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Acute Exposure to Noise Affects Sodium-Dependent High-Affinity Choline Uptake in the Central Nervous System of the Rat

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LAI, H. *Acute exposure to noise affects sodium-dependent high-affinity choline uptake in the central nervous system of the rat.* PHARMACOL BIOCHEM BEHAV **28**(2) 147–151, 1987.—Rats were acutely (45 min) exposed to white noise at intensity of either 70 or 100 dB. Sodium-dependent high-affinity choline uptake was determined in the striatum, frontal cortex, hypothalamus, and hippocampus immediately after exposure. The effects of noise on choline uptake varied according to the intensity of the noise and the brain area studied. Exposure to noise of 70 dB significantly increased the choline uptake in the frontal cortex, hypothalamus, and hippocampus as compared to the uptake of sham-exposed rats, whereas decreased choline uptake in the frontal cortex and hippocampus was observed after acute exposure to noise of 100 dB. No significant effect on choline uptake in the striatum was seen after exposure to noise of either intensity. In addition, pretreatment of the rats with the narcotic antagonist naltrexone (1 mg/kg, IP) before noise exposure blocked the effects of noise on choline uptake in the central nervous system. Changes in cholinergic activity in the central nervous system could be a response to the stress effect of noise and may be mediated by endogenous opioids.

White noise Choline uptake Central nervous system Naltrexone Endogenous opioids Stress

NOISE in the public and occupational environments has raised concern as a health hazard [2, 23, 24]. This problem is especially pertinent in occupational settings where short-term exposure to loud noise is unavoidable. On the other hand, it is possible that long-term exposure to environmental noise at moderate or low intensities is also detrimental to health. In addition to direct damage to the peripheral auditory system, noise could cause undesirable extra-auditory effects. For example, a popularly accepted notion is that noise is a "stressor," exposure to which can lead to detrimental health effects [28].

The effect of noise exposure on health is still not well studied. Epidemiological studies reported both positive [14,18] and no [5,9] cause-effect relationship between environmental noise and health. However, several experimental studies on humans [4,6] and animals [3, 13, 19] have shown that noise exposure affected endocrine functions, especially those of the hypothalamo-pituitary-adrenal axis. The effects are similar to those of stress.

No study has so far investigated the effect of noise exposure on neurochemical changes in the brain. Noise, in addition to its auditory sensory effect, can also elicit arousal responses. Thus, it is likely that noise can cause changes in neurochemistry in areas of the brain other than the auditory system. This paper reports the results of a series of studies to investigate the effects of acute white noise exposure on cholinergic activity in the central nervous system of the rat. Sodium-dependent high-affinity choline uptake was used as an index of cholinergic activity [29]. Four brain areas that contain the highest amount of cholinergic innervations, namely, the striatum, frontal cortex, hypothalamus, and hippocampus, were studied. The cholinergic system was studied since noise has been proposed to be a "stressor", and exposure to "stressor" is known to alter central cholinergic functions [8, 10, 11, 21]. The study examined the effects of acute exposure to two levels of white noise, a moderate noise intensity of 70 dB and an intense auditory stimulus of 100 dB. In addition, the possibility of endogenous opioid involvement in the effect of noise on central choline uptake was also studied, since endogenous opioids may be involved in the response of an animal to "stressor" [1]. The ability of a low dose of the narcotic antagonist naltrexone (1 mg/kg, IP) to block the effect of noise was used as the criterion of involvement of endogenous opioids.

METHOD

Animals

Male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratories, Bellevue, WA were used in the experiments. They were housed 4 to a cage in a temperature-controlled (22°C) vivarium that was maintained on a 12-hr light-dark cycle (lights on between 8:00 and 20:00 hr). They were provided with food and water ad lib. At least 24 hr before an experiment, rats were transported to the laboratory where they would be exposed to noise.

Method of Noise Exposure

Rats were exposed to noise in Plexiglas cylindrical cages (15 cm in diameter, 24 cm in length). Eleven rows of holes (1 cm in diameter, 6 holes per row) were drilled equally-spaced longitudinally on the wall of the cylinder. One end of the cage was sealed closed and the other end was a removable door. The cage was put on its side. A platform made of plastic rods was built longitudinally in the cylinder (3 cm from the lower side), through which waste could fall through. The rat had sufficient room to move freely inside the cage. A loudspeaker (Speakerlab, WA; Model KR 4580) was mounted at 30 cm above the cage and activated by a white noise generator (Lehigh Valley Electronics, Model 581-02) powered by an amplifier system (Hewlett-Packard 467A power amplifier and 6215A power supply). The frequency range of the noise generated was up to 40 kHz. Noise intensity was measured with a sound-meter (B & K, Inc.) and found to be uniform inside the cage. The background noise intensity inside the cage was 60 dB which came mostly from the ventilation system in the room.

