Pharmacology Biochemistry and Behavior, Vol. 55, No. 4, pp. 459–474, 1996

0091-3057/96 $15.00 + .00

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PII S0091-3057(96)00279-1

On Mouse Pups and Their Lactating Dams: Behavioral Consequences of Early Exposure to Oxazepam and Interacting Factors

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Received 8 April 1996; Revised 18 June 1996; Accepted 20 June 1996

LAVIOLA, G. *On mouse pups and their lactating dams: behavioral consequences of early exposure to oxazepam and interacting factors.* PHARMACOL. BIOCHEM. BEHAV. **55**(4) 459–474, 1996—Behavioral analysis in animal models appears to be a valuable and sensitive tool for detecting subtle alterations in CNS function, which can be produced by early exposure to small perturbations of sensory experience, hormonal milieu, or exposure to psychotropic agents devoid of major teratogenic potential. Concerning anxiolytics, the more recent work in mice, which is here summarized, was carried out by putting the emphasis on changes in naturally occurring species-typical social responses as a function of early exposure to benzodiazepines. For adult females, on the behavior expressed during the early postpartum period, whereas for infant subjects, on the ontogenetic stage of the establishment of social bonding. Critical issues such as the choice of fostering procedure and rearing conditions are also addressed. Evidences of relationships between changes in social milieu taking place during early rearing—i. e., when dramatic transitions in the neurochemical target system occur—and the adult behavioral response to challenges with BDZ agents are presented. These data strengthen the notion that the modes of reaction of adult animals to the joint influence of physiological and environmental (stimulus) variables are under the influence of events in early ontogenesis. Therefore, a better understanding of the mechanisms—as unveiled by an appropriate use of drug tools—that mediate such a plasticity might have considerable psychobiological and clinical-therapeutical relevance.

Benzodiazepines Developmental plasticity Neurobehavioral teratology Maternal care Playful behavior Mouse

OVER the past few decades, experimental study of young animals has provided major contributions toward important research objectives such as the identification of the causes (and potential treatments) of developmental disorders in humans, and the understanding of the basic mechanisms that lead to changes in motor, sensory or cognitive capacities with age. In young animals, small perturbations of sensory experience, hormonal milieu, as well as exposure to powerful substances have been documented to alter ontogenetic trajectories and to be susceptible to produce very large effects on behavior later in life (5). Because developmental trajectories have been proved to be more malleable at earlier than at later stages, much research has emphasized the usefulness of prenatal or neonatal manipulation or treatment in order to assess developmental outcomes in adulthood.

One of the aims of this kind of research is to provide a suitable model for understanding developmental processes in the child. However, this statement raises a deeper problem of what developmental researchers should expect from a model system. Some animal models are employed in the explicit attempt to duplicate the human situation and mimic human health concerns. However, a more fruitful objective of animal models is to employ analytic methods—including an appropriate use of drug tools—to understand control mechanisms underlying behavioral development in a relatively simple system. The findings concerning the model system can then be used to formulate specific hypotheses that can be evaluated in more complex systems, such as human subjects.

In the frame of the remarkable developmental plasticity of target systems, we have performed a series of mouse experiments aimed at challenging GABA-benzodiazepine (BDZ) systems of the CNS during development (60,63). A substantial part of this analysis was devoted at assessing the short-, medium-, and long-term consequences of prenatal oxazepam (a BDZ agonist) exposure. No attempt will be made here to provide a comprehensive account of the results so far obtained (1,8). Rather, the main purpose of the present paper is to address some interesting issues which have emerged in the

course of this series of studies, such as the finding i) of limited alterations in social interactions and play, following in utero exposure to benzodiazepines, in infant mice, i.e., during the ontogenetic stage characterized by the establishment of social bonding; ii) of marked changes in the postpartum emotional profile of lactating mouse dams either as a carry-over effect of developmental benzodiazepine exposure, or as a function of an anxiolytic drug treatment given to the mother until a few days before parturition; iii) and of changes in the behavioral response to benzodiazepine agents in adult animals as a function of manipulation of rearing conditions during infancy or prepuberty.

A brief survey of clinical-epidemiological data on the gestational benzodiazepine syndrome in human neonates and children precedes the experimental section in animal models.

Brief survey on the neurobehavioral consequences of maternal benzodiazepine exposure in human subjects. Anxiety is a major cause of psychiatric morbidity in the industrially-developed countries [(116); see also (12) for implications of gender differences]. In the 1960s benzodiazepines, a new class of tranquillizer, became one of the most commonly used group of drugs. With mounting evidence of their safety, an increase in use during pregnancy came to reduce anxiety or induce sedation (88). Some studies calling for attention to undesiderable side-effects on newborn infants began to emerge during the '70s (17), and symptoms such as hypotonia, low Apgar scores, hypothermia and respiratory depression in the newborn exposed *in utero* to BDZs were reported (34). These drugs are prescribed regularly and often chronically during pregnancy and early neonatal life to prevent epileptic seizures, febrile seizures, hyperbilirubinemia, and the stressful effects of labor (24,58). In addition, they have been shown to readily cross the placenta and to be secreted in the mother's milk (125).

During late '80s, developmental abnormalities—eg., dysmorphism and mental retardation—were reported in Sweden in children whose mothers had regularly taken high doses of benzodiazepines (diazepam or oxazepam) during pregnancy. However, the possible association between behavioral teratogenicity and the use of these drugs in humans is still controversial and not yet fully defined [for literature and discussion, see (124)]. More recently, in fact, the above-mentioned findings were criticized in a study examining the fetal outcomes of a large study considering approximately ten thousand women who took benzodiazepines during pregnancy in the USA, during the middle '80s. Most of the maternal records resulted to contain many potentially confounding diagnoses, such as maternal psychiatric problems, hypertension, diabetes, obesity, malnutrition, and elderly parity (striking risk factors for adverse pregnancy outcomes), which could confound any effect of the benzodiazepines (7). A further evaluation of the potential impact of fetal BDZ exposure on the newborn and young child was based on a longitudinal prospective study in Sweden of children born to mothers who used BDZ in prescribed doses throughout pregnancy (124). Only those women who reported the regular use of psychotropic drugs without the use of street drugs or the abuse of alcohol were included. These mothers were physically healthy, but all had a psychiatric diagnosis including anxiety disorder, predominantly panic disorder, or depressive disorder.

A tendency to decreased intrauterine growth and symptoms and signs of CNS depression, as well as CNS hyperirritability were found in infants exposed to BDZ. The BDZ-infants demonstrated at 10 and 18 months consistently lower developmental quotients for five scales assessing locomotor, personal-social behavior, hearing and speech, eye-hand coordination, and performance, as well as a general developmental quotient. Based on these data, the authors stressed the possibility that fetal exposure to BDZ may cause a general delay in mental development up to 18 months of age. The BDZ-children in most cases deviated by being inactive from normal activity than the children in the reference group. BDZ-newborns exhibited a set of symptoms that came to be known collectively as the floppy infant syndrome, which is characterized by hypotonia, hypothermia, sedation and poor sucking response (17). Some newborns of mothers who were chronically treated with DZP exhibited symptoms similar to those of narcotic withdrawal (96). Recently, Laegreid and colleagues (57) published data showing mainly sedation and withdrawal in infants of mothers taking benzodiazepines up to term.

Prospective, well designed studies of the risks to both mother and child of exposure to psychotropic drugs during pregnancy are neither feasible nor ethical. Thus, any appropriate data from unplanned exposures to drugs during pregnancy, animal studies, or observations about the nature and frequency of pregnancy and birth-related problems that occur in untreated women and their offspring must be accurately assessed. As stated above, there are few studies which addressed a reliable evaluation of the possible long-term alterations in children derived of maternal exposure to benzodiazepines during pregnancy. These few reports suggest that maternal exposure to BDZs can induce behavioral and cognitive alterations in children, although their subtle characteristics demand more powerful tools such as a more sensitive battery of neuropsychological tests for revealing the extent of the long-term consequences.

With respect to animal data, some of them, although far from dramatic, are suggestive of a production of selective changes in some regulatory mechanisms which serve important behavioral functions (1,8,36,49,58,121). This is an important issue, since most psychoactive drugs might interfere, as epigenetic factors, with the rigidly ordered temporal sequences of events that occur during the ontogeny of CNS, therefore leading to the onset of neurodevelopmental alterations (18).

