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Effects of Piracetam on Membrane Fluidity in the Aged Mouse, Rat, and Human Brain

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ABSTRACT. *In vitro* preincubation of brain membranes of aged mice with piracetam (0.1–1.0 mmol/L) enhanced membrane fluidity, as indicated by decreased anisotropy of the membrane-bound fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Piracetam had similar *in vitro* effects on brain membranes of aged rats and humans, but it did not alter brain membrane fluidity in young mice. Chronic treatment of young and aged rats with piracetam (300 mg/kg once daily) significantly increased membrane fluidity in some brain regions of the aged animals, but had no measurable effect on membrane fluidity in the young rats. The same treatment significantly improved active avoidance learning in the aged rats only. It is suggested that some of the pharmacological properties of piracetam can be explained by its effects on membrane fluidity. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 53;2:135–140, 1997.

KEY WORDS. piracetam; membrane; fluidity; aging; cognitive functions

A variety of experimental findings indicate that the cognition-enhancing properties of piracetam are usually more pronounced in aged than in young animals [1–4]. Therefore, it seems reasonable to assume that the mechanism of action of piracetam is at least partially related to biochemical alterations of the aged brain relevant for the impaired cognitive functions and that piracetam might restore or counteract these biochemical deficits of the aging brain.

Reduced fluidity of brain cell membranes probably represents an important mechanism explaining many functional alterations of the aged brain [5, 6]. Reduced fluidity of brain membranes has been shown for many species [7–11] and is usually explained by increased cholesterol to phospholipid ratios of brain membranes and by enhanced lipid peroxidation, leading to higher membrane concentrations of saturated fatty acids [6, 10, 12, 13]. Some previous observations seem to indicate that piracetam might interfere with mechanisms regulating integrity and composition of cellular membranes [14–16]. Thus, partial restoration of age-related changes in membrane fluidity could explain many of piracetam's effects on brain neurochemistry and behaviour in aging. The present paper reports some experiments that support this assumption and strongly suggest that piracetam directly alters membrane properties in the aging brain.

MATERIALS AND METHODS

Tissues

Female NMRI mice (3 and 22 months) and male Wistar rats (3 and 24 months) were killed by decapitation. The brains were quickly removed and homogenized in 20 mL 5 mmol/L Tris-HCl buffer (pH = 7.4). The homogenate was centrifuged at 40,000 × *g* for 20 min. The resulting pellet was resuspended twice in 20 mL Tris-HCl buffer (pH = 7.4) and centrifuged at 40,000 × *g* for 20 min. The final pellet was stored at −20°C until use in fluorescence polarization measurements. Human cortical tissue was obtained from autopsy cases without any mental diseases (age: 78 ± 10 years). For *ex vivo* experiments, young and aged rats were treated once daily (orally by stomach tube) with piracetam at the dose indicated.

Membrane Fluidity Measurements

Membrane fluidity of the individual brain homogenates was determined using DPH§ as a fluorescence probe. The samples were diluted with Tris-HCl buffer (5 mmol/L, pH 7.4, at 37°C) to give approximately 30 μg protein/100 μL. Samples (100 μL) were incubated with 900 μL Tris-HCl buffer and 1000 μL of a 1:150 DPH solution (prepared from a stock solution of 5 mmol/L DPH in tetrahydrofuran) for 60 min at 37°C or 25°C, after which time anisotropy was

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§ *Abbreviations:* DPH, 1,6-diphenyl-1,3,5-hexatriene; CR, conditioned response.

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stable. Fluorescence polarization was directly measured in an SLM 4800C Aminco spectrofluorometer, using excitation and emission wavelengths of 360 nm and 450 nm, respectively. As in other studies on the effect of aging on membrane properties [7, 12], the measured steady-state fluorescence polarization (P_s) was expressed as the anisotropy (r_s) of the probe using the following equation: $r_s = 2P_s/3 - P_s$.

Active Avoidance Learning

The rats were placed in the active avoidance learning box (31 × 35 × 50 cm), where a tone signal (1.6 kHz, 8 sec) (the conditioned stimulus) was followed by a foot shock (40 V, 12 sec) (the unconditioned stimulus) after 8 sec. The animals could avoid the shock by jumping onto a suspended pole. Avoidance learning was tested over 4 sessions (days), each comprising 10 consecutive trials. The test criterion for a CR was a rat's jumping onto the pole during the tone signal (8 sec). Further, the latency from the start of the tone to jumping onto the pole was registered. Treatment of the animals was performed as described under *Tissues*.

