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PRENATAL MALNUTRITION ALTERS DIAZEPAM-MEDIATED SUPPRESSION OF ULTRASONIC VOCALIZATIONS IN AN AGE DEPENDENT MANNER

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Abstract

The sensitivity of prenatally malnourished rats to the ultrasonic vocalization (USV) suppressant effect of diazepam (a non-specific benzodiazepine (BZ) receptor agonist) was investigated. Male offspring of dams provided with a protein deficient diet (6% casein) for 5 weeks prior to mating and throughout pregnancy were compared to the offspring of mothers provided with a diet of adequate protein content (25% casein). At postnatal day 7 or 11, pups were injected with vehicle or one of five doses of DZ (0.03, 0.1, 0.3, 1 or 3mg/kg) 30min after removal from their dam. Thirty minutes later they were subjected to 2min of cooling on a 20 degrees C surface and their USVs were quantified. DZ dose-dependently suppressed USV at both ages. At P7, the USV suppressant effect of DZ was the same for both groups. However, by P11 the prenatally malnourished rats showed significantly greater suppression of USV by 0.03 and 0.1mg/kg DZ than well-nourished controls. These differences were not related to degree of temperature loss or body weight. Thus, differential sensitivity to BZ receptor agonists develops in the second postnatal week in prenatally malnourished rats. This reflects either an altered program of development of the GABAergic system, or adaptive, compensatory changes in the GABAergic system in response to more extensive functional disturbances in the developing brain.

Keywords

Prenatal protein malnutrition; benzodiazepine; anxiolytic; $GABA_A$ receptor; ultrasonic vocalization; rat

INTRODUCTION

Behavioral assessments of the response to benzodiazepines, positive allosteric modulators of the $GABA_A$ receptor, have revealed significant alterations in sensitivity to these compounds in prenatally protein malnourished adult and juvenile rats. For example, prenatally malnourished rats display reduced sensitivity to the amnestic effect of systemic application of a moderate dose (5.6 mg/kg, i.p.) of the non-specific benzodiazepine (BZ) receptor agonist chlordiazepoxide (CDP) at both postnatal day 30 (P30) and P90 [34]. In adult prenatally malnourished subjects, direct application of CDP into the medial septum also results in lower

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sensitivity to its amnestic effects compared with well-nourished controls [36]. It has been demonstrated recently, however, that neither the direct $GABA_A$ receptor agonist muscimol nor the α1-subunit specific BZ receptor agonist CL218,872 differentiate between the two nutritional groups in Morris water maze performance in adulthood [33] suggesting that there is no lasting global dysfunction of the $GABA_A$ receptor, rather there is a difference in the way in which a subset of the $GABA_A$ receptors (i.e., those containing α2, α3 or α5 subunits) are modulated by BZs.

Many properties can be ascribed to BZs (e.g., they can produce anxiolytic, sedative, muscle relaxant, and anticonvulsant effects). To examine the behavioral sensitivity of prenatally malnourished rats to a different property of CDP, an operant drug discrimination paradigm was utilized [27]. Prenatally malnourished adult rats proved to have an increased behavioral sensitivity to the discriminative stimulus properties of CDP. Thus, the direction of the change in sensitivity to CDP induced by prenatal malnutrition is dependent upon the property of the drug that is studied.

Prenatally malnourished rats are fostered at birth to well-nourished dams, such that they receive adequate nutrition postnatally. Given the documented changes in the sensitivity to BZs in juvenile and adult rats, the question arises as to whether the prenatal development of the GABAergic system is particularly vulnerable to the prenatal insult (i.e., is subject to a pathological developmental disturbance) such that the greatest effect will be most evident close to the end of the insult (i.e., birth), with the impact perhaps becoming reduced postnatally as adaptive, compensatory responses occur in the CNS. A second possibility is that although the GABAergic system is impacted by the prenatal insult it is not expressed until later in the postnatal period. In other words the developmental program is disrupted. A third possibility is that there is a change in the way the GABAergic system is modulated during postnatal development. Conceptually, this could constitute one of the adaptive mechanisms that serve to compensate for developmental disturbances originating in other neurotransmitter systems. In the latter case, one might expect to observe greater behavioral dissociations between prenatally malnourished and well-nourished animals as they mature.

Taking advantage of the dose-dependent USV suppressing effects of the BZs [12,21,26,37] the present investigation examined the anxiolytic effect of diazepam (DZ) in prenatally malnourished and well-nourished pups. Two postnatal ages (P7 and P11) were studied to assess the possible postnatal development of altered sensitivity to BZs following prenatal protein restriction.

MATERIALS AND METHODS

All studies described below were carried out in an AAALAC approved animal facility and had been approved by the Boston University Medical School/Boston Medical Center Institutional Animal Care and Use Committee (approval #01-057).

Housing Conditions

The animal quarters were maintained at a temperature of 22.8°C (± 1.7) and at 45–55% humidity. A reverse 12h dark (8:00–20:00)/12h light (20:00–8:00) cycle accommodated behavioral observations during the active waking period of the rat. Red fluorescent lighting during the dark portion of the cycle provided continuous dim illumination.

