Chapter IV

Discussion
The nervous system is an extremely complex system. The brain is the command center of the body. It receives and integrates signals and then responds appropriately controlling all motor and sensory activities to maintain bodily function. Besides the involuntary processes such as breathing, circulation and peristalsis, the nervous system also controls voluntary processes such as movement, cognition and learning. During the last few decades there has been increased interest in the study of the nervous system as a target organ for toxicity (Weiss and Laties, 1969; Weiss and Laties, 1975; Mitchell, 1978; Geller et al., 1979; Weiss and Elsner, 1996).

Neurotoxicants may be naturally occurring in the environment (heavy metals like mercury, lead or manganese and plant origin neurotoxicants like *Lathyrus sativus*) or may be man-made (drugs, pesticides and insecticides, and several other chemicals). In the United States, approximately 800 neurotoxic chemicals can be found in workplaces. Also many house products contain neurotoxicants. About 2000 new chemicals are annually introduced into commerce (Office of Technology Assessment, Washington, 1990).
Behaviour is the final product of a highly complex nervous system. Any change in the behaviour of an animal is a reflection of the direct and/or indirect effects on the nervous system. Therefore behavioural changes are sensitive functional indicator of neurotoxicity. Behaviour represents the integration of motor, sensory, and associative neuronal functions which cannot be assessed using only neurochemical, histological or physiological techniques.

Study of behavioural alterations has been widely used for assessment of neurotoxicity of a variety of drugs and other chemicals. Dixon and co-workers (Dixon, 1982; Dixon et al., 1984; Dixon et al., 1990) provided exhaustive guidelines for an appropriate analysis of the effects of psychoactive drugs on rodent social and aggressive behaviour. Miczek and Krstiač (1979) and Miczek et al., (1984) reported drug-induced modifications in rodent social and agonistic behaviours. Maurissen et al., (1983) studied the somatosensory changes in monkeys on long-term exposure to acrylamide, while Di Giovanni et al., (1993) investigated the effect of gestational exposure to carbon monoxide on behaviour in rats. In humans,
long-term neurological and behavioural effects were seen in children born to mothers exposed to methylmercury (Marsh, 1987). Behavioural alterations in birds have also been reported (King et al., 1984; Hart, 1993).

Several workers (Kulig et al., 1996; Bignami, 1996; Kulig, 1996; O'Donoghue, 1996; Ulbrich and Palmer, 1996) have recommended the measure of behavioural variables to assess neurotoxicity of chemicals.

Pryor et al., (1983) made a comparative study of the neurotoxicity of eight chemicals, namely, acrylamide, methylmercury, chlordecone, tetracetyl tin, triethyl lead, lead acetate, arsenic and monosodium salicylate using a battery of neurobehavioural tests consisting of, among others, tests for motor activity, forelimb and hindlimb grip strengths, rotation orientation, thermal sensitivity, startle responsiveness to acoustic and air-puff stimuli, and performance of a multisensory conditioned pole-climb avoidance response task. Zbinden (1981) has reviewed the numerous experimental methods employed by
experimental psychologists and neurobehavioural toxicologists to study developmental toxicology.

Several studies have established the neurotoxicity of Al (Deibel et al., 1997; Golub et al., 1992a; Yokel, 2000 and 2001) and the US Agency for Toxic Substances and Disease Registry (ASTDR) has brought out a detailed Toxicological Profile for Aluminium (ASTDR, 1999).

Al has been implicated by several workers (Trapp et al., 1978; Crapper and Dalton, 1973; Crapper et al., 1973; Crapper et al., 1975; Crapper et al., 1976; Crapper et al., 1991; Perl and Brody, 1980) as a causative factor in Alzheimer's disease, though this is still not wholly proven and is highly controversial. Al is also thought to impair cognitive and memory functions and to cause neurological disorders such as encephalopathy syndrome, senile dementia of the Alzheimer's type, amyotrophic lateral sclerosis and parkinsonism-dementia (Perl et al., 1982; McLachlan et al., 1991; Zatta, 1993; Wakayama et al., 1996; Armstrong et al., 1996; Perl, 1985).
Ganrot, (1986) in an excellent review has discussed possible health effects of Al and presented a hypothetical model for the metabolism of Al. Ganrot also pointed out that lethal concentration of Al in brain exceeds the normal level by only a factor of 3-10, and that the time required in the normal course for the Al in the brain to reach lethal levels would be 100 to 150 years.

