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Microinfusion of clonidine and yohimbine into locus coeruleus alters EEG power spectrum: effects of aging and reversal by phosphatidylserine

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1 The behavioural and electrocortical (ECoG) power spectrum effects of clonidine, and yohimbine, an agonist and an antagonist at α_2-adrenoceptors, after their unilateral microinfusion into the rat locus coeruleus (LC) in young (50–70 days old) and old (13–15 months old) rats were studied.

2 Clonidine (0.09, 0.19, 0.28 and 0.56 nmol) microinfused into the LC of young rats induced dose-dependent behavioural and ECoG slow wave sleep (SWS) with a significant increase in total voltage power and power in the lower frequency bands. In contrast, yohimbine (1.3 and 2.6 nmol) infused into the LC of young rats produced ECoG desynchronization and a significant decrease in total voltage power.

3 In contrast to young rats, clonidine (0.19 and 0.28 nmol) given into the LC did not affect behaviour and the ECoG power spectrum in old rats. However, after higher doses of clonidine (0.56 and 1.2 nmol) a small and short-lasting period of behavioural and ECoG SWS was still evident. Similarly, in old rats yohimbine, at a dose (1.3 nmol) which was stimulative in young animals, did not significantly affect behaviour and ECoG power spectrum. Higher doses of yohimbine (2.6 and 5.2 nmol) were required to induce behavioural and ECoG changes similar to those observed with lower doses of yohimbine in young rats.

4 Chronic treatment with phosphatidylserine (30 mg kg^{-1}, orally, daily for 21 and 30 days), was able gradually to restore in old rats, in comparison with a vehicle-treated group, the responsiveness of α_2-adrenoceptors to clonidine and yohimbine given into the LC.

Introduction

The effects of aging on behaviour and electrocortical (ECoG) activity have been widely studied in several animal species (see Aporti *et al.*, 1982). ECoG alterations in old rats consist of spontaneous, asymptomatic, bilaterally symmetrical and synchronous bursts of epileptic-like spikes occurring prevalently from 6.8 to 9.4 Hz, the animals being immobile in a 'freezing'-like state. These typical behavioural and ECoG changes can be stopped abruptly with sound stimulation and no correlation between these changes and audiogenic seizures was observed (Aporti *et al.*, 1986). It has been suggested that these behavioural and ECoG alterations may be due to changes of neuronal membrane fluidity and consequently to alteration in neurotransmission in the brain (Crews *et al.*, 1981; Burchinsky, 1984; Schroeder, 1985; Toffano, 1985).

The locus coeruleus (LC) is a densely packed cell group located in the dorsal pons which contains approximately half of all noradrenergic neurones in the rat brain (Swanson & Hartman, 1975; Amaral & Sinnamon, 1977; Cedarbaum & Aghajanian, 1977). The main ascending and descending noradrenergic pathways in the brain originate from the LC (Dahlstrom & Fuxe, 1964; Moore & Card, 1984). In addition, it has been recognised for a long time that the LC is a crucial area in the control of the sleep-waking cycle (Steriade & Hobson, 1976; Clark, 1979; Ramm, 1979; Cespuglio *et al.*, 1982; Aston-Jones & Bloom, 1981a,b). Recently, we have shown that the α_2-adrenoceptor agonist and antagonist clonidine and yohimbine, microinfused into the LC, are able to

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affect sleep/arousal mechanisms in rats (De Sarro *et al.*, 1987a). Until now no data have been available in the literature concerning the behavioural and electrocortical changes produced by pharmacological manipulations of LC neurones in old animals.