EXPERIMENTAL SECTION IN ANIMAL MODELS

Treatment of pregnant female mice. A treatment aimed at a particular system(s) must be given at a time when significant developmental changes are known to occur in the system(s) in question (14,79). In the case of benzodiazepine binding sites, these changes are maximal in the CNS of rats and mice during the last days of pregnancy (32,121), which are marked by rapid growth of neural tissue and differentiation. In our benzodiazepine studies (1,4,8), the treatment schedule was chosen on the basis of current knowledge of relative potency and duration of various benzodiazepines, supplemented by data from preliminary studies using female mice of the same strain and PO treatment twice daily for five days by a wide range of oxazepam (OX) doses (a compound with an intermediate duration of action). Specifically, oxazepam (15 mg/kg) was given PO twice daily to the dams from the morning of day 12 (vaginal plug day 0) to the evening of day 16, that is, with an adequate interval between the last dose and parturition. The aim was to avoid i) cumulative effects which are often found with benzodiazepines metabolized more slowly than oxazepam, and ii) parturition in a state of sedation, or during behavioral disturbances sometimes occurring at about 24 h after treatment withdrawal. This treatment did not affect

a sensitive toxicity indicator such as pre-, peri-, and postnatal reproductive performance (1,4).

Changes in affiliative behaviors of developing mice as a function of prenatal BDZ exposure. More recently, the attention of psychobiologists and behavioral teratologists has shifted from documenting late-life effects of early perturbations to investigating the behavior expressed by young animals. This shift of emphasis is theoretically justified because the study of young animals, apart from being interesting in its own right, brings investigators a step closer to the mechanisms of developmental change (110). Appreciation that behavioral development emerges from the interaction between the organism and its environment has prompted a handful of laboratories to ask questions about the behavioral capacities of developing animals as a function of the environmental circumstances in which the young animal resides.

One way to investigate the role of a neurochemical regulatory system and to evaluate both the nature and specificity of the proactive developmental effects of any given type of agent is to study behavioral responses modulated by those mechanisms that are known to be affected by the agent. Benzodiazepines are reported to facilitate social interaction in several animal models (29), so that the study of social interactions appears to be a suitable animal model for the early assessment of subtle behavioral changes in the offspring after gestational drug exposure (68). While this variable has been successfully employed for young rats (87,89,130), our work has been the first attempt, to our knowledge, to investigate the topic on other altricial rodent such as the mouse.

A series of ethological studies has been concerned with alterations in specific aspects of the social behavior of juvenile and adult rodents following early pharmacological treatments (30,49,64). However, an area which has received little attention in the frame of the ethopharmacological approach is that concerning drug-induced changes in the earliest social interactions which develop in the period preceding weaning, when pups are still with their mother [for altricial rodents, this is the first three weeks of postnatal life (68)].

In human subjects, Rodning and colleagues (99,105) reported, in a study with toddlers, that a useful and sensitive index of the level of subtle behavioral disorganization induced by gestational exposure to different drugs is the quality as well as the quantity of playful activities. We examined the behavioral outcomes of early manipulation of the GABA/benzodiazepine receptor complex in the CNS of developing mice through prenatal exposure to oxazepam (119). The issue was addressed by characterizing the ethological repertoire of immature mice, which were observed in the home cage on postnatal days 16, 20 and 24. This consisted of social investigative/affiliative items, socially playful soliciting elements, and non-social behaviors. Since the focus of the experiment was on pups behavior, both treated and control animals were weaned at postnatal day 15 with the aim to avoid any bias derived by changes in maternal care (for a detailed discussion and references on the consequences of early weaning, see the study by Terranova & Laviola (117), which specifically addressed this issue). The aim was also to emphasize behavioral changes in the OX-treated offspring, as suggested by a human study (99), reporting that play behavior was particularly disorganized in children (exposed *in utero* to a mixture of various psychoactive agents) when observed in the absence of an adult.

Briefly, OX-exposed mice resulted to weigh slightly but constantly less than prenatal control mice, and this difference was particularly evident in the male group (see Table 1). The results regarding gender differences should be viewed in the light of literature data concerning both the interaction between the level of steroid hormones and the function of the GABA-BDZ systems (41,65,76), and the lasting influence of perinatal BDZ administration on the hormone-dependent sexual differentiation of physiological and behavioral parameters (54,107).

Prenatal OX exposure also produced fairly specific, though subtle consequences on neural and behavioral development—for transient changes in the maturation of neurobehavioral landmarks as well as alterations in baseline locomotory levels, habituation profile, and in the response to selected monoaminergic and opiatergic drug challenges (3,4)—with OX-mouse pups being apparently more involved than prenatal controls in behaviors related to the achievement and maintenance of a passive proximity with litter mates. By contrast, they were less interested in active investigation and solicitation of litter mates, in non-social behaviors such as locomotor-rotational play, and cage exploration, also showing a reduced frequency to approach and make contact with the novel object (see Figs. 1 and 2).

At first sight, this pattern of results may be suggestive of either an enhanced or a reduced level of fearfulness in the offspring of OX mothers. In this context, it has been shown that the joint expression of low environment exploration and high social interaction is a common correlate of a low level of fearfulness or anxiety (29). Thus, the present findings would be in agreement with other reports suggesting that perinatal BDZ exposure has long-term anxiolytic effects on social interaction of adult rats (30) and mice (64).

It should be noted, however, that the increased social behavior shown by OX mice only reflects a greater tendency to remain inactive in close proximity with litter mates, and that it was concomitant to an increased level of neophobia. Considering the age of the subjects, which had not yet developed the adult avoidance/defensive response to conspecifics (91), and their strict relatedness and familiarity with litter mates, it seems likely that they were adopting the social comfort response (89) as a behavioral strategy aimed at counteracting an anxiogenic situation posed by the novel object. Therefore, although the increased tendency to social inactivity might also be partially attributed to BDZ-induced alterations of thermoregulation (17,34,54,67,108), it seems reasonable to look at the present results as at an extension to infant subjects of previous reports on adult mice (64) and rats (77), indicating reduced control over emotional responses in animals prenatally exposed to BDZ and challenged by novel and intimidating environmental stimuli.

According to a different hypothesis, Kellogg and colleagues (53) outlined that prenatal BDZ exposure, acting during a sensitive developmental period, may impair, with long-lasting effects, early acquisition and/or processing of environmental information and, in particular, sensory functions (52,64). However, the two above hypotheses, i.e. increased emotionality and impaired processing of information, do not appear to be mutually exclusive, in that an interaction between the two factors may be hypothesized. In fact, it seems likely that, under certain circumstances, an impaired comprehension of environmental cues may lead to (or even follow on) an increased level of anxiety.

With respect to the mechanisms, chronic treatment with BDZ agents during a critical stage in development leads to a decrease of GABA binding sites in several CNS areas in rats (50,77,81). This supports the hypothesis that regulatory com-

TABLE 1

MEAN BODY WEIGHT GAIN IN GRAMS (± SEM) OF MALE AND FEMALE MICE AGED 16, 20, AND 24 DAYS, PRENATALLY EXPOSED TO EITHER OXAZEPAM (OX, 15 mg/kg TWICE/DAY ON PREGNANCY DAYS 12-16) OR TO VEHICLE (VEH)

	VEH♂	VEH♀	OX♂	OX♀
Day 16	8.74 ± 0.52	8.41 ± 0.49	7.70 ± 0.34	7.73 ± 0.33
Day 20	10.96 ± 0.61	10.38 ± 0.67	9.65 ± 0.69	9.53 ± 0.62*
Day 24	16.14 ± 1.22	15.50 ± 0.85	13.79 ± 1.06	13.54 ± 0.79*

$n = 7$ in each final group. *$p < 0.05$, VEH males vs VEH females. (Reprinted with permission from 119).

ponents of GABA receptor are involved in the action of BDZs. In a social situation, psychoactive agents which act by increasing the function of the GABA/BDZ receptor complex in the brain seem to have a fairly specific role in shifting the balance between approach and flight tendencies towards the former (29). We may expect, therefore, that prenatal exposure to a BDZ agent may result—via adaptive changes at the CNS level—in specific alterations in early social interactions with litter mates which are triggered into action during infancy (i.e., during an ontogenetic phase mostly devoted to early social bonding and attachment). In this context, a modification of GABAergic regulatory mechanisms (79), leading to an acceleration of behavioral responses, has been reported in three-week-old mice which have been exposed prenatally to BDZ (63). This effect was found in animals of the same age and mouse strain as that examined in the present study. The occurrence of behavioral disorganisation in the course of development—which can be detected at an early stage by monitoring the level of precocious social interactions and play—will probably be related to profound alterations in the establishment of normal social competence in adult life.

OX exposure *in utero* resulted to be associated with significant alterations in the general range of early investigative, affiliative, soliciting social interactions and play displayed by immature mice. With respect to mechanisms, the display of these behaviors is reported to be modulated, among others, by the activity of the endogenous opioid systems in the CNS (87,89). Thus, considering the interplay of CNS opioid and GABA/BDZ mechanisms in the brain, a speculative hypothesis can be a OX-induced change in the functional interaction between these two CNS regulatory systems. Previous studies reported a consistent reduction in the expression of opioid-mediated behavioral responses in both rats and mice exposed *in utero* to BDZ agents, which is consistent with this hypothesis (3,85).