Liu et al., (1996) reported elevated brain Al levels after administration of 10.8 and 5.6 mg Al/day/kg body weight in rats with compromised kidney function and low calcium diet. Florence et al., (1995) reported significant increase in the levels of Al in the various brain regions - frontal, temporal and parietal hippocampus, and in the liver and spleen in male Wistar rats on administration of 2 mg Al three times per week for a period of 4 to 8 weeks. The hippocampus region of the brain in particular showed 75% increase in Al levels as compared to the control animals.

Crammer et al., (1986) reported significantly increased body Al levels in fetal mice (2008 ± 05 mg/g dry wt compared to 592 ±
178 mg/g dry wt in control) following maternal exposure to 100 mg AlCla/day/kg on gestational days 7-16, while the placental Al levels was over ten-fold greater (27600 ± 6046 mg/g dry wt) than the controls (2677 ± 1804 mg/g dry wt).

There have been few studies investigating the effects of aluminium on the emotional reactivity and on learning and memory. An attempt was made in this study to investigate a few aspects in these areas.

In the present study adult male rats and the offspring of treated female rats were separately assessed for behavioural neurotoxicity on administration of Al. In the first set of experiments the four groups of pregnant female rats were administered 0, 5, 10 and 20 mg Al/kg body wt on days 14-16 (inclusive) of gestation. The offspring were tested for behavioural alterations in a battery of tests up to 100 days of age.

In another set of experiments four groups of adult male rats were similarly administered 0, 5, 10 and 20 mg Al/kg body wt for 10 days and then assessed for behavioural alterations in two
phases; Phase I beginning the second day after administration and Phase II from day 260 to day 264.

Developmental neurotoxicity of Al in rats and mice has been reported by several workers (Bernuzzi et al., 1986; Yokel, 1985; Rankin and Manning, 1993; Misawa and Shigeta, 1993; Gonda et al., 1996 and Golub et al., 1987). Domingo, (1995) has reviewed the reproductive and developmental toxicity of Al. Alterations in behavioural performance due to prenatal administration of Al have also been reported by Gonda and Lehotzky (1996) and Golub et al., (1995).

The offspring of Al treated pregnant rats in the present study were tested for behavioural alterations in a battery of tests up to 100 days of age. The results of the various tests reveal that aluminium did cause developmental neurotoxicity, especially at the highest 20 mg Al/kg dose. The dose levels as well as the duration of Al exposure were low compared to several other studies. For example, Gomez et al., (1991) administered 133 mg Al/kg/day by gavage for 10 days on gestational days 6 through 15, while Misawa and Shigeta (1993) administered pregnant rats
with a single dose of 900 or 1822 mg AlCl₃/kg on day 15 of gestation by gavage. Bemuzzi et al., (1986) orally administered 160 and 200 mg Al/kg to pregnant from 8th day of pregnancy to parturition (20th day). Yokel, (1985) administered rabbits with doses of 0.675, 2.700 and 10.800 mg Al/kg/injection in a staggered dosing regimen for 20 days between days 2 and 27 of pregnancy.

In the study of dam and pup parameters Gonda and Lehotzky (1996) have shown that gestational exposure of Al lactate in doses 2.45, 4.9 and 9.88 mg/kg had no effect on birth weight, mean litter size and the days of eye and ear opening of the mortality of pups. Similar results were reported by Gonda et al., (1996) and Muller et al., (1990). Donald et al., (1989) also reported little intra- or inter-group variability in the weights of dams and pups and birth parameters in mice given excess Al (5, 100 and 200 μg Al/kg/day) in diet from conception through weaning, though some variability was reported in the gestation length. Similar findings were shown by Muller et al., (1990) wherein rats were exposed to Al lactate during different lactation periods.
These findings were largely in agreement with the results in the present study wherein no significant effect was seen in the dam data parameters. There was no change in the duration of the gestation period and also no mortality in the treated pregnant rats. Also there was no significant gain in their body weight and no alteration in food and water consumption pattern. Al did not have any effect on the pup data parameters as well. There was no change on the days of pinna detachment and eye opening. But the Group I pups showed significantly increased body weight.

