive, in the offspring's behavioral development, altering the quality/quantity of environmental stimulation a mouse receives from each individual in its family unit. The manipulation of family-unit characteristics provided prepubertal mice with a set of different environmental cues which are usually associated with changes in social milieu, namely ultrasonic vocalizations (Naito & Tonue, 1987), physiological changes related to different olfactory inputs (Lupo di Prisco, Lucarini, & Dessi-Fulgheri, 1978; Vandenberg, 1989; Drickamer, 1992), and social experiences with littermates (Laviola & Alleva, 1995; Terranova & Laviola, 1995) or dam care (Moore & Morelli, 1979; Alleva et al., 1989).

The individual responses of animals to the pain-modulating action of CDP apparently reflect individual variation in the sensitivity of central mechanisms mediating the bidirectional action reported for different BDZ agents. It has been assumed by several authors that the putative endogenous ligand for BDZ binding sites may exert a pharmacological activity opposite to that of classical BDZ tranquilizers (Guidotti et al., 1983). Therefore, a tentative hypothesis is that the concentration of the putative endogenous ligand (in CNS) is higher in ecological stress (predatory odor) and CDP responders than in ecological stress and CDP nonresponders. Whether or not this is the case, the subtle experimental manipulation, namely the PSS procedure adopted here, offers an additional tool for analyzing the functional significance of the relationship between different ligand sites at the GABA/BDZ receptor complex (also see the discussion in Laviola & Loggi, 1992).

In the present study, the differences generated by PSS manipulation, namely both the absence of an analgesia response to an ecologically relevant stimulus and the modulatory effects of CDP can be tentatively attributed to an altered function of the GABA/BDZ receptor complex—a neural substrate considered to be an important participant in an organism's responding to environmental challenge (Bodnoff, Surany-Cadotte, Quirion, & Meaney, 1989; R. J. Blanchard et al., 1990; Drugan & Holmes, 1991; Laviola & Loggi, 1992). In agreement with this view, Primus and Kellogg (1990) reported that castration of male rats as juveniles (PND 19) counteracted the facilitative effect of diazepam on the adult response to an anxiogenic situation. An influence from gonadal steroids (or their metabolites) on function of the GABA/BDZ system has been extensively demonstrated (Harrison, Majewska, Harrington, & Barker, 1987; Lacau de Mendigo, Diaz-Torga, & Libertun, 1989; Majewska, Harrison, Schwartz, Barker, & Paul, 1986). Taken together, these reports indirectly suggest that prepubertal sexual segregation might interfere with gonadal hormones or their metabolites on pubertal development of the receptor complex (Drickamer, 1992; Lupo di Prisco et al., 1978; Sharpe, 1975; Vandenberg, 1989).

The fact that artificial manipulation of early social environment can qualitatively affect some aspects of the animal's behavioral repertoire in adulthood raises the question of whether comparable effects might occur naturally in view of the large differences in sex ratio of natural mouse litters. Perhaps quantitative and/or qualitative variation in social interaction with the mother as well as the opposite sex through infancy and adolescence is a contributor to the wide interindividual variation in capacity to cope with environmental challenges, including drug administration (File, 1983; Laviola & Loggi, 1992) or toxicant exposure.

Finally, one can attempt to justify these results from an evolutionary point of view. In the mixed-sex mice of both sexes, the shorter latency to leave the bright white area can be viewed as an increased "fearfulness" (Costall et al., 1989) which allows the animal to exhibit a prompt active reaction to a novel situation. In addition, the analgesia associated with predator stimuli may also represent an efficient response which increases individual survival. The fact that both such responses are performed by normally weaned and sex-balanced animals suggests that the value of the mixed-sex condition is greater than that of the unisexual one. In fact, the mixed-sex condition represents an increase of general variability into the family unit, and variability is well known to have an important evolutionary role in determining the adaptive success of a species. In this context, the differentiation of the sexual phenotype also contributes to overall fitness (see discussion in Laviola & Alleva, 1995, and Terranova et al., 1993).