Statistics

For statistical analysis (two-tailed *t*-test, ANOVA, correlation analyses), the SAS package was used.

RESULTS

As already shown for many species [6, 8, 11], brain membranes of aged mice showed significantly decreased fluidity, as indicated by enhanced fluorescence polarization (anisotropy) of membrane-bound DPH (Fig. 1). Preincubating membranes of the aged mouse brain with increasing concentrations of piracetam (0.1–1.0 mmol/L) decreased anisotropy (Fig. 1; Table 1). At 1 mmol/L piracetam, anisotropy values of aged membranes were no longer different from the values of young membranes (Fig. 1; Table 1). However, even at 1 mmol/L, piracetam did not alter fluidity of membranes of the young mouse brain, as indicated by the unchanged anisotropy of membrane-bound DPH (Fig. 1; Table 1). On the other hand, a comparable *in vitro* effect of piracetam (1 mmol/L) was demonstrated for membranes of the aged mouse, rat, and human brain, where piracetam significantly enhanced fluidity as indicated by decreased DPH anisotropy (Fig. 2).

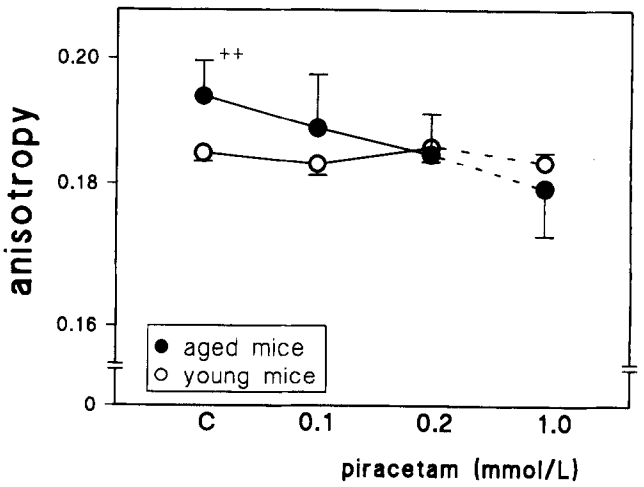

FIG. 1. Effects of piracetam (0.1–1.0 mmol/L) on brain membrane DPH anisotropy of young and aged mice at 37°C. Mouse brain membranes were incubated for 60 min (after which time the piracetam effect was maximal) with DPH alone or with DPH plus piracetam at the concentrations indicated, and membrane anisotropy was determined as indicated under Materials and Methods. Each point represents the mean ± SD of six determinations, each representing an individual animal. There was a significant treatment effect ($P < 0.02$, ANOVA) for the aged mice only. Versus young controls: ††$P < 0.01$.

TABLE 1. Effects of piracetam (1 mmol/L) and ethanol (0.2 or 1.0%) on fluidity parameters of brain membranes of young and aged mice

	37°C		
	r × 10^2	p × 10^2	η (poise)
Young	18.48 ± 0.13	25.37 ± 0.19	2.46 ± 0.08
Young + piracetam (1 mmol/L)	18.33 ± 0.16	25.19 ± 0.24	2.42 ± 0.01
Aged	19.38 ± 0.58	26.50 ± 0.87	2.72 ± 0.04
Aged + piracetam (1 mmol/L)	17.94 ± 0.70***	24.70 ± 1.05***	2.33 ± 0.06***
Young	18.21 ± 0.25	25.04 ± 0.37	2.39 ± 0.02
Young + ethanol (0.2%)	17.04 ± 0.39***	23.34 ± 0.58***	2.06 ± 0.03***
Young + ethanol (1.0%)	16.63 ± 0.39***	23.03 ± 0.58***	2.01 ± 0.03***
Aged	19.04 ± 0.47	26.08 ± 0.70	2.62 ± 0.03
Aged + ethanol (0.2%)	17.34 ± 0.62***	23.94 ± 0.93***	2.17 ± 0.04***
Aged + ethanol (1.0%)	16.73 ± 0.53***	23.16 ± 0.87***	2.03 ± 0.04***

Anisotropy (r) and fluorescence polarization (p) were obtained from DPH measurements as described under Materials and Methods. Microviscosity (η) was obtained using the relationship $\eta = 2P/(0.46 - P)$ [21, 33]. Data are mean ± SD of six experiments, each representing membranes of an individual animal.
***$P < 0.001$.

