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NEUROETHOLOGICAL STUDY OF ALCL3 AND CHRONIC FORCED SWIM STRESS INDUCED MEMORY AND COGNITIVE DEFICITS IN ALBINO RATS

ALBİNO RATLARDA ALCL3 VE KRONİK ZORLU YÜZME STRESİNE BAĞLI BELLEK VE BİLİŞSEL BOZULMALARIN NÖROETOLOJİK ÇALIŞMASI

Hira Rafi[1]*, Hamna Rafiq[1], Ruba Khan[1], Fahad Ahmad[1], Javaria Anis[1], Muhammad Farhan[1]

Abstract

Memory disorders could be caused due to occurrence of various neurological disorders including strokes, neurodegenerative diseases, trauma, anxiety or depressive disorders. Aluminium is a well-known neurotoxicant and is associated with several neurodegenerative diseases that caused memory deficits and CNS functional impairments related to memory and learning whereas chronic forced swim stress affects learning abilities and impaired various brain regions such as prefrontal cortex and hippocampus which are involved in memory and learning processes.

36 male Albino wistar rats were divided into three groups: Group I: Control (Water p.o.), Group II: AlCl3 (100 mg/kg bodyweight) (i.p.) test I and Group III: forced swim stress (20 oC, 10 minutes) test II for 14 days. Behaviors were assessed in Light/Dark transition Box, Open Field Test, Novel Object Recognition Test (NOR), T Maze Test and Morris Water Maze Test. Body weight and food intake were observed weekly.

Animals administered with AlCl3 and FSS revealed reduced body weight and food intake while decreased total numbers of square crossed with greater latency to move in open field test and reduced time spent and entries in bright area of light/dark box. Deficient spatial memory in Morris water maze and T maze test and reduced discrimination index along with decreased duration of interactions with novel object in NOR test were observed in learning and memory tests.

Present study determines that AlCl3 and FSS treatment could induce memory and cognitive impairment that lead to behavioral deficits and various other psychopathologies.

Keywords: Memory Impairment, Learning Responses, Behavioral Deficits, AlCl3, Forced Swim Stress.

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Öz

Bellek bozuklukları; inme, nörodejeneratif hastalıklar, travma, anksiyete veya depresif bozukluklar dahil olmak üzere çeşitli nörolojik bozuklukların ortaya çıkmasından kaynaklanabilir. Alüminyum iyi bilinen bir nörotoksiktir ve hafıza ve öğrenmeyle ilgili Santral Sinir Sistemi işlevsel bozukluklarına neden olan çeşitli nörodejeneratif hastalıklar ile ilişkilendirilir. Kronik zorlu yüzme stresi, prefrontal korteks ve hipokampus gibi öğrenme ve bellek süreçlerinde görev alan beyin bölgelerini etkiler. Bu araştırmada 36 erkek albino wistar rat üç gruba ayrılmıştır: Grup I: Kontrol (Su p.o.), Grup II: AlCl3 (vücut ağırlığına göre 100 mg / kg) (i.p.) testi I ve Grup III: zorlu yüzme stresi (20 oC, 10 dakika) 14 gün boyunca test II. Davranışlar Açık / Koyu Geçiş Kutusu, Açık Alan Testi, Yeni Nesne Tanıma Testi (NOR), T Labirent Testi ve Morris Su Labirent Testi'nde değerlendirilmiştir. Vücut ağırlığı ve besin alımı haftada bir gözlenmiştir. AlCl3 ve Zorlu Yüzme Testi uygulanan deneklerde vücut ağırlığı ve gıda alımının azaldığı, açık alan testinde hareket etmek öncesi daha uzun gecikme süresi kullandığı, geçilen kare sayısının azaldığı, açık / koyu kutuların aydınlık alanlarına girişlerin sayısının ve alanda geçirilen sürenin azaldığı ortaya konmuştur. Öğrenme ve bellek testlerinde Morris su labirentinde ve T labirent testinde uzamsal bellekte bozulma, Yeni Nesne Tanıma testinde yeni nesneyle etkileşimin süresinde azalma ve ayırt etme endeksinde azalma gözlenmiştir. Bu çalışma AlCl3 ve Zorlu Yüzme Testi uygulamalarının davranışsal bozukluklar ya da diğer psikopatolojilere yol açabilecek olan bellek kusurları ve bilişsel bozulmalar ilişkili olabileceğini göstermektedir.

Anahtar Kelimeler: *Bellek Bozukluğu, Öğrenme Yanıtları, Davranışsal Bozukluklar, AlCl3, Zorlu Yüzme Stresi.*

1. Introduction

Memory is the fundamental biological function vital for survival of an organism. It is the ability to preserve and retain gathered information, shape individuality, monitor thoughts and decision making ability, guide emotional reactions and recall it later. Memories depends on various neural systems. Different cellular processes are involved in acquisition, consolidation and evocation of memory (Kandel, 2001). Aluminium is a toxic metal that accumulates in various organs including brain, liver, bones and spleen and produces lethal effects (Willhite et al., 2014) with estimated seven years half-life for its elimination from human body (Yokel and McNamara, 2001). Previous studies described the role of aluminium as a major risk factor in the development of dementia, amyotrophic lateral sclerosis and Alzheimer's diseases (Singla & Dhawan, 2014). Aluminium is the most commonly known neurotoxicant (Hashmi et al., 2015; Mahboob et al., 2016) and studies evident it as an effective Alzheimer's disease inducing agent (Walton, 2014; Garcia et al., 2010). Aluminium is associated with the various neurodegenerative diseases progression including amyotrophic lateral sclerosis (Shiraki & Yase, 1975), Parkinsonism dementia (Shiraki & Yase, 1991), and Alzheimer's disease (Ferreira et al., 2008) that produces memory aberrations and numerous brain functions impairment associated with memory and cognition (Hardy & Selkoe, 2002; Wirths et al., 2004). The chief targeting organ for aluminium accumulation is brain (Kaneko et al., 2004), aluminium can cross blood brain barrier (Zatta et al., 2002) and store in entire brain most prominently in hippocampus (Kaur et al., 2006). The toxic effects of aluminium is associated with its bioavailability, animal model of aluminium chloride induced neurotoxicity is extensively used in several studies as it has high bioavailibity and certainly can administered orally or intra peritoneally (Hashmi et al., 2015). Aluminium exposure induces neurobehavioral, neurophysical, neuropathological and neurochemical changes in brain (Yuan et al., 2012; Walton, 2013). AlCl3 (50 mg/kg) for 14 days caused significant deficiency in spatial learning memory in mice (Al-Amin et al., 2019). Hippocampal aluminium accumulation causes neuroinflamation and β amyloid deposition, neuronal apoptosis that results in memory and cognitive impairments depending on hippocampus (Wang et al., 2014; Oshima et al., 2013).

