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ORIGINAL ARTICLE

Datumetine exposure alters hippocampal neurotransmitters system in C57BL/6 mice

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ABSTRACT
Our previous study showed that datumetine modulates NMDAR activity with long term exposure leading to memory deficit and altered NMDAR signaling. We aim to explore the neurotransmitters perturbations of acute datumetine-NMDAR interaction. Fifteen C57/BL6 mice were used for the study, they are divided into three groups of 5 animals each. Animals were administered DMSO (DMSO/Control), 0.25 mg/kg body weight of datumetine (0.25 Datumetine) and 1 mg/kg bodyweight of datumetine (1.0 Datumetine) intraperitoneally for 14 days. At the end of treatment, animals were euthanized in isofluorane chamber, perfused transcardially with 1XPBS followed by PFA. Immunofluorescence procedure was done to check the distribution of neurons, astrocytes, microglia and major neuronal subtypes in the hippocampus. Expansion and electron microscopy techniques were used to assess the condition of the synapses. Quantitative data were expressed as mean ± SEM and analyzed using ANOVA with Tukey post hoc using $p < 0.05$ as significant. Datumetine increased the expression of CD11b, GFAP, vGlut1, GABA, CHRNA7 and TH while expression of TrPH and NeuN were reduced in the hippocampus compared to control animals. Synaptic loss was evident in datumetine exposed animals with reduced synaptic vesicles accompanied by a thickness of postsynaptic density than that of control animals. This study concludes that acute datumetine exposure alters hippocampal neurotransmitter systems.

1. Introduction

Datura metel is a *Solanaceae* plant which contains different alkaloids (Prasad and Gowda, 2005). It is used locally in the treatment of asthma, bronchodilation, glaucoma, and antibiotics (Hilal *et al.* 2014) and cancer treatment (Devi *et al.* 2011). Recreation use of Datura plant is increasing among adolescents (Moses 2010). World drug report as produced by the United Nations Office of Drug and Crimes claimed that datura plant is one of the new psychoactive substance (NPS) recorded in Africa (United Nations Office on Drugs and Crime (UNODC), 2017, 2018).

In Nigeria, 27 thousand people had been reported to be exposed to the use of datura with 0.03 prevalence rate (United Nations Office on Drugs and Crime (UNODC) 2018). Despite this number, deaths have been reported in the national dailies where datura extract was added to the alcoholic drinks to increase intoxication. Long term use of the plant has been reported to induce psychosis in humans (Khanra *et al.* 2015). Recreational users of datura suffer memory loss during the phase of intoxication, this led to the hypothesis that datura may possess compound(s) that affect hippocampal functioning.

In our previous study, we found that datumetine which is one of the alkaloids found in *Datura metel* has the ability to bind with NMDA receptor at both its orthosteric and allosteric binding sites, alters NMDAR calcium current *in vivo* and long-term exposure induces memory deficit and altered NMDAR signaling in mice (Ishola *et al.* 2020). Our study is the first to show the biological effects of datumetine on animal which we proposed to be in part responsible for memory loss observed in *Datura metel* abusers. In this present study, we aim to further characterize the effects of acute datumetine exposure on the hippocampal neurotransmitter system *in vivo*.

2. Materials and methods

2.1. Ethical approval

Ethical approval was gotten from the University of Ilorin Ethical Review Committee (UERC) with approval number UERC/ASN/2018/1277 and protocol identification code UERC/BMS/108.

2.2. Purchase and preparation of datumetine

Datumetine (CAS No.: 67078–20-0) 98% purity was purchased from ChemFaces Biochemical Company (China). The compound was dissolved in dimethyl sulfoxide (DMSO) to achieve 1 mg/mL stock solution stored at 4 °C. It was then serially diluted based on the dosage of animals for working solution (kept at room temperature) to administer to animals.

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⊕ Supplemental data for this article is available online at here.

2.3. Animal care

Fifteen (15) adult male C57BL/6 mice procured from Jackson's Lab (Bar Harbor, ME, USA) were used for the experiment. They were housed in standard laboratory conditions of 12 hours alternating light and dark cycle. They were kept in standard cages of five animals/cage. The mice were allowed free access to feed and water. All animal handling protocol was approved by the Louisiana State University IACUC.

2.4. Animal grouping and treatment

The animals were divided into three groups of five (5) mice each. Animals were administered 0.25 mg/kg (0.25 Datumetine) and 1.0 mg/kg (1.0 Datumetine) body weight of datumetine and 0.1 mL of DMSO (DMSO) respectively. All treatments were done intraperitoneally for fourteen (14) days.

2.5. Animal sacrifice

The animals were sacrificed a day after the last administration. The animals were deeply anesthetized with isoflurane in gas chamber. They were then perfused transcardially with 10 mM phosphate-buffered solution (1X PBS pH 7.4) followed by 4% paraformaldehyde (PFA). The brains were then excised and stored in specimen bottle containing 4% PFA and stored at 4°C for 24 hours, these were then post-fixed in 4% PFA with 30% sucrose solution at 4°C till further processing. Samples for electron microscopy had their brains excised and the hippocampus dissected out on a cold surface after 1X PBS perfusion and was quickly transferred into electron microscopy (EM) fixative (1.6% paraformaldehyde, 2.5% glutaraldehyde, 0.03% Calcium chloride in cacodylate buffer pH 7.4) stored at 4°C till further processing.

2.6. Immunofluorescence (IF)

Brain samples for immunofluorescence were embedded in optimal cutting temperature (OCT) compound (4585 Fisher Healthcare Houston USA) in coronal plane on cryomold. They were serially sectioned at 60 μm thick sections on a cryostat (Leica CM 3050 S USA). Sections were then stored in 48 well microplates containing 1X PBS and stored at 4°C till further processing.

The fluorescence protocol was performed using the standard procedure of the laboratory has published in Shrestha

et al. (2019). Sections were washed with 1X PBS containing 0.5% tween 20 (1X PBST) twice for 10 minutes. Sections were then blocked in 5% goat serum (S-1000 Vector Laboratories USA) prepared in 1X PBST for 60 minutes at room temperature. Sections were then incubated in primary antibodies as stated in Table 1 (prepared in 5% goat serum) for either overnight at 4°C or for 120 minutes at room temperature. Sections were washed in 1X PBST twice for 10 minutes and then incubated in Alexa Fluor-488 goat anti-rabbit secondary antibody (2:500, A11034 Invitrogen Fisher Scientific USA); or Alexa Fluor-568 goat anti-rabbit (2:500, A11036 Invitrogen Fisher Scientific USA) for 60 minutes at room temperature in the dark. Sections were washed twice in 1X PBST for 10 minutes and then transferred to 1X PBS. Sections were mounted on gelatin-coated glass slides with antifade mounting medium with DAPI (VECTASHIELD H-1500 Vector laboratories the USA) and coverslipped and stored on slide tray in the dark. Slides were viewed and the image was taken using a Nikon fluorescent microscope attached with a CCD camera. The mean intensity of fluorescent expression was quantified in the region of interest (hippocampus) using NIS-Elements software Version 4 (Nikon Inc. USA).

2.7. Expansion microscopy

This was done to check for neural connections. This is done by embedding already stained sections in a gel polymer which is later digested and made to expand uniformly in water. This is to increase the clarity and magnification of the specimen. Immunofluorescence protocol was followed for the staining of neurofilament using rabbit anti-neurofilament-L (1:200 2837S Cell Signaling Tech. Danvas MA, USA) at 4°C overnight, followed by incubation in Alexa Fluor-568 goat anti-rabbit secondary antibody for 60 minutes at room temperature. Additional Expansion Techniques was done as follows;

- The stained sections were rinsed in 5% goat serum twice for 15 minutes each.
- Sections are placed in 1% acryloyl-X solution (prepared in 1X PBS) at 4°C overnight.
- Sections were rinsed twice in 1X PBS for 15 minutes each
- Sections were placed in gelling solution (94% monomer solution, 2% of 0.5% 4-hydroxytempo, 10% tetramethylethylenediamine – TEMED and 10% ammonium persulfate - APS) for 5 and 25 minutes at 4°C respectively.

Table 1. List of primary antibodies used and their dilution.

Probe	Antibody (Cat. Number)	Dilution	Company
Neurons	Anti-NeuN* (24307S)	1:100	Cell Signaling Tech. Danvas MA, USA
Astrocytes	Anti-GFAP (3670S)	1:300	
Microglia	Anti-ITGAM/CD11B (49420S)	1:300	
Dopaminergic neurons	Anti-TH (58844S)	1:100	
Glutamatergic neurons	Anti-vGlut2 (71555S)	1:150	
Serotonergic neurons	Anti-TPH-1 (12339S)	1:200	
Synapse	Anti-PSD-95 (3450S)	1:200	
Cholinergic neurons	Anti-CHRNA7 (PA5-37280)	1:300	Invitrogen Fisher Scientific USA
GABAergic neurons	Anti-GABA (PA5-32241)	1:5000	

*Incubation was done for 120 minutes at room temperature; others were overnight at $4\,^{\circ}$C.

- Each section was then incubated separately in a slide mounting chamber with a new gelling solution for 2 hours at 37°C.
- The excess gel was trimmed off and sections were placed in a digestion buffer (for digestion of the gelling polymer) at room temperature overnight.
- Sections were then rinsed in distilled water for 15 minutes for uniform expansion.
- Sections were then mounted on a glass slide and cover-slipped using DAPI mountant.

