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Prolonged perinatal AZT administration and early maternal separation: effects on social and emotional behaviour of periadolescent mice

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Abstract

Zidovudine (AZT) is an effective treatment in preventing perinatal transmission of HIV-1; however, a continuous re-evaluation of the risk–benefit ratio of human exposure to this drug is suggested by both clinical and animal studies. The objective of this study was to assess the medium and long-term effects of pre-postnatal AZT treatment on mouse social and emotional behaviour and the possible interactions between AZT exposure and disruptions in the mother–infant relationship. Pregnant CD-1 mice were administered per os with AZT (160 mg/kg) from pregnancy day 10, throughout delivery, to lactation day 10. In half of the litters, the offspring was separated from the mother for 3 h from postnatal days 2 (PND2) to PND14. On PND35, a 30-min social interaction test was performed and corticosterone levels were measured at the end of the session. On PND80, long-term effects of AZT on emotionality were assess by means of an elevated plus-maze. Results indicate that, on PND35, previous AZT exposure affected social behaviour of the experimental subjects, reducing aggressive interactions in males, while decreasing *investigative* behaviours in females. At adulthood, AZT inhibited exploratory behaviour in the plus-maze while increasing the frequency of risk-assessment postures in male mice. As for maternal deprivation, this early manipulation exerted a pro-aggressive effect in adolescent male mice, deprived subjects being overall characterised by higher activity levels and a deficit in habituation, an effect also observed in the plus-maze. A significant interaction between AZT and maternal deprivation was found for *affiliative* behaviours. As for corticosterone levels, no AZT effect was found, while maternal deprivation tended to reduce elevations of this hormone in response to stressful stimuli. Overall results from this study indicate that both AZT exposure and maternal deprivation induced gender-dependent changes in social and emotional behaviour both during adolescence and at adulthood.

Keywords: AZT; Social behaviour; Maternal deprivation; Plus-maze; Corticosterone; Behavioural teratology; Mouse

1. Introduction

In the last 10 years, zidovudine (AZT), a dideoxynucleoside inhibitor of retroviral reverse transcriptase, has become the main prophylactic therapy against vertical HIV-1 transmission (Centers for Disease Control and Prevention, 1998; Connor et al., 1994). Notwithstanding the unquestionable benefits deriving from AZT in preventing perinatal transmission of HIV (Gibb and Beatriz, 1999; Mofenson and McIntyre, 2000), an international agreement exists on the need to continuously re-evaluate the risk–benefit ratio of human exposure to antiretroviral therapy during development (Slikker et al., 2000).

Several investigations, using primate and rodent models, have suggested a number of potential adverse effects of administration of nucleoside reverse transcriptase inhibitors during development (Applewhite-Black et al., 1998; Busidan and Dow-Edwards, 1999; Calamandrei et al., 1999; Ha et al., 1998; Gerschenson et al., 2000; Rondinini et al., 1999; Slikker et al., 2000; Venerosi et al., 2000). This has become an important issue since the report by Blanche et al. (1999) of anomalies and evident mitochondrial disorders in uninfected children perinatally exposed to either AZT or AZT in combination with lamivudine (3TC). The literature on human data, however, appears controversial since a US retrospective study including 20,000 perinatal HIV–mother–infant pairs apparently did not reveal any progressive neurological disease or mitochondrial pathology in children exposed to AZT who died before 5 years of age (Perinatal Safety Review Working Group, 2000).

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Although for nonhuman mammals there are no reports of major teratological effects or evidence of dramatic CNS changes induced by AZT, yet some studies indicate long-term effects on behaviour associated with antiretroviral therapies given during pregnancy and/or lactation in rodents. In particular, a series of mouse studies assessing short-, medium- and long-term effects of perinatal AZT administration on neurobehavioural development reported selective changes in the intraspecific social/aggressive behaviour at adulthood in CD-1 mice (Calamandrei et al., 1999; Rondinini et al., 1999). Moreover, a clear change in the agonistic repertoire of adult males has been reported following repeated encounters (after a prolonged AZT treatment-gestational period throughout delivery and during lactation) (Venerosi et al., 2000; more details in discussion).

Variations in rearing conditions may represent a powerful procedure to highlight potential adverse effects of perinatal exposure to drugs or toxicants, as indicated by the exacerbation of the effects of prenatal alcohol exposure due to environmental factors such as a low socioeconomic status (Tronick and Beeghly, 1999). It is thus possible to hypothesise that socioanthropological and socioeconomical factors may represent important determinants of interindividual behavioural outcomes following perinatal AZT exposure (Harkness and Super, 1994; Tronick and Beeghly, 1999). Indeed, a positive correlation can be found between human health and social status, mainly depending upon access to nutritional food, especially early in life (Ellis, 1994). In particular, stillbirth, infant mortality and early childhood mortality have all been found to be inversely correlated with social status. In addition, life style and emotional stress factors appear to be strongly implicated. From a theoretical point of view, the individual must be viewed as embedded in a 'developmental niche,' a highly dynamic system where physical, cultural and psychological factors interact to produce unique contexts of development for children (Harkness and Super, 1994).

Animal models have been developed, mainly using rodents, to assess the consequences of disruptions in maternal care on behavioural and physiological responses of the offspring (Cirulli, 2001; Kaiser and Sachser, 2001; Levine, 1957; Meaney, 2001). Results from these studies indicate overall that while brief, repeated manipulations of the mother–infant dyad result in a more efficient coping with the environment, longer separations appear detrimental, leading to increased emotional responding of the offspring (Clausing et al., 2000; Plotsky and Meaney, 1993). In particular, rats exposed to repeated maternal separation of 180–360 min/day for the first 2 weeks of life show significantly increased plasma ACTH and corticosterone (CORT) response to stressful stimuli, compared to unseparated controls. The longer period of separation also results in decreased glucocorticoid receptor binding in both the hippocampus and the hypothalamus (Plotsky and Meaney, 1993). In the present study, in addition to AZT administration, mother–infant dyads were exposed to 3 h of daily separation from postnatal days 2 (PND2) to PND14 in order to produce a mouse model of reduced maternal care, e.g., due to stressful life events or paucity of resources, our hypothesis being that a disruption in the mother–infant relationship might magnify the effects of AZT exposure on the offspring's behavioural development.

