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# Vitamin D₃ Receptor Activation Rescued Corticostriatal Neural Activity and Improved Motor Function in −D₂R Tardive Dyskinesia Mice Model

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## Abstract

**Haloperidol-induced dyskinesia has been linked to a reduction in dopamine activity characterized by the inhibition of dopamine receptive sites on D₂-receptor (D₂R). As a result of D₂R inhibition, calcium-linked neural activity is affected and seen as a decline in motor-cognitive function after prolonged haloperidol use in the treatment of psychotic disorders. In this study, we have elucidated the relationship between haloperidol-induced tardive dyskinesia and the neural activity in motor cortex (M1), basal nucleus (CPu), prefrontal cortex (PFC) and hippocampus (CA1). Also, we explored the role of Vitamin D₃ receptor (VD₃R) activation as a therapeutic target in improving motor-cognitive functions in dyskinetic mice. Dyskinesia was induced in adult BALB/c mice after 28 days of haloperidol treatment (10 mg/Kg; intraperitoneal). We established the presence of abnormal involuntary movements (AIMs) in the haloperidol treated mice (−D₂) through assessment of the threshold and amplitude of abnormal involuntary movements (AIMs) for the Limbs (Li) and Orolingual (Ol) area (Li and Ol AIMs). As a confirmatory test, the dyskinetic mice (−D₂) showed high global AIMs score when compared with the VD₃RA intervention group (−D₂/+VDR) for Li and**

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Ol AIMs. Furthermore, in the behavioral tests, the dyskinetic mice exhibited a decrease in latency of fall (LOF; Rotarod-P < 0.05), climbing attempts (Cylinder test; P < 0.05) and latency of Turning (Parallel bar test; LOT-P < 0.05) when compared with the control. The reduced motor function in dyskinetic mice was associated with a decline in CPu-CA1 burst frequencies and an increase in M1-PFC cortical activity. However, after $VD_3RA$ intervention ($-D_2$/+VDR), 100 mg/Kg for 7 days, CPu-CA1 burst activity was restored leading to a decrease in abnormal movement, and an increase in motor function. Ultimately, we deduced that $VD_3RA$ activation reduced the threshold of abnormal movement in haloperidol induced dyskinesia.

## Keywords

Haloperidol, $VD_3R$, Dyskinesia, $D_2R$, Neural Activity, Corticostriatal

## 1. Introduction

Antipsychotics are often employed in the management of depression, schizophrenia and other neurological disorders; however, prolonged use of these drugs often results in tardive dyskinesia (TD) and other associated movement disorders [1]. The primary effects of these drugs involve the inhibition of dopaminergic $D_2$ receptor in the nigrostriatal system and cortical projections leading to persistent involuntary movements in the face, limbs, oral region, trunk and a decline in memory function [2] [3].

Previous studies have shown that haloperidol-induced motor disorders involve partial inhibition of dopamine receptive sites on $D_2$-receptor which prevents the heretomeric combination of $D_1$ and $D_2$ receptors during dopaminergic neurotransmission [4]. As a result of the inhibition of $D_1$-$D_2$ combination, calcium transport is impaired and this creates a state of $D_2$ receptor sensitivity and calcium-linked oxidative stress [4]-[6].

An important aspect of drug-induced dyskinesia is its effect on motor-cognitive function due to dopaminergic $D_2$ inhibition. Several studies have shown a decline in motor function and cognition after a prolonged use of these drugs in treatment of depression and schizophrenia [7] [8]. Going further, the observed behavioral deficits were attributed, in part, to loss of dopaminergic neurotransmission in the motor and memory neural systems, predominantly due to $D_2R$ inhibition and abnormal calcium currents in corticostriatal outputs [3] [5].

The significance of the $D_2R$ stimulation and calcium current supports the wide role of dopamine in various brain centers involved in motor and memory functions [5] [9]. Specifically, dopamine interacts with glutamate in hippocampal memory formation, striatal motor function, addiction and reward. In addition, glutamate-dopamine cross-talk has been described in glucose metabolism and oxidative stress in these brain centers [10]-[12].

Our previous experiments have shown that activation of Vitamin $D_3$ receptor ($VD_3R$) reduces calcium toxicity through central and peripheral mechanisms, and improves motor-cognitive function in mice after haloperidol induced parkinsonism [13]. In the present study, we investigate the link between haloperidol-induced dyskinesia and M1, CPu, PFC and CA1 neural activities *in vivo*. Furthermore, we studied the role of $VD_3R$ activation in improving motor-cognitive functions through restoration of epoch neural activities in the brain areas of dyskinetic mice.

## 2. Materials

All chemical reagents were sourced from Sigma-Aldrich, Germany. Haloperidol Injection was procured from Kanada Pharmacy, Nigeria and re-suspended in dextrose saline. $VD_3$ was procured form Standard Pharma, Nigeria and dissolved in normal saline. Haloperidol and $VD_3$ solutions were prepared weekly as needed and stored at 4°C.

### 2.1. Animal Treatment

BALB/c mice ($n = 24$) were separated into 4 groups based on body weight distribution. Dyskinesia was induced using 10 mg/Kg BW of dopaminergic $D_2$ blocker (Haloperidol i.p. 28 days) in $n = 12$ animals [13] [14]. Subsequently, $n = 6$ animals were treated with 100 mg/Kg BW (i.p. 7 days) $VD_3$ ($-D_2$/+VDR) while the remaining set

of $n = 6$ animals were untreated ($-D_2$). A separate group of $n = 6$ animals received $VD_3$ for 28 days (100 mg/Kg BW intraperitoneal; +VDR) while the control ($n = 6$) received normal saline for 28 days (NS; intraperitoneal).

