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Perinatal Diazepam Exposure: Alterations in Exploratory Behavior and Mesolimbic Dopamine Turnover

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GRUEN, R. J., A. Y. DEUTCH AND R. H. ROTH. *Perinatal diazepam exposure: Alterations in exploratory behavior and mesolimbic dopamine turnover*. PHARMACOL BIOCHEM BEHAV **36**(1) 169–175, 1990.—Perinatal exposure to diazepam has been shown to lead to alterations in motor activity and exploratory behavior in neonatal animals. Exploratory and locomotor behavior have been associated with changes in mesotelencephalic dopamine function. We have therefore examined the effects of perinatal diazepam administration on both exploratory behavior and mesotelencephalic dopamine turnover in the adult rat. Animals exposed to the benzodiazepine during the perinatal period engaged in significantly less exploratory behavior than did control subjects. The diazepam-induced alterations in behavior were developmentally specific: decreased exploratory behavior was observed at 90, but not 60, days of age. At 90 days of age, specific changes in dopamine turnover in diazepam-treated animals were restricted to mesolimbic (nucleus accumbens and ventral tegmental area) sites; alterations in dopamine turnover were not seen in other mesotelencephalic sites examined. The findings indicate that perinatal exposure to benzodiazepines leads to behavioral changes that are present in adulthood. These changes in exploratory behavior may be associated with alterations in mesolimbic dopamine function.

Dopamine GABA Benzodiazepine Ventral tegmental area Nucleus accumbens

PERINATAL exposure to benzodiazepines results in a variety of behavioral, biochemical, and physical abnormalities in humans and animals (4, 8, 12, 19, 22, 26, 30, 31, 43, 47, 50). Rat pups exposed in utero to diazepam (DZ) exhibit a variety of behavioral and physical sequelae, including alterations in locomotor activity (19,50), exploratory behavior (19), and acoustic startle response (27) during early development. Learning deficits (19,22) and disturbances in the development of auditory temporal acuity (26) have also been noted. These rodent studies indicate that perinatal exposure to DZ leads to behavioral disturbances during the neonatal period; however, it is unclear whether these behavioral deficits persist into adulthood.

The neurochemical bases underlying these developmental changes have not been clearly established. A number of studies have examined [^{3}H]GABA or [^{3}H]diazepam binding at different developmental points and reported that prenatal DZ exposure does not appear to result in changes in receptor density or affinity in forebrain regions (2, 28, 39). Other groups, however, have reported changes in benzodiazepine receptor density following prenatal diazepam treatment (1, 21, 33, 34). Studies of rats exposed to diazepam in utero suggest that noradrenergic function may be altered in the hypothalamus, but not in the hippocampus, cortex or brainstem of adult rats (47,51). Moreover, hypothalamic NE turnover is reduced after restraint stress in animals exposed to benzodiazepines during the prenatal period (52).

The mesotelencephalic DA system is thought to subserve locomotor and exploratory behavior (17, 18, 25). In particular, the nucleus accumbens septi (NAS) has been implicated as a critical mesolimbic DA terminal field subserving the expression of exploratory behavior (3, 24, 25, 53). Since prenatal exposure to DZ leads to alterations in exploratory behavior in neonates, and since benzodiazepines modulate stress-induced activation of the mesotelencephalic DA system (13, 32, 44, 48, 55), we have examined the behavioral and neurochemical consequences of perinatal DZ exposure of rats, focusing on changes in exploratory behavior and mesolimbic DA turnover.

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METHOD

Subjects

Pregnant Sprague-Dawley dams (Charles River Laboratories, Wilmington, MA) received either diazepam-loaded silastic pellet implants, empty pellet implants, or no treatment at all. There were 6, 6, and 4 dams in each of the three conditions, respectively. Subjects consisted of male offspring from dams in each group. There were three groups of subjects: offspring from dams exposed to DZ during the perinatal period (DZ-exposed); offspring from dams receiving empty pellet implants (pellet control); and offspring from dams who received no treatment at all (normal control). Offspring were group housed with their mothers; animals were maintained under a 12:12 light:dark cycle (lights on at 0600 hours), and allowed free access to food and water at all times. Handling was uniform and kept as unstressful as possible. On postnatal day 30, the mothers were removed from the cages and the female offspring culled from the the litter. Male offspring were group (N = 4) housed in breeding tubs until sacrificed at approximately 90 days of age.

For purposes of behavioral analyses there were 14, 21, and 21 subjects in the normal control, pellet control, and DZ-exposed groups, respectively. Biochemical measurements were performed on 7 animals randomly chosen from each treatment condition. Since animals were chosen at random (from the different litters of dams in the respective treatment groups) for the biochemical analyses, the potential confound of alterations attributable to sampling from a single litter was avoided.