Nutritional Treatment

Five weeks prior to mating, nulliparous female rats (Sprague-Dawley VAF plus; Charles River Laboratories, Kingston, MA) were obtained and allowed ad lib access to one of two isocaloric

diets (Teklad, Madison, WI). The diets were formulated to be of adequate protein (25% casein) or low protein (6% casein) content (detailed description given in [20]). Males obtained from the same source were acclimated to the experimental diets of the females for one week prior to mating. Each male was mated with 2 females receiving the same dietary treatment, over a period of 7 days. The presence of sperm in a vaginal smear determined whether mating had occurred. One week prior to the projected delivery date, the females were individually caged in polycarbonate breeding cages (51cm × 41cm × 21cm; Lab Products Inc., Maywood, NJ). Following parturition, litters were culled to 8 pups (6 males and 2 females) and fostered to well-nourished mothers who had given birth no more than 24h previously. Pups born to dams provided with the 6% casein diet and cross-fostered to lactating dams given the 25% casein diet were assigned the abbreviation “6/25” (prenatally malnourished). Pups born to dams provided with the 25% casein diet and fostered to other lactating dams fed the 25% casein diet were assigned the abbreviation “25/25” (controls). A more detailed description of the nutritional, mating, and fostering procedures is given in a previous paper [31]. All rats were weighed at birth (P0) and on the day of behavioral testing, using an electronic balance (Ohaus, GT 4000). The animals remained in their littermate groups throughout except when removed briefly for behavioral testing.

Subjects

A total of 276 pups were employed in this study. Two cohorts of prenatally malnourished and well-nourished litters were raised. Cohort 1 consisted of twelve 25/25 litters and twelve 6/25 litters, and these were the source of subjects tested at postnatal day 7. Cohort 2 consisted of eleven naïve 25/25 and eleven naïve 6/25 litters, and these were the source of subjects tested at postnatal day 11. In each cohort, six male pups from each litter were allocated to one of six DZ dose groups (0, 0.03, 0.1, 0.3, 1 or 3 mg/kg). No more than one pup per litter per dose was tested (though each litter was represented across all of the doses) and each pup was tested only once. Two 25/25 litters were excluded from analysis at P7, and two 25/25 litters and one 6/25 litter of P11 were excluded from analysis at P11, because of an unusually low rate of USV (<25 USV in 2 min) emitted by their vehicle injected controls. Male subjects were selected for consistency with earlier studies which focused on the behavioral sensitivity of juvenile and adult prenatally malnourished rats to drugs acting on the $GABA_A$ receptor complex [27,33, 34,36] and upon neurophysiological studies focused on inhibitory GABAergic neurotransmission at both the systems [4–9] and the cellular level [15,24].

Drugs

Diazepam (Sigma Chemicals, St Louis, MO) was suspended in vehicle (85% distilled water, 14% polyethylene glycol and 1% Tween 80) and administered in a volume of 1ml/100g by subcutaneous injection at one of six doses (0, 0.03, 0.1, 0.3, 1 and 3mg/kg) 30min prior to testing.

Separation & Cooling Procedure

The procedure was similar to that described previously [32]. Briefly, the mother was removed from her litter and placed in a temporary holding cage in the colony room. The litter was kept intact in the home cage and brought into the test room and placed on a pad (T/Pad TP-220, Gaymar Industries, NY) through which water was circulated using a pump (Gaymar T/Pump), maintaining a surface temperature of 33°C. Thirty minutes later a single male pup was selected, weighed, and its core body temperature taken using a Thermalert Model TH-8 temperature monitor. A very fine (0.41mm diameter), Teflon-sealed thermocouple microprobe (Type IT-21, Physitemp, NJ) was first lubricated with petroleum jelly and then inserted 1cm into the rectum. A stable reading was obtained within 5 s. The pup was then injected with one of the five doses of DZ, an identifying mark was placed upon the base of the tail using a non-toxic

marker and the pup was returned to the huddle of the litter. Thirty minutes later the temperature was retaken to determine the effect of DZ on body temperature and the pup was then placed directly onto a metal pan, the surface of which was maintained at 20°C by resting it on a water bath. The pan was located within a sound attenuated enclosure (Model 1101 Research Chest, Grason-Stadler Co., W. Concord, MA) to prevent other rats from hearing the USVs. Both the pan temperature and the temperature under the litter huddle were constantly assessed using the Physitemp temperature monitor connected to electronic surface probes. An ultrasonic microphone suspended 10 cm above the pan was connected to a bat detector (Pettersson Elektronik AB, Sweden; Model D940) located outside the chamber. Over the next two minutes, pup calls (in the 20–80 kHz range) were detected with the bat detector and the signal, with a frequency division setting of 1:10, was recorded on audiotape for later analysis (using a Sony TC-WE435 stereo cassette deck). Immediately after testing, the rectal temperature of the pup was measured again to determine the additional loss of body temperature with cooling. The pup was then placed back into the huddle of littermates. At carefully spaced intervals the five remaining male pups were injected with the other doses of DZ and tested using the same procedure as described above. Each litter was tested to completion prior to testing of another litter but the order was randomized between nutritional groups. Also, within a litter the order of injection was randomized across drug doses but was matched between nutritional groups. After testing the litter was returned to the colony room to be reunited with their mother.

