Psychomotor functions at various weeks of chronic renal failure in rats

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Abstract In chronic renal failure there is a gradual retention of substances in the tissues and body fluids, called as uremic retention toxins, which can bring about a number of biochemical activities in the body. Chronic renal insufficiency also leads to progressive behavioural conflict. Uremic toxins can affect both the central and the peripheral nervous system. Uremic encephalopathy is also associated with problems in cognition and memory. To study the psychomotor functional disorders in rats with progressive chronic renal failure surgical nephrectomy was done by resection method. The animals were grouped into two control groups, Sham control (SC) and normal control (NC) and two uremic groups, moderate uremia (G_M) and severe uremia (G_S). Psychomotor analysis was done by passive avoidance and open field in these animals at 4, 8, 12, and 16 weeks. After the incubation period, the nephrectomised groups (G_M and G_S) showed significant changes in exploratory, locomotor and emotional behaviour when compared to the controls (NC and SC). Psychomotor changes involve poor cognition, reduced memory, reduced

locomotor activity and decreased exploratory drive and emotional disturbance like increased fear during the initial stages. During the later stages a restless behaviour was noticed, associated with diminished fear.

Keywords Renal insufficiency · Uremic toxins · Nephrectomy · Psychomotor

Introduction

In chronic renal failure there is a gradual retention of substances in the tissues and body fluids, termed uremic retention toxins, which can bring about a number of biochemical activities in the body. Chronic renal failure results in profound biochemical disturbances affecting numerous organs and regulatory systems, including the functioning of the central nervous system (CNS) which can lead to progressive behavioural conflict. Uremic toxins can affect both the central and the peripheral nervous system. Uremic encephalopathy is also associated with problems in cognition and memory (Horl 1998; Brouns and De Deyn 2004).

Patients with uremic encephalopathy manifest disorientation, somnolence, asterixis and coma (Jeppson et al. 1982). Speech disorders, memory failure, myoclonic jerks, flapping tremor, restless legs, paresthesia and difficulties in walking are other serious features of chronic renal insufficiency (Yatzidis et al. 1984).

High rates of cognitive impairment and dementia have been reported in many studies of patients with end stage renal disease (Murray et al. 2006; Umans and Pliskin 1998). Several studies have reported an association between both dementia and cognitive impairment and moderate kidney dysfunction (Kurella et al. 2005a, b).

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Elderly individuals with elevated serum creatinine (indicating renal failure) were found to have an increased risk for dementia (Seliger et al. 2004).

Dementia and impaired memory are common complications in uremic patients. Further cognitive impairment, manifesting typically as vascular dementia, develops in a considerable proportion of patients on dialysis and improves with renal transplantation (Krishnan and Kiernan 2009).

Literature linking alterations in the nervous system functions in animal models of uremia were lacking. The present study was taken up to develop a similar condition in an experimental set-up and to develop similar psychomotor changes as seen in chronic renal failure.

Objective To study the psychomotor functional disorders in rats with chronic renal failure

Methodology Wistar Albino rats weighing 200–250 g, aged 3 months were used ($n = 10$ in each group). The study groups were gender matched with equal distributions of both genders in all groups. All rats were inbred and maintained in normal laboratory conditions such as 12 h dark and 12 h light cycle, in an air conditioned room ($24 \pm 1^\circ\text{C}$) and provided with standard rodent pellet diet and water ad libitum. Three rats were maintained in each cage to minimize the stress caused by overcrowding except post nephrectomy (isolated for 2 weeks, until the wound was healed). Rats were housed in polypropylene cages, ($22.5 \times 35.5 \times 15$ cm). All procedures for the treatment of animals were approved by the research committee of the institute and were done in accordance with the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health.

Develop experimental uremia in rats by a surgical procedure

Surgical nephrectomy (Iype et al. 2008)

Rats were anesthetized intraperitoneally with pentobarbitone sodium (40 milligrams per kilogram body weight). Under aseptic conditions a midline incision of the skin was made. Two models of uremia were created namely (1) moderate uremia (Group G_M) (2) severe uremia (Group G_S)

Moderate uremia

The lower pole of the left kidney was decapsulated. A clamp was placed diagonally along the lower pole of the left kidney and an area of one third of the total, distal to the clamp was resected. The clamp was left in place for 5 min

Fig. 1 Moderate nephrectomy: the dark areas in the figure represent resected renal tissue

Fig. 2 Severe nephrectomy: the dark areas in the figure represent resected renal tissue

to avoid bleeding. After 5 min the clamp was slowly released taking care to avoid bleeding. The right kidney was approached and the hilar region was lifted. A sterile suture was passed below the hilar region and lifted in order to obstruct the blood flow and tied. The kidney was placed back in the same position. This produced total nephrectomy on the right side. The animal was left with 2/3 of the normal functioning left kidney (Fig. 1).