Cognitive and learning functions are effected by chronic stress (McEwen et al., 2012; Hill, 2012) differently in normal cognitive subjects and with cognitive impairment. The levels of cortisol increases during stressful conditions that produces neurotoxicity over time which may leads to cognitive deficits. Chronic exposure to stress effects various brain regions including those which are essentially involved in emotional and learning responses such as the amygdala, prefrontal cortex and hippocampus (McEwen et al., 2012; Hill, 2012; Lupien et al., 2002). A proposed mechanism suggests that hypothalamic pituitary adrenocortical (HPA) axis releases glucocorticoids under threatening situation (McEwen et al., 1968). The glucocorticoids than bind to specific receptors in various brain regions such as hippocampus. The chronic release of glucocorticoids due to prolonged exposure to stress causes hippocampal structural damages (Lupien et al., 1998). Chronic forced swim stress was selected for the induction of memory impairment on the basis that the model of depression interconnected psychological (water and novelty) and physiological (exercise and water temperature) stressors with ease to repeat it frequently. The cause of stress in Forced swim stress water tank might be swimming for rats as it opposes their natural habitat (E. Badowska-Szalewska et al., 2010). Various studies have validated the importance of individuality that impact stress affected memory, learning and replenishment processes (Grootendorst et al., 2001). Several molecular mechanisms are involved in stress induced brain structure and cognition impairment. Specific neurotransmitters, neurotropic factors, adhesion molecules and signal transduction pathways have been associated with chronic stress effects on the brain (Sapolsky, 2000; Molteni et al., 2001). The forced swim test is believed to be an aversive stimulus and correlates to a psycho-physical stressor (Dayas et al., 2001), hence it is used for assessment of despair or depression like behavior as an experimental model (Muigg et al., 2007; Stone et al., 2007).

Thus, the present study was designed to determine the neurotoxic effects of AlCl3 and forced swim stress (FSS) on memory and cognition by using various behavior tests. The study also aimed to compare AlCl3 produced memory deterioration with stress induced deficits of memory and learning processes.

2. Material and methods

2.1. Animals

36 male adult albino Wistar rats weighing between (120-180 gm) were randomly divided into three group i.e. Group I: water-controls, group II: AlCl3 treated animals and group III: forced swim stress (FSS). Animals were kept at room temperature (25 ± 2 °C) under standard light/dark cycle of 12/12 h in separate cages with food and water ad libitum.

2.2. Treatment

Aluminium chloride (AlCl3) was purchased from Sigma Aldrich chemicals, USA and prepared in distilled water at w/v ratio of 1:1. AlCl3 (100 mg/Kg/Body Weight) was delivered intraperitoneally whereas, stressed tests animals were exposed to forced swim stress for 10 minutes in a transparent glass chamber (12 cm diameter and 22 cm height). The cylinder was filled up to 15cm with water (20 °C). Water as control was administered orally by an oral gavage for 14 days daily.

2.3. Procedure

36 rats were equally divided into three groups each that received the following treatments: Group I: Control (water was given orally) b. Group II: Aluminium chloride intraperitoneally (i.p.) 100 mg/Kg body weight (Gupta et al., 2017) and c. Group III forced swim stress (Ewa et al., 2010) for 10 minutes at 20 °C daily for 14 days. Behaviors were recorded in various paradigms post training period.

2.4. Behaviors

2.4.2. Body weight and food Intake

Weighed amount of fresh standard rodent diet cubes were given to each animal separately. Left over diet in cage hooper was weighed so that effects of neurotoxin and stress on feeding and satiety can be observed. Body weights of each rats were observed separately to determine the effect of AlCl3 and FSS on growth of animals.

2.4.2. Open field test

Open Field test is an uncomplicated and simple assessment of behaviors that does not require training to animals. The aversive behavior of rodents to bright, open and unfamiliar environment incorporates in various experimental testing in open field test (Choleras et al., 2001). The box consisted of 76 x 76 cm square area with 42 cm high opaque plastic walls. The floor was divided into 25 equal squares. Rat was placed in the center box of arena and exploration, anxiety and ambulation was observed in 5 minutes examination.

2.4.3. Light/Dark transition test

Light dark transition test is renowned for analyzing anxiety in rodents. The test is based on inborn aversive behavior of rodents to bright lit places and exploration in response to drugs or stresses (Crawley & Goodwin, 1980). The apparatus consists of two chambers of equal size made up of transparent and black opaque Plexiglas (20 x 30 x 30 cm). the partition is dividing the compartment has a 10 x 10 cm door in the middle of wall through which rat can move from one chamber to another. Single animal was positioned in the mid of bright chamber fronting towards opposite side from the middle wall opening. Behaviors measured were entries and time spent in light box for 05 minutes.

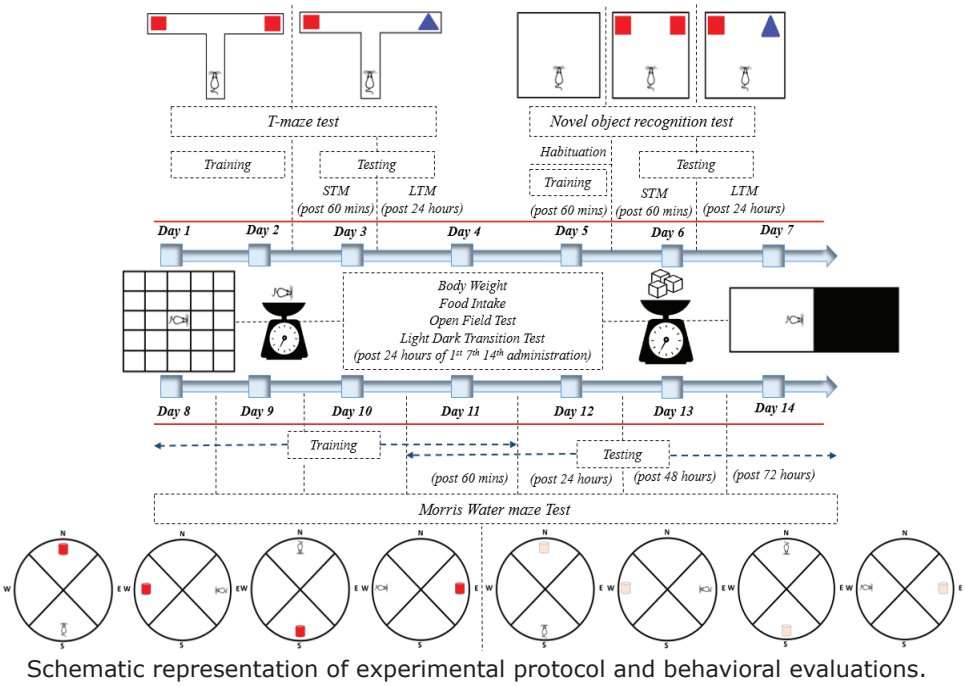
2.4.4. Novel object recognition test

Novel object recognition test is used to measure attention, anxiety, working memory and preference of novelty in animals (Goulart et al., 2010). The apparatus consists of wooden box of (40 x 40 x 40 cm) dimensions having floor covered with saw dust. The procedure comprises of three phases: habituation, familiarization and testing. Rat is freely allowed to explore NOR box in habituation phase for 15 minutes in the absence of objects. The experimental context is not considerably different during familiarization and testing phase. Each rat was placed for 15 minutes in training and test phase. During training phase, two identical objects were placed and animal was free to explore the objects whereas, in testing phase, an old object is replaced by a novel object and sniffing and discrimination index was observed as described by Line (Line, 2015) in five minutes duration.

$$\text{Discrimination Index} = \frac{\text{(Time devoted to novel object - Time devoted to familiar object)}}{\text{(Time devoted to novel object + Time devoted to familiar object)}}$$

2.4.5. Morris water maze

Water Maze was designed to observe spatial memory and learning. In 1984, Morris described procedures and



Schematic representation of experimental protocol and behavioral evaluations.

specifics for monitoring various forms of memory and cognition (Morris & Stewart, 1994). Primarily two axes bisect each other perpendicularly creating an imagined '+' sign that are labeled as North, South, East and West with N, S, E, and W symbols respectively. The apparatus used was stainless steel circular tank with the measurement of 37cm height, 45cm diameter and 12cm depth. The goal or platform was stainless steel cylindrical with diameter of 10 cm and immersed 2 cm below the surface. Water inside the tank was maintained with the temperature of 23 + 2°C and powdered milk was added in order to hide the goal for flawless rats tracking of the platform. Throughout the training of spatial working memory, the goal was visibly placed in one of the four possible locations (i.e. N, S, E and W) (Ouafa & Nour, 2008). Animal was given 120 sec in both phases of (STM and LTM) to discover the platform in testing phase whereas, experiments were designed to assess long term and short term memory.