Analysis of Vocalizations

Each signal from the audiotape was used as an input for the CoolEdit2000 program (Syntrillium software, Scottsdale, AZ) running on an IBM PC. A digital recording was made and visualized in a spectral view (sound frequency in Hz on the ordinate and time on the abscissa). Call rate (number of USVs) was quantified for the 2-min recording time from this visual record for all calls in the range of 30–50 kHz.

Data analysis and Statistics

Body weight was compared between groups taking litter as the unit for analysis (i.e., litter means were used). At birth, a one-way ANOVA was applied, and at postnatal days 7 and 11, a 2-way ANOVA (Nutrition x Age) was utilized. Initial analysis taking DZ dose as a factor showed no inadvertent body weight bias across any DZ dose. Thus, for clarity, only the Nutrition x Age ANOVA is reported. At each age, basal body temperature was analyzed using one-way ANOVAs (Nutrition), again taking litter as the unit for analysis (i.e., litter means were used). The degree of loss of body temperature from the basal temperature was analyzed using a 3-way ANOVA (Nutrition x Dose x Phase [following DZ or vehicle injection, and after additional cooling]. Phase was a within subjects factor). The basal rate of USV emission after vehicle injection was compared between nutritional groups (for each age, separately) using 1-way ANOVAs (Nutrition). Following DZ injection, the USV rate was expressed as a percentage of the rate exhibited by the vehicle injected pup from the same litter. These data were analyzed separately for P7 and P11 using 2-way ANOVAs (Nutrition x Dose).

RESULTS

Body Weight

The mean birth weight of all male pups tested at P7 and P11 was significantly less for 6/25 litters (M=5.51±012g, n=23) than for 25/25 litters (M=6.53 ± 0.10g, n=23), $F(1,44)=44.36$, $p<0.001$. The weight difference between groups was also evident at testing (P7: M=16.0 ± 0.7g vs M=19.7 ± 0.6g; P11: M=25.9 ± 1.0g vs M=28.2 ±0.8g for 6/25 and 25/25 litters, respectively), as indicated by a significant effect of Nutrition ($F(1,42)=14.95$, $p<0.001$). As expected, the pups weighed more as they matured, Age ($F(1,42)=141.92$, $p<0.001$) but there was no significant Nutrition x Age interaction.

Effect of DZ dose and Cooling on Body Temperature

P7: At P7 the 6/25 pups expressed significantly cooler basal body temperatures ($F(1,20)=4.61$, $p<0.05$) than the 25/25 pups (Figure 1 – upper left panel, baseline). Body temperature decreased after drug injection and after cooling (Figure 1 – upper right panels) in all groups. A significant effect of Phase ($F(1,120)=775.90$, $p<0.001$) indicated a greater loss of body temperature with cooling (M = −2.20°C) than with the vehicle or DZ injection alone (M = −0.56°C). A significant effect of Dose ($F(5,120)=3.10$, $p<0.02$) indicated a dose dependent reduction of body temperature (Figure 1, upper panels). Post hoc analyses (Tukey) revealed that those pups receiving 0.3 mg/kg (M= −1.78°C), 1 mg/kg (M= −1.78°C) and 3 mg/kg (M= −1.77°C) doses of DZ had significantly ($p<0.05$) lower body temperatures than those pups receiving the vehicle injection (M = −1.19°C). There was no significant effect of Nutrition, nor was there a Nutrition x Phase interaction. Similarly, no significant Dose x Nutrition or Dose x Nutrition x Phase interactions were indicated by ANOVA.

P11: At P11 the 6/25 pups tended to be slightly warmer than the 25/25 pups ($F(1,17)=3.81$, $p=0.068$) but the difference failed to achieve statistical significance (Figure 1 – lower left panel, baseline). A significant effect of Phase ($F(1,105)=430.14$, $p<0.001$) indicated a greater loss of body temperature with cooling (M = −1.04°C) than with the vehicle or DZ injection alone (M = −0.18°C). A significant effect of Dose ($F(5,102)=4.37$, $p<0.002$) indicated a dose dependent reduction of body temperature (Figure 2, lower right panels). Post hoc analyses (Tukey) revealed that those pups receiving 1 mg/kg (M= −1.06) and 3 mg/kg (M= −0.89°C) doses of DZ had significantly ($p<0.05$) lower body temperatures than those pups receiving the vehicle injection (M = −0.45°C). There was no significant effect of Nutrition, nor was there a Nutrition x Phase interaction. Similarly, no significant Dose x Nutrition or Dose x Nutrition x Phase interactions were indicated by ANOVA.

Modulation of USV by DZ

P7: At P7 there was no difference between 6/25 and 25/25 pups in the rate of emission of USV over the 2 min test period ($F(1,20)=0.82$, ns) following a vehicle injection (Figure 2 – upper left panel, VEH). USV data obtained following DZ injection were expressed as a percentage of the rate exhibited by the littermate that had been injected with the vehicle solution (Figure 2, upper right panel). After DZ injection there was a significant effect of Dose ($F(4,100)=7.62$, $p<0.001$) which indicated a dose-dependent suppression of USV production. Post hoc analyses (Tukey) revealed that the 3 mg/kg dose suppressed USV emission significantly more than the 0.03 mg/kg dose of DZ ($p<0.05$). However, there was no significant effect of Nutrition and no Nutrition x Dose interaction. Thus, DZ modulated USV equally in the two nutritional groups at this age.