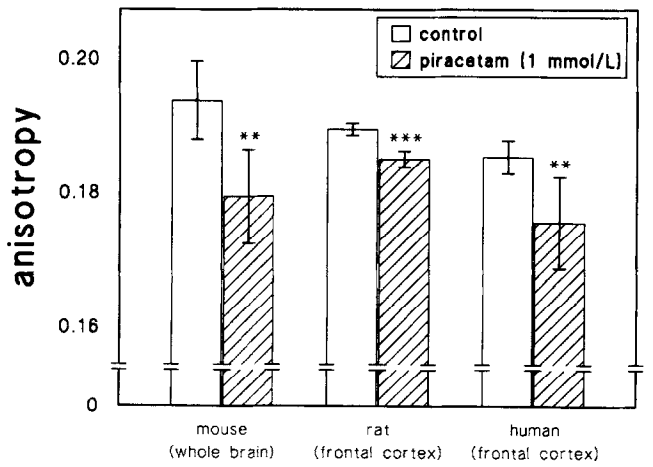

FIG. 2. Effects of piracetam (1.0 mmol/L) on brain membrane DPH anisotropy in aged mice (total brain), aged rats (frontal cortex), or aged humans (frontal cortex) at 37°C. Each point represents the mean ± SD of six determinations, each representing an individual animal. For further experimental details, see Fig. 1. Versus controls: **$P < 0.01$, ***$P < 0.001$.

In contrast to piracetam, ethanol concentrations as high as 0.2% and 1.0% decreased DPH anisotropy in membranes of young and aged mice in a similar fashion (Table 1). No effects of ethanol were observed at concentrations between 0.1 and 1.0 mmol/L (data not shown). Two structurally related piracetam-like nootropics (oxiracetam, pramiracetam) also increased fluidity of mouse brain membranes *in vitro* (Fig. 3). However, maximal effects were already seen at 0.1 mmol/L, and both drugs also increased membrane fluidity in the young mouse brain (Fig. 3).

We could confirm [6] that at temperatures below phase transition, anisotropy of membrane-bound DPH decreased in aging brain (Fig. 4). Piracetam again reversed the age-specific changes (Fig. 4). However, it also reduced DPH anisotropy in brain membranes of young mice (Fig. 4), indicating that its effect on membrane fluidity is not completely specific for the aged brain. The effect of piracetam on DPH anisotropy in aged brain membranes at 25°C was also different from the effect of ethanol (Fig. 4).

Age-specific and region-specific effects of piracetam on brain membrane fluidity could also be demonstrated after chronic treatment of rats (300 mg/kg piracetam daily for 8 weeks). Whereas piracetam had no effect on anisotropy values in all four brain regions of young animals, it did significantly decrease anisotropy values in the frontal cortex, the hippocampus, and the striatum of aged rats, but not in the cerebellum (Fig. 5). However, in all four regions, we observed an age-related decrease in membrane fluidity (Fig. 5).

Treatment of aged rats with piracetam (300 mg/kg for 8 weeks) significantly improved active avoidance learning (Fig. 6). Although the treatment did not show significant effects on active avoidance performance of young rats, it significantly improved latencies and the numbers of conditioned responses in the aged animals (Fig. 6). This is in agreement with other observations concerning the effects of piracetam on active avoidance learning [17].

DISCUSSION

Our data confirm several previous studies that have reported that brain membrane fluidity decreases with aging, as indicated by the elevated anisotropy of membrane-bound DPH [6, 7, 9–13]. The increase in DPH anisotropy with aging was comparable for membranes of the whole mouse brain and of all four rat brain regions (frontal cortex, hippocampus, striatum, cerebellum). Piracetam increased brain membrane fluidity not only after *in vitro* incubation within 1 hr (mouse, rat, human tissue) but also after *in vivo* treatment for several weeks (rats), indicating that this effect is also present under *in vivo* conditions, where piracetam improves cognitive functions. Piracetam had no measurable effects on brain membrane fluidity at 37°C in young ani-

