International Journal of Basic, Applied and Innovative Research
IJBAIR, 2016, 5(3): 100 - 113
ISSN: 2315 - 5388
www.arpjournals.com; www.antrescentpub.com
E-ISSN: 2384 - 681X

RESEARCH PAPER

NEUROBEHAVIOURAL ACTIVITY ON DECLARATIVE MEMORY IN THE NOVEL OBJECT RECOGNITION TASK FOLLOWING CONSUMPTION OF BEANS DIET IN SWISS WHITE MICE

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Received: 30th August, 2016 Accepted: 29th September, 2016 Published: 30thSeptember,2016

***Endorsed By**: Innovative Science Research Foundation (**ISREF**) and International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society; and IRMS Informatics India (J-Gate)*

ABSTRACT

Neuro-behavioural actions of serotonin include mood, memory, learning and sleep. Interestingly, beans -a stable diet of Nigerians, contain serotonin and its precursor -5-Hydroxytryptophan. This study investigated the effects of cooked and uncookedbeans on neurobehavioral parameters using 40 Swiss white mice. The mice were randomly assigned into four groups, viz; A –control; B -cooked beans diet (50% w/w); C -uncooked beans diet (50% w/w) and D placed on serotonin precursor (5-HTP) diet (0.2mg/50g w/w) (D) for thirty days. All the mice had access to clean drinking water *ad libitum*. The phytochemical properties of the beans as well as the LD_{50} of beans and serotonin precursor (5-HTP), were determined prior to the assessment of neurobehavioral parameters. Serotonin and serotonin precursor (5-HTP) concentration were measured in cooked and uncooked beans using High performance liquid chromatography (HPLC) analysis. The novel object recognition task (open field) was used for cognitive declarative memory. Results on cognitive declarative memory training showed that group B and C learnt better than the control (A) ($p<0.05$). Group D produced similar results observed in B and C; suggesting that serotonin may for the observation in B and C. Thus, serotonin in beans diet can improve cognitive declarative memory.

Keywords: Serotonin, 5-Hydroxytryptophan, NORT, Memory, Beans, Mice.

INTRODUCTION

Common bean (*Vignaunguiculata*) is a dicotyledon and belongs to the pea family (Gatel, 1994). Nowadays; there are many dry bean classes depending on the colour, shape and size of the beans. Some of the commonly consumed varieties are navy, black, kidney and pinto beans. The plant is edible for dry beans and green beans. Dry beans are the mature seeds, whereas green beans are the immature seeds wrapped in pods (Wortmann, 2006). Overall, common bean is a staple food in many parts of the world (Wader *et al*., 1998). It offers a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds (Adeyere, 1995). Researchers are particularly interested in the high antioxidant activities observed in beans. It is also a very nutritious food from many aspects and it is not surprising that nutritionists would characterize beans as a nearly perfect food (Shansuddin and Elsayed, 1998; Van der poel*et al*., 1990b).It has been reported that beans have ant carcinogenic, anti-mutagenic (Gref*and* Eaton, 1993; anti – inflammatory, anti-diabetic,hypoglycaemic, depurative, cardio-protective and antioxidant effects (Bennick*et al*., 2008).

It has also been reported that beans contain serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (Portas*et al.,* 2000).Beans contain other chemical compounds including saponins, tannins, glycosides, flavonoids etc. Among the array of chemical constituents, notably, serotonin has neurobehavioural actions such as mood, memory, learning, and sleep (Brunton *et al*.,2005).Serotonin has been shown to act (*Ceanorhabditiselegans*) as neurotransmitter to modulate behaviour in response to changing cues, acting on both neurons and muscles to affect egg laying, pharyngeal pumping, locomotion and learning(Daniel and zicheal,2007).Since beans contain neurotransmitters and chemicals that can potentially affect

behavioural patterns, it may be worthwhile to find out whether long-term consumption of cooked beans diet can affect behaviour. This was of interest when we consider the challenges that confront human behaviour and how behavioural disorders remain a global concern (Messman,2005). Human behaviour is believed to be influenced by endocrine and nervous system. The complexity in the behaviour of an organism is correlated to the complexity of its nervous system. Thus, organisms with more complex nervous systems (like the human) have a greater capacity to learn new responses and adjust their behaviour. This behaviour is influenced by physical and psychological changes that result from a complex state of feeling described as emotion (Cacioppoand Gardner, 1999).

We are aware physiologically, that to bring some emotion/behaviour under control is difficult, perhaps, owing to the paucity in connection between the limbic system(the part of the brain that controls our emotion) and the neocortex (the part of the brain whose activity can modify emotional behaviour).Furthermore, the prolonged after discharge in the limbic system following emotional stimulation makes emotional responses to outlast their stimuli (Ganong*et al.,*2010; Osim,2012).Therefore, owing to paucity of connections between the neocortex and the limbic system as well as the prolonged after discharge of the limbic system after stimulation, it is difficult to control our emotions.

In a series of talk presented at a conference, Osim(2012) noted that many people have devised various ways to help them control their emotion. They have explored methods such as music, yoga, exercise, drugs, alcohol, and religion all of which are believed to affect emotional state in one way or the other. Also, it has been noted that apart from the fact that it is quite expensive to manage behavioural conditions with medication, no social or behavioural concern will just vanish through medication. There is therefore the need to explore an alternative that will not leave us with deleterious side effects.

Sequel to these, Osim and his team have been investigating to find out if our common consumables (food substances) can affect our behaviour. They have shown that consumption of thermoxidized palm oil in the long term, increased fear and anxiety in animals (Osim,2012), common malaria drugs such as chloroquine increase anxiety and pain perception(Lelei*et al.,*2012),while artesunate decreases locomotion and exploration(Davies *etal.,*2013).

It is likely that behavioural changes can be associated with the consumption of stable foods like beans,since it contains neurotransmitters; especially serotonin and its precursor-5-Hydroxytryptophan, known to exhibit neurobehavioural actions. This study therefore, investigates the effects of cooked and uncookedbeans on neurobehavioral parameters using 40 Swiss white mice.