P11: There was no baseline difference between 6/25 and 25/25 pups in the rate of emission of USV over the 2 min test period ($F(1,17)$ <0.01, ns) following a vehicle injection (Figure 2 – lower left panel, VEH). USV data obtained following DZ injection were expressed as a percentage change from the rate exhibited by the littermate that had been injected with the vehicle solution (Figure 2 lower right panel). ANOVA carried out on these data indicated a significant effect of Dose ($F(4, 85)=11.41$, $p<0.001$), and Nutrition ($F(1, 85)=9.54$, $p<0.01$) and a significant Nutrition x Dose interaction ($F(4, 85)=3.95$, $p<0.01$). It can be seen from Figure 2 (lower right panel) that DZ dose-dependently reduced the rate of USV but that the 6/25 pups showed much greater suppression of USV at the lower doses than the 25/25 pups. Post hoc analyses (Tukey) revealed that the two nutritional groups were significantly different ($p<0.05$) at DZ doses of 0.03 and 0.1 mg/kg. A supplementary series of analyses (not reported here) in which the data was analyzed taking body weight and body temperature as covariates did not alter this pattern of findings. It is worth noting that the drug response in the 6/25 group

was very similar at both P7 and P11 while the 25/25 pups appeared to become less sensitive to the two lower doses at the older age (values around 100% of the vehicle value).

DISCUSSION

The present study established that a difference between prenatally malnourished and well-nourished male pups in their sensitivity to the USV suppressant effect of DZ develops as the rats mature. This occurs at a time when the pups are being raised under conditions of adequate nutrition, rather than being present at a time closer to the nutritional insult. Scrutiny of Figure 2 suggests it may be that the dose-response relationship to DZ changes with age in the well-nourished control group rather than the modification occurring in the prenatally malnourished group. In other words, there may be an important developmental change taking place in the well-nourished rats that is either delayed or impaired following prenatal malnutrition. Before proceeding with the discussion further it should be noted that this study only employed male subjects. For this reason, it is prudent to limit the following discussion to consideration of the effects of prenatal malnutrition on this gender. Female prenatally malnourished subjects may well differ in their later behavioral response to drugs compared to males [e.g., 30]. To begin to interpret the findings of the present study, the critical impact of subunit composition on the pharmacological properties of the $GABA_A$ receptor, as well as the developmental changes that normally take place in the GABAergic system during the early postnatal period of the rat, must be appreciated.

The $GABA_A$ receptor complex consists of 5 subunits assembled together from a subset of at least 6 known subunit classes [38] each possessing numerous isoforms (α1–α6, β1–β3, γ1–γ3, δ, ε, θ). In large part, BZ pharmacology (and other properties of the $GABA_A$ receptor) is determined by the $GABA_A$ receptor subunit composition. Receptors on the $GABA_A$ receptor complex that recognize the classical BZs (called “diazepam-sensitive”) contain α1, α2, α3 or α5 subunits while those that do not recognize these agents (called “diazepam-insensitive”) contain either the α4 or α6 subunit. Further, among the diazepam-sensitive receptors, those that contain the α1 subunit are said to be high affinity, whereas those that contain α2, α3 or α5 are considered to have lower affinity. Thus, different populations of $GABA_A$ receptors with different pharmacological properties (e.g., relative binding affinity for ligands acting upon those receptors) can be generated by altering the level of expression of different $GABA_A$ subunits, especially among α subunits.

During normal development of the rat brain, significant changes take place in the expression of the individual $GABA_A$ receptor subunits. Alpha 1 and α5 subunits have generally low levels of expression at birth and increase as the rat matures, while α2 and α3 subunits exhibit high levels of expression at birth and decline as the rat matures [10,17,22,25], although there are regional variations in this pattern. For example, expression of α1 subunit decreases rather than increases in the ventromedial nucleus [17]. Given the critical importance of $GABA_A$ receptor subunit composition to the properties of the receptor complex and the fact that the expression of the various subunits change as the rat matures, it is possible that differences in the developmental pattern of subunit expression may underlie the present findings. There is evidence that prenatal protein malnutrition does indeed alter $GABA_A$ subunit gene expression. This manifests itself in a region-specific [29] and developmentally-specific [28] manner. Of most relevance to the present findings, it was found that α3 subunit expression in the hippocampal formation was greater in prenatally malnourished rats at postnatal day 15 (the earliest age examined) than in controls, while α5 expression was reduced. In that α3 expression usually declines while α5 expression increases during the postnatal period, it is likely that prenatal malnutrition slows or prevents these developmental changes from taking place. Although it is not known which brain structures are critically involved in the suppression of USV by BZ agonists, the fact that prenatally malnourished pups exhibit higher α3 subunit

expression levels than controls may be important in light of the recent development of drugs having a high degree of subunit specificity. As mentioned earlier, classic BZ drugs have numerous behavioral effects not all of which are desirable in the clinical population. Hence, subunit specific drugs are under development in an attempt to limit their range of action. Recent evidence shows that agents with highly selective action on $GABA_A$ receptors bearing α3 subunits possess powerful anxiolytic effects without sedation [3,16,18,23], which has encouraged the synthesis and characterization of several more α3-selective compounds (e.g., [13]) as potential therapeutic, anxiolytic agents. Thus, if prenatal malnutrition were to have led to greater expression of α3 subunits at P11 than is developmentally appropriate, this may have resulted in a greater sensitivity to the anxiolytic effect of DZ. This would be consistent with their apparent lack of change in sensitivity to DZ between P7 and P11 (see Figure 2). In turn, it seems reasonable to propose that a developmental change (perhaps a decline in α3 expression with an increase in α1 expression) was taking place in the well-nourished rats leading to an apparent lack of sensitivity to the USV suppressing effects of DZ at the 0.03 and 0.1 mg/kg doses.

