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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Changes in behaviour, and cerebellar histology of Wistar rats exposed to refuse dump

Ijomone Meashack[1,3], Gilbert Waritimi[2], Felix Onyije[2], Atoni Atoni[2] and Polycarp Nwoha[3]*

[1]Department of Human Anatomy, Cross River University of Technology, Okuku, Cross River State, Nigeria.
[2]Department of Human Anatomy, Niger Delta University, Amassoma, Wilberforce Island, Bayelsa State, Nigeria.
[3]Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Open waste dump sites are a common spectacle in Nigeria. These sites may cause neurotoxicity to people dwelling in their vicinity. Hence, the present study investigated the effects of long-term habitation at the vicinity of refuse dump sites on motor, mood and cognitive behaviours in rats, as well as on cerebellar histology. Young adult male Wistar rats, approximately 70 days old, were used for this study. Rats were housed in the vicinity of a refuse dump site for 8 months. At the end of 8 months rats were subjected to neurobehavioural studies in the open-field test (OFT), elevated-plus maze (EPM) and Morris water maze (MWM). Following behavioural studies, the cerebellum was excised and prepared for routine Hematoxylin and eosin (H&E) staining protocol. Results showed significant difference (p<0.05) on motor parameters of the OFT between exposed rats and control. Also, exposed rats showed significantly lower (P<0.05) % time in open arms on the EPM, and significantly higher (p<0.001) latency in the MWM. Histological studies revealed alterations to the cerebellar white core as well as degenerative changes of the Purkinje cells. This study therefore shows that long-term habitation in the vicinity of refuse dump site may be injurious to brain structures accompanied with behavioural defects.

Key words: Refuse dump, behaviour, motor, cerebellum, rats.

INTRODUCTION

It is unavoidable that waste will continuously be generated by human population. However, management of these wastes is a global challenge (Kadafa et al., 2013). Waste management, which consists of generation, collection, processing, transport, and disposal of solid waste is of huge importance for both environmental and public reasons (Porta et al., 2009). The major methods of waste management include recycling, composting, sewage treatment, incineration and landfill (Rushton, 2003). Although considered as among the best practices of waste management, these methods have been shown to pose various health challenges (Rushton, 2003; Pheby et al., 2002). In many developing nations, landfill is the most commonly adopted practice of waste disposal (Alimba et al., 2012).

Landfill is the deposition of waste in an area specially designed for such (Porta et al., 2009; Rushton, 2003). Of all the methods mention earlier, this method is most similar

*Corresponding author. E-mail: polycarpnwoha@yahoo.com.

to open waste dump sites. However, modern landfill sites consist of a pre-constructed 'cell' enclosed in an impermeable layer and with controls to minimize emissions of gases (Rushton, 2003). In most instances, landfills are not designated for any specific type of waste. Therefore they may contain not just only household or municipal wastes, but also industrial or hazardous and biomedical or infectious wastes (Alimba et al., 2012; Abul, 2010).

In Nigeria, as in most developing countries, the prevalent form of solid waste disposal is "open dumping". Open waste dump sites are a common spectacle in various states of Nigeria, both within the residential areas and at outskirts of cities, towns and villages. These dump sites pose huge environmental effects, including water pollution from leachate (Alimba et al., 2012). Also, air pollution due to anaerobic putrefaction of organic matter results in the formation of methane, carbon dioxide, sulphur, nitrogen, and mixture of volatile organic compounds (Zmirou et al., 1994). Furthermore, these sites emit documented or suspected carcinogens or teratogens (such as nickel, chromium, arsenic, benzene, vinyl chloride and dioxins) (Harrad and Harrison, 1996) as well as accumulate other toxic substances such as cadmium, pesticides and asbestos (Harrad and Harrison, 1996). Other hazards may include dusts, odours and animal vectors (example, flies) (IEH, 1997). Epidemiological studies have linked exposure to landfill sites with increased birth defects and reproductive disorders as well increase in frequency of cancers (Portal et al., 2009; Rushton, 2003).

It is likely that exposure to open refuse dump would have disastrous consequences on the brain and behaviour. Yet many poor people live near these sites and rummage through the thick for recoverable they can market for money. The present investigation therefore, sets out the effects of long-term habitation at the vicinity of refuse dump sites on motor, mood and cognitive associated behaviours in rats, as well as on cerebellar histology.