2.4.6. T maze test

T Maze task has been widely used to investigate several functions of brain including spatial memory (Zimmerberg, Sukel & Stekler, 1991), conservations and long term memory (Olton & Papas, 1979). The apparatus consist of two closed arms (50 x 10 cm) with walls of 40 cm high and a central open arm (50 x 10 cm) in dimensions defining a T shape. All three arms were connected through a central square shaped opening (5 x 5 cm) and the apparatus was 60 cm high above the floor. The memory and learning were examined in training and test phases having same 5 minutes of cut off time. The training phase consists of two similar objects in both closed arms whereas, in test phases, a different object is placed which concludes long term and short term memory of animal.

2.5. Statistical analysis

The data obtained were analyzed by II way ANOVA repeated measure designs SPSS version 20, followed by Newman Keuls post hoc analysis test. Statistical Significance was considered as p less than 0.05. Data were described as means ± SEM.

3. Results

3.1. Body weight

Figure 1 explains the effects of AlCl3 and FSS induced memory impairment on body weight. Data obtained were analyzed by II way ANOVA. Statistical analysis determined the significant effects of days ($F(3, 33) = 15.836$, $p<0.05$), treatment ($F(2, 33) = 261.244$, $p<0.01$) and effects of days x treatment ($F(6, 33) = 52.123$, $p<0.01$). Post hoc analysis by Newman Keuls test determined that AlCl3 after 1st, 7th and 14th ($p<0.01$) administration and Forced swim stress after 7th and 14th ($p<0.01$) treatment decreased body weights significantly on the same day comparison with water controls. Whereas, when controls and tests were compared to their first administration, body weight increased after 7th and 14th ($p<0.01$) administration in controls and decreased after 7th and 14th ($p<0.01$) administration in AlCl3 and after 14th ($p<0.01$) treatment in FSS treated animals. Decreased body weight was observed in AlCl3 treated rats when compared with FSS group after 7th and 14th ($p<0.01$) day of treatment.

3.2. Food intake

Effects of AlCl3 and FSS induced memory impairment on food intake are described in Figure 2. All obtained data were analyzed by II way ANOVA that explained the significant effects of days ($F(2, 33) = 54.808$, $p<0.05$), treatment ($F(2, 33) = 390.773$, $p<0.01$) and days x treatment ($F(4, 33) = 35.537$, $p<0.01$). Post hoc analysis determined that AlCl3 and FSS after 1st, 7th and 14th ($p<0.01$) treatment decreased food intake significantly on the same day when compared with water controls. Whereas, food intake decreased after 7th ($p<0.01$) and increased after 14th ($p<0.01$) administration of water in controls while AlCl3 and FSS reduced food intake after 7th and 14th ($p<0.01$) administration when compared to their first administration. Body weight was observed significantly decreased in AlCl3 administered rats compared to FSS treated animals after 1st, 7th ($p<0.01$) and 14th ($p<0.05$) day.

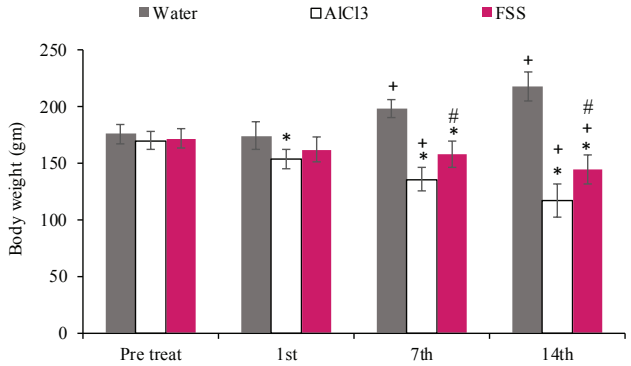


Figure 1. Effects of AlCl3 and FSS on body weight.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from pre-treatment; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

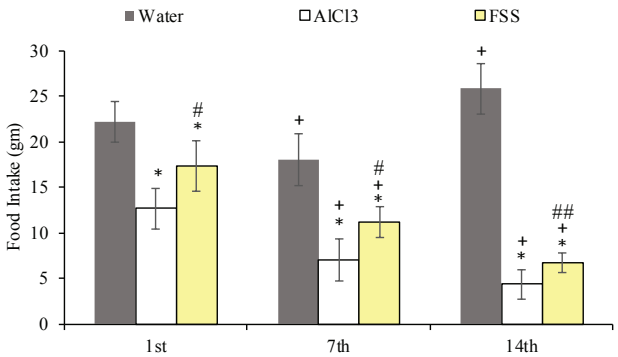


Figure 2. Effects of AlCl3 and FSS on food intake.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01, ##p<0.05 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

3.3. Open field test

The effects of memory deficits in open field test are shown in figure 3. Statistical analysis using II way ANOVA explained the significant effects of days ($F(2, 33) = 1118.741$, $p<0.01$), treatment ($F(2, 33) = 406.481$, $p<0.01$) and days x treatment ($F(4, 33) = 374.963$, $p<0.01$). Post hoc analysis determined that AlCl3 and FSS after 1st, 7th and 14th ($p<0.01$) treatment increased latency to move to the next box significantly when compared to water controls on the same day. Latency time increased significantly after 7th and 14th ($p<0.01$) treatment of AlCl3 and FSS from their first administration. Whereas, latency was observed increased after 7th ($p<0.01$) and decreased after 14th ($p<0.01$) treatment of FSS when compared with AlCl3 animals.

Total number of squares crossed in open field were analyzed by II way ANOVA that described the significant effects of days ($F(2, 33) = 1213.838$, $p<0.01$), treatment ($F(2, 33) = 1638.115$, $p<0.01$) and days x treatment ($F(4, 33) = 198.983$, $p<0.01$). Post hoc analysis explained that AlCl3 and FSS after 1st, 7th and 14th ($p<0.01$) treatment decreased total square crossed significantly on the same day when compared with water controls. Whereas, total square crossed decreased significantly after 7th and 14th ($p<0.01$) treatment of AlCl3 and FSS when compared to their first administration. Square crossed decreased significantly after 7th and 14th ($p<0.01$) FSS treatment compared with AlCl3 treated animals.