MATERIALS AND METHODS

Experimental animals/grouping: Forty (40) adult Swiss white mice weighing between 15-30g and procured from the disease-free stock of the animal house, Department of Physiology, University of Nigeria, Nsukka, were used for this research work. The animals were randomly assigned into four (4) groups of ten (10) animals each. Each mouse in a study group was individually housed in a plastic cage with iron gauze bottom grid and a wire screen top. The animal room was adequately ventilated, and kept at room temperature and humidity of 22 ± 3^0c and 40-70% respectively with 12-hour natural light-dark cycle.

Experimental Design: Theanimalswere weighed using a digital weighing balance. Identification of animals was simply done using identification cards attached to each cage, because animals were singly housed. The mice were grouped into four. Each of these groups consisted of ten (10) mice [group 1=control, group 2=cooked beans, group 3=uncooked beans and group 4= 5HTP]. In all, forty (40) mice were used for the experiments and the experiments lasted for thirty (30) days. The mice aged between 30 and 35 days and weighed between 15g and 30g. All the animals were clinically and andrologically examined and confirmed to be free from systemic disorders.

Preparation of feed: Ten cups of beans was procured but 5 cups were cooked, air dried, and blended into powder form, **while the other five cups were stored in a plastic container.**

Preparation of powdered beans diet: Fifty grams of powdered cooked beans was mixed separately with 50g of normal rodent chow making 50 %w/w) of beans diet. The diet was then used to feed the test groups.

Preparation of serotonin precursor diet: Synthetic serotonin precursor(5-Hydroxytryptophan) was obtained from May and Baker (M&B) limited, Enfield, Middle Sex, United Kingdom(UK), and used for the study. From the estimation of the powdered 5-Hydroxytryptophan (serotonin precursor) content of cooked beans according to the method of Feldman and M-

Lee (1995) as modified by Mosienko*et al.*, (2012), the serotonin precursor diet was prepared by mixing 20mg(0.04g) of the precursor in 100g of the feed. One gram (1g) of the mixture was mixed with 99g of the feed. So, the amount of 5HTP added was equivalent to that contained in the beans diet. An electric blender was used to blend the mixture to form the serotonin precursor diet.

PROCEDURE: The novel object recognition task was originally developed to test for declarative memory (Brown *et al*., 1999). Prior to testing, the mice were habituated to the apparatus for 5-min within 24-hours beforehand.The mice were carried to the test room within their cages and run individually**.** Mice were moved from their cages to the testing apparatus and back using a small container. After each 5-min trial, the mice were returned to their cages and the apparatus was cleaned with 70 % ethyl alcohol and permitted to dry between trials.

The mouse was scooped up from its cage in a yogurt container and placed in the middle of the open field arena. Each mouse was allowed to explore the arena and objects for 5-min. At the end of the trial the mouse was removed from the apparatus using the yogurt container and returned to its cage. After 15 minutes, inter-trial interval (retention period) the mouse was returned to the test apparatus (trial 2). The arena now contains the familiar object (01 or 02) in one of the two locations in trial 1 and a new object (N) that replaces O1 or O2. The same behaviours recorded for trial 1 was recorded for 5-min for trial 2.

BehaviouralMeasures: Thebehaviours scored using the Open Field (Brown et al, 1999; Podhorna& Brown, 2002) include:

1 Line Crossing: frequency with which the mice crossed one of the grid lines with all four paws.
2. Rearing: frequency with which the mice stood on their hind legs in the maze.
3. Rearing Against a Wall: frequency with which the mice stood on their hind legs against a wall of the open field.
4. Stretch Attend Postures: frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
5. Grooming: frequency and duration of time the animal spent licking or scratching itself while stationary.
6. Approaches to Each Object: directing the nose to the object at a distance of < 1 cm and/or touching it with the nose.
7. Time Spent with Each Object: sniffing or climbing the object.

RESULTS:

Frequency of exploration in two objects novel task during familiarization period (day 1): Figure 1 shows the comparison between the frequency of exploration in the two objects novel test during the familiarization period (day 1). There mean values are: 3.70 ± 0.70; 2.38± 0.63; 4.00 ±0.50 and 6.00 ± 0.49/5 min in object (A) fed with Normal, cooked, uncooked beans and serotonin precursor diets respectively. The values for the novel objects are: 8.00 ± 0.60; 4.00± 0.90; 5.88 ±0.52 and 3.71 ± 0.42/5 min for mice fed Normal, cooked, uncooked beans and serotonin precursor diets respectively.

In object A, the level of familiarization in the serotonin precursor fed mice was significantly higher ($P<0.05$) compared to control. However, the serotonin precursor group was also significantly higher when compared to cooked and uncooked beans group of mice.

In the novel object task the result showed that the cooked beans and serotonin precursor fed mice was statistically lower ($P<0.05$) compared to the control.

Duration of exploration in the novel objects recognition task during familiarization period (day 1): Figure 2 shows the comparison between the durationsof exploration in the two objects novel task during the familiarization period (day 1). There mean values are 14.5 ± 2.62; 17.4± 3.64; 20.3 ±1.75 and 27.57 ± 2.30/5 min in object (A) fed with Normal, cooked, uncooked beans and serotonin precursor diets respectively. The values for the novel objects are 31.02 ± 3.00; 30.75± 2.30; 26.54 ±1.30 and 16.78 ± 1.60/5 min for mice fed Normal, cooked, uncooked beans and serotonin precursor diets respectively.

In Object A, the duration of time it took for the animals fed with cooked, uncooked beans and serotonin precursor diet to explore the object was significantly higher ($P<0.05$) compared to control. Though the uncooked beans group tends to be significantly higher when compared to cooked beans group**.** The serotonin precursor group however, was significantly higher ($P<0.05$) compared to cooked and uncooked beans group of mice.

In the novel object task the uncooked beans and serotonin precursor group was significantly lower (P<0.05) compared to control. Though the uncooked beans group was statistically lower when compared to cooked beans group. However, the serotonin precursor fed mice was significantly lower than that of the cooked and uncooked beans group.

