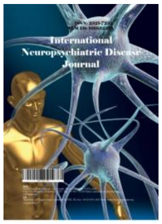

International Neuropsychiatric Disease Journal

14(4): 96-101, 2020; Article no.INDJ.63743
ISSN: 2321-7235, NLM ID: 101632319

Non-invasive Neurologic Assessment in Recovery and Treatment Phase Acrylamide Exposure in Ageing Wistar Rats

O. N. Ilochi[1*] and A. N. Chuemere[1,2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State, Nigeria.
[2]Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Choba, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/INDJ/2020/v14i430142
Editor(s):
(1) Hadaf Hashem Mohammed, Baghdad University, Iraq.
Reviewers:
(1) Bhavana Purushottam Khobragade, RDIK and NKD College, India.
(2) Ramin Seyedian, University of Medical Sciences, Iran.
(3) Hadaf Hashem Mohammed, Baghdad University, College of Veterinary Medecine, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63743

Original Research Article

Received 03 October 2020
Accepted 08 December 2020
Published 21 December 2020

ABSTRACT

Acrylamide is a chemical that naturally forms in starchy food products during every day high temperature cooking. The ingredients, storage and processing conditions greatly influence acrylamide formation in food. Home-cooking choices can have a substantial impact on the level of acrylamide humans are exposed to through the diet. This study scientifically determined the neurologic impact of acrylamide treatment and recovery in male wistar rats of different ages. A total of 20 male wistar rats were randomly sampled into 4 groups aged 45, 60, 75 and 90 days. The treatment dose was 50 mg/kg of acrylamide for 7 days. The study duration was 15 days with 2 phases; day 1 to 7 for exposure phase and day 8 to 14 as recovery phase after acrylamide exposure. Neurologic tests performed include open field test (OFT), buried reward test (BRT) and hand grip test (HGT). The study data was statistically significant at a confidence interval less or equal to 95%. Acrylamide adversely affected the tested physiologic responses in all ages during the phase of exposure. The distance covered and escape attempts were significantly reduced in all ages during acrylamide exposure phase with significant recovery in 45 and 60 days old

**Corresponding author: E-mail: Ilochiogadinma@gmail.com, Ilochi@madonnauniversity.edu.ng;*

animals. Group 45 and 60 days old animals had a wider field area during recovery phase but no observed improvement in exploratory behavior in ages 75 and 90 days. Olfactory and neuromuscular function was significantly improved in all age groups during recovery, however, the response of ages 45, 60 and 75 were similar during recovery. In this study, Acrylamide caused manifestations typical of a neurotoxic agent. The neurologic effect of acrylamide may be reversed if exposure period with the studied dose is within 7 days. Recovery in cognitive, exploratory, emotional, olfactory and neuromuscular function may be dependent on age during exposure.

Keywords: Acrylamide; maillard's; tests; aging; neurotoxicity.

1. INTRODUCTION

Acrylamide is a bioactive chemical that naturally forms in starchy food products during every day high temperature cooking (frying, baking, roasting and also industrial processing at +120°C and low moisture) [1,2]. It mainly forms from sugars [1] and amino acids [1,2,3] (especially asparagine) [1,2] that are naturally present in many foods. The chemical process that causes the formation of acrylamide is known as 'Maillard's Reaction' [1,3,4]. Acrylamide 'browns' food [4] and sweetens its taste [4,5]. It has been found in certain starchy foods [2,3] like potatoes [4,5] that have been cooked and processed at high temperatures [5,6]. Based on animal studies, previous evaluations reveal that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups [7]. Since acrylamide is present in a wide range of everyday foods, this concern applies to all consumers but children are the most exposed age group on a body weight basis [8]. Based on recent scientific data, ingested food was estimated to account for significant contribution to the total exposure of the general public to acrylamide [9]. The most important food groups contributing to acrylamide exposure are fried potato products, coffee, biscuits, crisp and soft bread [3,7,9]. The average intake for the general population was in the range of 0.2–0.9 µg of acrylamide per kilogram of body weight per day as estimated [9]. Polyacrylamide is also used in sugar refining, and residual amounts of acrylamide may remain in the final product [8]. The most essential source of drinking-water acrylamide contamination is the use of polyacrylamide flocculants containing residual levels of acrylamide monomer [7,8]. Acrylamide is of highly mobility in aqueous environments and readily leachable in soil [7,8]. As it has a higher mobility and lower rate of degradation in sandy soils than in clay soils [7], it may contaminate underground water [9]. Most of the acrylamide produced is used as a chemical intermediate or as a monomer in polyacrylamide production [9,10]. Both acrylamide and polyacrylamide are used mainly in the production of flocculants for potable water processing and in the treatment of industrial and municipal effluents [8-10]. They are also used as grouting agents in the construction of wells and reservoirs for drinking-water [10,11]. This study investigated the age-dependent neurologic changes in treatment and recovery from processed food chemical agent, acrylamide.