## 2.2. Motor Function

At the end of the treatment phase (Day 28 for Vehicle, $-D_2$, +VDR; Day 35 for $-D_2$/+VDR), the animals were examined in various tests for motor function. All animals were familiarized with the behavioral testing tools during the treatment phase and were moved to the testing area 72 hours before the commencement of the tests.

## 2.3. Motor Function Test for Dyskinesia

**Abnormal Involuntary Movements (AIMs):** Involuntary movements were accessed in AIMs (Orolingual and Limb AIMs) tests for dyskinesia. The values were recorded as amplitude of movement and basic movement using the methods of Cenci and co-workers [15]. Three experienced scientists assessed each animal independently using the grading scale of 1 - 4. The average score was adopted in each case for the amplitude and basic movement score at 0, 15 and 30 minutes respectively.

**Parallel Bar Test:** Motor coordination was accessed on two raised 1m long (1 mm) parallel bars (3 cm apart) mounted on the 60 cm high wooden frame. The animal was placed at the 0.5 m mark (center of the raised bars) following which we determined the duration taken by the mice to make a 90˚ turn (latency of turning; LOT) [16].

**Rotarod:** The test involved three trials of 3 minutes each ($T_1$, $T_2$ and $T_3$) separated by an inter trial time of 90 minutes. The time spent on the Rotarod in $T_1$, $T_2$ and $T_3$ was determined and averaged to calculate the latency of fall (LOF).

**Cylinder Test:** Each mouse was placed in a 500 ml transparent beaker (cylinder) and was allowed to explore the walls of the cylinder with the forelimbs while standing on the two hind limbs [15]. The number of times an animal explored the wall of the cylinder with the forelimb was counted to determine the average score of climbing attempts for each group.

**Bar Test:** the magnitude of motor impairment was also measured in the bar test [15]. The forelimbs of the animal were placed on a raised wooden bar for 3 minutes. The time taken by the animal to move the limb off the raised bar was measured for the treatment and the control groups.

## 2.4. Electrophysiology

Electrophysiological recordings of extracellular calcium hyperpolarization currents were obtained from the basal nucleus (CPu), Motor cortex (M1; L4 - L6), Hippocampus (CA1) and the Prefrontal cortex (PFC) using chronically implanted wire electrodes. Thirty minutes before the implant, animals received 2 mg/Kg i.p. meloxicam and were deeply anesthetized using 100 mg/Kg Ketamine and 5 mg/Kg Diazepam (i.p.) to keep the mice immobile but awake for basal motor functions (corneal reflexes and diaphragmatic movement) [17] [18]. Using a stereotaxic frame, the scalp was removed above the bregma to expose the cranium. Periosteal tissue was removed using hydrogen peroxide solution and a cotton bud. The M1, CPu, PFC and Hipp were located using a calibrated grid to determine the position and depth (electrode length) relative to the bregma [M1 (AP: +3.34 mm ML: +3 mm DV: +2.5 mm), CPu coordinates (AP: +2.28 mm ML: +3 mm DV: +6 mm), Hipp (AP: −4.4 mm ML: +2 mm DV: +3 mm), PFC (AP: +2.2 mm ML: +1 mm DV: +2.5 mm)]. A dental drill was used to make holes in the cranium following which insulated wire electrodes were inserted to the appropriate depth. The ground electrode was placed on the cranium of the contralateral side. Subsequently, the implant was covered by orthodontic resin to hold the electrodes in position during the recording procedure. The terminal wires of the electrodes were connected to the amplifier through small head-sockets in preparation for immobile awake recordings. The data from the amplifier (Spiker Box; Backyard Brains, Michigan, USA) was captured on the Audacity software v4.2 and analyzed in Sig View v2.1 (Signal Labs, USA) to determine the extracellular summation epoch neural activity (calcium signals) expressed as Frequency (Hz) per unit time (**Figure 1(g)** and **Figure 2(a)**).

## 2.5. Statistical Analysis

Data was presented as mean ± SEM; analyzed using ANOVA and Tukeys Post-Hoc test. Statistical Significance was set as $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$.

# 3. Results

## 3.1. AIMs Study for Dyskinesia

The global AIMs score for Limb (Li) and Orolingual (Ol) AIMs were assessed from 0 - 30 minutes after the animals were pretreated with 10 mg/Kg Haloperidol (intraperitoneal). Basic and amplitude scores were allotted by three independent scientists on a scale of 1 - 4 at 0, 15 and 30 minutes. Subsequently, the basic and amplitude scores were converted to the global AIMs score (Basic X Amplitude) for the $-D_2$ group after 28 days of haloperidol treatment to confirm dyskinesia, and $-D_2/+VDR$ treatments 7 days after $VD_3RA$ intervention (Day 35).

*Li AIMs*: Abnormal involuntary movement was observed in the limbs of the haloperidol treated mice after 28 days ($-D_2$). The frequency and threshold of such movements increased between 0 - 15 minutes after haloperidol treatment and decreased sharply between 15 - 30 minutes. After 7 days of $VD_3RA$ intervention ($-D_2/+VDR$), the AIMs score decreased significantly when compared with the $-D_2$ at 0 - 15 and 15 - 30 minutes (**Figure 3(a)**).

