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MEMORY ALTERATION AND POTENTIAL NON-ADDICTIVE EFFECTS INDUCED BY A CHRONIC INTAKE OF HOME MADE ALCOHOLIC DRINKING "ODONTOL"

AYISSI MBOMO Rigobert-Espoir[1*], TENE TADOUM Samuel Boris[2], NGUIDJOE Evrard Marcel[3], MOTO OKOMOLO Fleur Clarisse [1], NANGA Léopold Didier[4], NGOA MANGA Elisabeth Sylvie[4], NJAMNSHI KONGNYU Alfred[2]

[1]Department of Biological Sciences, Higher Teacher's Training College, University of Yaoundé I, Cameroon
[2]Department of Internal Medicine and Specialties, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon
[3]Department of Pharmacotoxicology and Pharmacokinetics, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon
[4]Department of Animal Biology, Faculty of Sciences, University of Yaoundé I, Cameroon.

***Corresponding Author: AYISSI MBOMO Rigobert-Espoir**

Senior Lecturer, Higher Teacher Training College, University of Yaoundé I, PoBox 47 Yaounde (Cameroon).

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ABSTRACT

The last decade in Cameroon has been the seat of increased consumption of artisanal drinks including *«Odontol»*. Appreciated by youngest and adults, this beverage has induced numerous deaths attributed to its poor manufacturing. Being in a context of emergency by 2035, this beverage of unknown composition and effects on human health still little explored. We have undertaken the study of possible addictive effects of *«Odontol»* as well as its effects on various essential functions for memory. Experimental analysis of behaviors associated with addiction and memory disorders in mice using various behavioral tests including conditioned place preference, object recognition and Y maze tests were performed. Results showed that the time spent in the *«Odontol»* associated compartment significantly decreases between the pre-conditioning and test (28.57%) in the conditioned place preference test, as well the time spent in the *«Odontol»* associated compartment compared to NaCl compartment during the test. In the object recognition test, animals of the test group spent less time exploring the new object than the control animals with a 57 times higher discrimination index when compared to test group animals. In the Y maze, test mice significantly spent less time (15.78%) in the new arm compared to control animal which spent 48.27% of time exploring the new arm. These results bring the light of possible aversion induced by *«Odontol»* and a severe attack of mnesic function in short and long term memories.

KEYWORDS: Object recognition, Y maze, Conditioned preference place, aversion, memory.

1. INTRODUCTION

A learning society is one that places learning at the heart of its objectives of social cohesion and the fulfillment of citizens.[1] Learning thus appears as an important factor of development to be considered and promoted in societies, more precisely in developing countries such as Cameroon. Cameroon is a booming country in all activity sectors; indeed, it contains a huge intrinsic potential that for its emergence requires qualified personnel, creative and able to innovate.[2] The development and expansion of business sectors such as transport, telecommunications, logistics, hydrocarbon exploitation, mining and many others require skilled Cameroonians. For this, it becomes crucial to focus on their training from their early childhood, especially since physical and mental health is a prerequisite to achieve this goal. This is all the more true since it has been observed that unhealthy behaviors have often been the cause of many school failures and low professional returns. In recent years in Cameroon, behaviors harmful to education and employment such as smoking and alcoholism have become widespread. According to some studies, Cameroon is ranked as the 12th country worldwide, the second in West and Central Africa consuming pure alcohol with around 5.64 L of alcohol/year/inhabitant over the age of 15 years old. There is also an increase in the consumption of home-made drinking spirits, such as *«Odontol»* which is very popular in the Central, Southern and Eastern regions of Cameroon.[3] Indeed, «*Odontol*» is a popular beverage with people because of its low cost, its high availability and its "liberating" and "enjoyable" effects. According to some apart from ethanol, the homemade *«Odontol»* also contains a methanol derivative, extremely toxic product resulting in methyl intoxication, because it is impossible to control the ingredients used, nor the conditions of

fermentation. This means that, unfortunately, this consumption is not without consequences. There are physiological disorders ranging from irritation of the throat to death. The national radio reported that 22 people were hospitalized after consuming this popular local drink; as a result, the authorities of the regions concerned have announced the prohibition of the production and the trade of this product which unfortunately continues to be sold in the informal circuits. This consumption, which has become recurrent among adolescents and young people, reveals a fairly linear correlation between alcohol consumption and learning failures in school and professional settings.[3] On the strength of this observation, we are entitled to ask ourselves if this increase in *«Odontol»* consumption is due to its low cost or to its probable addictive effects, and what would be the effects of its repeated consumption on the consumers' memory? The general objective of this work was the determination of the addictive and amnesic effects of chronic intake of *«Odontol»* in humans through modeling in mice, and more specifically, we have:

• Assessed the aversive effects of *«Odontol»* consumption through the conditioned place preference test (CPPT);
• Measured sensory memory and working memory disorders induced by the long-term "Odontol" consumption in the object recognition test (ORT);
• Measured the declarative memory disorders induced by *«Odontol»* taken repeatedly in the Y labyrinth test.

2. MATERIALS AND METHODS

2.1. Preparation of the alcoholic drinking *«Odontol»*

The alcoholic drinking used here was produced by artisanal distillation and rectification processes based on palm wine, sugar and stem bark of *Garcinia Lucida*. The mixing and distillation processes are only known by artisanal manufacturers.

2.2. Animals

The present experimental study was conducted at the Laboratory of Animal Physiology of the Higher Teacher's Training College of the University of Yaoundé I (ENS-UY1). A minimum of mice was recruited while respecting ethical principles and guidelines for care and use of Laboratory Mammals in accordance with National Ethic Committee (FWA-IRB00001954). Subjects included, young adult's mice of both sexes, aged of 2 months old, presenting no appearing pathological signs, with a good mobility. Mice were breed under the same environmental conditions at the animal room of ENS-UY1, where they were exposed to 12 hours light/dark cycle, at room temperature and humidity, and freely moved to get food and tap water.

2.3. Experimental protocol

2.3.1. Conditioned place preference test

Originally described by Beach in 1957, the CPPT is now broadly spread in many laboratories and researchers have made significant contributions, as well as a variety of different apparatus have been developed. This popular test characterizes the rewarding effect of drugs, because it represents a fair measure of the hedonic memory that comes with administration of the substance.[4] The paradigm used to perform the experiments was the three-chambers design, comprised two large compartments (28 × 28 × 28 cm) wood made, connected by a small central compartment made of glass. In the CPPT, each large compartment is associated with the administration of a specific substance. The floor and walls of the *«Odontol»* associated compartment was covered with a white paper to allow a visual and textual differentiation with the compartment associated to Nacl 0.9%. 8 mice were selected (4 males and 4 females) and submitted to the procedure comprising three main steps.

Step 1: Habituation to the paradigm. On day 1, the two communicating doors of the central compartment were opened so the animal could freely have access to the whole apparatus. The animals have not been subjected to any treatment, and individually each mouse was placed in the intermediary compartment, allow visiting the paradigm for 3 trials, in the cut off time of 15 minutes per trial. The time spent in each compartment was noted and was considered as the mean time spent in each compartment over the three trials. The time spent in *«Odontol»*'s associated compartment has been used to set the initial preference level.[5]

Step 2: Conditioning. From day 2 to day 9, each animal orally received alternately intragastric administration of 2.5cc/kg of NaCl solution or the alcohol *«Odontol»* before being placed for 15 minutes in the compartment associated with the administered substance without access to the others compartments.

Step 3 (test): On day 10, the animals were subjected to the same process as for the habituation consisting of one trial free access to the whole apparatus, then the time spent in each compartment was recorded. The time spent in the compartment associated with *«Odontol»* during the test was compared to the initial time to characterize the attractive or aversive effect of this drinking.

2.3.2. Object recognition test

Since the observations made by Berlyne in 1950, a new behavioral test was developed in the late 1980 s. Originally developed for use in rats,[6] and now spread to others rodents including mice,[7] the so call object recognition test is based upon the natural tendency of rodents to explore unknown object, then animal is placed in an enclosure that has already been explored, and dwell on the new objects present in it.[8] The apparatus used was a wooden box of (28 × 28 × 28 cm). The top of the apparatus was opened so that the experimenter can easily observe the bottom and record different parameters. The bottom representing the floor was covered by a piece of wood of 5 cm thick; according to the stage of interest, different types of objects were inserted on the floor. The objects used were of equivalent intrinsic attractiveness

(length, color), had sufficient weight so that it was not possible to be moved by mice, and the arena was spacious enough to allow the animal to freely move behind the objects without pushing them. According to the preliminary study, under normal conditions, all these three objects infallibly evoked equal amounts of explorations. Initially the animals were divided in test and control groups containing 8 mice (4 males and 4 females). Animal of the control group were naïve, whereas test group animals were subjected to chronic exposure to *«Odontol»*; this consisted of free intake of *«Odontol»* as the only drinking source for 14 consecutive days. At day 15, the animals were taken to the laboratory for 24 hours habituation without changing their diet. At day 16, they were subjected to the ORT which was conducted in 3 main steps: habituation, training and the test.

