Original Article

Access this article online

Quick Response Code:

Website: jecajournal.com

DOI: 10.4103/jeca.jeca_10_17

Role of gamma-aminobutyric acid ergic activation in pathology of −dopamine-2 receptors model of Parkinsonism in mice

Azeez Olakunle Ishola, Oladimeji Ogungbemi, Zaynab Abdulmalik, Ololade Boluwatife Faniran, Edem Ekpenyong Edem, Philip Adeyemi Adeniyi, Moyosore Salihu Ajao[1], Ogundele Olalekan Michael

Abstract

BACKGROUND: Blocking of dopamine-2 receptors (D_2R) in the brain showed motor symptoms seen in Parkinsonism. Since D_2R is excitatory in the brain and blocking it is like inhibition. This work is designed to show if activating gamma aminobutyric acid (GABA) system in the brain contributes to the pathogenesis of Parkinsonism seen in–D_2R model of Parkinsonism.

MATERIALS AND METHODS: Twenty male adult albino mice were randomly divided into four groups (Veh, –D_2R, +GABA, and –D_2R + GABA). Veh. animals were given 0.04 mL of normal saline, –D_2R were given 10 mg/kg body weight (BW) of haloperidol for 14 days, +GABA were given 10 mg/kg BW of diazepam for 7 days and –D_2R + GABA were given 10 mg/kg BW of haloperidol for 14 days with subsequent 10 mg/kg BW of diazepam for 7 days. Each group contains 5 animals and all treatment was done intraperitoneally. Motor activity of the animals was assessed using rotarod, Y-maze for spatial memory and elevated plus maze for anxiety and locomotion. At the end of treatment, the animals were anesthetized using ketamine and perfused transcardially with formal saline. Brains were then excised and fixed in formal saline. The prefrontal cortex (PFC) and hippocampus were processed for histological study using hematoxylin and eosin stain and immunohistochemistry for Lewy bodies. Data were expressed as mean ± standard error of mean and analyzed using analysis of variance with Tukey *post hoc* test significant level was set at $P < 0.05$.

RESULTS: Motor activity was significantly reduced in all treated groups (–D_2R, +GABA and –D_2R/+GABA) compared to the control (Veh) as they all have lower latency of fall and arm entries. Y-maze result shows that spatial memory was significantly reduced in –D_2R and –D_2R/+GABA groups but not + GABA. Anxiety-related behavior was high in all treated groups compared to control. Cellular distortion was observed in the PFC and hippocampus of all treated groups with –D_2R/+GABA group having a high level of distortion. Lewy bodies accumulation was absent in the brain regions observed from all the groups.

CONCLUSIONS: GABAergic activation aids motor and memory deficit and marked brain pathology in –D_2R model of Parkinsonism.

Keywords:

+gamma aminobutyric acid, dopamine-2 receptors, Lewy bodies, parkinsonism

Introduction

Parkinson's disease (PD) is a slowly progressive degenerative disease of the nervous system associated with the destruction of dopaminergic neurons in the substantia nigra pars compacta (Jason *et al.*, 2005). PD can be induced by long-term usage of certain antipsychotic drugs such

Department of Anatomy, Afe Babalola University, Ado-Ekiti, [1]Department of Anatomy, University of Ilorin, Ilorin, Nigeria

Address for correspondence:
Mr. Azeez Olakunle Ishola, Department of Anatomy, Afe Babalola University, Ado-Ekiti, Nigeria.
E-mail: ao.ishola@abuad.edu.ng

How to cite this article: Ishola AO, Ogungbemi O, Abdulmalik Z, Faniran OB, Edem EE, Adeniyi PA, *et al*. Role of gamma-aminobutyric acid ergic activation in pathology of −dopamine-2 receptors model of Parkinsonism in mice. J Exp Clin Anat 2017;16:93-102.

as haloperidol. Haloperidol is a fourth generation anti-psychotic drug which falls under the family Butyrophenones (Whalen *et al*., 2007). It functions by binding to and blocking dopamine-2 receptors (D_2R) receptors at clinically effective doses (Whalen *et al*., 2007) thereby providing relief for a patient suffering from psychosis. Despite its good effects, Haloperidol's affinity and binding with D_2R lead to these receptors being unavailable for binding with dopamine. These lead to the production of parkinsonian symptoms in most patients who were placed on the long-term prescription of haloperidol to relieve their psychosis (Whalen *et al*., 2007).

The Nigrostriatal pathway is one of the four major dopamine pathways and is the efferent connection between the susbtantia nigra and corpus striatum. It is particularly involved in the production of movement, as part of a system called the basal ganglia motor loop. Dopaminergic neurons of this pathway synapse onto gamma aminobutyric acid (GABAergic) neurons (Mattes *et al*., 1986; Conde *et al*., 1994). Loss of dopamine neurons in the substantia nigra is one of the main pathological features of Parkinson disease, leading to a marked reduction in dopamine function in the nigrostriatal pathway. Nigrostriatal pathway is also implicated in producing tardive dyskinesia, one of the side effects of antipsychotic drugs. This medication (particularly the older typical antipsychotics) blocks D_2-dopamine receptors in multiple pathways in the brain.

The role of the GABAergic system in PD pathology is not fully known. This study is designed to investigate if GABAergic activation (using benzodiazepine) has an active role in motor deficit and brain pathology observed in haloperidol-induced Parkinsonism.

Materials and Methods

Animal handling

Twenty animals in the range of 20–26 g were purchased from the animal house of University of Ibadan, Oyo state. They were transported in transport cages with minimal stress. They were housed in the animal house of Afe Babalola University Ado-Ekiti with standard cages. Food (10 mm fish feed) and drinking water were provided *ad libitum*. The animals were maintained at room temperature and were allowed to acclimatize for 10 days.

Drugs procurement

Haloperidol used to induce Parkinsonism was purchased from Juli Pharmacy Oja-Oba Market Ado-Ekiti. Diazepam used to activate GABAergic system was purchased from Reichpharm Pharmacy Ado-Ekiti through a Pharmacist colleague Dr. Olubiyi of Obafemi Awolowo University Ile-Ife.

