ECRONICON OPEN ACCESS

EC PSYCHOLOGY AND PSYCHIATRY
Research Article

Cognitive Impairment: Examining the Effect of Marijuana and Tramadol on Albino Rats

Chukwuemeka A F Okoye*, Cynthia N Udeze and Henry Nwaogu

Department of Psychology, Faculty of Social Sciences, Nnamdi Azikiwe University, Awka, Nigeria

***Corresponding Author**: Chukwuemeka A F Okoye, Department of Psychology, Faculty of Social Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

Received: November 22, 2017; **Published:** January 06, 2018

Abstract

This study examined the effects of marijuana and tramadol on cognitive impairment. Eight (8) participants (Albino Rats) formed the sample size of the experiment. They were three months old with a mean age of 3 and standard deviation of .3000, weighing about 300 - 350g. The albino rats were bought from a biochemist who owned an animal farm. Data were collected through experiment conducted by the researchers in which the participates were divided into four (4) experimental groups of 1a and 1b, 2a and 2b. Each of these groups were administered a certain level of marijuana and tramadol substance, with 3 ml as low dosage and 5 ml as high dosage. Radial Arm Maze was used to measure cognitive impairment, which was based on the rat's ability to visit the arms of the maze which contains the reward and ability to enter each arm a single time. The result of the 2-way ANOVA showed a significant effect of marijuana on cognition among albino rats, ($f = 5.44$, $p < .04$) and a non-significant effect of tramadol on cognition among albino rats, ($f = .08$, $p < .80$). Implications of the findings were highlighted and recommendations were made for further studies.

Keywords: *Cognitive; Impairment; Marijuana; Tramadol; Albino Rats*

Introduction

Background

There seems to be an unprecedented rise in the use of psychoactive substance among human all over the world with negative consequences on human's cognitive properties. This observation is supported pointers substance misuse which includes hallucination, euphoria feelings, disorientation in time and space, irregular dilation of the pupil, increase in heartbeat and pulse rate as well as disturbances in cognitive process such as material retention, memorization etc.

More importantly, psychologists hold that humans are information processing animals; therefore the simple S-R paradigm cannot explain why people show a progressive decline in cognitive activities (Craik and Jennings 1992), something must have happened in the information processing domain. These thought compelled with the fact that much of what is written on this subject matter in this milieu is speculative, makes an experimental study on the effect of psychoactive substance on cognitive processes very persuasive.

Cognition is the mental action or process of acquiring knowledge and understanding through thought, experience and senses. Cognition encompasses a process such as knowledge, attention, memory and working memory, judgment and evaluation, reasoning and "computation" problem solving and decision making, comprehension and production of language.

Shettleworth [1], described animal cognition as the mental capacities of non-human animals and the study of those capacities. The field developed by comparative psychology, including the study of animal conditioning and learning.

Cognition according to Blomberg [2], referred to the mental functions, mental processes (thought), mental representation and state of intelligent entities (humans, collaborative group, human organization, highly autonomous machines, and artificial intelligence).

Denis and Loomis [3], opined that spatial cognition concerns the acquisition, organization, utilization, and revision of knowledge about spatial environments.

Cognitive impairment or cognitive deficit according to Coren and Stanley [4] was an inclusive term to describe any characteristic that act as a barrier to the cognitive process.

Animal experiment and human studies have provided insight into the acute effects of psychoactive substance on cognitive impairments and into common factors underlying acute and chronic marijuana and tramadol intake and cognition. There are necessary exclusive reasons that the relationship between drug and cognition exist. A psychoactive, psychopharmacological or psychotropic drug is a chemical substance that changes brain function and results in alteration of perception, mood, consciousness or behaviour. This substance may be used medically, recreationally, to purposefully improve performance or alter ones consciousness or for research. One of the numerous psychoactive substances been abused that usually affect cognitive abilities is tramadol.

Tramadol is a centrally acting and chemically approved and used drug for point treatment [5]. Tramadol is a weak opioid (Opioid receptor) and analysis (pain killer) and sold under a brand name Ultram. Tramadol is an against (MOR), probably acting via its active metabolite O-desmethyltramadol (M) which has a 10 fold lower opinity for the U-Opioid than morphine [6,7]. Tramadol has two different mechanisms. First, it works by binding to the u-opioid receptor [6]. Secondly, it act as a serotonin-norepinephrine reuptake inhibitor (SNRI). Tramadol is a synthetic opioid of the benzenoid class. In the body, the liver metabolizes tramadol into desmetramadol, also known as o-desmethyltramadol, which is a more potent opioid.