REFERENCES

ABBREY, H., & HOWARD, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. Developmental Psychobiology, 6, 329-335.

ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1986). Postnatal social environment affects morphine analgesia in male mice. Physiology & Behavior, 36, 779-781.

ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1989). Litter gender composition affects maternal behavior of primiparous mouse dam (Mus musculus). Journal of Comparative Psychology, 103, 83-87.

BATISTON, P., MENDLE, M., & FEATHER, J. (1990). Play in the domestic cat is enhanced by rationing of the mother during lactation. Animal Behaviour, 40, 514-525.

BEKOFF, M. (1988). Motor training and physical fitness: Possible short- and long-term influences on the development of individual differences in behavior. Developmental Psychobiology, 21, 601-612.

BIJEK, L. T. A., & SADLER, D. (1987). Differences in maternal behavior of rats and the sociosexual development of the offspring. Developmental Psychobiology, 20, 85-99.

BLANCHARD, D. C., BLANCHARD, R. J., TOM, P., & RODGERS, R. J. ABBREY, H., & HOWARD, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. Developmental Psychobiology, 6, 329-335. ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1986). Postnatal social environment affects morphine analgesia in male mice. Physiology & Behavior, 36, 779-781. ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1989). Litter gender composition affects maternal behavior of primiparous mouse dam (Mus musculus). Journal of Comparative Psychology, 103, 83-87. BATISTON, P., MENDLE, M., & FEATHER, J. (1990). Play in the domestic cat is enhanced by rationing of the mother during lactation. Animal Behaviour, 40, 514-525. BEKOFF, M. (1988). Motor training and physical fitness: Possible short- and long-term influences on the development of individual differences in behavior. Developmental Psychobiology, 21, 601-612. BIJEK, L. T. A., & SADLER, D. (1987). Differences in maternal behavior of rats and the sociosexual development of the offspring. Developmental Psychobiology, 20, 85-99.

BLANCHARD, D. C., BLANCHARD, R. J., TOM, P., & RODGERS, R. J. ABBREY, H., & HOWARD, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. Developmental Psychobiology, 6, 329-335. ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1986). Postnatal social environment affects morphine analgesia in male mice. Physiology & Behavior, 36, 779-781. ALLEVA, E., CAPRIOLI, A., & LAVIOLA, G. (1989). Litter gender composition affects maternal behavior of primiparous mouse dam (Mus musculus). Journal of Comparative Psychology, 103, 83-87. BATISTON, P., MENDLE, M., & FEATHER, J. (1990). Play in the domestic cat is enhanced by rationing of the mother during lactation. Animal Behaviour, 40, 514-525. BEKOFF, M. (1988). Motor training and physical fitness: Possible short- and long-term influences on the development of individual differences in behavior. Developmental Psychobiology, 21, 601-612. BIJEK, L. T. A., & SADLER, D. (1987). Differences in maternal behavior of rats and the sociosexual development of the offspring. Developmental Psychobiology, 20, 85-99.
BLANCHARD, R. J., BLANCHARD, D. C., WEISS, S. M., & MEYER, S. (1990). The effects of ethanol and diazepam on reactions to predatory odors. Pharmacology, Biochemistry & Behavior, 35, 775-780.

BODNOFF, S. R., SURANY-CADOTTE, B. E., QUIRION, R., & MEANEY, M. J. (1989). Role of the central benzodiazepine receptor system in behavioral habituation to novelty. Behavioral Neuroscience, 103, 209-212.

CHIAROTTI, E, ALLEVA, E., BODNOFF, S. R., SURANY-CADOTTE, B. E., QUIRION, R., & MEANEY, M. J. (1989). Role of the central benzodiazepine receptor system in behavioral habituation to novelty. Behavioral Neuroscience, 103, 209-212.

CIRULLI, F., SANTUCCI, D., LAVIOLA, G., ALLEVA, E., & LEVINE, S. (1994). Behavioral and hormonal responses to stress in the newborn mouse: Effects of maternal deprivation and chloridiazepoxide. Developmental Psychobiology, 27, 301-316.