In addition to the initial study of the effects of perinatal diazepam exposure on exploratory behavior and mesotelencephalic DA turnover, we performed a replication of the behavioral experiment. In this replication, the number of groups and their treatments did not differ from that described above. However, in the replicate experiment, rather than removing tissue samples for measurements of DA function, a number of brain regions were removed and subjected to other biochemical and morphological studies. Since these studies are in progress, we will discuss only the behavioral data obtained from the replicate experiment.

Diazepam Treatment

DZ-containing silastic pellets were prepared and implanted on gestational day 8 following the procedure developed by Gallagher *et al.* (20). This method has been shown to result in the continuous release of DZ from implanted pellets for three weeks (average release of 5.0 mg/kg/24 hours); brain DZ levels are maintained between 200 and 300 ng/g wet weight. DZ exposure occurred from embryonic day 8 (E8) through the first postnatal week. Postnatal exposure occurred as a consequence of the transfer of DZ via the milk. The release of DZ from implanted pellets declines to minimal levels following the three-week exposure period (20).

Pieces of silastic tubing of 64 mm length were cut and sealed with silastic adhesive. Pellets for dams receiving DZ implants were filled with 90 mg recrystallized diazepam (Hoffmann-La Roche Inc.). One hour before implantation, the pellets were soaked in absolute ethanol for 30 minutes and in a 1% bovine serum albumin solution containing neomycin for 30 minutes. Animals were anesthetized with halothane (Ayerst Laboratories), and the skin overlying the interscapular space was locally anesthetized with carbocaine hydrochloride (Winthrop Breon). Three diazepam-loaded pellets were inserted subcutaneously, and the incision closed with wound clips. Pellet control animals were anesthetized as described above, and implanted with three empty silastic pellets; normal control animals were not anesthetized and did not receive pellet implants.

Exploratory Behavior

Exploratory behavior was assessed using a hole board apparatus (14,15); a dark grey box (floor dimensions: 66 × 54 cm; walls: 35 cm) with single holes (2.5 cm in diameter) drilled in each of the four sides at a point 3.5 cm above the floor and equidistant from each end. Nine equal size squares, delineated by 2.5 cm lines, were painted on the floor. Behavioral assessments were done at both 60 and 90 days after birth. All animals were tested between 1830 to 2100 hours to avoid errors attributable to variations in motor activity and head-dipping at different points during the activity cycle (14). The test room was darkened and the apparatus dimly illuminated with a red light placed 5 feet above the floor. The treatment condition of the subjects was not known to the examiner during testing. An animal was placed in the center square with its head facing away from the examiner and a number of behavioral parameters were recorded for 10 minutes (see below). The floor of the test apparatus was cleaned after each test session.

Five variables were scored. These included 1) the time elapsed before the animal left the center square when first placed in the box; 2) the time elapsed before the first head dip; 3) the total number of head dips made into any of the four holes; 4) the total time spent head dipping; and 5) total number of different holes into which the animal dipped its head at least once (range 1–4). A head dip was scored if the animal's eyes were not visible when it placed its head into a hole. Very brief head dips were arbitrarily given a value of 1 second due to the difficulty of accurately measuring the duration of such brief movements with a stopwatch (15,16). The first two variables have been suggested to reflect both locomotor and exploratory behavior (17,18), while the latter measures have been suggested to assess different aspects exploratory behavior alone (15,16).

Biochemical Determinations

Animals were sacrificed by decapitation at approximately 90 days after birth, and their brains rapidly removed and transferred to a chilled dissecting stage. The ventral tegmental area (VTA), nucleus accumbens (NAS), anteromedial prefrontal cortex (PFC), substantia nigra (SN), olfactory tubercle, and striatum were dissected as previously described (11). The septum was removed from the slice containing the striatum by placing a horizontal cut at the dorsal border of the anterior commissure and removing the tissue between the lateral ventricles by teasing it away from the overlying corpus callosum. The amygdala was punch (1.5 mm diameter) dissected from the next caudal slice. DA and DOPAC levels were measured by HPLC-EC as previously described (40); protein content was assessed using the method of Lowry *et al.* (36).

Data Analysis

Data were analyzed by means of analyses of variance (29). Planned comparisons (29) between normal control and pellet control subjects were conducted; in cases where group means did not differ, scores from the two control groups were collapsed. In the present study, alpha was set at $p = 0.05$. [In a previous report examining the effects of stress on animals exposed to DZ during the perinatal period (12) with a larger number of animals, alpha was set at $p = 0.01$ to control for experimentwise error (29).]