MATERIALS AND METHODS

Animal care and treatment

Thirteen young male adult Wistar rats, approximately 70 days old, were obtained from the animal holding of Department of Human Anatomy, Niger Delta University, Bayelsa, Nigeria, were used for this study. Some of the rats were housed in hut (built specifically for the purpose of the study) in the vicinity of a large site of refuse dump along Amassoma Road, outskirts of Yenagoa, Bayelsa. These served as the exposure group (n=7). The remaining animals served as control (n=6), and were housed in a similar hut but within the vicinity of the university. The rats were provided with food and water ad libitum. Control and exposed rats were left in their environments for 8 months. All animals were handled in accordance with the guidelines for animal research as detailed in the National Institutes of Health (NIH) Guidelines for the care and use of laboratory animals (NIH Publication 1985).

Neurobehavioural studies

At the end of the 8 month period, animals were returned to the animal holding and allowed to acclimatize. They were then subjected to various behavioural protocols associated with motor, mood and cognitive functions. Behavioural studies were performed across two days. On day one, animals were tested on the open-field box and elevated-plus maze. Morris water maze was done the following day. The protocols were performed as follows:

Open-field test (OFT): The OFT is commonly used to assess locomotor and exploratory activities in experimental rats and mice (Santiago et al., 2010; Brown et al., 1999). The apparatus consists of a box (72x72x36 cm) with the floor divided into 18x18 square units. The interior of the apparatus is painted white and the floor is covered with Plexiglas. The animals are placed in the centre of the box and allowed to freely explore the area for 5 min. The following parameters were obtained; line crossings (Frequency with which rats crossed one of the grid lines with all four limbs), and rearing against the wall (no of times the animals stood on their hind paws against the wall). The apparatus is cleaned with 5% ethanol before testing a new animal to eliminate possible bias due to odours left by previous animal.

Elevated-plus maze (EPM): The EPM is commonly used to assess anxiety-like behaviour in laboratory rodents (Sun et al., 2008; Casteller et al., 2006). The apparatus consists of two open arms (50 × 10 cm) crossed with two closed arms of the same dimensions with walls 30 cm high. The arms are connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. The maze is elevated 60 cm above the floor. Each rat was placed in the center of the elevated plus maze facing an open arm, and allowed 5 min free exploration. The following parameters were scored; open arm entry, closed arm entry, duration in open arm and duration in closed. %Open arm entries was thus obtained as percentage of open arm entry to total arm entries. %Time in open arms as percentage of time spent in the open arms to total time spent in both the open and closed arms. Arm entry is defined as when the hind paws of the rats are completely within the arm. The apparatus is cleaned with 5% ethanol before testing a new animal to eliminate possible bias due to odours left by previous animal.

Morris water maze (MWM): The MWM is one of the most widely used tasks for studying spatial learning and memory in laboratory rats and mice. Here, a single day protocol as previously described by Pierre and Debouzie, 2000, and adapted by Ijomone et al. (2012), was used. The apparatus consists of a large circular pool (tank) about 6 ft in diameter and about 3 ft deep. The inside of the tank was painted white and the outside brown. It was filled up with tap water, measuring about 25°C. A platform of 24 cm high and 10 cm in diameter was placed at one quadrant of the pool. During pre-training, it was exposed 1 inch above the water. This teaches the rat that there is a platform, and that it is the way to get out of the water. During the learning phase (training), the escape platform was 1 inch below the water, and the water was made opaque by adding milk. Pre-training and Learning phase (training) was done across a single day. Each animal underwent one session of three consecutive trials during pre-training. During training, each animal underwent four sessions of three consecutive trials per session. All animals completed a session before another session is carried out. The starting position was rotated across the four quadrants for the four sessions. The latency (time taken to reach the platform) was recorded manually with a stopwatch. If the animal failed to climb onto the platform within 60 seconds, the trial was stopped and the animal was removed from the water and placed on the platform. The inter-trial interval was 10 seconds, and the animal remains on the platform during this period before beginning the new trial. At the end of the three trials, the animal was removed and placed under a