COSTALL, B., JONES, B. S., KELLY, M. E., NAYLOR, R. S., DENENBERG, V., & DRAGAN, R. C., GUIDOTTI, A., FORCHETTI, C. M., CORDA, M. G., KONKEL, D., BENNET, D. M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. Pharmacology, Biochemistry & Behavior, 32, 777-785.

Dell’Omo, G., & ALLEVA, E. (1994). Snake odor alters behavior, but not pain sensitivity in mice. Physiology & Behavior, 55, 125-128.

Dell’Omo, G., FIORE, M., & ALLEVA, E. E. (1994). Strain differences in mouse response to odors of predators. Behavioural Processes, 32, 105-116.

DENENBERG, V. H., & MORTON, J. R. (1964). Infantile stimulation, prepubertal sexual-social interaction, and emotionality. Animal Behaviour, 12, 11-13.

DRINICKER, L. (1992). Premature and postweaning excretion of puberty-influencing chemosignals in house mice. Developmental Psychobiology, 25, 1-16.

DRUGAN, R. C., & HOLMES, P. V. (1991). Central and peripheral benzodiazepine receptors: Involvement in an organism’s response to physical and psychological stress. Neuroscience & Biobehavioral Reviews, 15, 277-298.

FANSELOW, M. S., & SIGNORET, R. A. (1986). Species-specific danger signals, endogenous anaglesia, and defensive behavior. Journal of Experimental Psychology: Animal Behavior Processes, 12, 301-309.

FILE, S. E. (1983). Variability in behavioral responses to benzodiazepines in the rat. Pharmacology, Biochemistry & Behavior, 18, 303-306.

GUIDOTTI, A., FORCHETTI, C. M., CORDA, M. G., KONKEL, D., BENNET, D. C., & COSTA, E. (1983). Isolation, characterization and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. Proceedings of the National Academy of Science, 80, 3531-3535.

HARRISON, N. L., MAJEWSKA, M. D., HARRINGTON, J. W., & BARKER, J. L. (1987). Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid-A receptor complex. Journal of Pharmacology & Experimental Therapeutics, 24, 346-353.

HAYASHI, S., & KIMURA, T. (1978). Effects of exposure to males on sexual preference in female mice. Animal Behaviour, 26, 290-295.

INSEL, T. R., HILL, J., & MAJOR, R. B. (1986). Rat pup ultrasonic calls: Possible mediation by the benzodiazepine receptor complex. Pharmacology, Biochemistry & Behavior, 24, 1263-1267.

INSEL, T. R., SCANLAN, J., CHAMPOUX, M., & SUOMI, S. (1988). Rearing paradigm in a non human primate affects response to β-CCE challenge. Psychopharmacology, 96, 81-86.

IZQUIERDO, I., CUNHA, C., & MEDINA, I. H. (1990). Endogenous benzodiazepine modulation of memory processes. Neuroscience & Biobehavioral Reviews, 14, 414-419.

KAVAILERS, M. (1988). Brief exposure to a natural predator, the short-tail weasel, induces benzodiazepine-sensitive analgesia in white-footed mice. Physiology & Behavior, 43, 187-193.

KEMBLE, E. D., & GIBSON, B. M. (1992). Avoidance and hypoalgesia induced by novel odors in mice. Psychological Record, 42, 555-563.

KRAKOV, S. (1992). Sex ratio manipulation in wild house mice: The effect of fetal resorption in relation to the mode of reproduction. Biology & Reproduction, 47, 541-548.

LACHAU DE MENGIDO, I. M., DIAZ-TORGA, G. S., & LIBERTUN, C. (1989). Diazepam: Endocrine effects and hypothalamic binding sites in the developing male and female rat. Life Science, 45, 567-575.

LAVIOLA, G., & ALLEVA, E. (1995). Sibling effects on the behavior of infant mouse litters. Journal of Comparative Psychology, 109, 68-75.