*Ol AIMs*: Haloperidol treatment ($-D_2$) caused abnormal involuntary movements of the Orolingual area and was characterized by uncontrolled chewy mouth movements and tongue protrusion. The threshold (AIMs score) was at its peak shortly after haloperidol was given intraperitoneally (0 - 15 minutes) and decreased significantly thereafter (15 - 30 minutes). Similar to the observations in Li AIMs, $VD_3RA$ intervention also reduced the severity of Ol AIMs when the $-D_2/+VDR$ treatment was compared with the $-D_2$ group. Lower AIMs scores were recorded in this group throughout the duration of the test (from 0 - 15 and 15 - 30 minutes) (**Figure 3(b)**).

Thus, $VD_3RA$ treatment after haloperidol induced dyskinesia significantly reduced the threshold of Li and Ol AIMs seen as a decline in the AIMs score from 0 - 30 minutes post haloperidol treatment. It is important to mention that the mice were also assessed for Axial AIMs (Ax) but showed no significant change in ipsilateral and contralateral turns when compared with the control (untreated animals).

## 3.2. Motor Function Tests

After establishing that dyskinesia was induced in the animals, the dyskinesia models ($-D_2$ and $-D_2/+VDR$) were compared with the VD3RA treated (+VDR) and untreated control groups (NS) in motor function tests.

**Rotarod:** After haloperidol induced dyskinesia ($-D_2$), the LOF decreased significantly on the treadmill when compared with the control ($P < 0.05$). Subsequent $VD_3RA$ intervention ($-D_2/+VDR$) caused an increase in the LOF (motor function) when compared with the $-D_2$ treatment and the control ($P < 0.05$). However, $VD_3RA$ treatment (28 days; +VDR), without prior induced dyskinesia, significantly increased the LOF (motor function); when this treatment was compared with the control and the $-D_2/+VDR$ ($P < 0.01$) (**Figure 1(a)**).
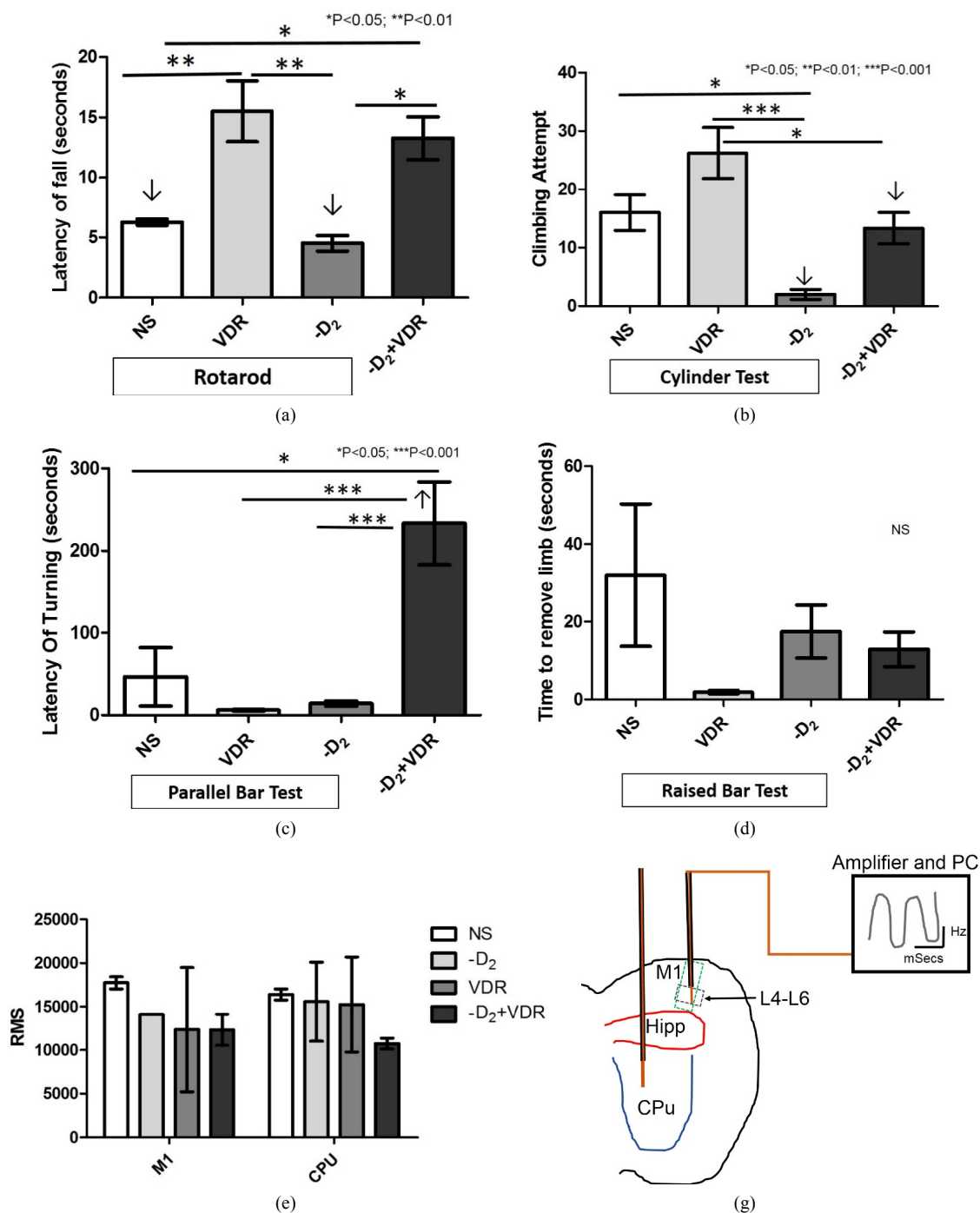
**Cylinder Test:** Motor activity was measured as a function of climbing attempts score. Haloperidol induced dyskinesia ($-D_2$) caused a decrease in motor activity which was seen as a decline in climbing attempts score versus the control (**Figure 1(b)**). $VD_3RA$ intervention in dyskinetic mice ($-D_2/+VDR$) increased the climbing attempts to match control scores as no significance was observed between the $-D_2/+VDR$ and the control (untreated) mice. Similar to our observations in the Rotarod test, $VD_3RA$ treatment (+VDR) without prior induced dyskinesia significantly increased the climbing attempts (motor function) when compared with the control and $-D_2/+VDR$ ($P < 0.05$) (**Figure 1(b)**).