2.8. Electron microscopy

Electron microscopy technique was done in accordance with the laboratory standard protocol as reported in Shrestha *et al.* (2019). Hippocampal samples for electron microscopy were trimmed into 1 mm sections using a sharp razor which were transferred into a fresh fixative for 2 hours at room temperature. The samples were washed in 0.1 M cacodylate buffer containing 5% sucrose and then fixed in 2% osmium tetroxide for 1 hour at room temperature. The sections were washed in water, in-block stained with 2% uranyl acetate prepared in 0.2 M sodium acetate buffer (pH 3.5), for 2 hours. Stained sections were dehydrated in ascending grades of alcohol and propylene oxide. The processed sections were then embedded in Epon-Araldite mixture and polymerized for 24 hours at 60°C. Tissue blocks were sectioned using a Leica Ultratome (Leica EM UC7). Thin (80 nm) sections were recovered and stained with lead citrate for 5 minutes. Transmission electron photomicrographs were obtained in a JEOL 1400 TEM microscope, equipped with a GATAN digital camera. All reagents for electron microscopy were from EMS (Hatfield, PA).

2.9. Statistical analysis

Quantitative data were expressed as mean ± SEM in bar charts. Data were analyzed using one- way analysis of variance (ANOVA) and Tukey *post-hoc* test was done when ANOVA shows significant p values was set at 0.05.

3. Results

3.1. Increased gliosis in the hippocampus of datumetine exposed animals

Hippocampal slices stained for neurons and glia (see Figures 1 and 2) showed that animals exposed to 0.25 mg/kg of Datumetine (0.25 Datumetine) expressed neurons in the cornu ammonis (CA) and dentate gyrus (DG) regions of the hippocampus, but little expression in the molecular layer (ML) of the CA region. Animals exposed to 1.0 mg/kg of datumetine expressed neurons in both the CA and DG parts of the hippocampus, but scanty expression seen in the ML of the CA region of the hippocampus. While animals exposed to DMSO expressed neurons in all parts of the hippocampus.

CD11b expression (microglia + myeloid marker) was observed in the ML of DG regions in the control animals. Similar expression was also observed in 0.25 Datumetine animals to the control while 1.0 Datumetine animals showed high expression of CD11b in all regions of the hippocampus (Figure 1). Immunofluorescence quantification revealed that, expression of CD11b were significantly increased in datumetine exposed animals than the control (Figure 1).

Astrocytic profile was also assessed in the hippocampus of experimental animals using ant-GFAP (Figure 2). Expression of GFAP positive cells in the control animals was more evident in the ML and part of DG of the hippocampus. 0.25 Datumetine animals showed GFAP positive expression in areas similar to the control animals (i.e., ML and part of DG of the hippocampus). Animals exposed to 1.0 mg/kg of datumetine showed expression of GFAP more in CA3 region, DG and ML of the hippocampus. Quantification of immunopositive cells showed that datumetine exposure significantly increased the expression of GFAP in the hippocampus of the exposed animals compared to the control animals.

3.2. Increased activity of excitatory and inhibitory neurons in datumetine exposed animals

Hippocampal slices stained for vGlut1 (glutamate neurons) showed that DMSO animals expressed vGlut1 in the granular cell layer of the CA region and DG indicating presence of glutamate neurons in the layer. Expression of vGlut1was increased in the hippocampus of datumetine exposed animals. Extracellular expression of vGlut1 (an expression not merging with DAPI staining) was also seen in datumetine exposed animals which indicates loss of glutamate neurons in the animals (see Figure 3).

Inhibitory neurons were stained using GABA. All parts of hippocampus showed increased expression of GABA in datumetine exposed animals than the DMSO animals. 1.0 Datumetine animals also showed no expression in some part of the molecular layer (ML) in the CA region (see Figure 4).

3.3. Hippocampal neuronal subtypes activity in datumetine exposure

Expression of CHRNA7 was observed in the CA region of the hippocampus together with the ML (Figure 5). 0.25 Datumetine animals showed CHRNA7 expression in the CA region and ML of the hippocampus which is more than that of the control animals. Similarly, 1.0 mg Datumetine animals had CHRNNA7 expression in all CA and ML regions of the hippocampus. Quantification of CHRNA7 fluorescence showed that, exposure to datumetine significantly (*$p < 0.05$) increased the expression of CHRNA7 than the control.

Dopaminergic terminals were labeled using anti-TH. Increased activity of dopaminergic terminals was seen in animals exposed to datumetine (see Figure 6).

Serotonergic neurons labeled with anti-TPH-1 showed reduce expression in 0.25 Datumetine animals in all the part of the hippocampus than the control. High extracellular expression of TPH-1 was seen in 1.0 Datumetine, indicating loss of serotonergic neurons. All animals exposed to datumetine showed no TPH-1 expression in the DG of the
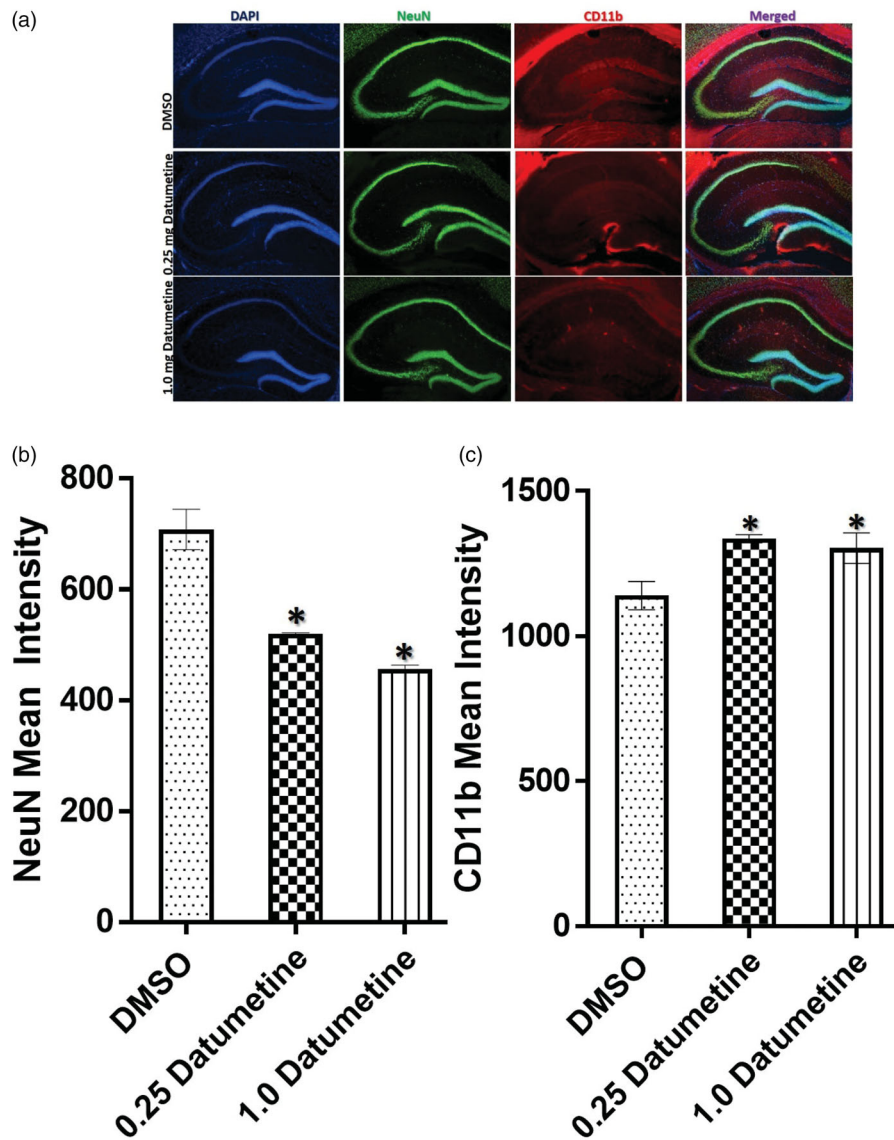
Figure 1. Immunofluorescence results showing (a) representative images of DAPI, NeuN, and CD11b immunostaining in the hippocampus of experimental animals. Graphical representation of flouresent quantification of (b) NeuN and (c) CD11b immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, F(2, 27)=31.85, $p = 0.0002$, *$p < 0.05$ one-way ANOVA with Tukey *post-hoc* test). See supplementary Figure 1 for higher magnification.

hippocampus (see Figure 7). All immunofluorescence quantitative data value were supplied in the table of supplementary file.

3.4. Datumetine altered neurofilament arrangement

Expansion microscopy technique was done to check for neural connections in the hippocampus. This was achieved by staining for neurofilament by immunofluorescence which serves as the structural protein of neurons in the hippocampus of experimental animals, and then expanding the slices to increase the magnification and clarity.

In all part of the hippocampus, arrangement and distinctiveness of neurofilament were altered in all animals exposed to datumetine compared to the control (Figure 8). In the CA regions, the lattice arrangement of neurofilament was altered and the organized arrangement of neurofilament in the DG was also disorganized in datumetine exposed animals compared to control.