Animal treatment

BALB/c male mice ($n = 20$) between 20 and 26 g with age 30–40 days were randomly separated into 4 groups [Table 1]. PD was induced after 14 days of treatment with 10 mg/kg body weight (BW) of haloperidol ($n = 10$). Subsequently, mice ($n = 5$) were treated with 10 mg/kg BW for 7 days of Diazepam ($-D_2R/+GABA$) while the remaining set of the haloperidol-induced mice ($n = 5$) were left untreated ($-D_2R$). A separate group of mice ($n = 5$) received diazepam for 7 days (10 mg/kg BW; +GABA) while the control mice ($n = 5$) received normal saline for 7 days (Veh). All administration was done intraperitoneally.

Behavioral studies

At the end of the treatment, animals were examined in various tests for motor coordination using rotarod, spatial memory using Y-maze, and anxiety using elevated plus maze.

Rotarod test (Ogundele et al., 2015)

This was done to assess the level of motor coordination in the experimental animals. Animals were placed on the rotarod bar and the animals were allowed to stable before switching on the machine to rotate. The rotarod machine was set at 5 rpm and to increase steadily to 30 rpm over a period of 5 min. The time taken the animals to fall off the rotating bar is recorded and termed latency of fall (LOF). Animals are assumed to fall off if they cling to the side of the machine. The duration of the test was for 5 min for each animal.

Y-Maze test (Adeniyi et al., 2016)

Spatial memory of the animals was assessed using the Y-maze test. The animals were placed at the junction of the Y-arms and allowed to explore the maze for 5 min. If the animals explore the three arms in succession is termed "Right Decision" but, if the animals explore one arm twice in three successions is termed "Wrong Decision." The spatial memory index termed as percentage of alternation is used as the function of memory and calculated as:

$$\%\ \text{Alternation} = \frac{\text{Number of right decision}}{\text{Number of right decision} + \text{Number of wrong decision}} \times 100$$

Elevated plus maze (Ogundele et al., 2015)

This is done to assess the locomotor activity and anxiety level in the animals. The animals are placed at the

Table 1: Summary of animal grouping and treatment

Days	Veh	$-D_2R$	+GABA	$-D_2R$/+GABA
0-7	NS	NS	NS	Haloperidol treatment
8-14	NS	Haloperidol treatment	NS	Haloperidol treatment
15-21	NS	Haloperidol treatment	Diazepam treatment	Diazepam treatment

NS - Normal saline, GABA - Gamma aminobutyric acid, D_2R - Dopamine-2 receptors

center of the maze facing the open arm and allowed to explore the maze for 5 min. The number of entries into the either closed or open arm is recorded (locomotor activities). The duration spent in the either closed or open arm (a function of anxiety) was also recorded. The number of time the animal looks down in the open arm (head dips) was recorded for the function of anxiety.

Animal sacrifice

A day after the last administration, the animals were anesthetized using 10 mg/kg BW ketamine. Subsequently, the animals were perfused (transcardially) through the left ventricle with normal saline to flush the blood after which a fixative (10% formo saline). The skull was then opened to harvest the whole brain following which it was kept inside specimen bottle containing formal saline. The brain was then dissected on a stereotaxic grid to expose the approximate prefrontal cortex (PFC) and Hippocampus in corona sections relative to the bregma using specific coordinates. The diced brain tissues were transferred into a freshly prepared cryopreservative (10% formalin + 30% sucrose) for 72 h at 4°C following which the brain tissue was processed to obtain paraffin wax embedded tissue blocks for histology and immunohistochemistry (IHC).

Histology (Adeniyi *et al*., 2016)

The brain slices were processed for histology using hematoxylin and eosin (H and E) stains. They are processed using the standard protocol in Anatomy Department, AfeBabalola University Ado-Ekiti.

Purchase of antibodies

Human/Mouse/Rat anti-alpha-synuclein primary antibody was purchased from R and D Systems (MAB13381) UK. Goat anti-rabbit/mouse (polyvalent) secondary antibody (ab64238) was purchased from Abcam and DAB substrate kit (ab93705) was also purchased from Abcam USA.

Immunohistochemistry protocol (Ishola et al., 2015)

IHC was done to the brain slices using heat method of antigen retrieval for paraffin embedded tissue. The slides were incubated in antigen retrieval solution at 70°C for 50 min. After which the slides are incubated with primary antibody (anti-alpha-synuclein) overnight at 10°C. Next day the slides were incubated with secondary antibody (goat anti-rabbit/mouse) for 30 min; then the color was developed following the protocol that came with the detection kit.

Statistical analysis

Data were expressed in mean ± standard error of mean. Data were analyzed using analysis of variance (ANOVA) to compare among the groups and Tukey *post hoc* test was used when ANOVA shows significant. *P* value was set at 0.05. This was done using GraphPd prism version 5.0 GraphPad Software Inc. USA.

Results

Behavioral results

Neurobehavioral activities of the animals were assessed to show if they exhibit cognitive and motor deficit. Motor activity was assessed using rotarod and elevated plus maze, spatial memory using Y-maze test and anxiety using EPM.

Motor activity

Haloperidol-induced PD ($-D_2R$) caused a decline in motor function seen as a reduction in LOF when $-D_2R$ treatment was compared with the control ($^*P < 0.05$). Subsequent treatment with GABA ($-D_2R$/+GABA) failed to improve motor function in this group as the mice also recorded a decrease in LOF when compared with the control (Veh) ($^*P < 0.05$). GABA treatment without prior induced PD also caused a decline in motor function when + GABA was compared with the control ($^*P < 0.05$). No significant difference was observed when $-D_2$, +GABA and $-D_2$/+GABA were compared with each other [Figure 1].

Locomotor activity was reduced in all treated animals as they have a significantly lower number of closed arm entries compared to the control ($^*P < 0.05$, $^{**}P < 0.01$). No significant difference was seen when the treated groups were compared with each other [Figure 2].