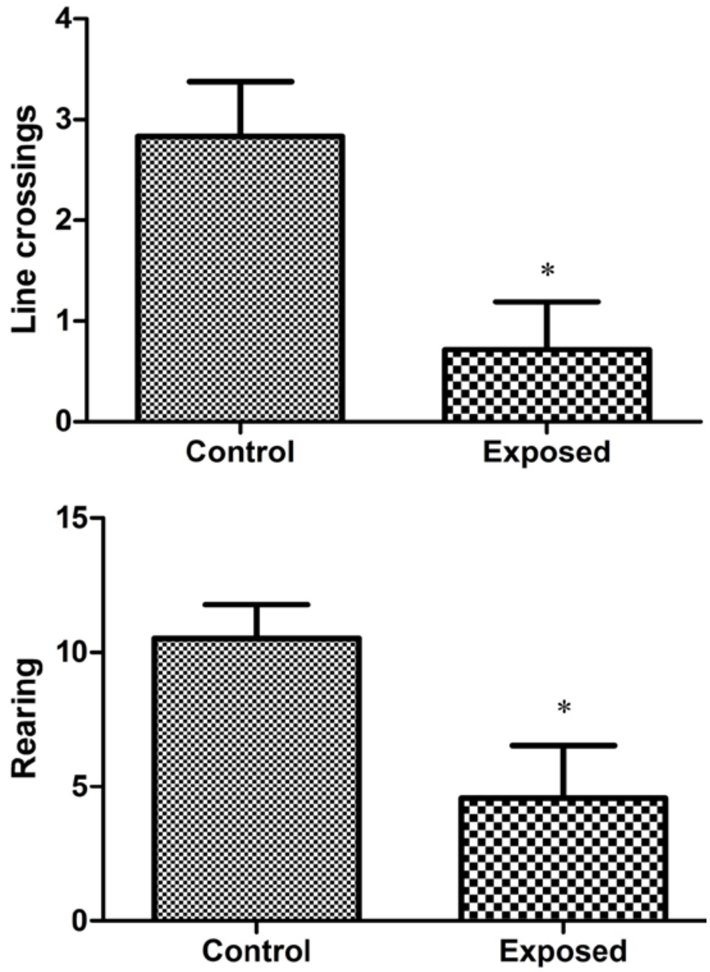

Figure 1. OFT of control (n=6) and exposed (n=7) rats. Bars are mean±SEM.
*P<0.05. Student's t-test.

lamp for warmth. The inter-session interval was 1 h. One probe trial was performed after training on the same day, in which the platform is removed from the pool. The probe trial was performed to assess that the animal recalls the location of the platform and the strategy that the animal follows when it discovers the platform is not there. The number of times the animal crossed the position of the removed platform was counted as probe trial scores.

Histological studies

Following behavioural studies, rats were sacrificed by cervical dislocation. Cerebellar tissues were excised and fixed in 10% formal saline. Tissues were processed for paraffin wax embedding and routine H&E staining protocol. Slides were viewed under a digital microscope (Leica DM750 with attached ICC50 camera) and digital photomicrographs were taken.

Statistical analysis

Data were expressed as mean±SEM and were analysed using student's t-test. GraphPad Prism 5 (Version 5.03, GraphPad Inc., USA) which was the statistical package used for data analysis. Significant difference was considered as P<0.05.

RESULTS

Neurobehavioural studies

Analysis of behavioural studies in the OFT showed that exposed rats had significantly lower line crossings compared to control rats (P<0.05). Also, exposed rats performed significantly lesser rearing compared to control (P<0.05) (Figure 1).

Behavioural studies on the EPM revealed no significant difference in %Open arm entries between control and exposed rats (P>0.05). However, %Time spent in open was significantly lower in exposed rats compared to control (P<0.05) (Figure 2). MWM studies, revealed that exposed rats had significantly higher latency compared to control (P<0.001). Though, there was no significant difference in