We demonstrate here that male Sprague-Dawley rats display, as they get older, a decreased responsiveness of the LC neurones to clonidine and yohimbine. We also examined whether chronic treatment with phosphatidylserine (PS), the most active fraction of brain cortical phospholipids (Bonetti *et al.*, 1983), was able to restore the behavioural and ECoG response to clonidine and yohimbine in old rats. Phosphatidylserine (PS) has previously been found to stimulate the adrenergic system *in vivo* (Calderini *et al.*, 1983). In addition, it has been shown that this compound is able to affect the activity of other neurotransmitters in the brain; for example, it induces dopamine release from dopaminergic terminals (Mazzari & Battistella, 1980), stimulates dopamine-dependent adenylate cyclase activity in the brain (Leon *et al.*, 1978) and increases acetylcholine output from the cerebral cortex (Casamenti *et al.*, 1979). It has been suggested that these effects may be due to a modulatory role of PS at the neuronal pre- and post-synaptic membranes where it can increase membrane fluidity (Gaiti *et al.*, 1981; Bruni & Toffano, 1982; Gaiti *et al.*, 1985; Toffano, 1985). Thus, since in old age there is a decrease in neuronal membrane fluidity in the brain (Nagy *et al.*, 1983; Schroeder, 1985; Toffano, 1985), it was of interest to examine whether long-term treatment with PS was able to affect in old rats the altered responsiveness to clonidine and yohimbine after direct microinfusion into the LC.

Methods

Adult young (50–70 days old; 200 ± 20.2 g) and old (13–15 months old; 670 ± 30.6 g) male Sprague-Dawley rats were purchased from Charles River, (Calco, Como, Italy) and housed in stable conditions of humidity (60 ± 5%) and temperature (22 ± 2°C). They were fed with standard diet and had water *ad libitum*. Animals were maintained on a 12 h light and dark cycle (lights on 6 h 00 min–18 h 00 min, off 18 h 00 min–6 h 00 min).

Rats were stereotaxically implanted under chloral hydrate anaesthesia (400 mg kg^{-1} i.p.; Carlo Erba, Milan) with permanent stainless steel guide cannulae (25 gauge), which had an angle of 5° from the vertical and the tip 2 mm from the LC. All coordinates were modified from the atlas coordinates of Paxinos & Watson (1982) (AP = 1.1 mm posterior to lambda, L = 0.5 mm lateral to the midline, H = 5.7 mm ventral to the skull surface).

After surgery a minimum of 48 h was allowed before experiments were carried out. All experiments were performed beginning at approximately 10 h 00 min. Freely moving rats were microinjected via an injector cannula (28 gauge) which extended approx 2 mm below the tip of the guide cannula.

Electrocortical (ECoG) activity was recorded via 4 chronically implanted steel screw electrodes inserted onto each fronto-parietal cortex (young rats: 2 mm behind the bregma and ±2 mm laterally to the midline; old rats: 2.2 mm behind the bregma and ±2.2 mm laterally to the midline) by a Stoelting stereotaxic frame. All electrodes and the injection guide cannula were anchored to the skull by acrylic dental cement. Electrocortical activity was recorded by means of an 8 channel EEG machine (OTE BIOMEDICA, Florence). For statistical purposes, the quantification of total voltage power (0.25–16 Hz) and of individual frequency bands (0.25–3; 3–6; 6–9; 9–12; 12–16 Hz) was carried out by using a Berg Fourier analyzer (OTE BIOMEDICA, Florence) according the method of Bricolo *et al.* (1978). ECoG spectra power were obtained by averaging spectra derived from 5 min ECoG epochs and the integrated energy signals were expressed as $\mu V^2 s^{-1}$; the time constant (0.03 s) was short enough to reduce the number of artefacts (HF cut-off = 5.3 Hz).

In some animals, for electromuscular (EMG) recordings, stainless-steel wires (200 μm diameter) were inserted into the right and left occipital muscles (at least 3 for each dose). In order to record EMG activity each rat was connected to an 8 channel EEG polygraph (OTE BIOMEDICA, Florence). Each recording session (5 h duration) started 60 min after the electrodes were connected.

The animals, placed individually in transparent cages (35 × 35 × 25 cm), were allowed 60 min before drug administration to acclimatize to the new environment. The behavioural changes and their onset and duration were recorded after drug injection. In particular, two independent observers followed gross behavioural changes consisting in eyes open or closed, locomotor activity, possible stereotyped movements, squatting posture and also they noted whether the rats concomitant to ECoG changes were alert, drowsy or sleepy.