RESULTS

Exploratory Behavior

At *60 days* of age (see Table 1) there were no significant

TABLE 1

THE EFFECTS OF PERINATAL DIAZEPAM EXPOSURE ON LOCOMOTOR AND EXPLORATORY BEHAVIOR AT 60 DAYS AFTER BIRTH

	1 Normal Control (n=14)	2 Pellet Control (n=21)	3 DZ-Exposed (n=21)	F
Latency out of center (seconds)	1.36 ± 0.20	2.20 ± 0.32	1.62 ± 0.22	2.53
Latency to first hole (seconds)	87.00 ± 9.92	71.00 ± 8.37	80.05 ± 9.42	0.70
Number holes explored	17.57 ± 1.52	12.57 ± 1.17	13.71 ± 0.92	4.32*
Contrast: 1 vs. 2				8.25†
1 vs. 3				4.91*
2 vs. 3				0.54
Total time spent exploring (seconds)	71.43 ± 12.22	37.38 ± 4.43	45.48 ± 5.01	5.85†
Contrast: 1 vs. 2				11.23†
1 vs. 3				6.52*
2 vs. 3				0.79
Number different holes explored	3.93 ± 0.07	3.95 ± 0.05	3.81 ± 0.11	0.89

Mean values (± S.E.M.) for normal control, pellet control, and DZ-exposed subjects are shown and the results of group comparisons are indicated.
*$p<0.05$; †$p<0.01$.

differences between normal control, pellet control, and DZ-exposed animals in latency out of the center square, latency to explore the first hole, and number of different holes explored. However, normal control subjects exhibited significantly higher scores than pellet control and DZ-exposed animals in the total number of holes explored and total time spent exploring.

At *90 days* of age (see Table 2), subjects in the three groups did not differ significantly in latency to leave the center square and latency to explore the first hole. Again, significant group differences in the total number of holes explored and the total time spent exploring were observed. Subjects in all three groups differed significantly from one another on both variables, with the following rank-ordering: normal control > pellet control > DZ-exposed. Subjects in the three groups also differed significantly with regard to the number of different holes explored such that normal control = pellet control > DZ-treated animals.

In the replication of the behavioral component of the original study, no significant differences were observed at *60 days* of age between normal control, pellet control, and DZ-exposed animals in any of the behavioral measures (data not shown). At *90 days* of age there was no significant differences between the three groups in latency to leave the center square ($F=0.39$, n.s.) and latency to explore the first hole ($F=0.30$, n.s.). However, DZ-exposed animals scored significantly lower than both normal and pellet control animals in the number of holes explored ($F=5.49$, $p\leq0.05$), total time spent exploring ($F=5.50$, $p<0.05$), and the number of different holes explored ($F=7.81$, $p<0.05$). Subjects in the normal control and pellet control groups did not differ significantly from one another on these measures.

Catecholamine Biochemistry

DOPAC/DA (an index of DA turnover), and DA concentrations did not differ significantly across treatment conditions in the olfactory tubercle, amygdala, striatum, septum, or prefrontal cortex (data not shown). Significant group differences in DOPAC/DA were observed only in the VTA and NAS (see Fig. 1). In the VTA, DOPAC/DA was significantly lower in DZ-exposed subjects compared to normal control and pellet control subjects, which did not differ significantly from one another. In the NAS, DOPAC/DA was significantly higher in normal controls than either pellet control or DZ-exposed subjects. DA turnover in DZ-exposed subjects tended to be lower than in pellet control animals, but this trend was not statistically significant.

Significantly higher concentrations of DA were found in the VTA and NAS of animals perinatally exposed to DZ than in normal control and pellet control subjects (see Table 3); concentrations of the two amines in the latter groups did not differ from one another. In contrast, in the SN of animals perinatally exposed to DZ, DA concentrations were significantly lower than in normal and pellet control subjects. DOPAC/DA did not vary across treatment groups in the SN.

DISCUSSION

Perinatal exposure to DZ resulted in long-lasting behavioral changes and concomitant alterations in DA turnover restricted to certain mesolimbic DA system sites. Rats treated with the anxiolytic benzodiazepine exhibited significantly less exploratory behavior than control animals. Perinatal DZ treatment also resulted in a significant decrease in DA turnover in the VTA of 90-day-old animals; changes in DA turnover in the NAS were also observed.