**Parallel Bar Test:** All groups were compared with the untreated control (standard) such that a significant increase or decrease in LOT score was considered a decline in motor function. LOT scores decreased after haloperidol induced dyskinesia when the $-D_2$ treatment was compared with the control, thus suggesting a decline in motor coordination ($P < 0.05$). Subsequent $VD_3RA$ intervention ($-D_2/+VDR$) significantly increased the LOT when compared with the control ($P < 0.05$) and $-D_2$ ($P < 0.001$). This outcome suggests a decline in motor coordination when compared with the control as the animals were characterized by freezing of movements on the raised bars. $VD_3RA$ treatment (+VDR), in non-dyskinetic control mice, caused a decline in LOT when compared with the control ($P < 0.05$) and was not significant versus $-D_2$ treatment (**Figure 1(c)**); thus suggesting a decline in motor coordination when compared with the control.

**Bar Test**: No significant change was observed in the bar test when the treatment groups were compared with the control. However, empirical data suggests that $-D_2$, $-D_2/+VDR$ and $VD_3R$ treatments did induce hyperkinesia, thus, causing a reduction in time taken for the mice to remove the limbs from the raised platform (versus the control; **Figure 1(d)**).

### 3.3. Motor Neural Activity (M1 and CPu) in −D$_2$ Induced Dyskinesia

Haloperidol induced dyskinesia (−D$_2$) caused a decline in motor function (increase in Ol/Li AIMs), abnormal neural activities in the M1 (L4 - L6) and CPu- when the −D$_2$ was compared with the control. Prominent changes in the neural spike trains involved an increase in M1 activity (**Figure 1(f)**) and a reduced threshold of CPu outputs when the dyskinetic group (−D$_2$) was compared with the control (**Figure 1(e)**). After VD$_3$RA intervention (−D$_2$/+VDR), an increase in motor activity (decreased threshold of Ol/Li AIMs; **Figure 3(a)** and **Figure 3(b)**) and CPu neural frequency bursts were observed in the VD$_3$RA intervention group (−D$_2$/+VDR; **Figure 1(f)**). In addition, the threshold of the M1 and CPu activities (RMS) for the intervention group (−D$_2$/+VDR) decreased
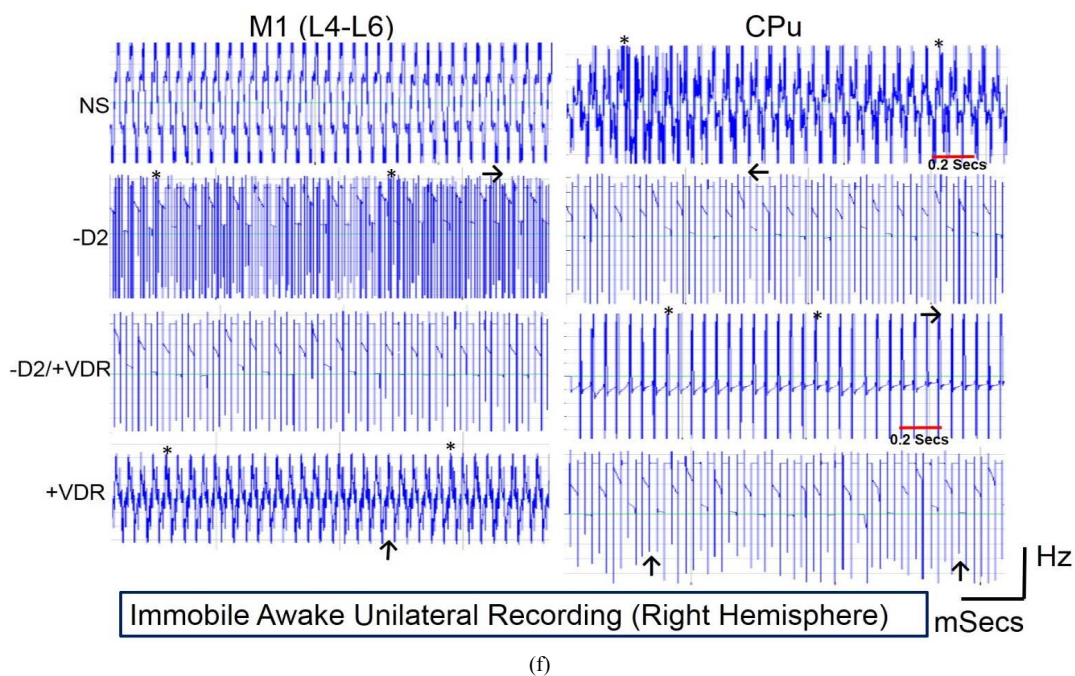


