Arousal deficit shown in aged rat's quantitative EEG and ameliorative action of pramiracetam compared to piracetam

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Summary. The basal EEG profile of the aged Fisher-344 rat was consistently different from that of the young rat, showing dominant high voltage slow-wave components. These slow waves were present in both the frontal cerebral cortex and dorsal hippocampus. Absent or greatly attenuated in the aged rat's hippocampal EEG was rhythmic theta activity, which was always dominant in the young awake rat's hippocampus. These EEG differences were clearly apparent only under basal test conditions, i.e., following habituation to the test situation. Pramiracetam sulfate acted strongly to normalize the aged rat's EEG, while the action of piracetam was weak and appeared to undergo tolerance development.

Key words. Pramiracetam sulfate; piracetam; cognition activator; nootropic; quantitative EEG; arousal or vigilance deficit; aged EEG; Fisher-344 rat.

Since the discovery of quantitative EEG analysis, very subtle changes in the EEG activity of brain can be measured. In recent years these methods have been applied in man to the study of all major classes of nootropic or encephalotropic agents. This recent work[1,2] has shown that one of the principal central actions of nootropic drugs in man is to promote cerebral vigilance.

Here we report that the aged rat, after habituation to the test situation, shows the characteristics of a vigilance deficit in both its cortical and hippocampal EEG. Moreover, the new cognition activator drug pramiracetam sulfate[3,4] was found to restore the aged rat's EEG to an essentially normal pattern.

Materials and methods. All tests were conducted on male Fisher-344 rats supplied by Harlan Laboratory of Indianapolis, Indiana. These animals lived in individual home cages in a room with 24-h general illumination. The animals weighed between 300 and 400 g and were 20 to 25 months old at the time of electrode implantation. Permanent recording electrodes were implanted bilaterally in the dorsal hippocampi (in the dentate region), and epidurally on the frontal cerebral cortices. The hippocampal electrodes were made from 30-gauge (A.W.G.) platinum wire with 0.5 mm of insulation scraped off at the tip. The frontal cortical electrodes consisted of a stainless steel screw 1.0 mm in diameter screwed into the skull and brought into contact with the surface of the dura mater. The animal was grounded through a similar stainless steel screw secured to the occipital bone of the skull. All leads were brought to a miniature plug implanted on the skull surface.

At the time of recording, a commutator device enabled the rat to move freely about the floor area of a test chamber 28.5 cm long, 21.5 cm wide, and 45 cm high. The wooden test chamber was painted flat beige, was open at the top, had a clear plexiglas front and a floor of metal rods; it was well-illuminated by room lighting entering from above and through the plexiglas front. Movement artifacts were eliminated by passing the insulated leads through a tygon tube filled with a saturated NaCl solution (electrically grounded). The recording system used was a 12-