In contrast intraperitoneal administration of high doses of AlCl₃ (75 to 200 mg/kg to rats in gestational days 9 to 13 or 14 to 18 resulted in lower weights, shorter crown-rump lengths, increased skeletal abnormalities and more resorptions (Benett et al., 1975). Bernuzzi et al., (1986) reported an increase in postnatal death rate without a dose-dependent effect in the offspring of rats intoxicated with 160 and 200 mg Al/kg/day from 8th to 20th day of gestation, though there was no effect on the number of young at parturition. Similar results have been shown by Bernuzzi et al., (1989a) and Yokel (1985). In contrast
there was no effect on the mortality rate in pups on treatment of Al on different gestational periods.

The offspring were also subjected to neurobehavioural tests such as righting reflex, placing reflex and auditory startle reflex, and tests like the suspension test, stress tolerance test and the motor coordination test.

The neurobehavioural tests employed in the present study has been validated and widely used by several workers to detect and assess the behavioural teratogenic effects of various chemicals (Pryor et al., 1983; Vorhees et al., 1979; Lehotzky et al., 1988; Lehotzky et al., 1990; Donald et al., 1989; Colomina et al., 1999a). Despite their apparent simplicity, these tests have been shown to be quite sensitive in detecting the neurotoxic effects of a number of compounds (Moser, 1989). Nevertheless, assessment of neurotoxicity of a chemical by measurement of behavioural responses is no easy task given the complexities involved. The task becomes more complex with a variety of factors influencing the dams and pups behaviour and the difficulty in detection and interpretation of the results due to the
subtleness of the effects of the neurotoxicants. It should be emphasized that the behavioural changes observed in teratological experiments are often not striking and therefore may easily be misinterpreted.

Yet another factor adding to the complexity of interpretation is the variability of behavioural responses of the animal—frequently, an animal responds differently, sometimes extremely so, even if all other conditions are similar. This is true of animals of both the Treated and Control Groups. Several underlying factors are in play in such a confounding situation, frequently producing contradictory and controversial findings (Werboff and Kesner, 1963 and Hoffeld and Webster, 1965).

Maternal exposure to Al in this study did not affect the performance of the offspring in the righting and placing reflex tests and the suspension test. All the animals passed these tests. But for the two of the eighteen animals in the Group II, all the animals also passed the auditory startle reflex test.
Misawa and Shigeta (1993) reported similar results in two groups of rat offspring whose mothers were administered a single dose of 900 and 1800 mg Al/kg respectively, on day 15 of gestation. The two Treated Groups did not show any change in the surface righting reflex. But the males in both the Treated Groups showed significant delay in the appearance of auditory startle. No change in the auditory startle was seen in the offspring of mice exposed to 25, 500, 1000 µg Al/g diet from conception to weaning (Donald et al., 1989; Golub et al., 1992b and Golub et al., 1995).

Delay in righting reflex was reported by Clayton et al., (1992) in pups of rats intraperitoneally exposed to 200 mg/kg aluminum sulphate on days 10-13 of gestation and by Colomina et al., (1999a) in pups of mice treated with 75 mg Al/kg/day on days 6-15 of gestation.

No significant difference was seen in the postural reflexes, suspension test and auditory startle test in the rat pups of mothers exposed to Al (2.5, 5 and 10 mg Al/kg subcutaneous doses) on 7th - 15th days of gestation (Gonda et al., 1996).
Similar findings were also reported by Muller et al., (1990). Bernuzzi and co-workers (1986), who had orally treated pregnant rats with 160 and 200 mg Al/kg from 8th day of gestation to parturition, reported impaired performance in the righting reflex, while in the suspension test, there were no significant differences.

Interestingly, the stress tolerance test which is frequently used to screen antidepressant drugs revealed that while the middle dose (10 mg Al/kg dose) group remained unaffected, the performance of the lower dose (5 mg Al/kg dose) and the higher dose (20 mg Al/kg dose) groups was negatively affected by the prenatal exposure.

This was in contrast with the results obtained by Gonda et al., (1996) wherein the Treated Groups showed no change.

In the present study, the offspring of the Treated rats were tested on the rotorod for motor coordination on PNDs 32, 45 and 100. The animals did not show significant change on PND 32
and PND 100, while on PND 45 the animals of the Treated Group spent significantly lesser time of the rotorod.