In each experiment, rats were exposed to the white noise at either 70 or 100 dB for 45 min. Control animals were sham-exposed, i.e., they were placed in the exposure cage and exposed to the ambient noise for 45 min. All exposures were done between 8:30 and 9:30 hr. In each experiment, the experimenter would put an animal in the cage, turn on the noise, and leave the room. At the end of the exposure period, he would remove and sacrifice the rat on a nearby workbench.

Method of Sodium-Dependent High-Affinity Choline Uptake Assay

A different experimenter, who was blind to the noise exposure condition of the rat, did the choline uptake assay from the brain tissues. Immediately after noise exposure, rats were sacrificed by decapitation and their brains were removed and dissected on ice into striatum, hypothalamus, and hippocampus according to the method of Glowinski and Iversen [12]. The frontal cortex, consisting of the cerebral cortex anterior to a coronal cut at the optic chiasma with the olfactory tubercles, septum, and frontal portion of the striatum removed, was also dissected out.

Sodium-dependent high-affinity choline uptake in the brain tissue was assayed by the method described by Zucker *et al.* [36] as follows. Brain tissue was homogenized in 2 ml of 0.32 M sucrose solution with a glass homogenizer. The homogenate was centrifuged at 1,000×g for 10 min. The supernatant was then recentrifuged at 17,000×g for 15 min, and the pellet was reconstituted in 2 ml of 0.27 M sucrose. Of this synaptosome preparation, 0.1 ml was added to each of a set of tubes containing 0.9 ml of a buffer (containing 4% dextrose, 126 mM NaCl, 1.28 mM Na_2HPO_4, 4.74 mM KCl, 1.27 mM $CaCl_2$, and 1.42 mM $MgCl_2$; pH 7.2), 0.3 μM choline chloride, and 0.4 μCi of ^{3}H-choline (80 Ci/mmol, New England Nuclear, Boston, MA). Nonsodium-dependent choline uptake was determined by addition of 0.3 μM of hemicholinium-3 (Sigma Chemicals, St. Louis, MO) to another similar set of tubes. Each brain sample was assayed in triplicate.

The samples were transferred from an ice-water bath to a water bath at 38°C for incubation for 4 min. Uptake was terminated by return of the samples to the ice-water bath. Synaptosomes were collected by centrifugation at 8,000×g for 20 min. The supernatant was discarded, and the pellet was washed with 1 ml of ice-cold 0.9% saline. The saline was removed, and the pellet was dissolved overnight with 0.7 ml of Protosol (New England Nuclear, Boston, MA). Protosol was then neutralized with 30 μl of glacial acetic acid, and 8 ml of Econofluor (New England Nuclear, Boston, MA) was added. Radioactivity was determined by liquid scintillation at 36% counting efficiency. High-affinity choline uptake was determined as the difference in uptakes in the absence and presence of hemicholinium-3, and was found to be approximately 40–50% of the total uptake. Protein concentration of the synaptosomal preparation was determined by the method of Lowry *et al.* [22] with bovine serum albumin as standards.

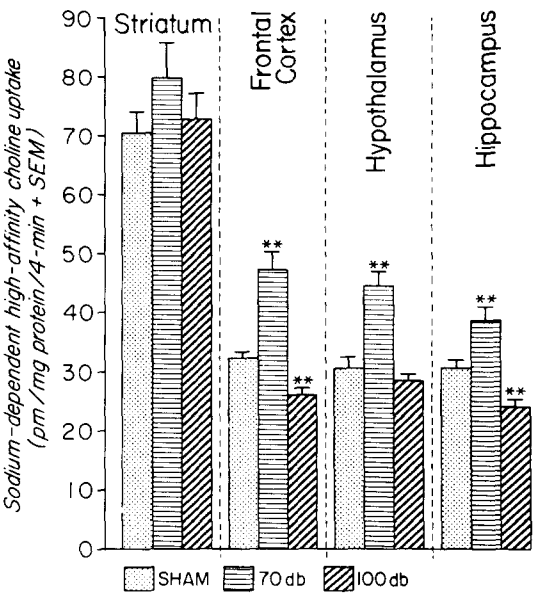

FIG. 1. Effects of acute exposure to 70-dB (n=12) or 100-dB (n=8) white noise on choline uptake in the striatum, frontal cortex, hypothalamus, and hippocampus. **Significantly different from uptake of sham-exposed controls (n=15) at $p<0.01$, as compared by the Newman-Keuls test.