The behavioral alterations seen in DZ-exposed subjects followed a developmentally specific pattern of change. At 90 days of age, DZ-exposed subjects engaged in significantly less exploratory behavior than normal control or pellet control subjects. In contrast,

TABLE 2

THE EFFECTS OF PERINATAL DIAZEPAM EXPOSURE ON LOCOMOTOR AND EXPLORATORY BEHAVIOR AT 90 DAYS AFTER BIRTH

	1 Normal Control (n=14)	2 Pellet Control (n=21)	3 DZ-Exposed (n=21)	F
Latency out of center (seconds)	1.21 ± 0.11	1.20 ± 0.09	1.47 ± 0.19	1.20
Latency to first hole (seconds)	44.77 ± 8.47	102.29 ± 17.21	108.47 ± 23.97	3.09
Number holes explored	8.08 ± 0.86	5.45 ± 0.85	2.88 ± 0.47	10.25‡
Contrast: 1 vs. 2				5.54*
1 vs. 3				20.28‡
2 vs. 3				6.46*
Total time spent exploring (seconds)	22.58 ± 3.27	13.60 ± 2.44	6.94 ± 1.34	9.51‡
Contrast: 1 vs. 2				6.69*
1 vs. 3				19.01‡
2 vs. 3				4.50*
Number different holes explored	3.64 ± 0.23	2.86 ± 0.29	2.15 ± 0.31	5.98†
Contrast: 1 vs. 2				3.36
1 and 2 vs. 3				9.82†

Mean values (± S.E.M.) in normal control, pellet control, and DZ-exposed subjects are shown and the results of group comparisons are indicated.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

DZ-exposed subjects did not differ from exploratory behavior, while both groups engaged in significantly less explatory behavior than normal controls. These data suggest that two factors may lead to alterations in exploratory behavior in the adult animal: 1) perinatal DZ exposure and 2) stress associated with the implantation procedure. Behavioral alterations seen only in DZ-treated subjects but not in animals in the two control groups (normal control or pellet control) probably reflect the effects of perinatal DZ exposure. In contrast, alterations seen in pellet control and DZ-exposed subjects but not normal control subjects probably reflect the effects of "prenatal stress." It is unclear to what degree the factor which is rather loosely defined as prenatal stress is specifically attributable to anesthesia as opposed to other aspects of the pellet implantation procedure (i.e., changes in maternal hormone levels associated with the stress of surgery which impact on fetal development); we have herefore chosen to use the general term prenatal stress. Thus, the alterations in exploratory behavior seen at 60 days (normal control > pellet control = DZ-treated) are probably a consequence of prenatal stress; behavioral alterations seen in DZ-exposed subjects at 90 days (normal control > pellet control > DZ-treated) are probably associated with both perinatal DZ exposure and prenatal stress.

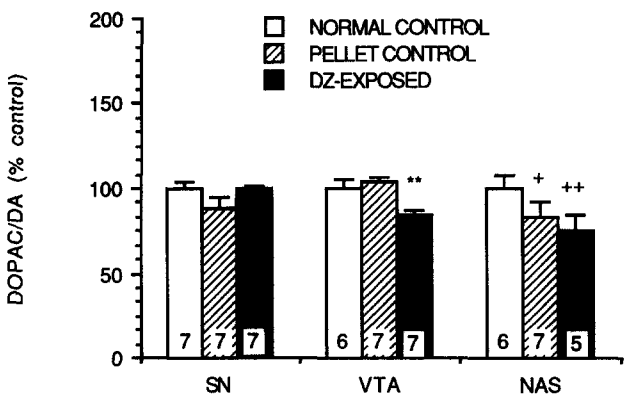

FIG. 1. The effects of perinatal diazepam exposure on DA turnover (DOPAC/DA) in the substantia nigra (SN), ventral tegmental area (VTA), and nucleus accumbens (NAS). The three bars in each brain region reflect mean DOPAC/DA (±S.E.M.) expressed as percent normal control for normal control, pellet control, and DZ-exposed subjects. Numbers in bars refer to the number of observations. None of the other sites examined showed an alteration in DA turnover (see text). **$p\leq0.01$, in reference to combined normal and pellet control groups; $^{+}p\leq0.05$, in reference to normal control group. $^{++}p\leq0.01$, in reference to normal control group.

In the replication of the original experiment, the same pattern of developmental specificity was observed with regard to alterations in exploratory behavior. In this latter study, however, pellet control and normal control subjects did *not* differ significantly from one another at 60 or 90 days, whereas in our initial study (see above), differences between the two control groups were apparent at both 60 and 90 days. The slightly different results in the replicate experiment (i.e., the lack of differences between the two control groups) probably reflect exposure of subjects in the two experiments to different degrees of prenatal stress. The salient point for purposes of the present discussion is that DZ treatment impacted on adult behavior in both the original study and the replication in a developmentally specific fashion. This indicates that perinatal DZ exposure has a significant and reliable effect on exploratory behavior in the adult rat. The present findings are in