2. MATERIALS AD METHODS

2.1 Toxicity Study

The Oral lethal dose 50 (LD_{50}) of acrylamide in wistar rats is 124mg/kg [10,11].

2.2 Study Design

This study was conducted within the matrices of the Biomedical Research Facility, Madonna University, Nigeria. A total of twenty (20) male wistar rats of different ages were sampled and exposed to same experimental procedures of regular circadian rhythm and feed *Ad libitum*. All animals were confirmed to be healthy with no previously observed physical or psychosocial disorder.

This study adopted non-invasive techniques. None of the animals were sacrificed neither was there a form of tissue invasion at any period of the experiment.

2.3 Neurologic Analysis

Neurologic tests performed include open field and buried food test.

2.4 Test for Exploratory Behavior

2.4.1 Open field test

Each animal was removed from its home cage and placed in the center of an open field. The duration to open field exposure and inter-trial interval was same for all animals. The behavior of each test sample was carefully monitored during each test period. Test period was 300 seconds per sample.

2.5 Test for Olfactory Response

2.5.1 Buried food test

The animals were fasted overnight before been introduced into clean cages 46cm x 23.5cm x 20cm. The rewarding (food) stimulus was buried in 3cm fresh cage bedding. Kellogg's® cookies was used for this test. Each animal was allowed to acclimate to the cage for 5 minutes after which the latency period (in seconds) taken to find the food stimulus was carefully recorded. This test was conducted before and after the experimental period. Latency of olfaction using buried food test was assayed twice; before and after treatment period.

2.6 Test for Neuromuscular Strength

2.6.1 Hand grip test

A grid was placed horizontally, with the animals carefully place on it ensuring tight grip with both fore and hind limbs. The grid was turned upside down and suspended 18" from surface. All aversive stimuli was removed from test area. The duration of time (in seconds) the animal cling to the grid before falling completely off the grid was recorded.

2.7 Statistical Analysis

In other to properly translate the outcome of this study, data collected from this study was analyzed using One-Way analysis of variance (ANOVA) and Post Hoc analysis with the aid of IBM®SPSS Version 20.0. The Percentage change (*%c*) was also presented in standard charts and tables, adopting methods used by Chuemere et al. [12].

Chart 1. Treatments details

Groups	Age (days)	Treatments
1	45	Acrylamide 50 mg/kg
2	60	
3	75	
4	90	
	N=5	

Chart 2. Study protocol

0	1-7	8-14	15
Start of observation	Period of Acrylamide treatment	Recovery phase of Acrylamide treatment	End of observation

3. RESULTS

The results of this study are presented in tables as shown below.

Table 1. Age-dependent differences in distance covered in an open field in acrylamide treatment and recovery

Age (days)	Distance covered (yd)		
	5	10	15
45	15.24±2.61	13.00±1.44[a]	15.16±0.18[b]
60	13.26±0.34	10.81±1.15[a]	16.61±1.17[a,b]
75	12.11±0.71	9.35±2.17[a]	8.37±0.05[a]
90	10.32±1.25	7.76±1.47[a]	6.21±0.11[a.b]
Total	50.93	40.92	46.35
Average	12.73	10.23	11.58

Key; Values are significantly different at P≤0.05; [a]=different compared to day 5,[b]=different compared to day 10

Table 2. Age-dependent differences in escape attempts in an open field in acrylamide treatment and recovery

Age (days)	Escape attempt (n)		
	5	10	15
45	19.64±1.10	15.13±1.64[a]	17.37±0.01[a,b]
60	25.27±0.27	19.25±0.03[a]	24.60±0.71[b]
75	14.29±1.15	9.71±1.01[a]	6.71±0.24[a,b]
90	8.41±2.21	4.26±0.07[a]	2.27±1.31[a,b]
Total	67.61	48.35	50.95
Average	16.90	12.08	12.73

Key; Values are significantly different at P≤0.05; [a]=different compared to day 5,[b]=different compared to day 10

Table 3. Age-dependent differences in Field area visited in an open field in acrylamide treatment and recovery

Age (days)	Field Area		
	5	10	15
45	CCo	CCo	CPCo
60	CoP	CoP	CPCo
75	CoP	Co	CoP
90	Co	Co	Co

Key; C=Central area, P=Perpheral area, Co=Corner

Table 4. Age-dependent differences in olfactory response in acrylamide treatment and recovery