(a)

(b)

(c)

(d)

(e)

(g)

(f)

**Figure 1.** ((a)-(d)) Motor function Tests for Dyskinesia and VDRA Treated Haloperidol-Induced dyskinesia Mice Model. (a) *Rotarod*: The latency of fall (LOF) was determined on the treadmill for a test duration of 3 minutes. The dyskinesia group ($-D_2$) recorded a reduction in the LOF when compared with the control and the +VDR groups (P < 0.01). However, $VD_3RA$ intervention in $-D_2$ mice increased the LOF significantly when compared with the $-D_2$ and the control group (P < 0.05). Similarly, $VD_3RA$ treatment (only) significantly increased motor function versus the control (P < 0.01); this showed the effect of $VD_3RA$ in increased motor function in normal and dyskinesia mice model; (b) *Cylinder Test*: The climbing attempts in the cylinder reduced significantly in the $-D_2$ mice when compared with the control (P < 0.05). $VD_3RA$ intervention group ($-D_2$/+VDR) recorded an increase in climbing attempts when compared with the $-D_2$ treatment and the control. Similar to our observations in the Rotarod test, $VD_3R$ (only) treatment significantly increased motor function as the +VDR group recorded the highest climbing attempts when compared with the $-D_2$ (P < 0.001) and $-D_2$/+VDR (P < 0.05); (c) *Parallel Bar Test*: The latency of turning (LOT) was reduced in the $-D_2$ treatment; the animals were unstable and constantly moving on the bars. $VD_3RA$ intervention ($-D_2$/+VDR), however increased the LOT when compared with the $-D_2$ (P < 0.001). The outcome for the $-D_2$/+VDR can be described as an increase in coordination but a reduction in motor activity when compared with the +VDR and $-D_2$ (P < 0.001); (d) *Raised Bar Test*: No significant change was recorded in this test for the treatment groups and the control. ((e)-(g)) Motor Neural Electrophysiological Recording for Dyskinesia Mice Model and $VD_3RA$ Intervention. ((e)-(f)) Haloperidol treatment induced dyskinesia in Mice after 28 days of intraperitoneal administration. This was characterized by a reduction in RMS in the M1 (L4 - L6), loss of burst activity and a reduction in RMS (*) in the CPu (**Figure 1(f)**). $VD_3RA$ intervention ($-D_2$/+VDR) reduced the RMS in the M1 and CPu when compared with the control (**Figure 1(e)**) spike pattern (**Figure 1(f)**). This was also associated with a reduction of Global AIMs score (Ol and Li) and an increase in motor coordination when the $-D_2$/+VDR treatment was compared with the $-D_2$ group. $VD_3RA$ (only) treatment caused an increase in motor activity in control animals when compared with the control (untreated) animals. Neural recordings for $VD_3RA$ treatment showed a decline in RMS in the M1 (L4 - L6) coupled with a decreased frequency of the spike train. However, this group recorded a slight decline in CPu RMS when compared with the control (**Figure 1(f)**). The decreased RMS in $VD_3RA$ treatment facilitated a loss of burst action potential in the spike pattern (**Figure 1(f)**). Thus, it was inferred that $VD_3RA$ treatment in control animals can cause neural changes seen as hyperactivity in behavioral studies. Similar to the observations in $-D_2$, $VD_3RA$ also caused a loss of burst pattern in the CPu; in both cases hyperkinesia was observed in the animals; (g) Schematic illustration of chronic unilateral electrode implants in the M1 (L4 - L6) and the CPu.

versus the control and dyskinetic mice ($-D_2$; **Figure 1(e)** and **Figure 1(f)**). Furthermore, haloperidol induced dyskinesia ($-D_2$) increased M1 but reduced CPu neural outputs and was associated with a decline in motor function, while $VD_3RA$ treatment (VDR+) of control mice decreased M1 activity and was associated with an increase in motor coordination. However, $VD_3RA$ intervention after haloperidol induced dyskinesia ($-D_2$/+VDR) decreased M1 activity and increased CPu outputs—when compared with $-D_2$—leading to an improvement in motor function. From these findings, we deduced loss of CPu burst frequencies was associated with abnormal movement in dyskinetic and $VD_3RA$ (only) treated mice. By contrast, $VD_3RA$ intervention reduced abnormal

movements (dyskinesia) by increasing CPu burst frequencies *in vivo*. From these findings, both M1 and CPu neural outputs were affected in dyskinesia and subsequent VD$_3$RA intervention.