More in detail, pregnant CD-1 mice were administered per os with AZT (160 mg/kg), from pregnancy day 10, throughout delivery, to lactation day 10. In addition, the offspring were separated daily for 3 h from PND2 to PND14. Aim of this study was to investigate possible interactional effects between AZT exposure and preweaning maternal separation, selecting as behavioural endpoint the behaviour shown during a social interaction test conducted at a very critical developmental age, such as middle adolescence. Rodents around this age are characterised by an enhanced tendency to socialise, showing a high degree of investigative behaviour as well as affiliative and playful social interactions (Spear, 2000; Terranova et al., 1993, 1999). Furthermore, at adolescence, gender-specific social behavioural patterns begin to emerge, thus changes in the typical sexual-dimorphic behavioural repertoire could reveal subtle alterations in neuroendocrine homeostasis induced by drug and/or the early separation procedure. In order to correlate behavioural and neuroendocrine responses to stress, circulating corticosterone levels were assessed in all subjects following the social interaction test.

Furthermore, in order to support previous evidence of changes in emotional behaviour induced by AZT at adulthood, CD-1 male mice were tested in an elevated plus-maze test (Pellow et al., 1985; Lister, 1987; File, 1992; Rodgers and Johnson, 1995). This test has been reported to be bidirectionally sensitive to manipulations designed to influence anxiety. Although at times controversial, the plus maze test can be informative if, in addition to more classical measures (such as open arm entries), other aspects of animal's behaviour are taken into account (Rodgers and Johnson, 1995).

2. Materials and methods

2.1. Animals and breeding procedures

Male and female mice of an outbred Swiss-derived strain (CD-1) weighing 25–27 g were purchased from a commercial breeder (Charles River, Calco, Italy). Upon arrival at the laboratory, the animals were housed in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%) with lights on from 2000 to 0800 h. Adult virgin males and females were housed in same-sex pairs in 33 × 13 × 14 cm Plexiglas boxes with a metal top and sawdust as bedding. Pellet food (enriched standard diet, Mucedola, Settimo Milanese, Italy) and tap water were continuously available. After 1 week of acclimatisation, breeding pairs were formed. Females were inspected daily for the presence of the vaginal plug (pregnancy day 0). On pregnancy day 10, males were

removed and 36 females were randomly assigned to AZT or control (SAL) groups ($n=18$ in each prenatal treatment group). At birth, all litters were culled to four males and four females. Eighteen litters belonging to each prenatal treatment group were randomly assigned to two different experimental conditions ($n=9$ for each condition): nondeprived (NDEP) and deprived (DEP). Pups were weaned on PND21 and the four males and the four females of each litter were housed, in same-sex pairs, in cages of the same type as the home cage. According to a split-litter design, each pup from each litter was assigned to one of the different groups in the social interaction test (see Section 2.4). The remaining male subject in each litter was used in the plus-maze test. Mean body weight data were collected at PND2, PND21, PND35 and PND80. No effects of treatment and condition or an interaction between these factors were found (data not shown).

All animal handling and experimental procedures were performed in accordance with the EC guidelines (EC Council Directive 86/609 1987) and with the Italian legislation (Decreto L.vo 116/92) on animal experimentation.

2.2. Treatments

AZT (3′-azido-3′-deoxythymidine, Sigma–Aldrich, Milano, Italy) was dissolved in 0.9% NaCl solution. Pregnant CD-1 mice were dosed intragastrically twice daily using a 3-mm in diameter, 3-cm long curved feeding needles attached to a 1-cm^3 syringe. AZT (160 mg/kg) or vehicle solution (0.9% NaCl) were administered between 0800 and 0900 h and 1900 and 2000 h both during pregnancy (from gestation day 10), throughout delivery, and until lactation day 10. The AZT dose was chosen on the basis of previous studies performed in our laboratory using the same mouse strain (Calamandrei et al., 1999; Venerosi et al., 2000), results in plasma AZT levels comparable to those measured in treated humans (Blum et al., 1988).

2.3. Maternal separation (deprivation procedure)

NDEP: all pups in the litter were left undisturbed with their mothers until the time of weaning, with the exception of PND 1–10, when the mothers had to be removed from the nest for about 30–60 s in order to be administered with the drug. DEP: all pups in the litter were daily separated from their dam from PND2 until PND14. DEP pups were removed from the home cage and housed in groups for 3 h (between 0930 and 1230 h) in an incubator maintained at a constant temperature (30 ± 1 °C). In the case of DEP animals, drug administration on PND2–10 occurred after placing pups in the incubator.

2.4. Procedure

On PND34, all subjects were weighed and housed individually with some of their own sawdust in a cage identical to the home cage for a 24-h period; this procedure increases the amount of social behaviour in the social interactions test. On PND35, after the 24 h of isolation, one male and one female from each litter were assigned to one of the following groups according to a split-litter design: (1) basal (BAS), immediately sampled for the detection of basal blood levels of corticosterone (CORT); (2) novelty (NOV), 30-min isolation period in a cage identical to the home cage with new sawdust as bedding. Two animals in each litter, one male and one female, were not used in the social interaction test. This condition served as control for the manipulation procedure; (3) social interaction (SI), 30-min period of social interaction with a same sex, same age and strain unfamiliar partner. The unfamiliar pair was placed in a cage identical to the home cage with new sawdust as bedding. At the end of the session, the experimental subjects were all sacrificed, blood collected and later assayed for CORT. Age of testing was chosen taking into account previous literature reports suggesting that a high degree of social interactions characterise rodents around PND35 (Spear, 2000; Terranova et al., 1993).

2.5. Behavioural observations

Behavioural testing of unfamiliar animals took place in an experimental room maintained at the same temperature and humidity conditions as the housing room, from 1000 to 1400 h, and the time of testing was counterbalanced between experimental groups. The behaviour of each pair was video-recorded by means of a video camera connected to a professional Sony videocassette recorder V0-5800PS. A software system for collection and analysis of observational data was used for scoring duration and frequency of each response (Observer 2.0, Noldus, 1991). A "focal animal-all occurrences" sampling method was used (see Altman, 1974).