Severe uremia

Total nephrectomy was done on the right side as described above. The lower pole of the left kidney was resected in the similar way as described under 'moderate nephrectomy'. After resecting the lower pole, a clamp was placed vertically and another one third of the total area was also resected as shown in the figure (Fig. 2). Then the animal was left with 1/3 of the left kidney functioning. The clamp was slowly released and the kidney was replaced back in its position.

Sham control (SC)

Sham operated animals served as additional controls. In these animals, a midline incision was done similar to those

Fig. 3 Study design and groups

in the experimental groups and one of the kidneys was handled and placed back in its position.

The degree of uremia (either moderate or severe) was standardized by serial estimations of serum urea and creatinine in each group.

Study groups

The number of animals in each group is 10 ($n = 10$).

Normal control (NC), Sham control (SC), Group moderate 1 (GM1, 2, 3 & 4) The animals underwent moderate nephrectomy and were incubated for 4, 8, 12 and 16 weeks. **Group severe 1 (GS1, 2, 3 & 4)** The animals underwent severe nephrectomy and were incubated for 4, 8, 12 and 16 weeks in this Group (Fig. 3).

Psychomotor analysis

The following psychomotor tests were done (a) Passive avoidance test (b) Open field tests.

Passive avoidance test

The passive avoidance apparatus was done by the method of Bures et al. 1983 (Fig. 4).

Procedure The experiment included three parts, (1) an exploration test, (2) an aversive stimulation and learning phase (passive avoidance acquisition), and (3) a retention test (Fig. 5).

During *exploration* the rat was kept in the centre of the larger compartment facing away from the entrance to the dark smaller compartment. The door between the two compartments was kept open. The rat was allowed to explore the apparatus (both larger and smaller compartments) for 3 min. In each trial, (1) the total time spent by the animal in the larger compartment and (2) the total time spent by the animal in the smaller compartment were noted. At the end of the trial, the rat was replaced in the home cage where it remained during an inter-trial interval of 5 min. This was repeated three times for each rat. The inter-trial interval was 5 min.

Fig. 4 Passive avoidance

After the last exploration trial, the rat was forced into the smaller compartment and the sliding door between the two compartments of the apparatus was closed. Three strong foot shocks (50 Hz, 1.5 mA, 1 s duration) were given at approximately 5-s intervals. The top cover was then opened and the rat was returned to its home cage.

Retention test was carried out 24 h after the acquisition test. The rat was kept in the centre of the larger compartment facing away from the entrance to the smaller compartment. The sliding door between the two compartments was kept open. The rat was allowed to explore the apparatus for 3 min. After 3 min the rat was returned to the home cage. This was repeated three times with an inter-trial interval of 5 min. In each trial the total time spent by the animal in the smaller compartment were noted.

Open field test

This assesses emotional and motor functions. The open field test was done by the method of Bures et al. 1983 (Fig. 6).

Fig. 6 Open field

Experimental procedure The rat was placed in the corner of the apparatus and its behavior was observed for 5 min. Whenever the animal entered a new square with both the forelegs, an event was recorded. Entering the 16 peripheral squares (outer squares) close to the wall was recorded separately from entering the central 9 squares (inner squares). Separate counts of outer and inner squares entered were noted.

The animal's behavior was quantified by noting the total number of crossings (locomotor activity), rearings (standing on hind legs either in the middle of the open field or sometimes leaning with forelegs on the wall) and grooming (to assess the exploratory behaviour) and counting the number of fecal pellets excreted (emotional activity) during this 5-min session. The test was repeated at the same hour on four subsequent days.