Tramadol is a racemic mixture of two active enantiomers [6]. The (+) – mi) are selective antagonist of the opioids receptor and have also serotonergic reuptake inhibitory effects (SRI); the (-) – reuptake inhibitors [8]. This activity profile suggests antidepressants potency and in animal paradigm, tramadol indeed shows antidepressants activity [9]. Recently, tramadol has been found to be useful in the treatment of premature ejaculations in humans. (Essa and El – Sharaly 2013).

Another psychoactive substance that tends to affect cognitive abilities is marijuana also known as cannabis. Marijuana plants is known to contain about 400 chemicals and about 40 of them are cannabinoids which are psychiatric components that are produced inside the body after cannabis or marijuana is metabolized. Cannabinoids is an active ingredient of marijuana. The most psychoactive cannabinoid chemical of marijuana that has the biggest impact on the brain is tetrahydrocannabinol (THC).

While marijuana has historically been denounced for being a social "menace" and for inducing sexually alternated behaviour and homicidal rages [10], recent research indicates that cannabis-intoxicated individual is in fact less likely to exhibit accurate cognitive orientation.

The debate regarding the issues of marijuana dates back decades and rages on today, while most of the contemporary antipathy shown towards cannabis revolves around its controversial status as a "gateway" drug [11], marijuana was originally maligned due to its purported effect or supposed effect on cognitive orientation. Subscribing to the notion of cannabis as a dangerous drug, the Bureau of Narcotics had soon thereafter established the marijuana Tax Act, which amounted to a ban of the drug. Several scientific studies have elicited the noxious effect of psychoactive substances on health and psychological processes especially marijuana on cognitive impairments and tramadol on general loss of inhibition. It is acceptable that the combination of these substances elevates or drops cognitive action and behavioural functioning.

Tramadol and marijuana use have become rampant of its known harm. These drugs-use poses a significant threat of cognitive development. Research believed that it kills brain cells, yet majority still abuse it which later affects their memory coordination and performance in school. Marijuana is believed to be correlated to poor academic performance.

According to Shehu., et al. (1996), Marijuana has a negative effect on academic performance which might be caused by persistent absenteeism of smokers of marijuana. The fact that the consumption of these substances is becoming trendy, the question of possible link between marijuana and tramadol on cognitive impairment becomes cogent. This is even more pressing in the light of extremely scarce literature on direct observation on the effect of the combination of these substances on behavior. If this lacuna in knowledge is attached, it is possible that a clue to the reduction of psychoactive substance misuse will be found.

In conclusion, in terms of direct causal relationship between a drug and increased likelihood of cognitive orientation, there is no doubt that marijuana is culpable. Marijuana seems to lead to cognitive impairment. What should now become the focus of research is the development of primary and secondary prevention programming to reduce the costs of marijuana related cognitive orientation.

Theoretically, several theories have been posited by scholars to explain cognitive learning behavour some of these Cognitive based theories include social cognitive theory by Pagares [2002] and Cognitive Behavioural Theory by Beck [12,13].

The social cognitive theory, stressed the importance of behavioral, environmental and Personal factors in cognitive leaning, These three variables in social cognitive theory are said to be interrelated with each other, causing learning to occur. An individual's personal experience can converge with the behavioural determinants and the environmental factors. In the person-environmental interest in human beliefs, ideas and cognitive competencies are modified by external factors such as supportive parents, stressful environments or hot climates. In person-behaviour interaction, the cognitive process of a person that affect or influences his or her behaviour; likewise,

performance of such behaviour can modify the way he or she thinks. Lastly the environment-behaviour interaction, external factors can alter the way you display a particular behaviour. Also your behaviour can affect and modify your environment. This theory implies clearly that for effective and positive learning to occur, an individual should have positive personal characteristics, exhibit appropriate behaviour and stay in supportive environment.

Secondly, cognitive behavioural theory describes the role of cognition i.e. knowing to determine and predict the behavioural pattern of an individual. This theory was developed by Aaron Beck. This theory conceives that individuals tend to form self-concept that affect the behaviour they display. These concept can be positive or negative and can be affected by a person's environment.

