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Aluminium-induced enhancement of ageing-related biochemical and electrophysiological parameters in rat brain regions

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We investigated alterations in the ageing-related parameters: multiple-unit action potentials, Na^+, K^+-ATPase activity, glutathione-s-transferase (GST) activity, glutathione peroxidase (GPx) activity, lipid peroxidation and lipofuscin contents in the brain regions cerebral cortex, striatum, hippocampus and thalamus, resulting from the chronic administration of aluminium chloride ($AlCl_3$) in drinking water to rats of 6 and 12 months of age. Aluminium treatment significantly depressed Na^+, K^+-ATPase, GST and GPx activities, elevated lipid peroxidation and lipofuscin contents, and produced intense epileptiform activity in the electroencephalograms of the studied brain regions together with a concomitant increase in the multiple-unit action potentials (MUA) indicating a vigorous neuronal epileptic hyperactivity. Taken together the aluminium-induced alterations in these parameters are indicative of an accelerated ageing process.

Keywords: Aluminium, brain ageing, Na^+, K^+-ATPase, multiple unit action potentials, antioxidant enzymes, lipofuscin, lipid peroxidation, epileptic activity, acceleration of ageing

Brain aluminium (Al) concentration is known to increase with normal ageing[1-5]. Some brain regions, particularly hippocampus and neocortex seem to be more affected by Al accumulation[6-8]. Al crosses the blood brain barrier and forms deposits in brain regions such as striatum, hippocampus and occipital cortex[9,10]. It is regarded as the etiological agent in pathogenesis of several degenerative disorders and can even be linked to the impairment in cognitive functions including long-term storage of memory[11,12]. Prolonged intake of even very low concentration of Al via drinking water and other sources may lead to ageing-related neurological dysfunction[13,14]. Al is potentially toxic and possibly augments the neurodegeneration resulting from other primary degenerative causes[15-18].

A number of findings support a role for Al in the acceleration of ageing process[2,15,19]. Young rats on exposure to Al for 10 weeks showed cell vacuolization, massive mitochondria swelling, demyelination, and increased accumulation of lipofuscin similar to what occurs in ageing[5]. In other studies, Al-induced ageing-related alterations such as cell loss[20], increased thiobarbituric acid products[21] were also reported. Ageing process-related biochemical and electrophysiological changes in the brain may vary from region to region[22]. For example, hippocampus shows greater age-related sensitivity while thalamus is less vulnerable to ageing[5,22]. Various brain regions also show differential vulnerability to Al[21,23]. Previous studies on regional effects of Al in the brain are limited. Al was found to increase lipid peroxidation in hippocampus to a greater extent than in striatum[21], and decreased NADPH diaphorase positive neurons in cerebral cortex, but not in periqueductal gray and spinal cord[23]. Al neurotoxicity may be mediated through oxidative damage via free radical production, and lipid peroxidation, etc.[18,21,24]. Al itself is without redox capacity, but it strongly enhances the peroxidant potential of iron[25]. It promotes aggregation and deposition of amyloid protein which induces oxidative stress[25] leading to neurotoxicity.

Previous studies[5,9,23] assessed the Al effects mostly on the animals of younger age groups (2-4 months). It would also be appropriate to investigate the effects of Al on ageing process-related changes in animals of higher age groups as strictly speaking at younger age, animals, are in developmental stage and thus not ageing. In rats, ageing process begins to manifest

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around 12 months of age. Therefore, we studied animals of higher age groups to focus on age-related effects of Al. We studied the oxidative stress-related parameters: lipid peroxidation, lipofuscin accumulation, glutathione-s-transferase (GST), and glutathione peroxidase (GPx) activities in brain regions of 6 and 12 months old rats, chronically fed with $AlCl_3$ in drinking water. In addition, two other ageing-related parameters Na^+, K^+-ATPase and multiple unit action potentials (MUA) were also studied as they are susceptible to lipid peroxidation[26-32]. MUA (action potentials derived simultaneously from many neurons) is an electrophysiological marker of spontaneous activity of a population of neurons[28-30]. In the present experiment, the effect of chronically administered Al on certain ageing-related biochemical and electrophysiological parameters in four brain regions, cortex, striatum thalamus hippocampus which differ in their vulnerability to ageing process, was evaluated in rats with a view to assess the Al-induced acceleration of ageing-related parameters.

Materials and Methods

Animals

Male Wistar rats (6 and 12 months of age) at the start of the Al treatment schedule were housed individually in propylene cages on 12L:12D cycle (0600 to 1800), and fed *ad libitum* on commercial rat food pellets. Water was available *ad libitum*. Animals were checked for their health status as in our previous work[28].

All chemicals used were purchased from Sigma Chemicals Co. U.S.A. Recording electrodes were obtained from Plastic One Company, Virginia (USA).

