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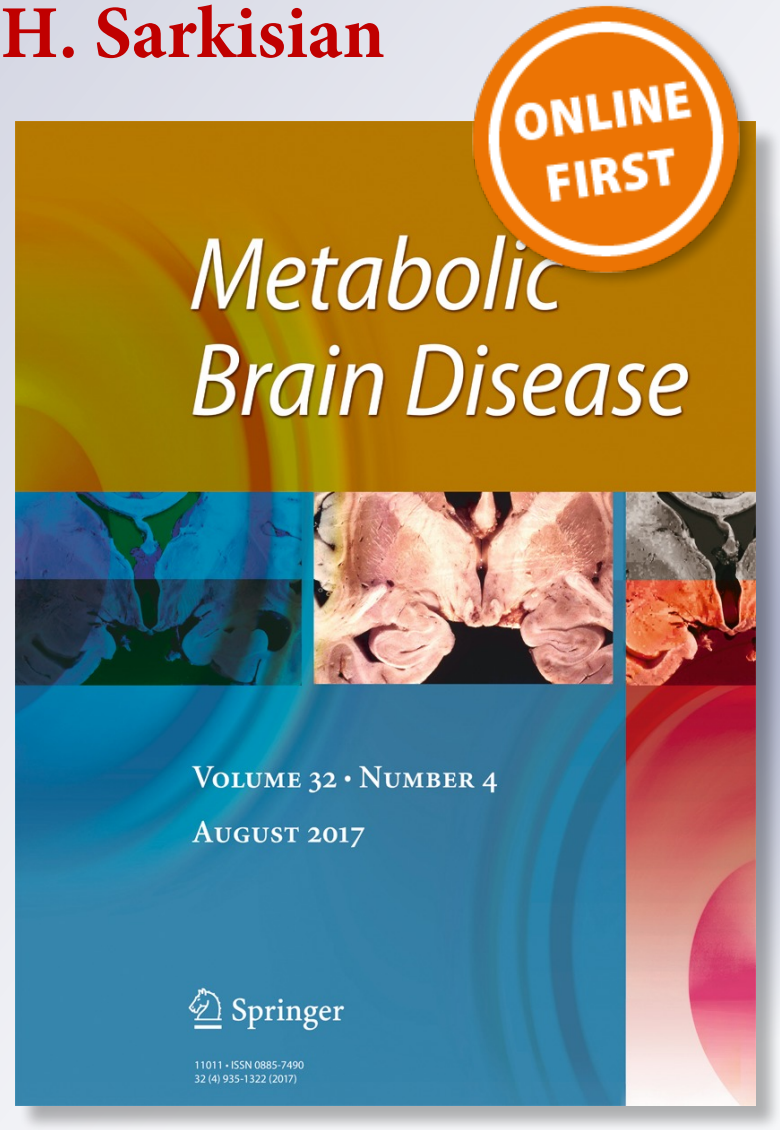
Metabolic Brain Disease

ISSN 0885-7490

Metab Brain Dis
DOI 10.1007/s11011-017-0060-y



Springer

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ORIGINAL ARTICLE

Protective effects of curcumin against rotenone-induced rat model of Parkinson's disease: in vivo electrophysiological and behavioral study

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Received: 31 March 2017 / Accepted: 22 June 2017

Abstract Curcumin is a naturally occurring phenolic yellow chemical isolated from the rhizomes of the plant *Curcuma longa* (turmeric), and is a major component of the spice turmeric. Curcumin has protective effects against rotenone-induced neural damage in Parkinson's disease (PD). The present study aims at providing new evidence for the validity of the rotenone rat model of PD by examining whether neuronal activity in the hippocampus is altered. Male albino rats were treated with rotenone injections (2.5 mg/ml intraperitoneally) for 21 days. We examined the effects of curcumin (200 mg/kg) on behavior and electrophysiology in a rat model of PD induced by rotenone. Motor activity was assessed by cylinder test. The electrical activity of neurons was measured in hippocampus. Rotenone causes significant reduction of neuronal activity. The results show that curcumin can improve the motor impairments and electrophysiological parameters and may be beneficial in the treatment of PD.