The social and nonsocial behavioural categories listed below, and their classification, are mainly based upon the ethological profiles of mouse behaviour described by Grant and Mackintosh (1963) and Terranova et al. (1993, 1998).

Social behaviours scored were: *agonistic behaviour*: *attack*, *aggressive grooming*, *tail rattling*, *offensive upright posture*, *defensive postures*—this item is comprehensive of two defensive elements: (i) the classical *defensive upright posture*, the animal's standing on its hindlimbs pushing the aggressive opponent with its forepaws; (ii) a *submissive posture*, the animal lies on its back, with its head directed backwards flat against the cage floor. The classical submissive postures, namely *submissive upright posture* and *crouched*, were not observed. *Affiliative behaviours*: *social rest*, *allogrooming*; *soliciting behaviours*: *push under*, *crawl*; *investigative behaviours I*: *follow*, *squire*, *mutual circle*; *investigative behaviours II*: *anogenital sniffing*, *body sniffing*.

Nonsocial behaviours were: *exploring*, *jumping*, *digging*, *self-grooming*.

2.6. Blood collection

Trunk blood was collected from all animals in centrifuge vials filled with EDTA (0.16 M) and centrifuged at 2000 rpm for 20 min to obtain cell-free plasma, and then frozen at −80 °C and later assayed for CORT using commercial radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA; sensitivity: 0.125 μg/dl).

2.7. Elevated plus-maze

On PND80, male mice ($n=8$ in SAL-NDEP group, $n=9$ in SAL-DEP group, $n=9$ in AZT-NDEP group, $n=8$ in AZT-DEP group) underwent an elevated plus-maze test in order to assess long-term effects of both deprivation and AZT treatment on animal's anxiety levels (Pellow et al., 1985; Rodgers and Johnson, 1995). Tests were conducted under red lights between 1030 and 1230 h. The apparatus was made of Plexiglas (black floor, clear walls) and elevated to a height of 60 cm above the floor level (for a detailed apparatus description, see Rodgers and Johnson, 1995). Mice were individually placed on the central platform facing an open arm and allowed to freely explore the maze for 6 min. Behavioural items recorded were: frequencies of total, open, central and closed arm entries (arm entry = all four paws into an arm); duration of the time spent in the total, open, central and closed arm of the maze, percentage of entries in the open arms, *rearing*, *immobility*, *self-grooming*, *head-dipping*, *stretched attend posture* (SAP). The behavioural items considered were mainly based upon the ethological profiles of mouse behaviour described by Pellow et al. (1985) and Rodgers and Johnson (1995).

2.8. Statistical analysis

Statistical analyses were performed using the BMDP statistical software package (version BMDP/dynamic 7.0, Berkeley, CA). Social interactions data were analysed by parametric analysis of variance for repeated measures. The model of such analysis included: treatment (AZT or SAL exposure) and deprivation (NDEP or DEP) as between litter factors, litter as random factor (nested under treatment and as block with respect to repeated measures) and repeated measures (three time block of 10 min) as fixed, within-subject factor. Preliminary observations indicated a main effect of sex on social interactions. Thus, male and female behavioural data were analysed separately and the Bonferroni correction applied. A value of $P \leq .025$ was accepted as statistically significant. Corticosterone data were analysed by means of a 2 Treatment (AZT or SAL exposure) × 2

