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L-NNA Decreases Cortical Vascularization, Alcohol Preference and Withdrawal in Alcoholic Rats

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LALLEMAND, F. AND PH. DE WITTE. *L-NNA decreases cortical vascularization, alcohol preference and withdrawal in alcoholic rats.* PHARMACOL BIOCHEM BEHAV **58**(3) 753–761, 1997.—Rats, which were made chronically alcoholic in combination with L-N^{o}-nitro-arginine (L-NNA) treatment (5 mg/kg/day), a nitric oxide (NO) synthase inhibitor, showed a significant decrease in their alcohol preference and hypermotility during the withdrawal period by comparison with chronically alcoholic rats. However, no difference in the global liquid consumption between treated and untreated rats during the withdrawal stage was identified. In addition, the hypervascularization of the cortical area observed after chronic alcoholism was significantly decreased in the rats that had received L-NNA during the alcoholism procedure and was comparable to control rats. Thus, L-NNA alters both the behavioral preference for alcohol after alcoholism and the hypermotility during alcohol withdrawal, thus supporting the hypothesis of a direct implication of NO in alcohol abuse and its withdrawal. © 1997 Elsevier Science Inc.

Alcohol L-NNA NOS inhibitor Alcohol preference Withdrawal hypermotility Brain vascularization

ARGININE, which is produced by the urea cycle (19), is the endogenous substrate for nitric oxide synthase (NOS), forming nitric oxide (NO) and citrulline. NO, which is generated within the brain cells from calcium-dependent isoforms of NOS, constitutive NOS, modulates the release of neurotransmitters such as dopamine, acetylcholine and norepinephrine (19) and excitatory amino acids such as glutamate and aspartate. NO also acts on glutamate presynaptic terminals, inducing glutamate liberation through guanylate cyclase. NO may mediate hippocampal long-term potentiation, a process involved in memory consolidation, again via an action of NO on the presynaptic neuron (19). Another isoform, endothelium NOS, is also present within cerebral vascular vessels, which controls vascular tone.

L-N^{o}-nitro-arginine (L-NNA) is an irreversible stereoselective NOS inhibitor that inhibits L-arginine oxidation, a reaction catalyzed by the calcium/calmodulin-dependent NOS (13). This inhibitor would presumably inhibit NOS isoforms present in constitutive and endothelial forms (22).

Ethanol induces contraction of blood vessels in vitro in a dose-dependent manner (20), although its mode of action remains undefined. It remains controversial as to whether the presence of the endothelium is essential for this effect. In one study, contraction of the blood vessels occurred in both the presence and absence of the endothelial cells within the cerebral (cortical) vessels (39); in another study, in which the aorta was investigated, the presence of the endothelium was essential for ethanol-induced contractions (20). Furthermore, in the latter study, the rats developed tolerance to such ethanol-induced effects after 2 days of ethanol administration, which could be reversed by the administration of N-nitro-L-arginine, thus implicating an involvement of NO in tolerance. It is unclear as to whether ethanol alters NO synthesis and production; acute ethanol administration has been reported to either increase (20) or decrease (33) its production.

Chronic alcoholism increases both the number of NMDA receptors and their sensitivity on the postsynaptic neuron (28). However, the postsynaptic calcium levels remains constant and NO production is not altered (5,27,29). In contrast, cultured cortical neurons respond to NMDA with excitotoxicity and cell death through the elevation of free intracellular calcium levels and activation of calcium-dependent reactions (26). Thus, the increase in the number of NMDA receptor/

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channel complexes in hippocampus, and possibly other brain regions such as cerebral cortex, striatum and thalamus, which had occurred during the chronic alcoholism period, may play a role in the generation or expression of ethanol-withdrawal seizure (15).

NOS inhibitors clearly alter working memory in rats (7), the prevention of acquired rapid and chronic tolerance to the effects of alcohol, (18) and a reduced alcohol intake in two strains of alcohol-preferring rats (34) in comparison with rats administered alcohol only.

The purpose of this study was to determine whether the inhibition of NOS in control rats and in rats during the chronic alcoholism procedure altered alcohol preference and withdrawal and caused perturbations of the cortical microvasculature.

MATERIAL AND METHODS

Chronic Alcohol Intoxication

Male Wistar rats (250 g), individually housed, were made alcoholic by breathing alcohol in an isolated plastic chamber (120 × 60 × 60 cm) that contained a mixture of alcohol and air. This mixture was pulsed into the chamber via a mixing system that allowed the quantity of alcohol to be increased every 2 days during the experimental procedure. The animals were kept for 30 days in the alcohol chamber (25).

Two groups of rats were made chronically alcoholic. The treated group (n = 17) received 5 mg/kg/day L-NNA in the drinking water bottle, and the untreated group (n = 16) received only water. Each day, the amount of liquid consumed by the rat on the previous day and the weight of the rat were assessed to calculate the amount of L-NNA to be added to the drinking water, to achieve a final intake of 5 mg/kg/day. The L-NNA-containing solution and water alone were changed every day to ensure a constant drug administration during the whole alcoholism procedure. The L-NNA treatment was stopped when rats were removed from the alcohol chamber. In another study, nonalcoholic rats received either L-NNA in their drinking water at a dose similar to the that of the alcoholized rats (5 mg/kg/day, n = 16) or water alone (n = 16).

Motility Recording

The motility of the rats during the withdrawal syndrome induced after 30 days of chronic alcohol treatment was assayed in an apparatus designed to detect motility operating on the principle of inertia. The forces generated by animal's movements are transmitted to a supporting plate that is