The following parameters were studied in the open field test:

- (i) For testing locomotor activity—number of crossings in the peripheral field

Fig. 5 Passive avoidance in a two compartment apparatus. E, exploration; L, learning; R, retention testing (Figure from: Bures et al. 1983)

- (ii) For testing exploratory behavior—number of rearing, number of grooming
- (iii) For testing emotional activity—number of fecal pellets excreted

Statistical analysis

Results were expressed as mean $\pm$ SD. A $p < 0.05$ was considered statistically significant. The results were analyzed by the one way ANOVA as well as repeated measure ANOVA. When the overall difference was significant, post hoc test (bonferroni) was used to find out the significance between and within groups. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 20, Chicago, USA).

Results

Results of body weight

An increase in body weights were seen in all groups including the controls (Table 1).

Results of urea and creatinine

One way ANOVA was found to be significant. As the uremia progressed there was a significant increase in the levels of urea in both G_M and G_S group when compared to the controls in almost all weeks. Creatinine levels showed a significant increase only from the 12th week when compared to the controls groups (Table 2).

Results of passive avoidance test

Exploration

Exploratory behavior was studied by comparing time spent in seconds in dark (small) compartment before and after incubation period following surgical nephrectomy. A significant difference in time spent was observed before and after incubation period in all groups including the controls. After the incubation period, the nephrectomised groups

(G_M and G_S) showed a significantly increased time spent compared to the controls (NC and SC) only in the 4th week group, but not in the other (8, 12 and 16-week) groups. In addition a significant increase in the time spent was observed after incubation in all groups except the 4 week control group. In the 4 week control groups a significant decrease was observed (Table 3).

Retention

The retention was studied by comparing time spent in seconds in dark (or small) compartment before and after nephrectomy. There was a decrease in the time spent by the control animals (both NC and SC) after the incubation period compared to before, at all weeks except at the 16 week. But none of them were statistically significant. In G_M group, there was an increase in time spent at 4th week of uremia, but it was not statistically significant when compared to the same before uremia. In the 8th, 12th and the 16th week groups there was an increase in the duration of time spent in the small compartment and this was statistically significant when compared to before the development of uremia and with NC ($p < 0.001$) as well as with SC ($p < 0.001$). In G_S group, there was an increase in time spent in the small compartment at all weeks of uremia and the values were statistically significant when compared to before the development of uremia ($p < 0.001$). The time spent in small compartment in these groups was also highly significant when compared with NC ($p < 0.001$) and SC ($p < 0.001$). In addition to the above, there was a significant increase in the duration of retention in G_S when compared to G_M group at 4 weeks of uremia ($p < 0.001$) (Table 4).

Results of open field test

The locomotor, exploratory and emotional behavioral activities were quantified by the following parameters: (1) number of crossings in peripheral squares (2) number of rearing (3) number of grooming (4) number of fecal pellets (defecation reflex) (Table 3).

Normally as soon as the animal was placed, it started moving along the walls. Most of initial exploration was limited to outer squares. The exploration was maximal in

Table 1 Body weight in grams at various weeks of uremia in adult male Wistar rats

Group	Weight				
	0	4	8	12	16
NC	232.2 $\pm$ 8.4	238.4 $\pm$ 8.9	256.4 $\pm$ 9.6	268.3 $\pm$ 9.9	281.8 $\pm$ 7.6
SC	229.5 $\pm$ 12.3	236.9 $\pm$ 8.1	246.4 $\pm$ 8.3	253.4 $\pm$ 7.5	259.3 $\pm$ 7.7
G_M	226.6 $\pm$ 10.6	242.6 $\pm$ 10.6	261.4 $\pm$ 13.9	268.4 $\pm$ 13.9	279.6 $\pm$ 11.8
G_S	230.6 $\pm$ 10.8	244.9 $\pm$ 13.3	263.9 $\pm$ 13.3	281.6 $\pm$ 7.8	294.1 $\pm$ 10.2

Results expressed as mean $\pm$ SD

Table 2 Serum urea (mg/dl) levels and creatinine levels (mg/dl) at various weeks of uremia in adult male Wistar rats