Drug Treatments

In some experiments, rats were injected with naltrexone hydrochloride (1 mg base/kg, IP; Du Pont Pharmaceuticals, Wilmington, DE) immediately before exposure. The drug was dissolved in pyrogen-free, sterile physiological saline and injected at a volume of 1 ml/kg. Some rats were injected with 1 ml/kg of the physiological saline intraperitoneally.

Data Analysis

Sodium-dependent high-affinity choline uptake is expressed as pmole/mg protein/4 min. Data were analysed by the one-way analysis of variance, and difference between two treatment groups was compared by the Newman-Keuls test. A difference with at least a $p<0.05$ was considered statistically significant.

RESULTS

Effects of acute exposure to 70- and 100-dB white noise on choline uptake in different areas of the rat brain are shown in Fig. 1. One-way ANOVA showed significant treatment effects in the frontal cortex, $F(2,32)=22.89$,

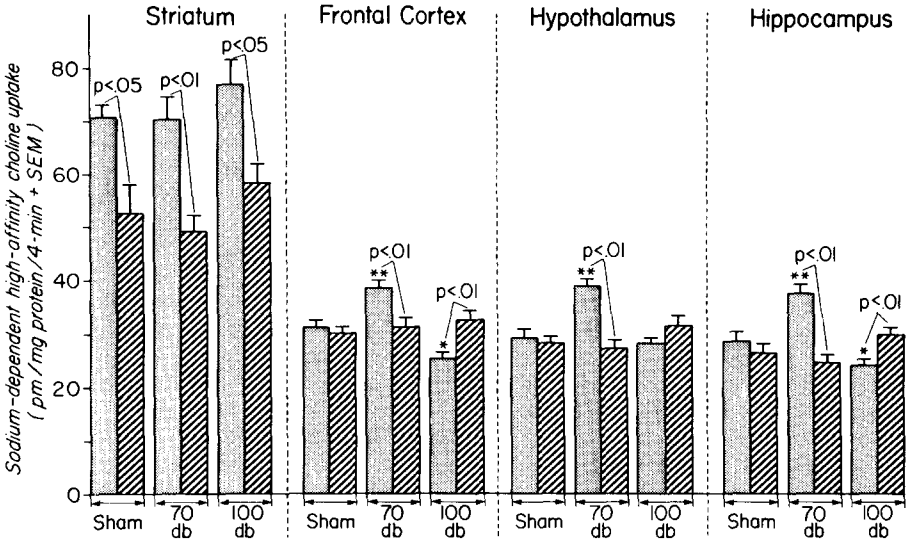

FIG. 2. Effects of pretreatment with naltrexone on choline uptake in different brain areas of rats exposed to 70- or 100-dB white noise (dotted bars=saline-treated; shaded bars=naltrexone-treated). *, **Significantly different from saline-treated, sham-exposed controls at $p<0.05$ and 0.01, respectively, as compared by the Newman-Keuls test. Each bar represents data from 8 rats.

$p<0.005$, hypothalamus, $F(2,32)=11.34$, $p<0.005$, and hippocampus, $F(2,32)=17.37$, $p<0.005$. No significant treatment effect was observed in the striatum, $F(2,32)=1.16$, nonsignificant. After exposure to the 70-dB noise, choline uptake in the frontal cortex, hypothalamus, and hippocampus was enhanced, whereas no significant effect was found in the striatum. Exposure to the 100-dB noise decreased choline uptake in the frontal cortex and the hippocampus and no significant effect was observed in the striatum and hypothalamus.