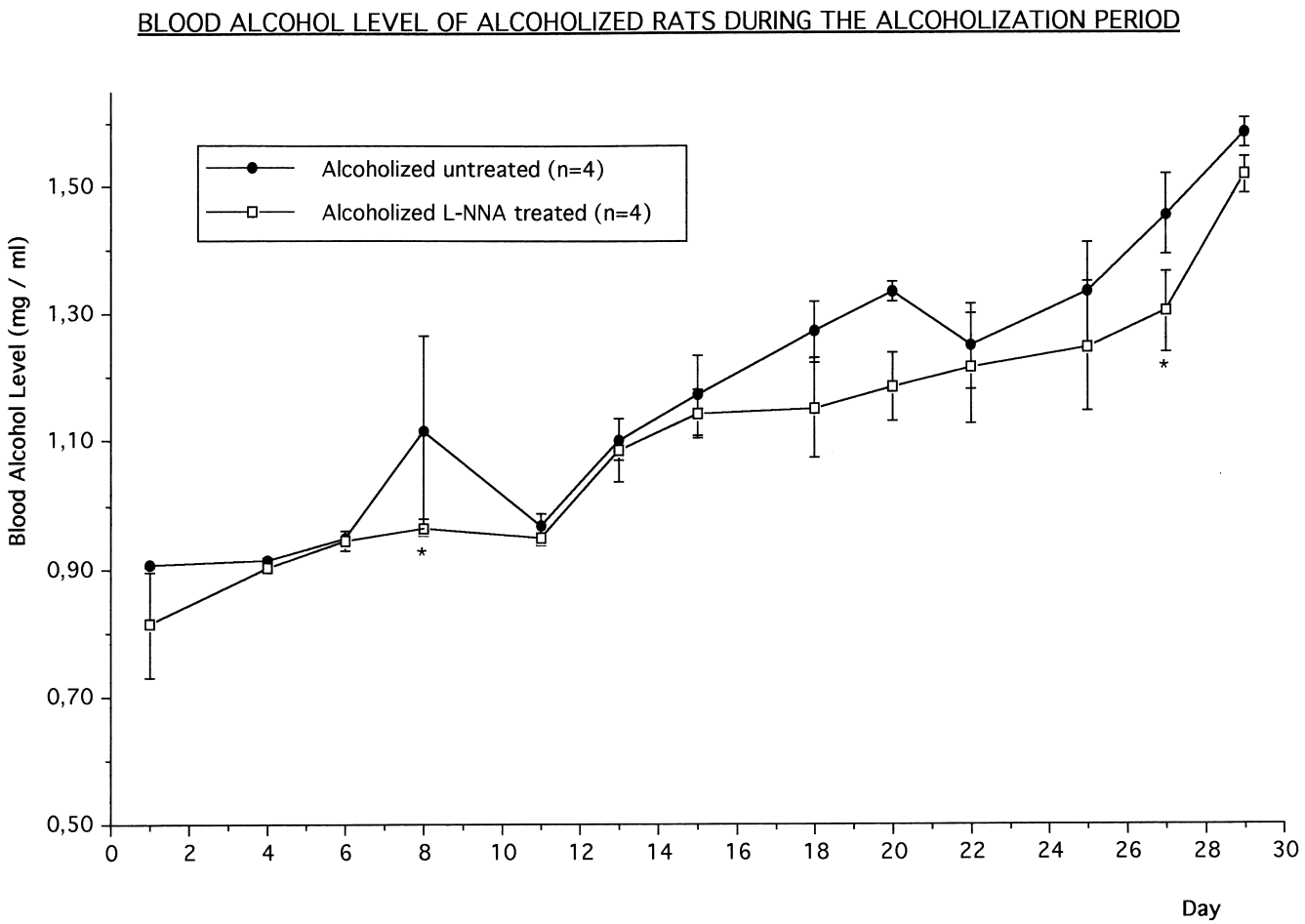

FIG. 1. Blood alcohol level of chronically alcoholic rats treated or not treated with 5 mg/kg/day L-NNA for 4 weeks. Results are presented as mean ± SE (NS).

mounted on ballbearings to minimize inherent frictions (38). Each rat was deprived of liquid for the last 18 h in the chamber before removal to the apparatus for 24 h.

Blood Alcohol Level

Blood was collected from the caudal portion of the rat tail, 3 times per week, during the chronic alcohol treatment period, from both groups of rats. The concentration of ethanol in the blood samples was assayed by the alcohol-dehydrogenase-based method (Boerhinger-Mannheim, Germany).

Free-Choice Paradigm

At the end of the chronic alcohol treatment period, rats from each group underwent three successive steps (24):

1. a full beverage deprivation (the last 18 h of the alcohol treatment period and the first 6 h of the withdrawal period),
2. presentation of 10% (v/v) ethanol solution as the sole drinking fluid during the following 18 h,
3. a free-choice beverage situation [water vs. 10% (v/v) ethanol solution] was presented until the animals showed a clear preference for water.

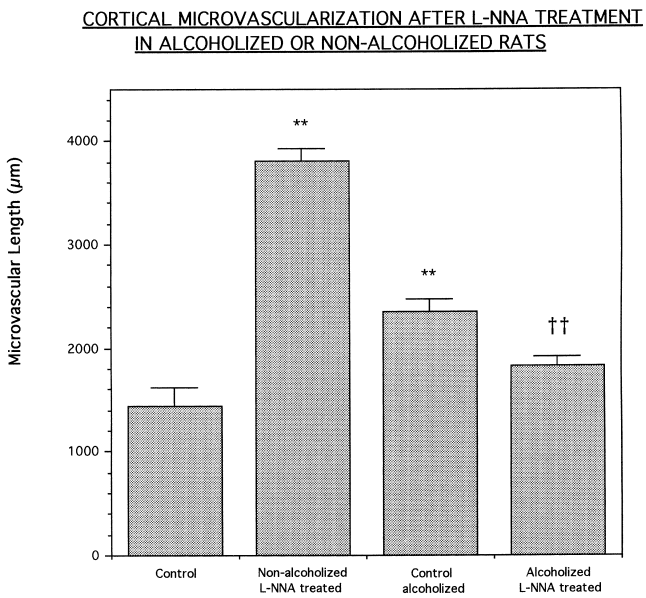

FIG. 2. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the frontoparietal cortical microvasculature in nonalcoholic and chronically pulmonary alcoholic rats. Results are presented as mean± SE. $^{**}p < 0.01$ vs. the control group; $\dagger\dagger p < 0.01$ vs. control alcoholic groups.