Age (days)	Latency (s)		
	5	10	15
45	424.21±5.61	470.76±0.90[a]	457.12±1.82[a,b]
60	460.38±2.28	530.73±7.63[a]	510.32±0.45[a,b]
75	481.56±3.71	570.21±5.10[a]	553.17±4.17[a,b]
90	580.12±0.81	598.00±2.21[a]	595.19±0.26[a]
Total	1946.27	2169.5	2115.8
Average	486.56	542.37	528.95

Key; Values are significantly different at P≤0.05; [a]=different compared to day 5,[b]=different compared to day 10

Table 5. Age-dependent differences on neuromuscular function in acrylamide treatment and recovery

Age (days)	Time (s)		
	5	10	15
45	17.46±0.08	16.22±0.47[a]	19.34±1.12[a,b]
60	18.84±0.19	17.01±1.30[a]	20.71±2.40[a,b]
75	10.23±1.17	9.62±0.57	11.63±0.09[a,b]
90	7.36±1.61	5.32±2.61[a]	7.91±0.05[,b]
Total	53.89	48.17	59.59
Average	13.47	12.04	14.89

Key; Values are significantly different at P≤0.05; [a]=different compared to day 5,[b]=different compared to day 10

4. DISCUSSION

This study investigated the changes in neurologic responses before and after acrylamide exposure. Acrylamide was administered through orogastric route. Only non-invasive tests were used. An open field test basically measures and translates neurologic behaviors like emotion, exploration and cognition [12]. The open-field test bas attained the status of one of the most widely used instruments in animal psychology [12,13]. From the results of this study, there was a significant improvement in distance covered during recovery phase for ages 45 and 60 days. The 5 days old animals showed a significant decline in distance covered from test day 5 to 10. Compared to day test day 10, there was a significant increase in distance covered on test day 15, which is similar to the distance covered on day 5. Age 60 days animals showed similar trend but distance covered on test day 15 was significantly higher compared to test days 5 and 10. The group of animals aged 75 and 90 showed a significantly progressive decline in distance covered in an open field from test days 5 to 15. It is a physiologic instinct to escape the test environment [12-14]. An initial significant reduction in attempts made to escape was observed in ages 45 and 60. The initial reduction in escape attempt from test day 5 to 10 was almost reversed from test day 10 to 15 in both age groups. Ages 75 and 90 reduced their attempts to escape progressively with the test days. The field area represents the area of the open field covered by the animals during each test period [15-17]. Rodents with high exploratory behavior tend to cover larger areas [18]. Exploratory behavior of these animals reflects their capacity to interact, learn and adapt to the novel environmental [19]. Exploratory behavior may increase after repeated exposure, a character that translates the cognitive ability of an animal. Test group with 45 days old animals showed progressive improvement in field area covered from day 10 to 15. They covered similar areas in days 5 and 10. A wider area was also covered on test day 15 of age 60. The groups aged 75 and 90 exhibited similar exploratory behavior. Areas covered in these groups were predominantly corners and peripheral with less escape tendency. A significant improvement in olfactory response from test day 10 to 15 was observed in tests groups aged 45 to 75 days. The latency of the animals during recovery was of less duration compared to the period of acrylamide treatment. The latency in both phases of exposure was significantly different. The change in latency of 90 days tests was non-significant in the recovery phase when compared to day 10. Acrylamide may have altered the mechanisms responsible for olfaction. This alteration may be at the level of the olfactory receptors in respiratory mucosa of the animals, at the region of the olfactory bulb or traced to the higher centers; the anterior olfactory nucleus, piriform cortex, olfactory tubercle, anterior cortical amygdaloid nucleus, periamygdaloid nucleus and entorhinal cortex [19,20]. Hand grip test was used to assess the neuromuscular strength of the animals [15]. A significant improvement in neuromuscular strength was peculiar to the test day 15 of 45, 60 and 75 days old animals with only a significant increase from test day 10 to 15 in 90 days old animals. Motor unit recruitment and neuromuscular transmission are altered by neurotoxicants [14].