### 3.4. –D$_2$ induced Dyskinesia Affects Cognition Related Brain Centers

The effect of haloperidol induced dyskinesia was further investigated on memory-related brain centers (PFC and Hipp). Unilateral electrode recordings in immobile awake mice showed a resting state PFC activity and Hippocampal CA1 burst in the control. Haloperidol induced dyskinesia increased the PFC frequency in immobile awake animals and reduced the CA1 burst activity when compared with the control (**Figure 2(c)**). This treatment was also
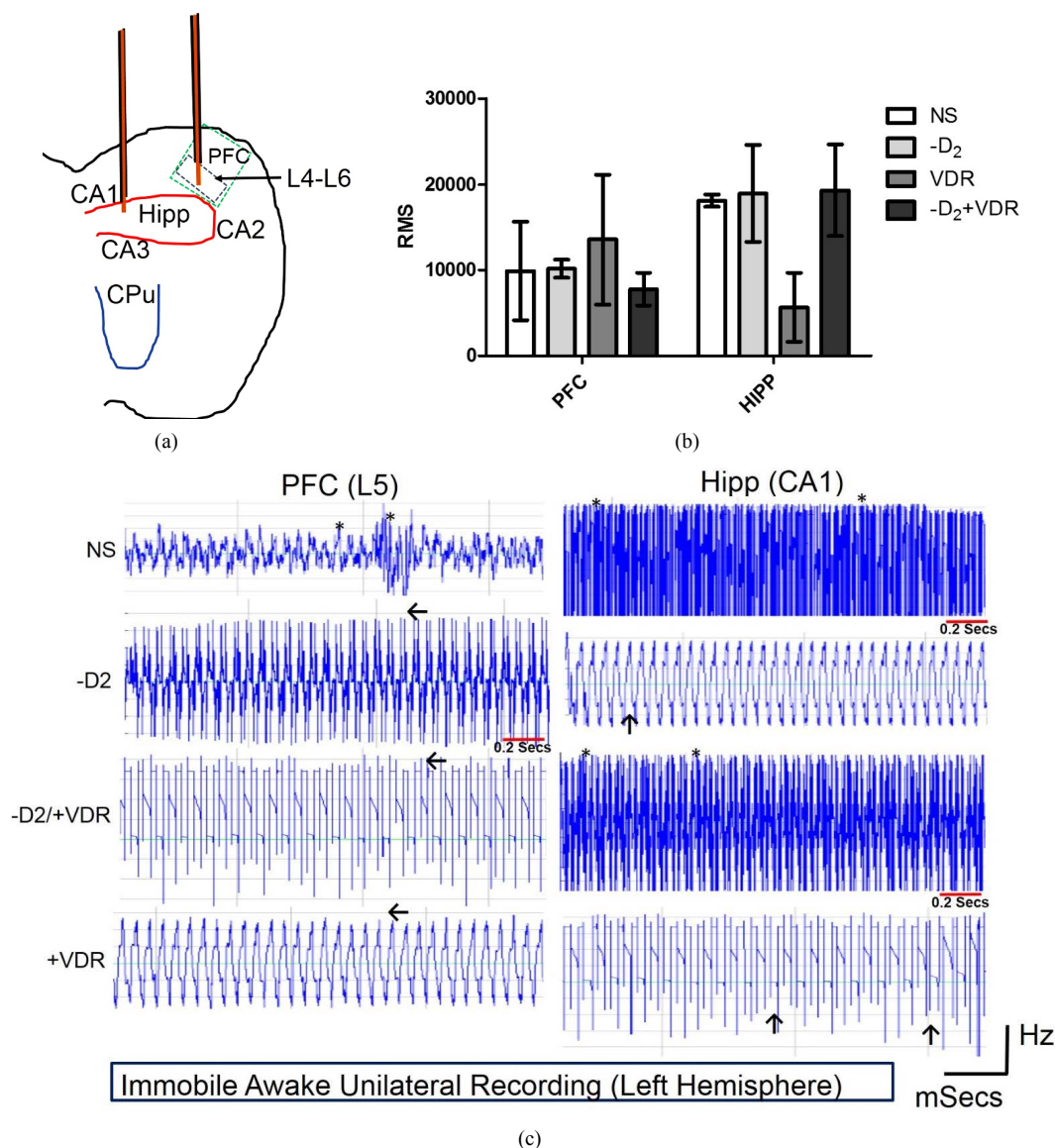


(a)                                         (b)

(c)

**Figure 2.** Neural Epoch Activity in the PFC and Hippocampus (CA1) of immobile awake dyskinesia and VD$_3$RA intervention mice. (a) Schematic illustration of electrode placement in the PFC and Hippocampus (CA1) regions; ((b)-(c)) The dyskinesia group (–D$_2$) recorded an increase in PFC activity but a decrease in CA1 burst when compared with the control (**Figure 1(c)**). VD$_3$RA intervention (–D$_2$/+VDR) facilitated an increase in CA1 burst when compared with the –D$_2$ and the control and was associated with an increased CA1 RMS (**Figure 2(b)**). However, the –D$_2$/+VDR treatment caused a decrease in PFC RMS when compared with the control (**Figure 2(b)**) and was associated with a decrease in spike train frequency. Prolonged VD$_3$RA treatment of control animals caused an increase in PFC activity and RMS; also a reduction CA1 burst and RMS (**Figure 2(b)** and **Figure 2(c)**).

characterized by an irregular RMS threshold for PFC and CA1 versus the control (**Figure 2(b)**). However, VD$_3$RA intervention in dyskinetic mice ($-D_2$/+VDR) reduced the PFC neural frequency (reduced RMS threshold) while restoring the CA1 burst pattern (increased RMS threshold). Thus we deduced that VD$_3$RA intervention can significantly restore memory alterations associated with dyskinesia by increasing CA1 burst and reducing PFC activity (opposite for dyskinetic mice). Interestingly, VD$_3$RA treatment of control animals (+VDR), however, increased PFC activity and reduced CA1 bursts similar to the effect of $-D_2$. Due to movement impairments, memory function was not assessed in these animals in behavioral tests (**Figure 2(b)** and **Figure 2(c)**).