3.5. Datumetine altered synaptic morphology with synaptic loss

Electron microscopy technique was done to check for viable synapse in the hippocampus. Viable synapse was identified by the presence of pre- and post-synaptic membrane, postsynaptic density (PSD) and presynaptic vesicles (Figure 9(a)). The number of viable synapses was reduced in animals exposed to datumetine; this effect was more in animals exposed to 1.0 mg/kg body weight of datumetine. The number of presynaptic vesicles identified in the viable synapse was observed to reduce significantly (*$p < 0.05$) in datumetine exposed animals compared to control with 1.0 Datumetine animals with the least count (Figure 9(b)). PSD thickness was also measured in viable synapse identified in the micrograph of the experimental animals. Datumetine significantly (***$p < 0.001$) increased the thickness of PSD in the hippocampus of experimental animals compared to control with 1.0 Datumetine animals having the highest PSD thickness (Figure 9(c)).
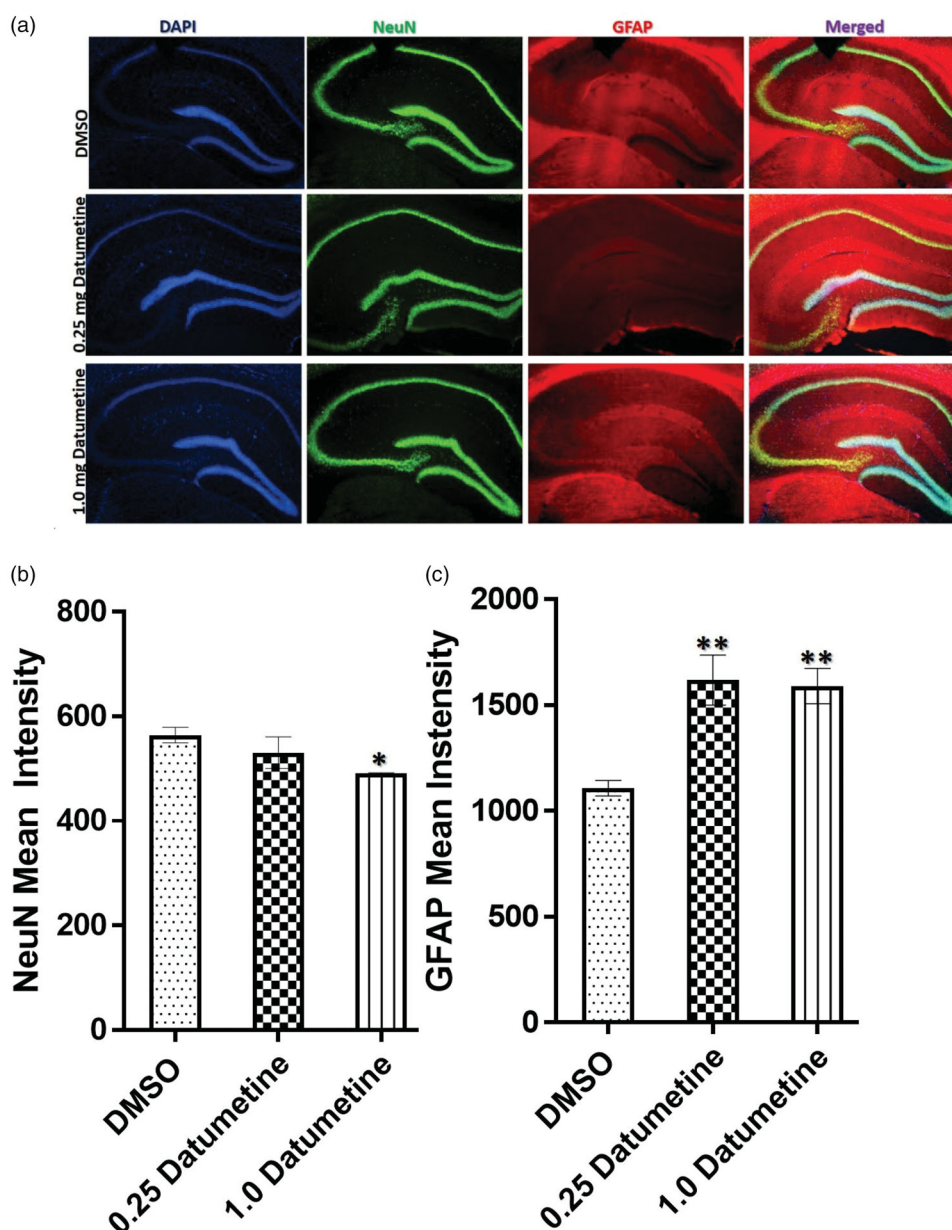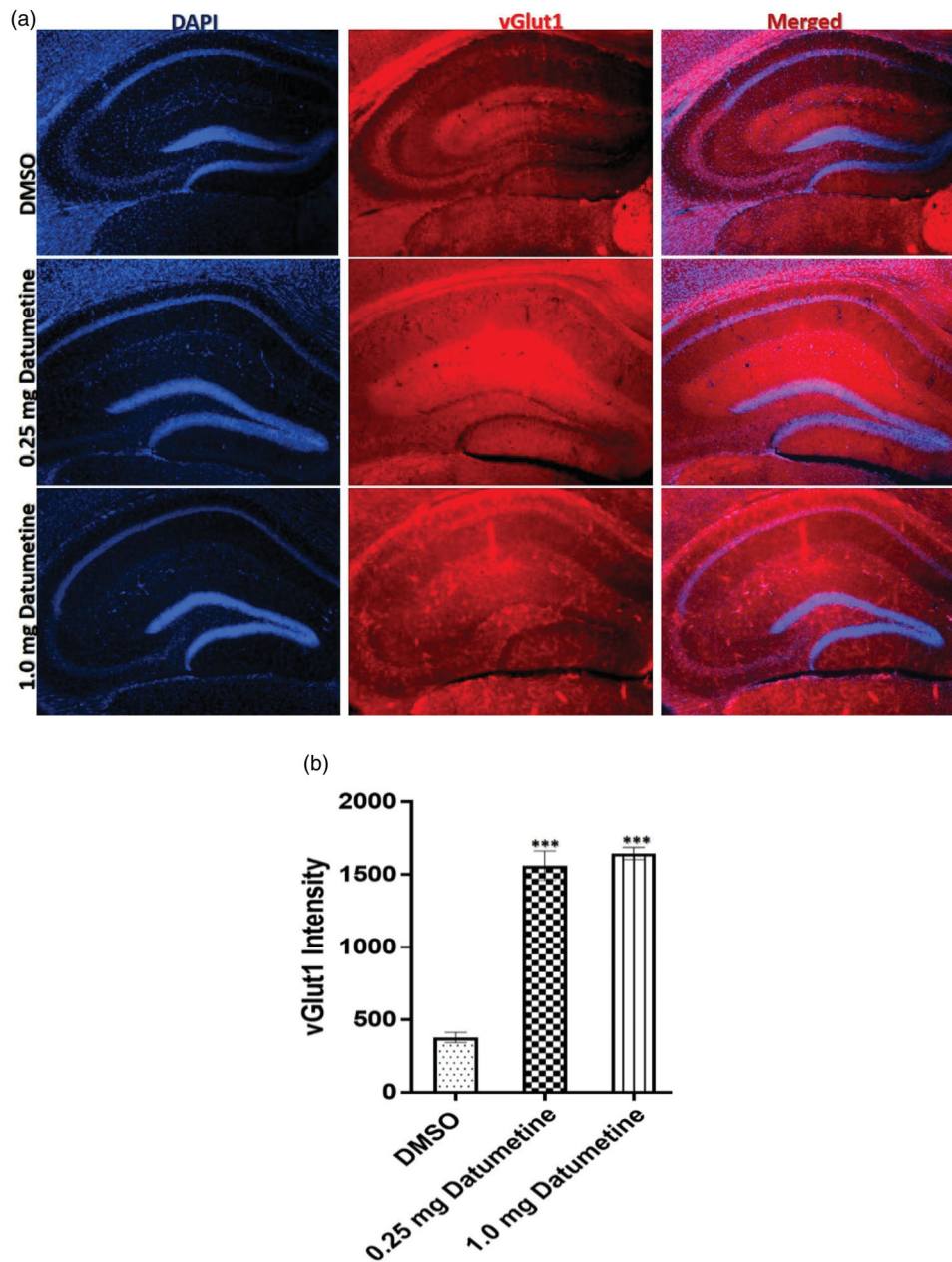
Figure 2. Immunofluorescence results showing (a) representative images of DAPI, NeuN, and GFAP immunostaining in the hippocampus of experimental animals. Graphical representation of flourescent quantification of (b) NeuN and (c) GFAP immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, $F_{(2, 27)} = 24.35$, $p = 0.0001$; $*p < 0.05$; $**p < 0.01$ one-way ANOVA with Tukey *post-hoc* test). See supplementary Figure 2 for higher magnification.

4. Discussion

Our earlier report showed that *Datura metel* contains datumetine which has the ability to modulate NMDAR activity, this brought about changes in NMDAR Ca^{2+} and inducing memory deficit together with altering NMDAR signaling (Ishola *et al.* 2020). This indicated that datumetine binding with NMDAR alters its activity leading to its memory deficit observed in our previous study.