Empirically several studies were reviewed: Anderson, Rizzo, Block, Pearlson, and O'Leary (2010) investigated sex, drugs, and cognition effects of Marijuana, involving 70 participants (male = 35, female = 35). Tasks were chosen to tap a wide variety of cognitive domains affected by sex and/or marijuana including attention, cognitive flexibility, time estimation, and visuospatial processing. As expected, acute marijuana use impaired performance on selective and divided attention, time estimation, and cognitive flexibility.

Schweinsburg, Brown, and Tapert (2007) in their study investigated the Influence of Marijuana Use on Neurocognitive Functioning in Adolescents. They carried out an experiment involving 33 participants (males only) with the mean of 18.1 and S.D of 0.7. The result showed that marijuana use have a long term effect on neurocognitive functioning but mild short term effect, also acute marijuana use causes a chronic effect on neurocognitive functioning among adolescents.

Hart, van Gorp, Haney, Foltin and Fischman [14], studied the effects of Acute Smoked Marijuana on Complex Cognitive Performance. Eighteen healthy research volunteers (8 females, 10 males), averaging 24 marijuana cigarettes per week, completed this three-session outpatient study; sessions were separated by at least 72-hrs. During this sessions, participants completed baseline computerized cognitive tasks, smoked a single marijuana cigarette (0%, 1.8%, or 3.9% Δ9-THC w/w) and completed additional cognitive tasks when compared to the 1.8% Δ9-THC condition, 3.9% Δ9-THC concentration cigarettes significantly increased the amount of time it took participants to finish the immediate recall and mental calculation tasks ($p < .05$). No significant differences were observed when accuracy of responding on attention, memory, visuospatial processing, reasoning, flexibility, and mental calculation tasks were analyzed as a function of Δ9-THC concentration.

In another study by Ebubechukwu (2015), on the effect of cannabis sativa and ethanol mixture on aggressive behaviour and cognition, involving 8 female guinea pigs with mean age of 5 and S.D of .5345. The calculated value of 0.78 and table value of .927 at .05 level of significant shows in her study that cannabis sativa does not have a significant effect on aggressive behaviour and cognition.

Hosseini-Sharifabad, Rabbani, and Narges Bagheri (2016) studied Acute and chronic tramadol administration on spatial memory impairment among wistar rat. Tramadol, 20 mg/kg, was injected intra-peritoneally as a single dose or once a day for 21 successive days considered as acute or chronic treatment respectively. The experiments were carried out on 28 male Wistar rats (weighing 220 - 230g) obtained from Animal House of School of Pharmacy at Isfahan University of Medical Sciences. The findings revealed that tramadol impaired memory when administered acutely or chronically. Single dose administration of tramadol showed more destructive effect than multiple doses of tramadol on the memory.

Purpose of the Study

The general purpose of this experimental study was to examine the effects of marijuana and tramadol on cognitive impairment of Albino rats.

Method

This chapter reports the details of this experimental research under four sections: Participants, Instrument, Procedure and design and statistics.

Participants

The subjects were Albino Rats, which were supplied by a Biochemist. They were nine (9) in number, but before they could be used for experiment, one (1) rat escaped from the laboratory. This was however not replaced because the number of subjects left could still be used for the experiment. All the participants were 3 months old and they weighed differently through similar weights (300 - 350g). The Albino Rats were kept in a cage with two different compartments; saw-dust were sprayed on the floor of the compartments and changed daily to maintain clean and comfortable environment.

Instruments

Cognitive impairment was tested using the radial arm maze. This was designed by Olton and Samuelson in 1976 to measure spatial learning and memory in rats. The original apparatus consists of eight equidistantly spaced arms, each about 4 feet long, and all radiating from a small circular central platform. At the end of each arm there is a food site (sugar in this experiment), the contents of which are not visible from the central platform. Two types of memory that were assessed during the performance in this experiment were reference memory and working memory. Reference memory is assessed when the rats only visit the arms of the maze which contains the reward.