3.4. Light/Dark transition test

Figure 4 explains the effects of AlCl3 and FSS on anxiety in light dark transition box. Number of entries in light box were analyzed by II way ANOVA repeated measure design. Statistical analysis determined the significant effects of days ($F(2, 33) = 185.490$, $p<0.01$), treatment ($F(2, 33) = 317.234$, $p<0.01$) and days x treatment ($F(4, 33) = 54.230$, $p<0.01$). Post hoc analysis determined that AlCl3 after 14th ($p<0.01$) and FSS after 1st, 7th and 14th ($p<0.01$) treatment decreased entries in light box significantly when compared to water controls on the same day. Water after 7th ($p<0.01$) while AlCl3 and FSS after 7th and 14th ($p<0.01$) administration decreased light box entries significantly comparing from their first administration. FSS decreased entries in light box significantly after 1st, 7th and 14th ($p<0.01$) treatment compared to AlCl3 group.

Time spent in the light box determining anxiogenic effects AlCl3 and FSS was observed in light dark transition test. The results were analyzed by II way ANOVA that explained the significant effects of days ($F(2, 33) = 136.145$, $p<0.01$), treatment ($F(2, 33) = 1470.330$,

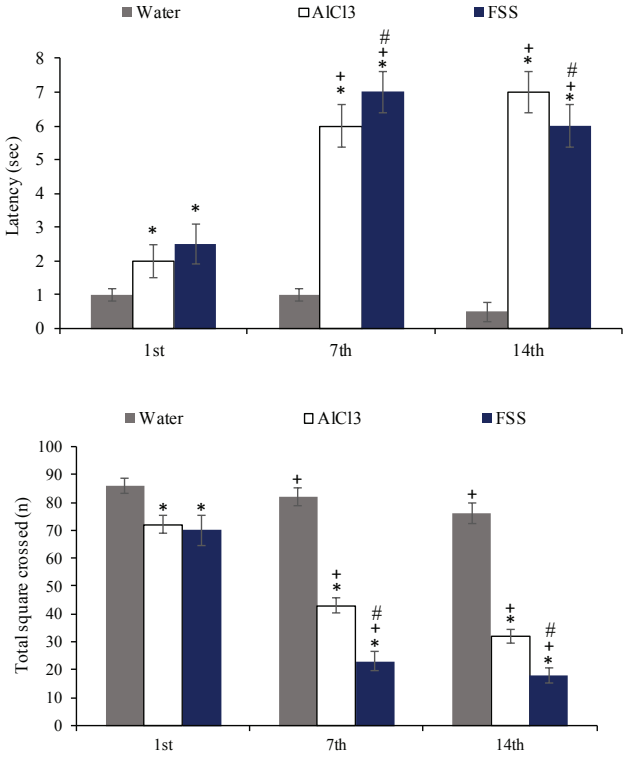


Figure 3. Effects of AlCl3 and FSS on locomotion and anxiety assessed in Open Field.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

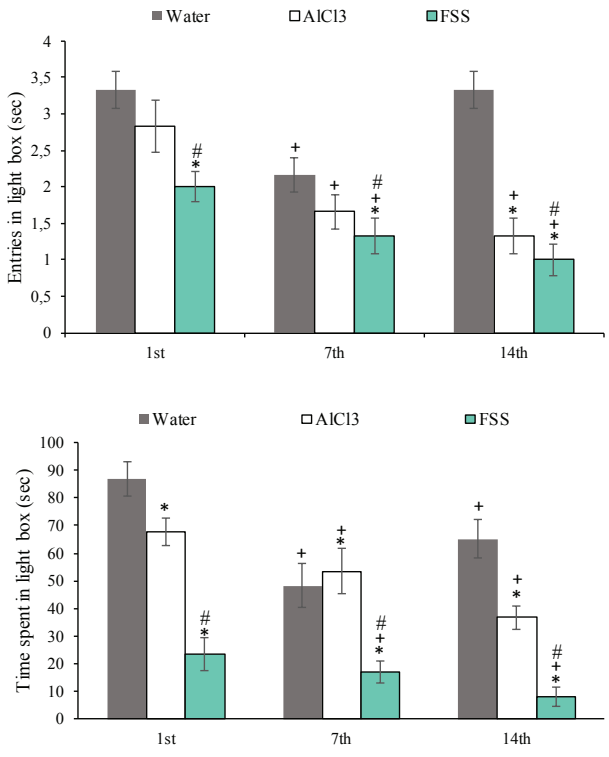


Figure 4. Effects of AlCl3 and FSS on anxiety assessed by Light/Dark transition test.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

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p<0.05) and days x treatment (F (4, 33) = 32.333, p<0.01). Post hoc analysis determined that AlCl3 and FSS after 1st, 7th and 14th (p<0.01) treatment decreased time spent in light box significantly on the same day comparison with controls. Water AlCl3 and FSS decreased time spent in light compartment significantly after 7th and 14th (p<0.01) administration when compared to their first administration. Whereas FSS significantly decreased time spent in light box after 1st, 7th and 14th (p<0.01) treatment when compared to AlCl3 administration.

3.5. Novel object Recognition test

The effects of AlCl3 and FSS induced memory deficits in NOR are explained in figure 5. Data were analyzed by II way ANOVA that described the significant effects of sessions (F (1, 33) = 151.671, p<0.10), treatments (F (2, 33) = 328.631, p<0.01) and effects of sessions x treatments (F (2, 33) = 11.253, p<0.10). Newman Keuls post hoc analysis determined that AlCl3 and FSS after 1 hour and 24 hours (p<0.01) sessions has significantly decreased discrimination index on the same day when compared to water treated controls whereas, decreased discrimination index were observed after 24 hours (p<0.01) testing sessions in controls, AlCl3 and FSS groups when compared with their respective first administration. Decreased discrimination index was observed in AlCl3 administered animals when compared with FSS treated rats post 1 hour and 24 hours (p<0.01) of training session.

Whereas, the obtained results of AlCl3 and FSS on animal interactions with novel object were analyzed by II way ANOVA that determined the effects of sessions (F (1, 33) = 340.230, p<0.05), treatment (F (2, 33) = 104.299, p<0.01) and sessions x treatment (F (2, 33) = 31.012, p<0.05) to be significant. Post hoc analysis explained that AlCl3 and FSS after 1 hour and 24 hours (p<0.01) sessions has significantly decreased time of interactions on the same day when compared to water treated controls. Whereas, when controls and AlCl3 and FSS tests animals were compared with their respective first administration, decreased time of interactions with novel object after 24 hours (p<0.01) testing session was observed. Interactions significantly decreased in AlCl3 treated animals when compared with post 1 and 24 (p<0.01) hours of FSS treatment.

3.6. Morris water maze

Morris water maze test determined the effects of AlCl3 and FSS on learning and memory impairment. Data were analyzed by II way ANOVA that described the significant effects of sessions (F (1, 33) = 405.596, p<0.05), treatment (F (2, 33) = 112.271, p<0.01) and sessions x treatments (F (6, 33) = 6.793, p<0.05). Post hoc analysis by Newman Keuls test explained that escape latency increased significantly after 1 hour, 24 hours, 48 hours and 72 hours (p<0.01) of training in AlCl3 treated rats and after 1 hour (p<0.01) in FSS group when compared with water treated controls on the same day. Whereas, decreased escape latency was observed after 24 hours, 48 hours and 72 hours (p<0.01) in water, AlCl3 and FSS treated animals when compared with their first administration respectively. We have observed increased escape latency in Morris water maze significantly in AlCl3 administrated animals when compared with post 24 hours, 48 hours and 72 hours (p<0.01) of training in FSS treated animals.