Colomina and co-workers (1999a) showed that intraperitoneal administration of Al (75 mg Al/kg/day) on days 6-15 of gestation reduced the time spent on a rotorod in mice. Bowdler et al., (1979) reported reduced rotorod time in 60-days old Al treated rats. Impaired performance of motor coordination was also reported by Muller et al., (1990) and Bernuzzi et al., (1989a).

In contrast Gonda et al., (1996) showed that there was little impairment by rats prenatally exposed to Al (2.5, 5 and 10 mg Al/kg daily during 7th – 15th days of gestation) on the rotorod tested at different ages.

The gestationally exposed rats and the male rats that were administered aluminum in adulthood were subjected to secondary behavioural tests. These tests are useful to quantify the toxicity and to evaluate the effects of a neurotoxicant on more complex behavioural processes such as emotional
reactivity, learning, memory and habituation or to provide information about the mechanism of action of the neurotoxicant.

Effect of Al on emotional reactivity has been evaluated by few workers – most reports appear to be on the effect of Al on learning and memory.

Measurement of emotional states like anxiety is useful for distinguishing anxiogenic and anxiolytic effects of chemicals and drugs. Several tests such as the measurement of ultrasonic vocalizations, the open field behaviour test and the elevated plus-maze test are used to assess emotional reactivity in rodents at different ages.

Ultrasonic vocalizations provide a useful model for investigating the ontogeny of emotionality (Winslow and Insel, 1991). It is used for assessment of emotional reactivity during early postnatal life providing a very useful tool in the assessment of neurotoxicity of chemicals.
On postnatal days 4, 10, 15 and 18, the pups of rats administered Al on Days 14, 15 and 16 of gestation were measured for ultrasonic vocalization and also for other parameters, that is, number of face washes, horizontal activity and vertical activity. Although a sensitive parameter and easy to measure, ultrasonic vocalization calling has been hardly employed in behavioral toxicity test battery. Few workers have used the measure of ultrasonic vocalization to assess toxicity of chemicals – a study by Rankin and Manning (1993) was the only report of measurement of ultrasonic vocalization calling to assess the effect of Al in mice pups that was found by the author.

In this study, in general, the Treated Groups showed significant reduction in the ultrasonic vocalization calling. Pups of dams exposed to the highest dose 20 mg Al/kg/body wt showed a pronounced reduction in the ultrasonic vocalization when compared to the Control Group on all the four testing days, while the pups in the lower dose groups (5 and 10 mg Al/kg/body wt) showed relatively lesser reduction. This was in agreement with the finding reported by Rankin and Manning.
(1993) wherein there was a considerable reduction in the production of ultrasounds by rat pups on administration of a single dose of 200 mg/kg body wt aluminium sulphate on Day 10 of gestation. The reduction in the USVs may have been due to delay in the maturation of the mechanisms responsible for ultrasonic vocalizations in the pups of the highest 20 mg Al/kg body wt dose group.

In a study, ultrasonic vocalization was induced in rat by cholinergic stimulation of the rat brain by injecting carbachol, an acetylcholine agonist, in the anterior hypothalamic/preoptic area, suggesting the involvement of the cholinergic system in the production of ultrasonic vocalizations (Brudzynski and Bihari, 1990). Hofer (1996) reported that a large number of neuromodulatory systems regulate the ultrasonic vocalization in infant rat. Increased ultrasonic vocalization was seen in Sprague Dawley rat pups on PNDs 9 and 11 as an effect of hypervitaminosis A (Adams, 1982) while no consistent effects on the ultrasonic vocalization rate and no effects on duration of the calls was observed in rat pups prenatally exposed to methylmercury (Adams et al., 1983).
Marked alterations in ultrasonic vocalizations have been reported in animals exposed to methylmercury and carbon monoxide (Elsner et al., 1990; Di Giovanni et al., 1993).

In the measure of face washes, horizontal activity and vertical activity (rearing) on PNDs 4, 10, 15 and 18, the findings in this study showed that gestational Al administration did not cause significant changes in the number of face washes; but significantly decreased horizontal activity was seen in the highest dose (20 mg Al/kg body wt) group indicating increased emotional reactivity and significantly reduced vertical activity was seen in Group I (5 mg Al/kg body wt) and Group III (20 mg Al/kg body wt) on PND 15 also indicating increased anxiety.