Frequency of exploration in the novel object recognition task during testing period (day 2): Figure 3 shows the comparison between the frequencies of exploration in the two objects novel task during the testing period (day 2). The mean values of object are 3.60 ± 0.85; 4.25± 0.88; 5.00 ±1.04 and 7.86± 0.51/5 min for mice fed Normal, cooked, uncooked beans and serotonin precursor diets respectively. The novel object values are 4.40 ± 0.56; 4.00 ± 0.82; 3.57 ±1.07 and 3.00 ± 0.69/5 min for mice fed with Normal, cooked, uncooked and serotonin precursor diet respectively.

In object A, the frequency of time it took the animals fed cooked, uncooked beans and serotonin precursor diet to explore the object was significant higher (P<0.05) compared to control. Though the serotonin precursor group was significantly higher when compared to the cooked beans group.

During the novel object test, the uncooked beans and serotonin precursor group showed a significantly lower level of exploration when compared to control (P<0.05). Though the uncooked beans group was significantly lower when compared to cooked beans group. The serotonin precursor group however, was significantly lower (P<0.05) compared to cooked and uncooked beans group of mice.

Frequency of line crossing during habituation, familiarization and testing periods of the nort: The frequency of line crossings during habitation, familiarization and testing period of the novel recognition test in trial 1 was 89.10 ± 10.19; 71.13± 7.12; 80.88±12.35 and 52.43± 10.06/5 min for mice fed with Normal, cooked, uncooked and serotonin precursor diets respectively.

In trial the values are 92.10 ± 10.92; 60.63 ± 11.58; 74.25±6.78 and 58.57 ± 6.18/5min for mice fed Normal, cooked, uncooked and serotonin precursor diet respectively.

While in trial 3, the mean values were, 93.90 ± 10.63; 73.13 ± 12.85 ± 63.50; 10.61 and 54.71 ± 8.05/5min for mice fed with normal, cooked, uncooked and serotonin precursor diet respectively.

The frequency of line crossings during the habitation, familiarization and testing period of the novel recognition test shows that in Trial 1, the serotonin precursor fed mice line crossings was significantly lower compared to control (P<0.05). In trial 2 the frequency of line crossings was also significantly lower in the cooked beans and serotonin precursor group compared to control. However, in trial 3, the uncooked beans and serotonin precursor group showed statistically lower level of line crossings compared to control (*See figure 4*).

Frequency of grooming in the nort: Figure 5 shows the comparison between the frequency of grooming in the novel recognition test during habitation, familiarization and testing periods.

In trial 1, the mean values are 3.29 ± 0.20; 3.00 ±0.13; 2.56 ± 0.15 and 2.10 ± 0.20/5min for mice fed normal, cooked, uncooked and serotonin precursor diet respectively. In trial 2, the values are 3.20± 0.28; 3.05 ± 0.22; 2.65 ±0.17 and 2.20 ± 0.23/5min for mice fed with normal, cooked, uncooked and serotonin precursor diet. Trial 3 values were, 3.40 ± 0.10; 3.20 ± 0.15; 3.00 ± 0.15 and 2.86 ± 0.12 for mice fed with normal, cooked, uncooked and serotonin precursor diet respectively.

The grooming frequency in trial 1 for mice fed with cooked, uncooked, and serotonin precursor diet was significantly lower compared to control (P<0.05). However, the serotonin precursor group was statistically lower (P<0.05) compared to cooked and uncooked beans group of mice.

In trial 2 and trial 3, the uncooked beans and serotonin precursor group was significantly lower (P<0.05) compared to control. Though the uncooked beans group was significantly lower compared to cooked beans group. However, the serotonin precursor group was significantly lower compared to cooked and uncooked beans group of mice.

Grooming duration in the nort: The grooming duration in the novel recognition test during habituation, familiarization and testing periods is shown in ((*See figure 6*).

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In trial 1, the mean values are 38.73± 3.00; 35.40 ± 4.41; 30.12±2.00 and 29.40 ± 1.20/5min for mice fed with normal, cooked, uncooked and serotonin precursor diet respectively. In trial 2, the values are, 37.40 ± 2.50 34.56 ± 4.30; 31.20 ± 2.30 and 29.67 ± 2.10/5min for mice fed with normal, cooked, uncooked beans and serotonin precursor diet respectively.
The grooming duration in trial 1 and trial 3 for mice fed with uncooked beans and serotonin precursor diet was significantly lower ($P<0.05$) compared to control. However, the serotonin precursor and uncooked beans fed mice was also significantly lower compared to cooked beans group of mice.

In trial 2, the grooming duration for mice fed with uncooked beans and serotonin precursor diet was significantly lower ($P<0.05$) compared to control. However, the serotonin precursor for mice was statistically lower when compared to cooked beans group ((*See figure 6*).

Stretch attend posture in the nort: In trial 1, the stretch attend posture (SAP) were, 2.30± 0.10; 2.00±0.12; 1.86 ± 0.12 and 1.20 ±0.14/5min for mice fed with normal, cooked, uncooked and serotonin precursor diet respectively. In trial 2, the values are 2.25 ±0.08; 2.12 ± 0.10; 1.87 ± 0.09 and 1.20±0.10/5min for mice fed with normal, cooked, uncooked and serotonin precursor diet respectively. In trial 3, the values were 1.13 ± 0.09; 1.00±0.10; 0.75±0.10 and 0.57±0.10/5 min for mice fed normal, cooked, uncooked and serotonin precursor diet.

In trial 1, the stretch attend posture shows that the mice fed cooked, uncooked and serotonin precursor diet was significantly lower ($P<0.05$) compared to control. However, the uncooked beans group was significantly lower than the cooked beans group while the serotonin precursor fed mice was also significantly lower ($P<0.05$) compared to cooked and uncooked beans group of mice.

In trial 2, the cooked, uncookedand serotonin precursor group was significantly lower ($P<0.05$) compared to control. However, the uncooked beans and serotonin precursor group was statistically lower when compared to cooked beans group, while in trial 3, the uncooked beans and serotonin precursor fed mice was significantly lower ($P<0.05$) compared to control. However, the uncooked beans group was statistically lower than the cooked beans group, while the serotonin precursor fed mice was significantly lower compared to cooked and uncooked beans group of mice (*See figure 7*).