Post-mortem histological examination confirmed the location of the guide cannula. Only animals in which the location of the injection site was confirmed histologically were used in the analysis of behavioural and ECoG data.

To quantify changes of total voltage power and of preselected bands of frequency induced by clonidine, yohimbine or saline, the area (expressed in mm^2) under the curve corresponding to plotted total voltage values during 60 min periods after each com-

Table 1 Effects of microinfusion of clonidine into the locus coeruleus on electrocortical (ECoG) total voltage power in young and old rats

Pretreatment (days)	*Clonidine* (nmol)	*Number of experiments*	*Age* (months)	*ECoG total voltage power* ($\mu V^2 s^{-1}$) *Control period*	*1 h after clonidine*	*2 h after clonidine*
None	0.09	8	2	101.7 ± 4.75	109.8 ± 6.52	102.1 ± 6.25
None	0.19	8	2	94.8 ± 3.26	113.3 ± 4.61	98.4 ± 7.02
None	0.28	10	2	102.1 ± 6.79	139.5 ± 5.72**	104.6 ± 7.72
None	0.56	9	2	96.5 ± 5.56	148.6 ± 6.05**	136.4 ± 6.25*
None	0.19	8	13–15	99.2 ± 6.51	101.6 ± 5.92	99.3 ± 6.5
None	0.28	8	13–15	100.3 ± 6.14	104.7 ± 6.27	101.2 ± 6.1
None	0.56	10	13–15	97.6 ± 7.95	117.5 ± 7.37	99.3 ± 7.29
None	1.2	10	13–15	101.2 ± 7.14	114.4 ± 5.66**	133.7 ± 7.63*
PS (1 day)	0.56	8	13–15	101.6 ± 6.72	122.2 ± 7.53	100.3 ± 6.97
PS (1 day)	1.2	8	13–15	98.7 ± 6.84	140.8 ± 6.84**	131.5 ± 7.06*

The results are presented as mean values ± s.e. of the ECoG total voltage power during the control period, 1 and 2 h after clonidine microinjection in young and old rats. Significant differences between control groups and clonidine-treated groups are denoted * $P < 0.05$ and ** $P < 0.01$ Student's paired t test). PS = phosphatidylserine.

pound was integrated by means of a Commodore computer and the percentage changes of the integrated area in comparison to the same interval area during the pretreatment period were calculated according to the 'trapezoidal rule' (Tallarida & Murray, 1981). To reduce inter-animal variation of baseline electrocortical activity and of single frequency bands the percentage changes following drug treatment were compared to the values of a corresponding period before treatment. In order to verify whether experimental groups were homogeneous, data obtained from young and old rats were analysed using a one-way analysis of variance followed, where appropriate, by analysis with Student's t test. ECoG spectrum data are presented in terms of means ± the standard errors (s.e.) of the variation of total voltage power and of preselected bands of frequency. The percentage changes following drug treatment were compared to the values of the corresponding period before treatment by use of Student's paired t test. In addition, we compared at several intervals of time (1, 2, 3 and 4 h) the changes of total voltage power occurring after chronic treatment with PS or vehicle (15, 21 and 30 days) in rats treated with clonidine, yohimbine or saline using the Mann-Whitney U test.

Drugs

Clonidine hydrochloride (Boehringer-Ingelheim, Germany) and yohimbine hydrochloride (Sigma St. Louis, Mo, U.S.A.) were infused into the locus coeruleus using a 5 μl Hamilton syringe, connected by a teflon tube to an injection cannula. The drug was infused in a volume of 0.5 μl at a rate of 0.2 $\mu l\,min^{-1}$ and the cannula left *in situ* for a further 1 min. Each animal was treated only once. Control infusions were carried out with the same volume of saline as that used to dissolve clonidine or yohimbine hydrochloride, and lacked effects on overt behaviour and electrocortical spectrum power activity. PS was dissolved as previously described by Aporti *et al.* (1986). The old rats were randomly divided into two groups, one received PS ($30\,mg\,kg^{-1}$) and the other vehicle orally each day for 30 days. The behavioural and ECoG testing was performed before treatment and 15, 21 and 30 days following the start of chronic PS treatment. The experiments with clonidine, yohimbine or saline were carried out 24 h after the last administration of PS.