Interestingly it was observed that while pups in the highest dose Group III showed decreased horizontal activity, there was increased horizontal activity in pups of the lower dose Group I than the Control pups on PNDs 10, 15 and 18 although it was not statistically significant. Likewise, while pups of Groups I and III showed decreased vertical activity on PNDs 10, 15 and 18, pups of Group II showed increased vertical activity.
Misawa and Shigeta (1993) reported that a single dose of prenatal Al treatment of 900 mg/kg body weight can reduce vertical activity (rearing) in rat. Markedly decreased activity was seen in adult rats after prenatal Al administration (Muller et al., 1990 and Cherroret et al., 1992). However, Colomina et al., (1999a) found no significant changes in the horizontal and vertical activity in mice exposed prenatally to 75 mg Al/kg/day on Days 6-15 of gestation. Gonda et al., (1996) reported significantly decreased horizontal activity on days 36 and 93 after prenatal Al exposure (9.8 mg/kg dose) which is in agreement with the findings in this investigation.

Effect of Al on emotional reactivity has been little studied – there have been few reports of work done in this area.

The open field test is one of the most widely used and validated test methods for the assessment of emotional reactivity – it is used to assess emotional states and exploratory behaviour in adolescent and adult animals.
In the open field test in the present study, different doses of Al seemed to have varyingly affected the time the gestationally exposed animals spent in the corner squares. In general, though the animals of all the Treated Groups spent more time in the corner squares than the animals of the Control Group, animals of Group I and II spent more time than animals of Group III indicating a state of increased anxiety. Rats of Group I spent significantly longer time on PNDs 98 and 99.

In the Al administered adult male rats, in Phase 1 (Day 1 to Day 5) the Treated Group animals generally spent more time in the corner squares than the Control Group indicating increased anxiety. But on Day 5 they spent significantly lesser time than the Control Group. The significantly longer time spent initially on Days 1 and 2 could have been due to the general effects of intraperitoneal administration of Al rather than the effect of Al toxicity. This observation is reinforced with the decrease seen in time spent in corner squares on Day 5.

In Phase 2 (Day 260 to Day 264) the Treated animals generally spent lesser time in the corner squares than the Control Group
thereby indicating increased tendency in the Treated animals to explore than in the Control Group. These differences though were not statistically significant.

In the gestationally exposed group, the horizontal activity was significantly decreased on PNDs 96, 97, 98 and 99 while the vertical activity was decreased in the lower dose groups in the present study. Rats in Group I appear to be most affected followed by those in Group II. This is in agreement with the finding of Gonda et al., (1996) who reported decreased horizontal activity in the rats prenatally treated with 10 mg/Al/kg body wt. Markedly reduced activity was reported in rats on developmental exposure to Al by Muller et al., (1990) and Cherroret et al., (1992) and by Misawa and Shigeta (1993) wherein females prenatally exposed to a single dose of 1800 mg/kg AlCl₃ showed decreased ambulation and rearings.

But Colomina et al., (1999a) reported no significant changes in the activities in the open field on PND 22 in mice prenatally administered 75 mg/kg/day AlCl₃ on days 6-15 of gestation.
In the Al administered adult male rats, the Treated Group of animals generally exhibited lesser horizontal and vertical activity than the Control Group in Phase 1 (Day 1 to Day 5). In Phase 1 (Day 260 to Day 264), Al did not appear to effect the performance of the Treated animals in horizontal and vertical activity.

The elevated plus-maze test is one of the methods used for assessment of emotional reactivity states like anxiety in rodents. It is based on the conflict between exploration and aversion, that is, on the capacity of situation aversiveness to reduce or block exploratory responses (Cuomo et al., 1996). It is frequently used for assessment of emotional changes produced in rodents by developmental exposure to drugs and chemicals.

In the present study in the gestationally exposed animals both the Treated and the Control Group rats exhibited variability in several measures over the five testing days thereby giving no clear indication of either increased or reduced emotional reactivity though other measures strongly reflected an indication of increased anxiety among the Treated animals. The male rats
exposed in adulthood also exhibited similar variability in different measures in the elevated plus-maze.

In rats, attempt to enter the open arms in the elevated plus-maze is indicative of conflict, that is, the tendency to explore open arms versus fear of the open and elevated spaces. Increase in the number of attempts to enter open arms indicates increased anxiety in the rats.