DISCUSSION:

The novel object recognition task (NORT) was originally developed for rats as a test for declarative memory, after it was discovered that rats will spend more time investigating a new object than a familiar one (Ennaceur and Delacour, 1988). It has since been validated as a test for recognition memory in mice (Brown *et al*., 1999; Podhorna and Brown, 2002; Sik et *al*., 2003). An advantage of the novel object recognition task over other tests of learning and memory is that it is less stressful for the mouse (there is no need for food deprivation as in the radial arm maze, or swim-stress as in the Morris water maze), as stress may interfere with memory performance (Sik et al., 2003). In the novel object recognition task. The frequency, duration of exploration in the novel object recognition task during familiarization period(day 1) and the frequency of exploration during the test period (day 2), showed that mice fed beans(cooked and uncooked) diets and serotonin precursor diet learned better than the control group of mice that consumed normal rodent chow. Also, the frequency of line crossings during habituation, familiarization and testing period of the NORT with the frequencies and duration of grooming in the NORT is also consistent with the results obtained above. Thus, indicating that beans(cooked and uncooked) diets and serotonin precursor diet fed mice takes lesser or shorter time to recognize the novel object when compared to the control, which means this group of mice learned better when compared to the control. However, the uncooked beans group and the serotonin precursor group of mice were able to learn better than the cooked beans group.

Beans are rich in vitamin B6 and contain serotonin (5-HT) as well as its precursor 5-Hydroxytryptophan (5-HTP) and tryptophan (Portas*et al*., 2000) in significant measures. Tryptophan hydroxylase converts tryptophan into 5-HTP which in turn is converted into serotonin (5-HT) by the enzyme aromatic amino acid decarboxylase that uses vitamin B6 as co-enzyme. Serotonin is a neurotransmitter that is known to improve learning and memory as well as cognitive functions (Portas*et al*., 2000; Walther *et al.,* 2003). Furthermore, this ability of beans (cooked) diet to improve declarative memory is further enhanced by the presence of these chemical and mineral compounds such as glutamic acid(Kovale*vetal*.,1989), magnesium, potassium, phosphorus and calcium, etc., which are known to enhance memory& learning.

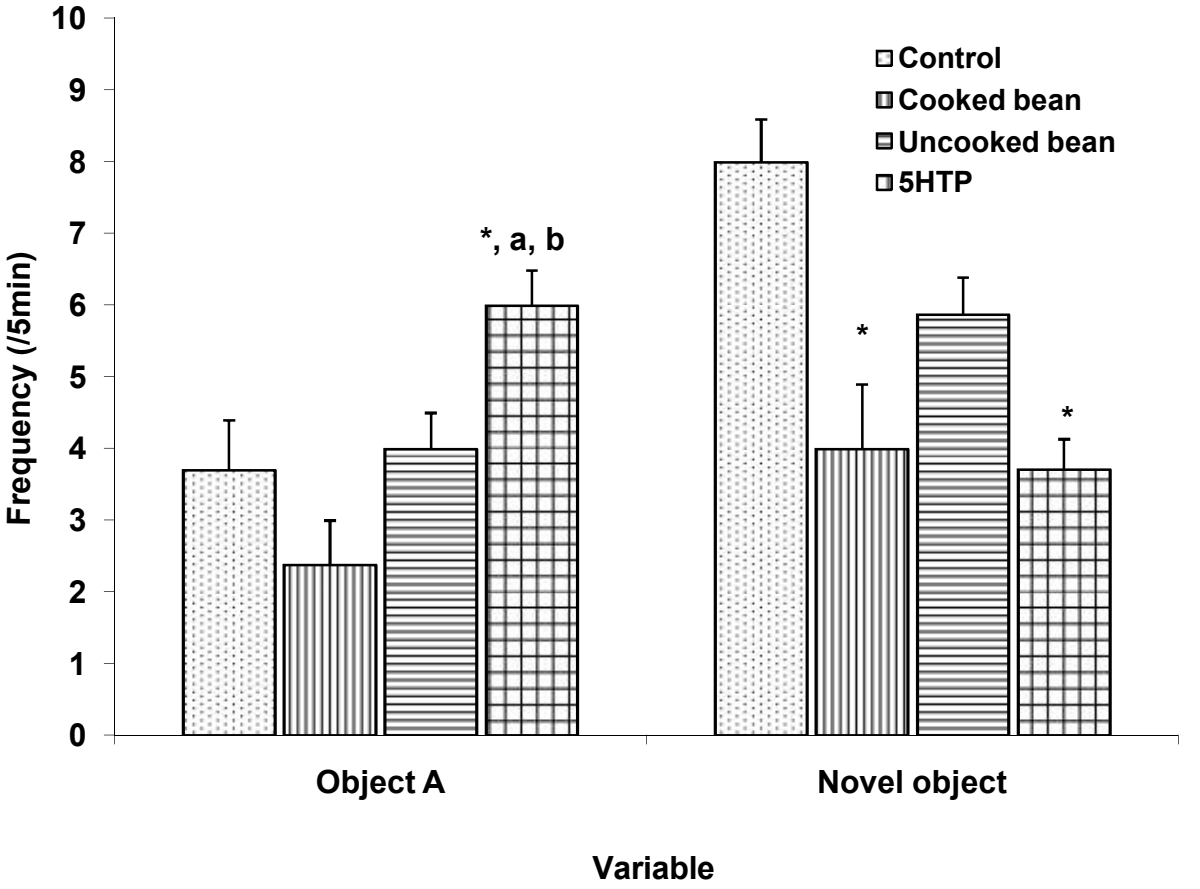

Figure 1: Comparison of frequency of exploration in two objects novel task among the different experimental groups during familiarization period (day 1).
Values are expressed as mean ± SEM, n = 10.