The carry-over effects of early BDZ exposure contribute to the notion that events in early ontogenesis can influence the subsequent mode of reaction at the juvenile as well as at the adult stage to the joint influences of physiological (i.e., hormone milieu) and environmental (stimulus) variables. Therefore, it appears that an appropriate use of drugs and tests can lead to a better understanding of the mechanisms that mediate such plasticity in the behavioral repertoire, which might have considerable heuristic value and clinical-therapeutical relevance. The present findings stress the importance of behavioral analysis as a valuable and sensitive tool for early

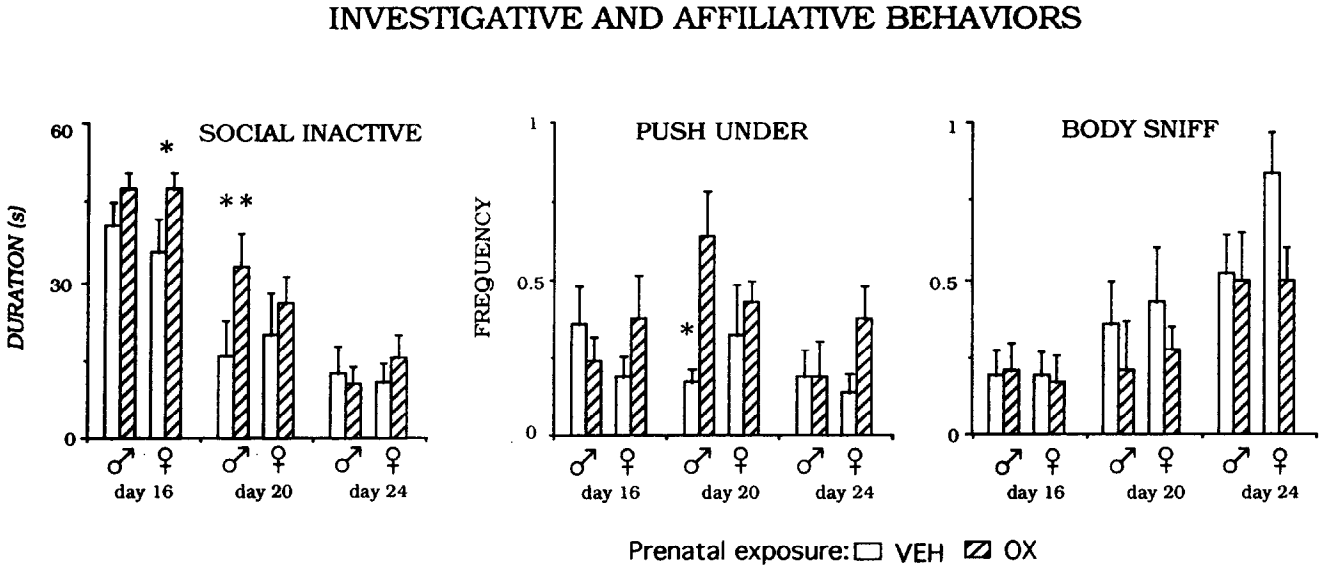

FIG. 1. Mean (SEM) frequency or duration of investigative and affiliative social behaviour in male and female mice aged 16, 20 and 24 days, prenatally exposed to either oxazepam or vehicle (15 mg/kg twice/day on pregnancy days 12-16). ($n = 7$ in each final group). Data refer to the same animals of Table 1. *$p < 0.05$, **$p < 0.01$, VEH vs OX. (Reprinted with permission from 119).

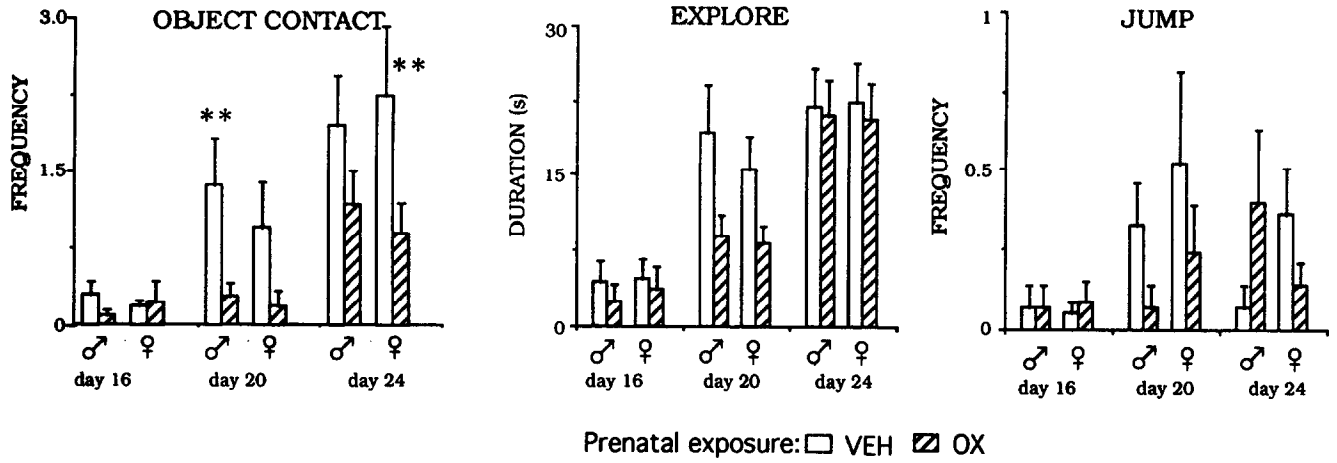

FIG. 2. Mean duration or frequency (SEM) of non-social behaviour and of approach to a novel object in male and female mice aged 16, 20 and 24 days, prenatally exposed to either oxazepam or vehicle. (n = 7 in each final group). **$p < 0.01$, VEH vs OX. Data refer to the same animals of Figure 1. (Reprinted with permission from 119).

detection of subtle changes in the CNS function of infant subjects. This is of particular relevance when substances of abuse or therapeutic drugs are taken by their pregnant mothers.

Changes of maternal care as a function of OX exposure until a few days before parturition and methodological issues associated to the choice of fostering procedure. The series of studies reviewed here was designed with the conviction that emphasis in the search for teratogenic effects should be placed on naturally occurring species-typical behavior that forms an integral part of the normal behavioral repertoire (8). From this viewpoint, it appears that the study of behavior of females of altricial rodents, such as mice and rats during a specific reproductive stage (i.e., the early postpartum period), can contribute significant information on the nature of changes in the emotional repertoire often characterizing this specific phase in the life cycle.

The administration to developing animals of psychoactive agents during their fetal life implicates a concomitant exposure for their pregnant mothers. Female rats and mice display following parturition an organized pattern of maternal care, which includes nest building, general body and genital licking of the young, retrieval of the young to the nest, and adoption of the lactating posture over the young (101). The display of this spectrum of maternal responses can be severely altered by dam exposure to drugs or toxicants (42,62,67).

Thus, it is possible that concomitantly to the effects on the immature animals, the psychoactive agent may also have short- or long-lasting behavioral and physiological effects on the dam. The quality of the offspring-mother interaction results to be affected by prior drug exposure, and so would be the course of developmental trajectories in the offspring (110,115). For these reasons, the usual procedure is to eliminate postnatal maternal effects by assigning at birth both treated and control litters to untreated-unhandled dams (62,114).

However, such influence appears to be still controversial, and the necessity for routine fostering has also been questioned (43,127). In this frame, Goodwing and colleagues (35) reported that when compared with control rats, animals exposed prenatally to the drug exhibited specific neurobehavioral deficits, which were exarcebated by allowing drug-exposed pups to be raised by their own and drug-exposed dams than by untreated foster dams. In other studies, such maternal effects appeared to be minimal or absent, leading to the conclusion that these factors may not be a source of experimental concern (4,127).

When dealing with such an important issue, it should be taken into account that any derived indication in animal models can be useful to shed more light on the interactions and variables at work with those human infants exposed to various psychoactive drugs during gestation. It is well known, for example that some of these subjects, particularly those born from mothers who are severely addicted to street drugs such as heroin, cocaine or crack, are raised at least for limited periods of time by individuals other than their biological mothers. So, the use of fostering procedures in the experimental condition may also approximate in some way the clinical situation.

We have recently addressed the issue of these maternally-mediated effects in dams exposed until a few days before parturition to oxazepam (for the rationale and procedure of drug exposure *in utero*, see the preceding section) and their offspring (62). In particular, since rodents maternal behavior is dependent upon specific sensory cues provided by the pups (2,66,90), we aimed at verifying the influence of the fostering variable by manipulation of the quality of pup-stimulation provided to the mother. Treatment/rearing groups included in this study were OX-exposed and control pups reared by foster mothers, which received the same treatment as the pups assigned to them until a few days before parturition, i.e., within 24 h after birth, entire litters were exchanged within treatments (in-fostered groups, IF); as well as OX-exposed and control pups reared by their own dams left undisturbed (un-fostered groups, UF). In addition, to determine whether maternal behavior in the previously OX-exposed and control dams might be differentially influenced by pups characteristics, foster control pups reared by prior OX-exposed mothers and foster OX pups reared by control dams (cross-fostered groups, CF) were also examined. We assessed various indices of maternal behavior, and what we found is shown in Figure 3.