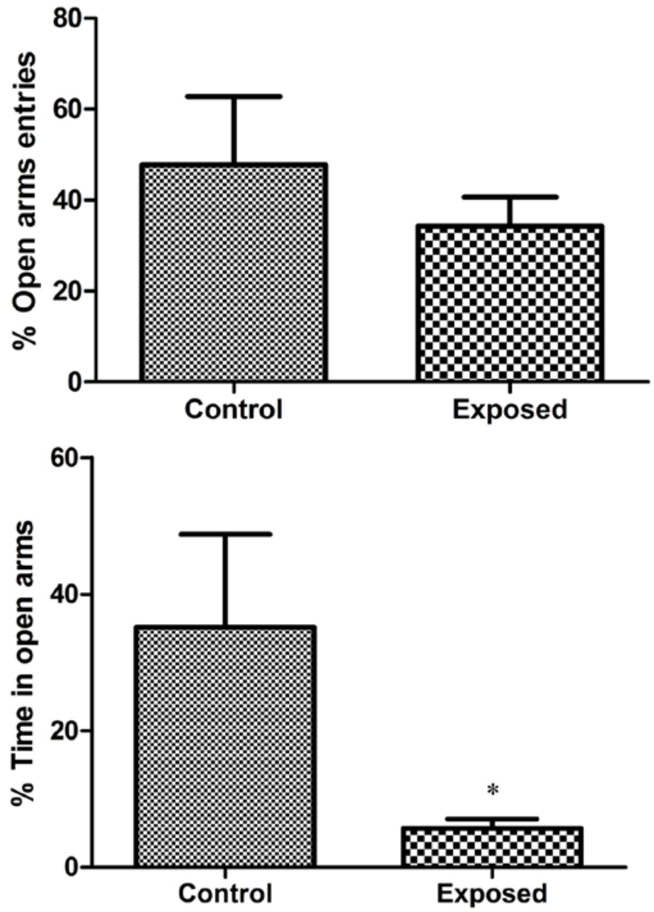

Figure 2. EPM of control (n=6) and exposed (n=7) rats. Bars are mean±SEM. *P<0.05. Student's t-test.

difference in probe trial scores between control and exposed rats (P>0.05) (Figure 3).

Histological studies

Control rats showed normal histology of the cerebellum with a branching central medulla (which is white matter consisting of large axonal tracts) and the three layers of the cortex. The layers are; molecular layer, granular cell layer and the single layer of large neurons (the Purkinje cells) between them. Exposed rats showed vacuolations in the medulla of the cerebellum. This may suggest damage to the white matter core of the cerebellum. Also, few classic appearance of degenerating Purkinje neurons with condensed nucleus and bright eosinophilic cytoplasm were seen (Figure 4).

DISCUSSION

Cancer, birth defects and reproductive disorders are the most common consequent effects on communities dwelling in the proximity of landfill sites. Pukkala and Ponka, (2001) demonstrated that landfills caused cancer or other chronic diseases amongst inhabitants dwelling within close proximity to such sites. They showed that the relative risk of developing cancers increased with the number of years lived in the area. However, in the review by Porta et al. (2009), the authors concluded that the evidence of an increased risk of cancer for inhabitants in proximity of landfill sites is inadequate. In another review, Saunders (2007), showed that while most studies, report a positive association between exposures to proximity of landfill sites with risk of birth defects and reproductive disorders, over half of studies reviewed reported no such association. Nevertheless, the present study has shown that exposures to open dump sites may produce behavioural alterations and neurotoxic effects.

The basic outcome of interest in the open field test is movement, which is greatly influenced by motor output and exploratory drive (Ajibade et al., 2011). Decrease in the parameters of line crossings and rearing are indicative of impaired motor functions and exploratory drive respectively, in the experimental rats (Santiago et