In further characterization of datumetine-NMDAR interactions, observations from the present study indicated that, long term exposure to datumetine increased the activity of glial cells (both astrocyte and microglia) in animals exposed to datumetine. Increased activity of these glial cells has been attributed to injury to the brain (Pekny *et al.* 1999, Sofroniew and Vinters 2010, Gorina *et al.* 2011, Zamanian *et al.* 2012, Paintlia *et al.* 2013), neuroinflammation (Takeuchi 2013,

Colombo and Farina 2016, Popichak *et al.* 2018) synaptic pruning (Paolicelli *et al.* 2011, Li *et al.* 2012b) and excitotoxicity (Matute *et al.* 2002; Rossi *et al.* 2004; Kauppinen and Swanson 2007). This indicates that prolonged datumetine exposure may be injurious to the hippocampus. Our previous study showed that datumetine has binding affinity to NMDAR at its orthosteric binding site (Ishola *et al.* 2020), this is the site where glutamate will normally bind (Luo *et al.* 2011), this indicates that prolong datumetine exposure is similar to glutamate excitotoxicity as it has been established that glutamate can exert both synaptic plasticity and excitotoxicity when is moderate and excess respectively (Szydlowska and Tymianski 2010, Vishnoi *et al.* 2015). Previous reports have shown that moderate activation of NMDAR is essential for cell survival, synaptic plasticity and LTP necessary for learning and memory (Traynelis *et al.* 2010,
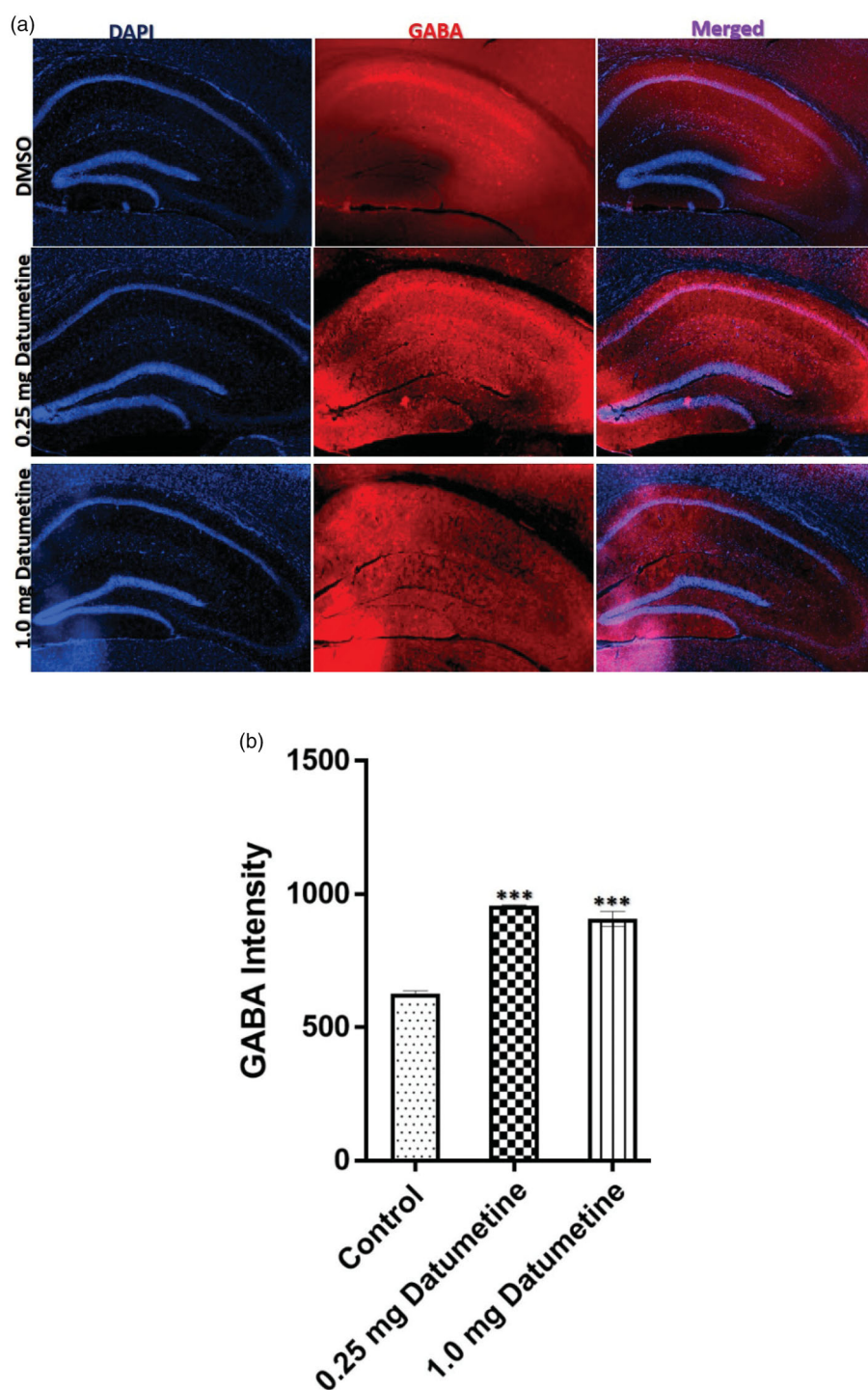
Figure 3. Immunofluorescence results showing (a) representative images of DAPI and vGlut1 immunostaining in the hippocampus of experimental animals, and (b) quantification of vGlut1 immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, $F_{(2, 27)} = 161.6$, $p = 0.0001$, ***$p < 0.001$ one-way ANOVA with Tukey *post-hoc* test).

Ahmad *et al.* 2012, Papouin *et al.* 2012, Iacobucci and Popescu 2017) whereas overactivation of NMDAR has also been implicated in cell death (Szydlowska and Tymianski 2010, Milnerwood *et al.* 2010, Xia *et al.* 2010). Studies on neurodegeneration diseases in rodents have also implicated impaired NMDAR function as part of the pathophysiology of the disease progression (Paoletti *et al.* 2013). Although NMDAR hyperactivation is not in all case responsible for glia activation (Subhramanyam *et al.* 2019).

Effect of datumetine on hippocampal neuronal subtypes was also assessed. Datumetine increases the expression of vGlut1. This indicates increased activity of glutamate transmission (Wojcik *et al.* 2004, Reimer, 2009, Reimer and Voglmaier, 2014). Unlike reports that indicated that vGlut1 is reduced when activity is increased (Seal 2016), our results showed that increasing NMDAR activity by datumetine also increases VGlut1. This difference may be in part as a result of neural loss in neurodegenerative disorders (Tabrizi *et al.* 2013, De Strooper and Karran 2016). Increased hippocampal excitation is well correlated to increase GABA activity (Xue *et al.* 2011, Yao *et al.* 2018). Together with the increased activity of vGlut1 expression of GABA too was increased in animals exposed to datumetine. Since our ligand docking result showed that datumetine is specific for NMDAR binding (Ishola *et al.* 2020), increased level of GABA will be as a result of increased NMDAR activity as it is well established that increased NMDAR activity leads to increase GABA release (Xue *et al.* 2011, Yao *et al.* 2018). Similar to our observations, administration of NMDAR agonist is reported to increase the level of GABA in cortical neurons (Drejer and Honore 1987).
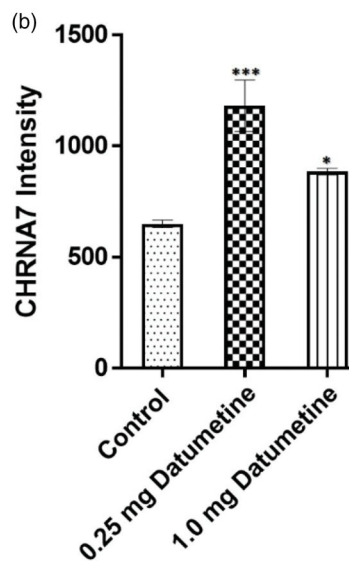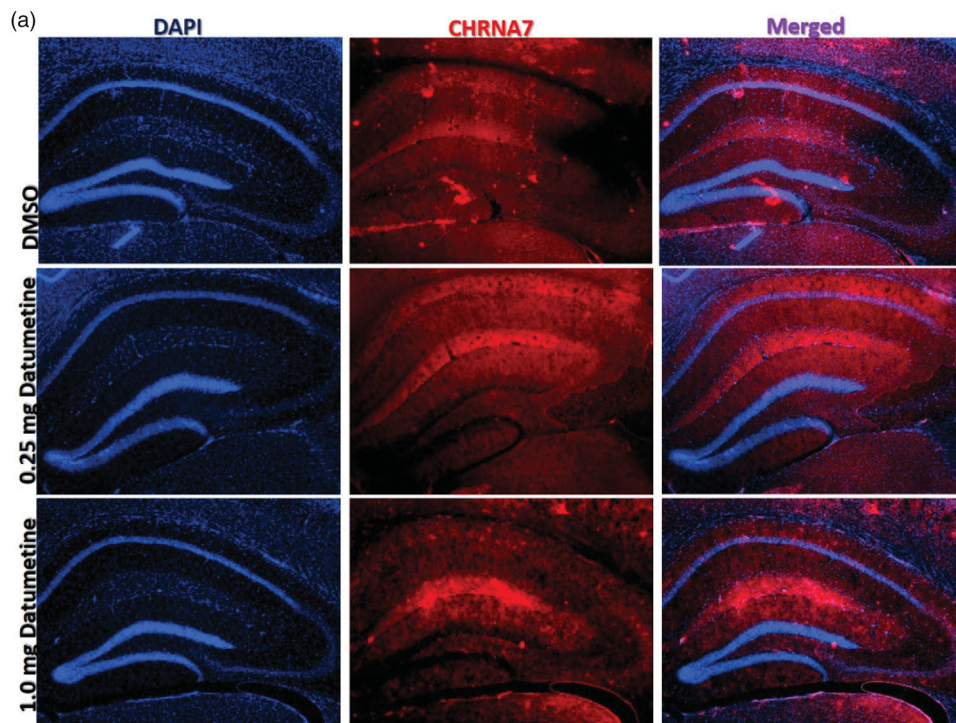
Figure 4. Immunofluorescence results showing (a) representative images of DAPI and GABA immunostaining in the hippocampus of experimental animals, and (b) quantification of GABA immunofluorescence intensity in the hippocampus of experimental animals. ($n = 10$ slices per group, $F_{(2, 27)} = 39.9$, $p = 0.0001$, ***$p < 0.001$ one-way ANOVA with Tukey *post-hoc* test).

This interaction is attributed in part to level of intracellular Ca^{2+} (Lindefors *et al.* 1997). Although the mechanism behind this regulation is still skeptical, possible mechanisms been put forward is that presence of glutamate receptors on GABAergic neurons or brain plasticity to ameliorate effect of hyperexcitation (Bavelier *et al.* 2010).

