SEXUALLY DIMORPHIC EFFECTS OF CHRONIC PRENATAL RESTRAINT STRESS INDUCED SPATIAL MEMORY IMPAIRMENT IN POSTWEANED MALE AND FEMALE WISTAR RATS

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ABSTRACT

Chronic or repeated stress during human fetal brain development has been associated with various learning, behavioral and mood disorders manifesting into adulthood. This study examined the effects of prenatal stress on the postnatal expression of sexually differentiated spatial memory in male and female wistar rats. Pregnant dams were subjected to restraint stress 6 hours per day during 11-21 days of pregnancy. The offspring of control and prenatally stressed dams were tested for spatial memory performance. Prenatally stressed male rats exhibited spatial memory deficits evidenced by longer target quadrant entry latencies and less time spent in the target quadrant. Prenatal stress had no effect on the spatial memory performance in female rats. Thus prenatal stress altered subsequent spatial memory performance in a sex-specific manner. These data reinforce the view that prenatal stress affects behavioral development interfering with sex differences. These data have implications for the effects of prenatal stress on the development of sexually dimorphic learning disabilities in a spatial memory task.

Keywords: Prenatal stress, Spatial memory, Morris water maze, Hippocampus.

INTRODUCTION

Prenatal environment can influence an individual's development profoundly, inducing changes lasting into adulthood. Numerous extrinsic and intrinsic adverse stressors constantly challenge the dynamic equilibrium that maintains the development of an offspring[1]. Deleterious life events during pregnancy induce neurobiological and behavioral defects in offspring, some of them involving the hippocampal formation[2]. A substantial body of evidence indicates that early adverse experiences such as prenatal stress significantly affect the development of brain and the organization of cognition[3,4]. Early environmental experiences have long-lasting effects on adult cognition in humans[5] and animals[6].

Prenatal stresses of different nature and duration applied during various gestational periods have shown to decrease the locomotor activity[7] and immobility in the constrained swim test[8]**.** Gestational stress is reported to increase the anxiety like behavior in elevated plus maze or in open field[9] and decrease the spatial learning and memory in T-maze[10]**,** diminution of time spent in target quadrant in the water maze and spontaneous alternation test in Y-maze[11]. Thus there are many instances in which neural function and cognition are either facilitated by prenatal stress[12] or even not affected[13]. In male rats, prenatal stress is reported to decrease the learning ability in water maze[14] and increase the tight rope test score[15]**,** increase the emotionality in open field, and depression like behavior in forced swim test[16]. In female rats, prenatal stress results in increased learning in water maze[17], decreased learning ability and memory[18] and elevated anxiety like behavior[19]. Both male and female prenatally stressed rats showed decreased performance in spontaneous alternation and delayed alternation in Y-maze[7], delayed memory deficit, spatial and non spatial memory and short and long term memories. Hence there exists sexual dimorphism in the effects of prenatal stress on postnatal cognitive behavioral literature.

Hence the present study was undertaken to assess the sexually dimorphic effects of prenatal stress on spatial memory and the underlying mechanisms accounting for it.

MATERIALS AND METHODS

Animals and Housing Conditions

In-house bred male and female Wistar strains of rats were used in the study. Animals were bred in Central Animal Research Facility of Manipal University, Manipal. Adult rats (3 months old) were housed in air conditioned animal rooms with constant light-dark cycle (12:12 h), controlled temperature (22±3ºC) and humidity (50±5%). Polypropylene cage with paddy husk as bedding materials was used for housing the rats. The animals had free access to food (Gold Mohur; Lipton India Ltd.) and water *ad libitum*. Breeding and maintenance of animals were done according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethical Committee (I.A.E.C) approval was obtained before the conduct of the study (IAEC/KMC/06/2005-2006) and care was taken to handle the rats in humane manner.

Details of experimental methods

Timed pregnancy in rats

To get the pregnant rats of known gestational days, all female rats were subjected to vaginal smear test[20]. The rats in the estrus cycle were mated with adult male rats overnight. Vaginal smear was examined within 12 hours after mating. The presence of sperms in the smear confirms the mating and that day was taken as day zero of pregnancy for further counting the days. Pregnant female rat was separated from other rats and housed individually with proper label indicating the day of conception. Pregnant females were assigned randomly into 'No stress' and 'stress groups' (n=6 in each group). The rats in 'No stress group' remained without any further procedures and allowed to deliver the pups. The rats in the 'Stress group' were subjected to restraint stress.