TABLE 3

THE EFFECTS OF PERINATAL DIAZEPAM EXPOSURE ON DOPAMINE (DA) AND DOPAC LEVELS IN THE SUBSTANTIA NIGRA (SN), VENTRAL TEGMENTAL AREA (VTA), AND NUCLEUS ACCUMBENS (NAS)

	Normal Control		Pellet Control		DZ-Exposed	
	DA	DOPAC	DA	DOPAC	DA	DOPAC
SN	6.5 ± 0.9	1.4 ± 0.2	7.7 ± 0.3	1.3 ± 0.1	5.0 ± 0.3*	1.1 ± 0.1
VTA	27.5 ± 4.5	5.6 ± 0.7	21.1 ± 1.7	4.8 ± 0.4	40.0 ± 1.9‡	7.0 ± 0.3†
NAS	56.3 ± 6.2	10.0 ± 0.6	45.9 ± 4.8	7.1 ± 0.6	73.3 ± 5.5*	10.1 ± 0.9

Mean values (± S.E.M.) for normal control, pellet control, and DZ-exposed subjects, expressed in ng/mg protein, are shown and the results of group comparisons indicated.

*$p \leq 0.05$, in reference to combined normal and pellet control; †$p \leq 0.01$, in reference to combined normal and pellet control; ‡$p \leq 0.001$, in reference to combined normal and pellet control.

accord with previous studies that indicate that perinatal exposure to DZ results in behavioral (26,28), biochemical (51), and receptor (46) changes in the rat that may not be apparent until later developmental stages.

The effects of perinatal DZ exposure on adult DA turnover were regionally specific: alterations in DOPAC/DA were observed in the VTA and the NAS, but not in the other sites examined. DOPAC/DA was lower in the VTA of DZ-exposed subjects as opposed to subjects in either the normal or pellet control groups; DA utilization between the two control groups did not differ significantly. These data suggest that exposure to DZ during the perinatal period results in decreased DA utilization in the VTA, and that the effect on DA utilization in the adult is not a consequence of prenatal stress. In contrast, DA turnover in the NAS was significantly lower in the DZ-treated and pellet control groups than in the normal control group. These data suggest that the observed decreases in DA turnover in the NAS may be associated with prenatal stress rather than perinatal DZ exposure. It should be noted that the interpretation of alterations in DOPAC/DA in the DA cell body regions (such as the VTA) may differ from that occurring in DA terminal field regions, such as the NAS. Nonetheless, the biochemical measurements are important in that the only significant changes observed in biochemical parameters of DA function were restricted to certain mesolimbic (NAS and VTA) sites.

One possible interpretation of the present results is that the DZ-elicited decrease in DA turnover in the VTA reflects a diminished autoregulatory tone, which is paralleled by a compensatory response of decreased turnover in the terminal field (NAS) region. This hypothesis would also account for the decrease in exploration, a NAS-mediated behavior. Further experiments will be required to specifically address this possibility.

The mechanisms through which perinatal benzodiazepine exposure may influence adult behavior and DA function are unclear, but may involve interactions of DZ with the benzodiazepine receptor/GABA receptor/chloride ionophore complex (54). It is possible that perinatal exposure to DZ results in changes in the density and/or affinity of the benzodiazepine/GABA receptor. While early studies did not reveal changes in [^{3}H]benzodiazepine or GABA binding following prenatal DZ exposure (2, 28, 39), more recent reports suggest that perinatal exposure to DZ may alter benzodiazepine receptor density in a regionally specific manner (33, 34, 45). Changes in the density or affinity of the benzodiazepine receptor could alter the dynamics of GABAergic neurons which modulate DA release and turnover (5, 42, 49). Alternatively, benzodiazepine receptors appear to be present on some midbrain DA neurons (10); alterations in the density or affinity of these receptors may directly affect DA function. Perinatal exposure to DZ has also been shown to lead to alterations in a low affinity form of the $GABA_A$ receptor (23); this may also be associated with corresponding changes in DA activity.

The localization of alterations in DA function to certain mesolimbic sites (NAS and VTA), but not other mesotelencephalic areas, is probably not due to regional differences in the distribution of the benzodiazepine/GABA receptor. Recent studies (10, 37, 57, 58) indicate a moderate to high benzodiazepine receptor density in all areas examined in the present study with the exception of the striatum and VTA, where the number of sites is somewhat lower.