Step 1: Habituation. During this step, each animal was individually left free to explore the enclosure for a cut off time of 360 seconds.
Step 2: Training. After habituation, the mice were individually placed in the arena in the presence of two different objects, including a rectangular object and a conical object for 360 seconds. The time spent exploring each object was recorded in seconds.
Step 3: Test. The conical or familiar object (FO) being left in the enclosure, and the rectangular object replaced by the pyramidal or new object (NO). The time spent exploring FO or NO determined, in a cut off time of 360 seconds.

Two different parameters were evaluated:
FT = time spent on the FO and NT = time spent on the NO

Several other parameters were then calculated:
The exploration index (EI) corresponding to the total time spent exploring the objects
EI = NT + FT
The recognition index (RI) representing the ratio of time spent on the NO, varying between 0 and 100%.
RI = [NT / (NT + FT)] x 100

The discrimination index (DI) corresponding to the proportion of time that the animal has passed in addition to exploring the NO; it therefore varies between - 100% (if the animal has only explored the FO) and 100% (if it only explores the NO).
DI = [(NT-FT) / (NT + FT)] x 100.

2.3.3. Y Maze test

The Y test is based upon the natural tendency of rodents to explore a new arm of the maze rather than returning to one that was previously visited, evaluate the various components of the episodic memory.[9] The paradigm of Y- shaped was wooden made and comprised 03 arms (50 × 32 × 16 cm), differentiated in BA (arm through which the animal is introduced into the apparatus), FA (familiar arm) and NA (new arm). The floor of the apparatus covered with a thin layer of chip was initially sprayed with 70% ethanol and changed after each trial to avoid olfactory traces that could skew the results.

The test was conducted in two main sessions:
Session 1: Acquisition. Mice where shared in two group, control group containing 8 naïve mice that didn't took any treatment; and a test group made of 8 mice subjected to *«Odontol»* for 14 consecutive days as the only drinking intake. Then, mice were individually introduced in the apparatus and allow exploring for a cut off time of 300 seconds. During this session, the NA was ceiled by a gate so that the animal only has access to the BA and FA.

Session 2: Recall. After a retention period of 1 hour (recall session), the animals individually were again allowed to explore the maze with the three arms being this time available. Here the time spent in each arm (BA, FA or NA) in seconds was noted.

2.4. Data processing and statistical analysis

Data of the various tests were recorded using a stopwatch and expressed as mean ± SD. The statistical analysis of the data was carried out using one-way ANOVA followed by Fischer's post-hoc, using the software Statistica 6.0. Differences in significance were realized at the threshold of 0.05.

3. RESULTS

3.1. Evaluation of the effect of *«Odontol»* in the conditioned place preference test

3.1.1. Time spent in the compartment associated with *«Odontol»* as a function of the experimentation phase

Figure 1 shows the time spent by each animal in the compartment associated with *«Odontol»* intake during pre-conditioning and test. From the data obtained, 06 animals out of the 08 tested during the test phase spent less time in the compartment associated with *«Odontol»* intake, that is to say 75% of aversion induced in the total population by the *«Odontol»* consumption.

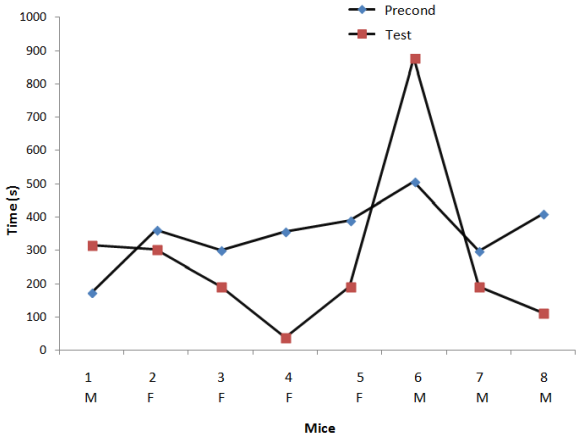

Figure 1: Variation of time spent in the compartment associated with *«Odontol»* according to the different experimental phases. Each point represents the time (in seconds) spent by each animal in the compartment

associated with *«Odontol»* intake during the two experimental phases, n=8, Precond: preconditioning; M: Male; F: Female.