Prenatal stress protocol

Pregnant rats in the 'stressed group' were subjected to daily restraint stress from 11th gestational day, till they deliver the pups. The pregnant rats were restraint stressed by placing them individually in a wire mesh restrainer, 6 hours per day[21]. This type of restrain is known to induce stress in rats as indicated by increased serum cortisol level and adrenal gland weight in them[22]. The wire mesh restrainer has a wooden base and stainless steel wire mesh restrainer hinged to the base. A padlock and latch will help to secure the rat in the restrainer. The restrainers of two different dimensions were used. The restrainer with 11cm (Length) × 6cm (Breadth) × 6cm (Height) dimensions for restraining the pregnant rats from E11-E17, and restrainer with 11cm (Length) x 8cm (Breadth) x 8cm (Height) dimensions was used to stress the pregnant rats from E18 till delivery[22].This type of restrainer claimed to restricts the animal's movement without any pain, discomfort or suffocation.

Experimental design

After weaning, two male pups, and two female pups were selected from each of the control mother and designated as normal control (NC, n=12) group. Similarly, two male pups and two female pups were selected from each of the stressed mother and designated as stressed (ST, n=12) group. Rats in both NC, and ST group were subjected to Morris watermaze test from 34th to 39th postnatal day as described below.

Morris water maze test

To test the spatial memory, rats were subjected to Morris water maze test[23] from 34th to 39th postnatal day. The water maze apparatus consists of a circular water tank of 1.83 meters in diameter, divided into 4 quadrants. There will be a 4''x 4'' size escape platform submerged in one of the quadrant, the target quadrant. The top surface of the platform was hidden approximately 1cm below the surface of the water. The pool is filled with water at a temperature of 18-26°C to a depth of about 40cm. Milk was added to the water just before the experiment to make the water opaque. Permanently positioned distinctive objects were placed for facilitating spatial orientation of the animal. Positions of the cues were kept unchanged throughout the period of training.The rats were trained in the water maze in 10 sessions on 5 consecutive days, two sessions on each day. Each session consists of 4 trials. In each trial, time taken to reach the hidden platform was recorded. If the rat was unable to find the platform within two minutes, the training session was terminated and a maximum score of two minutes was assigned. Twenty-four hours after the last session, rats were subjected to memory retention. This session was of 30 sec. duration. Here time taken to reach the target quadrant and time spent in the target quadrant were measured. Greater latency to reach the target quadrant and less time spent in the target quadrant suggests memory impairment.

RESULTS AND DISCUSSION

Latency to escape on to the escape platform during learning sessions.

Video tracking of representative rats in different groups during learning session is given in figure R1. As we can see, in the first session, rats in all groups went on swimming around water tank and failed to reach the escape platform. In the second session rats in all groups were able to reach the escape platform, though they took long time. In sessions 3, 4, and 5, rats in all groups (except stressed males) learnt to reach the escape platform quickly and escape there, as their escape latency decreased progressively from session to sessions (Table R1, Fig. R2). In all learning sessions, both stressed males and stressed females took significantly more time to escape on to platform compared to respective controls (STM:P<0.001, P<0.001, P<0.01, P<0.001, P<0.001 in 1st, 2nd, 3rd, 4th, and 5th learning sessions respectively; STF:P<0.01,P<0.01 in 1st ,2nd learning sessions respectively). Stressed females escaped quicker than stressed males (P<0.01, P<0.001, P<0.001, P<0.001, P<0.001 in 1st, 2nd, 3rd, 4th and 5th learning sessions respectively). Normal females took shorter time to escape than normal male rats (P<0.001, P<0.001, P<0.001 in 1st, 2nd and 5th learning sessions respectively, Table R1 and Fig. R1, R2).

Latency to enter target quadrant

Stressed male rats (STM) took longer time to reach the target quadrant during water maze retention test(probe test) 24 hrs after last learning session compared to control male rats (P<0.001,Table R2,Fig. R3, R4). However stressed females did not differ from control female rats. Stressed males took significantly more time to reach the target quadrant compared to stressed female rats (P<0.001, Table R2, Fig. R3, R4).