Results

Effect of clonidine

The microinfusion of clonidine into the LC of young rats (0.09, 0.019, 0.28 and 0.56 nmol) induced behavioural and electrocortical slow-wave sleep (SWS) within 1–2 min after the infusion which lasted for 13–114 min depending on the dose (Table 1) (at least 8 experiments for each dose). During sleep, rats showed a periodic increase of total voltage power, predominantly in 0.25–3, 3–6, 6–9 and sometimes 9–12 Hz frequency bands. In addition, muscular atonia was observed; sensory stimuli were able to produce behavioural and phasic electrocortical arousal.

In old rats the dose of 0.28 nmol did not significantly affect the behavioural and electrocortical activity, while the highest dose (0.56 nmol) induced a significantly shorter lasting (approx 30 min) behavioural and ECoG SWS (Table 1 and Figure 1) ($n = 8$ for each dose).

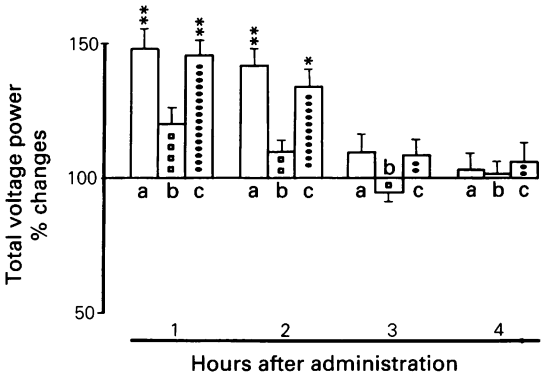

Figure 1 Effects of a single microinfusion into the locus coeruleus of clonidine (0.56 nmol) on electrocortical (ECoG) power spectrum of young rats (a), old rats (b) and old rats chronically treated with phosphatidylserine (c). Ordinates show the % changes of total voltage power in comparison to control period, abscissae show time. No significant changes of ECoG power spectrum changes were observed in old rats. The significance of the % changes of total voltage power between the pretreatment period and post-drug periods are denoted: * $P < 0.05$ and ** $P < 0.01$ (paired Student's t test). The total voltage power values observed during the control period in rats of various ages are presented in Table 1.

In order to get similar effects to those observed with clonidine (0.56 nmol) in young rats, a higher dose of clonidine (1.2 nmol) was required ($n = 10$ for each dose). The behavioural and ECoG sleep and the increase in total voltage power and power in the preselected frequency bands evoked by the clonidine infusion were significantly less marked and shorter lasting in the old than in the young rats (Table 1, Figures 1 and 2).

Effects of yohimbine

Yohimbine (1.3 and 2.6 nmol) given into the LC of young rats ($n = 8$ for each dose) induced arousal, an increase in locomotor and exploratory activity, stereotyped movements (sniffing, chewing and licking), tachypnoea which started within 3 min after the injection and lasted 60–160 min depending on the dose. Behavioural stimulation was accompanied by a significant decrease in total voltage power, in the 3–6, 6–9 and sometimes in the 9–12 Hz frequency bands (Table 2).

In contrast to young animals, the microinfusion of yohimbine (1.3 and 2.6 nmol) into the LC of old rats did not significantly affect the behavioural and electrocortical activity (Table 2 and Figure 3) ($n = 6$ for each dose). A higher dose of yohimbine (5.2 nmol) was needed in old rats to produce a behavioural and ECoG pattern similar to that observed after microinfusion of yohimbine (2.6 nmol) in young rats ($n = 6$–8 for each dose). Behavioural and ECoG changes seen after yohimbine in old rats were much less marked and shorter lasting than those observed in young ones (Table 2, Figures 3 and 4).