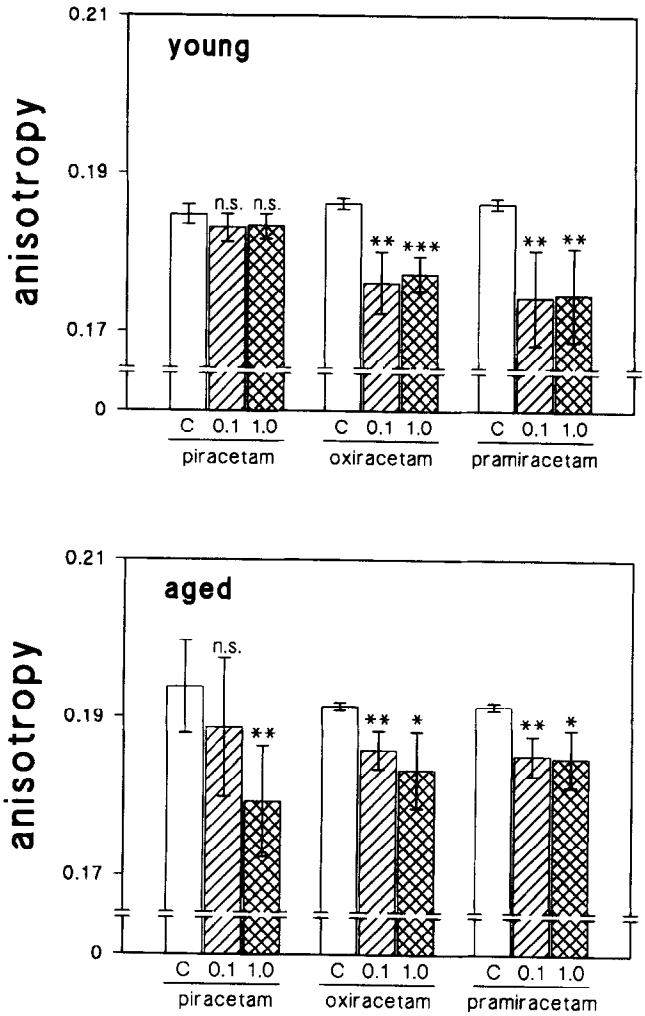

FIG. 3. Effects of piracetam, oxiracetam, and pramiracetam (0.1 and 1.0 mmol/L) on the fluidity of brain membranes of young and aged mice *in vitro* at 37°C. Each point represents the mean ± SD of six determinations, each representing an individual animal. For further experimental details, see Fig. 1. Versus controls: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

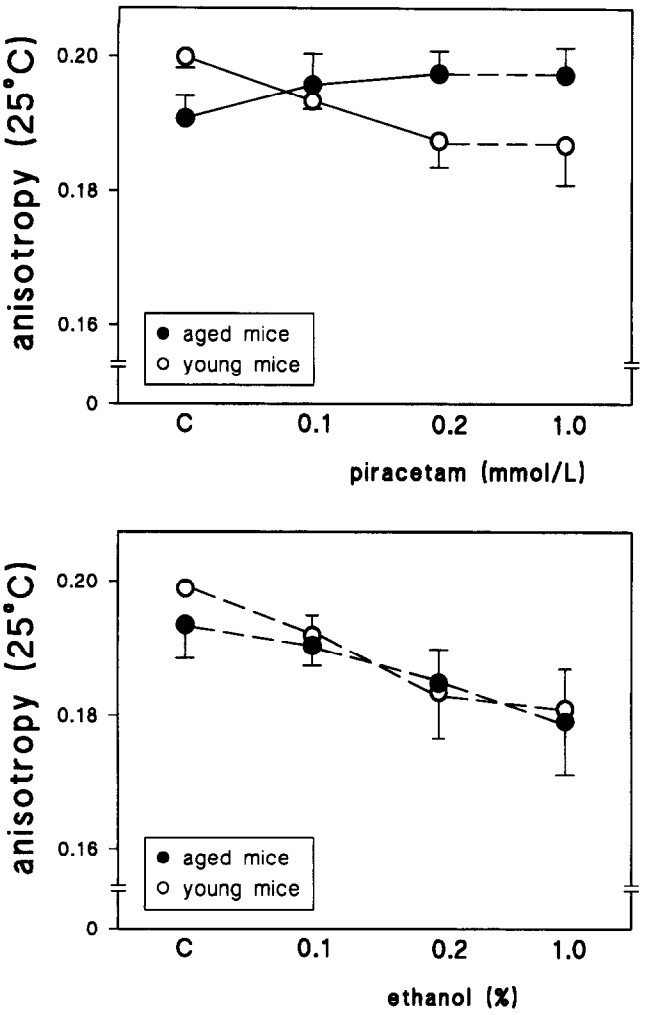

FIG. 4. Effects of piracetam (0.1 and 1.0 mmol/L) and of ethanol (0.1–1.0%) on brain membrane DPH anisotropy of young and aged mice at room temperature. Each point represents the mean ± SD of six determinations, each representing an individual animal. For further experimental details, see Fig. 1. There were significant (ANOVA) treatment effects for piracetam in young ($P < 0.001$) and aged mice ($P < 0.02$), and for ethanol in young ($P < 0.01$) and aged mice ($P < 0.05$).