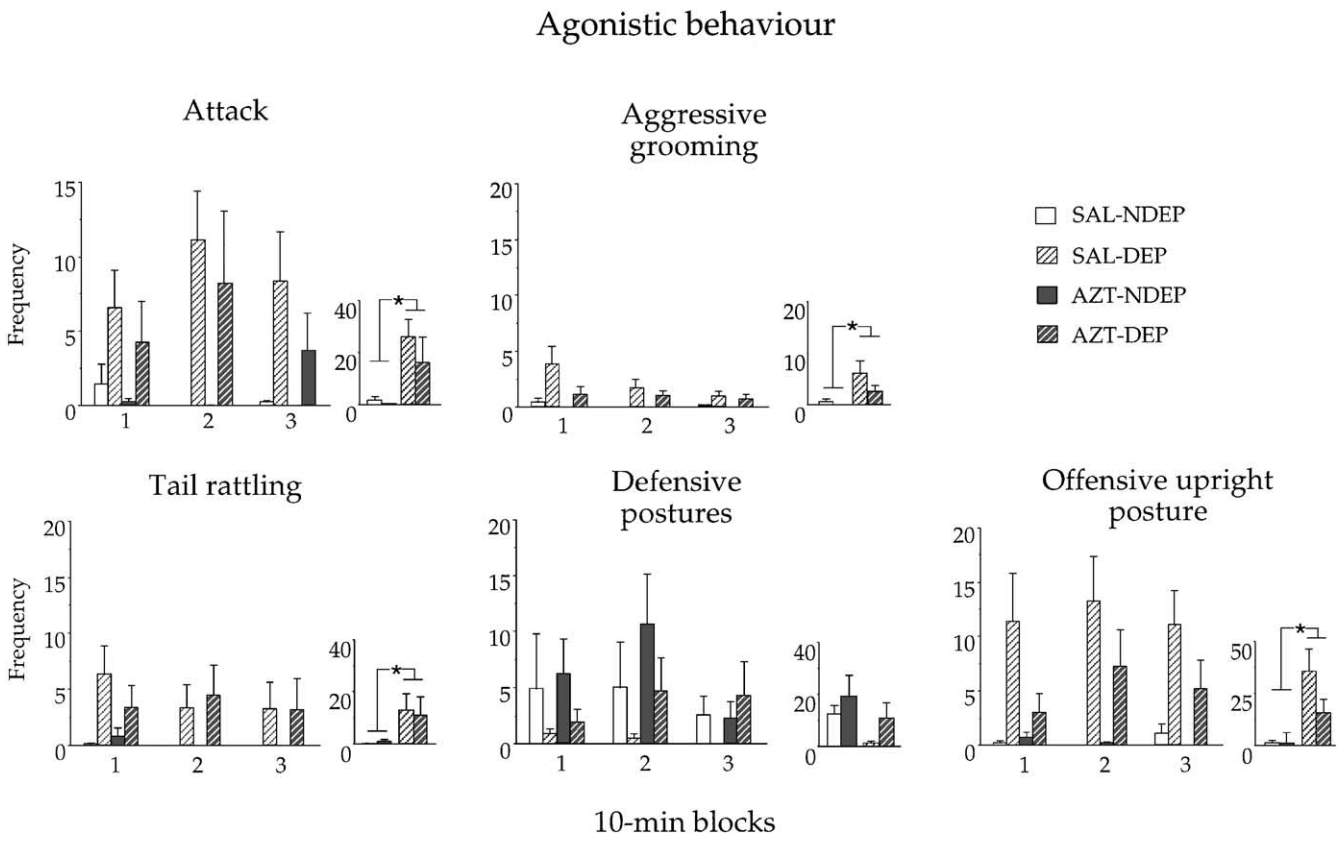

Fig. 1. Frequency of agonistic behavioural items displayed by deprived (DEP) and nondeprived (NDEP) control (SAL) and AZT-exposed (AZT) male offspring during a 30-min social interactions test on PND35. * indicates a significant difference between DEP and NDEP animals. Data are means ± S.E.M. $n=9$ in each treatment and condition group. See Section 3 for the description of main effects of treatment and deprivation.

Deprivation (NDEP or DEP) × 3 Conditions (BAS, NOV, SI) × 2 Sex (male or female) ANOVA.

Elevated plus-maze data were analysed by means of the above-described ANOVA model, but without repeated measures.

Post-hoc comparison was performed by Tukey's HSD test also in the absence of significant interactions (see Wilcox, 1987, p. 173).

3. Results

3.1. Social interaction test (PND35)

Data from male and female subjects were analysed separately because of a main sex difference in behaviour. In particular, most male mice performed agonistic behaviour and engaged in aggressive interactions (approximately 70% of the total time spent in social interaction was devoted to aggressive-type behaviours). Conversely, agonistic behaviour was never recorded during female interactions.

3.1.1. Males

3.1.1.1. Social behavior. Social interactions of adolescent CD-1 male mice were clearly affected by maternal separation, while only selective effects of perinatal AZT treatment were found.

The main effect of deprivation was to enhance the aggressive profile of social repertoire typical of male mice, which is reported to increase with age, reaching a detectable level around PND30 (Terranova et al., 1998). This facilitating effect of maternal separation was evident for frequency and duration of several of the agonistic items recorded: *attack* [$F(1,32) = 10.52$, $P < .01$; $F(1,32) = 8$, $P < .01$, respectively, for frequency and duration], *offensive upright posture*

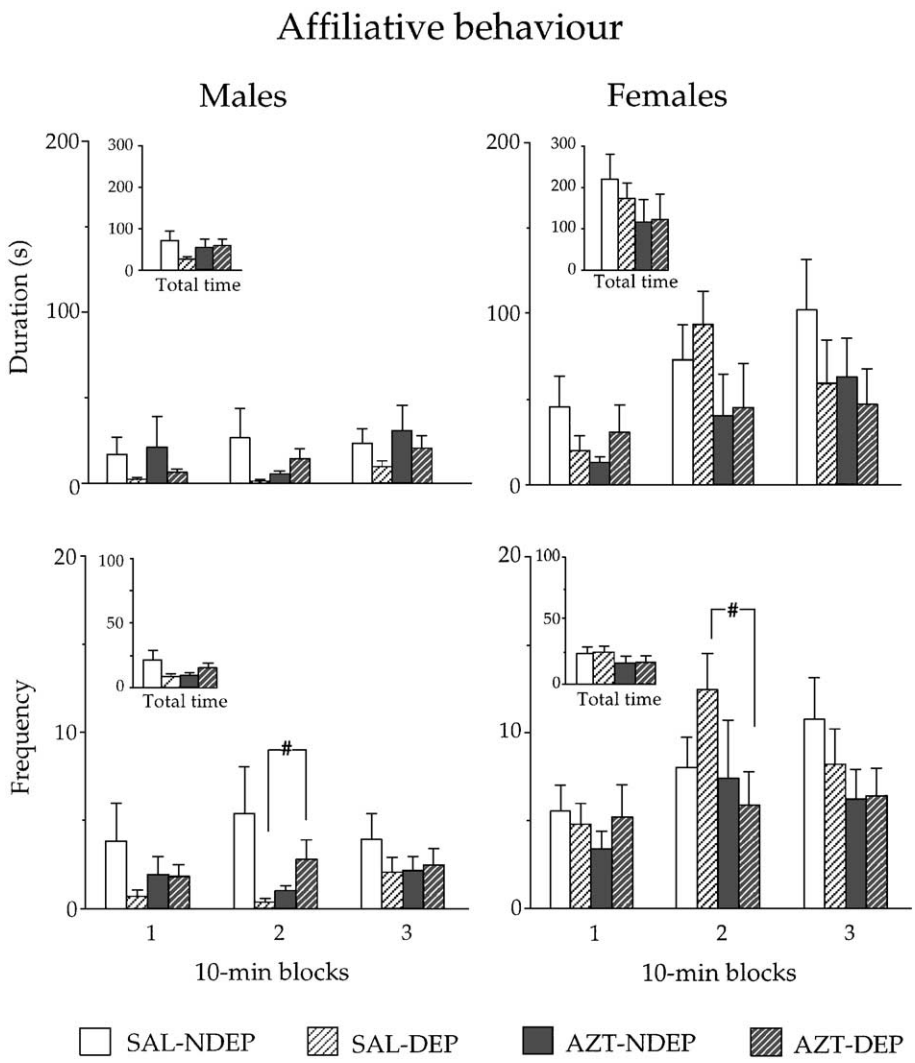

Fig. 2. Duration and frequency of *affiliative behaviours* performed by DEP and NDEP control (SAL) and AZT offspring during a 30-min social interaction encounter on PND35. * indicates significant differences between SAL-DEP and AZT-DEP (Treatment × Deprivation × Time Block interaction). Data are means ± S.E.M. $n = 9$ in each treatment and condition group.