Effect of clonidine and yohimbine in old rats chronically treated with phosphatidylserine

The chronic oral administration of PS (30 mg kg^{-1} daily for 30 days) produced a significant increase of the responsiveness to clonidine and yohimbine. No changes were observed, in comparison to untreated 13–15 month-old rats, in vehicle-treated animals or after a single administration of PS (30 mg kg^{-1}) (Table 1 and 2), or following chronic oral administration of PS (30 mg kg^{-1}) for 15 days. However, the behavioural and ECoG changes observed after clonidine and yohimbine in rats receiving PS for a longer

Table 2 Effects of microinfusion of yohimbine into the locus coeruleus on electrocortical (ECoG) total voltage power in young and old rats

Pretreatment (days)	*Yohimbine* (nmol)	*Number of experiments*	*Age* (months)	*ECoG total voltage power* ($\mu V^2 s^{-1}$) *Control period*	*1 h after yohimbine*	*2 h after yohimbine*
None	1.3	8	2	98.9 ± 6.72	76.8 ± 7.8	95.4 ± 8.4
None	2.6	8	2	102.1 ± 7.46	72.9 ± 8.3*	74.3 ± 8.5*
None	1.3	6	13–15	94.8 ± 7.05	80.9 ± 10.1	95.6 ± 8.7
None	2.6	8	13–15	101.5 ± 7.73	76.4 ± 8.9	96.4 ± 9.1
None	5.2	8	13–15	98.6 ± 6.25	69.5 ± 8.6*	72.4 ± 8.2*
PS (1 day)	2.6	6	13–15	101.1 ± 7.14	77.6 ± 8.9	99.8 ± 9.2
PS (1 day)	5.2	6	13–15	101.4 ± 8.11	72.7 ± 8.4*	74.2 ± 9.1*

The results are presented as mean values ± s.e. of the ECoG total voltage power during the control period, 1 and 2 h after yohimbine microinjection in young and old rats.
Significant differences between control groups and yohimbine-treated groups are denoted.
* $P < 0.05$ (Student's paired t test). PS = phosphatidylserine.

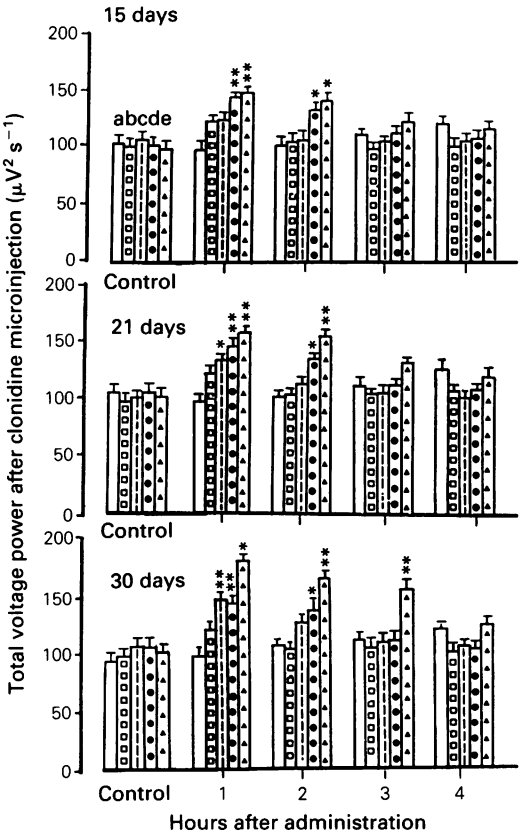

Figure 2 Changes of responsiveness to clonidine after chronic treatment with phosphatidylserine (PS) 30 mg^{-1} day^{-1} orally). The values are mean (at least 8 rats for each group) changes in total voltage power and bars show s.e.mean. Significance of the differences between saline and clonidine-treated groups of animals chronically treated with PS or vehicle are denoted: * $P < 0.05$ and ** $P < 0.01$. Columns (a) saline; (b) vehicle + clonidine 0.56 nmol; (c) vehicle + clonidine 1.2 nmol; (d) PS + clonidine 0.56 nmol; (e) PS + clonidine 1.2 nmol (Mann-Whitney U test).

period (21 and 30 days) were almost indistinguishable from those observed in untreated young rats.

