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Aging: Changes in a Passive-Avoidance Response With Brain Levels of Temazepam

H. L. KOMISKEY, A. RAHMAN AND K. L. MUNDINGER

Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford
1601 Parkview Avenue, Rockford, IL 61107

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KOMISKEY, H. L., A. RAHMAN AND K. L. MUNDINGER. *Aging: Changes in a passive-avoidance response with brain levels of temazepam.* PHARMACOL BIOCHEM BEHAV 31(3) 611–615, 1988.—Acute intravenous (IV) injections of temazepam were examined for the ability to impair the performance of young (3–4-month-old), mature (12–15-month-old) and old (28–30-month-old) male Fischer 344 rats in the step-down task relative to vehicle-injected controls. The effect of temazepam on the passive-avoidance response could be characterized as a U-shaped function of age. The performance of the mature rat was not significantly impaired by an IV injection of temazepam between 18 and 320 μg/kg. Temazepam was more effective in impairing the performance of the young and old rat. The brain levels of temazepam after a single IV injection of 18 μg/kg in mature and senescent rats, and 32 μg/kg in young rats were measured over a 2-hour time period. The brain of the mature rat was exposed to less temazepam between 0 and 120 minutes than the brain of the old rat. Therefore, the increased sensitivity of the senescent rat relative to the mature rat may in part be due to changes in the pharmacokinetics of temazepam. However, the inability of temazepam (between 18 and 320 μg/kg) to impair the performance of mature rats in the passive-avoidance task suggests that pharmacodynamic changes may be involved in the decreased sensitivity of mature rats relative to young and senescent rats.

Temazepam Rat Passive-avoidance Age-dependent

HUMANS and rodents show an age-related sensitivity to the behavioral effect of diazepam (7, 10, 17, 27). For example, the sensitivity of humans and the male Fischer 344 rat to the central nervous system (CNS) depressant effect of diazepam increased with age (10, 16, 27). Furthermore, senescent humans and rodents may have an increased sensitivity to the memory impairing effects of diazepam (17,25). In addition, New Zealand Black mice and male Fischer 344 rats show an age-related increased sensitivity to the diazepam-induced ataxia (7) and anticonflict effect (16), respectively.

Temazepam, a metabolite of diazepam, has been used as a hypnotic for five years in the United States (11). Although other benzodiazepines have been reported to produce age-related behavioral effects (21,25), no published information is available on the possible age-dependent effects of temazepam. Because diazepam has been shown to elicit an age-associated impairment of a passive-avoidance response in the male Fischer 344 rat (17), this strain of rat was examined for an age-related impairment of the step-down task by temazepam.

Age-dependent changes in the distribution of benzodiazepines are known to occur in humans and rats (14, 26, 35, 39). However, no one has investigated the kinetics of whole brain temazepam concentrations in relation to behavioral effects. Therefore, in the present study, young, mature and old male Fischer 344 rats were examined for age-associated changes in brain levels of temazepam after an IV injection of a dosage used in the passive-avoidance task.

METHOD

Animals

Young (3–4-month-old), mature (12–15-month-old), and senescent (28 plus-month-old) male Fischer 344 rats (Harlan Sprague Dawley and Charles River Breeding Laboratories from colonies sponsored by the National Institute on Aging) were housed one per cage. Water and food were available in the home cage. The vivarium had a 12 hr light/dark cycle and an ambient room temperature of 24°C.

Drugs and Reagents

Carbon-14 temazepam (8.1 and 12.5 mCi/mmol) was obtained from ICN Chemical and Radioisotope Division, Irvine, CA. Temazepam and Ro15-1788 were obtained from Hoffmann-La Roche Inc., Nutley, NJ. Ethyl ether and HPLC grade methanol were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ) and Burdick and Jackson Laboratories Inc. (Muskegon, MI), respectively.