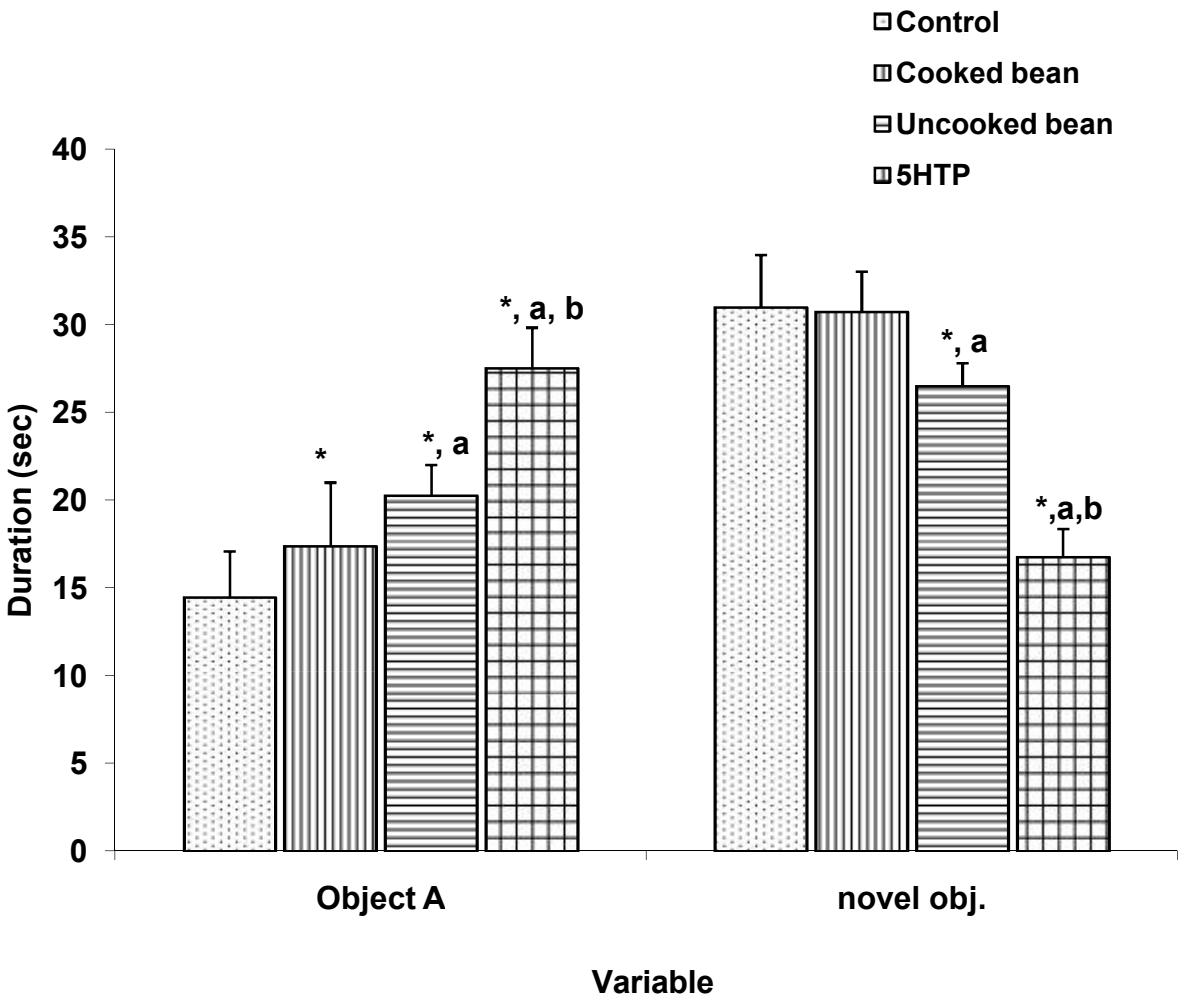

Figure 2: Comparison of duration of exploration in two objects novel task among the different experimental groups during familiarization period (day 1). Values are expressed as mean ± SEM, n = 10.
***significantly different from control at p<0.05;**
a = significantly different from cooked bean at p<0.05;
b = significantly different from uncooked bean at