Effects of pretreatment with naltrexone on the effect of white noise on choline uptake in the different areas of the rat brain are shown in Fig. 2. Effects of noise on central choline uptake were similar in the saline-injected animals and the animals that received no injection (cf., Fig. 1). One-way ANOVA showed significant treatment effects in the striatum, $F(5,42)=7.75$, $p<0.005$, frontal cortex, $F(5,42)=9.28$, $p<0.005$, hypothalamus, $F(5,42)=4.91$, $p<0.005$, and hippocampus, $F(5,42)=15.13$, $p<0.005$. Data in Fig. 2 show that in the striatum, no significant effect was seen after exposure to 70- or 100-dB noise (i.e., no significant difference compared to the uptake of saline-treated, sham-exposed animals). However, naltrexone treatment significantly reduced the choline uptake in the striatum irrespective of the exposure condition (sham or noise exposure).

Figure 2 also shows that naltrexone blocked the effect of 70- and 100-dB white noise on choline uptake in the frontal cortex. No significant difference in uptake was observed in the 70- or 100-dB noise-exposed naltrexone-treated rats compared to sham-exposed naltrexone-treated rats. However, naltrexone treatment did not significantly affect the frontal cortical choline uptake activity in the sham-exposed rats. Similar results were observed in the hippocampus; naltrexone pretreatment blocked the effects of noise of both intensities on choline uptake.

Pretreatment with naltrexone blocked the enhancement effect of 70-dB noise on choline uptake in the hypothalamus. Again, naltrexone treatment did not significantly affect choline uptake in the hypothalamus of the sham-exposed rats.

DISCUSSION

Data presented in this paper show that cholinergic activity in various regions of the brain of the rat is affected by acute exposure to white noise. The effects are dependent on the intensity of the noise and are blocked by pretreatment with narcotic antagonist. Furthermore, different areas of the brain respond differently.

Cholinergic innervations to the brain areas studied come from different sources: innervations to the hippocampus originate mainly from the medial septum/diagonal band of Broca, frontal cortical innervations originate from the nucleus basalis magnocellularis, cholinergic activity in the striatum are mainly from interneurons, whereas the hypothalamus contains intrinsic and extrinsic cholinergic innervations [7]. Activities of these cholinergic pathways are controlled by different neural mechanisms [26]. Thus, it is possible that they respond differently to acute noise stimulation.

The neural mechanisms by which noise affects central cholinergic activity are not known. Since central auditory system does not directly innervate the frontal cortex, hippocampus, and hypothalamus, the effects of noise on cholinergic activity in these brain areas would be indirect. Perhaps the arousal effect of noise affects the reticular formation which in turn modulates the cholinergic systems. Acute exposure to noise has been reported to lead to behavioral changes suggestive of general arousal. Acute noise exposure has been shown to increase open-field behavior [17]. Interestingly, this effect was also shown to be blocked by naltrexone treatment. Another behavioral effect indicative of arousal is that acute exposure to noise of various intensities has been shown to induce eating behavior in rats [20]. An interaction effect between noise exposure and tail-pinch on eating behavior has also been reported [33].

The cholinergic systems in the brain have been shown to be affected by restraint stress [8, 10, 11, 21]. Similar to the effect of noise, the effect of restraint on central cholinergic activity is also biphasic. Increase in choline uptake was seen in the hippocampus after short duration of restraint, and decreases in uptake in the hippocampus and frontal cortex were observed after a longer period of restraint. Biphasic behavioral responses to other "stressors" have also been reported. A series of experiments [15, 16, 27] has pointed out the different effects on behavior produced by acute and chronic exposure to "stressor." Acute exposure produces a behavioral activating effect whereas chronic exposure leads to a state of depression. However, it must be pointed out that in the above cases, biphasic response was observed after different durations of exposure to "stressor," whereas in the present study, biphasic response was a function of the intensity of noise. The neuromechanism of the biphasic response is not known. Endogenous opioids seem to play a role, since both components can be blocked by narcotic antagonist.