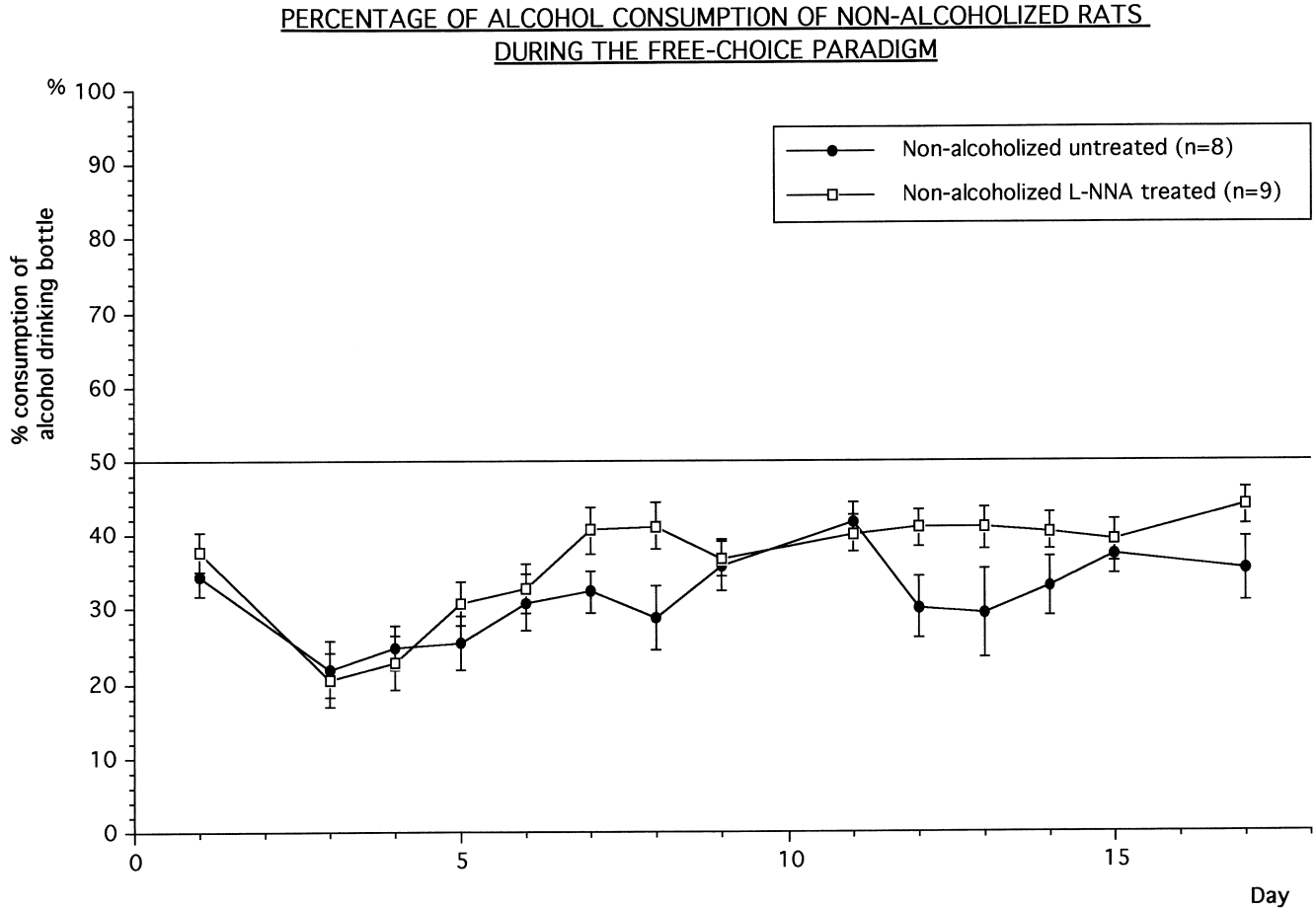

FIG. 3. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the alcohol 10% (v/v) consumption during a free-choice situation in nonalcoholic rats. Results are presented as mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control rats.

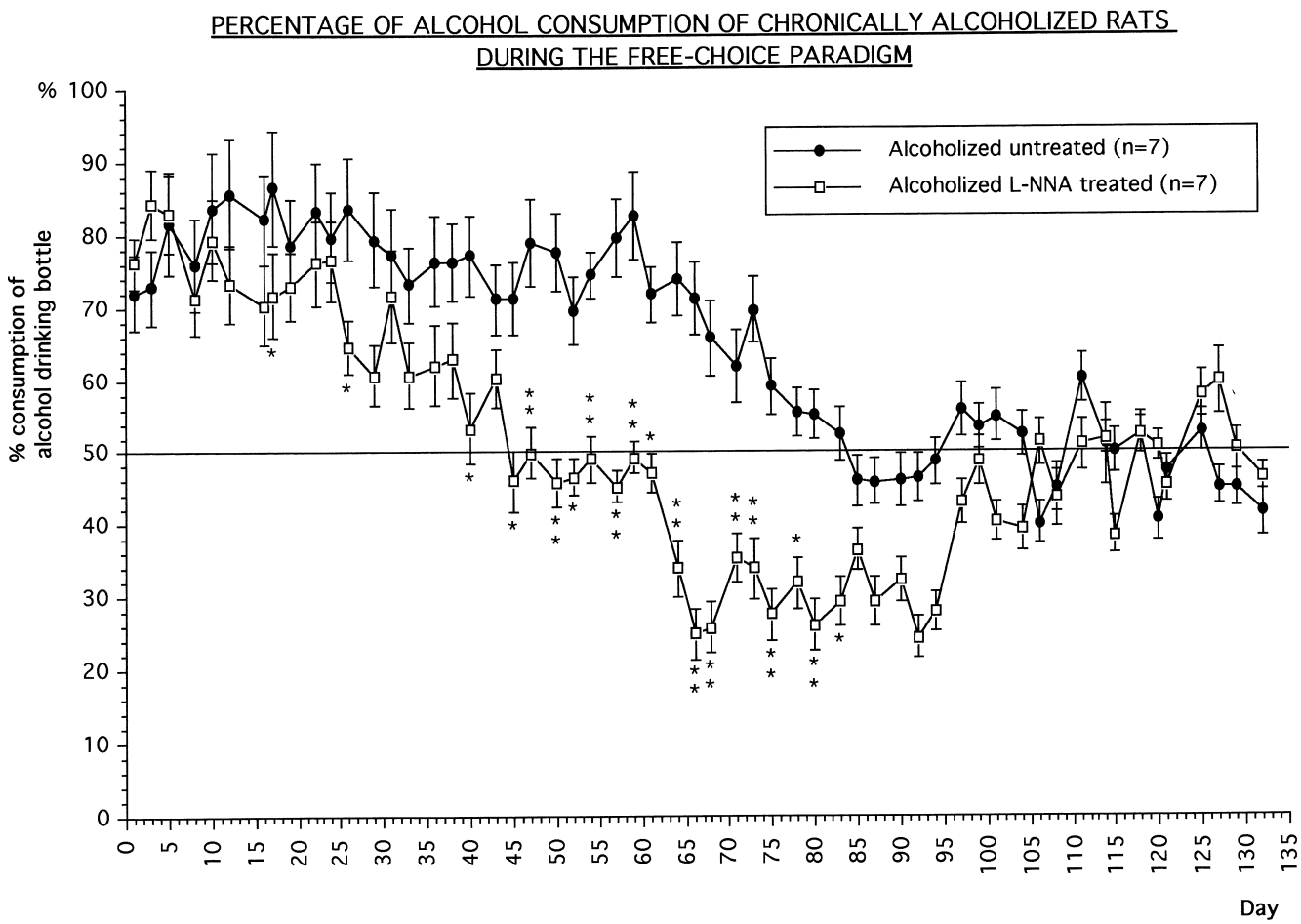

FIG. 4. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the alcohol withdrawal 10% (v/v) consumption during a free-choice situation in pulmonary alcoholic rats. *$p < 0.05$, **$p < 0.01$ vs. alcoholic untreated rats.

During the free-choice period, the fluid consumption was recorded daily. The position of the drinking bottle was changed randomly to avoid a position preference.