5. CONCLUSION

The neurologic effect of acrylamide is age-dependent. Significant recovery in exploratory, olfactory and motor function after acrylamide treatment is most common in animals aged less than 75 days. From physical observation of the various samples in this study, it can be stated that acrylamide formed via chemical modification of food molecules may adversely affect some neurologic responses. This effect is age-dependent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study followed the already existing principles and guidelines of animal use in research and was approved by Madonna University Research Ethics Committee, in November, 2019. The approved standard of animal use was developed by the University of Melbourne Animal Care and Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare and Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guo C, Xie YJ, Zhu MT, Xiong Q, Chen Y, Yu Q, Xie JH. Influence of different cooking methods on the nutritional and potentially harmful components of peanuts. Food Chem. 2020;316:126269.
2. Maan AA, Anjum MA, Khan MKI, Nazir A, Saeed F, Afzaal M, Aadil RM. Acrylamide formation and different mitigation strategies during food processing-A review. Food Rev. Int. 2020;1–18.
3. Capuano E, Fogliano V. Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT Food Sci. Technol. 2011;44:793–810.
4. Jozinovi´c A, Šarkanj B, Aˇckar D, Balenti´c JP, Šubari´c D, Cvetkovi´c T, Ranilovi´c J, Guberac S, Babi´c J. Simultaneous determination of acrylamide and hydroxymethylfurfural in extruded products by LC-MS/MS method. Molecules. 2019;24:1971.
5. Guo Q, Sun DW, Cheng JH, Han Z. Microwave processing techniques and their recent applications in the food industry. Trends Food Sci. Technol. 2017; 67:236–247.
6. Khan I, Tango CN, Miskeen S, Lee BH, Oh DH. Hurdle technology: A novel approach for enhanced food quality and safety—A review. Food Control. 2017;73:1426–1444.
7. Reverte-Ors JD, Pedreno-Molina J, Fernandez PS, Lozano-Guerrero AJ, Periago PM, Díaz-Morcillo A. A novel technique for sterilization using a power self-regulated single-mode microwave cavity. Sensors. 2017;17:1309.
8. Torres-Gonzalez JD, Dueik V, Carré D, Bouchon P. Effect of the addition of soluble dietary fiber and green tea polyphenols on acrylamide formation and in vitro starch digestibility in baked starchy matrices. Molecules. 2019;24: 3674.
9. Jing Y, Li X, Hu X, Ma Z, Liu L, Ma X. Effect of buckwheat extracts on

acrylamide formation and the quality of bread. J. Sci. Food Agric. 2019;99:6482–6489.
10. Mi´skiewicz K, Rosicka-Kaczmarek J, Nebesny E. Effects of chickpea protein on carbohydrate reactivity in acrylamide formation in low humidity model systems. Foods. 2020;9:167.
11. Stadler RH, Studer A. Chapter 1—acrylamide formation mechanisms. in acrylamide in food, analysis, content and potential health effects 2016; Academic Press Academic Press: Waltham, MA, USA. 2016;1–17.
12. Ogadinma Ilochi, Arthur Nwafor Chuemere. Neuroprotective potential of avocado peel correlates with antioxidant status in starvation and refeeding in wistar rats. World Wide Journal of Multidisciplinary Research and Development (WWJMRD). 2019;5(1):70-74.
13. Ilochi Ogadinma, Daniel Yaro Onoja, Chuemere Arthur Nwafor. Neurodynamic influence of hydrocortisone on rhinencephalic and telencephalic brain areas. International Neuropsychiatric Disease Journal. 2019;13(2):1-7. Article no.INDJ.52924.
ISSN: 2321-7235, NLM ID: 101632319.
14. Ilochi Ogadinma, Arthur Nwafor Chuemere, Ekwem Ikechukwu, Bassey Samuel. Neuroprotective and antitoxic potential of hydromethanolic extract of *Allium cepa* in experimental rats. European Journal of Pharmaceutical and Medical Research. EJPMR. ISSN: 2394-3211 EJPMR. 2018;5(9):138-143.
15. Ilochi ON, Kolawole TA, Oluwatayo BO, Chuemere AN. Starvation-Induced changes in memory sensitization, habituation and psychosomatic responses. International Journal of Tropical Disease and Health. 2019;35(3):1-7.
16. Michalak J, Gujska E, Czarnowska M, Klepacka J, Nowak F. Effect of storage on acrylamide and 5-hydroxymethylfurfural contents in selected processed plant products with long shelf-life. Plant Foods Hum. Nutr. 2016;71:115–122.
17. International Agency for Research on Cancer. IARC Acrylamide. In IARC Monographs on the Evaluation of Carcinogen Risk to Humans IARC: Some Industrial Chemicals; International Agency for Research on Cancer: Lyon, France. 1994;60:389–433.
18. Zamani E, Shokrzadeh M, Fallah M, Shaki F. A review of acrylamide toxicity and its mechanism. Pharm. Biomed. Res. 2017; 3:1–7.
19. Nguyen LT, Choi W, Lee SH, Jun S. Exploring the heating patterns of multiphase foods in a continuous flow, simultaneous microwave and ohmic combination heater. J. Food Eng. 2013; 116:65–71.
20. Tepe Y, Çebi A, Aydin H. Acrylamide content and color formation of hazelnuts roasted at different processing temperatures and times. Eur. Food Res. Technol. 2020;246:1543–1549.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63743