With respect to nursing responses, pups raised by oxazepam females in the UF and CF conditions (treated and control offspring, respectively) received less maternal care than those reared by control females; by contrast, treated-IF dams tended

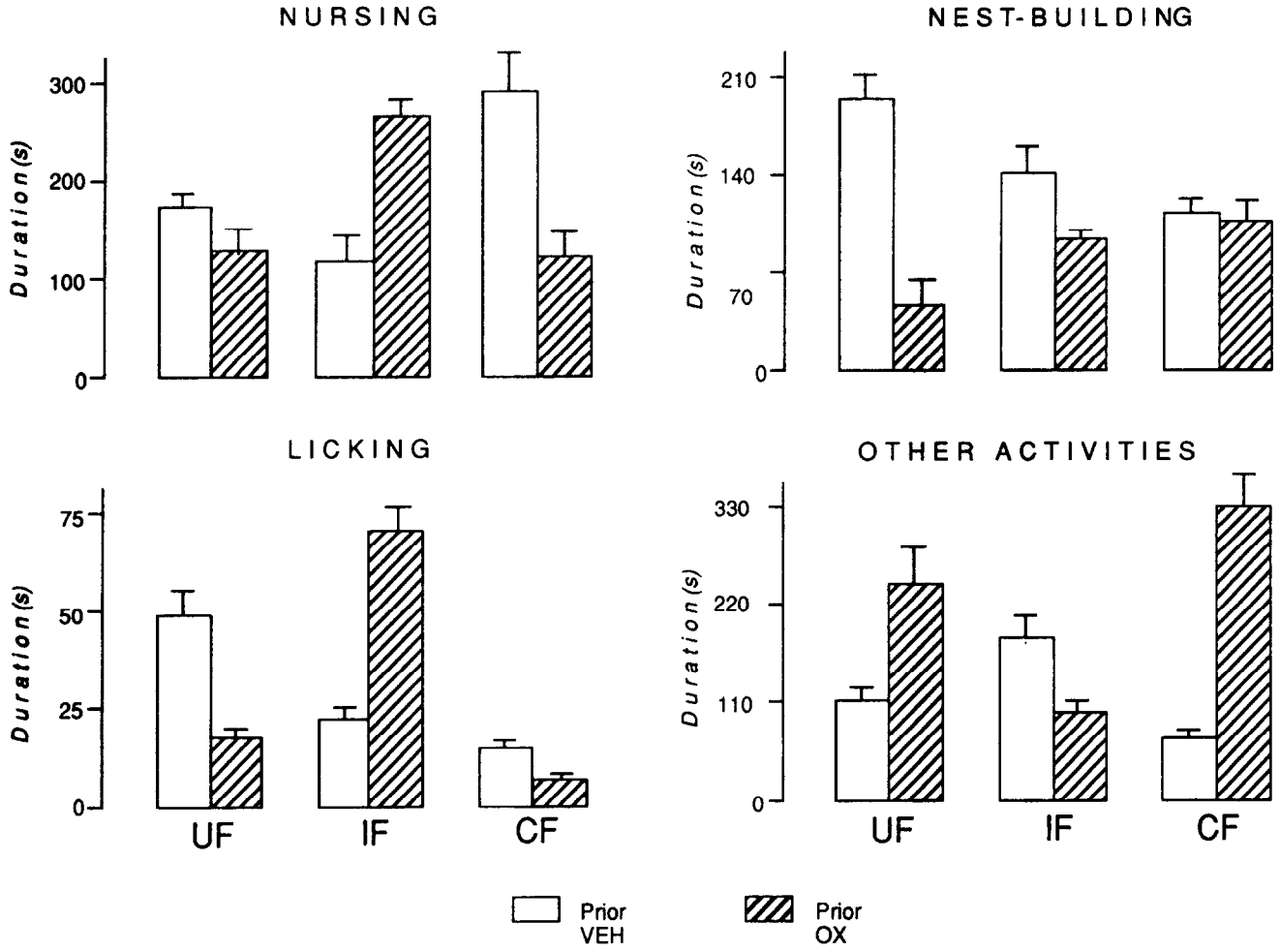

FIG. 3. Mean duration (SEM) of selected behavioral responses observed in mouse dams (single 10-min session) assigned to either the unfostered (UF), the in-fostered (IF), or the cross-fostered (CF) condition, after receiving either vehicle or oxazepam until few days before parturition (see text). Columns refer to data averaged over the three (4, 8, and 12) post-partum days. (Adapted with permission from 62).

to provide a normal or even an enhanced amount of care to treated offspring. Quite interestingly, the assignment of treated offspring (CF), instead of untreated offspring (IF), to control dams prevented the reduction of nursing duration observed in the IF condition. Considering that prenatal OX treatment causes a slight and transient retardation of postnatal body growth and neurobehavioral development (4), the results can be explained only by joint influences of several types of changes. Firstly, mildly impaired pups might constitute a stronger stimulus for the elicitation of maternal responses (66). Secondly, the exchange of litters *per se* appears to have dual effects, that is, an increase in the stimulus properties of the pups and a disturbing influence on the dam. Thirdly, the latter change seems to be prevented by anxiolytic treatment given until a few days before parturition. In other words, both OX dams and OX pups showed in one or more combinations of treatment and fostering procedure the maximal level of maternal care or capacity to elicit maternal care, respectively. By contrast, substantial reductions of such capacities can occur with other combinations of treated and control animals.

Such a profile would point to a joint role of prenatal effects and postnatal maternal effects in producing a dysfunction that results in measurable behavioral changes. This possibility could not be excluded *a priori* in the case of prenatal BDZ exposure, considering in particular that the changes in the target system, which start in late pregnancy, continue at a fast pace during the first two weeks of postnatal life (63).

The relation between CNS changes after BDZ exposure and changes in maternal behavior is not simple. What needs to be explained is the nature of the interaction between behavioral and CNS variables allowing BDZ treatment in late pregnancy to exert an enhancing rather than a depressing effect on maternal care when two additional conditions are satisfied: The first is the extra stimulation produced by an exchange of pups, which may have been modified by a residual anxiolytic action; the second condition is the stronger stimuli for the elicitation of maternal responses by pups with a mild impairment in neurobehavioral development (66,90); including a prolongation of ultrasonic calls in early BDZ-treated rat pups (14).

These data show that much more emphasis should be placed in the analysis of the experiential context in which treatment effects acquire an additional role in the modulation of sensory functions and responses to environmental stimuli,