Time spent in target quadrant

Stressed male rats spent significantly less time in the target quadrant compared to control male (P<0.001). Stressed males spent significantly less time in the target quadrant compared to stressed female (P<0.001, Table R2, Fig. R3, 4).

The results of the present study revealed that prenatal stress exposure affected the acquisition of learned responses in the Morris water maze (MWM) test. The stressed groups (both male and female) exhibited longer latency than the control animals to reach the hidden platform during the trial sessions. It was observed that the stressed males were more affected by the effects of maternal restraint stress when compared to the stressed females. In the retention test, their latency time to reach the target quadrant was longer and their time spent in the target quadrant was shorter. The stressed males were unable to recall the exact position of the hidden platform on the memory retention day conducted 24hrs after the last learning session, inspite of five training sessions on earlier days. This in turn points to the poor spatial navigation ability as well as the object-place configurations of the stressed group of animals which has affected the stressed male rats more significantly. The effects of prenatal stress on spatial memory, thus appears to be sex-specific.

Cognitive function is known to be influenced by stress, both in animals and in humans. MWM learning is an aversely motivated behavior, and, even when aversive factors are minimized to the extent possible, acquiring this task will always be stressful event for the animals involved. Under these conditions, prenatal stress exposure may revert profound effects on cognitive performance.

The hippocampus plays a major role in spatial memory. Learning is associated with an increase in the strength of synaptic connections in the cells of hippocampus[24]. One mechanism for synaptic strengthening is long term potentiation (LTP) that is, structural and functional changes in synapses due to repeated stimulation leading to an increase in the efficiency of the synapse[25,26]. Since neurons communicate via chemical synapses and because memories are believed to be stored within these synapses, LTP is widely considered one of the major cellular mechanism that underlie learning and memory. LTP may account for many types of learning, from the relatively simple classical conditioning present in all animals to the more complex, higher level cognition observed in humans[25].

It has been reported that chronic stress produces changes in the hippocampal morphology of rats and primates. These alterations include retraction of the apical dendrites in the CA3 region of the hippocampus[27-29]. Chronic stress can also modify hippocampal dendritic spine number and shape[30,31,22]. Prolonged stressful periods can result in cell death[32]. Collectively there is clear evidence that chronic stress can significantly alter hippocampal structure. Prenatal stress impairs LTP altering synaptic plasticity and enhances the effects of chronic maternal stress on synaptic plasticity in the hippocampus[33] which may be the mechanism for the impaired spatial learning and memory in the stressed group of rats.

Of particular interest is the finding that prenatal stress impaired male performance while enhancing female performance. Prenatal stress (PS) appears to have masculinized the female performance on the MWM, and this finding would be consistent with others who have observed PS-induced masculinization of the female offspring[34,35]. These masculinized daughters also display an up-regulation of androgen receptors and estrogen receptor in brain regions including the hypothalamus, thalamus, and CA1 region of the hippocampus[36]. In the guinea pig, it is hypothesized that the masculinization of daughters after PS is due to an increase in HPA axis activity, which increases both glucocorticoid (GC) and androgen secretion from the adrenal glands, which then cross the placenta and masculinizes vulnerable brain regions[35]. It seems feasible that a similar mechanism may mediate the apparent masculinizing PS effects on female spatial performance in the current study. Also there is evidence that the hypothalamic-pituitary adrenal (HPA) axis response to stress is greater in female than in male PRS rats[37,38], although PRS can switch the female response to stress into a male pattern, reducing the increase in corticosterone secretion induced by stress[39]. The effect of prenatal stress on spatial learning may also be the consequence of a deficit in neurogenesis, which can itself result from the dysfunction of the HPA axis. Indeed, cognition is modulated by corticosterone in a complex way[40], and high levels of corticosterone impair learning and memory[41,42]. Exposure to the water maze increases corticosterone secretion[43] and prenatally stressed animals show a delayed habituation of the corticosterone response to repeated exposure to stress[44]. Thus, it seems reasonable to hypothesize that the prenatal stress-induced cognitive impairments may result from a prolonged corticosterone secretion that inhibits cell proliferation in various regions of hippocampus.

These results have important implications for work on the effects of developmental stress in both humans and animals. More generally, they show the heuristic value of accurate animal models to better understand the mechanism by which early stress and epigenetic risk factors promote learning disabilities in children and also underscore the point that many effects of prenatal stress obtained in males cannot be generalized in females and highlight the need to investigate the stress response in both sexes.