The failure to do so will result in reference memory error. Working memory is assessed when the rats enter each arm a single time. Re-entry into the arms would result in a working memory error. The design ensures that, after checking for sugar at the end of each arm, the rat is always forced to return to the central platform before making another choice. As a result, the rat always has eight possible options. Elaborate controls are used to ensure that the rats are not simply using their sense of smell, either to sense unclaimed food objects or to sense their own tracks.

Other instruments used for this experiment included:

- Table
- Chair
- Stop wash
- A weighing balance that weigh above 1 gram and it was used for weighing the albino rats
- Permanent Marker: It was used to mark numbers on the body of the subjects in place of their names.
- Hand Towel: It was used to hold grip of the rats
- Heater: It was used to heat the marijuana and water to extract into the liquid content.
- 300 microns of standard test sieve: It was used to sieve the boiled marijuana
- A 5 ml and 3 ml syringe: It was used to administer the drug
- A 10 ml beaker: It was used to mix tramadol powder and fanta together
- A pipette: It is a long transparent object that is used to measure the quantity of the treatment to be administered.

Procedure

The participants (albino rats) were divided into 4 experimental groups, and the participants in each group were numbered with permanent marker on their tails for easy identification. The Albino Rats were kept in a cage with two different compartments; saw-dust were sprayed on the floor of the compartment and changed daily to maintain clean and comfortable environment. They were fed with poultry feeds, cake and popcorn daily, but they enjoyed cakes and popcorn more. This makes the researcher to supply the rats more of them. Their behaviour was observed daily for 2 weeks and the researcher changed their saw-dust daily in order to keep the cage neat and to ensure their food does not get contaminated with their urine.

However, the administration of the psychoactive substance was done orally, whereby the laboratory animals were held on by the neck region, thereby getting hold of the hands and also their legs. This was done in two ways: 3 ml and 5 ml. The 3 ml of the extracted liquid of marijuana was administered to group 1a, who were supposed to take low dosage of marijuana, while 5 ml was administered to group 1b, who were supposed to take high dosage of the marijuana. All these were done through the use of a syringe while their mouth is held open, even though little portion or amount may be vomited.

Also, 3 ml and 5 ml of tramadol powder mixed with Fanta due to its bitter taste were administered to group 2a and 2b respectively, through the use of the same procedure above. After the administration, the immediate action of the psychoactive substance on the laboratory rats was observed. Furthermore, because the immediate response of the treatment was needed for the experiment and not the long term effect and was based on the observational effect of the psychoactive substance on the ability of the rat to complete both reference and working memory. Thus, cognitive impairment was observed based on the rats' ability to visit the arms of the maze which contains the reward, and ability to enter each arm a single time.

Design and Statistics

The experiment made use of two independent variables with two groups each. Therefore it is a 2X2 factorial design. The 2-WAY ANOVA was used for data analysis.

Result

This chapter entails the result summary of the study analysis on effects of marijuana and tramadol on cognitive impairment among albino rats.

Tests of Between-Subjects Effects					
Dependent Variable: Cognitive Impairment					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	148.500a	3	49.500	2.176	.233
Intercept	3243.857	1	3243.857	142.587	.000
Marijuana	123.857	1	123.857	5.444	.041
Tramadol	1.714	1	1.714	.075	.797
Marijuana * Tramadol	1.714	1	1.714	.075	.797
Error	91.000	4	22.750		
Total	4024.000	8			
Corrected Total	239.500	7			
a. R Squared = .620 (Adjusted R Squared = .335)					

The ANOVA table showed significant effect of marijuana on cognition among laboratory animal (Albino Rats) ($f = 5.44$, $p < .04$). Therefore, the hypothesis which stated that "Marijuana would significantly impair cognition among laboratory animals', is hereby accepted.

On the contrary, the table revealed no significant effect of tramadol on the cognition of the laboratory animals (albino rats) ($f = .08$, $p < .80$). Therefore, the second hypothesis which stated that 'Tramadol would significantly impair cognition among laboratory animals', is hereby rejected.

The first hypothesis which was accepted was in-tandem with the result that was found out. Thus both the 3 ml and 5 ml of marijuana had an effect on the albino rat, unlike the 3 ml and 5 ml of tramadol which had no effect at all that was why the second hypothesis was rejected.