Group	Urea					Creatinine				
	0	4	8	12	16	0	4	8	12	16
NC	39.6 ± 6.3	39.6 ± 5.4	41.7 ± 7.5	41.1 ± 7.5	43.9 ± 8.6	0.4 ± 5.68E – 02	0.56 ± 0.17	0.69 ± 0.17	0.78 ± 0.1	0.8 ± 6.67E – 02
SC	42.5 ± 3.1	49.5 ± 6.9	49.8 ± 10.8	40.7 ± 6.8	39.6 ± 4.9	0.4 ± 4.22E – 02	0.5 ± 9.43E – 02	0.76 ± 0.16	0.61 ± 0.15	0.62 ± 0.14
G _M	39.5 ± 5.9	69.1 ± 10.1 ^c	62.7 ± 8.0	70.3 ± 7.8 ^{be}	73.2 ± 10.5 ^{bf}	0.5 ± 6.33E – 02	0.57 ± 0.17	0.85 ± 0.15	1.02 ± 7.89E – 02 ^{af}	1.01 ± 0.11 ^f
G _S	42.4 ± 2.4	90 ± 21.5 ^{cf}	79.1 ± 20.3 ^{ce}	91.4 ± 13.4 ^{cf}	94.8 ± 7.1 ^{cf}	0.5 ± 0.11	0.66 ± 0.15	0.9 ± 0.19	1.07 ± 0.2 ^{bf}	1.2 ± 0.15 ^{cf}

Results expressed as mean ± SD

Statistical significance of results (ANOVA): ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ when compared with NC and ^d $p < 0.05$, ^e $p < 0.01$, ^f $p < 0.001$ when compared with SC

the first few minutes and then gradually decreased. General exploratory behavior decreased on subsequent days, but visits to the inner squares became more frequent. At the same time, defecation rate was reduced. Correlation between the various parameters such as total number of crossings, the number of inner and outer squares visited, rearings, grooming and fecal pellets reflect the motor and emotional behavior of the animal (Table 5).

Number of peripheral crossings

The total number of peripheral squares crossed in 5-min session of observation was noted. In the 4-week nephrectomised rats, the peripheral crossings were significantly reduced in both G_M and G_S groups when compared to before nephrectomy ($p < 0.001$). The reduction in peripheral crossing was also seen between G_M (after) and G_S (after) with the NC (after) ($p < 0.001$) and SC (after) ($p < 0.001$). The reduction was more significant in the G_S group compared to the G_M group ($p < 0.001$). In the 8th week, there was a significant reduction ($p < 0.001$) before and after the incubation period in all groups including the controls. In addition, there was also a significant reduction in the number of peripheral crossings in the G_S (after) group when compared to NC, SC and G_M ($p < 0.001$), after groups. In the 12th week there was a significant reduction in the number of crossings before and after the incubation period in all groups including the controls ($p < 0.001$). In the group G_S there was significant reduction in the number of peripheral crossings when compared to NC and SC ($p < 0.01$) but not with G_M, after groups. In the 16th week there was a significant reduction in the number of crossings before and after the incubation period ($p < 0.001$).

Number of rearing

The total number of rearings during a 5-min session of observation was noted. In the 4 week group there was no significant difference before and after in NC and SC group, but in the G_M ($p < 0.05$) and G_S ($p < 0.001$) groups there was a significant decrease in the number of rearings after 4 weeks of nephrectomy. After nephrectomy a significant decrease in rearing was observed in G_M and G_S when compared to NC ($p < 0.001$) and SC ($p < 0.001$). In the 8 week group there was a decrease in the rearing in all groups except G_M which was not statistically significant, but in group G_M there was a significant increase in the rearing when compared to before nephrectomy ($p < 0.01$) and with NC ($p < 0.01$), SC ($p < 0.001$) and G_S ($p < 0.001$) after groups. In 12 week group there was a significant increase ($p < 0.001$) in the number of rearing after the incubation period in NC and SC group, but not in

Table 3 Time spent (seconds) in small compartment during exploration in the passive avoidance test in adult male Wistar rats