Aluminium dose and administration

Experimental animals were administered with $AlCl_3$ in drinking water (500 mg/l) daily for 6 months, according to procedure described earlier[9]. Water consumption was recorded to calculate the amount of Al ingested per rat per day[9,23]. In our experiments, daily Al intake calculated on the basis of water consumption was approx.13 mg per rat per day. The water consumption was found to remain similar in all the groups throughout the experimental period. Body weight of animals was recorded daily. The body weight of rats exposed to Al remained comparable to those of controls. The food and water intake by experimental animals did not differ significantly from that of controls.

Rats were randomly assigned to the following four groups. Group I (n=7) consisted of 6 months old rats which received Al in drinking water (as described above) for 6 months; Group II (n=7) consisted of 6 months old rats, which received normal drinking water for 6 months, these served as controls for group I animals; Group III (n=7) consisted of 12 months old rats, which received $AlCl_3$ in drinking water (as above) for 6 months; Group IV (n=7) consisted of 12 months old rats which received normal drinking water for 6 months, these served as controls for group III animals. In pilot experiments, when 18 months old animals were given the similar Al treatment, there was 100% mortality after 3 months of the treatment. When 6 and 12 months old rats were administered Al in drinking water for 6 months, there was about 20% to 30% mortality. Therefore, the present experiments were performed only on 6 and 12 months old animals.

Preparation of animals for electrophysiological recordings

One month before the end of 6-month period of Al administration, all the animals (including controls) were stereotaxically implanted with electrodes for recording electrical activity from the cortex, hippocampus (CA_3area), striatum and thalamus. The stereotaxic coordinates, respectively were: cortex 2.00 mm lateral to midline and 2.00 mm posterior to bregma, striatum AP-0.3 mm, L3.3 mm, V4.5 mm; thalamus AP-3.3 mm, L2.5 mm, V6.0 mm, and hippocampus AP-4.3 mm, L4.3 mm, V4.5 mm. The methods of the electrode implantation and electrophysiological recordings [electroencephalograms (EEG) and MUA] were as given in our earlier reports[26,28].

EEGs and MUA were recorded from conscious unrestrained animals by using a Grass EEG/polygraph. For MUA recordings, composite extracellular signals were routed through the high impedance probe (Grass HIP 511) and amplified and filtered by Grass P511J and 7P511L AC preamplifiers, electronically discriminated (WPI Window discriminator) and displayed on a storage oscilloscope. The standard output pulses from the window discriminator were counted by Tektronix counter and also simultaneously recorded on the polygraph. Recordings were limited to the awake immobile state in which a rat sits quietly, but remains awake[28]. Thus, all electrophysiological activity remained uncontaminated with movement related changes and artifacts. After completion of Al

administration, electrophysiological recordings were performed on all the animals. From each brain region, recordings were obtained for 2 hr per day for 5 days and the mean of MUA counts for every region was obtained. After collecting electrical activity data, animals were sacrificed to obtain tissues for biochemical assays. Brains were immediately taken out and cooled in a deep freezer. Brain regions were rapidly dissected out according to their stereotaxic coordinates[33]. Tissue samples were homogenized in 0.1 *M* phosphate buffer (*p*H 7.0). Lipofuscin concentration and lipid peroxidation levels were assayed in this homogenate. Crude synaptosomal and cytosol fractions were prepared further by differentially centrifuging the homogenate[34]. Various biochemical assays (n=5 per parameter per age-group) were performed as given below.

Biochemical assays

Na^+, K^+-ATPase (EC:3.6.1.3) activity was measured in the crude synaptosomal fraction as described earlier[31]. The reaction mixture contained 100 m*M* NaCl, 20 m*M* KCl, 5 m*M* $MgCl_2$, 3 m*M* ATP, 100 m*M* ouabain, 50 m*M* Tris-HCl buffer (*p*H 7.4), enzyme preparation and the desired reagent in 1.0 ml. Na^+, K^+-ATPase activity was calculated from the difference in the amounts of inorganic phosphate released in the absence and presence of ouabain.

Glutathione-s-transferase (EC:2.5.1.18) activity was assayed as described[35] in the cytosolic fraction[36]. Assay was based on glutathione conjugation to 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and measured spectrophotometrically. Specific activity of the enzyme was expressed as μ moles of CDNB-GSH conjugate formed/min/mg protein. Glutathione peroxidase (EC:1.11.1.9) activity was assayed as described[37], by following NADPH oxidation at 340 nm in the presence of H_2O_2, reduced glutathione and glutathione reductase, using a Shimadzu UV-260A spectrophotometer. Specific activity was expressed as μ moles of NADPH oxidized min/mg/protein.

Lipid peroxidation (thiobarbituric acid reactive substance) was measured[38] spectrophotometrically at 532 nm. Lipid peroxide levels were expressed as n moles of malonaldehyde formed/g/tissue. Lipofuscin was estimated spectrophotometrically[39]. Fluorescence intensity of chloroform-methanol extracted fluorescent chromolipids solution was measured with a Shimadzu spectrofluorimeter at an excitation wavelength of 360 nm and emission wavelength of 430 nm[40], using quinine sulphate as a standard. Lipofuscin accumulation was also assessed by fluorescence microscopy, using a Zeiss Orthmate microscope equipped with fluorescence attachment with Ploemipak Epi Illuminator; H2 cube (wide band) and excitation filter 390-490 nm.