3.1.2. Time spent in each compartment according to the experimentation phase

Figure 2 represents the average time spent by the animals in the different compartments as a function of the experimental period. In control group mice, the level of attraction of the two compartments was almost identical during the two experimental phases (pre-conditioning and test). During the test, the animals spent significantly more time in the compartment associated with Nacl solution compared to the compartment associated with *«Odontol»*. The time spent in the compartment associated with *«Odontol»* significantly decreased ($p<0.001$) for about 28.57% between pre-conditioning and the test.

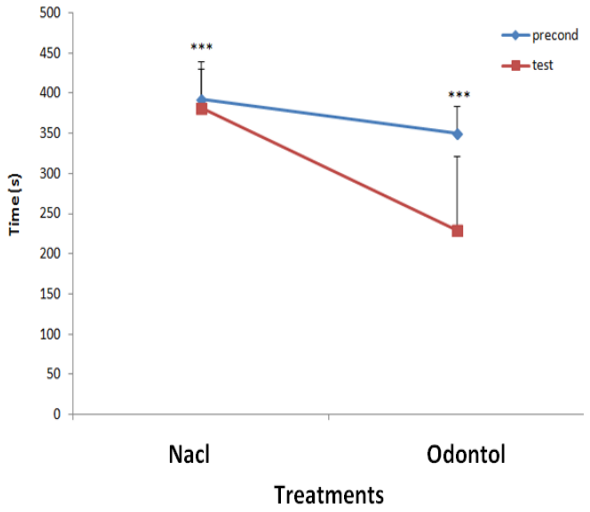

Figure 2: Time spent in each compartment according to the experimentation phase during the test of the conditioned period. Each point represents the average time (in seconds) spent in each compartment during the two experimental periods ± SD, n=8 *** P<0.001 significant difference compared to the time spent in the compartment associated with *«Odontol»* during the test. Precond: pre-conditioning phase.

3.2. Evaluation of the effects of *«Odontol»* on the memory using the new object recognition test

3.2.1. Exploration time of different objects

Figure 3 illustrates the effects of repeated intake of *«Odontol»* on the average time spent exploring the FO and NO in the various experimental groups. It appears from this figure that control group mice spent more time exploring the newly introduced object (which they do not know) compared to the familiar object which they recognized and therefore the interest becomes less.

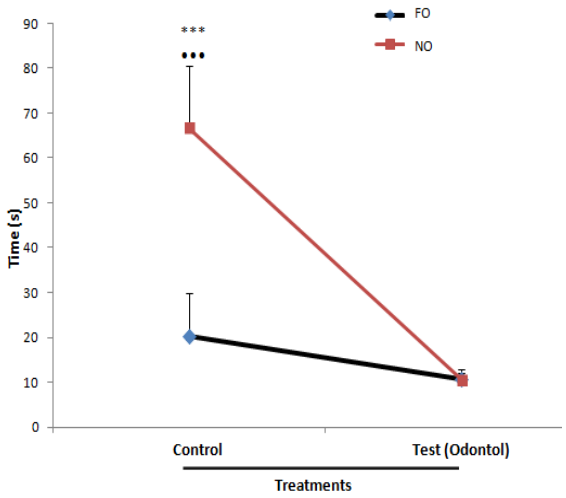

Figure 3: Exploration time of the different objects. Each point represents the average time (in seconds) for exploring FO or NO in the paradigm ± SD, n=8. ••• P<0.001: significant difference compared to the time spent exploring the familiar object in the control group and *** P<0.001: significant difference compared to the time spent exploring the new object in animals exposed to *«Odontol»*. Test: mouse having received prolonged exposure to *«Odontol»;* control: naive mice.

3.2.2. Recognition index and discrimination index

Figure 4 shows the values of the recognition index and the discrimination index in each group. From this figure, it appears that, unlike the values of the object recognition index, the discrimination index significantly decreased ($p<0.01$) by around 100% in the animals of the test group compared to the control group mice.