Drug Administration

Labelled and unlabelled temazepam were dissolved in a vehicle of 40% propylene glycol, 5% sodium benzoate and distilled water. The drug was injected via the lateral tail vein (0.05 ml/100 g of body weight). Control injections consisted of equal volumes of vehicles per body weight. A rigid plastic restrainer (E &M Instruments, Model 4) was used to provide

TABLE 1
EFFECT OF TEMAZEPAM ON PASSIVE-AVOIDANCE ACQUISITION

Treatment	Young N	Young Median min (25th–75th Percentile)	Mature N	Mature Median min (25th–75th Percentile)	Old N	Old Median min (25th–75th Percentile)
Control (Vehicle)	7	22.62(10.24–25.03)	7	12.53(10.61–25.90)	5	11.85(9.82–12.76)
3 μg/kg	3	6.27 (3.26– 9.28)	—	—	—	—
10 μg/kg	5	5.07 (2.56– 7.57)	—	—	3	3.39(2.64– 6.41)
18 μg/kg	5	6.43 (3.31– 9.56)	4	11.79 (8.46–15.12)	6	1.36(0.71– 4.44)*
32 μg/kg	7	2.71 (1.39– 4.97)*	3	7.86 (4.11–22.87)	4	3.42(1.76– 5.09)
56 μg/kg	3	7.64 (3.89–22.65)	4	6.87 (3.54–10.20)	—	—
100 μg/kg	6	4.61 (2.35– 9.88)	7	6.46 (4.27–10.22)	3	0.97(0.71– 2.04)
180 μg/kg	—	—	7	10.69 (7.62–26.47)	—	—
320 μg/kg	—	—	4	10.12 (5.12–20.13)	—	—

*Significantly different from control, $p<0.05$.

uniform restraint of the rats for approximately 1 min during the IV injection. A 28-gauge needle (Micro-fine III; Becton and Dickinson Consumer Products) was used in all the injections.

Step-Down Apparatus and Procedure

After a week of acclimation to the housing conditions, the animals were used in a step-down test conducted in a rectangular box (29 cm H × 29 cm W × 57 cm L) with an electrifiable grid floor and transparent Plexiglas® walls. A wooden disk 21 cm in diameter and 5 cm high was fixed in the center of the floor (on top of the electrifiable grid) of the rectangular box. A 23 cm high hollow Plexiglas® cylinder 21.5 cm in diameter was loosely fitted over the wooden disk. A white noise of approximately 80 dB (SPL) was used to mask any background noises.

Familiarization session(s). A naive rat was placed within the hollow cylinder on the wooden disk. The hollow cylinder was removed 15 sec later and the rat released. The animal was allowed 15 sec to explore and was then returned to its cage. The above procedure was repeated twice at 30 min intervals.

Training session. The training session started thirty min after the last familiarization session. A rat was placed within the hollow cylinder on the wooden disk 30 sec after it received an IV injection of either drug or the vehicle. Fifteen sec later the hollow cylinder was removed and the rat released. The time the rat remained on the wooden disk after raising the cylinder was recorded. After the rat had all four feet on the grid, a 1 mA shock was delivered by a Coulbourn shocker/distributor (E 13-16, Coulbourn Instruments, Inc.) through the electrifiable grid floor for 1 sec. The rat was then returned to its cage.

Test session. Fifteen hours after the training session the animal was placed within the hollow cylinder on the wooden disk. Fifteen sec later the animal was released by raising the cylinder. The time the rat remained on the wooden disk after raising the cylinder (maintained one foot or more on wooden disk) was recorded. The session was terminated if the rat remained on the wooden disk longer than 30 min.

Measurement of Brain Levels

Mature and senescent rats were injected with 18 μg ^{14}C-temazepam (1.5 μCi/ml)/kg. Similarly, young rats were injected with 32 μg ^{14}C-temazepam (1.7 μCi/ml)/kg. Rats of each age group were sacrificed 5, 10, 30, 60 and 120 min after the ^{14}C-temazepam injection by decapitation. Radiolabelled temazepam was extracted from the whole brain tissue above the cerebellum according to the method of Komiskey *et al.* (18). The extract was mixed with the required amount of internal standard (Ro15-1788). A 50 μl aliquot of the above mixture was analyzed for temazepam via high pressure liquid chromatography (HPLC). Reverse isotope dilution analysis was used to quantify temazepam in the above aliquot to a sensitivity of 1 pg/μl (18).

Data Analysis

Areas under the brain concentration-time curves (AUC) were determined by the Trapezoidal method (9). Age-associated changes in AUC were determined by analysis of variance. Significant differences between mean values were determined either by Student's *t* or Duncan's New Multiple Range Test (31).