Entries to the open arm of the maze were also significantly reduced in the treated groups compared to the control

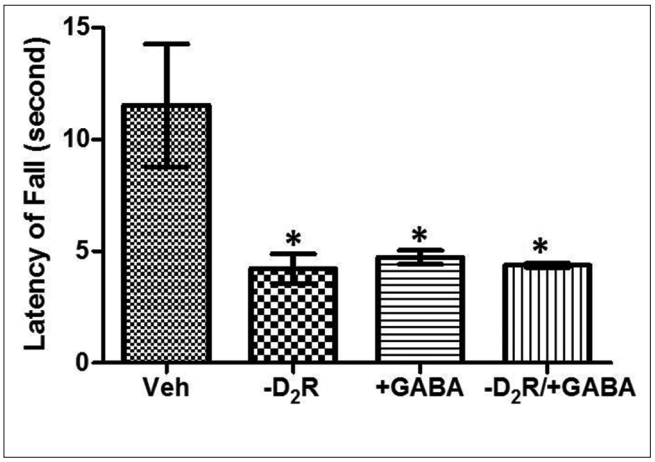

Figure 1: Latency of fall of experimental animals expressed in seconds. Veh; Control, - dopamine-2 receptors; Haloperidol-treated, +gamma aminobutyric acid; Diazepam treated, - dopamine-2 + gamma aminobutyric acid; haloperidol and diazepam treated. The graph shows that all treated group is significantly lower than the control $P < 0.05$. This shows that activating gamma aminobutyric acid nergic system does not alleviate motor coordination disorder experienced in Parkinsonism

group (**P < 0.01, ***P < 0.001). This shows that movement ability was impaired in all the treated group [Figure 3].

Spatial memory

Haloperidol-induced PD (–D_2R) caused a decline in spatial memory index seen as a reduction of percentage alternation when the –D_2R treatment group was compared with the control (*P < 0.05) and + GABA group ($^{\alpha}P$ < 0.05). Subsequent treatment with GABA (-D_2R/+GABA) failed to bring about significant improvement in spatial memory index in this group as the mice also recorded a decrease in percentage alternation when compared with the control (Veh) (*P < 0.05) and + GABA group ($^{\alpha}P$ < 0.05).

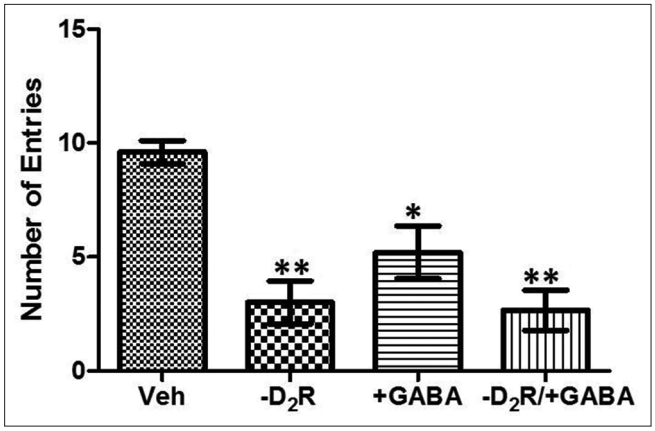

Figure 2: Graph of numbers of closed arm entries of the experimental animals on elevated plus maze. All treated animals have a significantly lower number of entries than the control, - dopamine-2 receptors, – dopamine-2 receptors/+gamma aminobutyric acid (**P < 0.01) and + gamma aminobutyric acid (*P < 0.05)

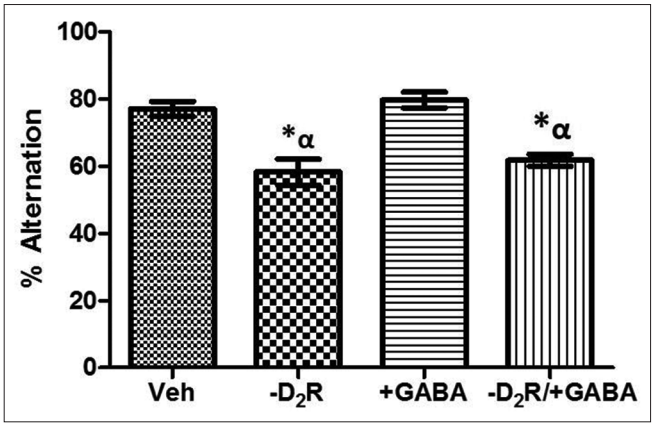

Figure 4: Graphical representation of spatial memory index (% alternation) from Y- maze. Animals with Parkinsonism (-dopamine-2 receptors) show a significant reduction in their memory compared to the control (Veh) (*P < 0.05) and animals treated with diazepam (+gamma aminobutyric acid) $^{\alpha}P$ < 0.05. Parkinsonism mice treated with diazepam (-dopamine-2 receptors + gamma aminobutyric acid) shows no improvement in spatial memory has it is significantly lower than the control (*P < 0.05) and diazepam treated only mice (+gamma aminobutyric acid) $^{\alpha}P$ < 0.05. There is no significant difference in the spatial memory of control and diazepam treated only mice, likewise, no significant difference between – dopamine-2 receptors and – dopamine-2 receptors/+gamma aminobutyric acid animals

GABA treatment without prior induced PD has no effect on spatial memory as the percentage alternation of the animals showed no significant difference compared to control (Veh) [Figure 4].

Anxiety test

The elevated plus maze (EPM) test is done to check anxiety level using number of head dips and duration spent in either closed or open arm.

Anxiety-related behavior which is assessed using the head dipping numbers (frequency of when the animals look downward in the open arm) was noted down. Less anxious animals have more head dips.–D_2R and–D_2R + GABA animals have reduced number of head dips which is significantly lower than the control (**P < 0.01). No significant difference was seen when all treated groups were compared against each other [Figure 5].

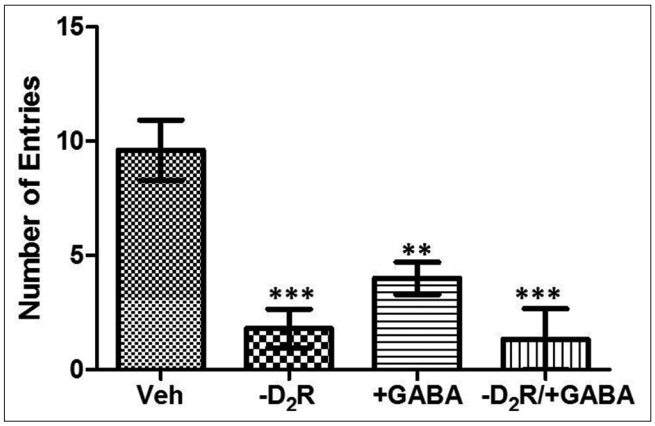

Figure 3: Graph showing number of open arm entries on elevated plus maze. All treated animals have a significantly lower number of entries than the control. **P < 0.01, ***P < 0.001