Another interesting finding of the present experiments is that the effect of noise can be blocked by pretreating the animals with a low dose of the narcotic antagonist naltrexone. However, naltrexone treatment has no significant effect on choline uptake in the frontal cortex, hippocampus, and hypothalamus in the sham-exposed animals. This finding would suggest that noise exposure activates endogenous opioids in the brain, which in turn cause the changes in cholinergic activity. In the striatum, where noise exposure has no significant effect, naltrexone treatment decreased cholinergic activity irrespective of the exposure condition. The cholinergic innervations in the striatum are probably under tonic control by endogenous opioids. Effects of opiates on striatal cholinergic functions have been reported. Morphine administration has been shown to enhance high-affinity choline uptake in the striatum of the mouse [32]. However, another study shows that the potent μ-agonist etorphine could suppress the turnover rate of acetylcholine in the striatum of the rat [34]. It is well known that cholinergic activity in the hippocampus is modulated by endogenous opioids. Administration of morphine or endogenous opioids to rats has been shown to change cholinergic activity in the hippocampus. However, both enhancement and depression in cholinergic activity have been reported, and are probably dependent on the dose of opiate administered [25,31]. Effect of endogenous opioids on hypothalamic cholinergic activity is less well studied. It is conceivable that opioids can modify cholinergic activity in the hypothalamus, since both transmitters are present in high concentrations in that brain area. The finding that the effects of noise on frontal cortical choline uptake were blocked by naltrexone suggests that endogenous opioids are involved in the control of frontal cortical cholinergic activity. Morphine has been shown to cause a naltrexone-reversible decrease in turnover rate of acetylcholine in the cerebral cortex [35]. However, another study [34] reported that various narcotic agonists had no significant effect on acetylcholine turnover rate in the frontal cortex of the rat. Furthermore, we [21] found that the effect of restraint stress on frontal cortical choline uptake was not significantly affected by treatment with narcotic antagonist. In this respect, the effects of noise and restraint are different.

Central cholinergic functions are involved in a wide variety of behavioral and physiological functions, such as learning, arousal, and motivational and consummatory behaviors [30]. The effect of noise on central cholinergic activity could result in detrimental behavioral effects. On the other hand, changes in cholinergic activity could be a direct consequence of the arousal effect of noise. The latter could be a coping mechanism to the stress.

The present study is only a preliminary experiment investigating the effect of acute noise exposure on brain functions. More questions can certainly be asked, e.g., what are the effects of varying the frequency of the noise, the effects of chronic exposure, the duration and reversibility of the effects after acute or chronic noise exposure, and the behavioral consequences of the effects? Further research on these aspects is certainly required.

REFERENCES

1. Amir, S., Z. W. Brown and Z. Amit. The role of endorphin in stress: Evidence and speculations. *Neurosci Biobehav Rev* **4:** 77–86, 1980.
2. Anticaglia, J. R. and A. Cohen. Extra-auditory effects of noise as a health hazard. *Am Indust Hyg Assoc J* **31:** 277–281, 1970.
3. Armario, A., J. M. Castellano and J. Balasch. Adaptation of anterior pituitary hormones to chronic noise stress in male rats. *Behav Neural Biol* **41:** 71–76, 1984.
4. Beardwood, C. J., C. A. Mundell and W. H. Utian. Gonadotropin excretion in response to audiostimulation of human subjects. *Am J Obstet Gynecol* **121:** 682–687, 1975.
5. Edmonds, L. D., P. M. Layde and J. D. Erickson. Airport noise and teratogensis. *Arch Environ Health* **34:** 243–247, 1979.
6. Favino, A., U. Maugeri, G. Kauchtschischvili, G. R. D. Cuna and G. Nappi. Radioimmunoassay measurements of serum cortisol, thyroxine, growth hormone and luteinizing hormone with simultaneous electroencephalographic changes during continuous noise in man. *J Nucl Biol Med* **17:** 119–122, 1973.
7. Fibiger, H. The organization and some projections of cholinergic neurons of the mammalian brain. *Brain Res Rev* **4:** 327–388, 1982.
8. Finkelstein, Y., B. Koffler, J. M. Rabey and G. M. Gilad. Dynamics of cholinergic synaptic mechanisms in rat hippocampus after stress. *Brain Res* **345:** 314–319, 1985.
9. Frerichs, R. R., B. L. Beeman and A. H. Coulson. Los Angeles airport noise and motality-faulty analysis and public policy. *Am J Public Health* **70:** 357–362, 1980.
10. Gilad, G. M., J. M. Rabey and L. Shenkman. Strain-dependent and stress-induced changes in rat hippocampal cholinergic system. *Brain Res* **267:** 171–174, 1983.
11. Gilad, G. M., B. D. Mahon, Y. Finkelstein, B. Koffler and V. H. Gilad. Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. *Brain Res* **347:** 404–408, 1985.
12. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain—1. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-DOPA in various regions of the brain. *J Neurochem* **13:** 655–669, 1986.
13. Henkin, R. I. and K. M. Knigge. Effect of sound on the hypothalamic-pituitary adrenal axis. *Am J Physiol* **204:** 710–714, 1963.
14. Herridge, C. F. Aircraft noise and mental hospital admission. *Sound* **6:** 32–36, 1972.
15. Katz, R. J. Animal model of depression: Pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav* **16:** 965–968, 1982.
16. Katz, R. J. and G. Baldrighi. A further parametric study of imipramine in an animal model of depression. *Pharmacol Biochem Behav* **16:** 969–972, 1982.