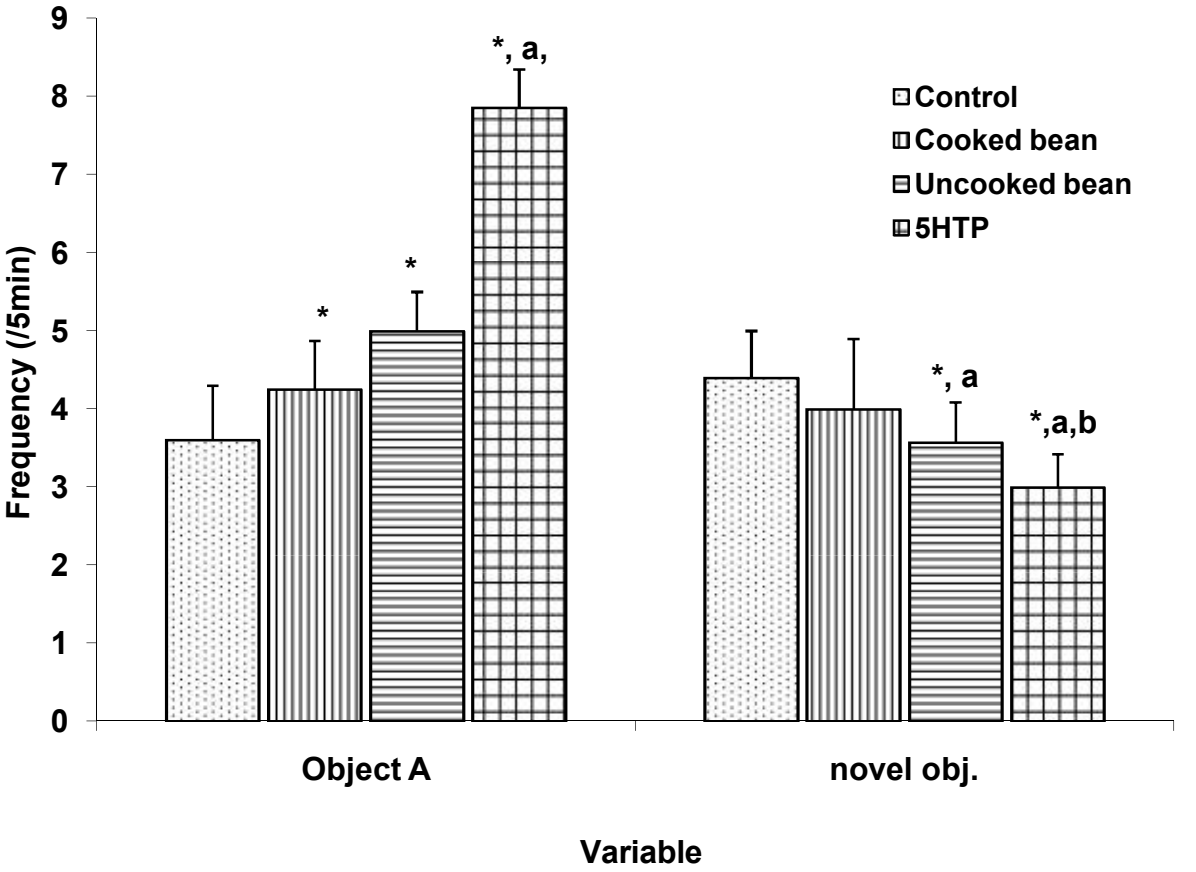

Figure 3: Comparison of frequency of exploration in two objects novel task among the different experimental groups during testing period (day 2). Values are expressed as mean ± SEM, n = 10.
***significantly different from control at p<0.05;**
a = significantly different from cooked bean at p<0.05;
b = significantly different from uncooked bean at

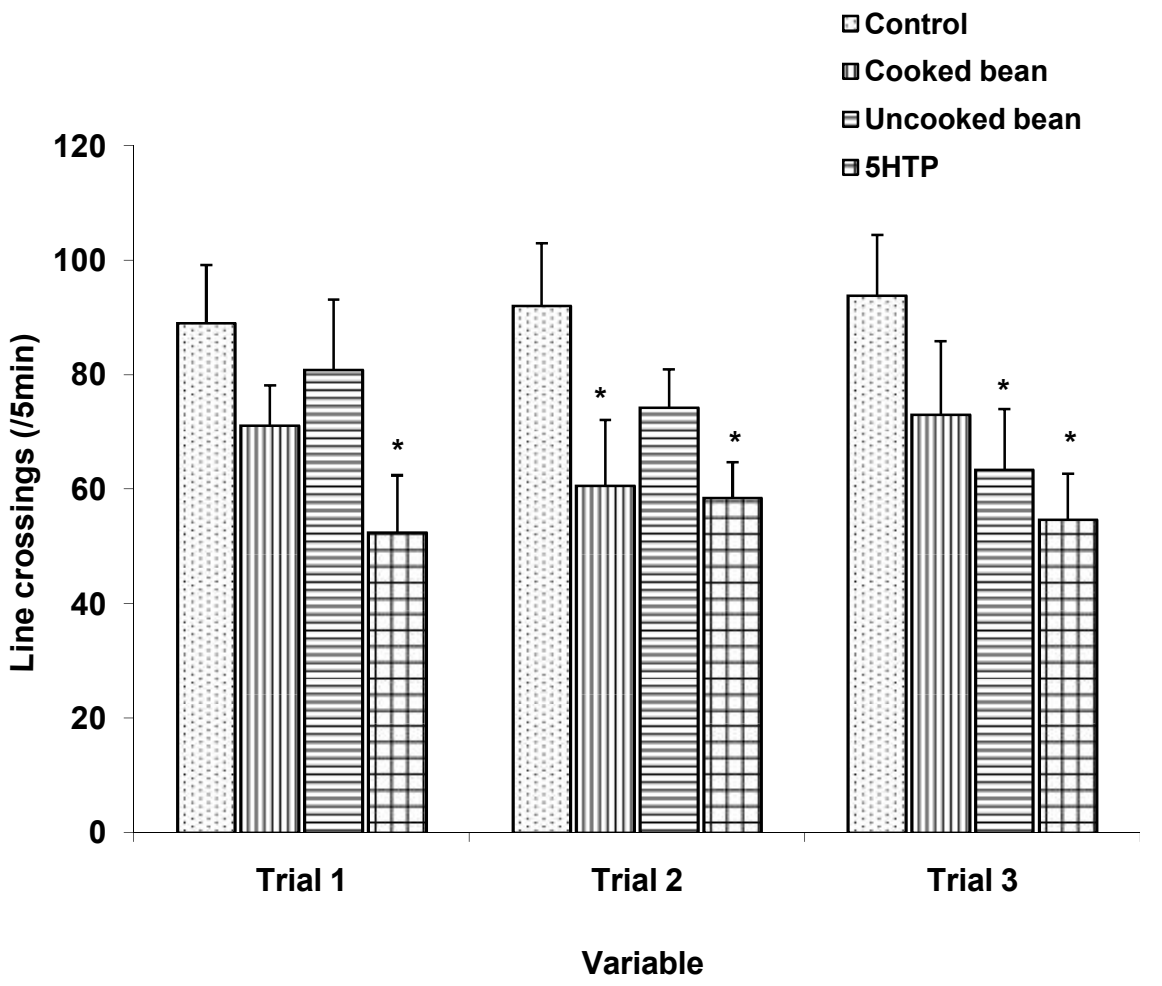

Figure 4: Comparison of frequency of line crossings among the different experimental groups during habituation, familiarization and testing periods of the novel object recognition test.
Values are expressed as mean ± SEM, n = 10.