In the gestationally exposed group the Treated animals made significantly fewer attempts to enter the open arms, that is, spent less time in conflict and decided more quickly than the Control animals to enter the open arms on all the five days. This could be interpreted as the Treated animals having lesser conflict than the Control Group and therefore having decreased anxiety. This interpretation is (partially) supported by the findings of lower first open arm latency in the Treated animals, particularly the animals of Groups I and II on PNDs 96, 98 and 100. But the inconsistency in the latency to the first open arm entry of all the animals does not provide a definitive support to the above assumption.
In the male rats exposed in the adulthood, except on Day 1 in Phase 1 (Day 1 to Day 5), the Treated rats made fewer attempts than the Control rats in sessions over the next four days. The animals of the highest dose group made the least number of attempts indicating that they spent comparatively less time in conflict. This implied that the Treated Groups, specially the highest dose (20 mg/kg) group was in lesser conflict and therefore had decreased anxiety levels than the Control Group.

But in Phase 2 (Day 260 to Day 264) the Treated animals particularly the animals of Group III generally made more attempts than Control Group animals to enter the open arms, that is, they spent more time in conflict and could decide less quickly than the Control Group animals to enter the open arms.

Though the difference was significant only on the first testing day of Phase 2, it provides an indication that the Group III animals had more conflict and therefore increased anxiety than the Control Group. But the variability in the first open arm latency by both the Control and Treated animals did not give
clear indication of either increased or decreased conflict and therefore increased or decreased anxiety.

There was variability in the number of open arm entries in the elevated plus-maze made by the animals of both the Control and Treated Groups in the gestationally exposed animals. The Treated Groups had higher number of open arm entries than the Control Group on PNDs 96, 98 and 99, and lower number of open arm entries on PND 100, while there was no difference on PND 97.

This feature was also reflected in the exploration of the open arms of the elevated plus-maze by the rats – measured by the percent of time the animals spent in the open arms. Increased exploration in the open arms is interpreted as a manifestation of decreased anxiety.

The Treated animals explored the open arms for higher percent of time than the Control Group initially on PND 96 as also on PNDs 98 and 99 indicating decreased anxiety, while on PND 100 the Control Group animals spent the higher percent of time in
the open arms. There was no difference on PND 97. For the most part, there was no definite indication of either increase or decrease in emotional reactivity. Adult male rats exposed in utero to diazepam spent significantly more time in the open arms than vehicle control animals indicating a decrease in the emotional reactivity in the gestationally exposed animals (Kellogg et al., 1991).

The absence of a definite indication of anxiety was correspondingly reflected in the number of closed arm entries made in the elevated plus-maze and the time spent in the closed arms by the animals of Treated Groups.

In case of animals that were administered Al in adulthood the Treated Groups generally made fewer open arm entries than the Control Group in Phase 1 (Day 1 to Day 5) of testing indicating increased anxiety. It must be noted here that the effect could be due to administration of Al. But variation was seen in the number of open arm entries made in Phase 2 (Day 260 to Day 264), though the Treated Groups, particularly the animals of Group III appeared to make more numbers of open arm entries
on Days 260 (significant), 261, 263 and 264 indicating decreased anxiety in the highest dose group.

This was correspondingly reflected in the number of closed arm entries made and the time spent in the closed arms of the elevated plus-maze by the animals of Treated Groups.

Head dipping is an index of risk assessment behaviour. Investigation over the edge of the open arm by the rat from an unprotected area of the open arm is termed as unprotected head dip. Increased investigations by the animal are indicative of decreased anxiety. In the gestationally exposed animals, the rats of all groups exhibited higher number of protected head dips than unprotected head dips, though the Treated animals generally had lower percentage of protected head dips than the Control animals. As in the measure of the amount of time spent in open arms, the Treated rats had a higher number of unprotected head dips initially on PND 96 as also on PNDs 98 and 99, while on PND 100 they had a lower number. There was no difference on PND 97. This variability again reflected neither increase nor decrease in emotional reactivity.
In case of male rats that were exposed to Al in adulthood, as in the gestationally exposed animals, the rats of all groups by large exhibited higher number of protected head dips than unprotected head dips in both Phase 1 (Day 1 to Day 5) and Phase 2 (Day 260 to Day 264).