Week	NC		SC		G _M		G _S	
	Before	After	Before	After	Before	After	Before	After
4	379.6 ± 24.6	306.8 ± 26.6 ^h	392.8 ± 35.2	332.4 ± 66.0 ^g	382.0 ± 43.7	511.9 ± 15.5 ^{icf}	377.4 ± 30.1	512.1 ± 11.1 ^{icf}
8	369.9 ± 31.3	510.1 ± 17.5 ⁱ	385.4 ± 20.5	514.0 ± 24.3 ⁱ	371.4 ± 24.1	510.2 ± 13.0 ⁱ	372.0 ± 28.1	516.3 ± 10.3 ⁱ
12	374.7 ± 31.6	496.4 ± 60.8 ⁱ	373.0 ± 38.4	516.3 ± 15.4 ⁱ	378.4 ± 35.7	505.9 ± 20.0 ⁱ	374.4 ± 30.4	509.9 ± 19.2 ⁱ
16	382.7 ± 46.4	510.6 ± 25.5 ⁱ	363.5 ± 44.5	507.1 ± 17.1 ⁱ	364.8 ± 26.7	501.9 ± 27.1 ⁱ	340.1 ± 100.0	521.0 ± 10.7 ⁱ

Results expressed as mean ± SD

Statistical significance of results (ANOVA): ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ when compared with NC

^d $p < 0.05$, ^e $p < 0.01$, ^f $p < 0.001$ when compared with SC and ^g $p < 0.05$, ^h $p < 0.01$, ⁱ $p < 0.001$ when compared before and after the nephrectomy

Table 4 Time spent (seconds) in small compartment during retention in the passive avoidance test in adult male Wistar rats

Week	NC		SC		G _M		G _S	
	Before	After	Before	After	Before	After	Before	After
4	72.0 ± 80.9	11.5 ± 15.7	55.7 ± 63.1	16.4 ± 14.5	40.6 ± 44.9	66.2 ± 29.8	48.4 ± 42.5	341.6 ± 200.4 ^{icf}
8	53.5 ± 46.8	24.0 ± 39.7	37.7 ± 36.9	9.3 ± 7.7	46.2 ± 27.0	508.8 ± 39.7 ^{icf}	55.9 ± 44.5	530.2 ± 7.2 ^{icf}
12	38.4 ± 41.1	23.4 ± 28.2	38.7 ± 42.5	34.9 ± 35.9	30.7 ± 38.1	494.9 ± 33.4 ^{icf}	42.3 ± 39.6	465.0 ± 132.5 ^{icf}
16	48.8 ± 41.7	62.2 ± 40.8	28.2 ± 35.7	47.2 ± 32.5	44.8 ± 39.1	511.9 ± 33.7 ^{icf}	47.4 ± 35.8	521.4 ± 15.0 ^{icf}

Results expressed as mean ± SD

Statistical significance of results (ANOVA): ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ when compared with NC ^d $p < 0.05$, ^e $p < 0.01$, ^f $p < 0.001$ when compared with SC ^g $p < 0.05$, ^h $p < 0.01$, ⁱ $p < 0.001$ when compared before and after the nephrectomy

G_M and G_S groups. When compared with NC and SC there was a significant decrease in the number of rearings in the G_M ($p < 0.001$, $p < 0.01$) and G_S ($p < 0.01$) after groups. In the 16 week group there was a significant increase in the number of rearings in all groups including the controls (NC $p < 0.01$, SC, G_M and G_S $p < 0.001$). After 16 weeks of nephrectomy, in group G_S ($p < 0.001$) there was a significant increase in the number of rearings when compared to the controls (NC and SC).

Number of groomings

The total number of groomings during a 5-min session of observation was noted. In the 4th week group there was no difference in the grooming behavior before and after the incubation period in both the controls. In the nephrectomised groups there was a significant decrease in the grooming when compared to before the nephrectomy (G_M $p < 0.01$ and G_S $p < 0.001$) as well as with NC and SC ($p < 0.001$) after groups. In the 8th week group there was a significant increase in the grooming in all the groups after the incubation ($p < 0.001$) of 8 weeks following nephrectomy. In the 16th week group there was a significant increase in the grooming in the nephrectomised groups (G_M and G_S) when compared to before the nephrectomy ($p < 0.001$). In the after groups, G_M showed a significant

increase in the grooming when compared to NC ($p < 0.01$), but not with SC. And G_S showed a significant increase in grooming when compared to both the controls, NC ($p < 0.001$) and SC ($p < 0.01$).

Number of fecal pellets (defecation reflex)

The total number of fecal pellets during a 5-min session of observation was noted. At 4 weeks of uremia the defecation scores were significantly increased in both the controls when compared to before the incubation period (NC, $p < 0.01$ and SC, $p < 0.001$), but not in the nephrectomised groups. In the nephrectomised groups after 4 weeks a significant decrease in fecal pellets were observed when compared to the controls ($p < 0.001$).