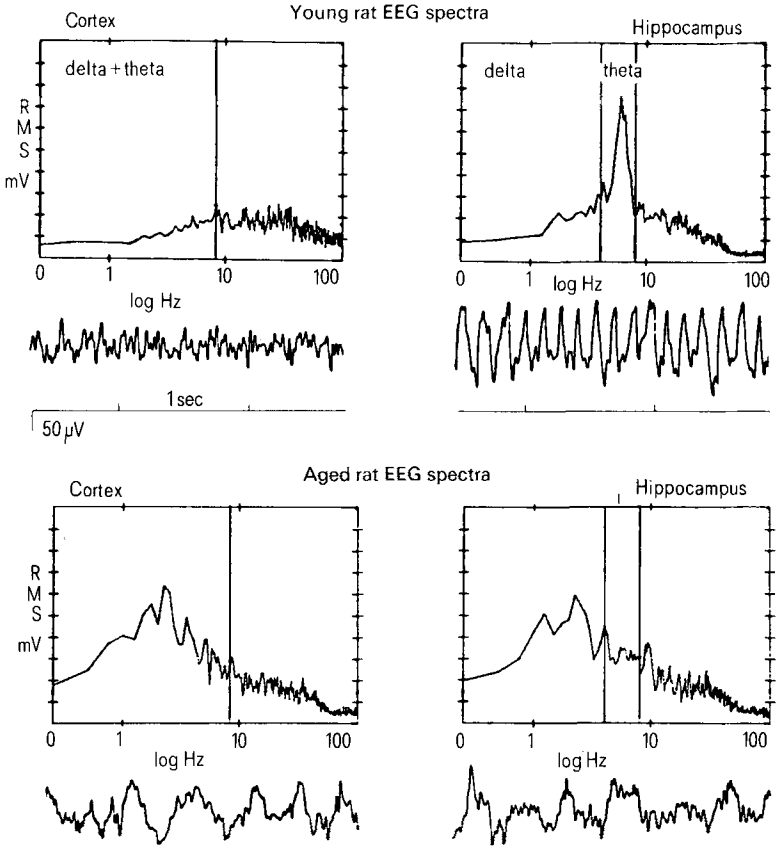


Figure 1. Representative spectral wave analyses from the frontal cortex and dorsal hippocampus of a young and an aged rat. A representative sample of actual EEG activity on which the spectral wave analysis was based is given below each spectral analysis.

channel, model 78D Grass polygraph, with outputs connected to a 12-channel, FM magnetic tape recorder. The analog signals were first electronically summed and then led through a digital FFT computing spectrum analyzer and averager (Nicolet model 446) and finally displayed by a digital oscilloscope and plotter with quantitative options. In the EEG spectra electronically placed windows were set as follows: 4–8 Hz in the hippocampus; 1–13 Hz in the cortex. The unit measured was millivolts root-mean-square (RMS mV). All rats served as their own controls for each test session, and the results were expressed as percentage change from the predrug control levels.

The test session was 180 min in length, with EEG samples taken every 30 min. These samples consisted of 32 successive 8 sec epochs analyzed with 400-line resolution. Each individual EEG spectral power analysis therefore represented 256 sec of the EEG tracing.

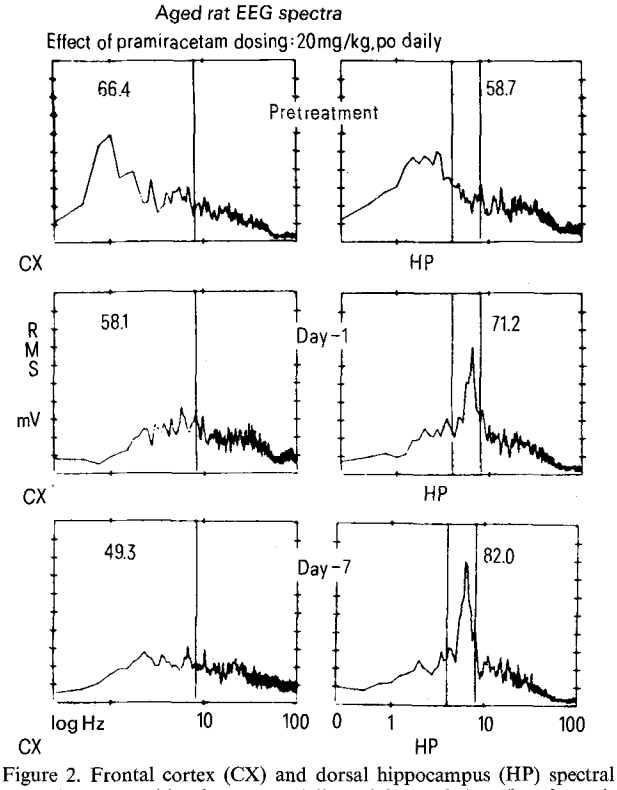
Prior to drug testing sessions, the rats were habituated to the test situation. This important phase of the method assured that the later EEG testing of drugs would be conducted under basal conditions. By basal condition is meant that the rat had become thoroughly familiar with being handled, with being orally dosed, with being in the test chamber, and with the normal background laboratory sounds. Moreover, the rat was moderately food deprived (20 h) during some of these acclimatization training sessions. Having the animals food deprived would be important in later drug testing sessions to: a) promote good absorption of drug following oral dosing, and b) to maintain wakefulness throughout the test sessions. About four weeks of daily 180-min sessions in the test chamber, 2–3 days per week, seemed required for establishing a totally stable basal EEG condition in our aged rats.