Differences between the time the controls (vehicle-injected) and temazepam-injected rats remained on the wooden disk during the test session were analyzed by the Kruskal-Wallis test (41). In addition, the Kruskal-Wallis test was used to analyze the differences among the times the three age groups remained on the wooden disk during the test session after vehicle injections. The significance of the differences between values of the temazepam-injected animals and the values of the controls were determined using Shaffer's extension of the Kruskal-Wallis procedure (19).

RESULTS

The effect of temazepam on the time young, mature, and old rats remained on the wooden platform during the testing session is illustrated in Table 1. In the young rats at 32 μg/kg, temazepam produced a significant reduction in the step-

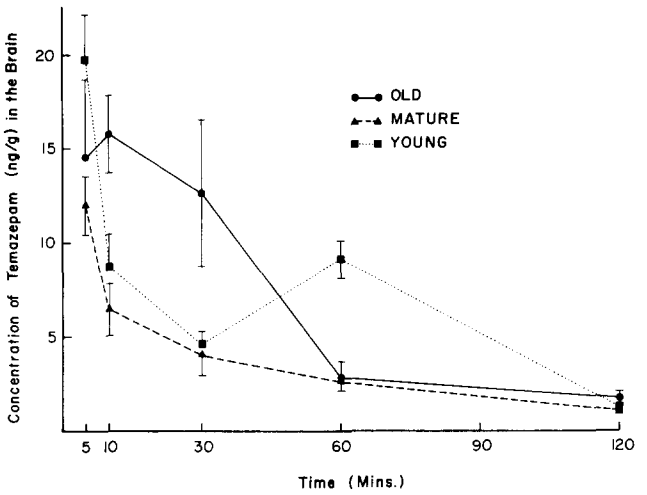

FIG. 1. Brain concentration-time profile of temazepam after intravenous injection of temazepam 32 μg/kg in young (■) and 18 μg/kg in mature (▲) and old (●) male Fischer 344 rats. Each data point is the mean±SE of four individual rats.

down latency relative to control rats. Temazepam was unable to significantly alter the step-down latency of mature rats between 18 and 320 μg/kg. On the other hand, temazepam caused a significant decrease in the step-down latency of old rats at 18 μg/kg.

The brain levels of temazepam at different time periods after an IV injection of 18 μg/kg in mature and senescent rats and 32 μg/kg in young rats are shown in Fig. 1. The brain AUCs between 0 to 30 min and 0 to 120 min after an IV injection of temazepam are shown in Table 2. The brain AUCs from 0 to 30 min and 0 to 120 min were significantly higher in the senescent rat relative to the mature rat. In addition, the brain AUC between 0 and 30 min in the senescent rat is significantly higher than in the young rat. On the other hand, the brain AUC from 0 to 120 min in the young rat is greater than in the mature rat.

TABLE 2

AREA UNDER THE BRAIN LEVEL TIME CURVE (0–30 AND 0–120 MIN) AFTER IV INJECTION OF TEMAZEPAM IN YOUNG, MATURE, AND OLD MALE FISCHER 344 RATS

Age Group	Dose (μg/kg)	0–30 min mean (ng·min/g) ±SEM*	0–120 min
Old	18	410 ± 30	800 ± 75
Mature	18	187 ± 22†	403 ± 18‡
Young	32	256 ± 12†	774 ± 35

*Mean ± standard error of the mean of four individual determinations.
†Significantly different from old, $p<0.05$.
‡Significantly different from young and old, $p<0.05$.

DISCUSSION

The ability of temazepam to impair passive-avoidance acquisition is consistent with previous studies with benzodiazepines (2, 6, 17, 20, 24). Furthermore, the increased sensitivity of old rats to the ability of temazepam to decrease passive-avoidance acquisition relative to the mature rats is in agreement with published results with diazepam (17). On the other hand, the inability of temazepam at the doses injected to decrease the step-down latency in mature rats is surprising. For example, the senescent rat is roughly ten times more sensitive to temazepam in the step-down task relative to the ability of IV diazepam to impair the performance of old rats of the same sex and strain in a step-through-type passive-avoidance task (17). In the latter step-through task, IV diazepam (360 μg/kg) caused a significant decrease in passive-avoidance acquisition in mature rats (17). Unfortunately, the solubility limits of temazepam in the vehicle prevented examination of the mature rat at higher doses of the drug. However, the present results also show that the young rat is more sensitive to the ability of temazepam to impair the passive-avoidance task relative to the effect in the mature rat. In summary, the age-related effect of temazepam on the step-down task can be characterized as a U-shaped function.