Discussion

Psychomotor functions

It is well known that many of the patients with uremia suffer from impaired functioning of the central nervous system (Brouns and De Deyn 2004; Burn and Bates 1998). The clinical manifestation and pathophysiology of uremic encephalopathy is poorly understood. Signs of uremic

Table 5 Number of crossings, rearings, grooming and fecal pellets at various weeks of uremia in adult male Wistar rats

Week	NC		SC		G _M		G _S	
	Before	After	Before	After	Before	After	Before	After
<i>Crossing in peripheral squares</i>								
4	90.8 ± 5.2	88.2 ± 8.9	84.1 ± 8.9	80.5 ± 5.9	81.9 ± 6.9	41.7 ± 5.2 ^{ic f}	84.7 ± 4.9	13.5 ± 3.9 ^{ic f}
8	88.9 ± 10.1	44.7 ± 16.0 ⁱ	89.3 ± 6.1	42.3 ± 18.3 ⁱ	89.8 ± 7.0	48.2 ± 17.3 ⁱ	85.5 ± 13.1	21.5 ± 14.2 ^{icf}
12	89.9 ± 4.9	51.3 ± 4.2 ⁱ	88.6 ± 4.2	50.9 ± 5.9 ⁱ	89.4 ± 4.8	44.4 ± 7.9 ⁱ	87.9 ± 6.2	33.9 ± 6.9 ^{ibe}
16	92.7 ± 7.0	57.2 ± 6.4 ⁱ	92.7 ± 5.1	58.7 ± 6.3 ⁱ	96.1 ± 5.7	47.9 ± 11.0 ⁱ	95.7 ± 7.3	47.9 ± 7.7 ⁱ
<i>Rearing</i>								
4	12.3 ± 2.3	14.5 ± 1.5	10.4 ± 4.3	12.3 ± 2.3	9.6 ± 3.6	5.6 ± 1.0 ^{gcf}	10.1 ± 2.2	4.0 ± 0.7 ^{icf}
8	9.1 ± 2.6	8.5 ± 3.6	9.7 ± 1.4	7.2 ± 2.3	8.6 ± 1.6	13.4 ± 4.4 ^{hbf}	8.4 ± 2.0	5.9 ± 2.1
12	6.6 ± 1.5	13.4 ± 3.4 ⁱ	7.4 ± 1.8	12.9 ± 1.7 ⁱ	7.2 ± 2.1	8.4 ± 2.9 ^{ce}	7.0 ± 1.8	8.4 ± 1.8 ^{be}
16	6.7 ± 1.7	10.6 ± 1.6 ^h	6.7 ± 1.7	11.0 ± 2.0 ⁱ	7.0 ± 2.8	14.0 ± 1.7 ⁱ	7.2 ± 1.8	17.5 ± 3.2 ^{icf}
<i>Grooming</i>								
4	9.0 ± 3.0	8.2 ± 1.3	8.1 ± 1.5	10.6 ± 1.5	7.2 ± 1.7	3.4 ± 1.5 ^{hcf}	7.8 ± 2.2	3.4 ± 1.1 ^{icf}
8	5.9 ± 1.0	12.4 ± 3.9 ⁱ	5.5 ± 1.4	11.4 ± 2.5 ⁱ	5.6 ± 1.4	13.3 ± 5.8 ⁱ	5.2 ± 0.9	10.8 ± 4.2 ⁱ
12	7.0 ± 2.0	6.6 ± 1.7	6.1 ± 0.9	6.7 ± 1.3	5.4 ± 0.9	6.4 ± 1.1	5.7 ± 1.2	6.5 ± 1.2
16	7.8 ± 1.9	7.7 ± 1.7	7.2 ± 2.1	8.3 ± 1.8	6.6 ± 2.2	11.5 ± 2.6 ^{ib}	6.5 ± 1.0	12.5 ± 1.9 ^{ice}
<i>Fecal pellets</i>								
4	4.0 ± 1.1	6.2 ± 1.3 ^h	3.3 ± 1.1	6.0 ± 1.2 ⁱ	3.6 ± 1.1	3.3 ± 0.9 ^{cf}	3.8 ± 0.9	2.3 ± 0.8 ^{cf}
8	3.4 ± 1.1	2.9 ± 1.4	2.7 ± 0.9	1.8 ± 0.7	2.3 ± 1.3	3.0 ± 1.3	2.6 ± 0.6	5.3 ± 1.8 ^{icf}
12	3.3 ± 1.1	2.0 ± 0.7	3.1 ± 1.4	2.1 ± 1.3	3.0 ± 1.3	4.0 ± 1.1 ^{ad}	2.4 ± 1.0	4.5 ± 1.0 ^{hcf}
16	3.7 ± 0.9	1.9 ± 1.3 ^g	3.8 ± 1.3	1.8 ± 0.4 ⁱ	3.4 ± 1.3	5.9 ± 1.0 ^{icf}	3.4 ± 0.7	6.6 ± 1.4 ^{icf}

