during the month of February (fig. 1) showed that ESPA fluctuated rhythmically throughout this time. A circadian rhythm was clearly demonstrated and the maximum latency to escape i.e. least sensitivity, could be seen in the early afternoon. A retrospective study of results obtained in afternoon experiments conducted over a year (fig. 2) revealed that ESPA was also altered in a rhythmic manner on an annual basis. The maximal effects were observed during the winter months. Significant ESPA could not be induced on summer mornings suggesting that this annual rhythm was probably not artefactual as a consequence of a circadian phase shift, although it cannot be excluded on the basis of this evidence alone.

Discussion. These findings clearly demonstrate the existence of rhythmic influences upon which ESPA depends. They may therefore account for some of the variability obtained with various types of electro-analgesia in both animals and man[6]. The variability is also due in part to biological variation between individuals contributing to the spread of results. The seasonal effect could be expected to be consequent to a phase shift of the circadian rhythm, but this may not be the case because ESPA was not readily induced on summer mornings. However only further extensive experiments in that season can finally validate this point. Some of the characteristics of ESPA are similar to those of morphine analgesia, for example antagonism of the antinociceptive effect by naloxone[4] and modulation by serotoninergic manipulations[7]. It is therefore of interest to note the close parallel between the circadian pattern of the analgesic effect of morphine in mice tested on the hot-plate[8] and the circadian dependence of the induction of ESPA as reported here. The escape reaction of mice on the hot plate has been associated more with an emotional component of the reaction to continued perception of a noxious stimulus and as such is prolonged both by morphine and by ESPA. At the present time the precise mechanism of ESPA is not fully elucidated, but it probably involves the enkephalins in a functional role as short-acting[9] endogenous antinociceptive substances released in response to harmful stimuli[10]. The rhythmic influences should also be of functional importance. In relationship to the circadian rhythm, recent studies[11] have shown that whole brain levels of methionine-enkephalin in mice subjected to noxious stimuli are significantly increased in the afternoons at the peak time of ESPA and of the analgesic activity of morphine, whereas in the mornings no changes are apparent. Similarly a circadian rhythm in rat brain opiate receptor has recently been reported[12] using [^{3}H]-naloxone binding, in which the number of recognition (binding) sites was maximal in the first half of the dark phase and minimal in the early morning hours correlating with minimal ESPA at this time.

Pharmacological studies in relationship to the seasonal control of ESPA remain to be explored, although difficulties with regard to serotoninergic mechanisms are anticipated in view of annual rhythms associated with that monoamine itself[13]. However the seasonal rhythm reported here is the first indication of such control within an antinociceptive system and, like the circadian rhythm, may partially contribute to an explanation of the varying results with regard to the pharmacology of stimulation-produced analgesia.

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Effects of centrophenoxine, piracetam and hydergine on rat brain lipid peroxidation

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Summary. The cerebral insufficiency improvers centrophenoxine, piracetam and hydergine were tested for their effect on lipid peroxidation of rat brain homogenates, both in vitro and in vivo. There was no effect either in vitro or after chronic 8 week dosing of animals.

There has been much discussion in recent years regarding the role of lipid peroxidation and its consequences in cellular deterioration. Thus, lipid peroxidation has been implicated as a causative agent in the ageing process, hyperbaric oxygen toxicity, ozone damage, the radiation syndrome and CCl_4 and $BrCCl_3$ toxicity[2-9]. Peroxidation of lipids or other molecules as a common aetiological factor in the above states is an attractive hypothesis, because for many years there has appeared to be a relationship between these diverse forms of cellular damage. However, as pointed out in the review by Plaa and Witschi[10] most of the evidence for such a relationship is indirect. The experimental evidence for a role of increased lipid peroxidation in the aging process is controversial with results ranging from significant increases in peroxidation[4,11,12] to no significant change or even decreases in peroxidation with increasing age[13,14].

Recently, there has been a resurgence of interest in a class of compounds loosely classified as cerebral insufficiency improvers. Compounds such as piracetam, hydergine and centrophenoxine have been used clinically in geriatric patients who present with symptoms such as confusion,

psychosomatic aesthenia and memory disturbances[15-21]. However, no biochemical basis for the action of these compounds has yet been established. Centrophenoxine is particularly interesting as there is much experimental evidence that this compound can reduce the quantity of lipofuscin present in brain tissue[17,22-24]. Lipofuscin, often referred to as aging pigment, is certainly known to increase in many brain areas with increasing age of animals, but its formation is as yet unclear. It has been suggested by several workers that lipid peroxidation of membranes of intracellular structures, such as mitochondria, may lead to the formation and subsequent accumulation of lipofuscin[2,25,26].