Statistics

Results were evaluated by Student's *t* test by comparing data from Al-treated animals of each age group with the respective controls[36,41,42].

Results

Aluminium-induced effects on MUA and EEG

In all the Al-treated animals of both the age groups (Group I and III), electroencephalographic recordings from the four brain regions showed the presence of intense epileptiform activity in their EEGs (Fig. 1). The spontaneous episodes of epileptiform activity consisted of spikes and spike-like wave complexes and appeared simultaneously in all the recorded regions. In Group I animals, these episodes were frequently interrupted by normal EEG activity; the epileptiform activity was often associated with minor behavioural convulsive activity, such as head nodding, movement of whiskers and gritting of teeth. However, in Group III animals the epileptiform activity episodes were of very long duration (Fig. 1), so that the EEG practically exhibited a continuous epileptiform activity. They showed tremors of fore limbs, rearing and grooming movements, head nodding, whisker movements, etc. In one animal, the behavioural convulsive movements did not occur. The control animals, however, showed no epileptiform activity in their EEGs, and exhibited no behavioural abnormalities.

MUA recordings in four brain regions of Al-treated animals of both age groups showed a considerable increase in MUA counts, compared to controls (Fig. 1 and 2). Percentage comparison of MUA increase in both age groups is presented in Table 1. Thus, in animals of both the groups, thalamus exhibited the lowest, while the hippocampus together with cortex showed the highest MUA increase. Also, in Group III (18 months old) animals, quantitatively Al-induced enhancement in MUA was higher in all the regions as compared to Group I (12 months old) animals. In Group I animals, concomitance between increase in MUA and epileptic bursts in EEG was conspicuous (Fig. 1). In Group III animals, MUA recordings showed a persistent increase in MUA counts due to epileptiform activity as compared to controls.