The actual drug testing was conducted as follows. Each individual EEG test session was run at about the same time in the afternoon, the rat having been deprived of food approximately 20 h earlier. The rat was connected to the recording leads, placed

Effect of repeated daily doses of pramiracetam, piracetam, and vehicle on hippocampal theta activity and cortical slow waves

Treatment and EEG source	Mean millivolts RMS (% change from day 0) day 0	day 1	day 3	day 7	n
Pramiracetam 5 mg/kg					
Hippocampus	52.6	70.4* (+33.8%)	75.0* (+42.6%)	82.9* (+57.6%)	4
Cortex	69.6	51.3* (−26.3%)	47.2* (−32.2%)	47.0* (−32.5%)	4
Pramiracetam 20 mg/kg					
Hippocampus	52.6	68.3* (+29.9%)	71.2* (+35.4%)	79.4* (+51.0%)	6
Cortex	75.7	60.5* (−15.2%)	55.0* (−27.3%)	50.3* (−33.6%)	6
Piracetam 200 mg/kg					
Hippocampus	53.0	58.6 (+10.6%)	63.6* (+20.0%)	57.6 (+8.7%)	4
Cortex	67.6	66.7 (−1.3%)	62.2 (−8.0%)	59.7 (−11.7%)	4
Piracetam 400 mg/kg					
Hippocampus	56.0	74.2* (+32.5%)	80.4* (+43.6%)	62.6* (+11.8%)	4
Cortex	61.0	57.4 (−6.3%)	48.3* (−20.8%)	56.0 (−8.2%)	4
Vehicle					
Hippocampus	53.4	54.4 (+1.9%)	53.9 (+0.9%)	53.7 (+0.6%)	6
Cortex	66.3	67.9 (+2.4%)	67.6 (+2.0%)	68.1 (+2.7%)	6

* Significantly different ($p < 0.05$) than corresponding day 0 reading by two-tailed Dunnett's t-test comparison (critical difference: cortex = 8.35; hippocampus = 7.19). Prior to this test, a repeated measures analysis of variance had demonstrated that effects due to treatment, day, and treatment × day, were all significant ($p < .001$) for both hippocampus and cortex.



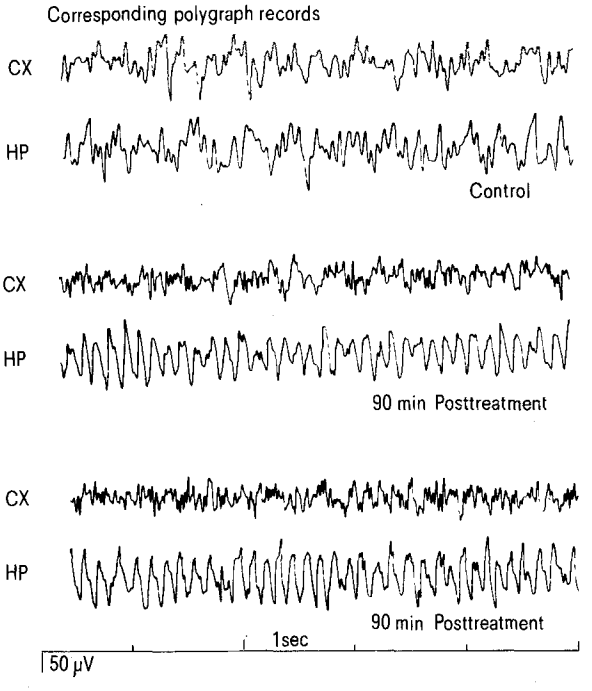


Figure 2. Frontal cortex (CX) and dorsal hippocampus (HP) spectral wave changes resulting from seven daily oral doses of 20 mg/kg of pramiracetam. Only the results from the pretreatment day, and from drug treatment days one and seven are shown. Numerical scores shown with each analysis represent the mean millivolts (root-mean-square) occurring within the indicated frequency band during the EEG sample. Representative samples of actual EEG activity on which the spectral wave analyses were based are shown.

into the test chamber, and allowed to acclimate to the situation for 1.0 h. Then a predrug control EEG sample was recorded. Immediately thereafter the animal was dosed by oral intubation and 30 min later the first posttreatment EEG sample was recorded. Similar EEG samples were repeated every 30 min for a total of seven per 180-min test session.

Pramiracetam was placed into solution in deionized water, which also served as the placebo substance. Similar studies on piracetam served to provide a reference standard. All drug doses are expressed in terms of the active drug moiety.

Results and discussion. A representative result showing the difference in EEG spectral profiles between a young and an old rat is shown in figure 1. A logarithmic scale has been imposed on this display in order to expand the important delta and theta bands into the center portion of the graph, thereby facilitating viewing.