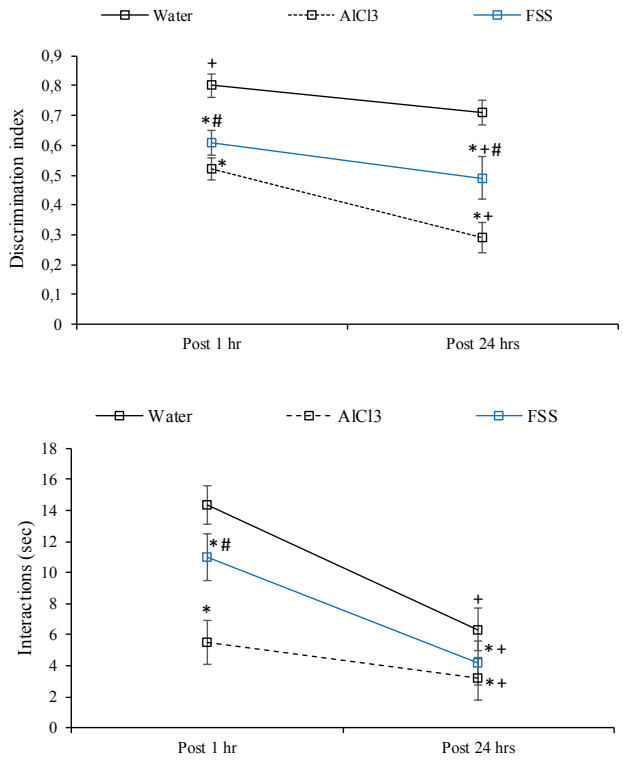

Figure 5. Effects of AlCl3 and FSS on memory and learning in Novel Object Recognition test.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

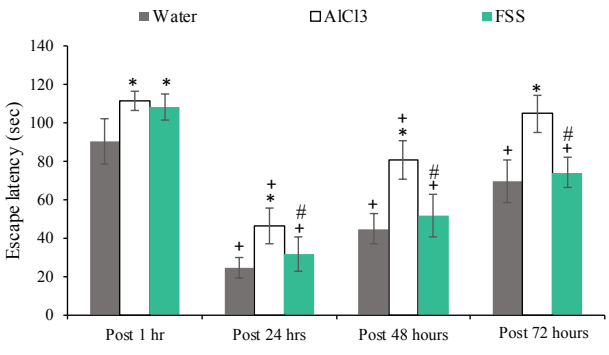

Figure 6. Effects of AlCl3 and FSS on memory and learning in Morris water Maze.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

3.7. T-maze

Figure 7 determine the effects of AlCl3 and stress induced memory deficits on correct arm choice in T-maze. Percent correct choice data were analyzed by II way ANOVA that described the effects of sessions ($F(1, 33) = 174.878$, $p<0.05$) and treatments ($F(2, 33) = 449.624$, $p<0.01$) to be significant while non-significant effects of sessions x treatments ($F(2, 33) = 0.210$) was observed. Newman Keuls post hoc analysis explained that AlCl3 and FSS after 1st and 24th hours ($p<0.01$) test sessions has significantly decreased percentage of correct arm choice on the same day when compared to water treated controls. % correct arm entries decreased after 24 hours ($p<0.01$) testing sessions in water, AlCl3 and FSS treated groups when compared with their respective first administration. Whereas AlCl3 significantly decreased percent correct choice when compared with post 1 and 24 ($p<0.01$) hours of FSS treatment.

Effects of AlCl3 and FSS on time spent in correct arm of T maze were analyzed by II way ANOVA that determined the significant effects of sessions ($F(1, 33) = 253.605$, $p<0.05$), treatment ($F(2, 33) = 427.441$, $p<0.05$) and sessions x treatment ($F(2, 33) = 21.203$, $p<0.05$). Post hoc analysis explained that AlCl3 and FSS after 1 hour and 24 hours ($p<0.01$) testing sessions has significantly decreased time spent in correct arm on the same day when compared with water treated controls. Whereas, significant decreased time spent in correct arm after 24 hours ($p<0.01$) testing sessions were observed in water, AlCl3 and FSS treated animal groups when compared with their respective first administration. Time spent in correct arm decreased in AlCl3 treated animals significantly when compared with post 1 and 24 ($p<0.01$) hours testing sessions of FSS animals.

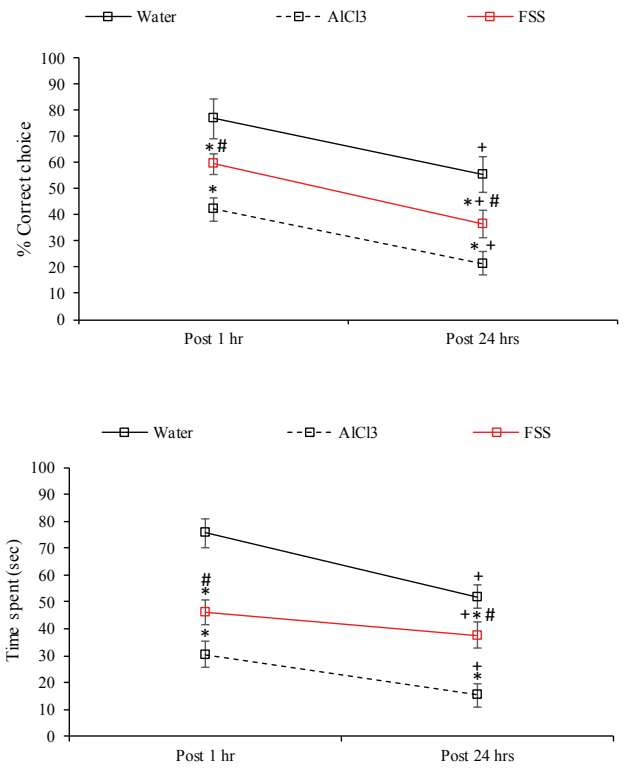

Figure 7. Effects of AlCl3 and FSS on memory and learning in T-maze.

Values are expressed as means + SEM (n=12). Significant differences by Newman-Keuls test: groups that differ significantly from respective water controls *p<0.01; + p<0.01 from similar drug administered groups that significantly differ from 1st administration; #p<0.01 from AlCl3 administration to forced swim stress on the same day following two way ANOVA (repeated measure design).

4. Discussion

We aimed to investigate the impact of AlCl3 and FSS on memory performance and cognition in rat models. Our study revealed important that aluminium exposure leads to spatial working memory impairment which is consistent with the study describing similar findings (Al-Amin et al., 2019). The present study also described the effects of forced swim stress that leads to deficits of working memory and learning. Moreover the study was designed to compare the interrelated and comparative behavioral deficiencies induced via AlCl3 and FSS exposure.

Memory and learning are essential aspects of human behavior, as they are involved in the regulation of actions and thought processes. Milner in 1998 reported that the modification of synaptic connections includes alternations in amount or structure of synapse that represent a particular mechanism of memory and cognition. The deviations in brain's signal transduction pathways, regulatory mechanisms of nucleus and changes in proteins synthesis can play a crucial part in learning and memory molecular mechanisms (Milner et al., 1998). Present study determines the deteriorating effects of AlCl3 and FSS on hunger and body weight. Food intake and body weight were decreased in animals exposed to aluminium and chronic force swim stress while a significant weight gain and food consumption was observed in control animals from first week of administration. Weight as well as food intake was reduced in aluminium treated rats when compared with FSS exposed animals revealing toxic effects of aluminium on growth of the subjects. These findings suggest that AlCl3 impacts more on hunger and growth as compared to forced swim stress and stress has multifarious stimulatory effects on body weight and food intake. These results are in line with previous studies describing the food and appetite patterns in stress (Meye & Adan, 2014; Tamashiro et al., 2011). Studies conducted on male and female wistar rats determined weight gain in water received animals whereas there was a significant weight loss in male and female wistar rats treated with aluminium (Buraimoh & Ojo, 2014).