mals *in vivo* or *in vitro*. The simplest explanation for the age-specific effects of piracetam on membrane fluidity would be modification of mechanisms responsible for the decrease in membrane fluidity in the aging brain. This explanation, however, is rather unlikely because of the rapid *in vitro* activity of piracetam, which occurs too quickly to significantly alter enhanced lipid and protein peroxidation or increased cholesterol/phospholipid ratios of aged brain membranes. Moreover, piracetam does not alter membrane fluidity in the aged rat cerebellum, although fluidity there is decreased in aged rats similar to all other brain regions investigated. Accordingly, as piracetam accumulates into brain membranes [18, 19], it might alter membrane fluidity by partitioning into the phospholipid bilayer. Piracetam seems to be different from other, nonspecific membrane fluidizing agents such as aliphatic alcohols, which alter membrane fluidity independent of age (see the experiments with ethanol). The assumption that piracetam might alter membrane properties by partitioning into the phospholipid bilayer is also supported by the experiments at temperatures below phase transition, where the effects of aging and piracetam are similarly altered. Moreover, recent findings using magnetic resonance spectroscopy have demonstrated that piracetam interacts with phosphate head groups of artificial phospholipid bilayers [20]. This is consistent with the assumption that DPH anisotropy is mainly sensitive to the angular reorientation of the lipid acyl chains [21, 22].

It has been speculated that the age-related decrease in brain membrane fluidity might be partially involved in behavioral deficits of aged animals [10, 11, 23]. In our experiments, the same piracetam treatment that significantly improved membrane fluidity in some regions of the aged rat brain also improved active avoidance learning in the aged animals only. Moreover, the two piracetam derivatives oxiracetam and pramiracetam, which are approximately 5–10 times more active (on a mg/kg basis) in behavioral experiments [4, 17, 24], were also approximately 5–10 times

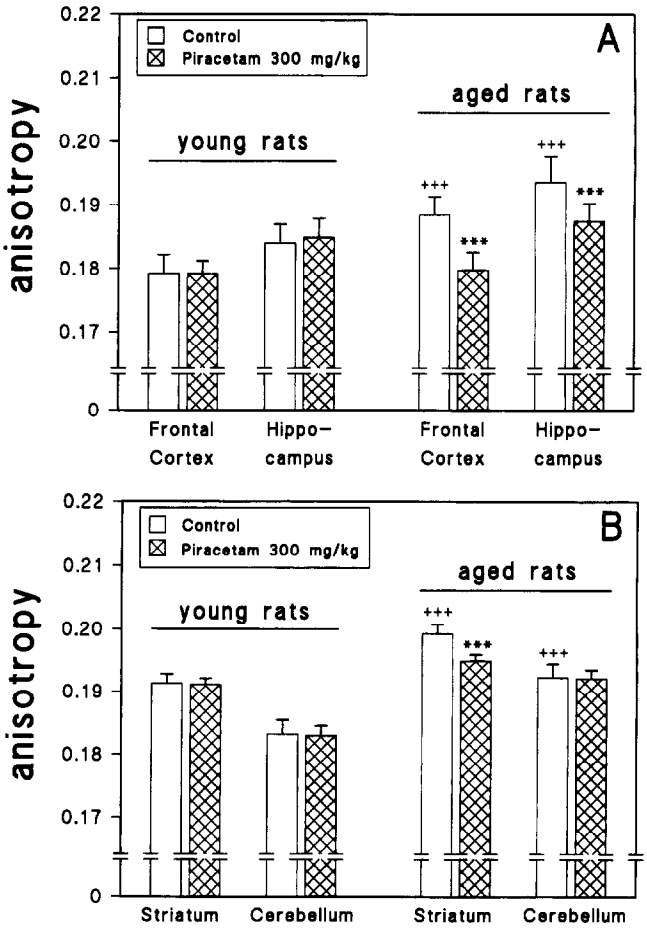

FIG. 5. Effects of chronic treatment of young (3 months) and aged (24 months) rats with piracetam (300 mg/kg once daily orally for 8 weeks) on the fluidity of membranes of four different brain regions. Data are the mean ± SD of 8–10 experiments, each representing an individual animal. †††$P < 0.001$ vs. young controls, ***$P < 0.001$ vs. aged controls.