Results expressed as mean ± SD

Statistical significance of results (ANOVA): ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ when compared with NC, ^d $p < 0.05$, ^e $p < 0.01$, ^f $p < 0.001$ when compared with SC, and ^g $p < 0.05$, ^h $p < 0.01$, ⁱ $p < 0.001$ when compared before and after the incubation period following nephrectomy

encephalopathy include disturbance in attention, cognition and emotionality, behavioural alteration, confusion and disorientation. No specific reason is attributable for all these signs (Burn and Bates 1998). In animals, psychomotor tests such as passive avoidance and open field test in animals provide good tools for studying these dysfunctions.

Exploration behaviour

In our study on passive avoidance, the exploratory behaviour reduced after the development of uremia in all groups. It was noticed that the locomotor activity is decreased in the initial stage of the disease which was similar to that reported by Raskin & Fishman (1976); they reported that CNS changes are more in the acute than in the chronic phase of the disease. In our study the initial stage could be compared to the acute phase which occurs at the onset of uremia or soon after nephrectomy. The changes in the initial stages could be due to the sudden disturbances caused by the nephrectomy. Later in the course of the disease, the exploratory activity was also found to be reduced and the animals immediately entered the dark compartment in all groups. This can be due to the ageing of the animals. We also found an increase in the body weight

in all groups. This could have reduced exploratory behaviour in the later weeks. Banchaabouchi et al. (1999) also reported a decrease in ambulation of mildly uremic mice for similar reasons.

Memory retention and cognition

We have observed a significant increase in the time spent by the animal in the dark compartment in all experimental groups following nephrectomy. It was also noticed that during the initial period after nephrectomy, the increase in retention time was less, compared to the later weeks after nephrectomy. This indicates that during the initial period of uremia, memory was unlikely to be affected, but as the condition progressed, we found that the memory deterioration was severe. The uremia is known to cause disturbances in neural pathways which control learning and short term memory. It could be that some of the uremic toxins may be affecting these pathways at various levels, like perception of impulse, integration of impulse and response behaviour (Fraser and Arief 1988). It could also be due to the interference of uremic toxins with the neurotransmitter involved in learning and memory. It is worthy to note that an optimum concentration of uremic toxin is required to

disturb these neural pathways. Our study indicates that this optimum concentration was attained in less than 8 weeks in moderate nephrectomy group and much earlier in severely nephrectomised rats. Retention was significantly impaired from 8 weeks until 16 weeks post nephrectomy in moderately uremic rats whereas, from 4 weeks onwards in severely uremic rats.

The memory of emotional events is modulated by neuronal activity in the amygdala (McGaugh et al. 1993; 1995). The strength of retention, in turn, is regulated by a variety of neurotransmitters, including norepinephrine (NE) and GABA, which influence the amygdala. NE agonists infused directly into the amygdala immediately after training enhanced retention (Liang et al. 1986, 1990; Ferry and McGaugh 1999; Ferry et al. 1999). GABA agonists infused directly into the amygdala immediately after training impaired retention (Castellano et al. 1989; Wilensky et al. 2000). Amygdala is reported to play a crucial role, preferentially in avoidance learning than spatial learning (Takashina et al. 1995) and is also involved with the formation of enhanced declarative memory for emotionally arousing events (Adolphs et al. 1997; Cahill and McGaugh 1998; Hamann et al. 1997). Declarative memory also depends on signals from the hippocampus and other parts of the medial temporal lobe of the brain for its retention. Neuropsychological studies have found loss of memory and defects in cognition as silent features of renal failure in patients (Hailpern et al. 2007). Another explanation for the association of the kidney with cognitive dysfunction is the accumulation of retention solutes. A study of patients who were on nocturnal hemodialysis revealed improvements in cognitive function after initiation of nocturnal dialysis (and thus removal of retention solutes), suggesting a reversible cause to the impaired cognitive function (Jassal et al. 2006). In our study, since the retention solutes are not being removed from the body of the rats, the loss of memory continued to progress as the uremia sustained. It may be hypothesised that the uremic toxins could have influenced functioning of amygdala or its connecting neural pathways in chronic renal failure. Further research needs to be conducted to make assertive conclusions in this regard.