Table 1. The effect of compounds in vitro on endogenous lipid peroxidation in rat brain homogenates incubated at 37 °C for 2.5 h

Treatment		N	MDA nmoles/ g wet brain
Controls		6	425 ± 22
Centrophenoxine	1 μM	3	397 ± 25
	10 μM	3	433 ± 37
	100 μM	3	435 ± 38
	1 mM	3	525 ± 40*
Controls		6	446 ± 22
Hydergine	1 μM	6	441 ± 20
	10 μM	6	457 ± 28
	100 μM	6	422 ± 34
Controls		5	499 ± 13
Piracetam	1 μM	3	499 ± 13
	10 μM	4	497 ± 14
	100 μM	4	521 ± 15
	1 mM	5	558 ± 25*
Controls		3	444 ± 39
α-Tocopherol	100 μM	3	397 ± 21
	1 mM	3	333 ± 31
Controls		3	435 ± 21
Pyrogallol	1 μM	3	359 ± 9*
	10 μM	3	160 ± 3**
	100 μM	3	40 ± 10**
	1 mM	3	23 ± 3**

* $p < 0.05$ from control values; ** $p < 0.001$ from control values. Results are expressed as mean ± SEM where N is the number of rats used for each determination. Concentrations of compounds are final concentration in 0.6 ml homogenate.

Table 2. Effect of piracetam, hydergine and centrophenoxine in vivo on endogenous rat brain lipid peroxidation after single doses or chronic drug treatment

Drug and administration	N	MDA nmoles/ g wet brain
Saline	4	519 ± 18
Hydergine 1 mg/kg single dose 1 h before assay	4	508 ± 13
Saline	4	412 ± 63
Piracetam 1 g/kg single dose 1 h before assay	4	411 ± 19
Saline	8	381 ± 9
Hydergine 0.4 mg/kg bi-daily, 4 weeks	8	381 ± 19
Piracetam 1 g/kg bi-daily, 4 weeks	8	399 ± 9
Saline	8	360 ± 6
Hydergine 0.4 mg/kg bi-daily, 8 weeks	5	352 ± 5
Piracetam 1 g/kg bi-daily, 8 weeks	5	389 ± 28
Saline	6	366 ± 11
Centrophenoxine 50 mg/kg bi-daily, 8 weeks	12	438 ± 20*

Control groups received an equivalent volume of saline. All drugs were administered i.p. and rats were sacrificed 1 h after the last injection. Results are expressed as in table 1. * $p < 0.05$ from control value.

If this were true, then centrophenoxine may act by inhibiting the initial lipid peroxidation and thus reduce the lipofuscin content.

In view of the large amount of interest in lipid peroxidation as a possible step in cellular aging and deterioration, we sought to establish whether centrophenoxine, piracetam and hydergine inhibit the production of brain lipid peroxides. For studies in vitro, male Füllinsdorf rats (220–250 g) were sacrificed by decapitation and the brain rapidly removed, weighed and placed in 20 vol. of cold 50 mM Tris buffer, pH 7.4 and homogenized. Centrophenoxine (Helfergin, Promonta Labs, Hamburg), hydergine (Sandoz) and piracetam (Nootropil, UCB 6215) (table 1) were incubated with 0.6 ml aliquots of brain homogenates for 2.5 h at 37 °C and the formation of lipid peroxides was measured as described by Boehme et al.[13]. Pyrogallol (Fluka), a well-known inhibitor of lipid peroxidation[27] and α-topopherol (Roche) which is generally regarded as the biological antioxidant, were also tested. α-Tocopherol was dissolved in acetone for in vitro experiments. Other compounds were dissolved in water. No more than 10 μl of acetone or water was added to the homogenate and control experiments showed that such concentrations of acetone had no effect on the measurements. All determinations were performed in duplicate. Lipid peroxides are expressed as the quantity of malondialdehyde (MDA) formed, assayed by the thiobarbituric acid method, using an extinction coefficient of 1.56×10^{-5} at 532 nm[14,28].

For chronic in vivo studies male Füllinsdorf rats of 130–150 g initial weight were dosed i.p., twice daily on weekdays and once daily at weekends, for 4 or 8 week periods. The dose levels of centrophenoxine, piracetam and hydergine were selected on the basis of previous reports for effects of these agents[22,29]. Control groups were injected with equivalent volumes of saline. Rats were weighed twice each week and the weight gain curves for all treated groups remained normal throughout the experiment, with final rat weights of approximately 320 g (range 288–345 g).