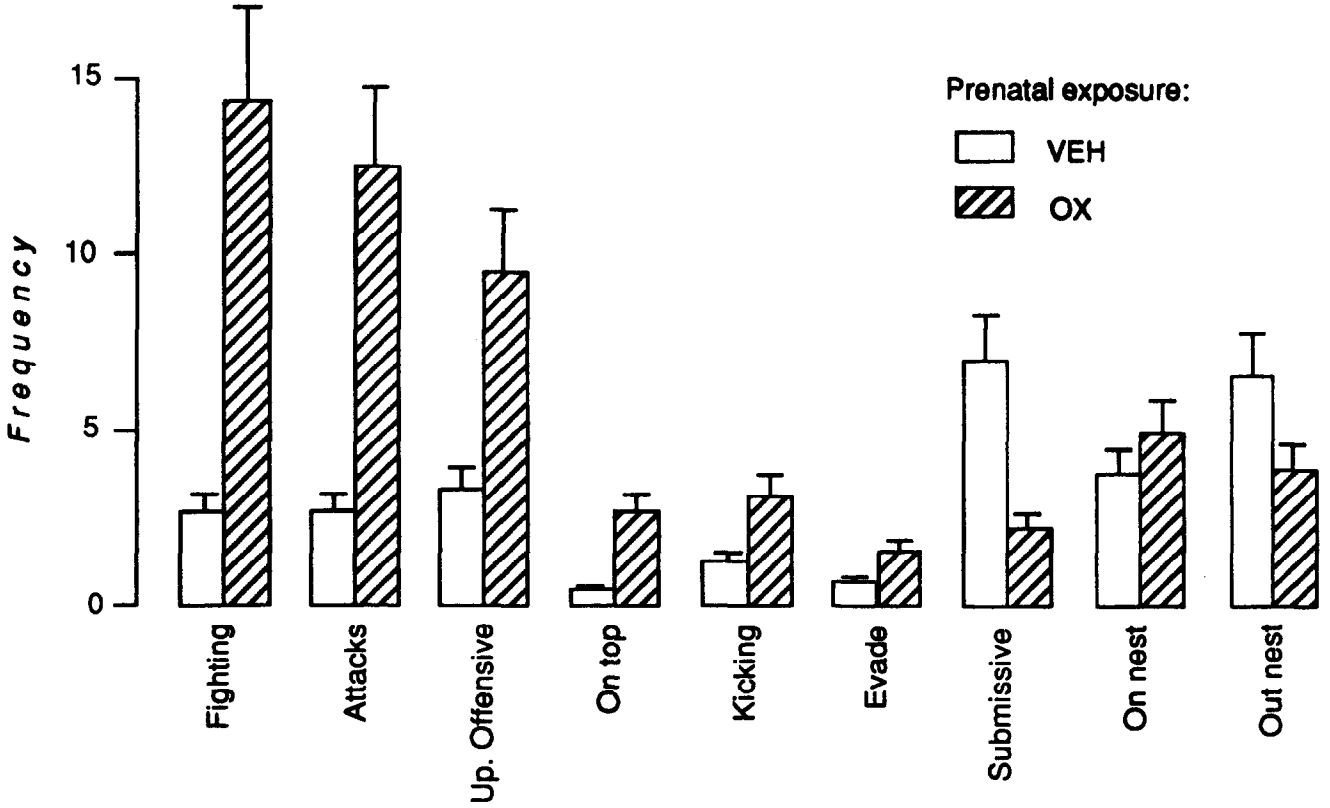

FIG. 4. Frequencies of selected behavioral responses recorded during 5-min maternal aggression tests (nursing female mice exposed to male intruders in the presence of their litters on postpartum day 6). The dams had been exposed prenatally to either oxazepam administered to their mothers (15 mg/kg twice/day on pregnancy days 12–16) or to vehicle. Data are means (SEM) of 16 animals per group. (Reprinted with permission from 64).

for example using different fostering procedures (i.e., manipulation of dam- and pup-related cues). Our findings do stress the need for a better understanding of dyadic mother/pup interactions in studies aimed at characterizing early drug and toxicant effects on animal and human development.

Changes of maternal agonistic behavior during the postpartum period as a function of developmental BDZ exposure. Another part of our analysis consisted of the assessment of maternal emotional changes during the early postpartum period, such as those expected to be expressed in a social interaction test between adult female offspring with a history of prenatal exposure to BDZ, as suggested by previous reports (27,30). This was accomplished by using the paradigm of a particular form of mouse aggressive behavior, namely, maternal aggression towards an unfamiliar male intruder in the nest area (64). In brief (see Fig. 4), when compared with control dams, lactating OX-treated females showed a prominent enhancement of aggressive responses. This effect was quite striking, given the high baseline of such response in control animals. On the other hand, when the same females were re-tested in the same paradigm on post-partum day 8 and challenged with chlordiazepoxide, the proaggressive effect of the BDZ agent given shortly before testing was not modified (data not shown).

Overall, the results apparently deny that the enhanced aggression was due to general changes in reactivity such as those which are usually ascribed to hyperarousal, since no significant changes in general activity level were found as a function of prenatal OX exposure. The basic change was rather in the relative prepotency of the various responses available within the species-specific aggressive/defensive repertoire. In particular, goal-directed (offspring-protective) responses were favored at the expense of fear/emotionality responses.

Previous models have postulated subtle modifications in sensory functions, such as altered auditory temporal resolution (52,77), in an attempt to explain some remarkable changes in fear expression and goal-directed behavior after an early history of BDZ exposure. Other explanations suggest that the functional value of the stimuli that contribute to the modulation of aggressive and defensive responses may show a marked variation during the animals lifetime (e.g., in relation to the reproductive cycle). [For literature and a more detailed discussion on this topic, see a specific section below].

Changes of maternal care as a function of developmental BDZ exposure. In the study just discussed we provided evidence that early manipulation of the GABA/BDZ neurochemical system by prenatal OX administration exerts a long-term influence on mouse maternal aggression. The latter represents an important aspect of the behavioral repertoire of lactating rats and mice which is directed at offspring protection.

An extension of this study was the assessment of possible influences of prenatal oxazepam treatment on the whole pattern of mouse maternal care (90). OX-treated female offspring were mated at the young-adult stage, and the display of their postpartum behavioral repertoire in a nursing context was scored. Briefly (see Fig. 5), when compared with control dams,

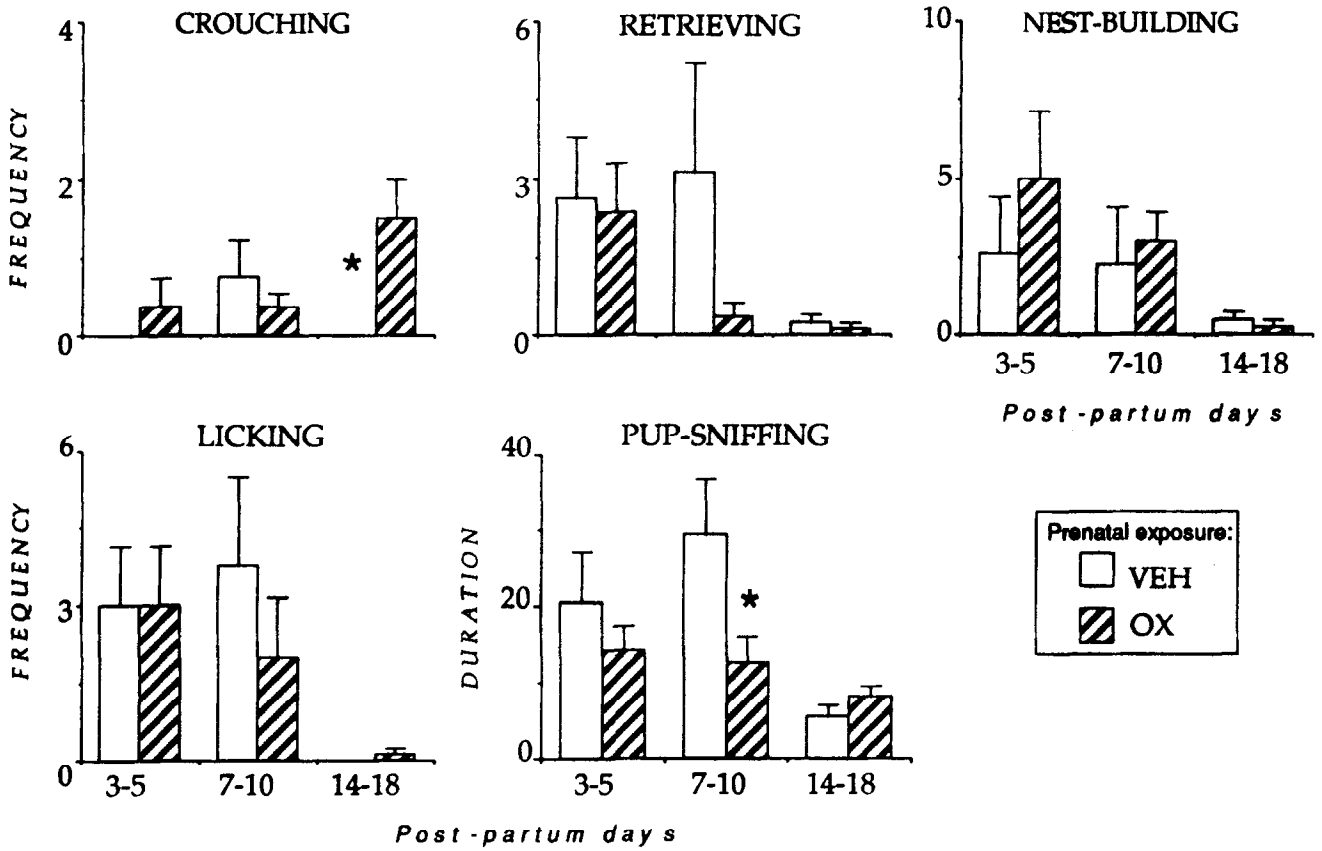

FIG. 5. Mean (SEM) frequency of pup directed activities (single 10-min session) observed in mouse dams treated prenatally with either oxazepam or vehicle. ($n = 8$). The data are pooled from observations carried out on postpartum days 3–5, 7–10, and 14–18. *$p < 0.05$. (Reprinted with permission from 90).

OX females showed a shorter duration of pup-sniffing at 7–10 days and enhanced crouching behavior when pups had reached the age of 14–18 days.

It appears that the slight changes found in the maternal behavior of OX females can be ascribed to modifications in their capacity to respond maternally towards the pups. In agreement with the results of the study reported above (64), the specificity of the oxazepam effects is further confirmed by the absence of changes in locomotion as well as in other non-pup-directed activities (data not shown). This argues one more against the view that the alterations of mouse maternal care can be ascribed to hyperarousal (77). Conversely, the limited changes in maternal care here observed contrast with the data reported above of major alterations in social responsivity (e.g. increased levels of maternal aggression). A more detailed discussion on hypotheses of mechanisms underlying these long-term BDZ effects is included at the end of this section.