LAVIOLA, G., CHIAROTTI, F., & ALLEVA, E. (1992). Development of GABAergic modulation of mouse locomotor activity and pain sensitivity after prenatal benzo diazepine exposure. Neurotoxicology & Teratology, 14, 1-5.

LAVIOLA, G., DEACETIS, L., BIGNANI, G., & ALLEVA, E. (1991). Prenatal oxazepam enhances mouse maternal aggression in the offspring, without modifying acute chloridiazepoxide effects. Neurotoxicology & Teratology, 13, 75-81.

LAVIOLA, G., & LOGGI, G. (1992). Sexual segregation in infancy and bidirectional benzodiazepine effects on hot-plate response and neophobia in adult mice. Pharmacology, Biochemistry & Behavior, 42, 865-870.

LAVIOLA, G., TERRANOVA, M. L., SEADOWPIA, K., CLAYTON, R., & MANNING, A. (1994). A mouse model of early social interactions after prenatal drug exposure: A genetic investigation. Psychopharmacology, 113, 388-394.

LUPO DI PRISCO, C., LUCARINI, N., & DESFI-FULGHERI, F. (1978). Testosterone aromatization in rat brain is modulated by social environment. Physiology & Behavior, 20, 345-348.

MAJEWSKA, D. M., HARRISON, N. L., SCHWARTZ, R. D., BARKER, J. L., & PAUL, S. M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science, 232, 1004-1007.

MOORE, C. L., & MORELLI, G. A. (1979). Mother rats interact differently with male and female offspring. Journal of Comparative Psychology, 93, 677-684.

MOORE, C. L., & POWELL, K. L. (1992). Variation in maternal care and individual differences in play, exploration and grooming of juvenile Norway rat offspring. Developmental Psychobiology, 25, 165-182.

MUSI, B., DEACETIS, L., & ALLEVA, E. (1993). Influence of litter gender composition on subsequent maternal behaviour and maternal aggression. Ethology, 95, 43-53.

NAITO, H., & TONE, T. (1987). Sex difference in ultrasonic distress call by rat pups. Behavioral Brain Research, 25, 13-21.

NAMIKAS, J., & WEIMER, F. (1978). Gender composition of the litter affects behavior of male mice. Behavioral Biology, 23, 219-224.

PRIMUS, R. J., & KELLOGG, C. K. (1990). Developmental influence of gonadal function on the anxiolytic effect of diazepam on environment-related social interaction in the male rat. Behavioural Pharmacology, 1, 437-446.

SHARP, R. M. (1975). The influence of the sex of litter-mates on subsequent maternal behaviour in Rattus norvegicus. Animal Behaviour, 23, 551-559.

SMITH, E. F. S. (1991). The influence of nutrition and postpartum mating on weaning and subsequent play behaviour of hooded rats. Animal Behaviour, 41, 513-524.

TERRANOVA, M. L., ALLEVA, E., & LAVIOLA, G. (1994). Affiliation and neophobia in developing mice prenatally exposed to oxazepam. Behavioural Pharmacology, 5, 52-60.

TERRANOVA, M. L., & LAVIOLA, G. (1995). Individual differences in mouse behavioural development: Effects of precocious weaning and ongoing sexual segregation. Animal Behaviour, 50, 1261-1271.

TERRANOVA, M. L., LAVIOLA, G., & ALLEVA, E. (1993). Ontogeny of amicable social behavior in the mouse: Gender differences and ongoing isolation outcomes. Developmental Psychobiology, 26, 467-481.

VANDENBERGH, J. G. (1989). Coordination of social signals and ovarian function during sexual development. Journal of Animal Science, 67, 1841-1847.

WILCOX, R. R. (1987). New statistical procedures for the social sciences: Modern solutions to basic problems. Hillsdale, NJ: Erlbaum.

ZANGROSSI, H., & FILE, S. E. (1992). Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Research Bulletin, 29, 381-388.

(Manuscript received August 31, 1995; revision accepted for publication February 27, 1996.)