[$F(1,32) = 14.7$, $P < .01$; $F(1,32) = 10.35$, $P < .03$, respectively, for frequency and duration], *tail rattling* [$F(1,32) = 5.53$, $P = .02$; $F(1,32) = 5.73$, $P = .02$, respectively, for frequency and duration] and *aggressive grooming* [$F(1,32) = 10.26$, $P < .01$; $F(1,32) = 10.7$, $P < .01$, respectively, for frequency and duration], as illustrated in Fig. 1.

Conversely, perinatal treatment with AZT did not affect significantly the agonistic behaviour of PND35 mice. How-

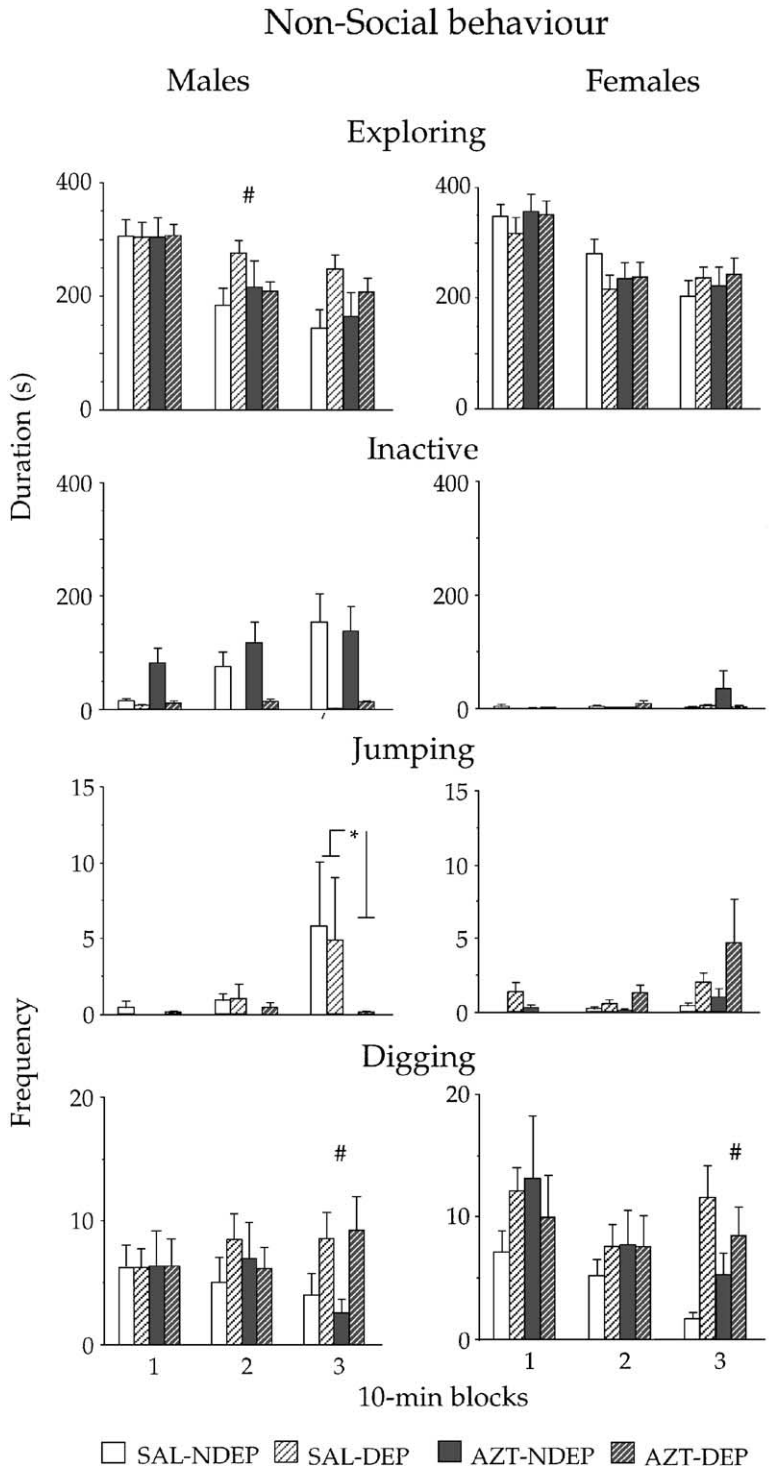

Fig. 3. Nonsocial behavioural items recorded for DEP and NDEP control (SAL) and AZT offspring during a 30-min social interaction encounter on PND35. * indicates a significant difference between SAL and AZT. # indicates a significant difference between DEP and NDEP subjects (Deprivation × Time Block interaction). Data are means ± S.E.M. $n = 9$ in each treatment and condition group.

ever, a significant Treatment × Time Block [$F(2,64)=3.99$, $P=.02$] was evident for the duration of *aggressive grooming*, corresponding to a lower time spent by AZT mice performing this behaviour during the first 10 min block compared to the SAL group ($P<.01$ by post-hoc, data not shown).

ANOVA revealed a Treatment × Deprivation × Time Block effect [$F(2,64)=3.97$, $P=.02$] on *affiliative behaviour*. Post-hoc comparisons showed an opposite effect of deprivation in the two treatment groups during the second time block. Specifically, deprivation decreased significantly the frequency of affiliative interactions in the SAL group ($P<.01$), while increased their frequency in the AZT-treated mice ($P<.05$) (Fig. 2).

3.1.1.2. Nonsocial behaviour. Early maternal separation resulted in an increase in nonsocial activities, as demonstrated by the lower frequency and duration of *inactive* [main effect of deprivation: $F(1,32)=5.75$, $P=.02$; $F(1,32)=7.82$, $P<.01$, respectively, for frequency and duration]. A significant interaction between deprivation and time block was evident for *digging* frequency [$F(2,64)=4.50$, $P=.01$], deprived male mice performing more of this behaviour at the end of the social interaction test (see Fig. 3). As for *exploration*, though in the presence of a nonsignificative Deprivation × Time Block interaction [$F(2,64)=2.99$, $P=.06$], post-hoc comparisons showed, in the second time block, that significant more time was spent rearing and moving around the cage by DEP compared to NDEP male mice.