Datumetine increased the activity of cholinergic and dopaminergic neurons but not serotonergic neurons. Cholinergic and dopaminergic transmission positively regulates the activity of NMDAR (Zweifel *et al.* 2008, Oswald *et al.* 2015). In this study, nAChR-α7 was used to identify cholinergic terminals. nAChR-α7 was reported to enhance glutamate transmission (Koukouli and Maskos 2015). nAChR-α7 was shown to directly control the gating of Ca^{2+} and upon activation can induce local depolarization which leads to glutamate release (Zhong *et al.* 2008, 2013). This process occurs presynaptically, while
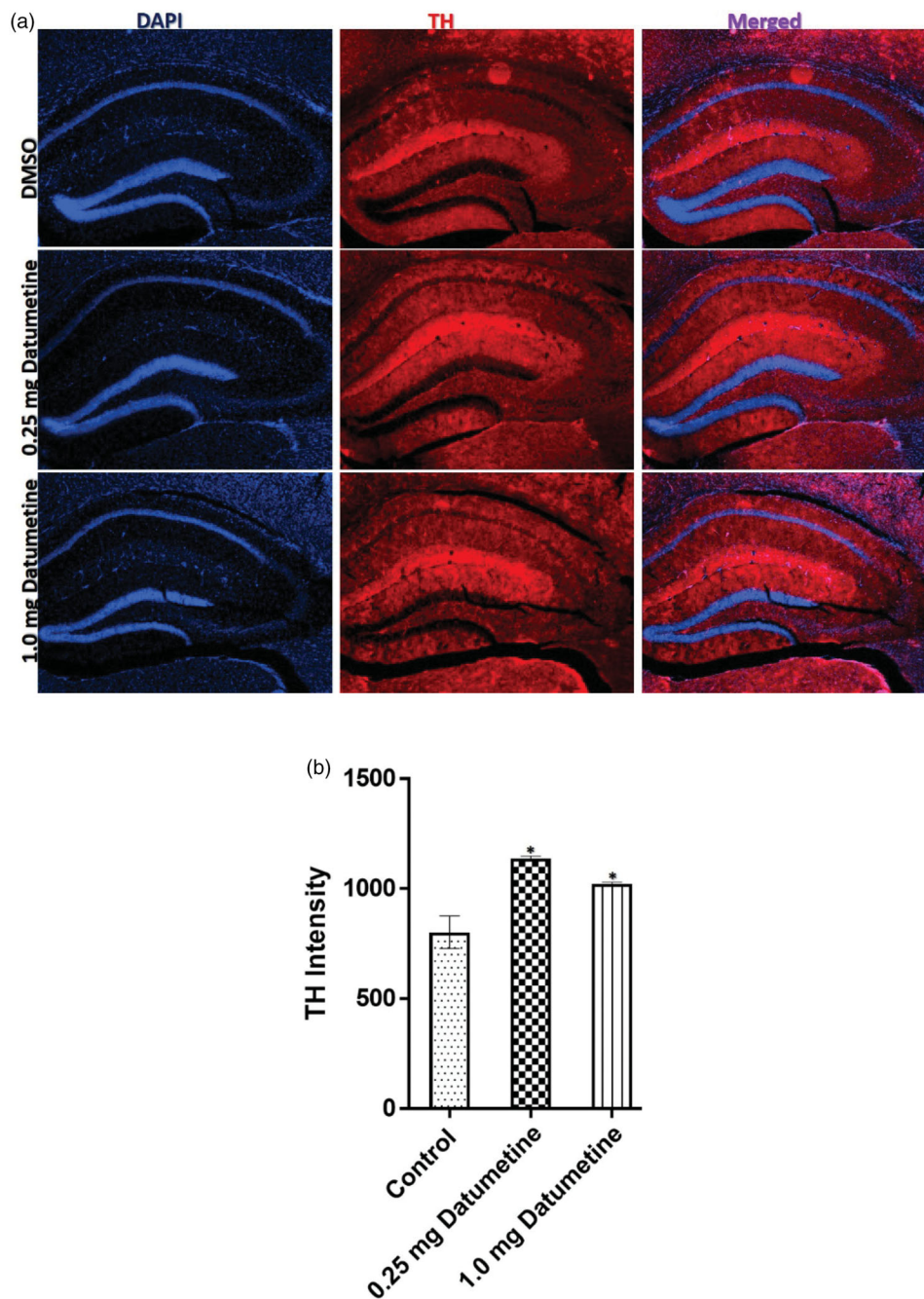
Figure 5. Immunofluorescence results showing (a) representative images of DAPI and CHRNA7 immunostaining in the hippocampus of experimental animals, and (b) quantification of CHRNA7 immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, $F_{(2, 27)} = 20.08$, $p = 0.0013$, $*p < 0.05$, $***p < 0.001$ one-way ANOVA with Tukey *post-hoc* test).

at the postsynaptic channel, nAChR-α7 induces glutamate release by amplification of weak signals (Ji and Dani 2000, Ji et al. 2001). It has been reported that nAChR-α7 and NMDAR directly interact in the hippocampus (Li et al. 2012a). This activity of nAChR-α7 may explain the increased level of vGlut1 observed in the treated animals. The activity of glutamate transmission and nAChR-α7 activity is closely related. Like the results obtained from this experiment, nAChR-α7 knock out mice showed reduced glutamate synapse (Koukouli and Maskos 2015), whereas activation of nAChR-α7 by nicotine increased glutamate synapse in cell culture (Lozada et al. 2012). The interaction of glutamate and

acetylcholine system is not unidirectional, blocking of NMDAR activity reduced the effects of nicotine (D'Souza and Markou 2011).

In a study conducted by Sing et al. glutamate and dopamine transmission is reported to be interrelated in which NMDAR activity modulate dopamine-1 (D1) receptor activity bidirectionally (Singh et al. 1990). They showed that increasing the activity of NMDAR increased dopamine transmission and signaling mediated by D1 receptors in the striatum and substantia nigra (Singh et al. 1990). Hippocampus receives dopaminergic inputs from the ventral tegmental area (VTA) (Weitemier and McHugh 2017) and is proposed that these

Figure 6. Immunofluorescence results showing (a) representative images of DAPI and TH immunostaining in the hippocampus of experimental animals, and (b) quantification of TH immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, $F(2, 27)=11.28$, $p = 0.0140$, $*p < 0.05$ one-way ANOVA with Tukey *post-hoc* test).

inputs play a role in addiction and memory (McNamara and Dupret 2017). In this study, datumetine which is proposed to enhance the activity of NMDAR also showed increased expression of dopaminergic terminals. Studies have shown the presence of different dopamine receptor subtypes in the hippocampus (Ishola *et al.* 2018). This observation may be in part due to the modulation of dopamine activity mediated by D1 as reported by Singh *et al.* (1990). Dopamine-2 (D2) receptor which is another subtype of dopamine receptor usually mediates the control of movement (Ishola *et al.* 2015, 2018) and is found mainly on medium spiny interneurons (Ishola *et al.* 2018). NMDAR activity is reported to have an

antagonistic effect on dopaminergic activity mediated by D2 receptors (Amalric *et al.* 1994). Blocking of NMDAR in the VTA is reported to attenuate the enhanced dopamine release and behavioral changes seen in animals exposed to psychostimulants (Kalivas and Alesdatter 1993). The interaction of glutamate and dopamine transmission system in the brain is not clear cut. Contrary to the result obtained, studies done on mice striatum showed that blocking NMDAR activity enhances locomotor activity similar to D2 agonist treatment (Mele *et al.* 1996) this action was mediated by D2 receptor activity while they also found out that memory impairment observed by blocking NMDAR was reversed by D1 agonist
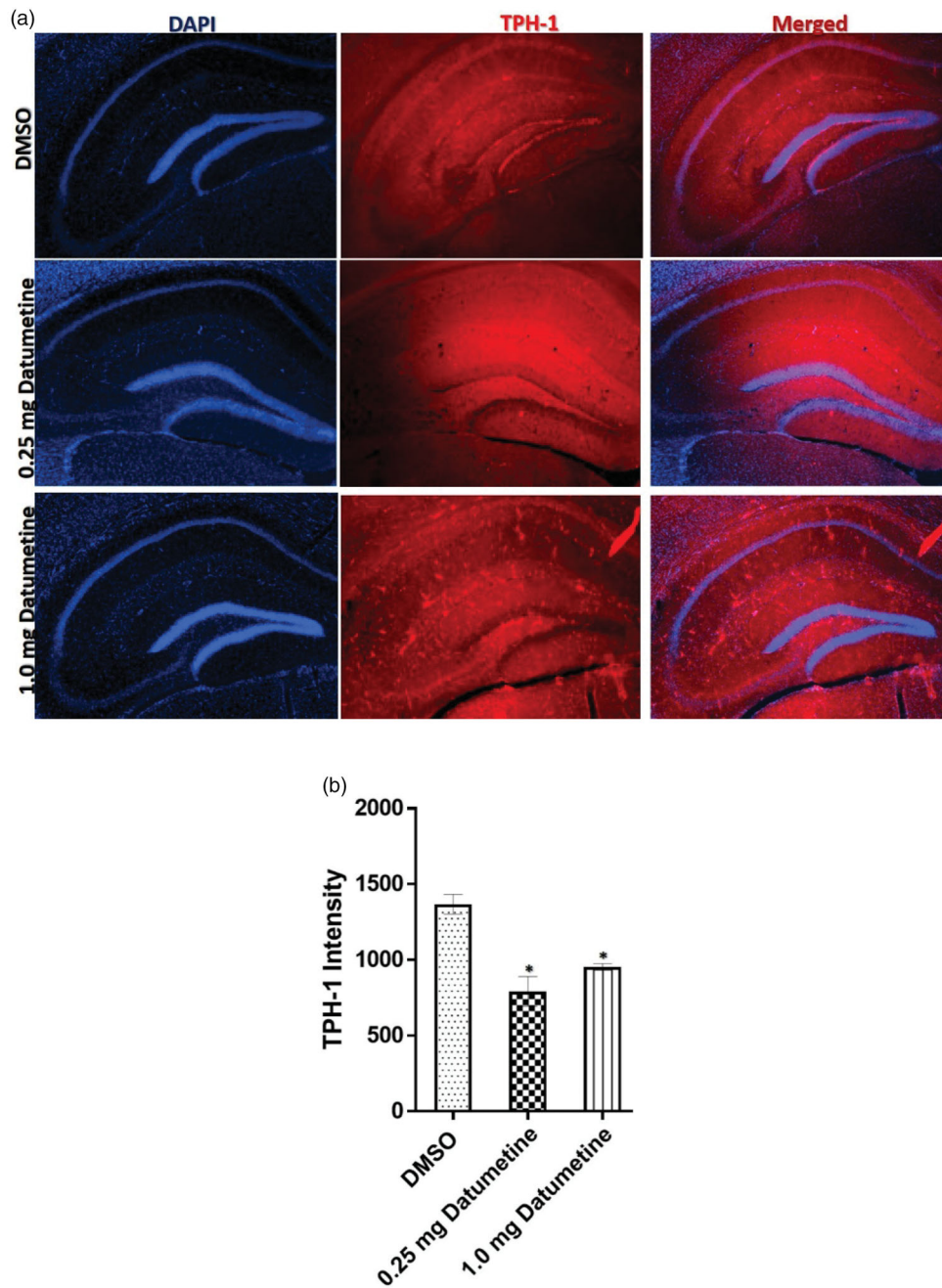
Figure 7. Immunofluorescence results showing (a) representative images of DAPI and TPH-1 immunostaining in the hippocampus of experimental animals, and (b) quantification of TPH-1 immunofluorescence intensity in the hippocampus of experimental animals ($n = 10$ slices per group, $F(2, 27)=22.49$, $p = 0.0016$, $*p < 0.05$ one-way ANOVA with Tukey *post-hoc* test).