Old rats chronically treated with PS (30 mg kg^{-1} daily for 30 days) showed, after LC microinfusion of clonidine (0.56 and 1.2 nmol), a behavioural and electrocortical SWS which lasted 80–165 min depending on the dose of clonidine (Figures 1, 2, 5 and 7) ($n = 8$ for each dose and group). In the vehicle-treated group the effects evoked by clonidine or yohimbine were similar to those observed in untreated 13–15 month-old rats (Tables 1 and 2) ($n = 8$ for each group).

Behavioural and electrocortical power changes were also evoked by yohimbine (2.6 and 5.2 nmol) in

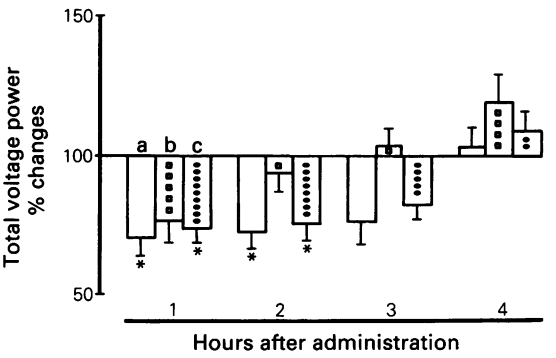

Figure 3 Effects of a single microinjection into the locus coeruleus of yohimbine (2.6 nmol) on electrocortical (ECoG) power spectrum of young rats (a), old rats (b) and old rats chronically treated with phosphatidylserine (c). Ordinate scale shows the % changes of total voltage power in comparison to control period; abscissae show time. Note the different ECoG power spectrum observed in the old rats. The significance of the % changes of total voltage power between pretreatment period and post-drug periods are denoted: * $P < 0.05$ (Student's paired t test). The total voltage power values observed during the control period in rats of various ages are presented in Table 2.

rats chronically treated for 30 days with PS (30 mg kg^{-1} daily) ($n = 6$ for each dose and group). Behavioural arousal and motor stimulation as well as ECoG desynchronization induced by yohimbine in these animals lasted 100–180 min depending on the dose (Figures 3, 4 and 6).

Discussion

De Sarro *et al.* (1987a) previously found that bilateral microinfusion of clonidine at very low doses (0.056 nmol) produced, in young rats, behavioural sleep accompanied by ECoG synchronization, increase in ECoG total voltage power and power in the lower frequency bands, whereas yohimbine produced behavioural arousal and ECoG desynchronization. The present experiments show that in older rats there is a decreased sensitivity to both clonidine and yohimbine. In fact, in old rats small doses of clonidine and yohimbine were ineffective in changing behaviour and ECoG activity, and after higher doses of clonidine only a small and shorter lasting behavioural sleep with an increase in ECoG total voltage power occurred. The reason for such reduced responsiveness to the α_2-adrenoceptor agonist and antagonist in old rats is not known at the present time, although one can hypothesize that behavioural and ECoG alterations occurring with aging may affect the behavioural and bioelectric responses to neurotransmitters. Thus it is not clear whether in old