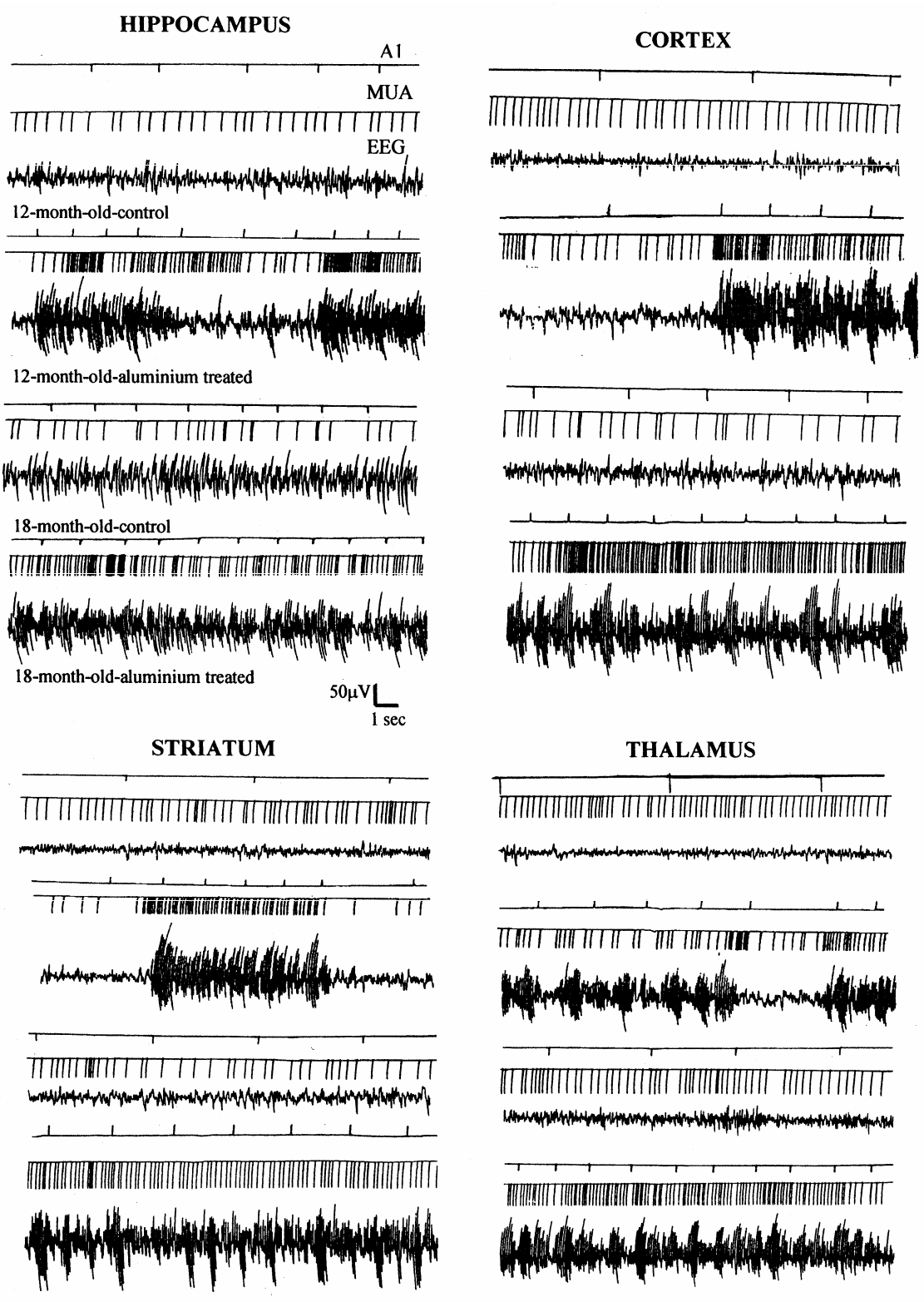


Fig. 1—Sample polygraph records of multiple unit action potentials (MUA) and EEG from four brain regions showing the effect of aluminium treatment in 12 and 18 months old rats [The traces above the MUA traces are of cumulative amplitude integration marks of corresponding EEG traces. The presence of epileptiform electrical activity in EEG traces (compared with corresponding controls) and increased MUA are evident in all four brain regions]

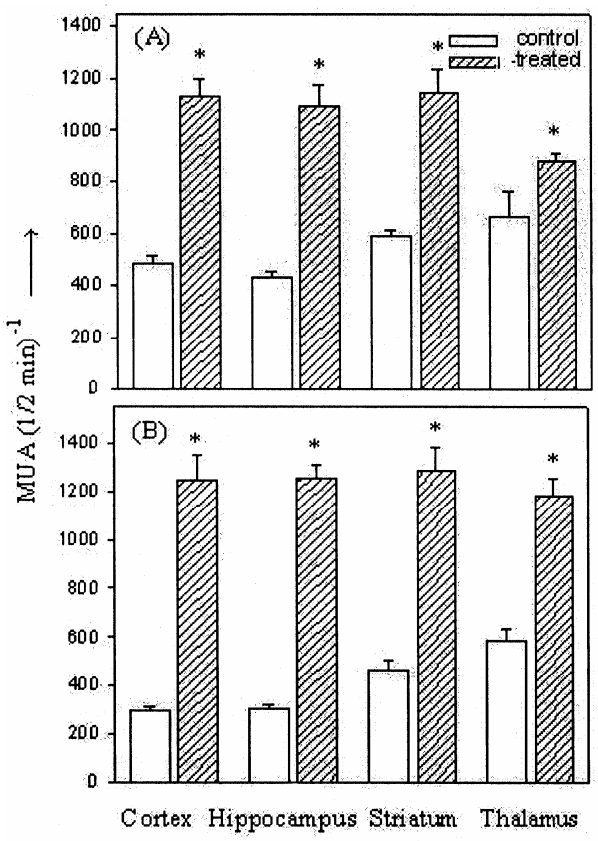


Fig. 2—Increase in MUA counts after Al treatment in brain regions [(A): in 12 months (▭) control; (▨) + Al-treated; and (B) in 18 months (▭) control; (▨) + Al-treated) age groups] MUA increases are significant compared with untreated age-matched controls in all the regions. *p<0.001; and $$p$<0.05)

Aluminium-induced effects on enzyme activities

Al treatment resulted in a significant decrease in Na^+, K^+- ATPase, GPx and GST activities in all four brain regions of the animals of both age groups (Group I and III), (Fig. 3) as compared to the respective controls.

Percentage comparison of inhibition of the enzyme activities in brain regions of both age groups is presented in Table 2. Increased inhibition of Na^+, K^+-ATPase in Group III, to greater extent than Group I indicates an age-related effect of Al. Also, the highest inhibition in Na^+, K^+-ATPase enzyme activity, was observed in hippocampus, compared to other brain regions. The magnitudes of inhibition of GPx and GST activities, appeared quantitatively similar in two age groups.

Aluminium-induced effects on lipid peroxidation and lipofuscin levels

In Al-treated rats of both the age-groups (Group I and III), lipid peroxidation and lipofuscin levels in different brain regions were significantly increased as compared to controls (Fig. 4). The magnitudes of elevations in two age groups (Table 1) appeared to be quantitatively somewhat similar indicating the absence of age-related effect of aluminium. Observations on fluorescence microscopic histological preparations also indicated an increase in intraneuronal lipofuscin deposits in Al-treated animals (Group I and III) as compared to controls (Fig. 5).