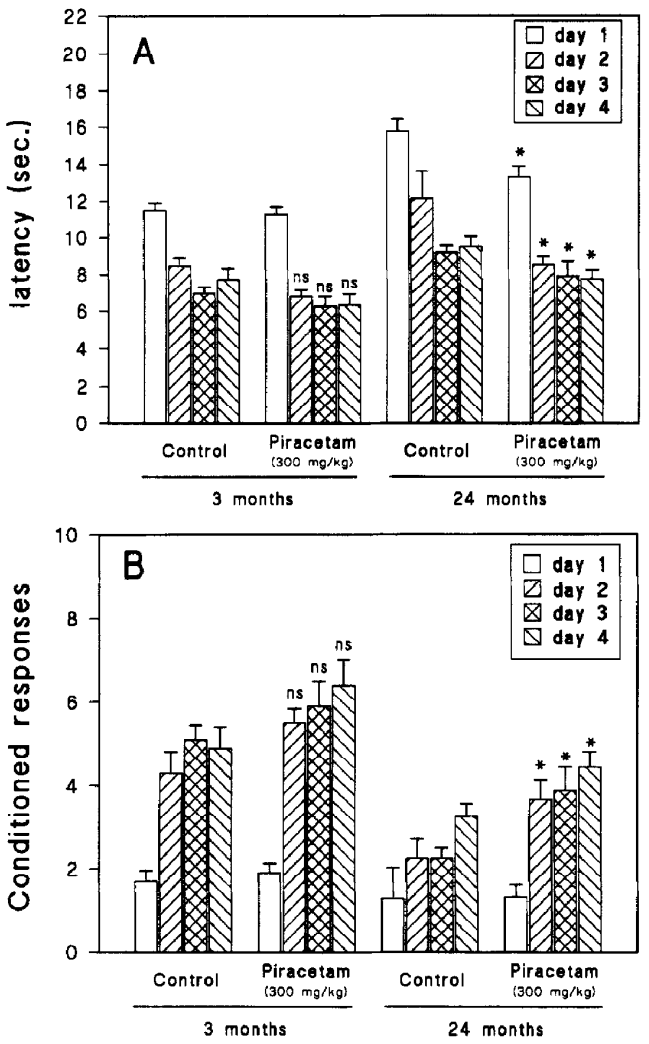

FIG. 6. Effects of subchronic treatment of young (3 months) and aged (24 months) rats with piracetam (see Fig. 4) on active avoidance learning. Data are given as latency values, i.e. the time in each trial needed to reach the criterion (A), or as the number of conditioned responses in each trial (B). There were significant effects of aging on latencies (F = 40.45; df = 1; $P < 0.0001$) and conditioned responses (F = 30.31; df = 1; $P < 0.0001$) and of treatment on latencies (F = 19.75; df = 1; $P < 0.0001$) and conditioned responses (F = 11.48; df = 1; $P < 0.002$). Age × treatment interaction did not reach significance for latencies (F = 2.30; df = 1; $P < 0.14$) or conditioned responses (F = 0.20; df = 1; $P < 0.65$). However, Bonferroni t-test (*$P < 0.05$) indicated a significant treatment effect for the aged animals only.

more active in increasing membrane fluidity *in vitro*. Both compounds were similarly effective in enhancing membrane fluidity in brains from young and aged mice. This again parallels behavioral data, as both drugs also seem to improve cognition in young rats and mice more than does piracetam [4, 17, 24]. Thus, our data are compatible with the assumptions that alterations in brain membrane fluidity might be involved in some of the behavioral effects of piracetam.

At the biochemical level, the decreased membrane fluidity of the aging brain has been thought responsible for deficits or dysfunctions of a large number of membrane-bound mechanisms of signal transduction, such as enzyme activities, receptor numbers, and receptor functions [5, 6, 12, 25]. Increasing the fluidity of aged brain membranes *in vitro* and *in vivo* with several fluidizing agents has been repeatedly shown to correct some of these deficits of signal transduction [7, 8, 26, 27]. Similarly, piracetam treatment with doses similar to the one used in the present experiments has been shown to specifically improve some deficits of receptor number or receptor function in the aging rat or mouse brain [28–32]. Thus, it seems possible that some of the nootropic properties of piracetam can be explained by effects on brain membrane properties in general and by modification of membrane-located mechanisms of central signal transduction.

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