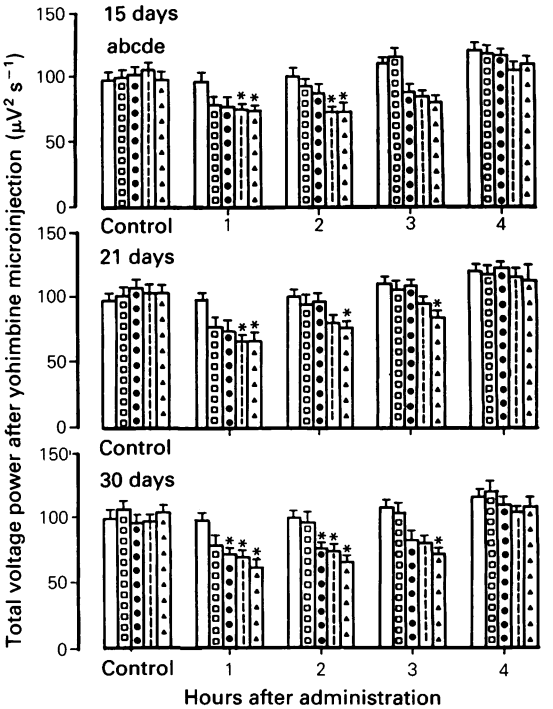

Figure 4 Changes of responsiveness to yohimbine after chronic treatment with phosphatidylserine (PS, 30 mg kg^{-1} day^{-1} orally). The values are mean (at least 6 rats for each group) changes in total voltage power and bars show s.e.mean. Significance of the differences between saline and yohimbine-treated groups of animals chronically treated with PS or vehicle are denoted: * $P < 0.05$ (Mann-Whitney U test). Columns (a) saline; (b) vehicle + yohimbine 2.6 nmol; (c) vehicle + yohimbine 5.2 nmol; (d) PS + yohimbine 2.6 nmol; (e) PS + yohimbine 5.2 nmol.

rats there is a decrease in the number of α_2-adrenoceptors in the LC and/or a decrease in their affinity for agonist and antagonist drugs. This phenomenon may not be specific to α_2-adrenoceptors in the brain. Evidence from a number of studies has suggested that an impairment of neurotransmission in the brain occurs in old rats (Bonetti *et al.*, 1983; Calderini *et al.*, 1983; Nagy *et al.*, 1983; Burchinsky, 1984; Toffano, 1985). In addition, it has been shown that β-adrencoeptor responsiveness is reduced in aged rats (see Fleisch, 1981) and that the number of α_1- and β-receptors is decreased in the cerebral cortex of 25 month-old rats as compared to 3 month-old rats (Misra *et al.*, 1980). However, little is known about changes in central α_2-adrenoceptors in aging, although in the vas deferens reduced sensitivity of prejunctional α_2-adrenoceptors was found in old rats (Docherty &

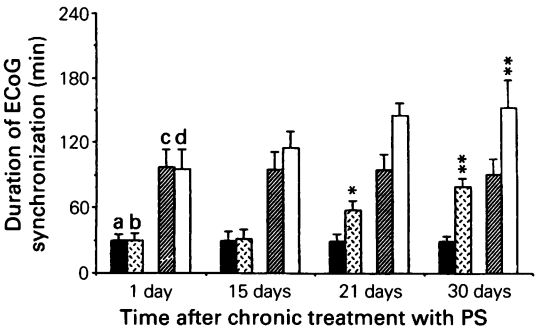

Figure 5 Changes of responsiveness to clonidine after a single dose and chronic treatment with phosphatidylserine (PS, 30 mg kg^{-1} day^{-1} orally). The values are mean (at least 8 rats for each group) duration of ECoG synchronization in min and bars show s.e.mean. Significance of the differences between concurrent control experiments (vehicle) and PS-treated groups: * $P < 0.05$, ** $P < 0.01$. Columns (a) vehicle + clonidine 0.56 nmol; (b) PS + clonidine 0.56 nmol; (c) vehicle + clonidine 1.2 nmol; (d) PS + clonidine 1.2 nmol.

Hyland, 1986). Other authors have demonstrated an age-dependent reduction of the number of α_2-adrenoceptors in human platelets (Brodde *et al.*, 1982) and rabbit brain (Hamilton *et al.*, 1984). A possible mechanism that might underly some of the age-related changes in receptor function is an alteration in lipid composition and fluidity of neuronal membranes. A reversal of the age-related decrease in membrane-mediated responses brought about by chronic treatment with PS is consistent with such a mechanism. Several groups of workers have demon-