Table 1—Percentage increase of MUA, lipid peroxidation and lipofuscin levels (from respective controls) in brain regions of two age groups

Parameter	Regions	Group I	Group III
MUA	Cortex	155	316
	Hippocampus	159	316
	Striatum	109	189
	Thalamus	38	95
Lipid Peroxidation	Cortex	76	79
	Hippocampus	74	89
	Striatum	75	79
	Thalamus	75	85
Lipofuscin	Cortex	75	81
	Hippocampus	77	79
	Striatum	79	83
	Thalamus	67	78

Discussion

In our study, the rats were exposed to Al feeding through drinking water for 6 months at doses comparable to those used in previous studies[9,23]. We have not measured accumulation of Al in the studied brain regions, although earlier studies have shown that ingested Al elevates the brain content of Al in various brain regions[5,9,23]. In humans also, increased accumulation of Al in the brain due to industrial exposure to Al dust and other sources has been reported[43,44]. At the doses used, chronic exposure to Al in rats do not produce appreciable neuropathological alterations[9,23], although impairments in some cognitive functions[9] occur. Although the mechanisms by which Al interacts with the nervous system are not understood, but *in vitro*[20] and electron microscope studies[5] show astrocytes as the principal target of Al's toxic action leading to neuronal cell loss and degeneration[20]. Electrophysiological studies indicate that Al impairs learning-related phenomenon such as hippocampal long-term potentiation[45], and interacts with glutamate receptors in hippocampus[46].

Electroencephalographic recordings in our study showed (Fig. 1) pronounced alterations in electrical

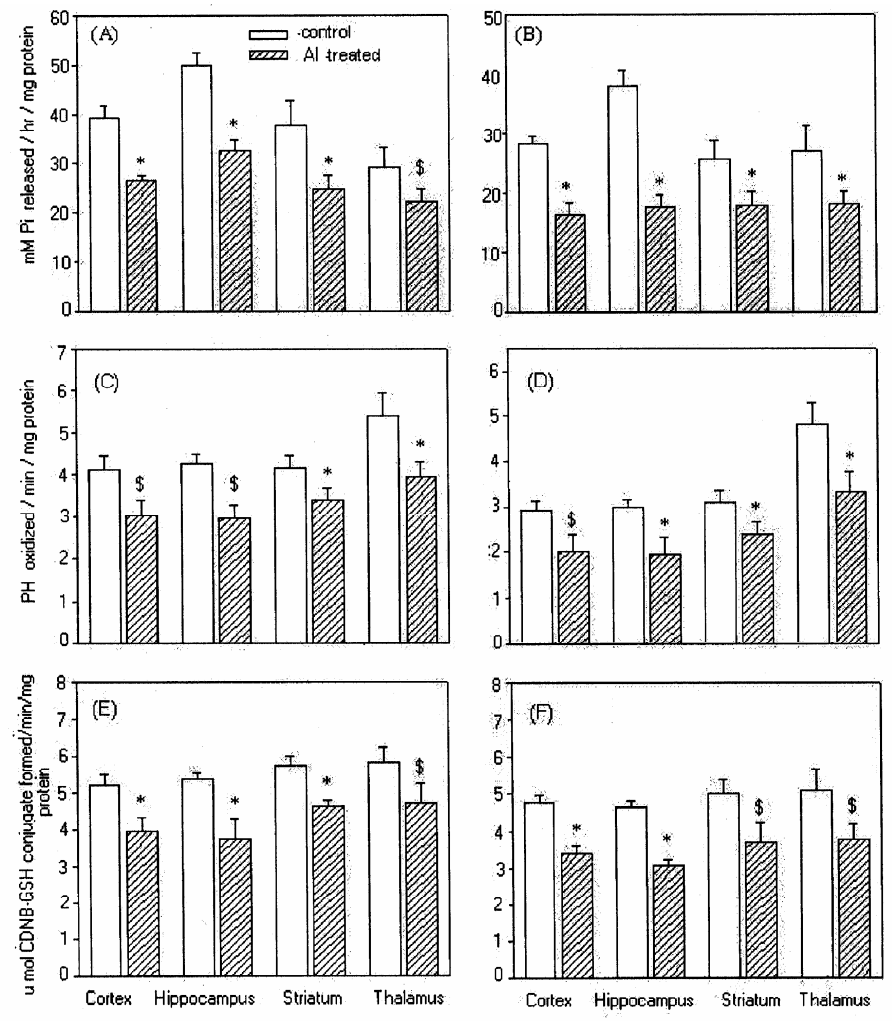


Fig. 3—Effect of Al treatment on the activities of Na^+, K^+-ATPase (A & B) glutathione peroxidase (C & D) and glutathione-s-transferase (E & F) in the four brain regions of 12 and 18 months age groups respectively. [Control (□); (▨) + Al-treated. Activities of all the three enzymes decreased in all four regions. Decrease of Na^+, K^+-ATPase was higher in 18 months age group. GPx and GST activity decreases were quantitatively similar in two age groups]