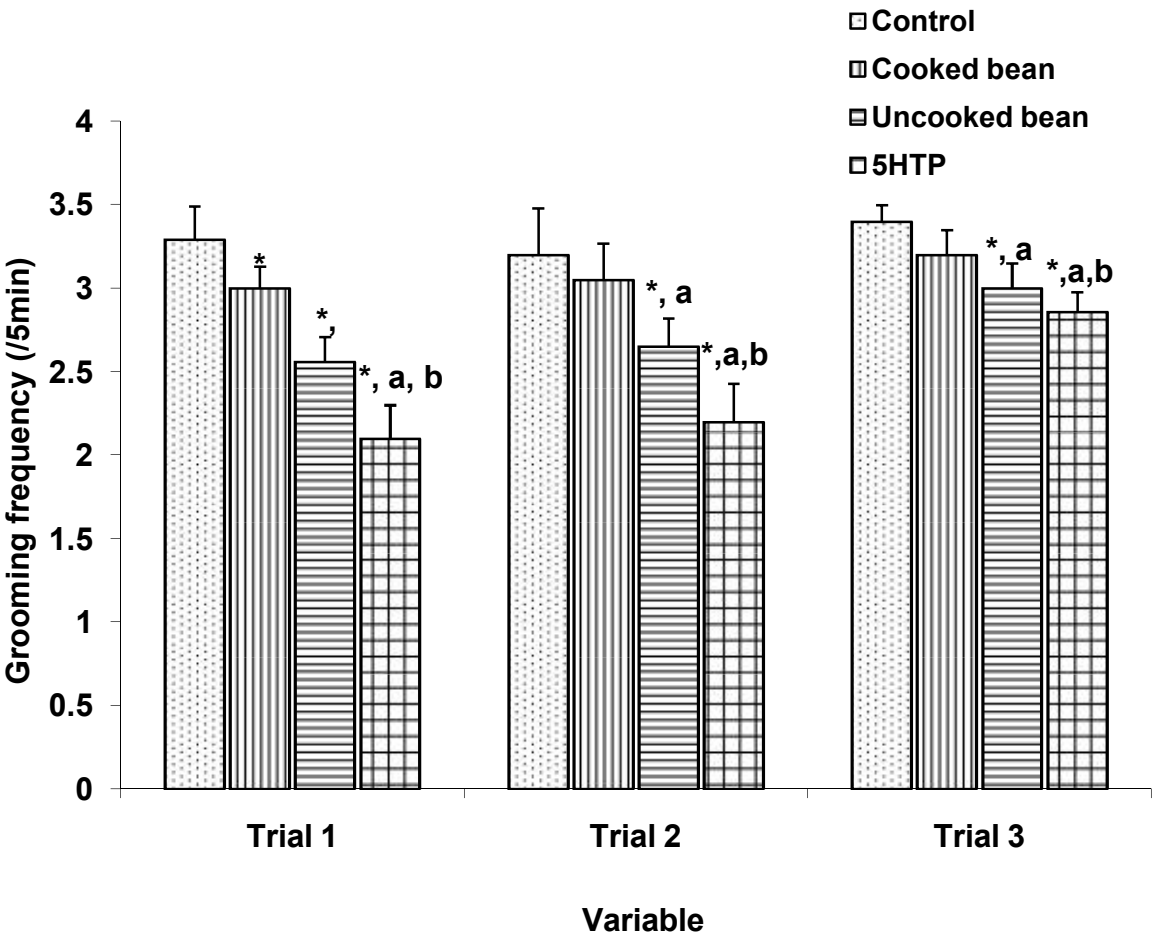

Figure 5: Comparison of grooming frequency among the different experimental groups during habituation, familiarization and testing periods of the novel object recognition test.
Values are expressed as mean ± SEM, n = 10.
***significantly different from control at p<0.05;**
a = significantly different from cooked bean at p<0.05;
b = significantly different from uncooked bean at