Microvascular Morphometric Quantification

At the end of the alcohol treatment period, rats that had received L-NNA ($n = 9$) or not ($n = 25$) were killed under ether anesthesia and perfused intraaortically with 30 ml of a wash solution (NaCl 0.8%, procaine hydrochloride 0.1%, glucose 0.1%) and with 50 ml of Ilford L4 nuclear emulsion in gel form diluted 1:4 with distilled water at 40°C and stored in an incubator at 37°C (9,37). The brain was rapidly removed and fixed by immersion in 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Fixed tissue was cut at 100 μm with an Oxford Vibratome. The sections were photographically developed for 5 min at room temperature in D19 revelator film (Kodak). After a short rinse in distilled water, the sections were fixed for 5 min in 30% thiosulphate, rinsed again in distilled water and mounted in Aquamount (Gurr). The length of the vascular network was measured in squares of 62,500 μm^2 in the frontoparietal cortex. All the vessel lengths, including terminal and lateral branches, were measured and collated. The measurements were assayed with a planimeter (MOP, Digiplan).

Experiments on Nonalcoholic Rats

A free-choice beverage situation [water vs. 10% (v/v) ethanol solution] also was conducted on nonalcoholic rats treated or not treated with L-NNA. The cortical microvascularization length on nonalcoholic rats treated ($n = 9$) or not treated ($n = 24$) with L-NNA was measured while the motility of nonalcoholic rats treated or not treated with L-NNA was recorded with the same apparatus as that used for the chronically alcoholic rats.

Statistical Analysis

Analysis of variance (ANOVA) followed by the Fisher LSD protected *t*-test (GB-Stat for Windows, Dynamic Microsystems) was used to assess the significance of difference in cortical microvasculature, withdrawal motility, preference, blood alcohol level and water consumption during the pulmonary alcohol treatment or chronic L-NNA treatment between groups. The levels of significance were set at $p < 0.05$ for alcohol treatment and $p < 0.01$ for L-NNA treatment.

Products

L-NNA was obtained from Sigma Chemicals (St. Louis, MO, USA); ethanol absolute GR, used in the free-choice situ-

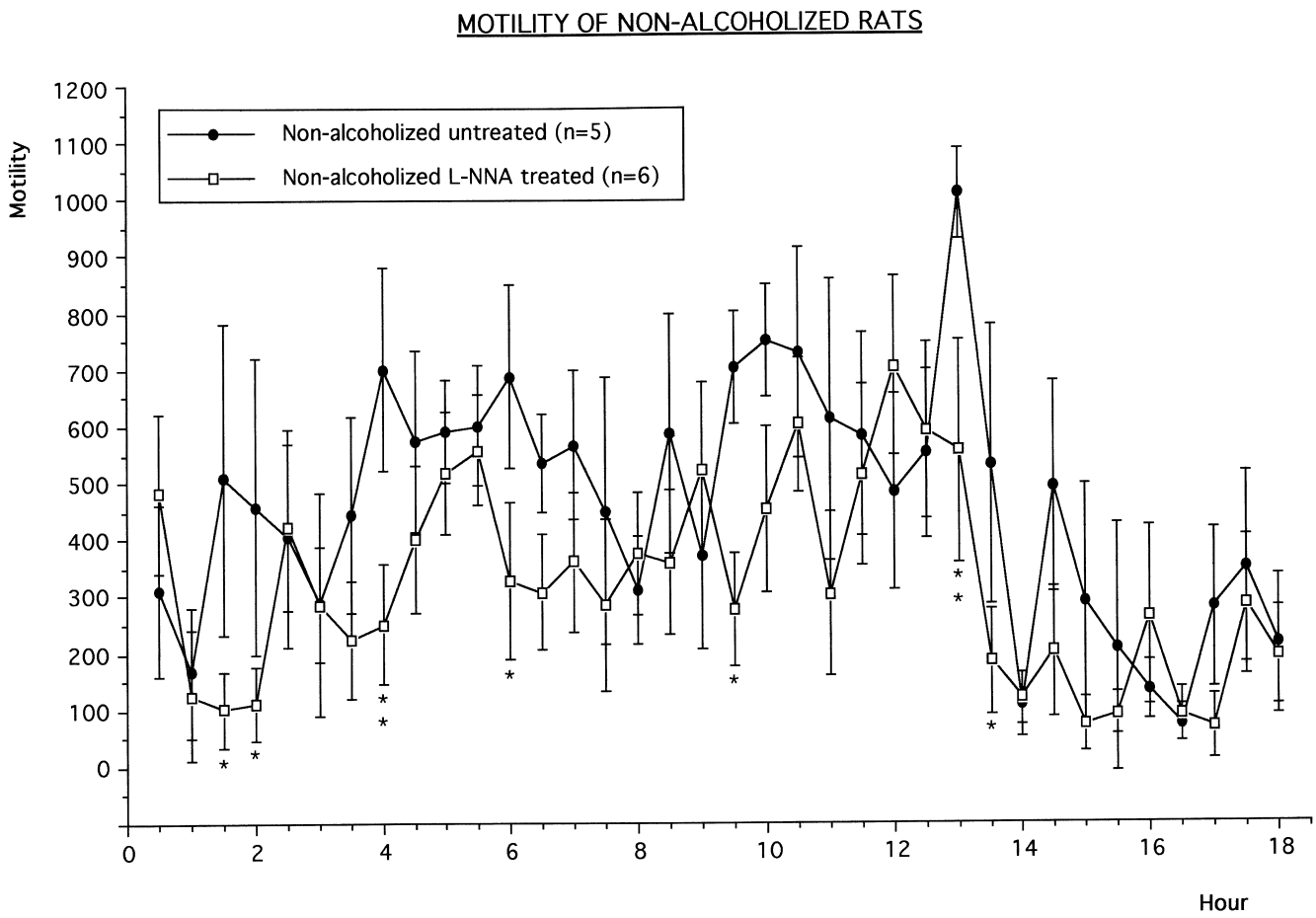

FIG. 5. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the motility in nonalcoholic rats. Results are presented as mean ± SE. *$p < 0.05$, **$p < 0.01$ vs. nonalcoholic untreated rats.

ation, was obtained from Merck (Germany); and ethanol 97% (Desinfect Concentrat) used during the pulmonary alcohol treatment was obtained from from Belgalco SA (Belgium), Aquamount (GURR) and Kodak D19 (Rochester, NY).

RESULTS

There was a steady increase in the mean blood ethanol concentration (0.06 g/L/day) in both groups of chronically alcoholic rats; administration of L-NNA during this alcohol treatment procedure did not significantly alter either the rate or final level of blood ethanol concentration [$F(12, 12) = 0.6134$, NS] (Fig. 1). Furthermore, no statistical differences were observed in the weights of any of the rats during the pulmonary alcohol procedure.

The cortical microvascularization was measured in three successive cortical depth regions, which have been averaged to give an overall mean for legibility of the graph. The results of the experiment were analyzed by two-way ANOVA to assay the interaction between the treatment with L-NNA and the treatment with ethanol. The mean cortical microvascularization length in the nonalcoholic rats administered the NOS inhibitor L-NNA vs. the control rats was significantly increased [$F(1, 63) = 31.666$, $p < 0.0001$] (Fig. 2). The alcoholic rats, which also were administered L-NNA, vs. the control rats showed a significant decrease in the microvascularization length [$F(1, 63) = 76.287$, $p < 0.0001$] (Fig. 2), and there was a significant increase of the microvascularization in the alcoholic rats in comparison with the nonalcoholic L-NNA-untreated rats [$F(1, 63) = 9.915$, $p < 0.0001$] (Fig. 2).