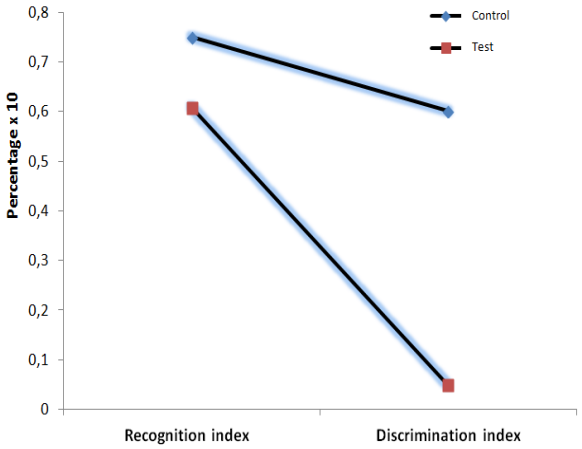

Figure 4: Recognition index (R) and discrimination index (D) of the objects according to the experimental group. Each point represents the recognition or discrimination index as a function of the experimentation phase.

3.3. Evaluation of the effect of *«Odontol»* on memory in the Y maze

3.3.1. Arms time

From figure 5 which presents the time spent by each group of mice in the different arms of the Y maze, the following observations were made: Animals in the control group explored the NA more than mice of the test group. Indeed, the percentage of NA time is 48.27% for the control group while it is only 13.04% for the test group. The animals of the test group spent less time 13.39 s in the NA compared to the other arms they have already explored including BA and FA where they spent about 100 s.

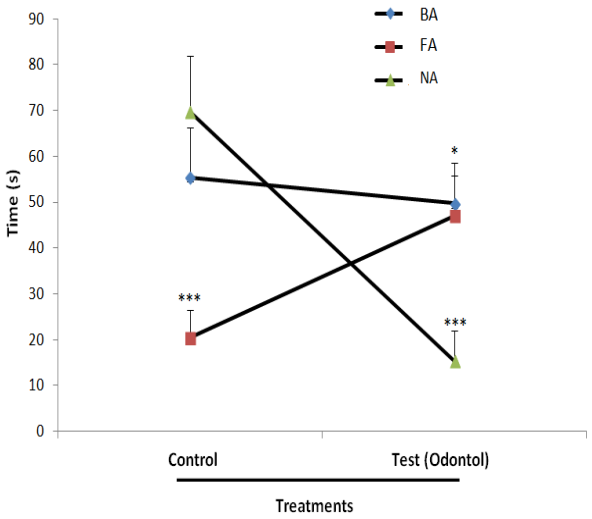

Figure 5: Exploration time of the different arms depending on the experimental group. Each point represents the average of the time (in seconds) to explore each arm of the paradigm ± SD, n=8; * P<0.05, *** P<0.001: significant difference compared to the NA in the control group. Test: mouse having received prolonged exposure to *«Odontol»*; control: naive mice. BA: starting arm; FA: familiar arm; NA: new arm.

3.3.2. Latency to leave the starting arm

Introduced into the Y labyrinth by the BA, during their exploratory activity, mice of the different groups then had to make the transition either to the FA or to the NA. This latency to leave the BA for one of the two other arms (FA or NA) very low (3.4 ± 0.5 s) in the control group increased to a significant (P <0.001) value of 26.2 ± 6.51 s in animals treated with *«Odontol»* (Figure 6).

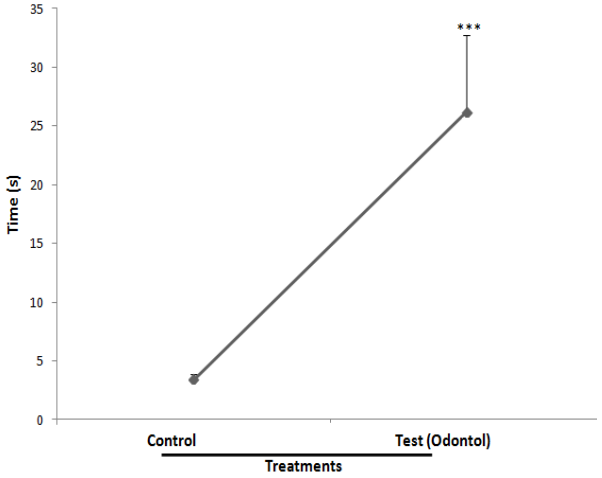

Figure 6: Latency between the starting arm (BA) and one of the others two arms (FA or NA) in each experimental group. Each point represents the average time in seconds to leave the BA and join either the FA or the NA ± SD, n=8. *** P<0.001: significant difference compared to the control group; Test: mice having received prolonged exposure to *«Odontol»*; Control: naive mice; BA: starting arm; FA: familiar arm; NA: new arm.