As for AZT treatment, ANOVA analysis of *jumping* frequency indicated a greater number of attempts to evade from the novel environment performed by SAL-treated mice in the last 10 min of observation [Treatment × Time Block effect: $F(2,64)=3.27$, $P=.04$ ($P<.01$ by post-hoc)] (see Fig. 3), while no main effect was found for any of the nonsocial behaviour recorded.

3.1.2. Females

3.1.2.1. Social behaviour. As observed for males, frequency and duration of *investigative behaviour* were not affected by previous maternal separation, as neither a main effect of condition nor a significant interaction with time block were found (see Fig. 2). This was also observed for the *soliciting* and *affiliative behaviours*. No main effect of treatment was found for frequency or duration of each of the behavioural categories considered. ANOVA on the duration of *investigative behaviours I* and *II* showed an interaction between Treatment × Time Block [$F(2,64)=2.96$, $P=.06$; $F(2,64)=3.28$, $P=.04$, respectively, for *investigative behaviours I* and *II*]. Post-hoc comparisons evidenced a significant difference between SAL and AZT females. In particular, AZT females spent less time following, with and without contact (*investigative behaviour I*), the standard opponent during the initial part of the social interactions compared to SAL females (first 10 min block, $P<.01$ by post-hoc). This pattern was reverted in the second 10 min block ($P<.01$ by post-hoc). The same temporal profile was apparent for the duration of *investigative behaviour II*, indicating that AZT females spent less time than controls in sniffing the standard opponent in the first time block ($P<.01$ by post-hoc) and increased this activity subsequently ($P<.01$ by post-hoc for both second and third 10 min block).

A three-way interaction was found for frequency of *affiliative behaviour* [$F(2,64)=3.93$, $P=.02$] with maternal deprivation increasing the number of affiliative interactions in SAL-exposed subjects, while decreasing them in AZT-treated subjects in the second time block ($P<.01$).

3.1.2.2. Nonsocial behaviour. As for the nonsocial behaviour repertoire, a main effect of deprivation was found for the frequency of *exploration* [$F(1,32)=6.21$, $P=.01$], DEP females showing a greater frequency of this behaviour compared to NDEP females independently from prenatal treatment. Maternal separation also increased *digging* frequency in the third time block [Deprivation × Time Block: $F(2,64)=3.18$, $P=.04$]. Neither a main effect of deprivation nor a significant interaction with treatment and time block was found for the other nonsocial behaviours (*inactive*, *self-grooming*, *jumping*). Overall, the ef-

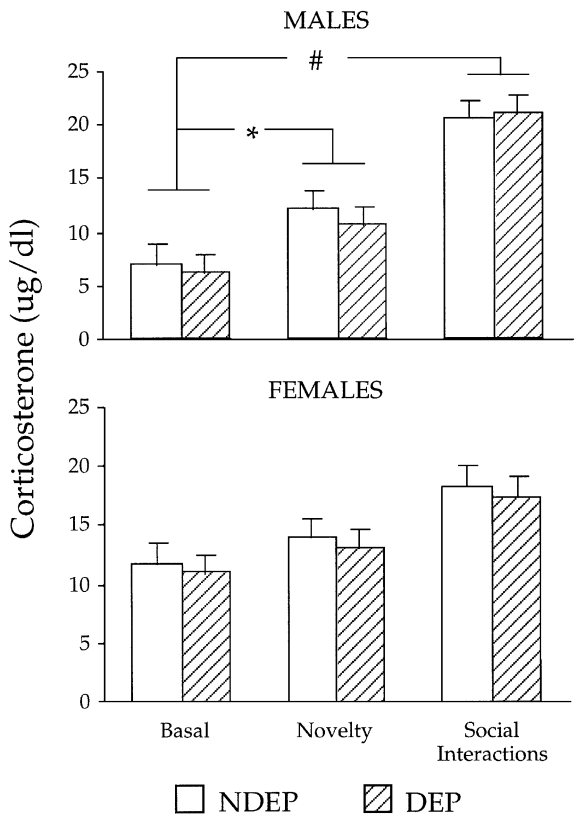

Fig. 4. Plasma corticosterone concentrations (μg/dl) detected in blood samples collected on PND35 from males and females undergoing the different experimental procedures. * and # indicate a significant difference with basal CORT levels. $n=18$ in each group. Data are pooled over treatment.

fects of maternal deprivation were less pronounced in AZT-exposed females.

3.2. Corticosterone

No main effect of AZT exposure was found on corticosterone levels, although deprived subjects showed overall lower CORT values than nondeprived [$F(1,30) = 3.30$, $P = .07$]. In addition, a main effect of challenge (social interaction test) [$F(2,60) = 33.16$, $P < .01$] and an interaction of challenge with sex [$F(2,60) = 4.95$, $P = .01$] were found, males showing lower basal levels than females ($P < .05$) and a significant increase in CORT secretion following NOV exposure ($P < .05$) or the social interaction test ($P < .01$) (see Fig. 4).

3.3. Elevated plus-maze test (PND80)

In the elevated plus-maze, both maternal separation and perinatal treatment induced changes in the behaviour of CD-1 adult male mice but did not interact with each other. Deprivation affected the frequency of *close arm entries* [$F(1,30) = 7.85$, $P < .01$] and *central zone entries* [$F(1,30) = 12.95$, $P < .01$], but not the number of *open arm entries* [$F(1,30) = 3.10$, $P = .08$] or percentage of entries in the *open arm* [$F(1,30) = 3.37$, $P = .08$] (see Fig. 5).