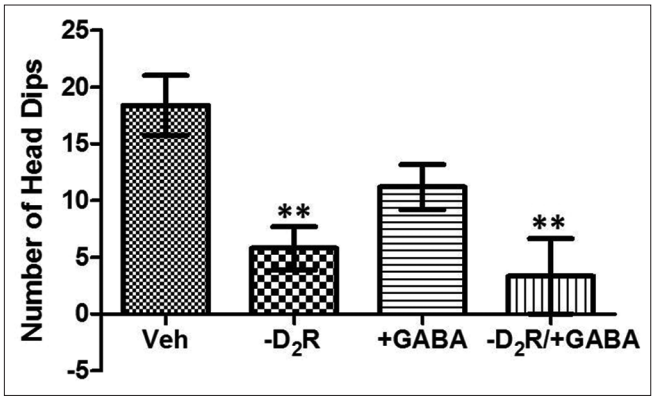

Figure 5: Graph showing the number of head dips observed on the elevated plus maze. Parkinsonism (-dopamine-2 receptors) and Parkinsonism treated (-dopamine-2 receptors/+gamma aminobutyric acid) shows a significant reduction in the number of head dips (**P < 0.01) than the control. No significant difference observed between the control and diazepam treated group i.e. normal saline versus + gamma aminobutyric acid

Time spent in the closed arm which is also a function of anxiety as anxious animals spent more time in closed arm compared to the open arm. Control animals spend less time in the closed arm has they have the minimum value.–D_2R/+GABA animals spent much time in the closed arm (anxious). No significant differences were seen when the values were compared using ANOVA [Figure 6].

Open arm durations were also recorded and compared. Control animals spend much time in the open arm than other groups (opposite of closed arm duration). No significant difference was observed when values were compared using ANOVA [Figure 7].

Histology and immunohistochemistry

Histological evaluation of PFC and hippocampus was done to check the neural architecture using H and E. Lewy bodies deposition was also assessed in the brain areas using the immunohistochemical method.

Neurohistology

Histological slides of the PFC of the control and treated groups showed neurodegeneration mainly in the haloperidol (–D_2R) treated group and combined GABA-treated group (–D_2R + GABA) observed as large empty spaces and distortion in the shape of pyramidal neurons. These are mostly evident at the outer pyramidal layer observed. This shows that while haloperidol (–D_2R) treated group brings about neurodegeneration, further treatment with + GABA fails to bring about improvement in the parkinsonian group.

Histological slides of the dentate gyrus (DG) region of the hippocampus of the control and treated groups showed the presence of wide empty space beneath the granule cells of DG seen in all the treated groups. Axons of the neurons are mean to protrude toward the interior and the presence of this wide space signifies axonal breakage in all the treated groups. The presence of the space-penetrating into the neuronal layers in the –D_2R only group and to a lesser extent –D_2R + GABA group shows that there is cellular distortion in the –D_2R group and to a lesser extent –D_2R + GABA group. This signifies that + GABA treatment to the parkinsonian group brings about no improvement.

Histological slides of the cornu ammonis (CA) region of the hippocampus of the control and treated groups showed distortion of Granule cells in the treated groups as the granule cells appeared to be compressed together. These affected the thickness of the granular cell layer as it was reduced greatly in –D_2R + GABA and + GABA groups.

Neuro immunohistochemistry

Deposition of Lewy bodies is part of the pathological hallmark of Parkinsonism. Blocking of D_2R to mimic Parkinsonism did not lead to accumulation of Lewy bodies as it is not shown in the part of the brain (PFC and hippocampus) observed. Activating GABAergic system after blocking of D_2R and activation of GABA alone also did not lead to Lewy bodies' accumulation.

Discussion

Motor and locomotion deficit

Motor deficit is the major signs of PD and other Parkinsonism related disorders (Litvan *et al*., 2009). Several studies have pointed to the fact that lack of dopamine in the system from the substantia nigra leads to lack of motor modulation in the brain (Eitan *et al*., 2014; Ogundele *et al*., 2016). This study showed that animals treated with haloperidol (–D_2R) have a decline in motor function (reduced LOF) when compared with the control [Figure 1]. This is in line with our previous

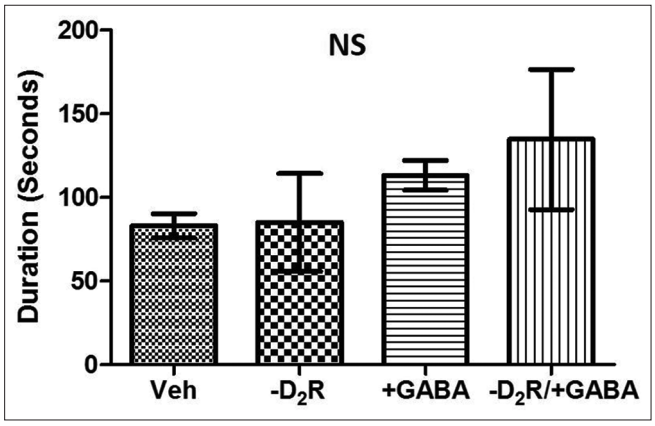

Figure 6: Graph showing total time spent in the closed arm of the elevated plus maze. – Dopamine-2 receptors/+gamma aminobutyric acid animals have the highest value but no significant difference was observed when compared with the control and other groups

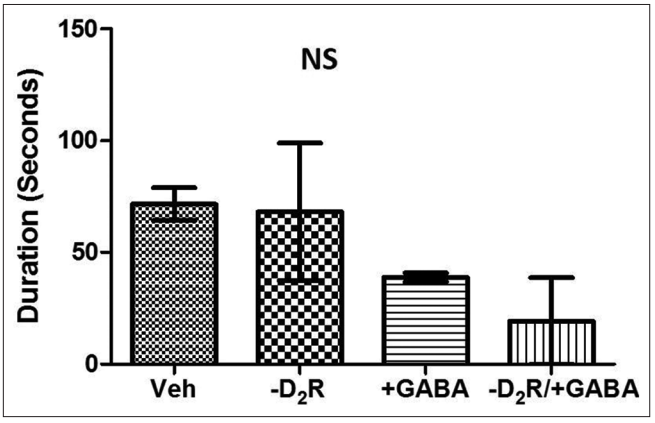

Figure 7: Graph showing total time spent on the open arm of the elevated plus maze. Control and Parkinsonism induced mice (i.e. normal saline and – dopamine-2 receptors) has high value but no significant difference was observed among all the groups