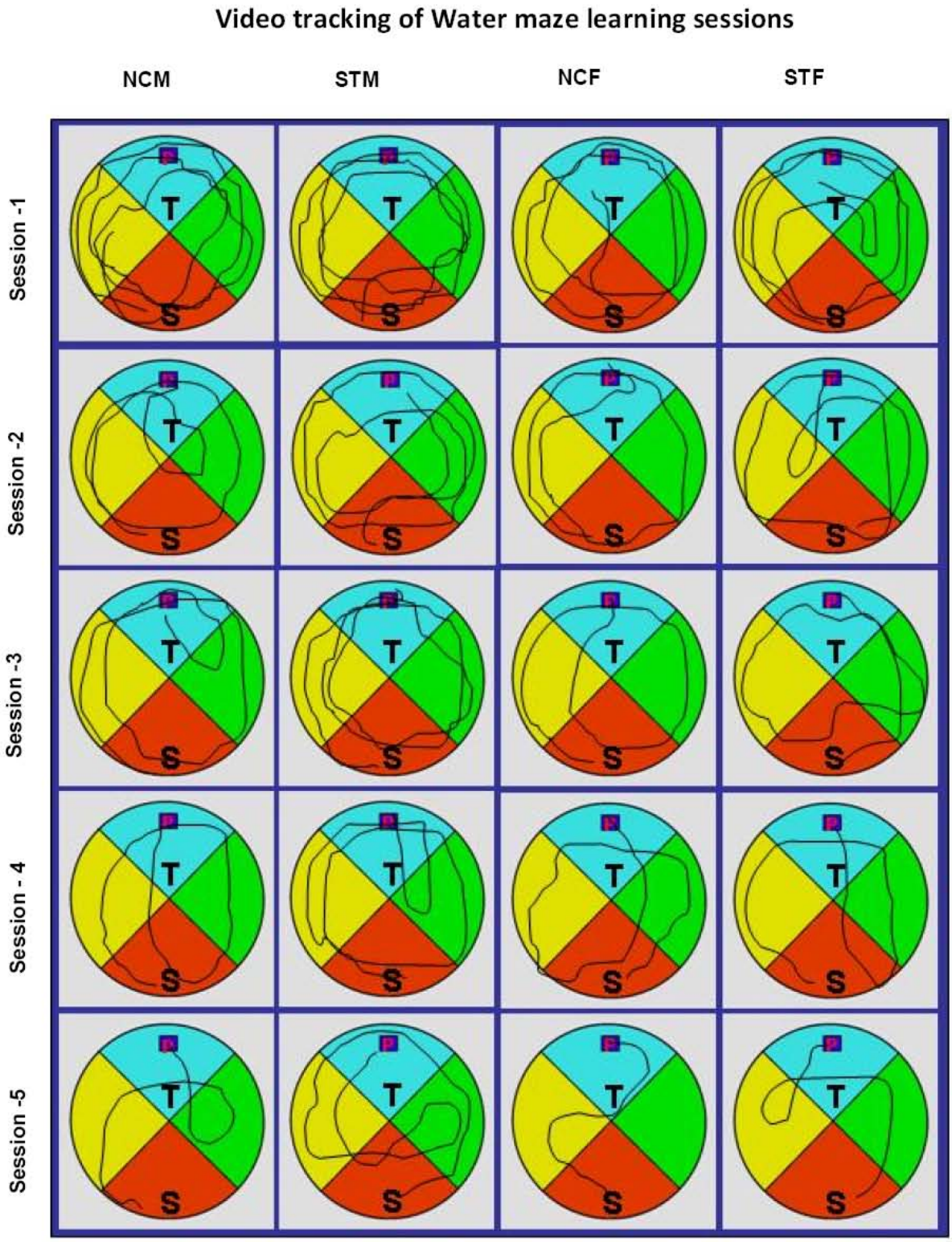

Figure R 1: Video tracking of representative rats belonging to different groups during learning sessions1-5 in Water maze. NCM-normal control male, NCF- normal control female, STM- stressed male, STF-stressed female, S-starting quadrant- target quadrant-escape platform. Note rat in the NCM, NCF, STF groups learnt to reach the target quadrant quickly by 3/4 sessions. However rats in STM group, even in 5th session took long time to escape onto the platform. In any given sessions stressed rats (STM, STF) took longer time to escape on to platform compared to normal rats (NCM, NCF).