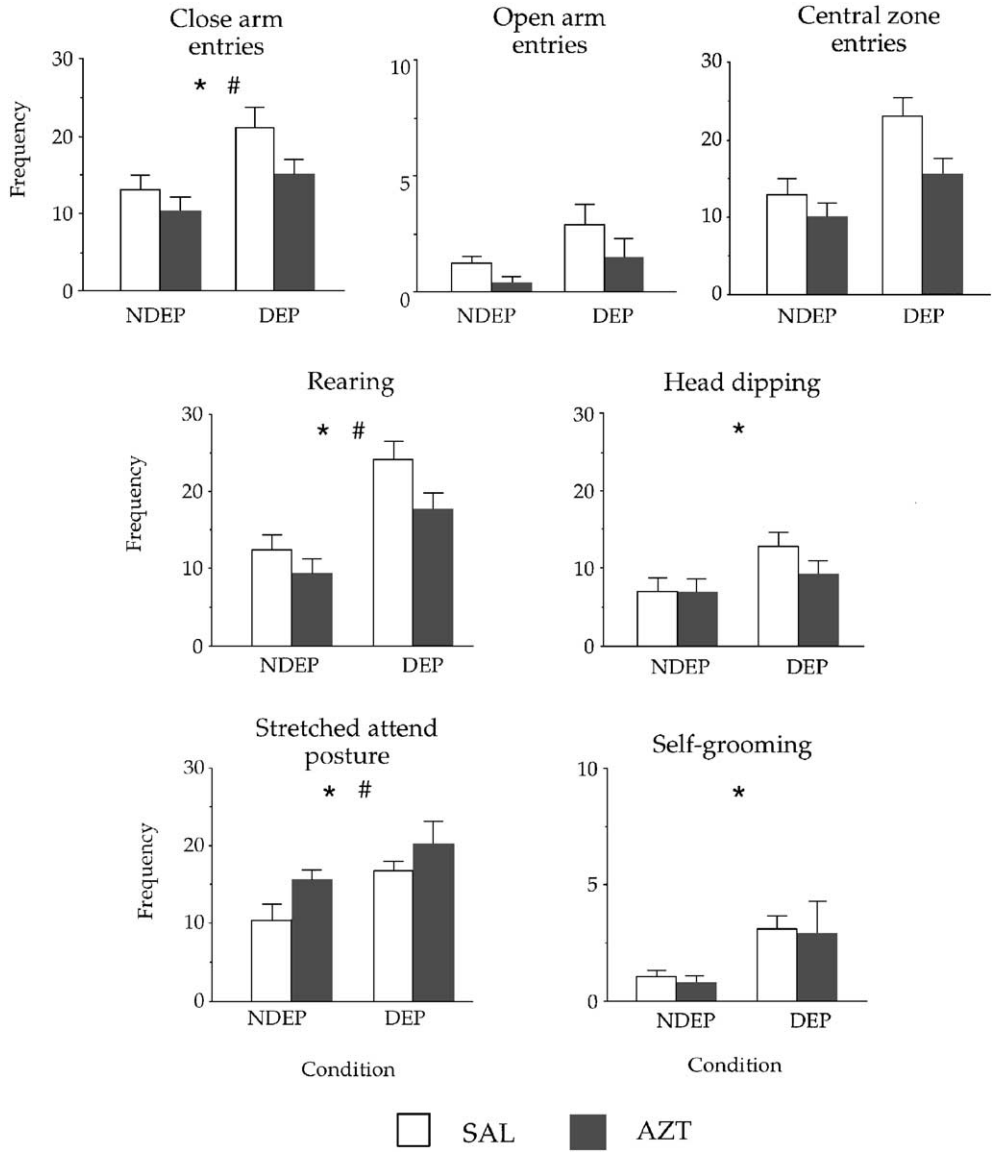

Fig. 5. Mean frequency of behaviours performed on PND80 during a 6-min plus-maze test by DEP and NDEP control (SAL) and AZT male offspring. $n = 8$ in SAL-NDEP group, $n = 9$ in SAL-DEP group, $n = 9$ in AZT-NDEP group, $n = 8$ in AZT-DEP group. * indicates a main effect of maternal deprivation; # indicates a main effect of AZT.

Perinatal treatment also affected behaviour in the plus-maze, with AZT decreasing frequency of entries both in the *close arm* [$F(1,30)=3.89$, $P=.05$] and in the *central zone* of the maze [$F(1,30)=5.75$, $P=.02$]. This effect did not extend to *open arm entries* [$F(1,30)=2.06$, $P=.16$] or percentage of entries in the *open arms* [$F(1,30)=1.77$, $P=.19$] as illustrated in Fig. 5. Neither deprivation nor AZT treatment affected the time spent in each zone of the maze considered. Overall, no differences in the time spent in the open arms suggest no effect of AZT/DEP on behaviours which are considered as standard anxiety indices. Nonetheless, the above results indicate opposite effects of maternal separation and AZT treatment on locomotor activity which is reflected in total and close arm entries: the former increasing, the latter decreasing it. Similarly, an increase in *rearing* and *head dipping* in DEP animals [main effect of deprivation: $F(1,30)=20.91$, $P<.01$; $F(1,30)=5.19$, $P=.03$, respectively, for *rearing* and *head dipping*] was found. These two measures represent, respectively, an index of vertical activity and of exploratory behaviour. A significant inhibitory effect of AZT was evident only for *rearing* [frequency: $F(1,30)=4.73$, $P=.03$].

Interestingly, as illustrated in Fig. 5, perinatal AZT treatment increased the frequency of the *stretched attend posture* [$F(1,30)=10.39$, $P<.01$], an effect also present in deprived animal [$F(1,30)=23.32$, $P<.01$], reflecting increased risk assessment. Finally, a main effect of deprivation [$F(1,30)=6.53$, $P=.01$] was found for *self-grooming* (see Fig. 5).

4. Discussion

Results from this study indicate long term effects of perinatal AZT treatment on mouse social behaviour. Differently from what was expected, however, no interaction between AZT and maternal separation was found. Nonetheless, the more novel finding appears to be the effect of repeated maternal separations on the social behaviour resulting in a pro-aggressive effect on social repertoire typical of male mice and, overall, in reduced emotionality.

One of the main aims of this study was to investigate early changes in social behaviour, using periadolescence as a critical developmental point for the emergence of affiliative/agonistic behaviour in rodents. In the present study, AZT exposure induced behavioural changes in adolescent subjects that were gender-dependent. In males, AZT induced some selective alterations of agonistic behaviour, as indicated by a significant reduction in the amount of *aggressive grooming* performed by AZT-treated mice. Compared to previous studies using adult subjects AZT effects on periadolescent rodents appear less pronounced. This might be explained by taking into account that adolescent male mice are characterised by a relatively immature form of aggressive behaviour (Terranova et al., 1998), thus a direct comparisons of aggressive behaviour between young and adolescent subjects cannot be drawn. Nonetheless, results obtained in this study confirm previous evidence of significant effects of perinatal AZT treatment on mouse social behaviour (Calamandrei et al., 1999; Rondinini et al., 1999; Venerosi et al., 2000).