In light of the rather uniform distribution of the benzodiazepine receptor across the mesotelencephalic sites examined, the regionally restricted effects of the perinatal diazepam treatment suggest that perinatal DZ exposure may have a differential impact on the ontogeny of the benzodiazepine/GABA receptor in discrete brain regions. In the present study, DZ exposure occurred from E8 through the first postnatal week. This period appears to overlap with the ontogenesis of the benzodiazepine/GABA receptor. However, the synaptic arrangements of cellular elements (including GABAergic neurons) develop over very different time courses in different brain regions (e.g., substantia nigra and cortex), including an extended postnatal developmental sequence; it is possible that the perinatal DZ treatment differentially alters synaptic arrangements in different brain regions. Similarly, while the perinatal exposure to the benzodiazepine overlaps with the ontogenesis of mesencephalic DA neurons, the time course associated with the final density and arrangement of telencephalic DA projections differs considerably across forebrain sites.

The present data suggest that perinatal exposure to DZ is associated with significant alterations in both exploratory behavior and DA turnover in certain mesolimbic brain areas. The developmental course associated with the behavioral disturbances bears an interesting resemblance to the point in time at which initial psychotic breaks generally occur in schizophrenia (typically the period of transition from adolescence to young adulthood). Moreover, recent data suggest that perinatal factors (such as long labor and perinatal hypoxia) may be more closely associated with the subsequent development of schizophrenia than previously realized (9,41). Further, it has been suggested that benzodiazepines or benzodiazepine augmentation of neuroleptic medications may be therapeutically advantageous in certain cases in the treatment of schizophrenic patients (7,56). These observations suggest that it will be critical to define perinatal events which may alter, in a developmentally specific fashion, behavior and the neural substrates of behavior.