In addition, OX treated dams used significantly more cotton and were assigned higher scores for nest construction than control females (data not shown). This activity is an useful indicator in mice of hormonal or drug-induced changes in maternal care (67). The difference between groups appeared only in the presence of their offspring, i.e., after the birth of the pups, suggesting an active role of mother-pups relationship, with the behavior of OX dams being in some way imbalanced by pup presence (67).

With respect to mechanisms, acute benzodiazepine treatment is known to cause hypothermia in adult animals (15). However, the hypothesis that the highest levels of nest construction found in OX females might result from altered thermoregulatory functions (71,108) is weakened by other evidences that prenatal BDZ treatment does not affect the development of thermoregulation in rats (54). [For hypotheses concerning the involvement of changes in hormonal regulations see discussion in a specific section below].

Changes of maternal care as a function of developmental BDZ exposure are dissociated from pup-related stimulus perception. There is evidence that maternal behavior of altricial rodents depends upon sensory information provided by the pups and it is also a function of the females condition, e. g. reproductive state or prior drug exposure (42,62,66,67,90,101).

Concerning the second point, we could expect on the basis of literature data, that prenatal OX exposure produces specific changes in sensory function and stimulus perception in female mice (51,62,64). These alterations were assumed at least to be partially responsible for the changes in maternal care. Thus, we thought it would have been interesting to investigate possible OX-induced changes in pup-stimulus perception (90). To this aim, lactating females prenatally treated with oxazepam were challenged in sequence on postpartum day 8 with a pattern of pup-related stimuli differing in complexity and intensity. Overall, no reliable or statistical significant carry-over effects of prenatal dam treatment were found (data not shown).

These results served to draw the suggestion that the reported alterations in the level of maternal care due to early BDZ exposure cannot be ascribed to changes of stimulus perception (at least for the portion of stimuli here investigated). In fact, the two groups of females were not separated in the response to different patterns of pup-related stimuli.

General discussion on the complex interrelationship of behavioral as well as endocrine/neurotransmitter factors in the mediation of BDZ-related changes in maternal care. As a general rule, the study of emotional disorders in the life cycle of female mammal demands attention to endocrine-behavioral interaction, since some of the most stressful events in life (i.e., pregnancy, labor or lactation) occur simultaneously with marked fluctuations in the level of circulating steroid hormones (75). The rapid shifts in the levels of such hormones, as progesterone and estrogen, occurring at the puerperium (72) may underlie a borderline endocrine/neurotransmitter. This can be precipitated to some extent by environmental factors, resulting in a phase in the female life span of functional vulnerability (59). [For a more detailed discussion on this topic, see below].

A tentative explanation could involve changes in the regulation of GABA systems in the CNS. Benzodiazepines act by facilitating GABAergic neurotrasmission (95), while the functional links between the components of the large GABA/BDZ receptor complex are still poorly understood, it may have at least heuristic value to focus attention on possible functional alterations at the level of such a system when attempting to understand the effects of anxiolytic or antiepileptic agents. In altricial rodents such as rats and mice, mother/offspring interactions during the postpartum period have been shown to modulate the brain GABAergic activity of lactating dams. Specifically, the cerebrospynal fluid concentration of GABA in the mother, which profoundly influences the behavior and the neuroendocrine phenomena associated with lactation, is markedly increased by pup-related stimuli, reaching very high levels while the mother is nursing her pups, and very low levels following pup removal (93). Moreover, the activity of glutamic acid decarboxylase (a GABA biosynthesis enzyme) is increased in the mediobasal hypothalamus of lactating rats (94), and GABA mechanisms in rat mediobasal hypothalamus are known to affect prolactin output during suckling stimulation (94). In mice prolactin reportedly facilitates some items of maternal care such as retrieving and crouching (126).

We can therefore postulate a positive interaction between alterations in GABAergic neurotrasmission, which are reported in animals chronically treated with BDZ agents (either in adult subjects or after prenatal exposure), and the physiological enhancement in GABAergic activity associated with the presence of the pups during postpartum and lactation. What has been observed at a behavioral level are i) changes in mother–young interaction (i.e., more nursing behavior and better nestbuilding scores) in the group of dams treated prenatally with OX, while ii) a more complex picture of changes in the capacity of the prior BDZ-treated dams to provide maternal care and in the capacity of the mildly impaired OX-pups, which possibly constitute a stronger stimulus, to elicit such care. Also intriguing appear the long-term changes in maternal aggression following developmental BDZ exposure, which might be interpreted by postulating a subtle change in the balance between different responses that can be triggered by the approach/avoidance conflict created by the testing situation (51,64,98,123). These findings are consistent with reports that early BDZ exposure of rats affects adaptive behaviors in the exposed animals as adults (19,30,36,54,64,78). Female protection and care of young is in fact very adaptive for species survival.

Lactating female rats and mice are reported to show an integrated behavioral picture consisting of altered emotional responses. Specifically, female rats eat more, are less fearful or anxious (27), and appear more reactive and freeze less in response to a sudden environmental change (i.e., an auditory signal) during lactation than during other stages of the reproductive cycle (40). Such a profile suggests a functional shift in affective patterns to less fear-emotionality responses.

There is evidence suggesting a complex interaction between female hormones and the GABA/BDZ system in the brain (72,76,82,100). The ovarian sex steroids estradiol and progesterone are known to affect GABA/BDZ receptors in several brain areas (72). Moreover, progesterone metabolites have been shown to increase the binding of the BDZ flunitrazepam to benzodiazepine sites, and to be potent barbiturate-like ligands of the GABA receptor complex (76,82), while progesterone produces anxiolytic effects in the female, but not in the male rat (10,76). Therefore, since, in animal models, nest construction and maintenance are progesterone-dependent activities (106), changes in progesterone levels or responsivity may have contributed to the altered expression of this complex behavioral pattern in lactating dams exposed *in utero* to BDZ (see above).

As it is well known, in several species, including rats and mice, the stimuli provided by the pups are very important for the development and maintenance of luteal function after post-partum ovulation. Therefore, with respect to the results of one of the experiments discussed above, which specifically addressed the issue of fostering procedure, it is not unlikely that progesterone functions may also be indirectly affected by the stimulus variables indicated above (namely, exchange of pups and mild pup impairment), resulting in a higher average level in the OX-IF condition, namely, the group of females experiencing both a BDZ treatment until a few days before parturition and the fostering of OX treated pups. At present, however, it appears difficult to make a definite choice among a variety of possible mechanisms by which changes in progesterone functions might result in an enhancement of maternal care, including effects on non-behavioral or behavioral thermoregulation and neural mechanisms underlying maternal responses.

There is also evidence, on the basis of psychopharmacological observations in animal models (mostly rats and mice) that activity in the GABA/benzodiazepine receptor complex in specific brain areas is implicated in the display of maternal behavior (27,39,93). In fact, the expression of maternal care in the lactating female rat is blocked by the administration of BDZ antagonists, while BDZ themselves elicit this behavior in non maternal rats (38). In this regard, it has also been shown that pregnancy related to the onset of natural maternal behavior, produces a decrement in the density of $GABA_a$ receptors and an increase of its affinity in the female rat forebrain. This has been considered the probable consequence of the elevated levels of placental and adrenal endogenous steroids that occur during pregnancy (75).

Interestingly, analogous changes in the density and affinity of $GABA_a$ receptors are obtained as a result of prenatal treatment with diazepam, a direct BDZ agonist (50). In this frame, a facilitation of maternal behavior has been demonstred in adult virgin female rats after developmental administration of diazepam (19). It seems that developmental BDZ administration to the female rat induced an imprinting of the func-

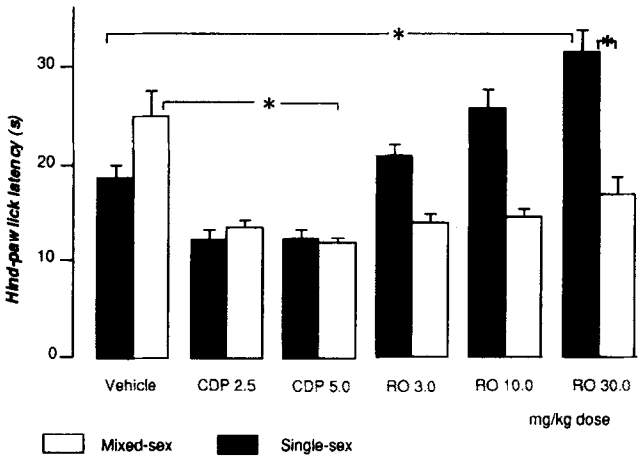

FIG. 6. Mean latency (SEM) to lick a hind-paw in a hot-plate test (set at 55 ± 1°C) of adult female mice, which were either sexually segregated (single-sex litters) or reared in mixed-sex conditions during infancy (birth to postnatal day 21). Since weaning to adulthood (time of test), all mice were re-housed according to sex. At adulthood (postnatal day 70 ± 1), animals were challenged with either vehicle or a BDZ agonist chlordiazepoxide (CDP at 2.5 or 5.0 mg/kg dose) or with a BDZ receptor partial inverse agonist Ro 15-3505 (RO at 3, 10 or 30 mg/kg dose). (n = 12). *$p < 0.05$, **$p < 0.01$. (Reprinted with permission from 65).

tional status of these receptors in a similar way to that found after post-partum due to changes in the levels of endogenous steroids.