The behavioral preference to alcohol and the motility of these rats were ascertained after the different treatments. The results were analyzed by two-way ANOVA for the behavioral preference to assay the interaction between the two parameters, namely treatment with L-NNA and time. Nonalcoholic L-NNA-treated rats showed no behavioral preference to alcohol when compared with the nonalcoholic untreated rats [$F(13, 13) = 0.4292$, NS] (Fig. 3). Alcoholic rats administered with L-NNA showed a significant decrease in preference to alcohol in comparison with the alcoholic untreated group [$F(56, 56) = 2.0487$, $p < 0.0001$] (Fig. 4).

The motility was analyzed by two-way ANOVA. Nonalcoholic rats administered L-NNA showed no statistical significant effect in motility [$F(35, 35) = 1.191$, NS] in comparison with the nonalcoholic untreated rats (Fig. 5). Rats administered both alcohol and L-NNA showed a significant decrease in withdrawal motility in comparison with the alcoholic untreated group [$F(47, 47) = 1.5871$, $p = 0.0104$] (Fig. 6).

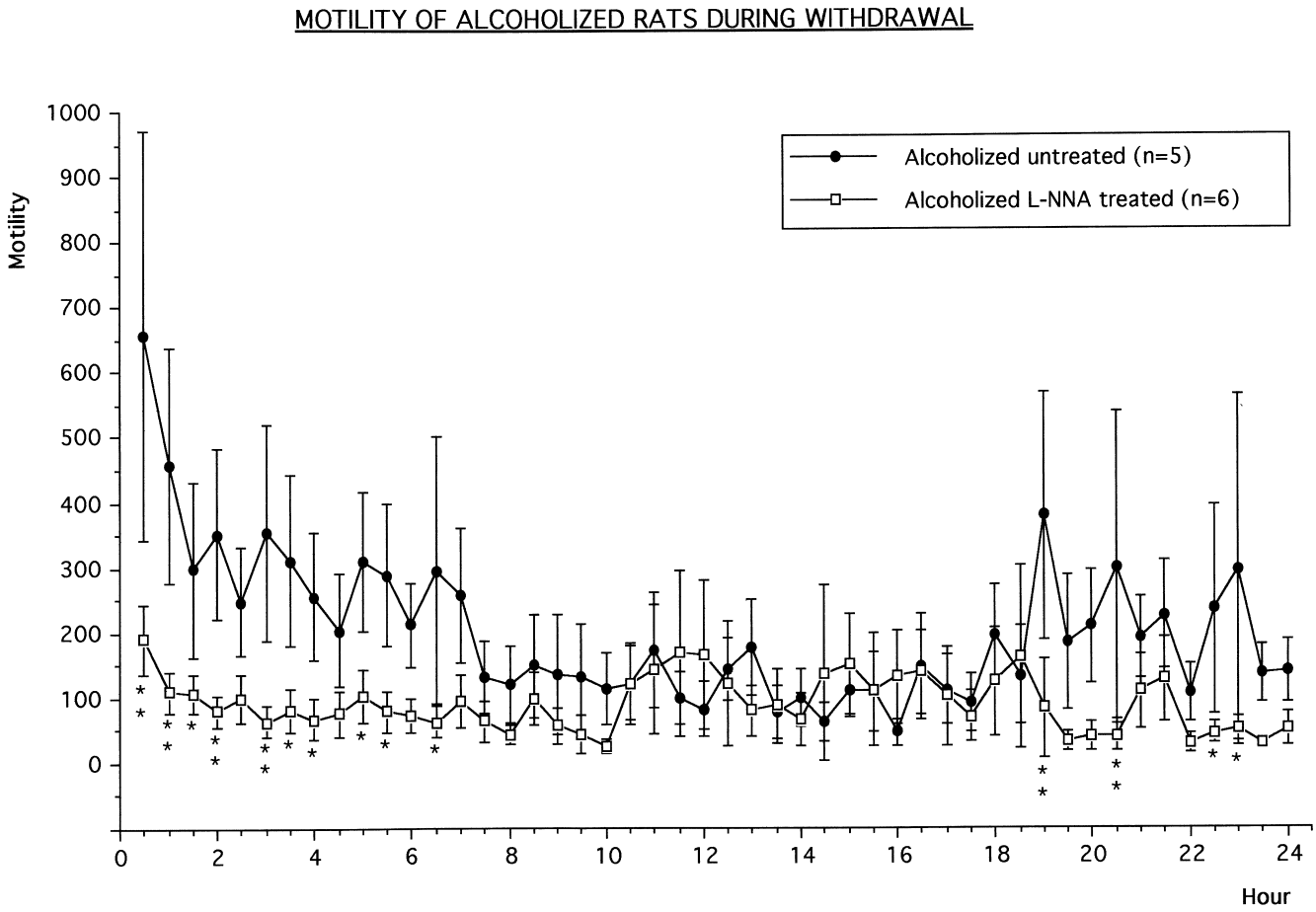

FIG. 6. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the alcohol-withdrawal motility in pulmonary alcoholic rats. Results are presented as mean ± SE. *$p < 0.05$, **$p < 0.01$ vs. alcoholic untreated rats.

The water consumption of the different treated groups was analyzed by two-way ANOVA to assay the interaction between L-NNA treatment and time. Although ANOVA analysis did not show any overall significant change in alcohol consumption across treatment groups, it was clear that at certain time points there were significant changes in the amount of liquid consumed in the different groups (Figs. 7, 8).

DISCUSSION

In the present study, we have shown that the NOS inhibitor L-NNA, a stereoselective inhibitor of the cerebral constitutive type 1 NOS (11,13), has several effects on behavior and anatomical changes induced by chronic ethanol consumption.

We have identified structural modifications of cortical blood vessels after the administration of the NOS inhibitor L-NNA alone and during the chronic alcohol treatment procedure. Previous studies have shown that NOS inhibitors evoke structural modifications of blood vessels, e.g., an increase in the media lumen ratio and a thickening of the media, which often will result in arterial hypertension in vitro (8), particularly when high doses are administered, e.g., >50 mg/kg L-NNA (35). Therefore, in these present studies, rats were administered L-NNA at a relatively low dose, 5 mg/kg o/s, for 4 weeks to prevent such hypertension from occurring. Even at these relatively low doses, a highly significant increase in the microvascularization of the cortex was observed, indicating that changes in vascular tone had occurred, possibly due to diminution of NO release. Chronic alcoholism also increased cortical microvascularization, as previously reported (14), although this was not to the same extent as the rats administered L-NNA alone. This result may indicate that chronic alcoholism affects NO production. Such a constant NOS inhibition appears to reduce the excitotoxicity induced by chronic alcoholism, in particular the NMDA excitotoxicity (23), which could contribute to the hypermicrovascularization (14). Previous studies have clearly identified that chronic ethanol administration induces reductions in cerebral blood flow in both man and animals (3), although the fundamental scientific basis has not been studied.