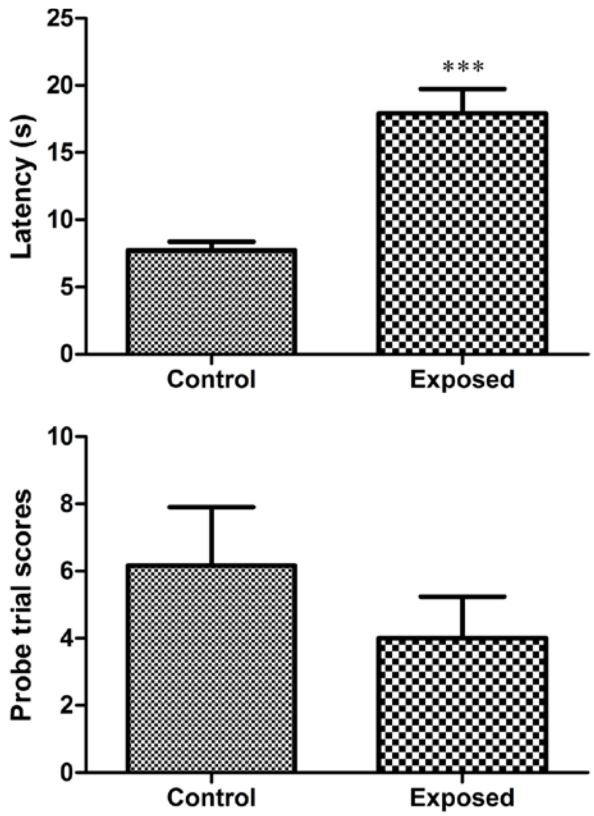

Figure 3. MWM of control (n=6) and exposed (n=7) rats. Bars are mean±SEM. ***P<0.001. Student's t-test.

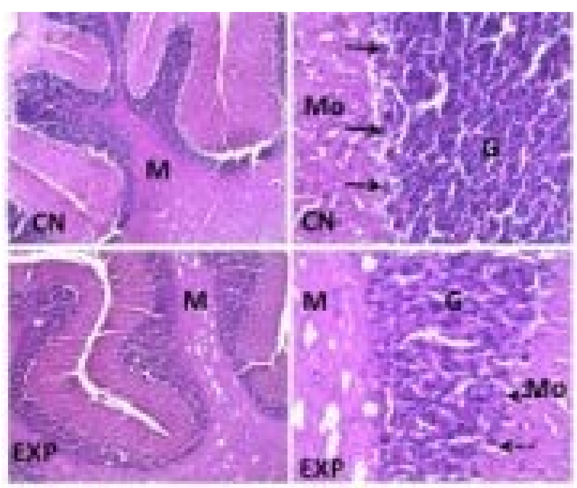

Figure 4. Representative micrographs of cerebellum of control (CN) and exposed (EXP) rats. H&Ex100 (left panel), H&Ex400 (Right panel). Observe the normal cerebellum as seen in control, with branching central medulla (M) and three layers of cortex; molecular (Mo), Granular (G) and Purkinje cells (arrows) layer. In exposed group, observe the presence of numerous vacuolations in the medulla. Also there are few Purkinje neurons showing classic appearance of degenerating neurons, with condensed nucleus and bright eosinophilic cytoplasm (dash arrows).

al., 2010; Brown et al., 1999). In effect, this study shows such long-term exposure to open dump sites impaired the rats motor output and exploratory drive. The EPM is the most frequently used tool to study anxiety trait in rodents (Tejada et al., 2009; Walf and Frye, 2007). Anxiety can be referred to as behavioural, physiological and psychological state induced in animals and humans by stress resembling conditions, characterized by feelings of fear or dread. Fear is a motivational state stimulated by specific stimuli that result in escape or defensive behaviours (Navqi et al., 2012). Rodents normally prefer to spend more time in the closed arms, and make more entries into the closed arms than open arms. Increase in time spent in open arms and/or entries into the open arms points to anti-anxiety (anxiolytic) behaviours (Tejada et al., 2009; Walf and Frye, 2007). Anxiogenic (anxiety-causing) behaviour is thus associated to reduce time spent in open arms and/or open arm entries. Thus, our present data suggest that long-term exposures to refuse dump site results in anxiogenic behaviours in Wistar rats.

Increased latency (time taken to reach the platform) during the training phase of the MWM test point to reduced or impaired learning abilities in rodents. Meanwhile, probe trial scores points towards animals' abilities to recall position of the platform. This parameter adjudges working or short-term memory in a single-day MWM test (Ijomone et al., 2012; Ijomone et al., 2011; Scerri, 2011). The present results thus suggest that exposed rats may have shown an impaired ability to learn though short-term recall of previously learnt location (of the platform) is not altered. Histological studies, revealed some microscopic alterations to the cerebellum of exposed rats. This is suggestive of potential neurotoxicity that may arise from such long-term exposures. Also, considering that the cerebellum coordinates muscular activities of the body, it is possible that alterations to its microarchitecture may have resulted in the observed motor deficiencies during the OFT tests.

It is important to note that many of the substances emitted from these open dump sites have been reported to have neurotoxic effects. Arsenic (Vahidnia et al., 2007), cadmium (Wang and Du, 2013), nickel (Das et al., 2008) as well as carbon dioxide (Dimakakos et al., 1998) have been associated with neurotoxicity. It is likely that a combination of these substances may result in the behavioural modifications and histological alterations shown in this study. However, it is also possible that other harmful substances emitted from dumps as earlier outlined, amongst other hazards such as odours and animal vectors may cause metabolic conditions that may affect behavioural performance.