Latency to escape on to the platform during learning sessions in water maze (sec)

Groups	Learning sessions				
	1	2	3	4	5
NCM(n=12)	65.88±6.23	36.08±5.63	17.58±2.34	17.42±2.07	14.67± 1.45
STM(n=12)	99.63 ± 12.58***	55.92±6.34***	31.04±2.38**	31.88± 2.16***	40.22± 5.61***
NCF(n=12)	49.46±4.16 †††	17.63±2.63 †††	16.09±2.04	13.55±1.25	8.05 ± 0.61 †††
STF(n=12)	58.67±5.21 $^{\#\#,\$\$}$	27.13±2.47 $^{\#\#\#,\$\$}$	17.49±1.35 $^{\#\#\#}$	18.7±2.26 $^{\#\#\#}$	6.41± 0.71 $^{\#\#\#}$
F value	7.92	12.54	11.47	16.17	28.45
Anova Significance	P<0.01	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Table R1: Latency to escape on to the platform during learning sessions in water maze test (sec) by rats in different groups. NCM-normal control male, NCF- normal control female, STM -stressed male, STF-stressed female. Note (i) stressed males took longer time to escape on to the platform on all learning sessions compared to control males, but stressed females learned to escape by 3rd session onwards like the control females, (ii) Stressed females took relatively less time to escape compared to stressed males, and (iii) normal females took shorter time to escape compared to normal males. NCM vs STM: **P<0.01, ***P<0.001; NCF vs STF: $^{\$\$}$ P<0.01; STM vs STF: $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001, NCM vs NCF: †††P<0.01. (One way ANOVA, Bonferroni's test. Each data represents mean±SEM).

Water maze test performance during retention test

	Male		Female		F value	ANOVA significance
	Normal (NCM, =12)	Stressed (STM, n=12)	Normal (NCF, n=12)	Stressed (STF, n=12)		
Latency to enter the target quadrant(sec)	2.66 ± 0.21	7.33 ± 0.49***	3.83 ± 0.60	4.50 ± 0.42$^{\#\#\#}$	18.89	P<0.0001
Time spent in target quadrant(sec)	22.83 ± 1.13	9.00 ± 0.57***	19.5 ± 1.72	16.67 ± 2.3$^{\#}$	14.03	P<0.0001

Table R2: Latency to enter the target quadrant, and time spent in the target quadrant by rats in different groups during water maze retention test. NCM-normal control male, NCF- normal control female, STM -stressed male, STF-stressed female. Note (i) stressed male rats took significantly longer duration to reach the target quadrant, compared to control males, unlike stressed females which took almost same time as control females to reach the target quadrant (ii) stressed male rats spent significantly less time in the target quadrant, compared to control males, unlike stressed females which spent almost the same time as control females. Stressed females deferred significantly from stressed males both in latency to enter the target quadrant and time spent in target quadrant. NCM vs STM: ***P<0.001; NCF vs STF: not significant; STM vs STF: $^{\#}$ P<0.05, $^{\#\#\#}$ P<0.001, NCM vs NCF: not significant. (One way ANOVA, Bonferroni's test. Each data represents mean ± SEM).