NO may mediate some of the effects induced by chronic ethanol administration, in particular the compensatory enhancement of NMDA receptor channel expression and function. Although such ethanol induced changes in glutamate receptors numbers may increase Ca^{2+} influx into the cell (16,31,36), the present results and those of Fitzgerald et al. (12) indicate that even, if such a rise in calcium did occur during the chronic alcohol procedure, there was no evidence to

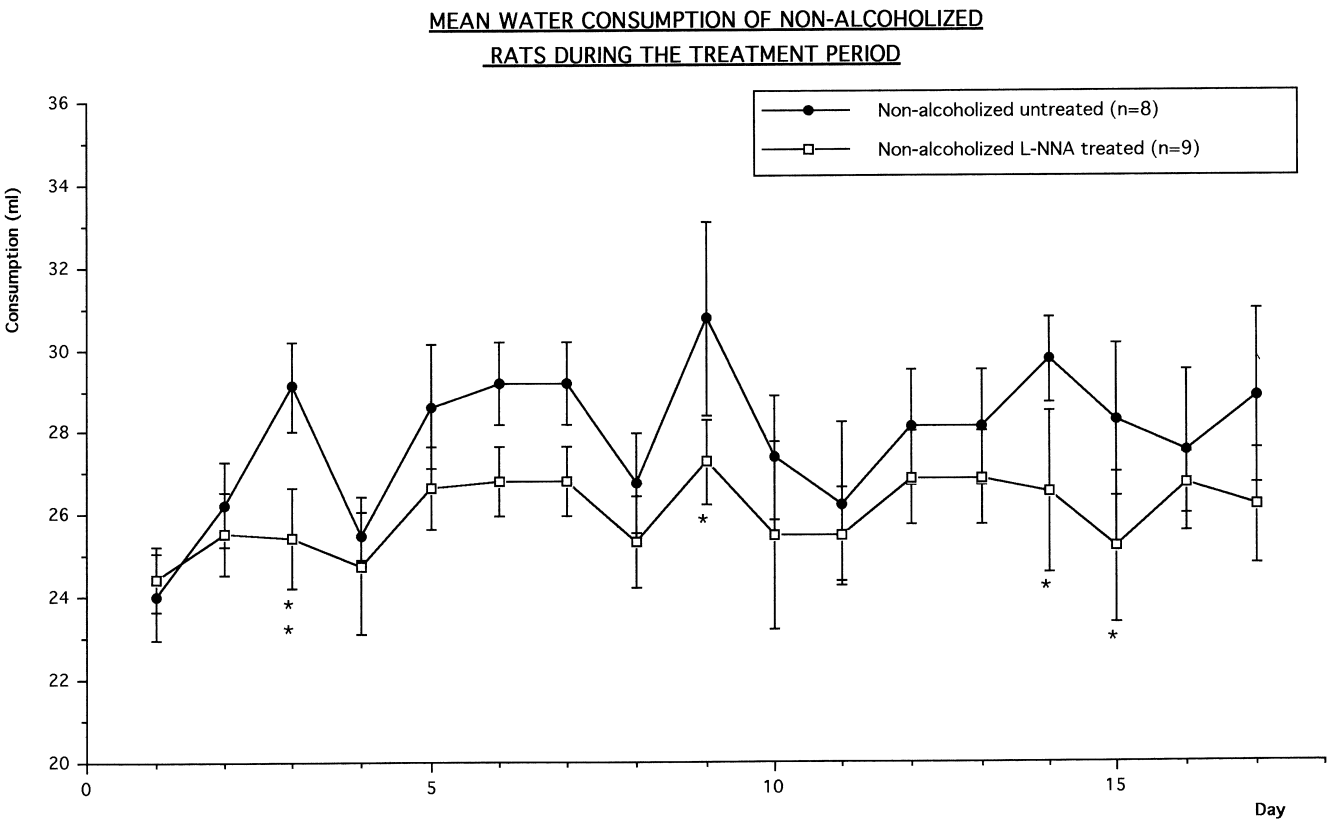

FIG. 7. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the liquid consumption during the treatment period in nonalcoholic rats. Results are presented as mean ± SE. *$p < 0.05$, **$p < 0.01$ vs. alcoholic untreated rats.

suggest that there was increased generation of NO. When chronic ethanol was administered with L-NNA, the effect of L-NNA alone was not observed in the cortical microvascularization, thus indicating a modulatory effect of ethanol on the inhibition of NOS induced by L-NNA administration. Thus, we can assume that a constant NOS inhibition appears to reduce the excitotoxicity induced by chronic alcoholism.

There was no significant change in the weight of the rats administered L-NNA alone for 4 weeks or in the alcoholic rats with and without L-NNA. This is an important observation because high doses of L-NNA, e.g., 25–50 mg/kg (30), significantly decrease food intake, indicating that NOS, in some undefined manner, plays a physiological role in the regulation of food intake. Diminution of NO release induced by this compound or its methyl ester, particularly at high doses, limits food intake in both mice (30) and chickens (6).

In the present study, the rats administered L-NNA during the chronic alcohol procedure showed a preference for water after 40 days of withdrawal; in contrast, the chronically alcoholic rats had a preference for water 85 days after cessation of the chronic alcohol treatment. In a previous study (34), in which two strains of alcohol-preferring rats, Fawn-Hooded and alcohol-preferring rats, received a single dose of a less potent NOS inhibitor, L-NAME, either 30 mg/kg or 60 mg/kg, the amount of alcohol consumed in a free-choice situation was severely limited by approximately 50%. In addition, injection of L-NAME 30 mg/kg for 4 consecutive days significantly reduced alcohol intake, although tolerance developed after 3 days of treatment. Such results indicate an effect of NO on drinking behavior, although an explanation for such an effect, such as changes in the flux of calcium through neuronal channels or a direct effect of L-NAME on neurotransmitters involved in alcohol drinking, remains to be elucidated. Whether NO can act as a neuromodulator, possibly by modulating dopamine release (10,21), remains unknown because other studies (4) have reported that NO was not involved in the dopamine-dependent rewarding effect of electrical brain stimulation.

NO has been implicated in ethanol tolerance by its possible action on NMDA synapses (17). Acute injection of L-NNA (5 mg/kg intraperitoneally) into normal rats reduces the activity of NOS by approximately 50% (11,13), although no studies have assayed its tissue activity after chronic administration of both L-NNA and ethanol. In the present study, L-NNA administration in conjunction with chronic alcohol treatment reduced ethanol preference in rats by approximately 40 days, thus showing a clear relationship between NOS inhibition and ethanol preference because L-NNA alone produced no change in the natural preference for water.