Discussion and Conclusion

Discussion

The finding of the study reveals a significant effect of marijuana on cognitive impairment. Thus validating the hypothesis which stated that marijuana would significantly impair cognition among laboratory animals (albino rats). Evidence from this finding seems to be in line with literary assertions relating to the studied variable, which claims that the consumption of marijuana or other psychoactive substance disrupt the coordination of the nervous system as well as the executive functions of the brain which are the cognitive abilities that helps one to plan, organize, reason, achieve goals, control emotions and also control behavioural tendencies.

More specifically, this finding is indeed in tandem with the work done by Anderson, Rizzo, Block, Pearlson and O'Leary (2010) in their study investigating sex, drugs, and cognition. Tasks were chosen to tap a wide variety of cognitive domains affected by sex and marijuana including attention, cognitive flexibility, time estimation, and visuospatial processing. As expected, the result shows acute marijuana use impaired performance on selective and divided attention, time estimation, and cognitive flexibility.

It also agrees with the research done by Schweinsburg, Brown, and Susan Tapert (2007) in their study investigating the influence of Marijuana use on Neurocognitive Functioning in Adolescents, and found that marijuana use have a long term effect on neurocognitive functioning but mild short term effect, also acute marijuana use causes a chronic effect on neurocognitive functioning among adolescents.

On the contrary, it disagrees with the research done by Hart, Wilfred, Haney, Richard and Marian [14], who studied effects of Acute Smoked Marijuana on Complex Cognitive Performance, and observed no significant differences when accuracy of responding on attention, memory, visual-spatial processing, reasoning, flexibility, and mental calculation tasks were analyzed as a function of Δ9-THC concentration.

It also disagrees with the research study done by Ebubechukwu (2015) on the Effect of Cannabis Sativa and Ethanol Mixture on Aggressive Behaviour and Cognition, and found that cannabis sativa does not have a significant effect on aggressive behaviour and cognition.

On the contrary, the finding shows non-significant effect of tramadol on cognition among laboratory animals (albino rats). This contradicts the second hypothesis, which stated that 'Tramadol would significantly impair cognition among laboratory animals. This is in disagreement with the study of Hosseini-Sharifabad, Rabbani, and Bagheri (2016) who studied effect of Acute and chronic tramadol administration on spatial memory impairment among wistar rat, and found that tramadol impaired memory when administered acutely or chronically. Single dose administration of tramadol showed more destructive effect than multiple doses of tramadol on the memory [15-30].

Limitation of the Study

The major limitation of this study is the small sample size used for the experiment. The population of the albino rats employed for the experiment is small. Due to financial crises the researcher adopted few albino rats for the experiment.

Suggestion of Further Studies

The researchers suggested that there is need for a repeat experiment of the same research topic but using tramadol alone as the independent variable with higher number of subjects. Results gotten from this experimental study and subsequent studies would be very helpful and experiments should be channeled on the effect of tramadol on the short term memory. Also NDLEA should see the publication of this work as a wakeup call to eliminate the proliferation of marijuana and tramadol use in the country because of its effect on cognitive functioning.

Implication of the Study

Cognitive deficits associated with specific parameters of cannabis and tramadol use and its interactions with neurodevelopmental stages and neural substrates will better inform our understanding of the nature of the association between cannabis and tramadol use and psychosis. Further research in this field will enhance the knowledge of underlying pathophysiology and improving treatments for substance abuse and mental illness. Cognitive functions may provide a guide to treating marijuana and tramadol addiction and furthermore insight in the cognitive motivational processes related to cannabis and tramadol use in cognitive impairment treatment strategies.

Conclusion

This experimental study explains the prevalence rates that marijuana and tramadol use have increased in recent years and as such, chronic, heavy cannabis and tramadol use is a growing health concern. This research on the effects of marijuana and tramadol on cognitive impairment has shown that marijuana significantly impaired cognition while tramadol have no chronic significant effect on cognition. This showed the effect of marijuana on executive functions. However, this research concludes that when interpreting effect of tramadol on executive cognition, there are some important methodological differences to take into consideration such as the recency, amount, duration and age of onset of those psychoactive substance uses.

Bibliography

1. Shettleworth S J. "Cognition, Evolution and Behavior (2nd edition). Oxford Press, New York (2010).

2. Blomberg O. "Concepts of cognition for cognitive engineering". *International Journal of Aviation Psychology* 21.1 (2011): 85-104.