As seen in table 1, there was no decrease in lipid peroxide formation in vitro by brain tissue homogenates incubated with centrophenoxine, piracetam or hydergine. Indeed, at 1 mM concentrations centrophenoxine and piracetam caused slight increases in lipid peroxidation while pyrogallol showed significant inhibition at concentrations of 1 μM and above. The results obtained with α-tocopherol and pyrogallol were very similar to those obtained by Kappus et al.[27] for inhibition of microsomal lipid peroxidation. 3 separate control experiments in which the highest concentrations of drugs used were added immediately after the incubation period showed no differences from control values, indicating the absence of artefacts.

There was a similar lack of effect of these compounds in vivo (table 2). Piracetam or hydergine injected as a single dose 1 h before sacrifice had no effect on the endogenous concentration of peroxides (table 2). Chronic treatment for up to 8 weeks with centrophenoxine, piracetam or hydergine, similarly did not decrease lipid peroxidation in rat brain homogenates. This lack of reduction of lipid peroxidation was particularly interesting for centrophenoxine which is known to reduce neuronal lipofuscin content in several species, including rats, when given chronically at these doses for 2 months.

Thus centrophenoxine, piracetam and hydergine did not affect the endogenous lipid peroxidation of rat brain homogenates in our experiments. If lipofuscin accumulation is a result of lipid peroxide formation, the well-documented action of centrophenoxine in reducing the lipofuscin content of brain cells[17,18,23,24] does not appear to be due to an initial effect in inhibiting peroxidation.

Note added in proof. Since completion of these experiments we have carried out in vivo trials in older animals (16-month-old rats at commencement of the trial), with piracetam and centrophenoxine, using similar dosage schedules to those described. Again, there was no reduction in brain lipid peroxidation after drug treatment in these aged rats.

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Naloxone reduces abdominal muscle tone in mice and rats

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Summary. The effect of naloxone on muscle tone was investigated in mice and rats at various times after administration. The naloxone effect was also tested in diazepam-pretreated animals. Naloxone was found to display muscle relaxant activity. This effect appeared to be additive to that of diazepam.

The demonstration of endogenous opioid peptides in the mammalian organism has led to intense efforts to elucidate the physiological significance of the endorphinergic system(s). The opiate antagonist naloxone should represent a convenient tool for such investigations, since this compound is believed to compete selectively for opioid binding sites when administered at low doses[2]. Indeed, several reports have demonstrated certain effects of naloxone in the absence of exogenous opiates[3–6], pointing to a regulatory function of endogenous opioids in somatic and psychic activities.

Catatonia and muscle rigidity, apart from analgesia, are among the most prominent effects observed on the administration of opioid agonists to animals[7]. Whether or not endogenous opioids are involved in the physiological control of muscle tone is not, as yet, known. The present study examines the possible muscle relaxant activity of naloxone in mice and rats not previously treated with opiates. The effects observed were compared to those obtained with diazepam, a centrally acting compound with pronounced muscle relaxant activity. The combination of naloxone plus diazepam was also studied.

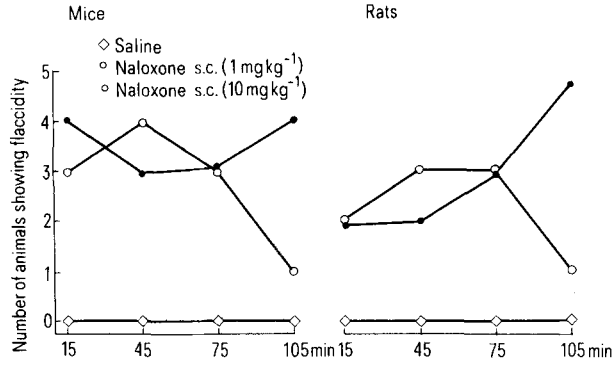

Figure 1. Effect of 1 and 10 mg kg^{-1} naloxone s.c. on the abdominal muscle tone of mice and rats at different times after administration, in comparison to saline-treated controls. The points represent the number of animals, out of a total number of 10 tested, showing a muscle relaxation. This effect of naloxone was shown to be significant by use of the Fisher-test 2×2 tables (2.5% for 1-side hypothesis), when compared to saline controls.