The hyperactivity induced by alcohol withdrawal in the alcoholic L-NNA-treated rats decreased when compared with alcoholic untreated rats. Other studies in which L-NAME was administered during alcohol withdrawal also have identified significant reductions in hyperactivity, tremors and rigidity (1,2,32,33). Further studies are clearly required to ascertain the activity of NOS in specific brain regions during alcoholism and withdrawal.

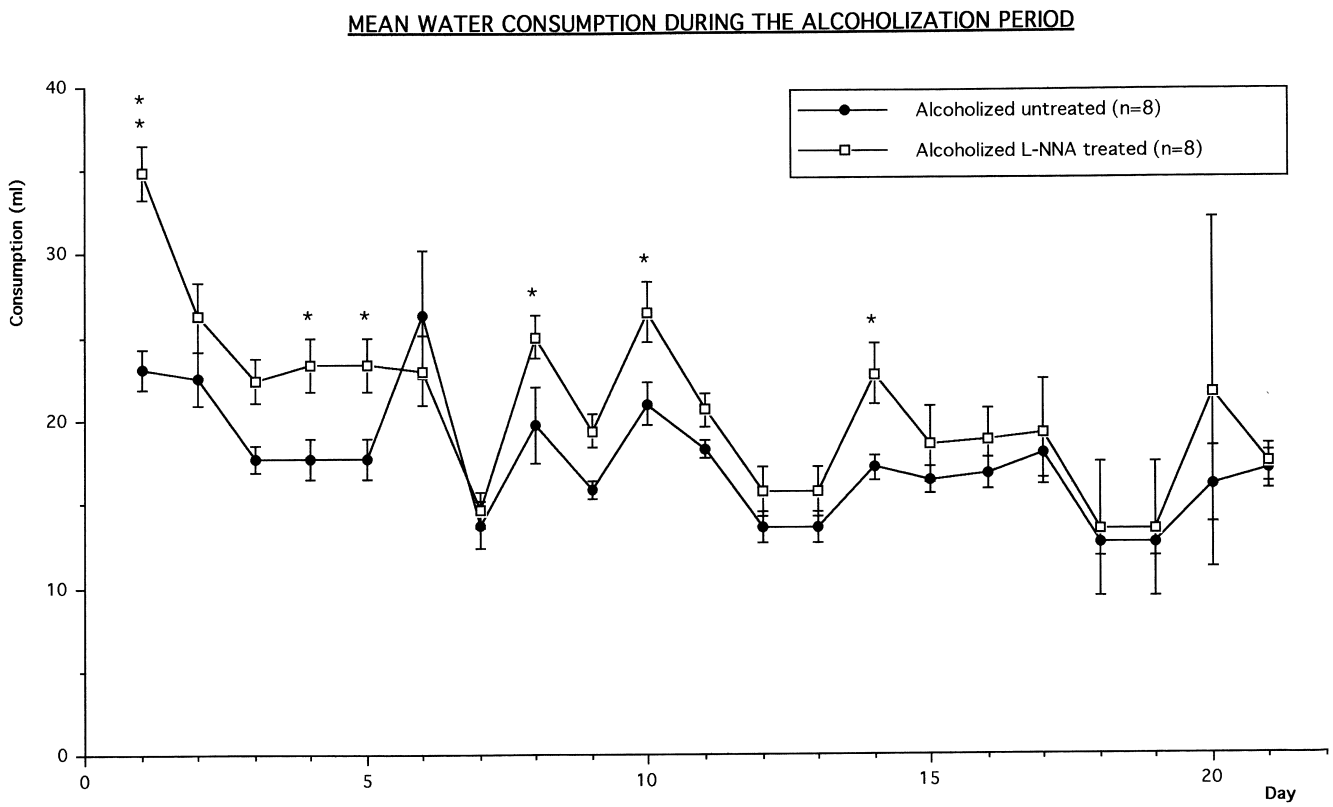

FIG. 8. Effects of NOS inhibition by L-NNA (5 mg/kg/day) on the liquid consumption during the treatment period in chronically alcoholic rats. Results are presented as mean ± SE. *$p < 0.05$, **$p < 0.01$ vs. alcoholic untreated rats.

In conclusion, we have observed that the administration of a NOS inhibitor during chronic pulmonary alcohol treatment diminished cortical microvasculature, the preference for alcohol and alcohol-withdrawal motility. These results suggest that the L-arginine–NOS–NO pathway could represent a valuable therapeutic target for decreasing alcohol preference and some of the adverse ethanol-induced effects during withdrawal.

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REFERENCES

1. Adams, M. L.; Meyer, E. R.; Sewing, B. N.; Cicero, T. J.: Effects of nitric oxide-related agents on alcohol narcosis. Alcohol. Clin. Exp. Res. 18:969–975; 1994.
2. Adams, M. L.; Sewing, B. N.; Chen, J.; Meyer, E. R.; Cicero, T. J.: Nitric oxide-related agents alter alcohol withdrawal in male rats. Alcohol. Clin. Exp. Res. 19:195–199; 1995.
3. Altura, B. M.; Altura B. T.: Alcohol, the cerebral circulation and strokes. Alcohol 1:325–331; 1984.
4. Bozarth, M. A.; Pudiak, C. M.; Morris, M.: Nitric oxide synthesis inhibition does not affect brain stimulation reward. Pharmacol. Biochem. Behav. 48:487–490; 1994.
5. Brennan, C. H.; Crabbe, J.; Littleton, J. M.: Genetic regulation of dihydropyridine-sensitive calcium channels in brain may determine susceptibility to physical dependence on alcohol. Neuropharmacology 29:429–432; 1990.
6. Choi, Y.-H.; Furuse, M.; Okumura, J.-I.; Denbow, D. M.: Nitric oxide controls feeding behavior in the chicken. Brain Res. 654: 163–166; 1994.
7. Cobb, B. L.; Ryan, K. L.; Frei, M. R.; Guel-Gomez, V.; Mickley, G. A.: Chronic administration of L-NAME in drinking water alters working memory in rats. Brain Res. Bull. 38:203–207; 1995.
8. Deng, L. Y.; Thibault, G.; Schiffrin, E. L.: Effect of hypertension induced by nitric oxide synthase inhibition on structure and functions of resistance arteries in the rat. Clin. Exp. Hypertens. 15: 527–537; 1993.
9. de Parmentier, F.; Heuschling, P.; Knoops, B.; Jansens de Varebeke, P.; van den Bosch de Aguilar, P.: A new model for quantification of microvascular regeneration after a lesion of the rat cerebral cortex. Brain Res. 398:419–424; 1986.
10. DiChiara, G.; Imperato, A.: Drugs abused for humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85:5274–5278; 1988.
11. Dwyer, M. A.; Bredt, D. S.; Snyder, S. H.: Nitric oxide synthase: Irreversible inhibition by L-N^G-nitro-arginine in brain in vitro and in vivo. Biochem. Biophys. Res. Commun. 176:1136–1141; 1991.
12. Fitzgerald, L. W.; Charlton, M. E.; Duman, R. S.; Nestler, E. J.: Regulation of neuronal nitric oxide synthase by chronic ethanol ingestion. Synapse 21:93–95; 1995.
13. Furfine, E. S.; Harmon, M. F.; Paith, J. E.; Garvey, E. P.: Selective inhibition of constitutive nitric oxide synthase by L-N^G-nitroarginine. Biochemistry 32:8512–8517; 1993.
14. Gewiss, M.; Heidbreder, Ch.; Juge, J.; De Witte, Ph.: Modifica-