Keywords Curcumin · Parkinson's disease · rotenone · hippocampus · cylinder test

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Background

Parkinson's disease (PD) is a debilitating neurodegenerative disorder that results from the loss of or damage to dopaminergic cells in the substantia nigra. The major pathological hallmark of Parkinson's disease is the presence of insoluble, fibrous aggregates, composed of α-Syn in intraneuronal inclusions of Lewy bodies (LBs) and Lewy neuritis (LNs) (Cookson 2005). The agricultural chemicals, such as rotenone, evoke degenerative changes in human central nervous system. Rotenone's effect has been attributed to inhibition of mitochondrial complex I (Betarbet et al. 2000), the release of NADPH oxidase-derived superoxide from activated microglia (Gao et al. 2002) and possibly alteration of glutamate transmission (Moussa et al. 2008). Rotenone also causes inflammation, which appears to contribute to the formation of PD (Chinta et al. 2013). The animal models that express human α-Syn indicate the direct involvement of α-Syn aggregation in PD pathogenesis (Lansbury and Brice 2002). This pathology can be accelerated by exposure to environmental toxins, excitotoxicity, oxidative stress, or mutations in the α-synuclein gene, which encodes a protein found in Lewy bodies in idiopathic PD lesions. Due to the loss of dopaminergic neurons in the substantia nigra, striatal cholinergic neurons are disinhibited, leading to an imbalance of dopaminergic/cholinergic neurotransmission (Meissner et al. 2011). Clinical and experimental findings support the view that the hippocampus, a temporal lobe structure involved in physiological learning and memory, is also implicated in the cognitive dysfunction seen in some patients with Parkinson's disease. Patients with Parkinson's disease dementia were differentiated by a significant reduction in hippocampal cholinergic activity, by a significant loss of non-pigmented lateral A10 dopaminergic neurons and Ch4 cholinergic neurons (30 and 55% cell loss, respectively, compared with neuronal

Published online: 10 July 2017

preservation in control subjects), and by an increase in the severity of α-synuclein pathology in the basal forebrain and hippocampus (Hall et al. 2014). Previous studies have reported that micromolar concentrations of rotenone induce neuronal degeneration in primary neuronal and hippocampal slice cultures (Xu et al. 2003) and disrupt basal synaptic transmission in acute hippocampal slices (Costa et al. 2008). In addition, studies have demonstrated that rotenone PD model presents significant reduction of dopamine levels in striatum and hippocampal formation (Li et al. 2004; Ulusoy et al. 2011). Furthermore, aged Lewis rats exposed to 2 mg/kg/day rotenone demonstrated increased aggregation of the Aβ peptide, hyperphosphorylated TAU, and alpha-synuclein in the substantia nigra (Almeida et al. 2016). Curcumin ((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), generically known as turmeric, is a well-known polyphenolic compound that has preventive properties and therapeutic potential for treating neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (Giri et al. 2004). Curcumin crosses the blood-brain barrier and curcumin preparations with enhanced bioavailability (delivered orally) can achieve therapeutic concentrations in the brain (Filippov et al. 2014; Akram et al. 2010). Pre-treatment with curcumin protects brain mitochondria against various oxidative stress (Mythri et al. 2007). Curcumin was found to be pharmacologically safe in human clinical trials with doses up to 10 g/day (Chainani-Wu 2003). In PD, curcumin has been shown to inhibit the α-Syn aggregation in vitro (Ahmad and Lapidus 2012) and attenuate the α-Syn oligomer toxicity in cells (Liu et al. 2011), which are considered to be a more toxic specie compared to mature fibrils. It has been suggested that α-Syn oligomers are much more toxic species compared to mature fibrils (Winner et al. 2011) and curcumin has been reported to attenuate the toxicity of the oligomers (Wang et al. 2010). In addition to the reported benefits of curcumin in traditional Chinese and Indian medicine, the beneficial effects of curcumin have been demonstrated in a wide variety of cells, including neurons (Ye and Zhang 2012), astrocytes (Lavoie et al. 2009) and microglia (Karlstetter et al. 2011). Effects have also been tested in primary cell cultures from different regions of the central nervous system, including cortical (Wang et al. 2012), mesencephalic (Ortiz-Ortiz et al. 2010), hippocampal (Ye and Zhang 2012). Curcumin also confers protection against MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP^+ (1-methyl-4-phenylpyridinium) induced apoptosis in PC12 cells through the Bcl-2-mitochondria-ROS-iNOS pathway (Chen et al. 2006), and by the inhibition of the JNK pathway, which contributes to preventing dopaminergic neuronal death (Yu et al. 2010). Curcumin was able to decrease the oxidative stress, inhibit the activity of monoamine oxidase B, suppress apoptosis, inhibit protein nitration, increase levels of glutathione and decrease the activity of mitochondrial complex I induced by MPTP treatment (Monroy et al. 2013). Administration of natural phenolic anti-oxidants including curcumin in a 6-OHDA rat model significantly attenuated the loss of DA neurons in SN (Zbarsky et al. 2005). In addition, the curcumin treatment can protect hippocampal neurons against excitotoxic and traumatic injury and the enhancement of hippocampal neurogenesis by curcumin was reported (Kim et al. 2008). In the present study we utilize a rotenone induced PD rat model to investigate the effects of curcumin and rotenone on hippocampal neurons' synaptic activity and to test whether curcumin protects or reverses synaptic dysfunction in the hippocampus.