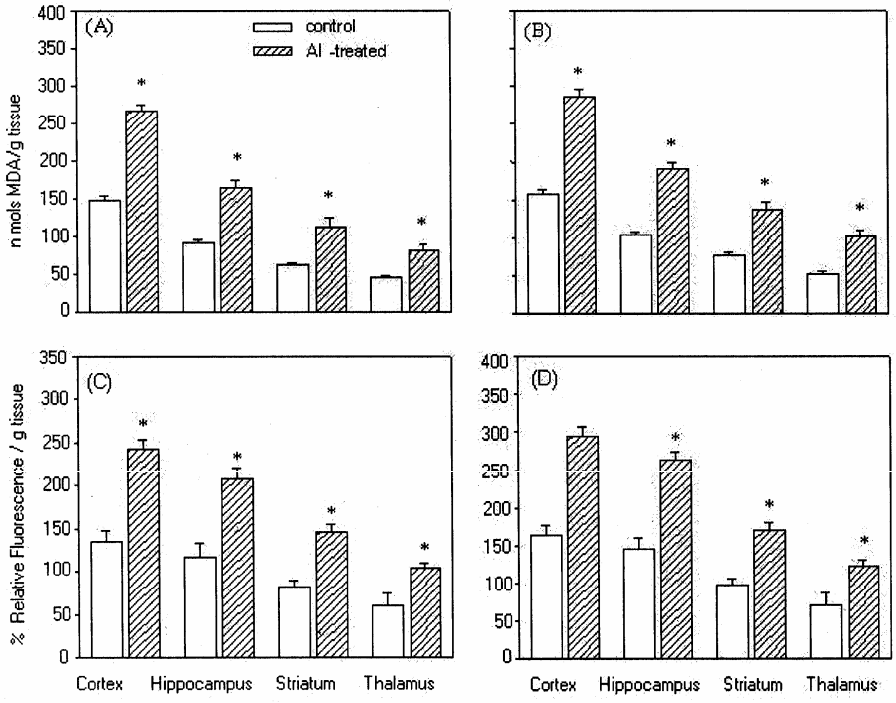


Fig. 4—Elevation in the levels of lipid peroxidation (A & B) and lipofuscin (C & D) in the four brain regions of two age groups after Al treatment [Control (□); (▨)+ Al-treated]

Table 2 — Percentage inhibition in enzyme activities (from respective controls) in brain regions of two age groups

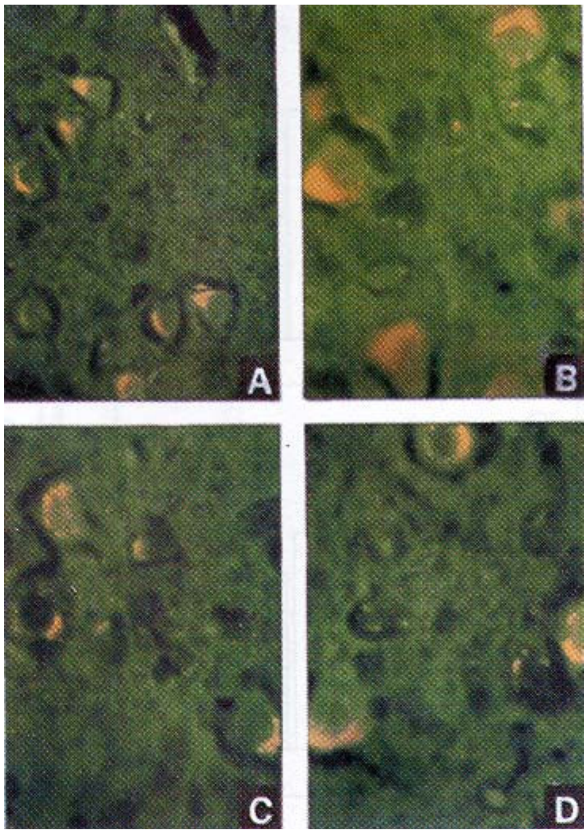
Parameters	Regions	Group I	Group III
Na^+, K^+-ATPase	Cortex	30	46.4
	Hippocampus	36	60.5
	Striatum	34	28
	Thalamus	17.8	42
Glutathione peroxidase	Cortex	25	25
	Hippocampus	30	36
	Striatum	20	26
	Thalamus	27.2	31
Glutathione-s-transferase	Cortex	25	29
	Hippocampus	30	33
	Striatum	21	30
	Thalamus	20	30

activity of brain regions in Al-treated animals. Prominent epileptiform activity in the form of intermittent prolonged epileptic bursts appeared in EEG recordings. Our results also showed that Al produced more intense changes in electrical activity in rats of higher age group compared to lower age group, indicating an age-related effect of Al. Neurological abnormalities, such as generalized convulsions and seizures along with EEG abnormalities (paroxysmal high voltage EEG discharges) have been reported in humans, due to Al exposure[43,44,47]. Al can be epileptogenic in brain tissue, and experimental animal epilepsy model based on the topical application of alumina cream in brain[48] is in use.