observations that blocking D_2R with haloperidol has been used as a model for studying Parkinsonism (Ogundele *et al.*, 2014; Ishola *et al.*, 2015; Bankole *et al.*, 2015). Haloperidol is said to bind irreversibly to D_2R in the striatum and other brain areas with the D_2R inhibiting the action of dopamine (Reavill *et al.*, 1999; Bertran-Gonzalez *et al.*, 2009) and causing hyperpolarization in the motor cortex and striatum (Ishola *et al.*, 2015). This inhibition leads to movement disorder as dopamine from the substantia nigra cannot exert its action on this brain areas leading to a lack of modulation of motor signals. This is considered as extrapyramidal symptoms. Activating GABAergic system fails to ameliorate the motor deficit seen in haloperidol-induced PD [Figure 1]. D_2R is located on small interneuron in rats (Conde *et al.*, 1994) and mice in all cortical layers (unpublished data). It is hypothesized that D_2R located on the interneurons in the cortex help in modulating output from the pyramidal neurons (Horn, 1990). Activating the GABAergic system which will inhibit the pyramidal neurons together with blocking of D_2R leads to hyper inhibition (long term depression) in the cortex affecting the outflow to the muscle for movement.

Activating the brain inhibitory system alone also shows a decline in motor deficit [Figure 1]. This may also be due to the action of signal inhibition as the basal ganglia also receive input from GABAergic neurons (Siegel and Sapru, 2011). Inhibiting the basal ganglia and the action of interneurons in the cortex may be responsible for the motor decline in this group.

Locomotor activity was also reduced in the treated groups as seen from results obtained from EPM [Figures 2 and 3]. Since motor coordination has been affected seen from the LOF, ability of the animal to move muscle will also be impaired. Similar symptoms are seen in patient with PD as their movement is impaired with on and off period (Murray *et al*., 2012).

Spatial memory function

Memory decline is another symptom associated with PD (Litvan *et al*., 2009). Ishola *et al.*, (2015) and Ogundele *et al.*, (2014) have shown that blocking D_2R in mice not only mimic motor deficit seen in PD but also memory decline. This study also shows that Haloperidol-induced PD ($-D_2R$) caused a decline in spatial memory index seen as a reduction of percentage alternation when the-D_2R treatment group was compared with the control [Figure 4]. In rodents, the most behavioral tests involved the movement of the animals during a test trial. Since animal's movement are impaired in induced PD, this may explain why they perform lower in memory test as they tend to make errors. Another possible explanation is that D_2R is reported to be located on hippocampal neurons as well (Kohler *et al.*, 1985). D_2R has been shown to be important in memory consolidation in the hippocampus (Torres *et al.*, 2003). Blocking of D_2R in hippocampus will inhibit memory consolidation which may lead to spatial memory decline. GABAergic activation (+GABA) fails to produce spatial memory decline seen in $-D_2R$ and $- D_2R$/+GABA [Figure 4]. This shows that D_2R is key to hippocampal function as the only group treated with haloperidol shows decline in memory. This strengthens the report that D_2R is key to memory formation in the hippocampus (Torres *et al.*, 2003).

Anxiety

This study shows that + GABA and$-D_2R$/+GABA spent more time in the closed arm than the open arm with fewer head dips in the open arm than the control group [Figures 5-7] evidenced from the EPM test. Haloperidol-induced PD ($-D_2R$) caused an increase in the anxiety level of parkinsonian mice model seen as a decrease in the number of head dips out of the open arms during the EPM test (less anxious animals have higher head dips) when the $- D_2R$ treatment group was compared with the control group. This is due to the inability of the animals to coordinate their movement. Comparing these parameters with the duration spent in the closed arm [Figure 6],

$-D_2R$/+GABA spent the highest time in the closed arm with little time in the open arm [Figure 7]. Although no significant difference was observed, studies have shown that anxiety and depression serve as symptoms in the pathology of PD (Dissanayaka *et al.*, 2011; Barone, 2011; Schwarz *et al.*, 2011). Some scientist has argued that it is the inability of patients with PD to move when they intend to move that leads to depression and the fear of not been able to coordinate movement when asked to causes the anxiety. Dissanayaka *et al.* (2011), Leentjens *et al.* (2011), Eskow-Juanarajs *et al.* (2011) reported that in some pathology of PD anxiety and depression may have occurred earlier even before evidenced motor disorder seen in PD.

Engin and Treit (2007) showed that GABA type A receptor agonist administration to animals could be a possible reason for the increase in anxiety levels, no work has directly linked haloperidol's effect on the hippocampus to increase in anxiety levels. They also showed that gamma-aminobutyric acid type A receptor agonist, both directly and indirectly, reliably inhibit a number of animals' untrained anxiety reactions when microinfused into the hippocampus, whereas GABA-A receptor antagonists do not. Two hypotheses were postulated in response to their experiment. The first hypothesis is that anxiety is functionally segregated within the hippocampus, with ventral subregions more involved in anxiety-related processes, and dorsal subregions more

involved with cognitive processes. Another possibility is that different hippocampal functions (e.g., memory and anxiety) are mediated by different neurotransmitter systems and/or different receptor subtypes within the hippocampus. While further experiments will be required to verify both hypotheses, we could possibly deduce from our experiment that haloperidol's effect on the hippocampus brings about an increase in anxiety level, an effect which is higher than that of + GABA only administration to the mice model and further administration of + GABA to the parkinsonian mice group brings about further increase in anxiety levels.

Cellular distortion and neuro-degeneration

Haloperidol-induced PD (-D_2R) mice group showed distortion in the shape of granule cells of the CA of the hippocampus when H and E slides of the parkinsonian induced mice model were compared with that of the control. Histological slides of the control group showed well-spaced clusters of the granule cells in contrast with the tightly packed and in some cases merged cells of the parkinsonian group. Subsequent treatment of the parkinsonian group with + GABA not only failed to bring about improvement in the shape of the cells but also lead to a more compact and less thick granular cell layer when compared with the histological slides of both the control and the parkinsonian only group [Figures 8-10]. Haloperidol has been reported not to only block dopamine receptors but also induces neuroinflammation (Bishnoi *et al.*, 2008; Voronkov *et al.*, 2013). It has been hypothesized that this can be achieved by production of reactive oxygen species (ROS) in the neuron (Byron *et al.*, 2010). ROS accumulation in the neuron have been reported to leads to microtubule collapse, cytoskeletal disorganization (Hasbi *et al.*, 2009; Delotterie *et al.*, 2010; Benitez-King *et al.*, 2010).