Anxiety is a widely known threat for the development of stress related mental illnesses like depression in humans (Gladstone & Parker, 2006) and it has been related with higher degrees of memory cognitive impairment resulting in chronic stress. Our study revealed the anxiogenic behavior in Open field test in relation to memory impairment. fig 3 determined the significant decrease in numbers of squares crossed and increased latency to move in both aluminium and forced swim stress exposed rats when compared with water treated controls whereas, daily treatment of AlCl3 and FSS caused less exploration and higher anxiety in animals represented by less numbers of square crossed and more latency when

assessed weekly. Herrero and hi co-workers in 2006 determined peributal anxiety in rats (43 days old) using open field and elevated plus maze shown the detrimental effects of 21 days long restraint stress on spatial memory depended on hippocampus (Herrero et al., 2006). Our study reveals anxiogenic behavior in AlCl3 and FSS treated rats in light dark transition test which shown the significant decreased time spent and entries in light box comparing with water controls while weekly assessment also demonstrated decline entries and time spent in light compartment from 1st day treatment. Lesser time spent in light box and entries are observed in chronic stress treated group than AlCl3 administered animals. The findings from the present study described the reducing time spent and entries in bright box which is due to the anxiogenic behavior caused by neurotoxicity of AlCl3 or chronic stress induced memory impairment.

The novel object recognition test is commonly used for the assessment of memory changes (Antunes & Biala, 2012). Our study demonstrates that the discrimination index and the time spent in interacting with novel objects were decreased post one hour in aluminium chloride and stress treated rats when compared with controls whereas after 24 hours of training, the interactions and discrimination index were reduced significantly in both AlCl3 and FSS animals. AlCl3 significantly decreased discrimination and interactions as compared to FSS. Silvers in 2007 described that the recognition of novel object involves more cognitive abilities for the subject than the novel environment exploration or a particular object (Silvers et al., 2007), it also explained the retention of familiar object in the animal memory (Ennaceur, 2010). Various behavioral and pharmacological studies described an inverted U shape interrelation between stress and memory retention. High stress levels form strong and long lasting memories that are proportionally more persistent whereas, too intense or chronic stresses cause memory impairments. Therefore, it has been observed that high levels of noradrenaline and glucocorticoids (stress hormones) that are released in stress response, intervene and modulate memory preservation according to U shape inverted curve (Roozendaal & McGuagh, 2004, Sandi & Pinelo-Nava, 2007). In 1995, Luis and his co-workers conducted a study which explained that forced swim stress (20 oC) was retaining latency immediately or post 1 hour of training but recover post 2 hours of training of swim stress concluding that 2 hours are required for acquired information consolidation (Luis J et al., 1995).

Figure 6 explained the effects of memory impairment in the Morris water maze and the obtained results revealed the significant decrease in latency time to reach goal in a specific quadrant of water maze after 60 minutes testing in both AlCl3 and FSS groups whereas, time required to reach the platform increased after 24 hours, 48 hours and 72 hours of training session. Significant increase in escape latency was also observed in aluminium treated rats comparing with water controls while no visible difference were observed in forced swim stressed groups from during all four test periods. Escape latency significantly decreased in FSS animals when compared with AlCl3 administered tests. These findings are consistent with the previous reports that described aluminium oral administration reduced spatial working memory in mice (Al-Amin et al., 2016). Different Studies conducted on Aluminium treated mice and rats revealed a significant impairment in spatial memory and learning demonstrating a reduced escape latency of rodents compared with controls (Ouafa & Nour, 2008; Miu et al., 2013; Kaneko et al., 2006; Roig et al., 2006). Numerous studies validated that rats that were treated with chronic stress exhibits impairment in learning and memory in various spatial tasks such as the Y maze (Conrad et al., 1996), the radial arms maze (Luine et al., 1994) and the Morris water maze (Sandi et al., 2003; Venero et al., 2002). The findings of our study described the discriminating behavior of rats in novel spatial context of T maze paradigm (Figure 7). Both test groups of AlCl3 and chronic stressed rats shown significant decrease in correct choice of arm and time spent to explore novel object compared with water treated controls and when compared with post 60 minutes of testing with respective treatment groups. T maze is based on the inborn behavior of rodents of preferring novel environment in contrast to known one (Zhang et al., 2008). A great adaptive and ecological importance is required to distinguish novel spatial context, effects that are non-specific and unrelated to the memory behavior are minimized in T maze test as this test does not require reward or punishment which are usually used in various memory tests. Our findings are in line with a previous describing memory and learning performance (Ritter & Cummings, 2015).

5. Conclusion

To sum up, our study indicated that both AlCl3 and forced swim stress (FSS) induce memory and cognitive impairment that leads to behavioral disorders and deficits whereas AlCl3 deteriorates memory and learning process immensely as compared to chronic forced swim stress.

Patient informed consent: Informed consent was obtained.

Ethics committee approval: Ethics committee approval was obtained.

Conflict of interest: There is no conflicts of interest to declare.

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Referanslar

Al-Amin, M. M., Chowdury, M. I. A., Saifullah, A. R. M., Alam, M. N., Jain, P., Hossain, M., Alam, M. A., Kazi, M., Ahmad, A., Raish, M., Alqahtani, A. and Reza, H. M., (2019). Levocarnitine Improves AlCl3-Induced Spatial Working Memory Impairment in Swiss albino Mice. Fronteiers in Neuroscience, 13,278. doi: 10.3389/fnins.2019.00278

Al-Amin, Md. Mamun & Reza, Hasan & Saadi, Hasan & Mahmud, Waich & Gambool, Abdirahman & Alam, Musrura & Kabir, Nadia & Saifullah, A.R.M. & Tarannum, Sarjana & Ruhul Quddus, A. H. M. (2016). Astaxanthin ameliorates aluminum chloride-induced spatial memory impairment and neuronal oxidative stress in mice. European journal of pharmacology, 777, 60-9. doi:10.1016/j.ejphar.2016.02.062

Antunes, M. & Biala, G. (2011). The novel object recognition memory: Neurobiology, test procedure, and its modifications. Cognitive processing, 13(2), 93-110. doi: 10.1007/s10339-011-0430-z

Milner, B., Squire L. R., Kandel E. R. (1998). Cognitive neuroscience and the study of memory. Neuron, 20(3), 445-68. doi. 10.1016/S0896-6273(00)80987-3

Bolla, K. I., Briefel, G., Spector, D., Schwartz, B. S., Wieler, L., Herron, J., & Gimenez, L. (1992). Neurocognitive effects of aluminum. Archives of Neurology, 49(10), 1021-1026. doi: 10.1001/archneur.1992.00530340037015

Buraimoh A. A., Ojo, S. A. (2014). Effects of Aluminium chloride exposure on the body weight of Wistar rats. Annals of Biological Sciences, 2(2), 66-73. doi: 10.9790/3013-0230525533