The present results showed that in Al-treated animals, synaptosomal Na^+, K^+ -ATPase activity was inhibited in all four brain regions of both the age groups. Earlier we have found[49] that normal ageing from 6 to 12 months does not result in inhibition of Na^+, K^+-ATPase activity in all the four regions. The present data showed that Al treatment at 6 to 12 months of age caused a significant inhibition of this enzyme even in 12 months old animals, indicating Al's enhancement of this age-related parameter.

Normal ageing from 12 to 18 months depresses Na^+, K^+-ATPase activity in hippocampus, striatum and cortex[49]. However, Al treatment during 12 to 18 months of age, significantly inhibit the enzyme activity in all four regions indicating Al's enhancing effect on the ageing process. Al inhibited the enzyme activity even in thalamus where normal ageing does not have an effect. This is indicative of an augmentation of this ageing-related parameter by Al.

The present data also clearly showed that regional inhibition of the enzyme (Na^+, K^+-ATPase) activity after treatment with Al was much higher in 18 months



age group than in 12 months age group, showing a greater vulnerability of higher age group to Al. Hippocampal enzyme activity showed the highest Al-induced inhibition, compared to other brain regions, thus showing a higher vulnerability of hippocampus to Al. The *in vitro* studies have shown both inhibitory[50,51] and stimulatory[52] effects of Al on Na^+, K^+-ATPase activity. *In vivo* studies[9] on Al effects in whole rat brain homogenates, however, showed an inhibitory effect on the enzyme[9]. In monkey brain regions also, an *in vivo* inhibitory effect of Al on Na^+, K^+-ATPase activity was found[53]. Our study shows the effect of Al on this enzyme activity in rat brain regions of two different age groups, where of inhibition was higher in brain regions of higher age vulnerability, as well as in older age group. Our data thus show age-related effects of Al and regional vulnerability of Al effect.

There is an increased incidence of epilepsy in elderly human population[54]. Thus, in advanced age the central nervous system may become more susceptible to producing epileptiform activity. The ageing process is, therefore, likely to be associated with increased occurrence of epileptiform electrical

activity. Al's induction of epileptiform activity in the present study would indicate an Al-induced increase in this aspect (epileptogenicity) of ageing process. The Na^+, K^+-ATPase activity inhibition, is implicated in epileptogenesis[48]. Thus, generation of epileptiform activity in Al-treated animals may be due to Al-induced inhibition of Na^+, K^+-ATPase activity. Decrease in Na^+, K^+ pump activity increases neuronal sensitivity to glutamate, thus making the cells more excitable[48]. Also, N-methyl-D-aspartate (NMDA) receptors become more sensitive during ageing[55]. These findings taken together support the concept of a higher propensity of aged nervous tissue for epileptogenesis. Al's ability to depress Na^+, K^+-ATPase activity thus may augment ageing-related susceptibility of aged central nervous system to epileptogenesis. The greater epileptic electrophysiological activity in Al-treated 18 months age group may be related to the higher inhibition of Na^+, K^+-ATPase activity compared to younger age group.

During normal ageing from 6 to 18 months, lipid peroxidation is not increased in thalamus, but is elevated in other three studied regions by about 30%[49]. The present data showed that Al treatment resulted in a significant elevation of lipid peroxidation in all the four regions of both age groups. It is, thus, of interest to note that in thalamus, where normal ageing does not elevate lipid peroxidation, Al significantly enhanced the lipid peroxidation. Earlier, Al-induced increase in lipid peroxidation is reported in chick brain[56], and whole rat brain[9]. Elevated lipid peroxidation is also reported in hippocampus, cortex and striatum of very young rats administered Al glutamate[21]. Al potentiates stimulation of lipid peroxidation induced by iron *in vitro,* since it itself is without any redox activity[57]. Increased lipid peroxidation after *in vivo* Al-treatment should, therefore, be due to potentiation of iron-induced lipid peroxidation that may occur during normal ageing. Furthermore, decrease in Na^+, K^+-ATPase activity after Al administration could also be due to Al associated increase in lipid peroxidation.

Normal ageing is also associated with elevation of lipofuscin contents in four studied brain regions[49]. During ageing from 6 to 18 months, the elevation is about 20% to 30%, while it is more pronounced during ageing from 18 to 24 months (40-45%). The present biochemical data showed that Al treatment increased lipofuscin content by 65 to 80%, the increase was, however, quantitatively similar in both age groups. Amongst various regions, elevation was greater in cortex and hippocampus, compared to other regions. This was true for lipid peroxidation also. Histochemical study also indicates that neuronal lipofuscin accumulation was significantly increased after Al treatment. In previous electron microscopic observations[5], Al was found to increase lipofuscin content of astrocytes. Our light microscopic observations (Fig. 5), however, showed intraneuronal increase in lipofuscin contents indicating intraneuronal action of Al.