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REFERENCES

1. Avnimelech-Gigus, N.; Feldon, J.; Tanne, Z.; Gavish, M. The effects of prenatal chlordiazepoxide administration on avoidance behavior and benzodiazepine receptor density in adult albino rats. Eur. J. Pharmacol. 129:185–188; 1986.
2. Braestrup, C.; Nielsen, M.; Squires, R. F. No changes in rat benzodiazepine receptors after withdrawal from continuous treatment with lorazepam and diazepam. Life Sci. 24:347–350; 1979.
3. Brudzynski, S. M.; Morgenson, G. J. Association of the mesencephalic locomotor region with locomotor activity induced by injections of amphetamine into the nucleus accumbens. Brain Res. 334:77–84; 1985.
4. Buttar, H. S. Effects of chlordiazepoxide on the pre- and postnatal development of rats. Toxicology 17:311–321; 1980.
5. Cheramy, A.; Nieoullon, A.; Glowinski, J. GABAergic processes involved in the control of dopamine release from nigro-striatal dopaminergic neurons in the cat. Eur. J. Pharmacol. 48:281–295; 1978.
6. Christmas, A. J.; Maxwell, D. R. A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behaviour in mice and rats. Neuropharmacology 9:17–29; 1970.
7. Cohen, S.; Khan, A. Adjunctive benzodiazepines in acute schizophrenia. Neuropsychobiology 18:9–12; 1987.
8. Cree, J. E.; Meyer, J.; Hailey, D. M. Diazepam in labour: Its metabolism and effect on the clinical condition and thermogenesis of the newborn. Br. Med. J. 4:251–255; 1973.
9. DeLisi, L. E.; Dauphinais, I. D.; Gershon, E. S. Perinatal complications and reduced size of brain limbic structures in familial schizophrenia. Schizophr. Bull. 14:185–191; 1988.
10. Deutch, A. Y.; Roth, R. H. The determinants of stress-induced activation of the prefrontal cortical dopamine system. Prog. Brain Res.; in press.
11. Deutch, A. Y.; Tam, S.-Y.; Roth, R. H. Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Res. 333:143–146; 1984.
12. Deutch, A. Y.; Gruen, R. J.; Roth, R. H. The effects of perinatal diazepam exposure on stress-induced activation of the mesotelecephalic dopamine system. Neuropsychopharmacology 2:105–114; 1989.
13. Fadda, F.; Argiolis, A.; Melis, M. R.; Tissari, A. H.; Onali, P. L.; Gessa, G. L. Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in n. accumbens: Reversal by diazepam. Life Sci. 23:2219–2224; 1978.
14. File, S. E.; Day, S. Effects of time and day and food deprivation on exploratory activity in the rat. Anim. Behav. 20:758–762; 1972.
15. File, S. E.; Wardill, A. G. The reliability of the hole-board apparatus. Psychopharmacologia 44:47–51; 1975.
16. File, S. E.; Wardill, A. G. Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacologia 44:53–59; 1975.
17. Fink, J. S.; Smith, G. P. Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res. 199:359–384; 1980.
18. Fink, J. S.; Smith, G. P. Mesolimbic and mesocortical dopaminergic neurons are necessary for normal exploratory behavior in rats. Neurosci. Lett. 17:61–65; 1980.
19. Gai, N.; Grimm, V. E. The effect of prenatal exposure to diazepam on aspects of postnatal development and behavior in rats. Psychopharmacology (Berlin) 78:225–229; 1982.
20. Gallagher, D. W.; Malcolm, A. B.; Anderson, S. A.; Gonsalves, S. F. Continuous release of diazepam: Electrophysiological, biochemical and behavioral consequences. Brain Res. 342:26–36; 1985.
21. Gavish, M.; Avnimelech-Gigus, N.; Feldon, J.; Myslobodsky, M. Prenatal chlordiazepoxide effects on metrazol seizures and benzodiazepine receptor density in adult albino rats. Life Sci. 36(18): 1693–1698; 1985.
22. Grimm, V. E. A review of diazepam and other benzodiazepines in pregnancy. In: Yanai, J., ed. Neurobehavioral teratology. Amsterdam: Elsevier Science Publishers; 1984:153–162.
23. Gruen, R. J.; Elsworth, J. D.; Roth, R. H. Regionally specific alterations in the low affinity $GABA_A$ receptor following perinatal exposure to diazepam. Brain Res.; in press.
24. Herman, J. P.; Choulli, J.; Abrous, N.; Dulluc, J.; LeMoal, M. Effects of intra-accumbens dopaminergic grafts on behavioral deficits induced by 6-OHDA lesions of the nucleus accumbens or A10 dopaminergic neurons: A comparison. Behav. Brain Res. 29:73–83; 1988.
25. Kelley, A. E.; Stinus, L. Neuroanatomical and neurochemical substrates of affective behavior. In: Fox, N. A.; Davidson, R. J., eds. The psychobiology of affective behavior. Hillsdale, NJ: Lawrence Erlbaum Assoc.; 1984:1–75.
26. Kellogg, C.; Ison, J. R.; Miller, R. K. Prenatal diazepam exposure: Effects on auditory temporal resolution in rats. Psychopharmacology (Berlin) 79:332–337; 1983.
27. Kellogg, C.; Tervo, D.; Ison, J.; Parisi, T. Prenatal exposure to diazepam alters behavioral development in rats. Science 207:205–207; 1980.
28. Kellogg, C. K.; Chisholm, J.; Simmons, R. D.; Ison, J. R.; Miller, R. K. Neural and behavioral consequences of prenatal exposure to diazepam. Monogr. Neural. Sci. 9:119–129; 1983.
29. Keppel, G. Design and analysis: A researcher's handbook. Englewood Cliffs, NJ: Prentice-Hall Inc.; 1973.
30. Kolata, G. B. Behavioral teratology: Birth defects of the mind. Science 202:732–734; 1978.
31. Lauer, J. A.; Adams, P. M.; Johnson, K. M. Perinatal diazepam exposure: Behavioral and neurochemical consequences. Neurotoxicol. Teratol. 9:213–219; 1987.
32. Lavielle, S.; Tassin, J.-P.; Theirry, A.-M.; Blanc, G.; Herve, D.; Barthelemy, C.; Glowinski, J. Blockade by benzodiazepines of the selective high increase in dopamine turnover induced by stress in mesocortical dopamine neurons of the rat. Brain Res. 168:585–594; 1979.
33. Livezey, G. T.; Marczynski, T. J.; Isaac, L. Prenatal diazepam: Chronic anxiety and deficits in brain receptors in mature rat progeny. Neurobehav. Toxicol. Teratol. 8:425–432; 1986.
34. Livezey, G. T.; Marczynski, T. J.; Isaac, L. Enduring effects of prenatal diazepam on the behavior, EEG, and brain receptors of the adult cat progeny. Neurotoxicology 7:2:319–334; 1986.
35. Livezey, G. T.; Marczynski, T. J.; McGrew, E. A.; Beluhan, F. Z. Prenatal exposure to diazepam: Late postnatal teratogenic effect. Neurobehav. Toxicol. Teratol. 8:433–440; 1986.
36. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin reagent. J. Biol. Chem. 193:265–275; 1951.
37. Marcel, D.; Weissmann-Nanopoulos, D.; Mach, E.; Pujol, J. F. Benzodiazepine binding sites: Localization and characterization in the limbic system of the rat brain. Brain Res. Bull. 16:573–596; 1986.
38. Marczynski, T. J.; Hawkins, M. C.; Swann, P. G.; Krivograd, A. F.; Patel, M. K.; Dugich, M. Perinatal upregulation of benzodiazepine receptor ontogenesis: "Fearless" and more efficient goal-directed behavior of adult rat progenies. Neurotoxicol. Teratol. 10:101–111; 1988.
39. Massotti, M.; Alleva, F. R.; Balazs, T.; Guidotti, A. GABA and