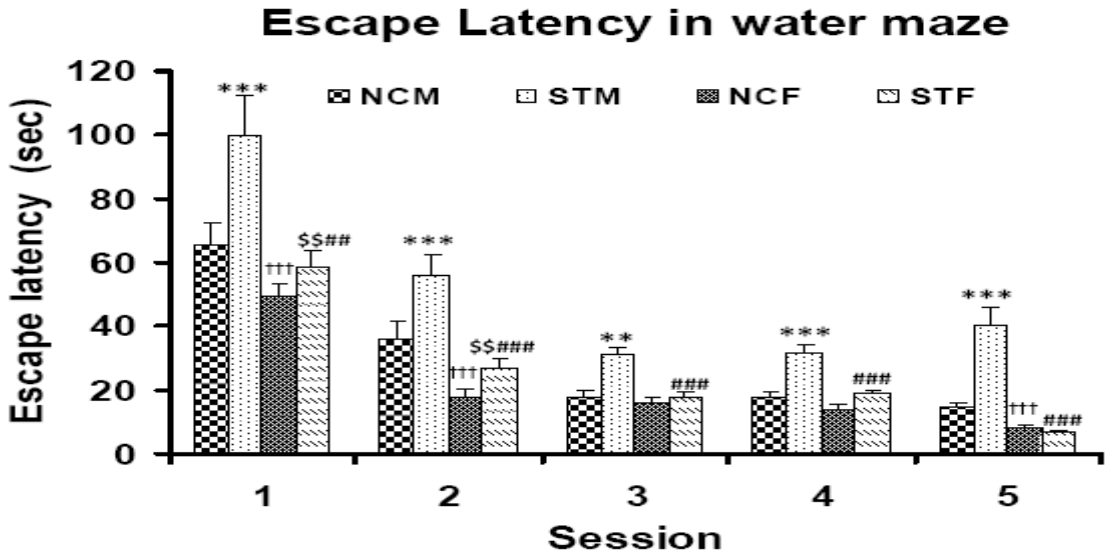

Figure R2: Latency to escape on to the platform during learning sessions in water maze test (sec) by rats in different groups. NCM-normal control male (n=12), NCF- normal control female (n=12), STM -stressed male (n=12), STF-stressed female(n=12). Note (i) stressed males took longer time to escape on to the platform on all learning sessions compared to control males, but stressed females learned to escape by 3rd session onwards like the control females, (ii) Stressed females took relatively less time to escape compared to stressed males, and (iii) normal females took shorter time to escape compared to normal males. NCM vs STM: **P<0.01, ***P<0.001; NCF vs STF: $^{\$\$}$ P<0.01; STM vs STF: $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001, NCM vs NCF: †††P<0.01. (One way ANOVA, Bonferroni's test. Each bar represents mean±SEM).

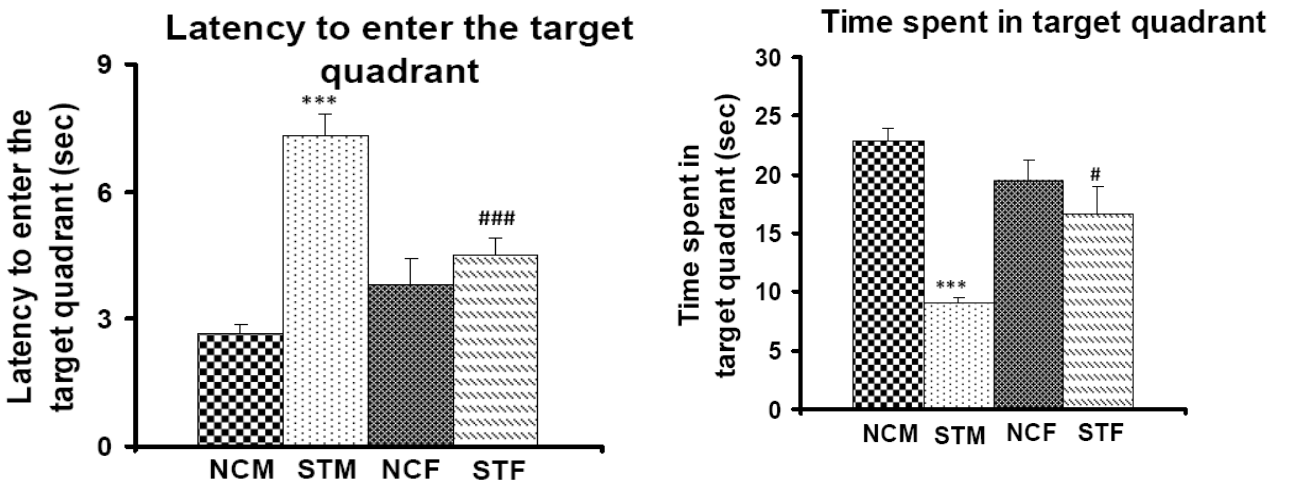

Figure R3: Latency to enter the target quadrant, and time spent in the target quadrant by rats in different groups during water maze retention test. NCM-normal control male(n=12), NCF- normal control female(n=12), STM -stressed male(n=12), STF-stressed female(n=12). Note (i) stressed male rats took significantly longer duration to reach the target quadrant, compared to control males, unlike stressed females which took almost same time as control females to reach the target quadrant (ii) stressed male rats spent significantly less time in the target quadrant, compared to control males, unlike stressed females which spent almost the same time as control females. Stressed females deferred significantly from stressed males both in latency to enter the target quadrant and time spent in target quadrant. NCM vs STM: ***P<0.001; NCF vs STF: not significant; STM vs STF: $^{\#}$ P<0.05, $^{\#\#\#}$ P<0.001, NCM vs NCF: not significant. (One way ANOVA, Bonferroni's test. Each bar represents mean±SEM).