Choleris, E, Thomas, A. W., Kavaliers M, Prato F.S. (2001). A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neuroscience & Biobehavioral Reviews, 25(3), 235-60. doi: 10.1016/S0149-7634(01)00011-2

Conrad, C. D., Galea, L. A. M., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine treatment. Behavioral Neuroscience, 110(6), 1321-1334. doi: doi: 10.1037/0735-7044.110.6.1321

Crawley, J. N., Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacology Biochemistry and Behavior, 13, 167-170. doi: 10.1016/0091-3057(80)90067-2

Dayas, C. V., Buller, K. M., Crane, J. W., Xu, Y., & Day, T. A. (2001). Stressor categorization: Acute physical and psychological stressor elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. European Journal of Neuroscience, 14(7), 1143-1152. doi: 0.1046/j.0953-816x.2001.01733.x

Ennaceur, A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. Behavioral Brain Research, 215(2), 244–254. doi: 10.1016/j.bbr.2009.12.036

Ewa Badowska-Szalewska, Edyta Spodnik, Ilona Klejbor and Janusz Moryś. (2010). Effects of chronic forced swim stress on hippocampal brainderived neutrophic factor (BDNF) and its receptor (TrkB) immunoreactive cells in juvenile and aged rats. Acta neurobiologiae experimentalis, 70, 370–381.

Ferreira, P. C., Piai Kde A, Takayanagui, A. M., Segura-Muñoz, S. I. (2008). Aluminium as a risk factor for Alzheimer's disease. Revista Latino-Americana de Enfermagem, 16(1), 151–7. doi: 10.1590/S0104-11692008000100023

Garcia, T., Esparza, J. L., Nogues, M.R., Romeu, M., Domingo, J. L., Gomez, M. (2010). Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminium. Hippocampus, 20(1), 218-25. doi: 10.1002/hipo.20612

Gaurav Gupta, Dinesh K. Chellappan, Mohit Agarwal, Madhu Ashwathanarayana, Srinivas Nammi, Kavita Pabreja, Kamal Dua. (2017). Pharmacological evaluation of the recuperative effect of morusin against aluminium trichloride (AlCl3)-induced memory impairment in rats. Central Nervous System Agents In Medicinal Chemistry, 17(3), 196-200. doi: 10.2174/1871524917666161111095335

Gladstone, G. L., Parker G. B. (2006). Is behavioral inhibition a risk factor for depression? Journal of Affective Disorders, 95(1-3), 85-94. doi:10.1016/j.jad.2006.04.015

Goulart, B. K., de Lima, M. N., de Farias, C. B., Reolon, G. K., Almeida, V. R., Quevedo, J., Kapczinski, F., Schröder, N., Roesler, R. (2010). Ketamine impairs recognition memory consolidation and prevents learning-induced increase in hippocampal brain-derived neurotrophic factor levels. Neuroscience, 167(4), 969-73. doi:10.1016/j.neuroscience.2010.03.032

Grootendorst, J., Oitzl, M.S., Dalm, S., Enthoven, L., Schachner, M., de Kloet, E. R., Sandi, C. (2001). Stress alleviates reduced expression of cell adhesion molecules NCAM, L1, and deficits in learning and corticosterone regulation of apolipoprotein E knockout mice. European Journal of Neurosciences, 14(9), 1505-1514. doi: 10.1046/j.0953-816x.2001.01766.x

Hardy, J., Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, 297(5580), 353–3566. doi:10.1126/science.1072994

Hashmi, A. N., Yaqinuddin, A., Ahmed, T. (2014). Pharmacological effects of ibuprofen on learning and memory, muscarinic receptors gene expression and APP isoforms level in pre-frontal cortex of AlCl3-induced toxicity mouse model. International Journal of Neurosciences, 125(4), 277-287. doi:10.3109/00207454.2014.922972

Herrero, A. I., Sandi, C., Venero, C. (2006). Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. Neurobiology of Learning and Memory, 86(2), 150-159. doi: 10.1016/j.nlm.2006.02.001

Hill, M. N. (2012). Introduction to the special issue on stress, emotional behavior, and the endocannabinoid system: a decade of research. Neuroscience, 204, 1-4. doi:10.1016/j.neuroscience.2012.01.038

Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. Science, 294(5544), 1030-1038. doi:10.1126/science.1067020

Kaneko, N., Takada, J., Yasui, H., Sakurai, H. (2006). Memory deficit in mice administered aluminium–maltolate complex. BioMetals 19(1), 83-89. doi: 10.1007/s10534-005-6965-7

Kaneko, N., Yasui, H., Takada, J., Suzuki, K., Sakurai, H. (2004). Orally administrated aluminium-maltolate complex enhances oxidative stress in the organs of mice. Journal of Inorganic Biochemistry, 98(12), 2022-2031. doi: 10.1016/j.jinorgbio.2004.09.008

Kaur, A., Joshi, K., Minz, R.W., Gill, K. D. (2006). Neurofilament phosphorylation and disruption: a possible mechanism of chronic aluminium toxicity in Wistar rats. Toxicology, 219(1-3), 1-10. doi: 10.1016/j.tox.2005.09.015

Luine, V., Villegas, M., Martinez, C., McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. Brain Research, 639(1), 167-170. doi: 10.1016/0006-8993(94)91778-7

Luis Jodar, Masakatsu Takahashi and Hiroshi Kaneto. (1995). Effects of Footshock-, Psychological- and Forced Swimming-Stress on the Learning and Memory Processes: Involvement of Opioidergic Pathways. The Japanese Journal of Pharmacology, 67(2), 143-147. doi: 10.1254/jjp.67.143

Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P., Thakur, M., McEwen, B. S., Hauger, R.L., Meaney, M. J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nature Neuroscience 1(1), 69–73. doi: 10.1038/271

Lupien, S. J., Wilkinson, C. W., Briere, S., Menard, C., Kin, N. M., Ng Ying, Nair N. P. (2002). The modulatory effects of corticosteroids on cognition: studies in young human populations. Psychoneuroendocrinology, 27(3), 401–416. doi: 10.1016/S0306-4530(01)00061-0

Mahboob, A. Farhat, S. M. Iqbal, G. Babar, M. M. Nabavi, S. M. Ahmed, T. (2016). Alpha-lipoic acid-mediated activation of muscarinic receptors improves hippocampus-and amygdala-dependent memory. Brain Research Bulletin, 122, 19-28. doi: 10.1016/j.brainresbull.2016.02.014

McEwen B. S, Eiland L, Hunter R. G, Miller M. M. (2012). Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology,62(1),3-12. doi: 10.1016/j.neuropharm.2011.07.014

McEwen BS, Weiss JM, Schwartz LS. (1968). Selective retention of corticosterone by limbic structures in rat brain. Nature, 220, 911–912. doi: 10.1038/220911a0

Meye, F. J, Adan, R. A. (2014). Feelings about food: the ventral tegmental area in food reward and emotional eating. Trends in Pharmacological Sciences, 35(1), 31–40. doi: 10.1016/j.tips.2013.11.003

Miu, A.C., C.E. Andreescu, R. Vasiu and A.I. Olteanu. (2003). A behavioural and histological study of the effects of long-term exposure of adult rats to aluminium. International Journal of Neurosciences, 113(9), 1197-1211. doi: 10.1080/00207450390232292

Molteni, R., Fumagalli, F., Magnaghi, V., Roceri, M., Gennarelli, M., Racagni, G., Melcangi, R. C., Riva, M. A. (2001). Modulation of fibroblast growth factor-2 by stress and corticosteroids: from developmental events to adult brain plasticity. Brain Research Reviews, 37(1-3), 249-258. doi: 10.1016/S0165-0173(01)00128-X

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods, 11(1), 47–60. doi: 10.1016/0165-0270(84)90007-4

Morris, R. G. M. (1993).An attempt to dissociate 'spatial-mapping' and 'working-memory' theories of hippocampal function. In: Seifert, W., editor. Neurobiology of the Hippocampus, 405-432.