Although the effects of temazepam on the step-down task have been shown to vary with the age of the rat, the exact behaviors affected by temazepam were not identified. Because temazepam was injected prior to the training session, the above nonlinear age-related changes on the step-down task could be produced by a variety of variables. For example, it has been hypothesized that the effects of a drug given prior to passive-avoidance training may be due to changes in learning, memory, and stimulant activity (4). Other possible explanations of anxiolytic-induced suppression of passive-avoidance behavior include modification of "fear" reactions (13,34), state-dependent learning (2,24), sedation (21), and motivation (22). All of the above explanations or their interactions could be applied to the present data.

The behavior(s) altered by temazepam to suppress passive-avoidance responding may be dependent on the age of the animal. Although the sex of the rats was not specified, Fischer 344 rats have been reported to have an age-related reduction in step-through acquisition (20). In the present study, vehicle-injected mature and senescent rats relative to vehicle-injected young rats showed a nonsignificant reduction in step-down acquisition. The latter finding agrees with the results of other studies demonstrating that passive-avoidance responding does not significantly differ with age in the rat when measured within 1 day of the training sessions (5,30). Methodological difference may account for the above differences in results (28). However, visual and locomotor skills are known to decline markedly with age in the Fischer 344 rats (5). The age-dependent changes in visual and locomotor skills with or without lifelong sensory and social deprivation (rats housed singly) may elicit age-related variation in behavior of rats in the passive-avoidance task (28). The above age-dependent sensory and motor skills may also influence the drug-induced responses in the task.

The large fluctuations in the concentration of temazepam in the brain of young rats is not surprising. Previously, investigators have reported large fluctuations in benzodiazepine concentrations over time in the brain of young rodents after acute injections of the drug (8,40). The above phenomenon may be related to the large fluctuations in plasma concentrations of benzodiazepines reported after acute injections of the drug (1, 8, 36). The large fluctuations

in plasma concentrations of benzodiazepines have been theorized to be due to enterohepatic circulation of benzodiazepines and to redistribution of the drugs (12,36). However, it is unlikely that the free temazepam concentration in plasma water is the only factor influencing the age-associated AUCs of temazepam in the brain. For example, the unbound plasma concentration of diazepam did not show the age-related changes that occurred in the brain of male Fischer 344 rats after a single IV injection of the drug (26). Recently, Rahman *et al.* (26) have reviewed mechanisms that may account for the elevated brain concentrations of benzodiazepines in senescent rats.

The possible usefulness of AUC as a pharmacokinetic index to the behavioral effect of psychoactive drugs is illustrated in this study. As previously pointed out, the chemotherapeutic and teratogenic effect of some drugs correlates well with the AUC of the drug (26, 33, 38). Although it remains to be proven that the AUC for temazepam during a particular period of time is a good index of the drug's ability to impair performance on the step-down task, the present results may help guide future investigations. In the present study, a behavioral active dose of temazepam in old rats produced a significantly larger AUC of the drug in senescent rats relative to the same dose in mature rats. Even though the behaviorally active dose of temazepam in the passive-avoidance task varied in young and old rats, a similar AUC from 0 to 120 min was produced in each age group. In summary, the age-associated AUCs of temazepam indicates that the age-related effects of temazepam in the step-down task may in part be due to age-dependent changes in the pharmacokinetics of the benzodiazepine.

The biological bases for the age-related temazepam-induced impairment of passive-avoidance responding remains to be clarified. As discussed above, an age-related increase in brain levels of temazepam may be involved in the temazepam-induced impairment of passive-avoidance responding. Because benzodiazepine-induced impairment of passive-avoidance responding is partially blocked by a selective benzodiazepine antagonist (3, 32, 37), the temazepam-elicited impairment may be mediated through the benzodiazepine/GABA receptor complex. Therefore, age-related pharmacodynamic changes in the benzodiazepine/GABA receptor complex elicited by single IV benzodiazepine injections in naive rats may also be involved in the above age-dependent behavioral effects (15).