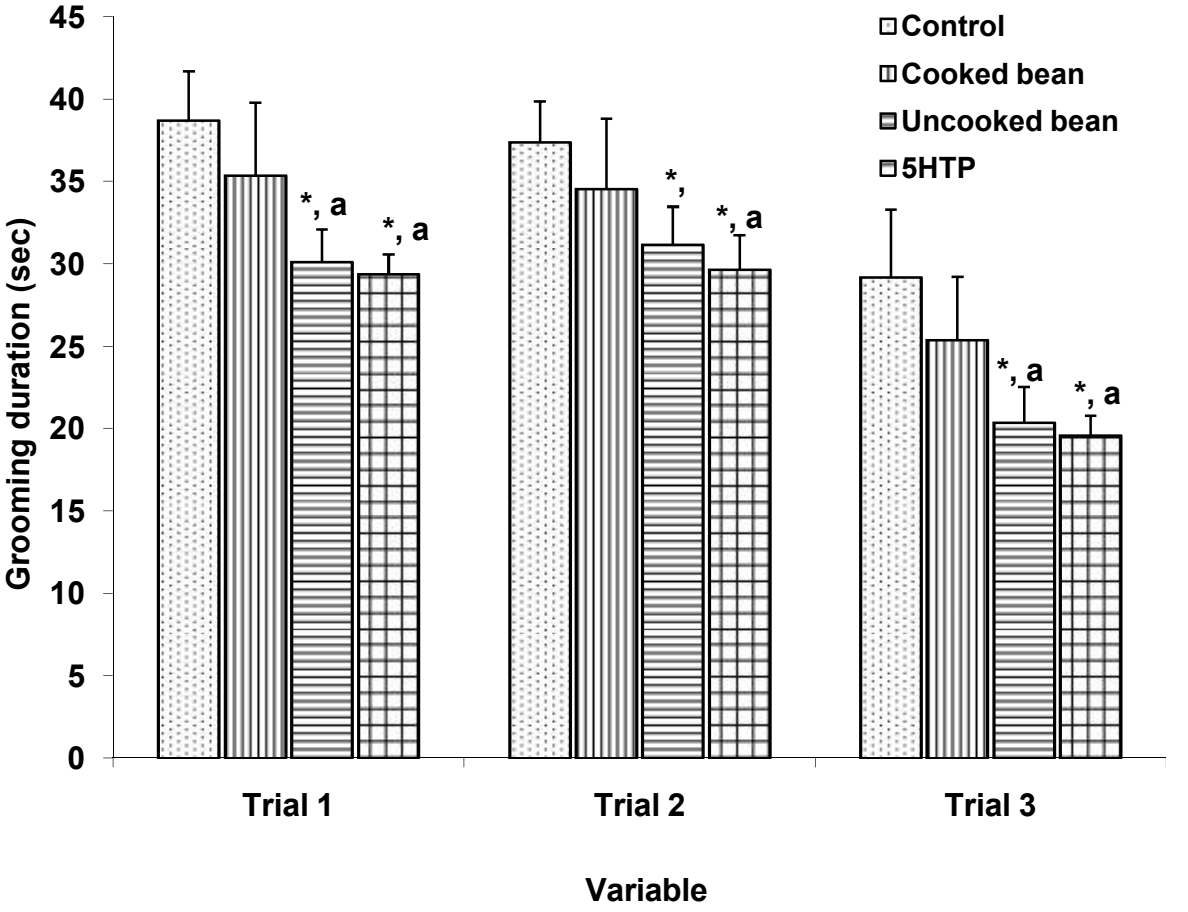

Figure 6: Comparison of grooming duration among the different experimental groups during habituation, familiarization and testing periods of the novel object recognition test.
Values are expressed as mean ± SEM, n = 10.
***significantly different from control at $p<0.05$;**
a = significantly different from cooked bean at $p<0.05$.

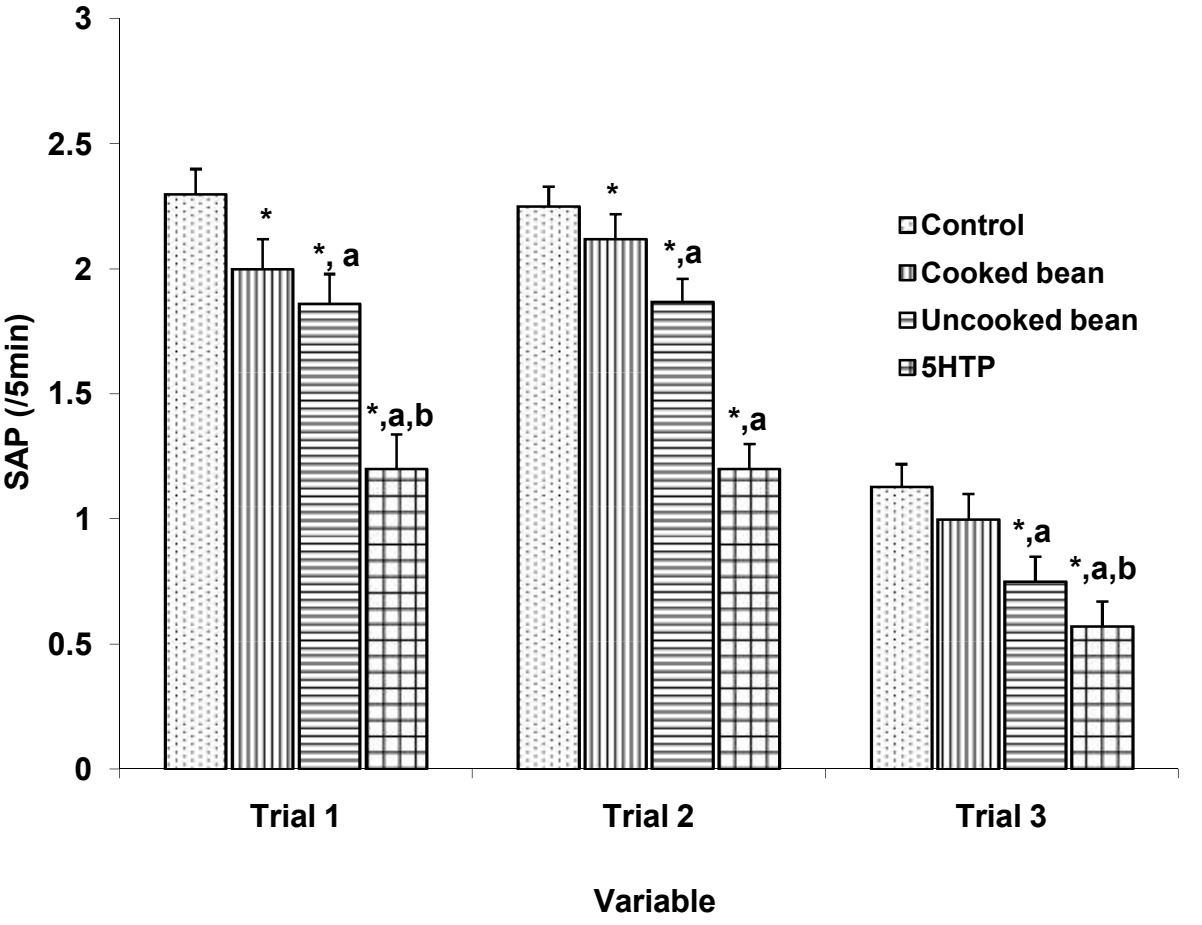

Figure 7: Comparison of stretch attend postured among the different experimental groups during habituation, familiarization and testing periods of the novel object recognition test.
Values are expressed as mean ± SEM, n = 10.
***significantly different from control at p<0.05;**
a = significantly different from cooked bean at p<0.05;
b = significantly different from uncooked bean at

Our finding therefore suggests that long term consumption of beans diet enhances cognitive declarative memory. If this research can be extrapolated to humans, we recommend that the serotonergic potential of beans may be harnessed in the prevention, treatment and management of behaviour/mental disorders as this could potentially open up a vista for improved mental health status in populations globally.

Our finding therefore suggests that consumption of beans diet enhances declarative memory. If this research can be extrapolated to humans, we recommend that the serotonergic potential of beans may be harnessed in the prevention, treatment and management of behaviour/ mental disorders as this could potentially open up a vista for improved mental health status in populations globally.

ACKNOWLEDGEMENT

We acknowledged Pa and MrsB.A.Aduema, Mr.Iwasam Joshua, Dr.Nmaju, Prof.E.E.Osim and Associate Prof.A.A.Nwankwo for their priceless support.

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