During normal ageing from 6 to 12 months, GPx activity is inhibited by about 26% in cortex, hippocampus and striatum, and by about 10% in thalamus[49]. At age of 18 months, the enzyme activity becomes further lower and falls to about 47% of 6 months values in cortex, hippocampus and striatum and to about 18% in thalamus. The present data showed that in Al-treated rats, GPx activity decreased further by about 25 to 35% in all four regions, showing Al's inhibitory effect on the enzyme activity, the magnitude of inhibition was quantitatively similar in two age groups indicating the absence of an age-related effect. Furthermore, amongst various brain regions, the magnitude of inhibition did not differ significantly from each other. In a previous study[58], GPx activity was found to decrease in brain, following a 4 weeks Al treatment. However, no other studies of Al's effects of GPx activity in brain regions have been reported.

We have previously shown that GST activity during normal ageing first increases from 6 to 12 months by about 45 to 60% in the four studied regions[36,49], and then falls during ageing from 12 to 18, and 18 to 24 months. The present data showed that Al exposure during 6 to 12 months of age lowered the normal ageing-related increase of GST in all four studied brain regions, and accentuated normal ageing-related decrease that occurs during 12 to 18 months of age. On the whole, Al treatment decreased the activity significantly by about 25 to 30%. The magnitude of inhibition was quantitatively similar in both age groups, and in four studied brain regions. Earlier[24], a non-significant decrease of GST activity was found in whole brain. The present data would thus indicate that Al-induced changes are more conspicuously detected in discrete brain regions than in whole brain. No other studies of Al's effects on the GST activity have been reported. Al's ability to depress the enzymes GPx and GST together with its

ability to elevate lipid peroxidation and lipofuscin levels would thus indicate its capacity to lower the antioxidant capacity of the tissue indicating Al's ageing accelerating influence on the ageing process.

In some studies, a decrease in the average life span after Al feeding is reported, which may be due to Al-induced acceleration of the rate of ageing[59,60]. In these studies, experimental animals of $AlCl_3$-fed group died at younger ages than those of control group. In our experiments, some mortality occurred in Al-fed groups, which obviously would be related to Al-induced acceleration in the rate of ageing. Al administration chronically to rats in drinking water alters iron homeostasis that could lead to free radical damage[16]. It also alters glycolytic pathway, protein phosphorylation and protein kinase activities in brain[17,61]. All these factors may contribute to increased rate of ageing. In contrast to previous studies[5] in which Al was fed to rather younger animals (e.g., 8 weeks old), our experiments were performed on relatively higher age group animals. It would, thus appear that younger animals withstand long-term Al treatment without mortality, whereas in older animals Al treatment may result in some mortality. For example, Lal *et al.*[9] reported no mortality in experimental animals even after 6 months of Al feeding, because Al was given to animals of less than 6 months of age. In our present experiments, mortality was 20 to 30% in 6-12 months age group and was much higher in 18 months age group. However, in either case, mortality occurred in the latter half of treatment i.e., animals did withstand 3 months or more of Al treatment. More severe changes appear to be caused by Al in older animals than in younger ones. For instance, in our experiments, Na^+, K^+-ATPase was inhibited to a higher extent in 18 months, than 12 months old ones, and more severe epileptiform activity occurred in the former than in the latter.

It will also be relevant to consider the implications of a long-term *in vivo* Al treatment. In our experiments, Al-feeding for 6 months would appear to simulate the prolonged Al exposure that may be involved in humans in normal ageing and pathological ageing such as Alzheimer's disease. The daily intake of Al through various sources in humans may be around 10-20 mg/day throughout the life time[18], and such prolonged exposure might be implicated in the development of Alzheimer's type pathology[62]. However, experiments have been performed in which Al has been fed to animals for various longer or shorter durations: for example, two and a half months to two years[5,23,61], and acceleration of ageing-related parameters was found even in experiments where Al was given for 5 weeks[23] or two and a half months[5].

It will also be of interest to consider whether effects of Al exposure would be reversible. Although we have not studied reversibility of Al effects, previous studies indicate that the effects may be reversible: Al-induced enhancement in blood-brain barrier permeability showed reversibility in 24 hr[63], and Al-induced deficit in conditioned avoidance behavioural response was also reversible[64]. Since Al's effect may be attributed to its augmentation of oxidative stress resulting from activation of oxidant enzymes, stoppage of Al treatment (i.e., decrease in the tissue concentration of Al) is likely to result in reversal of oxidative effects. In our own studies (to be published), we have found that, acetyl-L-carnitine[49] and L-deprenyl[41], which are antioxidative in their action, prevent Al-induced augmentation of oxidative stress. In this context, however, it would be of interest to explore further whether oxidative stress effects of Al are reversible.

In summary, data derived from present work show that chronic administration of $AlCl_3$ in drinking water of rats resulted in a distinct acceleration of brain ageing process, as evidenced by the increase in lipid peroxidation and lipofuscin accumulation, decrease in the activities of GST, GPx and Na^+, K^+-ATPase, and the increased epileptogenicity of brain.

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