## 4. Discussion

Taken together, the outcome of this study confirms the role of Dopaminergic $D_2$ receptor ($D_2$R) blockade in tardive dyskinesia after prolonged intraperitoneal haloperidol treatment. Similar to the findings in L-DOPA induced dyskinesia model (6-hydroxydopamine; 6-OHDA lesion) [15], haloperidol induced dyskinesia caused abnormal involuntary movements in the Orolingual region and Limbs. In this study, we observed no prominent change in *axial movement* when compared with the described symptoms for L-DOPA induced dyskinesia. The main abnormal movement observed in the orolingual region and limbs can be described as hyperkinesia (**Figure 3(a)** and **Figure 3(b)**).
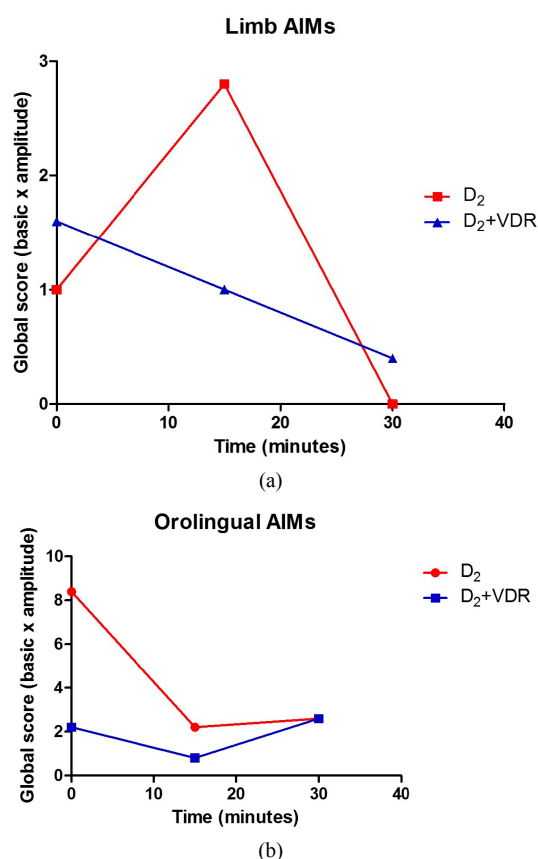


(a)



(b)

**Figure 3.** Abnormal Involuntary Movements (AIMs) Study for Haloperidol induced Dyskinesia and VDRA intervention. (a) *Limb AIMs* (*Li*): Intraperitoneal Haloperidol treatment for 28 days ($-D_2$) induced prominent limb dyskinesia in the animals 30 minutes after the treatment. The peak of limb AIMs was observed at 15 minutes and declined rapidly between 20 - 30 minutes. Interestingly, we observed that VD$_3$RA intervention for 7 days after dyskinesia ($-D_2$/+VDR) significantly reduced the severity of the symptoms in these animals. A decline in the global AIMs score was also recorded from 0 minutes to 30 minutes; (b) *OrolingualAIMs* (*Ol*): Haloperidol induced dyskinesia significantly increased the global AIMs score for *Orolingual AIMS* with prominent constant movement of the mouth and chewy activities. These symptoms were most noticeable after haloperidol was administered (0 Minutes) and gradually reduced by 15 - 30 minutes. The $-D_2$/+VDR treatment showed very limited signs of uncontrolled Orolingual movements throughout the duration of the experiment and recorded minimal global AIMs score when compared with the $-D_2$ treatment

Subsequent VD$_3$RA intervention in dyskinetic mice reduced the threshold and amplitude of the Orolingual and Limb aims observed over a duration of 30 minutes post haloperidol treatment. The dyskinetic mice (–D$_2$) showed a steady increase in abnormal Orolingual movement (Li) from 0 - 15 minutes and this declined rapidly between 15 - 30 minutes. Similarly, the global AIMs score for Li was highest within the first 5 minutes after haloperidol treatment and reduced significantly between 15 - 30 minutes. However, VD$_3$RA treatment reduced the threshold and amplitude of Li and Ol AIMs when the intervention group (–D$_2$/+VDR) was compared with the dyskinetic group (–D$_2$) at 0, 15 and 30 minutes respectively (**Figure 3(a)** and **Figure 3(b)**).

## 4.1. Haloperidol Induced Dyskinesia

After confirming the role of haloperidol in –D$_2$ induced dyskinesia, and the effect of VD$_3$RA in ameliorating the dyskinesia-linked movement disorders, we examined the general effects of prolonged D$_2$R blockade and VD$_3$RA intervention on motor function in these animals using arrays of motor function tests.