3.3.3. Arms entries

In the Y labyrinth test, the number of entries in the different arms is presented in Figure 7. The observation of this figure shows the existence of a significant decrease (p <0.001) in the number of entries in the NA, from an approximately value of 8 for mice of the control group to a minimum value of 1 observed in animals treated by *«Odontol»*. In fact, animals of the control group explored much more the NA to the detriment of the FA and BA, while those in the test group almost independently visited the different arms (BA, FA and NA) (Figure 7).

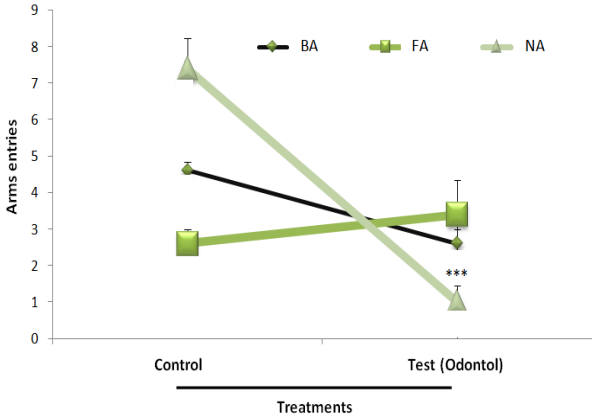

Figure 7: Number of entries in the different arms of the Y labyrinth. Each point represents the value of the average number of passages in each of the three arms of the maze ± SD in the different experimental group, n=8 *** P<0.001 significant difference in comparison with the number of new arms entries in the control group. BA: starting arm; FA: familiar arm; NA: new arm.