3. Denis Michel and Loomis Jack. "Perspectives on Human Spatial Cognition: Memory, Navigation, and Environmental Learning". *Psychological Research* 71 (2007): 235-239.

4. Coren Stanley., *et al*. "Sensation and Perception". Harcourt Brace (1999): 9.

5. Shipton E A. "Tramadol present and future". *Anaesth Intensive Care* 28.4 (2000): 363-374.

6. Frink M C., *et al*. "Influence of tramadol on neurotransmitter systems of the rat brain". *Arzneimittelforschung* 46.11 (1996): 1029-1036.

7. Minami K., *et al*. "What is the main mechanism of tramadol"? *Naunyn-Schmiedeberg's Archives of Pharmacology* 388.10 (2015): 999-1007.

8. Matthiesen T., *et al*. "The experimental toxicology of tramadol: an overview". *Toxicology Letters* 95.1 (1998): 63-71.

9. Rojas-Corrales M O., *et al*. "Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats". *Life Sciences* 72.2 (2002): 143-152.

10. Julien R M. "A primer of drug action". New York: Freeman (1992).

11. Fergusson D M and Horwood L J. "Does cannabis use encourage other forms of illicit drug use?" *Addiction* 95.4 (2000): 505-520.

12. Beck A T. "Cognitive therapy and emotional disorder". New York: International University Press (1976).

13. Beck A T. "Cognitive therapy". *American Psychologist* 46.4 (1991): 368-376.

14. Carl L Hart., *et al*. "Effects of Acute Smoked Marijuana on Complex Cognitive Performance". *Neuropsychopharmacology* 25.5 (2001): 757-765.

15. Ase A R., *et al*. "Altered serotonin and dopamine metabolism in tie CMS of serotonin 5-HT1A or 5-HT1B re: Knockout mice". *Journal of Neurochemistry* 75 (2000): 2415-2426.

16. Cannon W B. "Bodily changes in pain, hunger, fear and rage: An account of recent researches into the function of emotional excitement". New York: Appleto J., *et al*. Age-dependent changes of brain GABA levels, turnover rates and Shock -induced aggressive behavior in inbred strains of mice. Biochemical Pharmacology 26 (1987): 83-88.

17. Darwin C. "Origin of species". New York: Modem Library (1871/1948).

18. Dauda U. "Drug Abuse, Alcolism: A Case Study of Zaria Local Government" (1999).

19. Hinde R A. "Animal behavior". New York: McGraw-Hill (1970).

20. Peterson J B., *et al*. "Acute alcohol intoxication and cognitive functioning". *Journal of Studies on Alcohol* 51.2 (1990): 114-122.

21. Pfaff D W Drive. "Neurobiological and Molecular Mechanisms of Sexual Motivation". (MIT Press, Massachusetts, USA)" (1999).

22. Post R B., *et al*. "An effect of alcohol on the distribution of spatial attention". *Journal of Studies on Alcohol* 57.3 (1996): 260-266.

23. Ramboz S., *et al*. "5-HT1B receptor knock out-behavioral consequences". *Behavioural Brain Research* 73 (1996): 305-312.

24. Rojas-Corrales M O., *et al*. "Tramadol induces antidepressant-type effects in mice". *Life Sciences* 63.12 (1998).

25. Rush C R., *et al*. "Acute behavioural and cardiac effects of alcohol and caffeine, alone and in combination, in humans". *Behavioral Pharmacology* 4.6 (1993): 562-572.

26. Timbergen N. "The curious behavior of the Stickleback". *Scientific American* 187.6 (1952): 22-26.

27. Tiplady B., *et al*. "Effects of ethanol and temazepam on episodic and semantic memory: A dose-response comparison". *Human Psychopharmacology* 14 (1999): 263-269.

28. Tzambazis K and Stough C. "Alcohol impairs speed of information processing id simple and choice reaction time and differentially impairs higher-order cognitive abilities". *Alcohol and Alcoholism* 35.2 (2000): 197-201.

29. Viding E., *et al*. "Aetiology of the relationship between callous unemotional traits and conduct problems in childhood". *British Journal of Psychiatry* 190 (2007): 33-38.

30. WHO. "World health organization expert committee on problems related to alcohol consumption". Geneva, Switzerland, World Health Organization 2 (2007).

Volume 7 Issue 1 January 2018