Neurodegeneration was also identified in histological slides of the PFC and the DG region of the hippocampus of the parkinsonian mice group when compared to that of the control. These slides showed the presence of wide empty spaces (PFC and dentate gyrus) and change in shape of neurons from pyramidal to amoebic (PFC) when compared with those of the control. Subsequent treatment of the parkinsonian group with + GABA failed to bring about any improvement as both the wide spaces and change in shape of neurons were observed in histological slides of-D_2R + GABA group when compared with the control [Figures 8-10]. Synaptic denervation is another mechanism in which haloperidol induces its damage on the brain (Cazorla *et al.*, 2014). This is evidenced in the DG of the hippocampus has the regions beneath the granule cells were disconnected from the granular cell layer.

Protein aggregation

The deposition of Lewy bodies is part of the pathological hallmark of Parkinsonism (Kosaka, 2013). Alpha-synuclein a small soluble protein expressed primarily at presynaptic nerves in the central nervous system is found in abundance as inclusions of Lewy bodies during PD (Kosaka, 2013). In contrast to earlier studies carried out involving haloperidol-inducement of Parkinson's disease, IHC slides of the brain of-D_2R only group showed no signs of Lewy bodies presence when its slide was compared with that of the control. A similar observation was made when studying the slides of subsequent treatment of Parkinsonian group with + GABA which also failed to shows signs of Lewy bodies [Figure 11]. This shows that blocking D_2R for the period used in this experiments did not lead to accumulation of Lewy bodies in the brain. These developments led to the questioning of the long-held

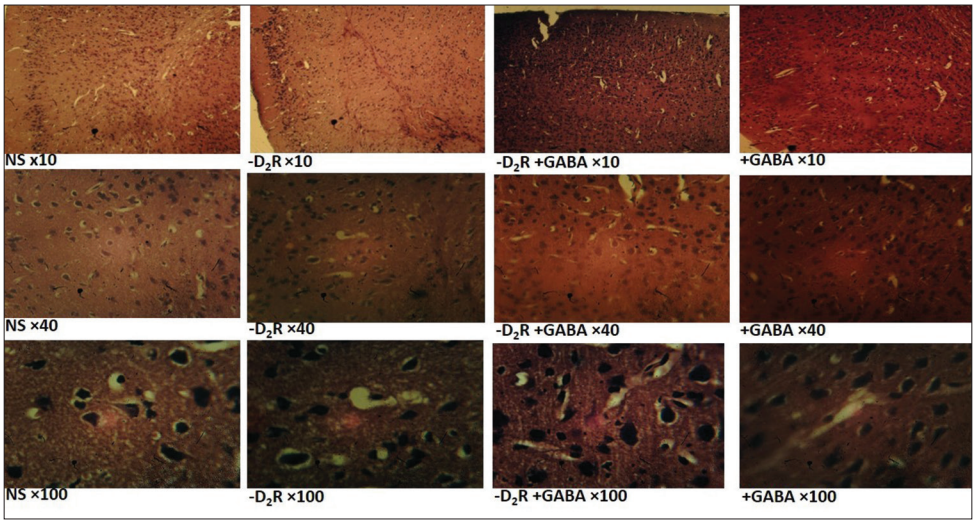

Figure 8: The Histological slide of the prefrontal cortex H and E stains of the experimental animals show at different magnification of the objective lens. Neuronal degeneration is observed mainly in the haloperidol (-dopamine-2 receptors) treated group and combined gamma aminobutyric acid-treated group (-dopamine-2 receptors + gamma aminobutyric acid) which is most evident at the outer pyramidal layer observed

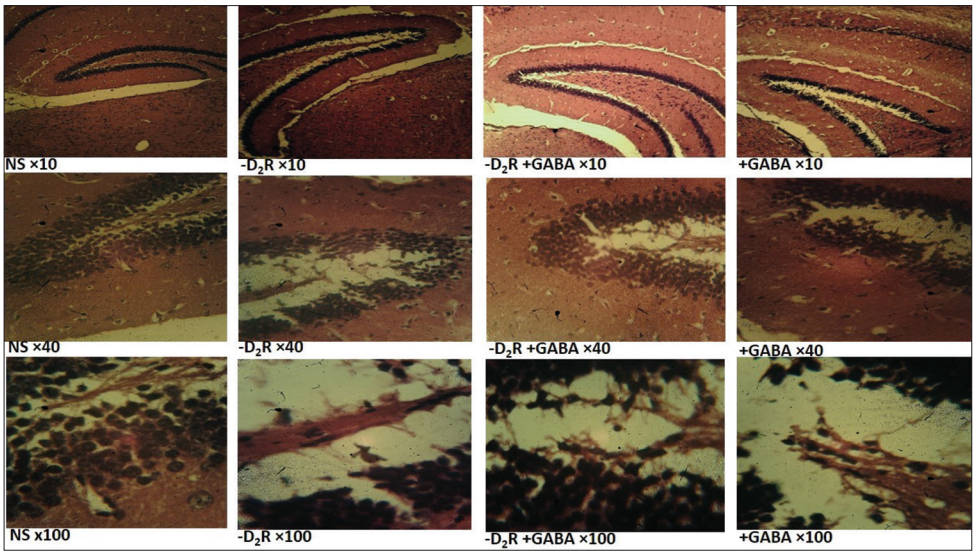

Figure 9: Histological slides of the dentate gyrus of the hippocampus H and E stains of experimental animals shown at a different magnification of the objective lens. Axonal breakage is observed in the dentate gyrus of the hippocampus of all the treated group evidenced by wide space beneath the granule cells of dentate gyrus. - Dopamine-2 receptors group also has cellular distortion has the degeneration enter deep into the granule cells, while – dopamine-2 receptors + gamma aminobutyric acid group also has cellular distortion with less degree compared to – dopamine-2 receptors only. +gamma aminobutyric acid group has the widest space beneath the granule cells but no granule cells distortion