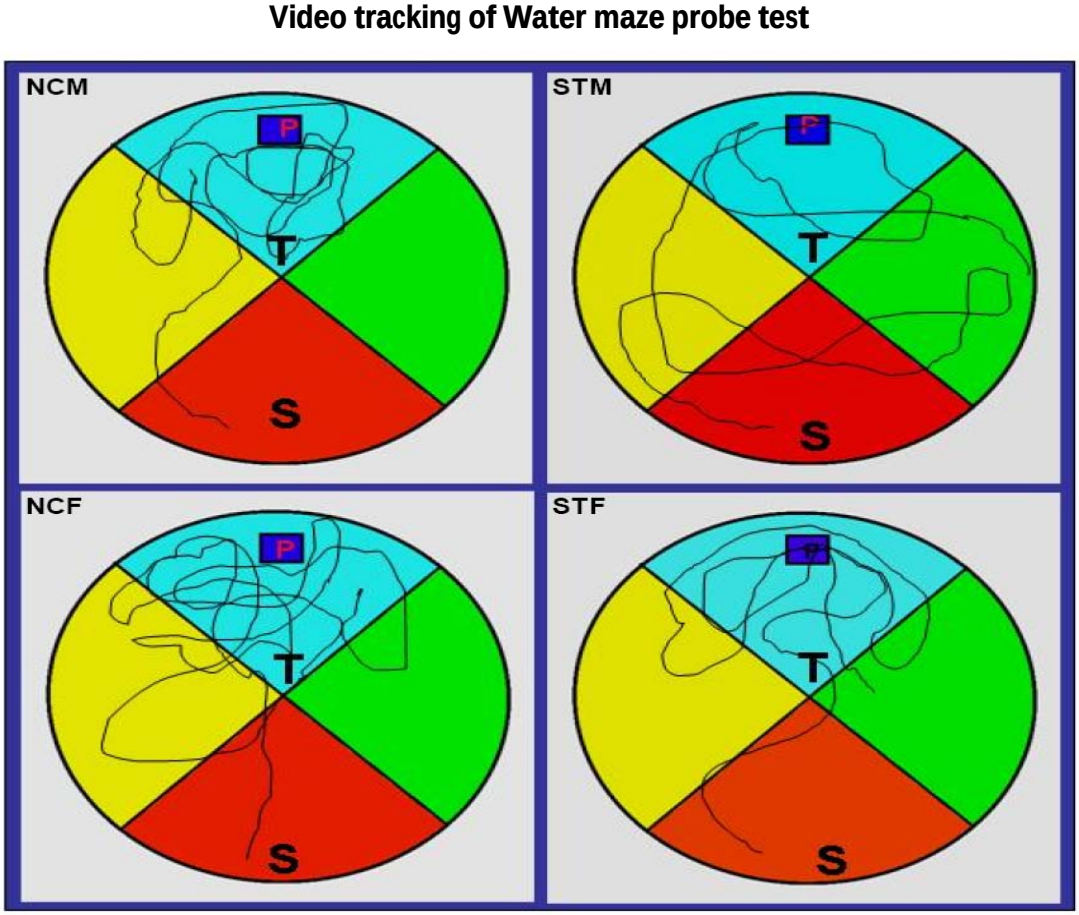

Figure R4: Video tracking of representative rats belonging to different groups during probe test (memory retention test) 24 hours after last learning session in Water maze. NCM- normal control male, NCF- normal control female, STM- stressed male, STF-stressed female, S-starting quadrant- target quadrant-escape platform. Note rats in the NCM, NCF, and STF groups reached the target quadrant quickly and spent most of their time swimming in the target quadrant, indicating good memory retention. However rat in STM group, failed to reach target quadrant quickly, and spent their time swimming in all quadrants.

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