It is also interesting to note that a drop in the density of $GABA_a$ receptors and an increase of its affinity, as a consequence of pregnancy, have been proposed to be the basis of the so called postpartum blues or milk blues (75). The use of these terms derives from an assumed relationship between the onset of lactation (on approximately the third day) and major emotional upheaval and postpartum depression (129). Both the cause and the significance of the described postpartum emotional vulnerability are largely unexplored. A complex interrelationship of emotional and endocrine factors suggests the possibility of a borderline endocrine/neurotransmitter condition, which can be precipitated to some extent by environmental factors.

In this frame, an intriguing possibility concerns the involvement of changes in the levels of neuroactive steroids (6,92). In fact, elevated concentrations of these compounds have been found during pregnancy in the maternal plasma and brain of many mammalian species, including humans, due to the extremely high levels of their precursor hormones, progesterone and deoxycorticosterone, and due to increased activity of their synthesizing enzymes in the placenta and fetal tissues (74).

The biosynthesis of neuroactive steroids has been shown to be influenced by diet or markedly increased by the administration of hypnotic and anticonvulsant drugs such as phenytoin, barbiturates, or diazepam. These compounds have been shown to modulate bidirectionally the function of the GABA-a receptor and affect neuronal excitability (10,33,92). During pregnancy high levels of these compounds may also contribute to the behavioral profile, which is characteristic of pregnant women. On the other hand, the anxiety and depression associated with the post-partum period may represent physiological withdrawal from several-month exposure to the endogenous anxiolytics.

The effects of early exposure to BDZs may interact with the effects of rearing conditions on development of the receptor complex at which OX acts (65,75). Thus, the following section may serve to raise the attention at these subtle interactions when dealing with behavioral teratological investigations.

Changes in behavioral response to challenge with GABA-BDZ agents in adulthood as a function of manipulation of social milieu during infancy or prepuberty. GABA-BDZ regulatory systems, i.e., those CNS systems which are the specific targets for anxiolytics, seem particularly useful for an ontogenetic and psychobiological analysis, for at least two main reasons. Firstly, the different mechanisms by which these systems exert a direct or indirect modulation of several behavioral responses, including those involved in pain reactivity, spontaneous locomotor activity, and exploration, have been quite well characterized (13,16,23,28,47,61,65). Secondly, the modes of reaction of these CNS systems to a wide range of environmental perturbations are at least partially understood, particularly those produced by different types of stressful experiences, including social interactions, etc. (48,69,73,97,128).

Several studies also indicate that GABA-BDZ regulatory systems show a remarkable plasticity during development (63,91,95,103). Administration of BDZ agents during an early developmental stage has been shown to affect a wide range of aspects of both the young and the adult behavioral repertoire in several animal models, as well as the response to the same drugs in adultood (see above for a survey of the results obtained by our and several other groups in the research context of neurobehavioral teratology, specifically in the topic of the prenatal benzodiazepine syndrome). The same systems are markedly reactive to several environmental stimuli, particularly those which can affect the threshold of pain sensitivity (e.g., Environmentally Induced Analgesia). In fact, the portion of the endogenous pain-inhibiting systems in which BDZ pathways are involved, interact with the social environment, and social agonistic interactions may promote analgesia, which is reversed by BDZ antagonists (98). In this frame, there is now substantial evidence for a role of endogenous brain BDZ in the regulation of social responses (16,45,80,84), and several experiments have shown an influence of social environment on BDZ system functioning (44,46). In fact, prolonged isolation has been demonstrated to alter BDZ receptor density in the brain of adult mice (25). Individually housed mice show differential effects of Ro 15-1788 (a BDZ antagonist) and are also less sensitive than group-housed subjects to diazepam (a BDZ agonist)-induced impairment of rotarod performance (98,109). Moreover, 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 (47). Therefore, it seems quite possible that alterations in an organism's social environment may be an important factor in the genesis of anxiety related disorders (23,31,116).

There is also evidence that these CNS systems are activated during critical stages of physical and social development (55,68,103,111,116). The BDZ receptor matures early in ontogeny (4,63) and thus may play an important physiological role in the mediation of affiliative bonds during the course of development (16,45,65,68,119). In this context, a series of experiments by our group has shown long-term influences of early social events on the function of GABA-BDZ receptor systems in the CNS (65,69). This approach is aimed at better understanding some of the features underlying the individual variability exhibited by adult subjects to the behavioral and

physiological effects of various psychoactive agents, including benzodiazepines and drugs of abuse (28,65,69).

Putting together all these considerations, we came to test the hypothesis that the manipulation of the social characteristics of early environment obtained by raising infant animals in condition of sexual segregation (117) would affect in some way the development and subsequent functioning of this important CNS regulatory system (see below). This was addressed by searching for possible changes in the behavioral response expressed by adult animals when challenged with drugs acting on the GABA/BDZ receptor complex (120).

What we found is shown in Fig. 6. Adult female mice raised during infancy within litters of a mixed gender composition showed the expected hyperalgesia profile (measured by the latency to lick a hind-paw in a hot-plate test) in response to challenge with chlordiazepoxide (CDP) (102). On the other hand, females raised in all-female litters were totally unresponsive to drug-induced changes in behavior. Interestingly, the sexually segregated female group exhibited a prominent dose-dependent analgesia in response to Ro 15-3505 (RO), and this was not the case for females reared in sex-balanced condition. Interestingly, no significant or reliable changes were found for males. Even if the mechanisms by which Ro 15-3505, a BDZ receptor partial inverse agonist, increased hot-plate latencies are not clear, its ability to induce analgesia is especially interesting in light of the very recent literature on stress-induced analgesia (73,104). In fact, RO may be viewed as a pharmacological stressor activating these same systems. Supporting this hypothesis, it has been shown that just as increases in stress severity can increase analgesia, RO too can potenziate stress-induced analgesia (see the dose-response profile of the single-sex reared group in Fig. 6).

In the same study, adult male mice reared in gender-balanced conditions showed more exploratory behavior when tested for the approach to a novel object than unisexually reared males. Sexual segregation in infancy was apparently responsible for the increased neophobia exhibited by unisexually reared animals. It also altered the function of the GABA/BDZ receptor complex as revealed by the restriction to mixed-sex reared males of the facilitative effects of both CDP and RO in the test with the approach to a novel object (data not shown).

The different individual response of animals to the hyperalgesic action of CDP or to the analgesic effect of RO possibly reflects the individual variation—which was apparently acquired during development—in sensitivity of central mechanisms mediating the bidirectional pain-modulating action of BDZ agents. It has been recently assumed by several authors that the putative endogenous ligand for BDZ binding sites may exert a pharmacological activity opposite to that of classical BDZ tranquillizers (37). Therefore, a tentative hypothesis is that in RO-responders the concentration of the putative endogenous ligand (in CNS) is higher than in RO-nonresponders. If it is the case or not, the developmental sexual segregation procedure, which is here proposed as a means to have adult animals characterized by a different set up of specific CNS regulatory systems, seems to offer an additional tool for analyzing the functional significance of the relation between different ligand sites at the GABA/BDZ receptor complex.

At the present stage of the work it is impossible to determine which factor was responsible for the differences related to early social environment condition. An individual variation in adult behavioral response to challenge with Beta-carbolines (BDZ inverse agonist and putative anxiogenic agents) has been shown in primates (46). This effect has been interpreted in terms of long-term influence of early life events on the function of the GABA/BDZ receptor system. With respect to our results, one working hypothesis may be that the nature of the interactions among siblings of the same litter [e.g., different levels of social grooming or playful interactions in infancy as a function of sex of littermates (61,117)] can affect the development and subsequent function of the BDZ system (91). On the other hand, both rat and mouse dams discriminate and interact differently with male and female offspring and possibly behave differently with litters of differing sex ratios. Such a sex-biased maternal behavior has been shown to affect several aspects of the offspring's behavioral repertoire (2,83).

With the aim to discriminate, among the different behavioral interactions, those more responsible for the functional canalization, i.e., the differential (and/or individual) response to challenge with BDZ agents (considered as an index of functional changes of the corresponding regulatory systems), we performed an extension of the study described above. In this experiment two important social variables were manipulated during development. Firstly, adult male and female mice which underwent either a mixed or a unisexual rearing from prepubertal day 15 to 25 were used. In addition, the inclusion in the experimental design of a group of mice precociously separated from the mother enabled us to try to separate the causal factors discussed above (61,117).

At adulthood mice were tested 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) (69,120). Animals were also injected with chlordiazepoxide and challenged by a naturalistic threatening predatory stimulus, i.e., cat urine. In fact, the application of a naturalistic approach allows the 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 (11,20,26,48,128).