4. DISCUSSION

A behavior of living organisms is a visible manifestation of the activity of the central nervous system, and appetitive or aversive stimuli are typically used to motivate an animal to perform a particular behavior. Many researchers have so far attested the addictive potential of industrial processed alcohols and most of them have well controlled ethanol content.[10] Ethanol is the psychoactive substance of the alcoholic drinking responsible in general for depressant effects on the nervous system, while stimulating the reward system.[11] Usually, the observed effects are proportional to the percentage of ethanol consumed in the drinking, the frequency of consumption, the state and physiological predispositions of the subject.[10] The rudimentary and uncontrolled process of *«Odontol»* manufacturing does not easily make possible the evaluation of ethanol and others alcohols like methanol percentages in the mixture. In the CPPT, the animals treated with *«Odontol»* showed not an attraction but rather a strong aversion induced by the consumption of this drinking. According to previous studies, an increase time spent in the compartment associated with the administration of ethanol indicates a preference for this conditioned space, therefore an attractive effect of ethanol. It is also important to mention that, this test works for all addictive drugs, but the results are more marked with opiates and psycho-stimulants. According to Ericksson, the aversive behavior related to *«Ondontol»* associated compartment could be attributed at first glance to its strong and unpleasant odor as well as to its taste and irritant effects on the palate.[12] Indeed, during the experiment, it could be observed that *«Odontol»* consumption was followed by a general condition of animal very characteristic of the effects of ethanol on the nervous system. As previously described by many researchers, ethanol by its physiological action causes a general state of calm, drowsiness, relaxation, reduced mobility, slower breathing rate and even coma. This sheds light on the actual presence of ethanol in the *«Odontol»* mixture in a sufficient percentage to cause depression of the nervous system, but also clearly reveals the aversive effect of *«Odontol»*, probably due to either its ethanol percentage not sufficient to induce attraction, either to the palatability and odor of *«Odontol»* probably due to the presence of others alcohols (methanol) at higher percentage in the mixture. However, it is not possible to clearly speak about the non addictive power of *«Odontol»*. Indeed, addiction is essentially characterized by a conversion of the reward system with the intake of the psychoactive substance as only element rewarding.[13] Thus, in this test, it is possible that the hedonic reminder of *«Odontol»* consumption prevents the expression of the attack on the reward system by the ethanol presents in this beverage; since the test used in this work is not a decision-making test. The above-mentioned effects of *«Odontol»* are distributed unevenly across the population of mice used. It was noted that some mice were not affected, other mice affected mostly among the females. These results are then consistent with those of many studies conducted on alcohol and its vulnerability factors including gender and biological or genetic vulnerability to alcohol.[14,15] There is a variety of tests to evaluate various modalities of memory to characterize the mnesic state of a subject. Here we used the Y maze test, to measure parameters of the spatial recognition reflecting the different components of episodic memory: what, where and when. As previously suggested, in this test, the behavior associated with a normal memory function is a higher exploration of the NA. In fact, the spatio-temporal data acquired during the training stage after the retention period, are stored in the episodic memory. The animal thus has the ability to remember already visited arms associated with their spatial (where) and temporal (when) components and thus to explore more the unknown arm.[16] The results provided by this test show that mice subjected to *«Odontol»* consumption spent less time exploring the NA compared to other arms, whereas control group mice discriminate the NA from the FA, in accordance with the previous work.[17] Animals treated with *«Odontol»* showed a variable exploration of different compartments of the Y maze, and it appears that *«Odontol»* acted as an amnesic substance and thus erased any remembrance of the information acquired during the training stage. The chronic intake of *«Odontol»* alters the episodic memory of individuals. This test also evaluates the working memory characterized by short-term retention of information and its immediate use to express appropriate behavior, as seen here as further exploration of the least-visited arm.[18] The animals whose working memory is fully functional explore the least frequently visited arm and thus show a high degree of alternation exploring the three arms. This is consistent with the results of our experiment in which animals of the control group showed higher exploration of the different arms compared to animals of the test group which achieved only few entries into the NA. On the other hand, these results reflect the reduction of the general locomotion induced by *«Odontol»* consumption because the total distance traveled by mice of the control group is clearly greater than those of the test group. About the latency to leave the BA, its analysis provides a lot of information concerning the working memory in animals. Indeed, the higher latency with the test group suggests a higher decision-making time in comparison with the control group.[18] The working memory allows the recording of information whose subsequent use (two hours later) requires a mobilization of this information in order to make the appropriate decision. The test animals take longer, reflecting the slowness of the mental processes responsible for the booster including the slow synaptic transmission which is one of the effects of ethanol in the central nervous system. The results provided by the object recognition test give information on the short-term memory, purely based on the innate preference of the rodent to explore the novel object (NO) rather than the familiar one (FO). Animals of the control group showed a normal physiological reaction after introduction of the new object in the arena. Mice not responding to this

change showed a major cognitive deficit affecting short-term memory. As previously stated by Ennaceur et al., in 2005, a larger or even exclusive exploration of the NO during the test is refers as a normal memory function allowing the recall of the information recorded in the short term.[19] Indeed, the information acquired during the training may have suffered damage during the recording or encoding, making the recall impossible or slower, and even a defect occurring during the recall phase of memory information yet well recorded. The discrimination index and the recognition index are variables involving in the characterization of sensory memorization processes. An object being a set of sensory impressions, FO and NO recognition are based on a set of sensory elements such as color, shape, texture, appreciable dimensions through sense organs.[20] The deficit observed in the test animals could therefore be the result of a lack of encoding of the sensory information of the different objects causing the recognition disorders. The object recognition test has been used to investigate number of different brain regions underlying recognition memory, including insular cortex,[21] perirhineal cortex,[22] and ventromedial prefrontal cortex,[23] thus the loss of short-term memory highlighted here by the decrease of the time spent exploring the NO could result from the lesions induced by repeated exposure to «Odontol», on at least one component of this neural network.

CONCLUSION

At the end of this study which allowed us to study the potential addictive effects of *«Odontol»* as well as its probable memory effects, the main observation that emerges is that *«Odontol»*, an alcohol, does not only induce aversive character for the consumer due to its hedonic properties but also constitutes a destructive agent of memory functions. As the experiments carried out did not allow us to be certain about the real addictive power of this beverage and the memory structures targeted by its amnesic action, this study therefore opens the door to several questions that may be the subject of future studies.

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