Locomotor, exploratory and emotional activity

The open field test is a classical behavioural test which has been widely used to study the ‘emotionality’ of laboratory mice and rats. We have observed a decrease in number of crossings in the initial stage of uremia, but as uremia progressed, the crossings in the peripheral squares were almost similar to those observed in the normal controls, both in the moderate and severe nephrectomy groups. It was also evident that the animals were found to occupy the

corner squares for a longer time, especially in the severely nephrectomised group. The decrease in locomotor and exploratory activity was also observed by Topczewska et al. (2001) after evaluation for 8 weeks following nephrectomy. We have observed similar results at 8 weeks post nephrectomy, but the locomotor activity improved after 12 weeks until 16 weeks post nephrectomy. These changes were also associated with a high emotional reactivity in the initial stages of post nephrectomy. Our animals showed thigmotaxis and the exploratory drive was less in the initial stages of the disease which indicates that the animals were less anxious and more fearful (Gould et al. 2009). The lesser time spent in the periphery and number of fecal pellets can be interpreted with emotionality (Gould et al. 2009). Initially, the animals were found to sit only in the corner peripheral squares, continuously grooming. Fecal boli were also large in the initial stage of severe nephrectomy. Initially, a decrease in exploratory activity and increased fear were observed in the experimental animals. As the uremia progressed, increase in exploratory activity and decreased fear were noticed.

The study of grooming has become the subject of extensive research, primarily because of its usefulness in modelling hierarchical motor control (Fentress 1988). In rodents, grooming is a complex ritual, which normally proceeds in a cephalocaudal direction and consists of several stages, including licking the paws, washing movements over the head, fur licking, and tail/genitals cleaning (Berridge and Aldridge 2000a, b; Eguibar and Moyaho 1997).

In our study, grooming in the nephrectomised groups lasted for a long duration and sometimes even for a minute. Many neuromediators and hormones as well as multiple regions in the brain appear to be involved in the regulation of grooming behaviour (Bolivar et al. 1996; Bressers et al. 1998; Cromwell et al. 1998; Van Erp et al. 1995). The sequential coordination of grooming depends on dopamine neurotransmission in basal ganglia circuits, as the pattern is disrupted by dopaminergic nigrostriatal lesions (Berridge 1989), and the pattern is made more rigid by the transgenic elevation of neostriatal extracellular dopamine (Berridge et al. 2005). The dopamine level in renal failure was reported to be low and therefore, the mechanism of grooming appears to be much more complex in these animals (Pestana et al. 1988). Future studies focussing on the role of dopamine on grooming could throw more light on this mechanism.

Many studies have shown a progressive decline in cognitive functions with progressive renal failure (Kurella et al. 2005b; Davey et al. 2013). Emotional changes, anxiety and depression have also been reported in cases of chronic renal failure (Montinaro et al. 2010). Our study is consistent with these findings and hence our model can be

used as an excellent tool to understand the pathophysiology and progression of such changes seen in chronic renal failure. Further experimental measures can be instituted to ameliorate the psychomotor and cognitive changes in relation to human beings. These experiments will be useful as chronic renal failure is an ever increasing problem and hemodialysis has become the norm of treatment.

In conclusion psychomotor changes involve poor cognition, reduced memory, reduced locomotor activity and decreased exploratory drive and emotional disturbance like increased fear during the initial stages of uremia (up to 8 weeks). During the later stages a restless behaviour was noticed which was associated with diminished fear which is consistent with changes seen in chronic renal failure. As this study was done on animal models, there is always a probability of renal regeneration. Hence more research is warranted to determine if these cognitive changes are permanent or if they can be reversed. Such an understanding may lead to new therapies.

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