17. Katz, R. J. and C. P. Manik. Endogenous opiates and stress: behavioral activation elicited in the rat and effect of naltrexone, diprenorphine and morphine. *Neuropharmacology* **23:** 1425–1430, 1984.
18. Knipschild, P., H. Meijer and H. Salle. Aircraft noise and birth weight. *Int Arch Occup Environ Health* **48:** 131–136, 1981.
19. Kraicer, J., G. Beraud and D. W. Lywood. Pars intermedia ACTH and MSH contents: effect of adrenalectomy, gonadectomy and a neurotropic (noise) stress. *Neuroendocrinology* **23:** 352–367, 1977.
20. Kupfermann, I. Eating behavior induced by sound. *Nature* **201:** 324, 1964.
21. Lai, H., J. Zabawska and A. Horita. Sodium-dependent high-affinity choline uptake in hippocampus and frontal cortex of the rat affected by acute restraint stress. *Brain Res* **372:** 366–369, 1986.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein determination using the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
23. Miller, J. D. Effects of noise on people. *J Acoust Soc Am* **56:** 729–764, 1974.
24. Moller, A. R. Occupational noise as a health hazard: physiological viewpoints. *Scand J Work Environ Health* **3:** 73–79, 1977.
25. Moroni, F., D. L. Cheney and E. Costa. The turnover rate of acetylcholine in brain nuclei of rats injected intraventricularly and intraseptally with alpha- and beta-endorphin. *Neuropharmacology* **17:** 191–196, 1978.
26. Robinson, S. E. Cholinergic pathways in the brain. In: *Central Cholinergic Mechanisms and Adaptive Dysfunctions*, edited by M. M. Singh, D. M. Warburton and H. Lal. New York: Plenum Press, 1985, p. 37.
27. Roth, K. A. and R. J. Katz. Stress, behavioral arousal and open-field activity: A re-examination of emotionality in the rat. *Neurosci Biobehav Rev* **3:** 247–263, 1979.
28. Schell, L. M. and L. S. Lieberman. Noise stress and cancer. In: *Stress and Cancer*, edited by K. Bammer and B. H. Newberry. Toronto: C. J. Hogrefe Inc., 1981, p. 202.
29. Simon, J. R., S. Atweh and M. J. Kuhar. Sodium-dependent high-affinity choline uptake: a regulatory step in the synthesis of acetylcholine. *J Neurochem* **26:** 909–922, 1976.
30. Singh, M. M., D. M. Warburton and H. Lal. *Central Cholinergic Mechanisms and Adaptive Dysfunctions*. New York: Plenum Press, 1985.
31. Vallano, M. L. and T. K. McIntosh. Morphine stimulates cholinergic neuronal activity in the rat hippocampus. *Neuropharmacology* **19:** 851–853, 1980.
32. Vallano, M. L., M. T. Spoerlein and C. Van Der Wende. Morphine enhances high-affinity choline uptake in mouse striatum. *Pharmacol Biochem Behav* **17:** 419–423, 1982.
33. Wilson, J. F. and M. B. Cantor. Noise-induced eating in rats facilitated by prior tail-pinch experience. *Physiol Behav* **37:** 523–526, 1986.
34. Wood, P. L. and L. M. Stotland. Actions of enkephalin, μ, and partial agonist analgesics on acetylcholine turnover in rat brain. *Neuropharmacology* **19:** 975–982, 1980.
35. Zsilla, G., G. Racagni, D. L. Cheney and E. Costa. Constant rate infusion of deuterated phosphoylcholine to measure the effects of morphine on acetylcholine turnover rate in specific nuclei of rat brain. *Neuropharmacology* **16:** 25–31, 1977.
36. Zucker, J. R., H. Lai and A. Horita. Intraseptal microinjections of substance P and analogs potentiate pentobarbital-induced narcosis and depression of hippocampal cholinergic activity. *J Pharmacol Exp Ther* **235:** 398–401, 1985.