implicating role of D1 receptors in memory formation (Mele *et al.* 1996). Although locomotor activity was not assessed in this experiment, and the striatum of the animals was not assessed, future studies need to examine the effects datumetine enhancement of NMDAR may have on the D1 receptor in the hippocampus as well as the behavioral implication.

Loss of serotonergic neurons was prominent in the DG region of the hippocampus of datumetine exposed animals. Serotonin activity bidirectionally modulates NMDAR functions (Maura *et al.* 2000, Yuen *et al.* 2005). Blocking of NMDAR is well correlated with increased serotonin transmission (Smith

et al. 1981, Loscher and Honack 1992, Lindefors *et al.* 1997). The interplay between NMDAR and serotonin is reported to be contradictory in the periaqueductal gray (PAG) area (Moraes *et al.* 2008) which is similar to our present observation in the hippocampus. Several reports conducted on the interplay of NMDAR and serotonin activity revealed that effects of upregulating activity of serotonin was counteracted by NMDAR agonist (Schmitt *et al.* 1995, De Souza *et al.* 1998, Carobrez *et al.* 2001, Moraes *et al.* 2008).

Increased excitatory transmission leads to synaptic pruning (Henson *et al.* 2017, Inquimbert *et al.* 2018) to regulate brain
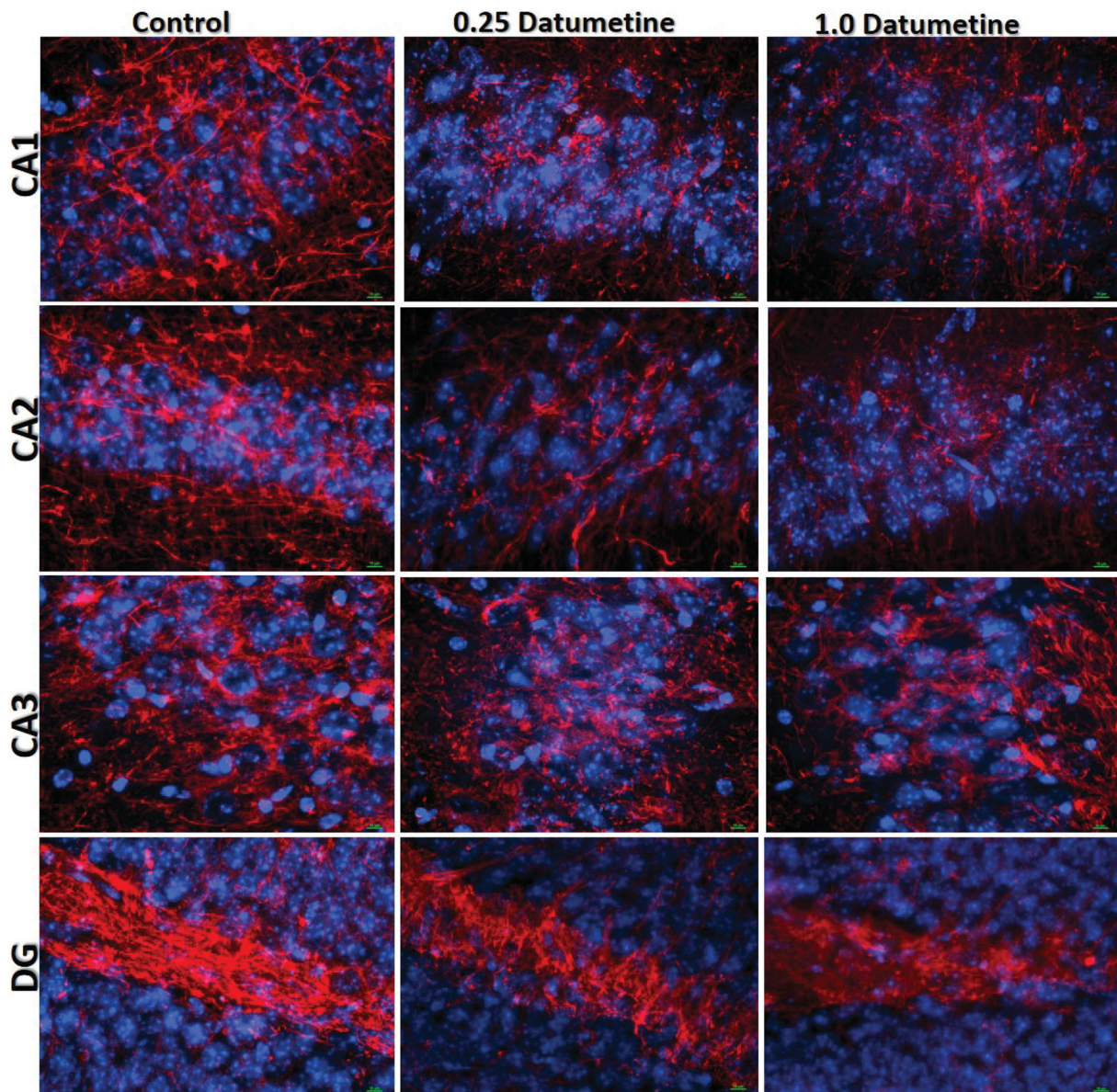
Figure 8. Representative expansion microscopy images of the hippocampus of experimental animals showing neurofilament (red) and DAPI (blue). Treated animals showed altered neurofilament expression and arrangement.

activities. The neural connection was assessed using expansion microscopy techniques stained for neurofilament. Datumetine induces loss of neural connections in all regions of the hippocampus. This may be due to the increased excitatory transmission (Sattler *et al*. 1999, Dong *et al*. 2009, Zhou *et al*. 2013) induced by datumetine. Glia cells also play a major role in synaptic pruning (Paolicelli *et al*. 2011, Li *et al*. 2012b), as stated earlier datumetine exposure increased the activities of glia. This together showed that prolong exposure of datumetine increased NMDAR activities leading to hyper-excitation in the hippocampus.

Electron microscopy studies on the synapse revealed that datumetine exposed animals showed a reduction in the number of viable synapses with 1.0 mg/kg Datumetine animals showing the greatest reduction compared to controls. It is on