As can be seen from both the spectrum analysis and from the brief sample of actual EEG tracing, the cortical EEG of the aged rat is dominated by very slow wave (< 4 Hz), high voltage activity. The young rat's cortical EEG samples do not show this kind of slow-wave activity. Equally well, the aged rat's hippocampal EEG activity differs from that of the young rat's; that is, the theta rhythm which is so dominant in the young rat's hippocampus is largely absent in the old rat's hippocampus. Moreover, the spectrum analysis shows clearly that slow-wave activity (< 4 Hz) is predominant also in the hippocampus of the aged rat. These findings indicate that the aged rat's brain suffers from a deficit in vigilance, which is revealed clearly by testing under basal conditions. It should be added that these animal findings, plus the concept of a vigilance deficit, are consistent with work done in man[1,2]. Of course, the human studies could not investigate the electrical activity of the hippocampus for obvious reasons.

Figure 2 presents a representative result showing how pramiracetam affected an aged rat's cortical and hippocampal EEG. In these tests, 20 mg/kg of pramiracetam was administered orally once per day for seven consecutive days. The drug effects shown are for the EEG sample 90-min posttreatment, which is the time of peak action of pramiracetam following oral dosing.

As can be seen from figure 2, 20 mg/kg of pramiracetam produced cortical and hippocampal activation on the first day of dosing and also on the seventh day. These arousal effects can be seen in the shape of the spectral profile as well as in the millivolt score associated with the electronic window sampled in each spectral analysis. It is important to note that after seven days of treatment the arousal effect in cortex and hippocampus were stronger than on the first day. In fact, by the seventh day the spectral wave analyses appear indistinguishable from a young rat's. This finding shows that rapid tolerance does not develop to the central effect of pramiracetam.

The table shows the results of similar studies in a number of aged rats employing daily doses of vehicle (deionized water), the reference drug piracetam at two doses, and pramiracetam at two doses. The findings are presented as mean millivolts RMS and also as the mean percent change from the initial baseline level (all measured in the same manner as previously described for the 90-min posttreatment EEG sample).

The results for vehicle shown in the table indicate that the largest change associated with vehicle was +2.7%. The changes associated with pramiracetam treatment were very much larger than this, and all attained statistical significance. Generally, piracetam produced results similar in pattern to pramiracetam, although the effects were smaller in magnitude despite employing much greater doses. Moreover, the cortical arousal effect of piracetam was always rather weak compared to pramiracetam's, and by the seventh day of dosing piracetam's effects on hippocampus and cortex had largely disappeared (apparent tolerance development), while pramiracetam's seemed to be still increasing. Thus, the present method enables one to distinguish between drugs in terms of the arousal patterns they produce in the aged brain after acute doses and also after a period of daily doses.

1 Bente, D., Glatthaar, G., Ulrich, G., and Lewinsky, M., Arzneimittel-Forsch. *28* (1978) 1529.
2 Saletu, B., Grünberger, J., and Linzmayer, L., Meth. Find. exp. clin. Pharmac. *2* (1980) 269.
3 Poschel, B.P.H., Marriott, J.G., and Gluckman, M.I., Drugs exp. clin. Res. *IX* (1983) 853.
4 Butler, D.E., Nordin, I.C., L'Italien, Y.J., Zweisler, L., Poschel, B.P.H., and Marriott, J.G., J. med. Chem. *27* (1984) 684.

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Increased formation of arginine deiminase by *Clostridium perfringens* FD-1 growing in the presence of caffeine

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Summary. Caffeine slowed growth and markedly increased the formation of arginine deiminase in growing *C. perfringens* FD-1 when dextrin, but not maltose or maltotriose, served as the energy source. It is postulated that the ability of caffeine to induce arginine deiminase is related to an inhibition of polysaccharide utilization, resulting in a shift-down condition known to induce arginine deiminase and other enzymes in bacteria.

Key words. Clostridium perfringens; caffeine; arginine deiminase; shift-down; dextrin.

Several isolated reports have implicated caffeine in the induction of gene-expression of luciferase[1], adenylosuccinate lyase[2], and λ-prophage[3] in prokaryotes, ornithine decarboxylase[4] and tobacco mosaic virus[5] in eukaryotes. In no case was the mechanism elucidated. Caffeine and related methylxanthines have been shown to stimulate sporulation in certain strains of *C. perfringens*[6–11]; sporulation is considered to be a primitive form of differentiation[12]. This caffeine effect does not appear to be related to increased levels of cyclic AMP[13] resulting from inhibition of cAMP phosphodiesterase by the methylxanthine. We report here that induction of arginine deiminase in *C. perfringens* FD-1 is greatly increased by caffeine, an effect pronounced when dextrin serves as the energy source but slight in the presence of maltose or maltotriose.

Materials and methods. Strain; spore stocks. *C. perfringens* strain FD-1 was obtained from S.M. Harmon, Food and Drug Ad-