As in the measure of the amount of time spent in the open arms of the elevated plus-maze, in Phase 1, the Treated Groups had lower number of unprotected head dips than the Control Group rats on all the five testing days indicating increased anxiety in the Treated animals. But in Phase 2 (Day 260 to Day 264), the Treated rats, the animals of Group I and Group III in particular, had more unprotected head dips than the Control animals indicating decreased anxiety. The lower number of unprotected head dips seen in Phase 1 is probably due to the general effects of intraperitoneal administration of aluminium rather than its toxicity.

Vertical activity (rearing) by a rat is indicative of exploration in a novel environment. Lower number of rears indicates increased anxiety. Though not significantly so, the rats of all the Treated
Groups in the gestationally exposed animals generally had a lower number of rearing on all testing days, with the animals of Group I having lower number of rears on PND 98, 99 and 100, and rats of Group II on PNDs 96, 97 and 100.

Therefore in contrast to indications of increased anxiety by other measures, the lower number of rears indicated decreased anxiety in the rats of Treated Groups in the gestationally exposed animals.

In the male rats which were administered Al in adulthood, in Phase 1 (Day 1 to Day 5) the rats of the Treated Groups made fewer rears than the Control Group animals indicating increased anxiety except Group II rats which had higher number of rears on Days 4 and 5.

In phase 2, only the animals of Group III made more rears than the Control Group on all five days indicating decreased anxiety in the highest 20 mg/Al/kg body wt dose group rats.
In the gestationally exposed rats, increased anxiety is also indicated by the increased number of freezes exhibited by the rats of the Treated Groups. Freezing is a defensive behaviour and is indicative of increased anxiety. Treated animals of Groups I and III exhibited significantly higher number of freezes on PNDs 97 and 98. Animals of Group III continued to show higher number of freezes on PNDs 99 and 100 although it was not statistically significant.

In the male rats exposed in adulthood, in Phase 1 (Day 1 to Day 5) all the Treated Group animals exhibited fewer freezes than the Control Group thereby indicating decreased anxiety. In contrast, in Phase 2 (Day 260 to 264) the Treated animals generally exhibited higher number of freezes indicating increased anxiety.

The interpretation of increased anxiety in the Treated rats in the gestationally exposed animals was also supported by the grooming behaviour in them. Significantly higher number of grooms is seen in rats of Group I and III on all the five testing days.
In the adult exposed animals in Phase 1 (Day 1 to Day 5) only rats of Group I exhibited higher number of groomings indicating increased anxiety. In Phase 2 (Day 260 to 264), Group I animals exhibited higher number of groomings as did the rats of Group II, again indicating increased anxiety.

The Morris water maze is a behavioural test procedure used to assess spatial memory and learning ability using negative reinforcement. Learning may be defined as 'an enduring change in the mechanisms of behaviour that results from experience with environmental events' or a 'relatively permanent change in an organism's potential for responding that results from prior experience or practice.'

In the present study, in the gestationally exposed animals, there was some variability in the escape latencies of rats of all the groups including the Control Group. It was generally observed that animals of both Control and Treated Groups could learn and remember the visible platform test of the Morris water maze quickly; by the 5th trial on PND 90 the animals had achieved
lower escape latencies than those achieved during habituation sessions and initial trials.

Peculiarly, it appeared that higher doses of A1 seemed to improve the performance of the rats in the Morris water maze – the rats in Group II and III generally achieved lower escape latency reflecting better learning ability than the Control Group rats on the five testing days – PND 90 to PND 95, while it appeared that the animals of Group I had longer escape latencies.

In the no cue probe in Trial 4 on PND 95, the results showed that the rats – both Control and Treated, exhibited lengthened escape latency when no visible cue marked the escape platform suggesting that the rats did not develop any alternative exploratory strategy and based their platform search on the visible cue during all the trials throughout the testing period.

The rats had to necessarily base their platform search on the visible cue due to change in the starting position and the position of the escape platform at each trial. Again it appeared
that the Group I animals took longer time to reach the escape platform.

As in the gestationally exposed groups, the male rats exposed to Al in adulthood also exhibited variation in performance in the Morris water maze. It was observed that Al did not seem to affect the escape latencies – both the Control and the Treated rats achieved comparable escape latencies. As in the gestationally exposed animals, the animals of both Control and Treated Groups could learn and remember the visible platform test of the Morris water maze quickly; by the 5th trial on Day 2 of the acquisition phase the animals had achieved lower escape latencies than those achieved during habituation sessions and initial trials on Day 1 and Day 2. Their performance remained unchanged at the initial stages in the retention trails.