tions similaires du réseau microvasculaire cortical chez le rat âgé et chez le rat intoxiqué par l'alcoolisation pulmonaire. Circul. Métabol. Cerv. 6:231–240; 1989.
15. Gulya, K.; Grant, K. A.; Valverius, P.; Hoffman, P. L.; Tabakoff, B.: Brain regional specificity and time-course of changes in the NMDA receptor-ionophore complex during ethanol withdrawal. Brain Res. 587:129–134; 1991.
16. Iorio, K. R.; Reinlib, L.; Tabakoff, B.; Hoffman, P. L.: Chronic exposure of cerebellar granule cells to ethanol results in increased N-methyl-D-aspartate receptor function. Mol. Pharmacol. 41:1142–1148; 1994.
17. Khanna, J. M.; Morato, G. S.; Shah, G.; Chau, A.; Kalant, H.: Inhibition of nitric oxide synthesis impairs rapid tolerance to ethanol. Brain Res. Bull. 32:43–47; 1993.
18. Khanna, J. M.; Morato, G. S.; Chau, A.; Shah, G.: Influence of nitric oxide synthase inhibition on the development of rapid tolerance to ethanol. Brain Res. Bull. 37:599–604; 1995.
19. Kerwin, J. F. Jr.; Heller, M.: The arginine-nitric-oxide pathway: A target for new drugs. Med. Res. Rev. 14:23–74; 1994.
20. Knych, E. T.: Endothelium-dependent tolerance to ethanol-induced contraction of rat aorta: Effect of inhibition of EDRF action and nitric oxide synthesis. Alcohol. Clin. Exp. Res. 16:58–63; 1992.
21. Kuhar, M. J.; Pogun, S.: Modulation of dopamine transport by nitric oxide (NO). Soc. Neurosci. Abstr. 19:937; 1993.
22. Lambert, L. E.; Whitten, J. P.; Baron, B. M.; Cheng, H. C.; Doherty, N. S.; McDonald, I. A.: Nitric oxide synthesis in the CNS, endothelium and macrophages differed in its sensitivity to inhibition by arginine analogues. Life Sci. 48:69–75; 1991.
23. Lancaster, F. E.: Nitric oxide and ethanol-induced brain damage—A hypothesis. In: Hunt, W. A.; Nixon, S. J., eds. Alcohol induced brain damage. NIH Publication No. 93-3549. Bethesda, MD: National Institute of Health; 1993:373–386.
24. Le Bourhis, B.: Sur l'établissement de la dépendance des rats à l'égard de l'alcool. Physiol. Behav. 18:475–478; 1977.
25. Le Bourhis, B.: Alcoolisation du rat par voie pulmonaire. Comptes Rendus Séances Soc. Biol. 169:898–904; 1975.
26. Lustig, H. S.; Chan, J.; Greenberg, D. A.: Ethanol inhibits excitotoxicity in cerebral cortical cultures. Neurosci. Lett. 135:259–261; 1992.
27. Messing, R. O.; Sneade, A. B.; Savidge, B.: Protein kinase C participates in up-regulation of dihydropyridine-sensitive calcium channels. J. Neurochem. 55:1383–1389; 1990.
28. Michaelis, E. K.; Mulvaney, M. J.; Freed W. J.: Effects of acute and chronic ethanol intake on synaptosomal glutamate binding activity. Biochem. Pharmacol. 27:1685; 1978.
29. Michaelis, E. K.; Freed, W. J.; Galton, N.; Foye, J.; Michaelis, M. L.; Phillips, I.; Kleinman, J. E.: Glutamate receptor changes in the brain synaptic membranes from human alcoholics. Neurochem. Res. 15:1055–1063; 1990.
30. Morley, J. E.; Farr, S. A.; Suarez, M. D.; Flood, J. F.: Nitric oxide synthase inhibition and food intake: Effects on motivation to eat and in female mice. Pharmacol. Biochem. Behav. 50:369–373; 1995.
31. Ortiz, J.; Fitzgerald, L. W.; Charlton, M. E.; Lane, S.; Trevisan, L.; Guitart, X.; Shoemaker, W.; Duman, R. S.; Nestler, E. J.: Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine pathway. Synapse; in press.
32. Persson, M. G.; Gustafsson, L. E.: Ethanol can inhibit nitric oxide production. Eur. J. Pharmacol. 224:99–100; 1992.
33. Persson, M. G.; Cederqvist, B.; Wiklund, C. U.; Gustafsson, L. E.: Ethanol causes decrements in airway excretion of endogenous nitric oxide in humans. Eur. J. Pharmacol. 270:273–278; 1994.
34. Rezvani, A. M.; Grady, D. R.; Peek, A. E.; Pucilowski, O.: Inhibition of nitric oxide synthesis attenuates alcohol consumption in two strains of alcohol-preferring rats. Pharmacol. Biochem. Behav. 50:265–270; 1995.
35. Ribeiro, M. O.; Antunes, E.; de Nucci, G.; Lovisolo, S. M.; Zatz, R.: Chronic inhibition of nitric oxide synthesis: A new model of arterial hypertension. Hypertension 20:298–303, 1992.
36. Trevisan, L.; Fitzgerald, L. W.; Brose, N.; Gasic, J. P.; Heinemann, S. F.; Duman, R. S.; Nestler, E. J.: Chronic ingestion of ethanol up-regulates NMDAR1 subunit immunoreactivity in rat hippocampus. J. Neurochem. 62:1635–1638; 1994.
37. Van-Reempts, J.; Haseldonckx, M.; Borgers, M.: A simple technique for the microscopic study of microvascular geometry and tissue perfusion, allowing simultaneous histopathologic evaluation. Microvasc. Res. 25:300–306; 1983.
38. Weyers, M. H.; Annys, M.: Two apparatus for detection of motility and water consumption of rodents. Physiol. Behav. 29:759–762; 1982.
39. Zhang, A.; Altura, B. T.; Altura, B. M.: Ethanol-induced contraction of cerebral arteries in diverse mammals and its mechanism of action. Eur. J. Pharmacol. 248:229–236; 1993.