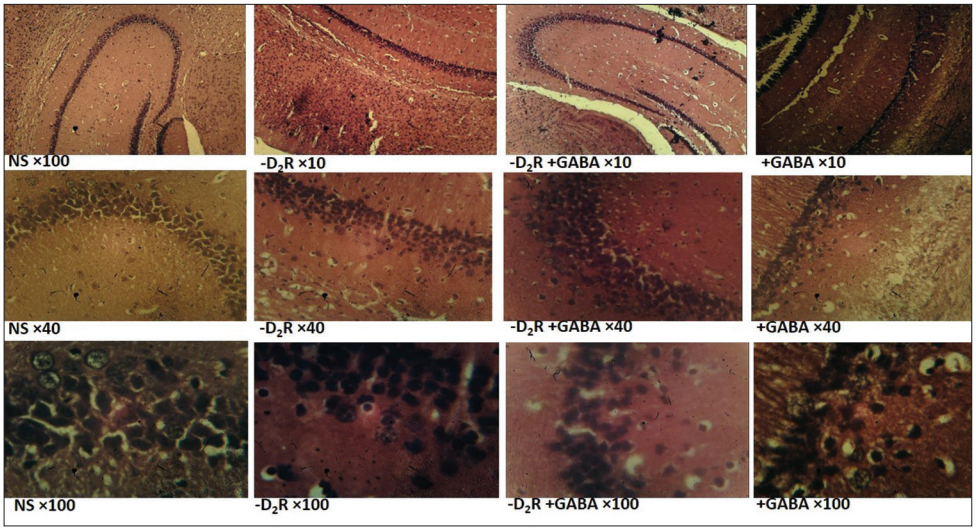

Figure 10: Histological slides of cornu ammonis region of the hippocampus H and E stains of the experimental animals shown at a different magnification of the objective lens. Granule cells distortion was also observed in the cornu ammonis region of all the experimental group. The thickness of the granular cell layer also reduced greatly in – dopamine-2 receptors + gamma aminobutyric acid and + gamma aminobutyric acid groups

theory that drug-induced Parkinsonian models tend to have Lewy bodies in their brain, though further experiments will be required to contradict these.

Conclusions

Haloperidol-induced Parkinsonism leads to motor and spatial memory deficit. GABAergic activation in Parkinsonism fails to reverse the memory and motor deficit but further elevate the deficits seen in motor coordination and memory. Activating GABA system alone increase the anxiety level of the animals and further increase as well in Parkinsonian mice.

The GABAergic system plays a synergistic role in the motor and memory deficit seen in haloperidol-induced PD. Studies can be done to inhibit GABA as another form of therapeutics in ameliorating the symptoms seen in PD.

Acknowledgment

The authors would like to thank the technologists of anatomy department, Afe Babalola University for technical support in carrying out the immunostain procedure.

Financial support and sponsorship

Nil.

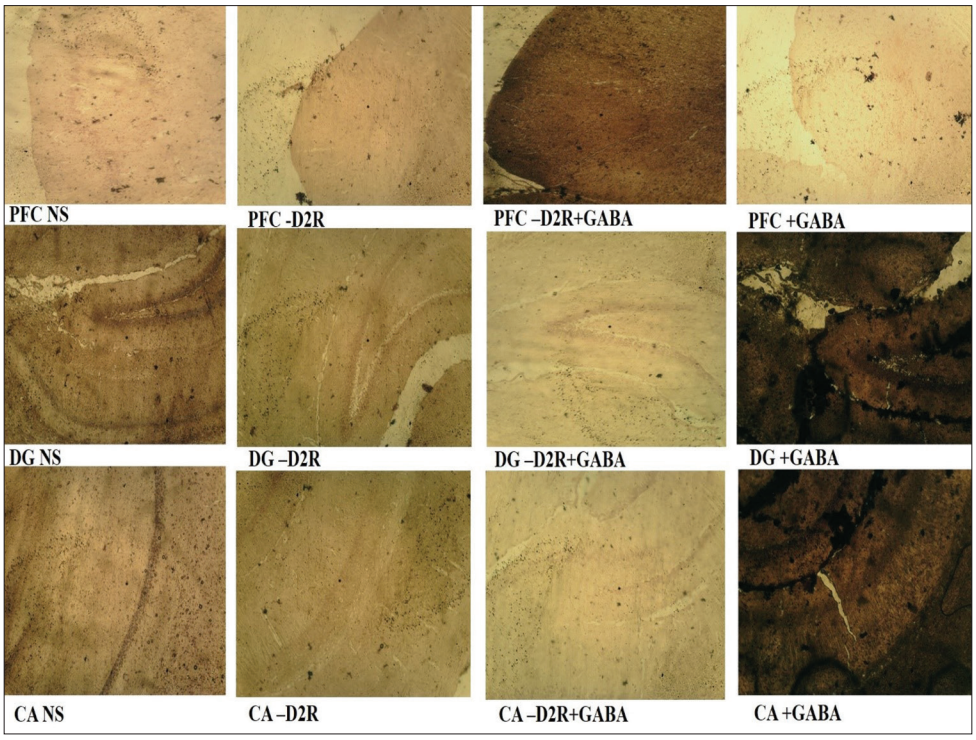

Figure 11: The immunohistological slide of prefrontal cortex and Hippocampus for synuclein-alpha (Lewy bodies) deposition in the experimental animals at × 10 objective. It is shown that no deposition of Lewy bodies was seen in the area of the brain observed

Conflicts of interest

There are no conflicts of interest.