The results obtained clearly indicated that adult male mice sexually-segregated during pre-puberty had a significant longer latency to leave a bright white area in the Black/White exploration test than did males reared in a balanced-gender condition [18.0 ($\pm$2.3) and 12.2 ($\pm$1.2) min, respectively]. No such a difference appeared in the group of females. In the hot-plate test (see Fig. 7), both male and female mice raised in a balanced-gender condition showed a prominent analgesia (measured as the latency to lick a forepaw in a hot-plate test, which is well known to be considerably shorter than a hindpaw response) in response to a potential threatening stimuli such as cat's urine. As expected on the basis of previous reports (65), prior CDP administration dose-dependently reduced this response (11,23,48). Interestingly, neither the simple response to the odor nor the CDP effects, were found in animals raised during pre-puberty in sexual segregation.

Half of the animals were also weaned precociously at two weeks of age (see above), and this manipulation affected the behavioral profile in adulthood, with animals reared in mixed-sex condition and weaned precociously not showing the cats' odor-induced analgesia, nor any response change to CDP. Also, precocious weaning strongly reduced the long-term segregation-related differences in behavior [see the profile of regular weaning group in the left panel of Fig. 7] providing an indication for a major modulatory role of the mother early in development in the production of segregation-related effects (117).

As for the possible mechanisms underlying the above segre-

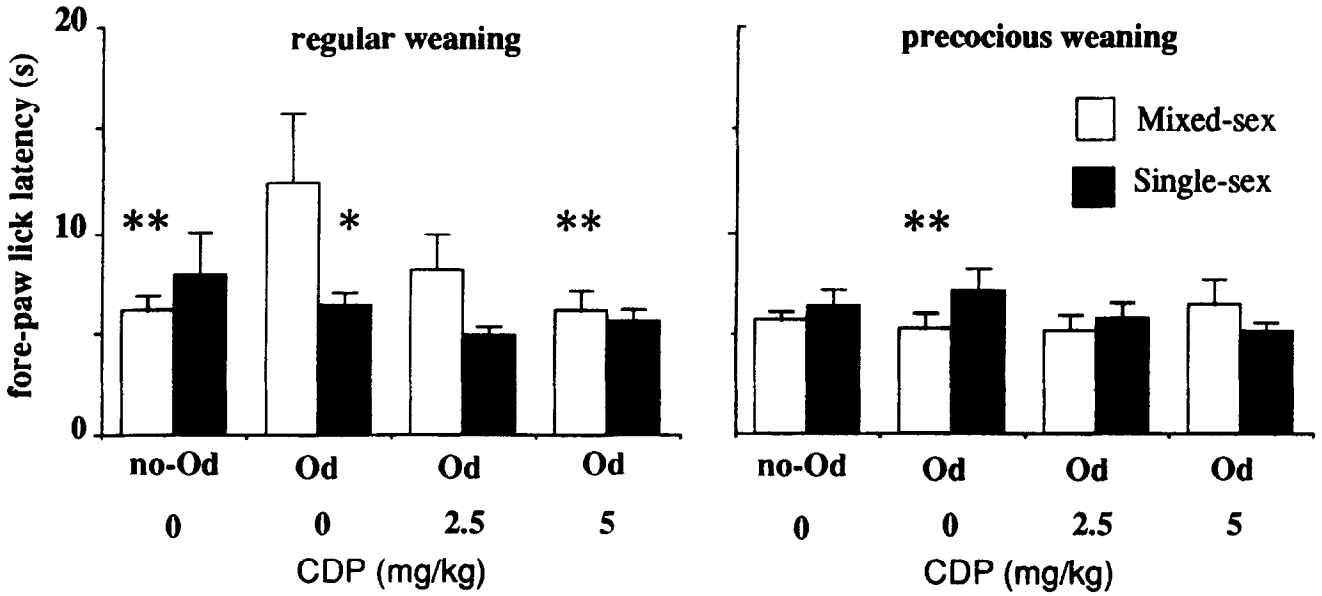

FIG. 7. Mean latency (SEM) to lick a fore-paw in a hot-plate test (set at 55 ± 1 °C) of adult mice, which were either sexually segregated (single-sex litters) or reared in mixed-sex conditions from postnatal day 15 to 25. Half of the animals were weaned on postnatal day 15 (Precocious weaning, PW), or remained with the dam until day 25 (Regular weaning, RW). At adulthood (postnatal day 70 ± 1), animals were challenged with either vehicle or CDP at 2.5 or 5.0 mg/kg dose, 30 min before to be exposed for 2 min in a chamber with cat odor (Od), while the control group was put in a similar chamber without odor (CDP 0, no-Od). (n = 12). *$p < 0.05$ refers to comparison of mixed-sex vs single-sex reared animals, made within the regular weaning group: **$p < 0.01$, refers to comparisons of the regularly weaned and mixed-sex, CDP 0-Od group vs other experimental groups. (Reprinted with permission from 69).

gation 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, namely, ultrasonic vocalizations, physiological changes related to different olfactory inputs (22,70,122), dam care (2), and social experiences with litter mates (21,61,117).

With respect to the latter, we have previously reported that mice experiencing ongoing unisexual rearing were less involved in affiliative and huddling-like behavior compared to pups reared in sex-balanced litters (117) and it is most possible that such a less varied and affiliative social milieu (61, 117) has a long-term influence on systems which modulate both the process of coping with a threatening natural stimuli such as a predatory odor, and the results of it on the response to painful stimulation.

In general, the data discussed above suggest that quantitative and/or qualitative variation in social interaction with the mother as well as with the opposite sex through infancy and adolescence, may well be a contributor to the wide interindividual variation in the capacity of coping with environmental challenges, including drug administration (28,65) or toxicant exposure. Moreover, since in the present study the interval between the end of exposure to different social conditions and testing was relatively long (approximately 50 days), the results suggest that manipulation of social milieu during development has a kind of organizational influence on these systems.

In the present series of experiments, the differences generated by sexual segregation during development 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 reactivity to environmental challenges (11,13,23,65). In agreement with this view, Primus and Kellogg (91) reported that castration of male rats as juveniles (postnatal day 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 demonstered (41,56,76,91). Changes of testosterone metabolism in the CNS have been reported in rats reared in sexual segregation (70), as well as changes of reproductive performance in both adult male and female rats (61,117). Taken together these reports indirectly suggest that pre-pubertal sexual segregation may interfere with gonadal hormones or their metabolites on pubertal development of the receptor complex (22,70,122).

FINAL COMMENTS AND CONCLUSIONS

The work summarized and discussed in the present report, together with a growing literature on the same and related topics (see reviews quoted in the introductory section), indicates that the developmental psychobiological approach has made available to behavioral teratologists the fine tuning of both strategy and model tools that serve to define short-, medium-, and long-term effects of early treatments in terms of the behavioral processes which are affected (112). An expanded view of teratogenic effects on development points to pay research attention to the animal-context synergistic process, rather than simply to those aspects of the organism that the teratogen directly affects (86). This therefore includes a large class of variables that have traditionally been ignored in explanations of the effects of teratogens on behavior.

As outlined by Bignami and colleagues (8,9), this is made possible by moving up from individual response changes to

considering clusters of response changes. The identification of correspondences between clusters of response changes—defined in terms of affected behavioral processes—and clusters of CNS changes appears to be much more helpful for the purpose of understanding treatment effects and mechanisms of action. The studies so far reviewed were based on the effects of a single treatment given during a particular developmental stage in an outbred strain of mice of one of many altricial rodent species. It appears, however, that by appropriate modifications, the adoption of the strategy so far illustrated can be extended to the analysis of many similar problems encountered in a much vaster area.

In summary, there are a number of critical issues associated with studies of environmental perturbations or psychoactive drugs in developing mammals. The issue of fostering remains controversial. Certainly, in the absence of adopting such a procedure, one can not assume that the observed effects in the offspring are related to the consequences of the prenatal exposure *per se* (35,42,62,111,114,115,127). Further, whereas traditionally tests in the behavioral teratological approach have focussed largely on adult testing, increasingly more interest is being directed in testing early in life than in adulthood (1,50,55,68,99,111,112,119). Last but not least, the issue of rearing conditions is intriguing and needs to be watched carefully in the frame of an analysis of the modes of reaction of the target system to perturbations a mouse receives during development.

ACKNOWLEDGEMENTS

I am indebted to Enrico Alleva, Giorgio Bignami, Flavia Chiarotti, Linda P. Spear, and Aubrey Manning for their helpful discussions and feedback over the years. The original research reported in this article was made possible by the effort given by a number of young colleagues such as Simona Petruzzi, M. Livia Terranova, Giacomo Dell'Omo, and Giampietro Loggi. We received support by the Subproject on Behavioural Pathophysiology (Project on Non-infectious Pathology) of the Istituto Superiore di Sanità, Roma, Italy and by the Italian National Health System Project "Risk Factors in Maternal and Child Health."

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