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REFERENCES

1. Baird, E. S.; Hailey, D. M. Plasma levels of diazepam and its major metabolites following intramuscular administration. Br. J. Anaesth. 45:546–547; 1973.
2. Berger, B. D.; Stein, L. Asymmetrical dissociation of learning between scopolamine and Wy4036, a new benzodiazepine tranquilizer. Psychopharmacologia 14:351–358; 1969.
3. Bonetti, E. P.; Pieri, L.; Cumin, R.; Schaffner, R.; Pieri, M.; Gramzu, E. R.; Muller, R. K.; Haefely, W. Benzodiazepine antagonist Ro15–1788: Neurological and behavioral effects. Psychopharmacology (Berlin) 78:8–18; 1982.
4. Butler, D. B.; Poshel, B. P.; Marriott, J. G. Cognition-activating properties of 3-(aryloxy)pyridines. J. Med. Chem. 24:346–350; 1981.
5. Campbell, B. A.; Krauter, E. E.; Wallace, J. E. Animal modes of aging: Sensory-motor and cognitive function in the aged rat. In: Stein, D. G., ed. The psychobiology of aging: Problems and perspectives. Amsterdam: Elsevier/North Holland Inc.; 1980:201–226.
6. Cole, S. O. Effects of benzodiazepines on acquisition and performance: A critical assessment. Neurosci. Biobehav. Rev. 10:265–272; 1986.
7. Forster, M. J.; Retz, R. C.; Popper, M. D.; Lal, H. Age-dependent enhancement of diazepam sensitivity is accelerated in New Zealand Black Mice. Life Sci. 38:1433–1439; 1986.
8. Friedman, H.; Abernathy, D. R.; Greenblatt, D. J.; Shader, R. I. The pharmacokinetics of diazepam and desmethyldiazepam in rat brain and plasma. Psychopharmacology (Berlin) 88:267–270; 1986.
9. Gibaldi, M.; Perrier, D. Estimation of areas. In: Pharmacokinetics. 2nd ed. New York: Marcel Dekker Inc.; 1982:445–449.
10. Giles, H. G.; MacLeod, S. M.; Wright, J. R.; Sellers, E. M. Influence of age and previous use on diazepam dosage required for endoscopy. Can. Med. Assoc. J. 118:513–514; 1978.
11. Greenblatt, D. J.; Shader, R. I.; Abernathy, D. R. Current status of benzodiazepines. N. Engl. J. Med. 309:354–358; 1983.
12. Guentert, T. W. Pharmacokinetics of benzodiazepines and of their metabolites. Prog. Drug Metab. 8:241–386; 1984.
13. Kameyama, T.; Nabeshima, T.; Kozawa, T. Step-down-type passive-avoidance and escape learning method. J. Pharmacol. Methods 16:39–52; 1986.
14. Klotz, U.; Avant, G. R.; Hoyumpa, A.; Schenker, S.; Wilkinson, G. R. The effects of age and liver disease on the disposition and elimination of diazepam in adult man. J. Clin. Invest. 55:347–350; 1975.
15. Komiskey, H. L. Aging: Effect on ex-vivo benzodiazepine binding after a diazepam injection. Neurochem. Res. 12:745–749; 1987.
16. Komiskey, H. L.; Buck, M. A.; Mundinger, K. L.; McSweeney, F. K.; Farmer, V. A.; Dougan, J. D. Diazepam elicits an age-related anticonflict and CNS depressant effect in the rat. Fed. Proc. 45:675; 1986.
17. Komiskey, H. L.; Cook, T. M.; Lin, C. F.; Hayton, W. L. Impairment of learning or memory in the mature and old rat by diazepam. Psychopharmacology (Berlin) 73:304–305; 1981.
18. Komiskey, H. L.; Rahman, A.; Weisenburger, W. P.; Hayton, W. L.; Zobrist, R. H.; Silvius, W. Extraction, separation, and detection of ^{14}C-diazepam and ^{14}C-metabolites from brain tissue of mature and old rats. J. Anal. Toxicol. 9:131–133; 1985.
19. Levy, K. J. Nonparametric applications of Shaffer's extension of Dunnett's procedure. Am. Stat. 34:99–102; 1980.
20. Lippa, A. S.; Pelham, R. W.; Beer, B.; Critchett, D. J.; Dean, R. L.; Bartus, R. T. Brain cholinergic dysfunction and memory in aged rats. Neurobiol. Aging 1:13–19; 1980.
21. Lister, R. G. The amnesic action of benzodiazepines in man. Neurosci. Biobehav. Rev. 9:87–94; 1985.
22. Mondadori, C. Avoidance conditioning: Methods and problems. Pharmacol. Ther. 5:423–425; 1979.
23. Overton, D. A. Experimental methods for the study of state-dependent learning. Fed. Proc. 33:1800–1813; 1974.
24. Patel, J. B.; Ciofalo, V. B.; Iorio, L. C. Benzodiazepine blockade of passive-avoidance task in mice: A state-dependent phenomenon. Psychopharmacology (Berlin) 61:25–28; 1979.