References

1. Adeniyi P.A., Ishola A.O., Laoye B.J., Olatunji B.P., Bankole O.O., Shallie P.D., *et al*. (2016). Neural and behavioral changes in male periadolescent mice after prolonged nicotine-MDMA treatment. Metab Brain Dis 31:93-107.
2. Bankole O.O., Laoye B.J., Sirjao M.U., Ishola A.O., Oyeleke D.E., Balogun W.G., *et al*. (2015). Vitamin D3 receptor activation rescued corticostriatal neural activity ad improved motor function in-D2R tardive dyskinesia mice model. J Biomed Sci Eng 8:520-30.
3. Barone P. (2011). Treatment of depressive symptoms in Parkinson's disease. Eur J Neurol 18:11-5.
4. Benitez-King G., Dominguez-Alonso A., Ramirez-Rodrigue G. (2010). Haloperidol causes cytoskeletal collapse in N1E-115 cells through tau hyperphosphorylation-induced by oxidative stress: Implications for neurodevelopment. Eur J Pharmacol 44:24-31.
5. Bertran-Gonzalez J., Hakansson K., Borgkvist A., Irinopoulou T., Brami-Cherrier K., Usiello A., *et al*. (2009). Histone H3 phosphorylation is under the opposite tonic control of dopamine D2 and adenosine A2A receptors in striatopallidal neurons. Neuropsychopharmacology 34:1710-20.
6. Bishnoi M., Chopra K., Kulkarni S.K. (2008). Activation of striatal inflammatory mediators and caspase-3 is central to haloperidol-induced orofacial dyskinesia. Eur J Pharmacol 590:241-5.
7. Byron K.Y., Bitanihirwe B.K., Tsung-Ung W.W. (2010). Oxidative stress in schizophrenia: An integrated approach. Neurosci Biotehav Rev 35:878-93.
8. Cazorla M., de Carvalho F.D., Chohan M.O., Shegda M., Chuhma N., Rayport S., *et al*. (2014). Dopamine D2 receptor regulate the anatomical and functional balance of basal ganglia circuitry. Neuron 81:153-64.
9. Conde F., Lund J.S., Jacobowitz D.M., Baimbridge K.G., Lewis D.A. (1994). Local circuit neurons immunoreactive for calretinin, calbindin D-28k, or parvalbumin in monkey prefrontal cortex: Distribution and morphology. J Comp Neurol 341:96-116.
10. Delotterie D., Ruiz G., Brocard J., Schweitzer A., Roucard C., Roche Y., *et al*. (2010). Chronic administration of atypical antipsychotics improves behavioral and synaptic defects of STOP null mice. Psychopharmacology 208:131-41.
11. Dissanayaka N.W., Selbach A., Sinburn P.A., O'Sullivan J.D., Marsh R., Mellick G.D. (2011). Factors associated with depression in Parkinson disease. J Affect Disord 132:82-8.
12. Eitan E., Hutchison E.R., Mattson M.P. (2014). Telomere shortening in neurological disorders: An abundance of unanswered questions. Trends Neurosci 37(5):256-63. [Doi: S0166-2236 (14) 00026-5].
13. Engin E., Treit D. (2007). The role of hippocampus in anxiety: Intracerebral infusion studies. Behav Pharmacol 18:365-74.
14. Eriksen J.L., Wszolek Z., Petrucelli L. (2005). Molecular pathogenesis of Parkinson's disease. Arch Neurol 62:353-7.
15. Eskow-Juanraja K.L., Angoa-Perez M., Kuhm D.M., Bishop C. (2011). Potential mechanisms underlining anxiety and depression in Parkinson's disease: Consequences of L-DOPA treatment. Neurosci Biobehav Rev 35:556-64.
16. Hasbi A., Fan T., Alijaniaram M., Nguyen T., Perreault M.L., O'Dowd B.F., *et al*. (2009). Calcium signaling cascade links of dopamine D1-D2 receptors heteromer to striatal BDNF production and neuronal growth. Proc Natl Acad Sci USA 106:21377-82.
17. Horn A.S. (1990). Dopamine uptake: A review of progress in the last decade. Prog Neurobiol 38:387-400.
18. Ishola A.O., Laoye B.J., Oyeleke E.D., Bankole O.O., Sirjao M.U., Cobham E.A., *et al*. (2015). Vitamin D3 receptor activation rescued corticostriatal neural activity and improved motor-cognitive function in-D2R Parkinsonian mice model. J Biomed Sci Eng 8:601-15.

19. Kohler C., Hall H., Ogen S.O., Gawell L. (1985). Specific *in vivo* and *in-vitro* binding of 3H-raclopride, a potent substituted drug with high affinity for dopamine D2 receptors in rat brain. Biochem Pharmacol 34:2251-9.
20. Kosaka K. (2013). Lewy body dementia. Brain Nerve 65:1521-7.
21. Leentjens A.F., Dujardin K., Marsh L., Richard I.H., Starkstein S.E., Martinez-Martin P., *et al*. (2011). Anxiety waiting skills in Parkinson disease; a validation study of the Hamilton anxiety rating scale, the Beck anxiety inventory and the hospital anxiety and depression scale. Mov Disord 26:407-15.
22. Litvan I., Jahanshahi M., Krack P., Rodriguez-Oroz M.C., Macias R., Bezard E., *et al*. (2009). Initial clinical manifestations of Parkinson's disease: Features and pathophysiological mechanisms. Lancet Neurol 8:1128-39.
23. Mattes M.P., Bouthenet M.L., Sales N., Sokoloff P., Schwartz J.C. (1986). Widespread distribution of brain dopamine receptors evidenced with[1251] iodol sulpiride, a highly selective ligand. Science 228:752-5.
24. Murray E.D., Buttner E.A., Price B.H. (2012). Depression and psychosis in neurological practice. Bradley's Neurology in Clinical Practice 2012: Expert Consult-Online and Print, 6e (Bradley, Neurology in Clinical Practice edition 2v Set). 6th ed. Elsevier/ Saunders, Philadelphia, PA, p. 102-3.
25. Ogundele O.M., Nanakumo E.T., Ishola A.O., Ogbende O.M., Enye L.A., Balogun W.G., *et al*. (2015). NMDA R/+VDR pharmacological phenotype as a novel pharmaceutical target in relieving motor-cognitive impairments in Parkinsonism. Drug Chem Toxicol 38(4):1-13.
26. Reavill C., Kettle A., Holland V., Riley G., Blackburn T.P. (1991). Attenuation of haloperidol-induced catalepsy by a 5-HT2c receptor antagonist. Br J Pharmacol 126:572-4.
27. Schwarz J., Oden P., Buhmann C., Csoti I., Jost W., Wüllner U., *et al*. (2011). Depression in Parkinson disease. J Neurol 258:S336-8.
28. Siegel A., Sapru H.N. (2011). Essential Neuroscience. 2nd ed. Lippincott Williams and Wilkins, Hong Kong; China.
29. Torres G.E., Gainetdinov R.R., Caron M.G. (2003). Plasma membrane monoamine transporters: Structure, regulation, and function. Nat Rev Neurosci 4:13-25.
30. Voronkov D.N., Khudoerkov R.M., Dovedova E.L. (2013). Changes in neurological interactions in the cerebral nigrostriatal structures in a model of dopamine system dysfunction. Zh Neurol Psikhiatr Im S S Korsakova 113:47-51.
31. Whalen K., Finkel R., Panavelil T.A. (2007). Lippincott's Illustrated Reviews: Pharmacology. 6th ed. Wolters Kluwer, New York; USA.