But in the later stages (Days 49, 70 and 260) of the retention phase, the animals of the three Treated groups took longer time to reach the escape platform than the Control Group animals. Significantly longer time was taken to reach the escape platform by the animals of Groups I and II on Day 49, and the Group I
animals on Day 70 of the retention phase. It is possible that the effects of aluminium on the memory processes are not immediately manifested.

In the three no cue probes on Days 7, 75 and 265, the Treated Groups had longer escape latencies than the Control Group rats (significantly longer in Trial 4 on Day 7) when no visible cue marked the escape platform, suggesting the possibility of development of an alternative strategy by the Control rats and the lack of same in the Treated rats: they did not seem to develop any alternative exploratory strategy and presumably based their platform search on the visible cue.

No reports were found on the assessment of effect of prenatal Al administration in rats or mice on the performance of in the Morris water maze. Contrary to expectations, in the gestationally exposed group in the present study, the Treated animals especially the rats in Group II and III generally achieved lower escape latency reflecting better learning ability than the Control Group rats. Similar results were reported by Golub et al., (2000) wherein mice fed on high aluminum diets (1000 µg
Al/g diet) throughout their life span showed enhanced performance on the Morris water maze. Connor et al., (1988) suggested that addition of 0.3% Al to the diet of 120-130 g rats for one month induced facilitation of learning in a passive conditioned avoidance response (CAR) task thus implying that Al enhanced learning. But the working memory, as assessed on one way active CAR and 8-arm radial maze were not effected by Al exposure.

Notably in the present study, the escape latencies in the rats of both the Control and Treated Groups did not change significantly even between trials separated by intervals of one, two and three weeks, and remarkably, by interval of 27 weeks, showing excellent long-term memory in the animals.

Long-term reference memory is thought to be hippocampal independent phenomenon (Squire, 1992). The non-spatial visible platform task is also thought to be a hippocampus-independent learning task. (Grant et al., 1992; Silva et al., 1992a; Silva et al., 1992b;). The fact that the escape latencies in the non-spatial visible platform task did not change even after
long intervals suggests that exposure to Al did not alter the non-hippocampal functions in the rats.

The hippocampus plays a role in a number of adaptive phenomena associated with gathering of information about the environment – in exploration of novelty and learning multiple relationships of cues: it is thought to play a role in forming a spatial map of the environment. (O'Keefe and Nadel, 1978). The activity of hippocampal place cells remains remarkably specific and stable even when a large number of environmental (spatial) cues are removed, (O'Keefe and Speakman, 1978) suggesting that the hippocampus does not simply rely on the input of cues but uses an internal map, probably stored in higher cortical areas (Squire, 1992).

Accumulation of Al in hippocampus region of brain may therefore cause behavioural alterations.

Protein phosphorylation is a key cellular process in cell signaling. Protein phosphorylation can control and regulate
complex functions of the mammalian central nervous system (CNS) by altering the function of the target proteins. Al intoxication has been shown to result in altered protein phosphorylation (Wisniewski et al., 1980; Leterrier et al., 1992).

Several neurotoxicants exert their effects on the CNS by regulating the intracellular concentrations of specific protein kinases and/or phosphatases.

Al is known to inhibit protein kinase C (PKC) activity (Katsuyama et al., 1989; Cochran et al., 1990).

Protein kinase C (PKC), an enzyme, is present in the brain in high concentrations (Costa, 1990; Chuang, 1989; Fowler and Tiger, 1991; Fisher et al., 1992). It has been involved in the release of several neurotransmitters, an effect strongly correlated with phosphorylation of a synaptic protein known as B-50 or GAP43 (Dekker et al., 1989). This action may be linked to the role of PKC in the maintenance of long-term potentiation (a possible functional equivalent of memory storage), suggesting a
role for this enzyme in the process of memory (Chiarugi et al., 1989).

It is possible that Al exerts its effects by inhibition of PKC activity. Blocking of long-term potentiation could cause learning and memory deficits. Disruption in the release of neurotransmitters by the inhibition of PKC could also affect the emotionality in the animal causing alterations in its anxiety levels. Further investigations are required in this area before definite conclusions can be drawn on the subject.