Conclusion

Long-term exposure to refuse dump site may be injurious to brain structures with resultant detrimental psychological and motor effects.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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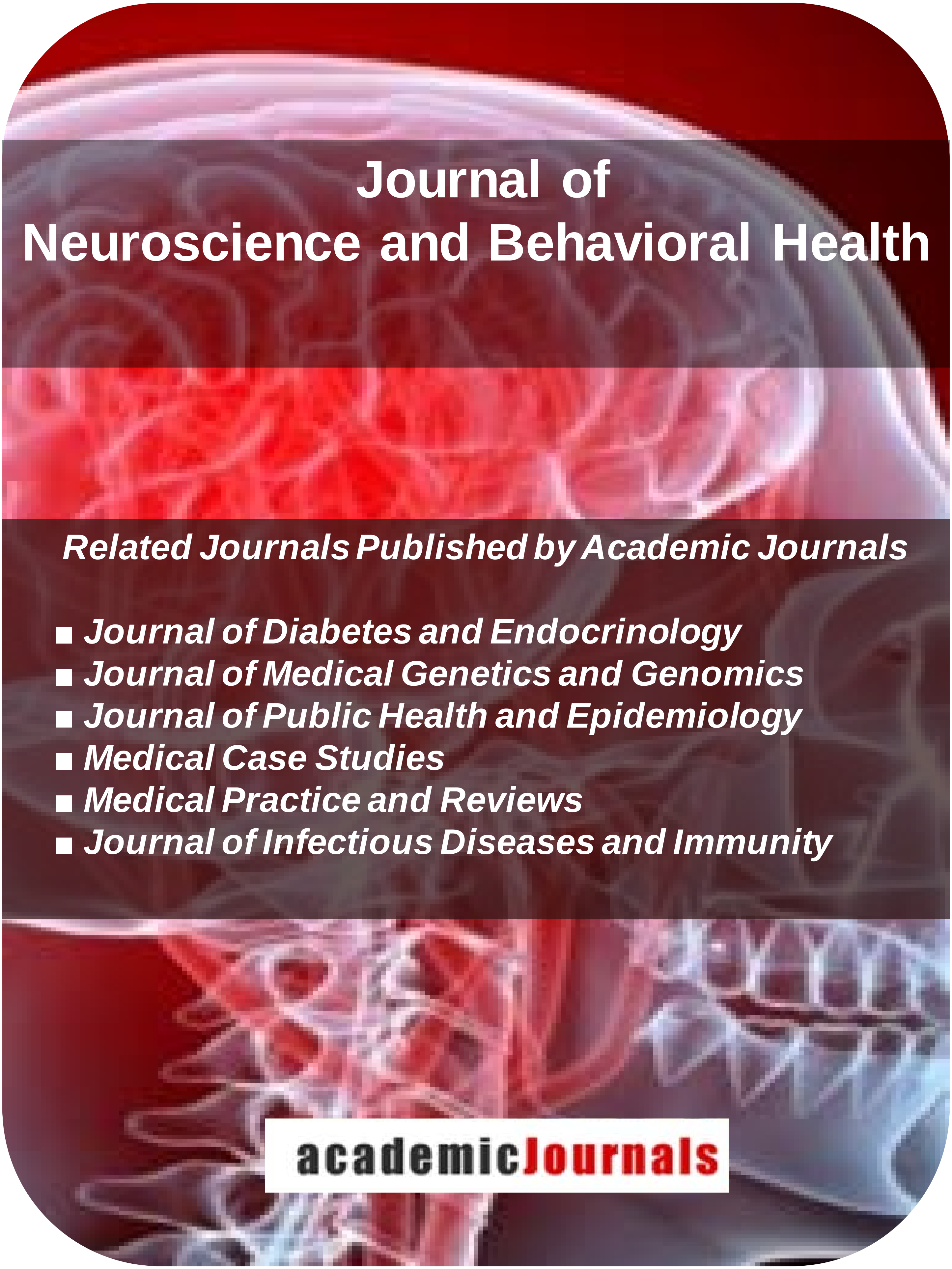
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