Dyskinetic mice (–D$_2$) showed a decline in motor function in behavioral tests; Rotarod, Cylinder test, parallel bar test but exhibited no significant change in the raised bar test when compared with the control. The dyskinetic mice recorded a decline in latency of fall (LOF) when compared with the control (P < 0.05; **Figure 1(a)**). Similarly, these animals scored lower in climbing attempts when assessed in the cylinder test versus control (untreated animals) (P < 0.05; **Figure 1(b)**). Subsequent analysis of motor coordination on raised parallel bars revealed a decline in motor coordination when compared with the control and was seen as a significant decrease in latency of turning (LOT) (**Figure 1(c)**; P < 0.05). Surprisingly, these animals showed no significant change in motor function when examined for limb removal in a raised bar test and the outcome was rather inconclusive. However, this can be attributed to the abnormal movements associated with the limbs (due to tardive dyskinesia) rather than a swift removal of limbs as a result of motor coordination (**Figures 1(a)-(d)**).

Although the role of VD$_3$RA in Parkinsonism has been studied extensively, its importance in dyskinesia remains poorly explored. Other studies have favored experiments on the effect of Vitamins (Vitamin E) in reducing the threshold of tardive dyskinesia [19]. The anti-dyskinetic effect of Vitamin E was attributed mostly to its antioxidant properties and its role in radical detoxification [19] [20]. Similarly, VD$_3$ deficiency has been linked to the cause and progression of various movement disorders [21]; including PD. By virtue to its role in radical detoxification, calcium-related signaling and general brain health, VD$_3$ depicts a potential therapeutic target in reducing dyskinetic symptoms similar to the effects Vitamin E [22] [23]. In support of this hypothesis, 7 days of VD$_3$RA intervention in the dyskinetic mice reduced Ol and Li AIMs, and was associated with an overall improvement in motor function when compared with the untreated dyskinetic mice (–D$_2$) (**Figure 3(a)** and **Figure 3(b)**, **Figure 1(a)** and **Figure 1(b)**). In the Rotarod test, the intervention group (–D$_2$/+VDR) recorded an increase in LOF when compared with the –D$_2$ (untreated dyskinesia) (P < 0.05; **Figure 1(a)**). Similarly, these animals recorded an increase in climbing attempts score when compared with the untreated mice (–D$_2$) (**Figure 1(b)**). These outcomes suggest that VD$_3$RA can reduce dyskinesia significantly, similar to what was described for Vitamin E [19]-[21].

## 4.2. Neural Epoch Activity in Dyskinesia

The abnormal involuntary movement observed after prolonged haloperidol treatment (**Figure 3(a)** and **Figure 3(b)**; **Figures 1(a)-(d)**) were associated with prominent changes in the M1 and CPu neural outputs in deeply sedated (immobile awake) mice. Haloperidol induced dyskinesia was characterized by an increase in M1 and CPu outputs when compared with the control. This increase in cortical and striatal outputs was attributed to the observed hyperkinesia (AIMs) in this dyskinetic mice; similar to the reports by Lindenbach and Bishop [24]. By contrast, VD$_3$RA intervention decreased the M1 cortical output , facilitated CPu burst frequencies and was associated with a decrease in global AIMs scores (improved motor function) in the –D$_2$/+VDR. Thus, these observations, and other reports [25], suggests that a reduction in CPu burst frequency and an increase in M1 cortical outputs were associated with the observed motor deficits seen in Dopaminergic receptor inhibition.

## 4.3. Corticostriatal Outputs in Dyskinetic Mice

Haloperidol induced dyskinesia (–D$_2$) decreased CA1 burst and reduced PFC cortical outputs in resting mice when compared with the control (**Figure 2(c)**). Subsequent VD$_3$R intervention (–D$_2$/+VDR) improved motor function and was associated with a restoration of hippocampal bursts while reducing the PFC cortical outputs

when compared with the untreated dyskinetic mice (–$D_2$; **Figure 2(c)**). From these observations, we deduced that changes in neural activities in the PFC and CA1 were associated with haloperidol induced dyskinesia when the M1-CPu was compared with the PFC-CA1 outputs (**Figure 1(f)** and **Figure 2(c)**).

In addition, haloperidol induced dyskinesia increased M1 and PFC (cortical outputs) but decreased the CA1 and CPu burst frequencies. A similar effect was described by Wang and Goldman-Rakic on the role of $D_2R$ in control of burst activities through its long term depression (LTD) effects on glutamergic systems; often explored in treatment of schizophrenia [26]. However, after VD$_3$RA intervention (–$D_2$/+VDR), restoration of CA1 and CPu burst frequencies were observed. This was associated with an increase in motor activity and a decrease in the threshold of abnormal movements when the animals were assessed in behavioral tests for motor function. These observations suggests that $D_2R$ inhibition affects motor and cognitive brain centers sequel to drug induced dyskinesia, through disruption of CA1 and corticostriatal projections to the PFC and M1.

## 5. Conclusion

Haloperidol induced dyskinesia was associated with a loss of CPu-CA1 burst pattern and increased M1 neural frequencies. VD$_3$RA intervention improved motor function and reduced AIMs score through reversal of M1, CPu and CA1 outputs in dyskinetic mice, specifically, restoration of CPu-CA1 burst frequencies.

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## Author Contributions

OOM and OOB initiated and designed the study. BJL, MUS, AOI, DEO, WGB, AA, and AEC participated in the implementation, design of apparatus for electrophysiology and behavioral tests. OOM, IDA and AOI analyzed the data. OOM, BJL, OOB and AOI compiled the reports and interpreted the data for write up.

## Conflict of Interest

The Authors hereby declare there is no conflict of interest associated with this study or any of the procedures and materials used for the purpose of the study.

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