Other questions raised by the present findings are: what (if any) is the biological significance of an altered development of the GABAergic system following prenatal malnutrition and, are there any other factors contributing to this pattern? Our previous study [32] identified significant differences in the ultrasonic call characteristics of prenatally malnourished pups (as detected by sonographic analysis) over this same developmental period (postnatal day 7 – 11). Specifically, prenatally malnourished pups exhibited a significantly higher mean sound frequency for their USV calls (irrespective of sub-type) at postnatal day 7. Constant sound frequency vocalizations were also of longer duration. Prenatally malnourished pups also emitted a smaller variety of calls over the age range studied, with fewer ascending frequency vocalizations and either significantly fewer (P9) or greater (P11) descending frequency calls. While it might be controversial to suggest that the type of USV may influence the behavior of the mother, it is an established fact that prenatally malnourished rats are groomed more than controls by their foster dam over the first 10 days of life [20]. Experience of high levels of maternal licking and grooming over the first 8–10 days of life has been associated with changes in central BZ receptors [11] and a life-long reduction in stress response and anxiety-related behaviors in normal rat populations [14]. Thus, it is possible that the greater levels of licking and grooming experienced by the prenatally malnourished pups (whether it is due to their USV characteristics or some other feature) may exert an epigenetic influence on the development of the GABAergic system (e.g., subunit expression) leading to reduced anxiety in classic anxiety tests such as the elevated plus maze [1] and the elevated T-maze, later in life [2]. It should be noted, however, that prenatally malnourished rats do not always exhibit evidence of low anxiety and indeed demonstrate heightened physiological responsiveness in some stressful test situations (e.g., [19,35]).

In summary, the present study demonstrated that an age dependent difference in sensitivity to the USV suppressant effects of DZ developed following prenatal malnutrition. Whether this altered program of development of the $GABA_A$ system is a direct result of the nutritional insufficiency, a result of epigenetic programming mediated by the mother, or constitutes adaptive, compensatory responses of the $GABA_A$ system, simply cannot be determined from the present study. These important questions must therefore await the results of further research.

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References

1. Almeida SS, Tonkiss J, Galler JR. Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus maze. Physiol Behav 1996;60:675–680. [PubMed: 8840934]
2. Almeida SS, Tonkiss J, Galler JR. Prenatal protein malnutrition affects avoidance but not escape behavior in the elevated T-maze test. Physiol Behav 1996;60:191–195. [PubMed: 8804663]
3. Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM. TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo [4,3-b]pyridazine], an agonist selective for alpha2- and alpha3-containing GABAA receptors, is a nonsedating anxiolytic in rodents and primates. J Pharmacol Exp Ther 2006;316:410–422. [PubMed: 16183706]
4. Austin KB, Beiswanger C, Bronzino JD, Austin-LaFrance RJ, Galler JR, Morgane PJ. Prenatal protein malnutrition alters behavioral state modulation of inhibition and facilitation in the dentate gyrus. Brain Res Bull 1992;28:245–255. [PubMed: 1596745]
5. Austin KB, Bronzino JD, Morgane PJ. Prenatal protein malnutrition affects synaptic potentiation in the dentate gyrus of rats in adulthood. Dev Brain Res 1986;29:267–273.
6. Bronzino JD, Austin-LaFrance RJ, Morgane PJ, Galler JR. Diet-induced alterations in the ontogeny of long-term potentiation. Hippocampus 1996;6:109–117. [PubMed: 8797012]
7. Bronzino JD, Austin-LaFrance RJ, Mokler D, Morgane PJ. Effects of prenatal protein malnutrition on hippocampal long-term potentiation in freely moving rats. Exp Neurol 1997;148:317–323. [PubMed: 9398474]
8. Bronzino JD, Austin-LaFrance RJ, Morgane PJ, Galler JR. Effects of prenatal protein malnutrition on kindling-induced alteration in dentate granule cell excitability. I. Synaptic transmission measures. Exp Neurol 1991;112:206–215. [PubMed: 1645274]
9. Bronzino JD, Austin-LaFrance RJ, Morgane PJ, Galler JR. Effects of prenatal protein malnutrition on kindling-induced alteration in dentate granule cell excitability. II. Paired-pulse measures. Exp Neurol 1991;112:216–223. [PubMed: 1903711]
10. Brooks-Kayal AR, Sumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. Gamma-amino butryric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. J Neurochem 2001;77:1266–1278. [PubMed: 11389177]
11. Caldji C, Diorio J, Anisman H, Meaney MJ. Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. Neuropsychopharmacol 2004;29:1344–1352.
12. Carden SE, Hofer MA. Independence of benzodiazepine and opiate action in the suppression of isolation distress in rat pups. Behav Neurosci 1990;104:160–166. [PubMed: 2107826]
13. Carling RW, Russell MG, Moore KW, Mitchinson A, Guiblin A, Smith A, Wafford KA, Marshall G, Atack JR, Street LJ. 2,3,7-Trisubstituted pyrazolo[1,5-d][1,2,4]triazines: Functionally selective GABA(A) alpha3-subtype agonists. Bioorg Med Chem Lett 2006;16:3550–3554. [PubMed: 16621541]
14. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 2003;79:359–371. [PubMed: 12954431]
15. Chang Y-M, Galler JR, Luebke JI. Prenatal protein malnutrition results in increased frequency of miniature inhibitory postsynaptic currents in rat CA3 interneurons. Nutr Neurosci 2003;6:263–267. [PubMed: 12887143]
16. Crawforth J, Atack JR, Cook SM, Gibson KR, Nadin A, Owens AP, Pike A, Rowley M, Smith AJ, Sohal B, Sternfeld F, Wafford K, Street LJ. Tricyclic pyridones as functionally selective human GABAA alpha 2/3 receptor-ion channel ligands. Bioorg Med Chem Lett 2004;14:1679–1682. [PubMed: 15026049]
17. Davis AM, Penschuck S, Fritschy JM, McCarthy MM. Developmental switch in the expression of GABA(A) receptor subunits alpha(1) and alpha(2) in the hypothalamus and limbic system of the rat. Dev Brain Res 2000;119:127–138. [PubMed: 10648879]

18. Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson GR, Reynolds DS. Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005;25:10682–10688. [PubMed: 16291941]
19. Durán P, Galler JR, Cintra L, Tonkiss J. Prenatal malnutrition and sleep states in adult rats: Effects of restraint stress. Physiol Behav. 2006in press
20. Galler JR, Tonkiss J. Prenatal protein malnutrition and maternal behavior in Sprague-Dawley rats. J Nutr 1991;121:762–769. [PubMed: 2019885]
21. Gardner CR, Budhram P. Effects of agents which interact with central benzodiazepine binding sites on stress-induced ultrasounds in rat pups. Eur J Pharmacol 1987;134:275–283. [PubMed: 3032656]
22. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 1992;12:4151–4172. [PubMed: 1331359]
23. Lewis RT, Blackaby WP, Blackburn T, Jennings AS, Pike A, Wilson RA, Hallett DJ, Cook SM, Ferris P, Marshall GR, Reynolds DS, Sheppard WF, Smith AJ, Sohal B, Stanley J, Tye SJ, Wafford KA, Atack JR. A pyridazine series of alpha2/alpha3 subtype selective GABA A agonists for the treatment of anxiety. J Med Chem 2006;49:2600–2610. [PubMed: 16610803]
24. Luebke J, St John J, Galler JR. Prenatal protein malnutrition results in increased frequency of miniature inhibitory postsynaptic currents in rat CA1 pyramidal cells. Synapse 2000;37:23–31. [PubMed: 10842348]
25. McKernan RM, Cox P, Gillard NP, Whiting P. Differential expression of GABAA receptor alpha-subunits in rat brain during development. FEBS Lett 1991;286:44–46. [PubMed: 1650715]
26. Olivier B, Molewijk E, van Oorschot R, van der Heyden J, Ronken E, Mos J. Rat pup ultrasonic vocalization: effects of benzodiazepine receptor ligands. Eur J Pharmacol 1998;358:117–128. [PubMed: 9808260]
27. Shultz PL, Galler JR, Tonkiss J. Prenatal protein malnutrition enhances the stimulus properties of CDP, but not a CDP/THIP combination, in a drug discrimination paradigm. Pharm Biochem Behav 2002;73:759–767.
28. Steiger JL, Alexander MJ, Galler JR, Farb DH, Russek SJ. Effects of prenatal malnutrition on GABAA receptor alpha1, alpha3 and beta2 mRNA levels. Neuroreport 2003;14:1731–1735. [PubMed: 14512847]
29. Steiger JL, Galler JR, Farb DH, Russek SJ. Prenatal protein malnutrition reduces beta(2), beta(3) and gamma(2L) GABA(A) receptor subunit mRNAs in the adult septum. Eur J Pharmacol 2002;446:201–202. [PubMed: 12098603]
30. Tonkiss J, Almeida SS, Galler JR. Prenatally malnourished female but not male rats show increased sensitivity to MK-801 in a DRL task. Behav Pharmacol 1998;9:49–60. [PubMed: 9832948]
31. Tonkiss J, Galler JR. Prenatal protein malnutrition and working memory performance in adult rats. Behav Brain Res 1990;40:95–107. [PubMed: 2126733]
32. Tonkiss J, Bonnie KE, Hudson JL, Shultz PL, Duran P, Galler JR. Ultrasonic call characteristics of rat pups are altered following prenatal malnutrition . Develop Psychobiol 2003;43:90–101.
33. Tonkiss J, Shultz PL, Bonnie KE, Hudson JL, Duran P, Galler JR. Spatial learning deficits induced by muscimol and CL218,872: Lack of effect of prenatal malnutrition. Nutr Neurosci 2003;6:379–387. [PubMed: 14744042]
34. Tonkiss J, Shultz PL, Shumsky JS, Fiacco TA, Vincitore M, Rosene DL, Galler JR. Chlordiazepoxide-induced spatial learning deficits: Dose-dependent differences following prenatal malnutrition. Pharmacol Biochem Behav 2000;65:105–116. [PubMed: 10638643]
35. Tonkiss J, Trzcinska M, Galler JR, Ruiz-Opazo N, Herrera VLM. Prenatal malnutrition-induced changes in blood pressure: Dissociation of stress and non-stress responses using radiotelemetry. Hypertension 1998;32:108–114. [PubMed: 9674646]
36. Tonkiss J, Trzci ska M, Shultz PL, Vincitore M, Galler JR. Prenatally protein malnourished rats are less sensitive to the amnestic effects of medial septal infusions of chlordiazepoxide. Behav Pharmacol 2000;11:437–446. [PubMed: 11103910]