benzodiazepine receptors in the offspring of dams receiving diazepam: Ontogenetic studies. Neuropharmacology 19:951–956; 1980.
40. Michaud, R. L.; Bannon, M. J.; Roth, R. H. The use of C8-octyl columns for the analysis of catecholamines by ion pair reverse-phase liquid chromatography with amperometric detection. J. Chromatogr. 225:325–34; 1981.
41. Nassrallah, H. A. The differential diagnosis of schizophrenia: Genetic, perinatal, neurological, pharmacological and psychiatric factors. In: Nassrallah, H. A.; Weinberger, D. R., eds. Handbook of schizophrenia, vol. 1: The neurology of schizophrenia. Amsterdam: Elsevior; 1986:49–63.
42. Palfreyman, M. G.; Hout, S.; Lippert, B.; Schecter, P. J. The effect of λ-acetylenic GABA, an enzyme-activated irreversible inhibitor of GABA-transaminase, on dopamine pathways of the extrapyramidal and limbic systems. Eur. J. Pharmacol. 50:325–336; 1978.
43. Rementeria, J. L.; Bhatt, K. Withdrawal symptoms in neonates from intrauterine exposure to diazepam. Pediatr. Pharmacol. Ther. 90: 1:123–126; 1977.
44. Roth, R. H.; Tam, S.-Y.; Ida, Y.; Yang, J.-X.; Deutch, A. Y. Stress and the mesocorticolimbic dopamine systems. Ann. NY Acad. Sci. 537:138–147; 1988.
45. Rothe, T.; Bigl, V. The ontogeny of benzodiazepine receptors in selected regions of the rat brain: Effect of perinatal exposure to diazepam. Neuropharmacology 28:503–508; 1989.
46. Rothe, T.; Langer, M. Prenatal diazepam exposure affects β-adrenergic receptors in brain regions of adult rat offspring. J. Neurochem. 51:1361–1366; 1988.
47. Ryan, C. L.; Pappas, B. A. Intrauterine diazepam exposure: Effects on physical and neurobehavioral development in the rat. Neurobehav. Toxicol. Teratol. 8:279–286; 1986.
48. Scatton, B.; D'Angio, M.; Driscoll, P.; Serrano, A. An in vivo voltametric study of the response of mesocortical and mesoaccumbens dopaminergic neurons to environmental stimuli in strains of rats with differing levels of emotionality. Ann. NY Acad. Sci. 537:124–137; 1988.
49. Scheel-Kruger, J.; Cooks, A. R.; Honig, W. Muscimol antagonizes the ergometrine-induced locomotor activity in nucleus accumbens: Evidence for a GABA-dopaminergic interaction. Eur. J. Pharmacol. 42:311–313; 1977.
50. Shore, C. O.; Vorhees, C. W.; Bornschein, R. L.; Stemmer, K. Behavioral consequences of prenatal diazepam exposure in rats. Neurobehav. Toxicol. Teratol. 5:565–570; 1983.
51. Simmons, R. D.; Kellogg, C. K.; Miller, R. K. Prenatal diazepam exposure in rats: Long-lasting, receptor-mediated effects on hypothalamic norepinephrine-containing neurons. Brain Res. 293:73–83; 1984.
52. Simmons, R. D.; Miller, R. K.; Kellogg, C. K. Prenatal exposure to diazepam alters central and peripheral responses to stress in adult rat offspring. Brain Res. 307:39–46; 1984.
53. Taghzouti, K.; Simon, H.; Louilot, A.; Herman, J. P.; LeMoal, M. Behavioral study after local injections of 6-hydroxydopamine into the nucleus accumbens in the rat. Brain Res. 344:9–20; 1985.
54. Tallman, J. F.; Gallager, D. W. The GABAergic system: A locus of benzodiazepine action. Annu. Rev. Neurosci. 8:21–44; 1985.
55. Thierry, A. M.; Tassin, J. P.; Blanc, G.; Glowinski, J. Selective activation of the mesocortical DA system by stress. Nature 263: 242–244; 1976.
56. Wolkowitz, O. M.; Breier, A.; Doran, A.; Kelsoe, J.; Lucas, P.; Paul, S. M.; Pickar, D. Alprazolam augmentation of the antipsychotic effects of fluphenazine in schizophrenic patients. Arch. Gen. Psychiatry 45:664–671; 1988.
57. Young, W. S.; Kuhar, M. J. Radiohistochemical localization of benzodiazepine receptors in rat brain. J. Pharmacol. Exp. Ther. 212(2):337–346; 1980.
58. Young, W. S.; Niehoff, D.; Kuhar, M. J.; Beer, B.; Lippa, A. S. Multiple benzodiazepine receptor localization by light microscopic radiohistochemistry. J. Pharmacol. Exp. Ther. 216:425–430; 1981.