As for females, which did not show overt aggression, AZT exposure affected *investigative* and *affiliative* behaviours, especially in the first portion of the social interaction test. These data are corroborated by previous observations reporting a dampening of sexual differences in investigative behaviour in juvenile mice exposed in utero to AZT (Calamandrei et al., 1999). Changes in female social behaviour have also been observed upon in utero exposure to the combined treatment of AZT with 3TC, another nucleoside analogue acting as reverse transcriptase inhibitor (Venerosi et al., 2001). AZT + 3TC overall increased the latency to interact with a novel social stimulus, and depressed, in females, investigative and affiliative components of social behaviour (Venerosi et al., 2001).

The altered "coping style" showed by AZT-treated mice when presented with a new social stimulus may result from the interference of this drug with maturation of those neural mechanisms involved in emotional behaviour. However, no significant differences in basal or stimulus-induced corticosterone secretion were found as a result of perinatal AZT exposure suggesting that other systems might be the target of this drug.

While AZT effects on adolescent behaviour appeared limited, this drug exerted clear-cut effects in the plus-maze test performed at adulthood. Perinatal AZT-exposure did not affect the percent of *open arm entries*, a classical index of anxiety. However, this treatment increased the frequency of risk assessment postures, such as the *stretched attend posture*. This behaviour is classically described as an index of risk assessment, which should facilitate the information gathering in potentially dangerous environments and which is reported to be very sensitive to anxiolytic and anxiogenic drugs (File, 1992, 1993; Pellow et al., 1985). It is worth to notice that AZT treatment inhibited both locomotor activity (frequency of closed arm entries) and explorative behavior (*rearing*) of adult male mice (Rodgers and Johnson, 1995). AZT effects cannot be ascribed to an indirect effect on body growth since longitudinal assessment of body weight from PND2 to PND80 did not reveal any significant difference between AZT-treated and control mice.

In this study, we introduced a maternal-separation paradigm in order to produce a chronic disruption in the mother–infant relationship (Cirulli et al., 1997; Plotsky and Meaney, 1993). The main effect of maternal deprivation was to enhance the aggressive profile of the social repertoire typical of male mice, which has been reported to increase with age, reaching a measurable level around PND30 (Terranova et al., 1998). Early maternal separation exerted a marked 'pro-aggressive' effect, increasing both frequency and duration of the agonistic items recorded. In addition, as far as nonsocial behaviours are concerned, DEP subjects

were characterised by high levels of activity, mostly accounted for a deficit in habituation, rather that an absolute increase in activity levels, compared to nondeprived subjects. In fact, while in the first 10 min of observation, the expression of *exploration* was very similar in both the conditions considered, subsequently, only NDEP mice decreased this behaviour, DEP male mice showing the same amount of exploration in the following time block. The same considerations also apply to another index of exploration such a *digging* behaviour. As for females, which did not show an aggressive behavioural profile, in accordance with male data the main effect of deprivation was to increase exploratory activity, while decreasing habituation in behavioural items such as *digging*.

Results in the plus-maze indicated greater locomotor activity of adult DEP mice, while suggesting lower anxiety levels, if one takes into account a trend to show a higher percentage of entries in the open arms by these subjects, compared to NDEP. Furthermore, deprivation increased explorative behaviour, as shown by a greater number of *rearing* and *head dipping*.

The increase in aggression and exploration overall characterising DEP subjects could reflect greater arousal, although CORT levels measured following the social interaction test did not differ in DEP and NDEP animals. An alternative explanation is that DEP animals are characterized by a decreased neophobia, which could explain both their greater explorative activity in the plus maze and the higher levels of aggressive behavior. These data appear in contrast with previous findings in rats indicating that maternal separations (3–6 h) lead to increased CORT secretion in response to stressful stimuli such as restraint or novelty exposure, compared to NDEP or handled subjects (Plotsky and Meaney, 1993). In this study, DEP subjects did not differ from NDEP animals in terms of CORT secretion following novelty or the social interaction test. If anything, DEP mice tended to secrete less CORT in response to stressful stimuli while showing increased exploration (Levine and Broadhurst, 1963; Meaney et al., 1991). This discrepancy might be due to species differences, since in previous studies rats were the experimental subjects, or to subtle differences in methodology. In fact, it should be mentioned here that the NDEP group did receive a minor handling for the first 10 days of life since, in order to be administered AZT or the control treatment, the mothers had to be removed from the nest for about one min. In addition, differently from previous studies (Plotsky and Meaney, 1993) during the separation procedure, the mother was left in the home cage instead of being removed.

Our working hypothesis was that disruption in the mother–infant relationship might exacerbate the effects of AZT exposure on the offspring's behavioural development. This premise was not proved since in just one case a significant interactional effect between perinatal AZT treatment and preweaning maternal separation emerged. In males, which showed mainly agonistic behaviour, previous maternal deprivation increased the frequency of *affiliative behaviours* in AZT-treated mice, reducing it in control subjects. In females, mainly showing affiliative-type behaviours, an opposite effect was observed: maternal separation decreased the frequency of *affiliative behaviours* in AZT-treated females, increasing it in controls. Thus, maternal deprivation did not exacerbate the effects of AZT and the interaction between these two treatments appears more like an additive effect.

Given the data currently present in the literature and the present results, AZT is still a treatment of choice in preventing mother-to-child HIV transmission. Nonetheless, since the early signs characterising many CNS disorders can be relatively negligible, it is cautious to advice clinicians to monitor exposed children in order to develop specific preventive and protective interventions once a negative outcome has been identified (French Perinatal Cohort Study Group, 2002). As for the maternal separation paradigm used in this study, differently from what was expected based on the literature, in mice, this procedure appears to produce animals, which are less emotional and more explorative than controls (Levine, 1957). Indeed, other recent reports seem to indicate that the separation paradigm adopted in this study results in neurobiological effects that are in the same, rather than the opposite direction to those characterising handled subjects (Lehmann et al., 2002). Further studies are needed to assess more thoroughly the neurobehavioural and neuroendocrine sequelae of the early manipulations employed in this study.

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