25. Pomara, N.; Stanley, B.; Black, R.; Guido, J.; Stanley, M.; Greenblatt, D. J.; Newton, R. E.; Gershon, S. Diazepam impairs performance in normal elderly subjects. Psychopharmacol. Bull. 20:137–139; 1984.
26. Rahman, A.; Komiskey, H. L.; Hayton, W. L.; Weisenburger, W. P. Aging: Changes in distribution of diazepam and metabolites in the rat. Drug Metab. Dispos. 14:299–302; 1986.
27. Reidenberg, M. M.; Levy, M.; Warner, H.; Countinko, C. B.; Schwartz, M. A.; Yui, G.; Cheripko, J. Relationship between diazepam, dose, plasma level, age and central nervous system depression. Clin. Pharmacol. Ther. 23:371–374; 1978.
28. Rigter, H. Pitfalls in behavioral aging research in animals. In: Gispen, W. H.; Traber, J., eds. Aging of the brain. New York: Elsevier Science Publ.; 1983:197–208.
29. Rigter, H. Vasopressin and memory: The influence of prior experience with the training situation. Behav. Neural Biol. 34:337–351; 1982.
30. Rigter, H.; Martinez, J. L.; Crabbe, J. C. Forgetting and other behavioral manifestations of aging. In: Stein, D. G., ed. The psychobiology of aging: Problems and perspectives. Amsterdam: Elsevier/North Holland Inc.; 1980:161–175.
31. Steel, R. G.; Torrie, J. H. Principles and procedures of statistics. 2nd ed. New York: McGraw-Hill Book Co.; 1980.
32. Thiebot, M. H.; Childs, M.; Soubrie, P.; Simon, P. Diazepam-induced release of behavior in an extinction procedure: Its reversal by Ro15–1788. Eur. J. Pharmacol. 88:111–116; 1983.
33. Thorn, C. D. Linear pharmacokinetic systems. Fed. Proc. 39:2443–2449; 1980.
34. Treit, D.; Lalordo, V. M.; Armstrong, D. E. The effects of diazepam on "fear" reactions in rats are modulated by environmental constraints on the rat's defensive repertoire. Pharmacol. Biochem. Behav. 25:561–565; 1986.
35. Tsang, C. F. C.; Wilkinson, G. R. Diazepam disposition in mature and aged rabbits and rats. Drug Metab. Dispos. 10:413–416; 1982.
36. Van der Kleijn, E.; van Rossum, J. M.; Muskens, E. T.; Rijntjes, M. V. Pharmacokinetics of diazepam in dogs, mice, and humans. Acta Pharmacol. Toxicol. 29(Suppl. 3):109–127; 1971.
37. Venault, P.; Chapouthier, G.; Carvalho, L. P.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. Benzodiazepine impairs and B-carboline enhances performance in learning and memory tasks. Science 321:864–866; 1986.
38. Weiss, M. On pharmacokinetics in target tissues. Biopharm. Drug Dispos. 6:57–66; 1985.
39. Wilkinson, G. R. The effects of liver disease and aging on the disposition of diazepam, chlordiazepoxide, oxazepam and lorazepam in man. Acta Psychiatr. Scand. Suppl. 274:56–74; 1978.
40. Wilks, L.; File, S. E.; Martin, I. L. Evidence of strain differences in GABA-benzodiazepine coupling. Psychopharmacology (Berlin) 93:127–132; 1987.
41. Zar, J. H. Biostatistical analysis, Englewood Cliffs, NJ: Prentice-Hall Inc.; 1974:139–142.