37. Vivian JA, Barros HMT, Manitiu A, Miczek KA. Ultrasonic vocalizations in rat pups: Modulation at the gamma-amino butyric acid$_A$ receptor complex and the neurosteroid recognition site. J Pharmacol Exp Ther 1997;282:318–325. [PubMed: 9223570]

38. Wingrove PB, Safo P, Wheat L, Thompson SA, Wafford KA, Whiting PJ. Mechanism of α-subunit selectivity of benzodiazepine pharmacology at γ-aminobutyric acid type A receptors. Europ J Pharmacol 2002;437:31–39.

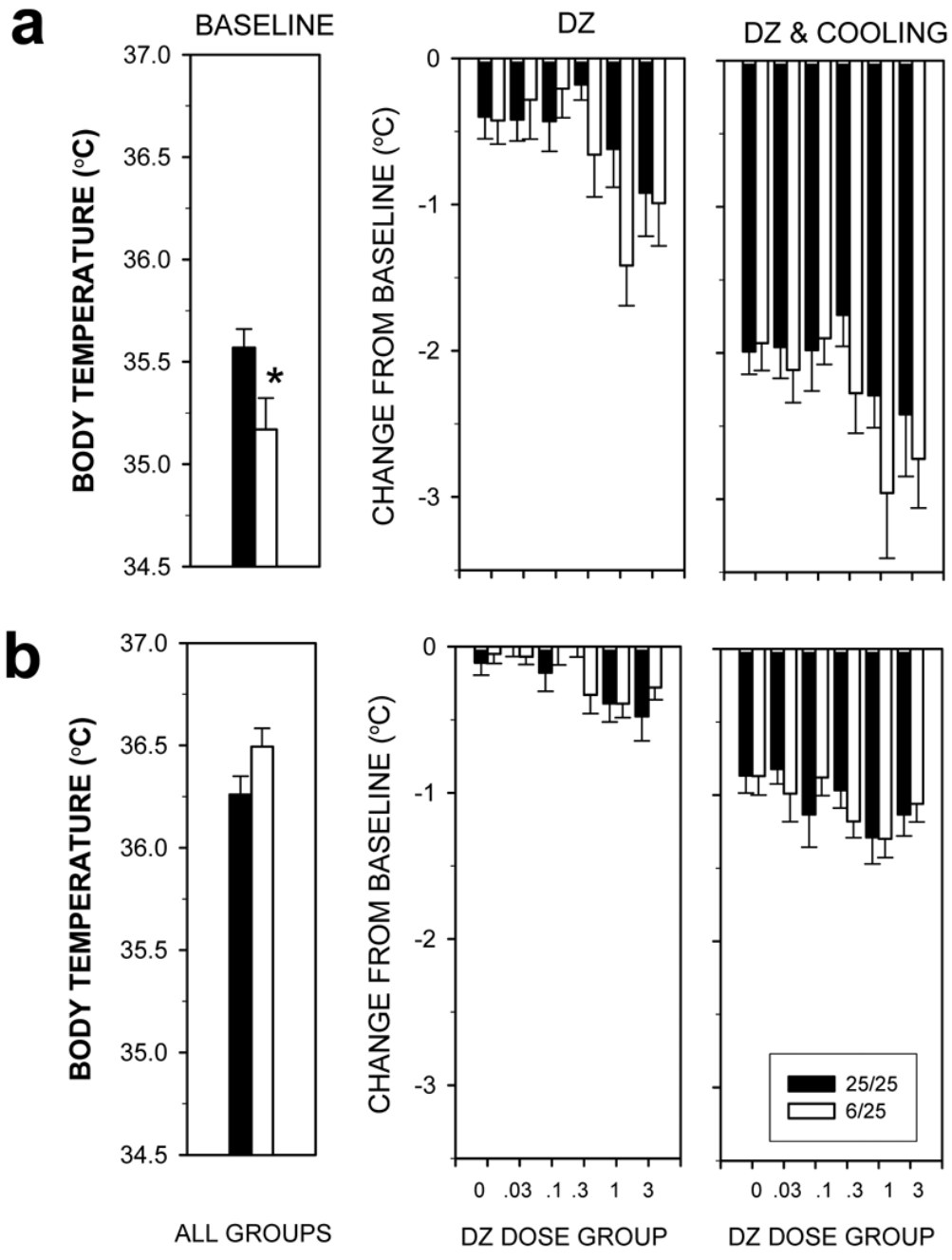

FIGURE 1.