Muigg, P., Hoelzl, U., Palfrader, K., Neumann, I., Wigger, A., Landgraf, R., Singewald, N. (2007). Altered brain activation pattern associated with drug-induced attenuation of enhanced depression-like behavior in rats bred for high anxiety. Biological Psychiatry, 61(6), 782–796. doi: 10.1016/j.biopsych.2006.08.035

Olton, D. S., Papas, B. C. (1979). Spatial memory and hippocampal function. Neuropsychologia, 17(6), 669-82. doi: 10.1016/0028-3932(79)90042-3

Oshima, E., Ishihara, T., Yokota, O., Nakashima-Yasuda, H., Nagao, S., Ikeda, C., Naohara, J., Terada, S., Uchitomi, Y. (2013). Accelerated tau aggregation, apoptosis and neurological dysfunction caused by chronic oral administration of aluminium in a mouse model of tauopathies. Brain Pathology, 23(6), 633-644. doi: 10.1111/bpa.12059

Ouafa Rebai and Nour Eddine Djebli (2008). Chronic Exposure to Aluminium Chloride in Mice: Exploratory Behaviors and Spatial Learning. Advances in Biological Research, 2 (1-2), 26-33.

Ritter, A., and Cummings, J. (2015). Fluid biomarkers in clinical trials of Alzheimer's disease therapeutics. Frontiers in Neurology, 6,186. doi: 10.3389/fneur.2015.00186

Roig, J. L., S. Fuentes, M. Teresa Colomina and P. Vicens. (2006). Aluminium, restraint stress and aging: behavioral effects in rats after 1 and 2 years of aluminium exposure. Toxicology, 218(23), 112-124. doi: 10.1016/j.tox.2005.10.006

Sandi, C., Davies, H. A., Cordero, M. I., Rodriguez, J. J., Popov, V. I., Stewart, M. G. (2003). Rapid reversal of stress induced loss of synapses

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in CA3 of rat hippocampus following water maze training. European Journal of Neuroscience, 17(11), 2447-2456. doi: 10.1046/j.1460-9568.2003.02675.x

Sapolsky, R. M. (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biological Psychiatry, 48(8), 755-765. doi: 10.1016/S0006-3223(00)00971-9

Shiraki, H., Yase, Y. (1975). Amyotrophic lateral sclerosis in Japan. In: Vinken PJ, Bruyn GW (eds.). Handbook of Clinical Neurology, System disorders and atrophies II. Elsevier Science Publishers, Amsterdam, 22, 353–419.

Shiraki, H., Yase, Y. (1991). Amyotrophic lateral sclerosis and Parkinsonism-dementia in the Kii peninsula: comparison with the same disorders in Guam and with Alzheimer's disease. Handbook of Clinical Neurology, 15, 273–300.

Silvers, J. M., Harrod, S. B., Mactutus, C. F., Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. Journal of Neuroscience Methods, 166(1), 99–103. doi: 10.1016/j.jneumeth.2007.06.032

Singla, N., Dhawan, D. K. (2011). Zinc modulates aluminium-induced oxidative stress and cellular injury in rat brain. Metallomics, 6(10), 1941–1950. doi: 10.1039/c4mt00097h

Stewart, C. A., Morris, R. G. M. (1993).The watermaze. In: Sahgal, A., editor. Behavioural Neuroscience, a Practical Approach. IRL Press at Oxford University Press; Oxford, 1, 107-122.

Stone, E. A., Lehmann, M. L., Lin, Y., Quartermain, D. (2007). Reduced evoked fos expression in activity-related brain regions in animal models of behavioral depression. Progress in Neuropsychopharmacology and Biological Psychiatry, 31(6), 1196–1207. doi: 10.1016/j.pnpbp.2007.04.010

Tamashiro, K. L., Sakai, R. R., Shively, C. A., Karatsoreos, I. N., Reagan, L. P. (2011). Chronic stress, metabolism, and metabolic syndrome. Stress, 14(5), 468–474. doi: 10.3109/10253890.2011.606341

Venero, C., Tilling, T., Hermans-Borgmeyer, I., Schmidt, R., Schachner, M., Sandi, C. (2002). Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience, 115(4), 1211-9. doi: 10.1016/S0306-4522(02)00543-2

Walton, J. R. (2013). Aluminium involvement in the progression of Alzheimer's disease. Journal of Alzheimer's Disease, 35(1), 7-43. doi: 10.3233/JAD-121909

Walton, J. R. (2014). Chronic aluminium intake causes Alzheimer's disease: applying sir Austin Bradford Hill's causality criteria. Journal of Alzheimer's Disease, 40(4), 765-838. doi: 10.3233/JAD-132204

Wang, L., Hu, J., Zhao, Y., Lu, X., Zhang, Q., Niu, Q. (2014). Effects of aluminium on β-Amyloid (1–42) and secretases (APP-Cleaving Enzymes) in rat brain. Neurochemical Research, 39(7), 1338-1345.

Willhite, C. C., Karyakina, N. A., Yokel, R. A., Yenugadhati, N., Wisniewski, T. M., Arnold, I. M., Momoli, F., Krewski, D. (2014). Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminium, aluminium oxides, aluminium hydroxide and its soluble salts. Critical Reviews in Toxicology, 44(Suppl.1), 80. doi: 10.3109/10408444.2014.934439

Wirths, O., Multhaup, G., Bayer, T. A. (2004). A modified β- amyloid hypothesis: intraneuronal accumulation of the β-amyloid peptide—the first step of a fatal cascade. Journal of Neurochemistry, 91(3), 513–20. doi: 10.1111/j.1471-4159.2004.02737.x

Yokel, R. A, McNamara, P. J. (2001). Aluminium toxicokinetics: an updated mini review. Pharmacology & Toxicology, 88(4), 159-67. doi: 10.1034/j.1600-0773.2001.d01-98.x

Yuan, C. Y., Lee, Y. J., Hsu, G. S. (2012). Aluminium overload increases oxidative stress in four functional brain areas of neonatal rats. Journal of Biomedical Science, 19, 51. doi: 10.1186/1423-0127-19-51

Zatta, P., Kilyen, M., Kiss, T. (2002). In vivo and in vitro effects of aluminium on the activity of mouse brain acetylcholinesterase. Brain Research Bulletin, 59(1), 41-45. doi: 10.1016/S0361-9230(02)00836-5

Zhang, J., He, J., Chen, Y., Wang, J., & Ma, Y. (2008). Morphine and propranolol coadministration impair consolidation of Y-maze spatial recognition memory. Brain Research, 1230(16), 150-157. doi: 10.1016/j.brainres.2008.06.061

Zimmerberg, B., Sukel, H. L., Stekler, J. D. (1991). Spatial learning of adult rats with fetal alcohol exposure: deficits are sex-dependent. Behavioural Brain Research, 42(1), 49-56. doi: 10.1016/S0166-4328(05)80039-7