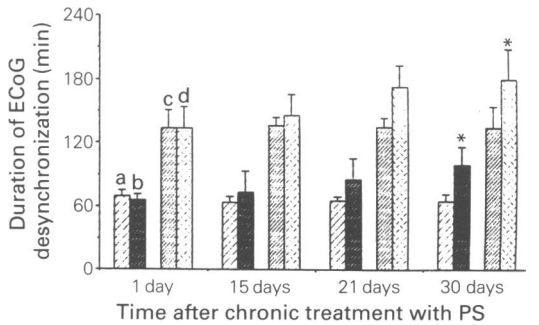

Figure 6 Changes of responsiveness to yohimbine after a single dose and chronic treatment with phosphatidylserine (PS, 30 mg^{-1} day^{-1} orally). The values are mean (at least 6 rats for each group) duration of ECoG desynchronization in min and bars show s.e.mean. Significance of the differences between concurrent control experiments (vehicle) and PS-treated groups: * $P < 0.05$. Columns (a) vehicle + yohimbine 2.6 nmol; (b) PS + yohimbine 2.6 nmol; (c) vehicle + yohimbine 5.2 nmol; (d) PS + yohimbine 5.2 nmol.

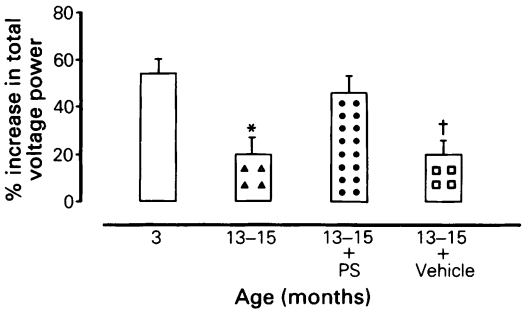

Figure 7 Percentage increase in total voltage power 1 h after clonidine (0.56 nmol) in young and old rats treated with vehicle or phosphatidylserine (PS) for 30 days. Values are expressed as the mean of 8–10 experiments for each group; bars show s.e.mean. * Significant difference ($P < 0.01$) between the groups of 3 month- and 13–15 month-old rats. † Significant changes in total voltage power between vehicle and PS-treated groups ($P < 0.01$).

strated that PS is able to increase the *in vivo* incorporation of unsaturated fatty acids (Gaiti *et al.*, 1984) and to stimulate, *in vitro*, their release from phospholipids (Gatti *et al.*, 1985). These two effects may be important in aged cerebral membranes where the uptake and reutilization of these fatty acids is reduced (Gaiti *et al.*, 1981; 1985). Previous pharmacological studies have shown that in brain of old rats PS normalizes the cholesterol/phospholipid molar ratio and the Na^+, K^+-ATPase activity which are modified during the aging process (Bonetti *et al.*, 1983; Calderini *et al.*, 1983). PS is also able to increase acetylcholine output from rat cerebral cortex (Casamenti *et al.*, 1979) and to antagonize the effects of haloperidol and scopolamine on both brain electrical activity and spontaneous behavioural alteration in the rat (Pepeu *et al.*, 1980; Aporti *et al.*, 1982; 1985). Recently, it has been shown that chronic treatment with PS is able to restore the electrically-evoked acetylcholine release from cortical slices in 24 month-old rats (Pepeu *et al.*, 1986), to increase the sensitivity of pituitary dopamine receptors to dopamine in aged rats (Di Renzo *et al.*, 1987), and to reduce the number of ECoG power changes and epileptic spikes observed in old rats (De Sarro *et al.*, 1987b). A reduction of prolactin secretion in rat lactotrophs during aging was also obtained after chronic treatment with PS (Toffano & Bruni, 1980; Zauli *et al.*, 1984; Toffano, 1985). The changes in lactotrophs membrane fluidity during aging has been shown to affect phosphatidylinositol (PI) metabolism; PS was found to reduce *in vitro* ^{32}P incorporation into PI and such an effect was associated with the inhibition of prolactin secretion (Bonetti *et al.*, 1987).

In conclusion, the present experiments provide evidence that there is a decreased responsiveness of LC α_2-adrenoceptors in old rats, that these changes, at least in rats of 13–15 months, are not irreversible, and that treatment with PS is able to restore the behavioural and electrocortical responses to α_2-adrenoceptor agonists and antagonists.

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