record that overactivation of NMDAR leads to synaptic loss (Talantova *et al*. 2013, Zhou *et al*. 2013, Lewerenz and Maher 2015). This observation may be due to the persistent interaction of datumetine with NMDAR (Ishola *et al*. 2020). The postsynaptic density was thicker in datumetine exposed animals with a great reduction in presynaptic vesicles. Chemical neurotransmission is through the release of synaptic vesicles (Trkanjec and Demarin 2001, Ikeda and Bekkers 2009) which are tightly regulated by re-uptake back to the presynaptic neurons (Piedras-Renteria *et al*. 2004, Dickman *et al*. 2012, Davis and Muller 2015). Datumetine greatly reducing the number of synaptic vesicles showed that either reuptake of the vesicles is altered, or rate of production is not balanced with the rate of release (Wang *et al*. 2016, Li and Kavalali 2017). Another possible explanation may be that NMDAR
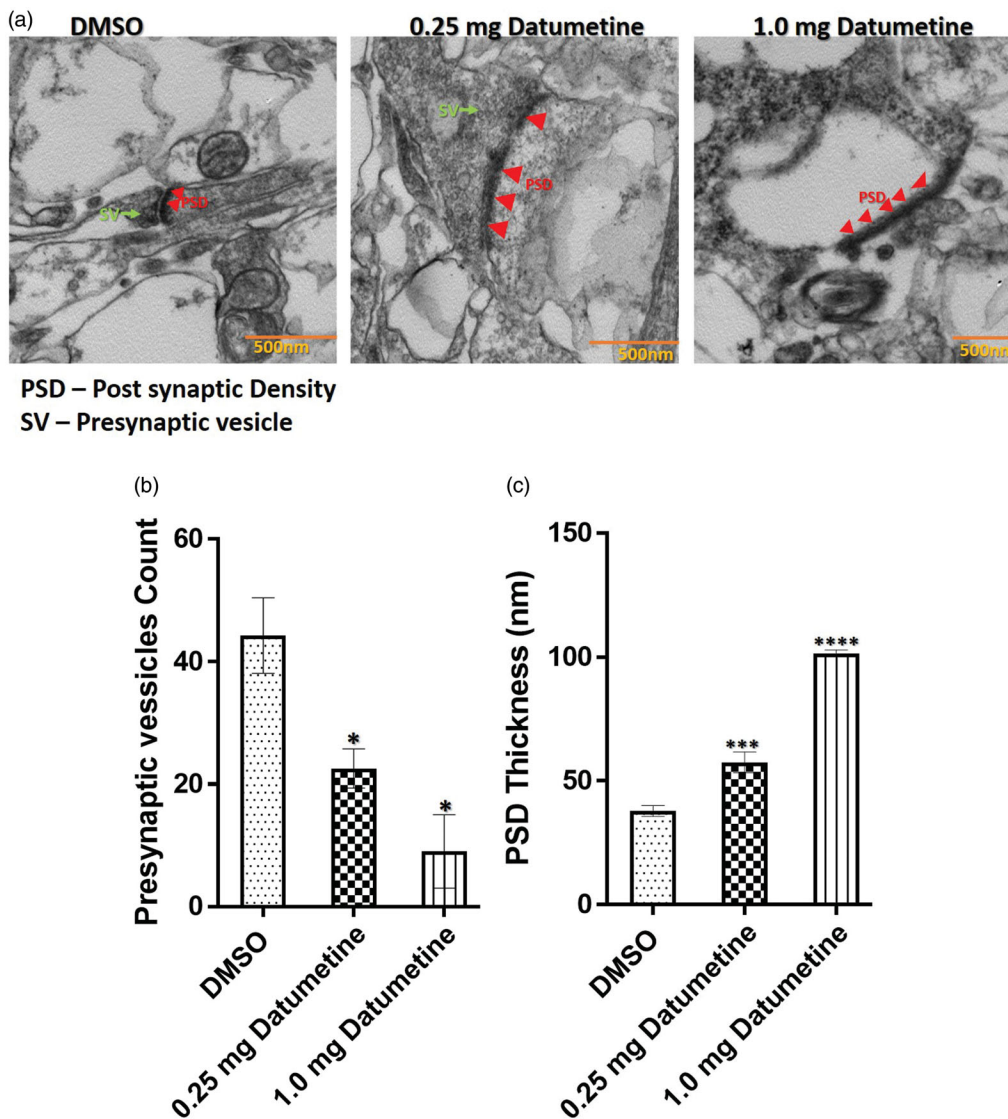
Figure 9. (a) Representative electron micrograph of the hippocampus of experimental animals; (b) quantification of presynaptic vesicles; (c) quantification of postsynaptic density.

binding with datumetine increases the affinity of presynaptic NMDAR for glutamate thereby increasing the release of neurotransmitters (Reimer *et al.* 1998, Takamori 2016).

5. Conclusion

This study concludes that acute datumetine exposure altered hippocampal neurotransmitter systems, neurofilament and synaptic morphology which may predispose to neurodegeneration.

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References

Ahmad, M., *et al.*, 2012. Postsynaptic complexin controls AMPA receptor exocytosis during LTP. *Neuron*, 73 (2), 260–267.

Amalric, M., *et al.*, 1994. Functional interactions between glutamate and dopamine in the rat striatum. *Neurochemistry International*, 25 (2), 123–131.

Bavelier, D., *et al.*, 2010. Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 30 (45), 14964–14971.

Carobrez, A.P., Teixeira, K.V., and Graeff, F.G., 2001. Modulation of defensive behavior by periaqueductal gray NMDA/glycine-B receptor. *Neuroscience and Biobehavioral Reviews*, 25 (7–8), 697–709.

Colombo, E., and Farina, C., 2016. Astrocytes: key regulators of neuroinflammation. *Trends in Immunology*, 37 (9), 608–620.

Davis, G.W., and Muller, M., 2015. Homeostatic control of presynaptic neurotransmitter release. *Annual Review of Physiology*, 77, 251–270.

De Souza, M.M., Schenberg, L.C., and de Pádua Carobrez, A., 1998. NMDA-coupled periaqueductal gray glycine receptors modulate anxio-selective drug effects on plus-maze performance. *Behavioural Brain Research*, 90 (2), 157–165.

De Strooper, B., and Karran, E., 2016. The cellular phase of Alzheimer's disease. *Cell*, 164 (4), 603–615.

Devi, M.R., *et al.*, 2011. Neurotoxic and medicinal properties of *Datura stramonium* L. – review. *Assam University Journal of Science and Technology*, 7 (1), 139–144.

Dickman, D., Tong, A., and Davis, G.W., 2012. Snapin is critical for pre-synaptic homeostatic plasticity. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32 (25), 8716–8724.

Dong, X.X., Wang, Y., and Qin, Z.H., 2009. Molecular mechanisms of exci-totoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica*, 30 (4), 379–387.

Drejer, J., and Honore, T., 1987. Phencyclidine analogues inhibit NMDA-stimulated [3H]GABA release from cultured cortex neurons. *European Journal of Pharmacology*, 143 (2), 287–290.

D'souza, M.S., and Markou, A., 2011. Neuronal mechanisms underlying development of nicotine dependence: implications for novel smoking-cessation treatments. *Addiction Science and Clinical Practice*, 6, 4–16.

Gorina, R., *et al.*, 2011. Astrocyte TLR4 activation induces a proinflamma-tory environment through the interplay between MyD88-dependent NFκB signalling, MAPK, and Jak1/Stat1 pathways. *Glia*, 59 (2), 242–255.

Henson, M.A., *et al.*, 2017. Long-term depression-associated signaling is required for an *in vitro* model of NMDA receptor-dependent synapse pruning. *Neurobiology of Learning and Memory*, 138, 39–53.

Hilal, T., *et al.*, 2014. Comparative study of phytochemical screening, anti-oxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *Journal of King Saud University – Science*, 26 (3), 237–243.

Iacobucci, G.J., and Popescu, G.K., 2017. NMDA receptors: linking physio-logical output to biophysical operation. *Nature Reviews. Neuroscience*, 18 (4), 236–249.

Ikeda, K., and Bekkers, J.M., 2009. Counting the number of releasable syn-aptic vesicles in a presynaptic terminal. *Proceedings of the National Academy of Sciences of the United States of America*, 106 (8), 2945–2950.

Inquimbert, P., *et al.*, 2018. NMDA receptor activation underlies the loss of spinal dorsal horn neurons and the transition to persistent pain after peripheral nerve injury. *Cell Reports*, 23 (9), 2678–2689.

Ishola, A.O., *et al.*, 2018. Age-dependent dopamine-2 receptor (D$_2$R) plas-ticity in mouse motor cortex and hippocampus. *Nigerian Journal of Neuroscience*, 9 (1), 1–8.

Ishola, A.O., Imam, A., and Ajao, M.S., 2020. Effects of novel alkaloid from Datura metel on hippocampal NMDAR activity. *Toxicology Reports*, (In press).

Ishola, A.O., *et al.*, 2015. Vitamin D$_3$ receptor activation rescued cortico-striatal neural activity and improved motor-cognitive function in -D$_2$R Parkinsonian mice model. *Journal of Biomedical Science and Engineering*, 08 (09), 601–615.

Ji, D., and Dani, J.A., 2000. Inhibition and disinhibition of pyramidal neu-rons by activation of nicotinic receptors on hippocampal interneurons. *Journal of Neurophysiology*, 83 (5), 2682–2690.

Ji, D., Lape, R., and Dani, J.A., 2001. Timing and location of nicotinic activ-ity enhances or depresses hippocampal synaptic plasticity. *Neuron*, 31 (1), 131–141.

Kalivas, P.W., and Alesdatter, J.E., 1993. Involvement of NMDA receptor stimulation in the VTA and amygdala in behavioral sensitization to cocaine. *Journal of Pharmacology and Experimental Therapeutics*, 267, 486–495.

Kauppinen, T.M., and Swanson, R.A., 2007. The role of glia in excitotoxic-ity and stroke. *In*: A. Lajtha, and P.H. Chan, eds. *Handbook of Neurochemistry and Molecular Neurobiology*. Boston, MA: Springer.

Khanra, S., Khess, C.R., and Srivastava, N., 2015. Chronic non-fatal Datura abuse in a patient of paranoid schizophrenia: a case report. *Addictive Behaviors*, 43, 39–41.

Koukouli, F., and Maskos, U., 2015. The multiple roles of the α7 nicotinic acetylcholine receptor in modulating glutamatergic systems in the normal and diseased nervous system. *Biochemical Pharmacology*, 97 (4), 378–387.

Lewerenz, J., and Maher, P., 2015. Chronic glutamate toxicity in neurode-generative diseases-what is the evidence? *Frontiers in Neuroscience*, 9, 469.

Li, S., *et al.*, 2012a. The α7nACh-NMDA receptor complex is involved in cue-induced reinstatement of nicotine seeking. *Journal of Experimental Medicine*, 209 (12), 2141–2147.

Li, Y., *et al.*, 2012b. Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. *Developmental Cell*, 23 (6), 1189–1202.

Li, Y.C., and Kavalali, E.T., 2017. Synaptic vesicle-recycling machinery com-ponents as potential therapeutic targets. *Pharmacological Reviews*, 69 (2), 141–160.

Lindefors, N., Barati, S., and O'Connor, W.T., 1997. Differential effects of single and repeated ketamine administration on dopamine, serotonin and GABA transmission in rat medial prefrontal cortex. *Brain Research*, 759 (2), 205–212.