Mean body temperature of prenatally malnourished (6/25, open bars) and well-nourished (25/25, solid bars) rat pups 30 min after removal from the mother and confinement in a warmed cage (Baseline); 30 min after an injection of diazepam at one of five doses (DZ); and, 2 min after additional cooling on a 20°C surface (DZ & Cooling) at **(a)** Postnatal day 7 (25/25 group, n=10; 6/25 group, n=12 for each DZ dose group), or **(b)** Postnatal day 11 (25/25 group, n=9; 6/25 group, n=10 for each DZ dose group). Vertical bars represent the SEM. *6/25 significantly less than 25/25, $p<0.05$ (Tukey)

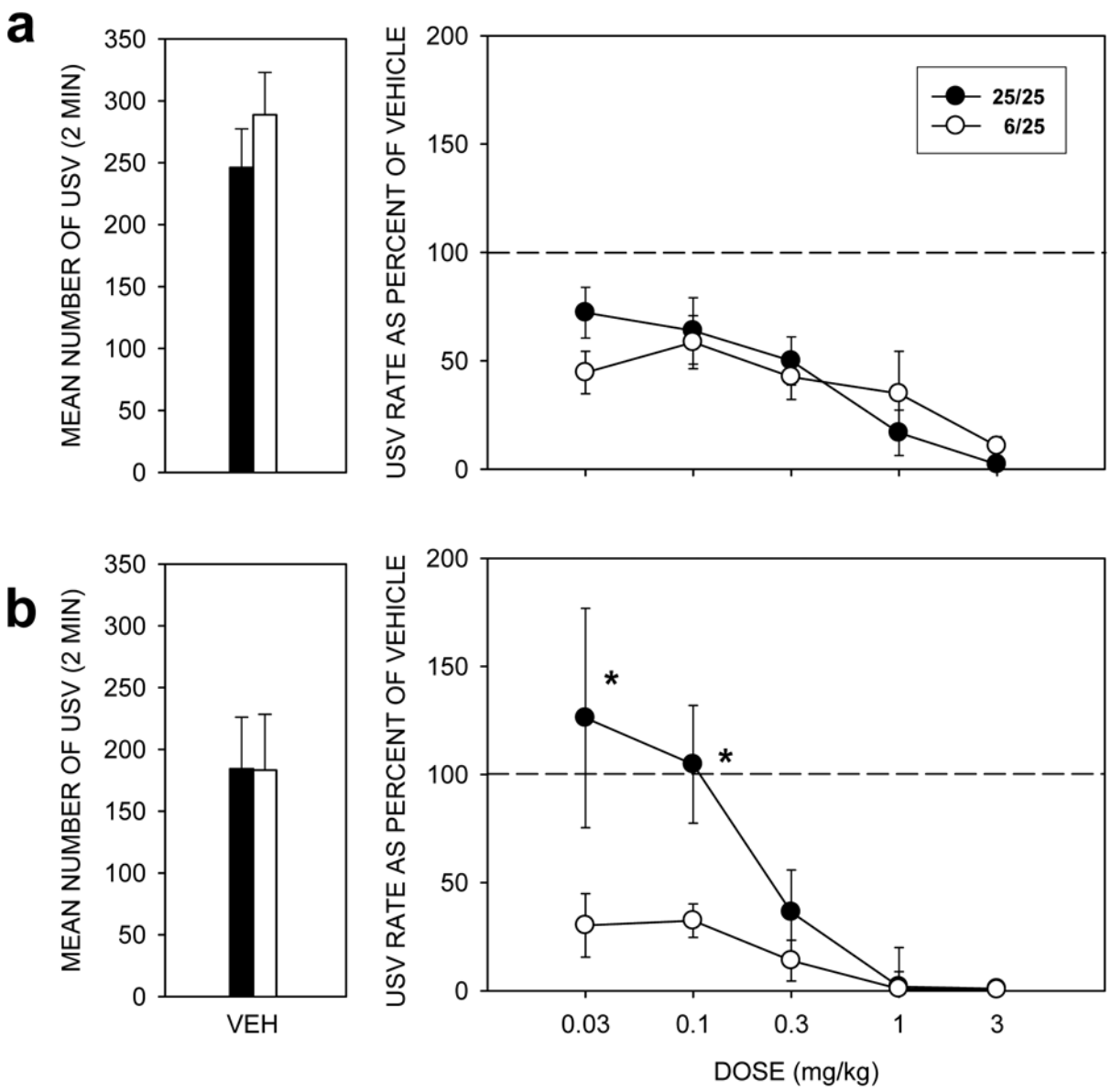

FIGURE 2.

Left panels - Mean number of ultrasonic vocalizations (USV) emitted by prenatally malnourished (6/25, open bars) and well-nourished (25/25, solid bars) pups over a 2 min period of cooling on a 20°C surface 30 min after an injection of vehicle (VEH). Right panels – Mean USV rate following five doses of diazepam (DZ) expressed as a percentage of the vehicle control pup for the same litter **(a)** Postnatal day 7 (25/25 group, n=10; 6/25 group, n=12 for each DZ dose group), or **(b)** Postnatal day 11 (25/25 group, n=9; 6/25 group, n=10 for each DZ dose group). Vertical bars represent the SEM. *6/25 significantly less than 25/25, $p<0.05$ (Tukey).