Loscher, W., and Honack, D., 1992. The behavioural effects of MK-801 in rats: involvement of dopaminergic, serotonergic and noradrenergic systems. *European Journal of Pharmacology*, 215, 199–208.

Lozada, A.F., *et al.*, 2012. Glutamatergic synapse formation is promoted by α7-containing nicotinic acetylcholine receptors . *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32 (22), 7651–7661.

Luo, T., Wu, W.H., and Chen, B.S., 2011. NMDA receptor signaling: death or survival? *Frontiers in Biology*, 6 (6), 468–476.

Matute, C., *et al.*, 2002. Excitotoxicity in glial cells. *European Journal of Pharmacology*, 447 (2–3), 239–246.

Maura, G., *et al.*, 2000. Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: involvement of 5-HT(2C) and 5-HT(1A) receptors . *British Journal of Pharmacology*, 130 (8), 1853–1858.

McNamara, C.G., and Dupret, D., 2017. Two sources of dopamine for the hippocampus. *Trends in Neurosciences*, 40 (7), 383–384.

Mele, A., *et al.*, 1996. Dopamine-N-methyl-D-aspartate interactions in the modulation of locomotor activity and memory consolidation in mice. *European Journal of Pharmacology*, 308 (1), 1–12.

Milnerwood, A.J., *et al.*, 2010. Early increase in extrasynaptic NMDA recep-tor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron*, 65 (2), 178–190.

Moraes, C.L.K., Bertoglio, L.J., and Carobrez, A.P., 2008. Interplay between glutamate and serotonin within the dorsal periaqueductal gray modu-lates anxiety-related behavior of rats exposed to the elevated plus-maze. *Behavioural Brain Research*, 194 (2), 181–186.

Moses, A., 2010. Drug abuse and drug dependence treatment situation. *Activity Report*, GL0/J71. Available from: https://www.unodc.org. [Accessed 10 February 2019].

Oswald, M.J., *et al.*, 2015. Potentiation of NMDA receptor-mediated trans-mission in striatal cholinergic interneurons. *Frontiers in Cellular Neuroscience*, 9, 116.

Paintlia, M.K., *et al.*, 2013. S-nitrosoglutathione induces ciliary neuro-trophic factor expression in astrocytes, which has implications to pro-tect the central nervous system under pathological conditions. *The Journal of Biological Chemistry*, 288 (6), 3831–3843.

Paoletti, P., Bellone, C., and Zhou, Q., 2013. NMDA receptor subunit diver-sity: impact on receptor properties, synaptic plasticity and disease. *Nature Reviews. Neuroscience*, 14 (6), 383–400.

Paolicelli, R.C., *et al.*, 2011. Synaptic pruning by microglia is necessary for normal brain development. *Science*, 333 (6048), 1456–1458.

Papouin, T., *et al.*, 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell*, 150 (3), 633–646.

Pekny, M., *et al.*, 1999. Abnormal reaction to central nervous system injury in mice lacking glial fibrillary acidic protein and vimentin. *The Journal of Cell Biology*, 145 (3), 503–514.

Piedras-Renteria, E.S., *et al.*, 2004. Presynaptic homeostasis at CNS nerve terminals compensates for lack of a key Ca^{2+} entry pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 101 (10), 3609–3614.

Popichak, K.A., et al., 2018. Glial-neuronal signalling mechanisms underlying the neuroinflammatory effects of manganese. *Journal of Neuroinflammation*, 15, 324.

Prasad, C.S., and Gowda, N., 2005. Dietary level and plasma concentration of micronutrients in crossbred dairy cows fed finger millet and rice straw as a dry roughage source. *Indian Journal of Dairy Science*, 58 (2), 109–112.

Reimer, R.J., Fon, E.A., and Edwards, R.H., 1998. Vesicular neurotransmitter transport and the presynaptic regulation of quantal size. *Current Opinion in Neurobiology*, 8 (3), 405–412.

Reimer, R.J., and Voglmaier, S.M., 2014. Key molecules: vesicular neurotransmitter transporters. *Reference Module in Biomedical Research*. doi: 10.1016/B978-0-12-801238-3.04801-7

Reimer, R.J., 2009. Vesicular neurotransmitter transporters. *In*: Squire L, ed. *Encyclopaedia of neuroscience*. Elsevier BV.

Rossi, D., et al., 2004. Contribution of astrocyte glutamate release to excitotoxicity. *In*: C. Ferrarese and M.F. Beal, eds. *Excitotoxicity in neurological diseases*. Boston, MA: Springer.

Sattler, R., et al., 1999. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science*, 284 (5421), 1845–1848.

Schmitt, M.L., et al., 1995. Anxiogenic-like effect of glycine and d-serine microinjected into dorsal periaqueductal gray matter of rats. *Neuroscience Letters*, 189 (2), 93–96.

Seal, R.P., 2016. Do the distinct synaptic properties of VGLUTs shape pain? *Neurochemistry International*, 98, 82–88.

Shrestha, A., et al., 2019. SK channel modulates synaptic plasticity by tuning CamKIIα/β dynamics. *Frontiers in Synaptic Neuroscience*, 11, 18.

Singh, N.A., et al., 1990. Dopamine-mediated changes in central nervous system neurotensin systems: a role for NMDA receptors. *European Journal of Pharmacology*, 187 (3), 337–344.

Smith, D.J., et al., 1981. Properties of the optical isomers and metabolites of ketamine on the high affinity transport and catabolism of monoamines. *Neuropharmacology*, 20 (4), 391–396.

Sofroniew, M.V., and Vinters, H.V., 2010. Astrocytes: biology and pathology. *Acta Neuropathologica*, 119 (1), 7–35.

Subhramanyam, C.S., et al., 2019. Microglia-mediated neuroinflammation in neurodegenerative diseases. *Seminars in Cell & Developmental Biology*, 94, 112–120.

Szydlowska, K., and Tymianski, M., 2010. Calcium, ischemia and excitotoxicity. *Cell Calcium*, 47 (2), 122–129.

Tabrizi, S.J., TRACK-HD Investigators., et al., 2013. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. *The Lancet Neurology*, 12 (7), 637–649.

Takamori, S., 2016. Presynaptic molecular determinants of quantal size. *Frontiers in Synaptic Neuroscience*, 8, 2.

Takeuchi, H., 2013. Roles of glial cells in neuroinflammation and neurodegeneration. *Clinical and Experimental Neuroimmunology*, 4 (1), 2–16.

Talantova, M., et al., 2013. Aß induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proceedings of the National Academy of Sciences of the United States of America.*, 110 (27), 2518–2527.

Traynelis, S.F., et al., 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews*, 62 (3), 405–496.

Trkanjec, Z., and Demarin, V., 2001. presynaptic vesicles, exocytosis, membrane fusion and basic physical forces. *Medical Hypotheses*, 56 (4), 540–546.

United Nations Office on Drugs and Crime (UNODC). 2017. *Global synthetic drugs assessment: amphetamine-type stimulants and new psychoactive substances*. UNODC Research. Available from: https://www.unodc.org. [Accessed 10 February 2019].

United Nations Office on Drugs and Crime (UNODC). 2018. *World drug report: executive summary conclusions and policy implications*. UNODC Research. Available from: https://www.unodc.org. [Accessed 10 February 2019].

Vishnoi, S., Raisuddin, S., and Parvez, S., 2015. Modulatory effects of an NMDAR partial agonist in MK-801-induced memory impairment. *Neuroscience*, 311, 22–33.

Wang, X., Pinter, M.J., and Rich, M.M., 2016. Reversible recruitment of a homeostatic reserve pool of synaptic vesicles underlies rapid homeostatic plasticity of quantal content. *Journal of Neuroscience*, 36 (3), 828–836.

Weitemier, A.Z., and McHugh, T.J., 2017. Noradrenergic modulation of evoked dopamine release and pH shift in the mouse dorsal hippocampus and ventral striatum. *Brain Research*, 1657, 74–86.

Wojcik, S.M., et al., 2004. An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proceedings of the National Academy of Sciences of the United States of America*, 101 (18), 7158–7163.

Xia, P., et al., 2010. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 30 (33), 11246–11250.

Xue, J.G., et al., 2011. NMDA receptor activation enhances inhibitory GABAergic transmission onto hippocampal pyramidal neurons via presynaptic and postsynaptic mechanisms. *Journal of Neurophysiology*, 105 (6), 2897–2906.

Yao, L., et al., 2018. Higher ambient synaptic glutamate at inhibitory versus excitatory neurons differentially impacts NMDA receptor activity. *Nature Communications*, 9, 4000.

Yuen, E.Y., et al., 2005. Serotonin 5-HT$_{1A}$ receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 25 (23), 5488–5501.

Zamanian, J.L., et al., 2012. Genomic analysis of reactive astrogliosis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32 (18), 6391–6410.

Zhong, C., et al., 2008. Presynaptic type III neuregulin 1 is required for sustained enhancement of hippocampal transmission by nicotine and for axonal targeting of alpha7 nicotinic acetylcholine receptors. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 28 (37), 9111–9116.

Zhong, C., Talmage, D.A., and Role, L.W., 2013. Nicotine elicits prolonged calcium signaling along ventral hippocampal axons. *PLOS One*, 8 (12), e82719.

Zhou, X., et al., 2013. Involvement of the GluN2A and GluN2B subunits in synaptic and extrasynaptic N-methyl-D-aspartate receptor function and neuronal excitotoxicity. *The Journal of Biological Chemistry*, 288 (33), 24151–24159.

Zweifel, L.S., et al., 2008. Role of NMDA receptors in dopamine neurons for plasticity and addictive behaviors. *Neuron*, 59 (3), 486–496.