Materials and methods

Chemicals

Curcumin and rotenone were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Other chemicals were provided by local commercial sources.

Experimental animals

All of the experimental protocols were approved by the Committee of Ethics of the Yerevan State Medical University (YSMU) (Yerevan, Armenia). All animal procedures were carried out in accordance with the European Communities Council Directive 2010/63/UE and the local Animal Care Committee. Adult male Wistar albino rats weighing 200 ± 30 g were purchased from the experimental center of Orbeli Institute of Physiology NAS RA. The experiments were performed at the same time period of the day (09:00–12:00 h) and during the light period of the light–dark cycle. The animals were maintained at 25 ± 2 °C, 12 h light – dark cycle and lights on 07:00–19:00 h. Food and water ad libitum was provided to the animals. All experiments were carried out in separate and isolated laboratories, which have the same environmental conditions as the colony room.

Experiment design

The animals were divided randomly into 4 groups (n = 6): rotenone (n = 6), sunflower oil (n = 6), dimethyl sulfoxide (DMSO) (n = 6), or curcumin (n = 6). A total of 6 male albino rats received intraperitoneal (IP) injections with 2.5 mg/kg rotenone in Sunflower oil once a day for 3 consecutive weeks. An equal number of animals were injected with Sunflower oil (1 ml/kg) alone (sham-treated) for up to 3 weeks. DMSO (1 ml/kg) and curcumin (200 mg/kg) was given to the male rats per day for up to 3 weeks. DMSO was used as a vehicle for curcumin. Vehicle of rotenone (Sunflower oil) and vehicle of curcumin (DMSO) were injected IP, respectively. Rotenone

was dissolved in Sunflower oil one day before beginning treatment.

Group I - Rotenone, 2.5 mg/kg (IP) for 21 days
Group II- Sunflower oil, 1 ml/kg (IP) for 21 days
Group III - Rotenone (21 days) + DMSO, 1 ml/kg (IP) for 21 days
Group IV - Rotenone (21 days) + Curcumin, 200 mg/kg (IP) for 21 days
Group V-norm group

In groups 3 and 4 curcumin (200 mg/kg) and DMSO were administered 3 weeks after PD induction.

Animals were studied after 6 weeks and *in vivo* electrophysiological analysis was done. The behavioral (cylinder test) parameters were studied on daily basis.

In vivo electrophysiology and data analysis

In acute experiment the animals were anesthetized (Urethan 1.2 g/kg), immobilized with 1% ditiline (25 mg/kg i/p), fixed in a stereotaxic apparatus and were transferred to artificial respiration. The sample of isolated rat brain was obtained by transection of spinal cord (T2 – T3). The rat skull was orientated according to Paxinos and Watson stereotaxic atlas. The stimulating electrode was repeatedly inserted into the ipsilateral entorhinal cortex (EC) according to stereotaxic coordinates (Paxinos and Watson 2005) (AP – 9, L ± 3.5, DV +4.0 mm) and a glass recording microelectrode (1–2 μm tip diameter) filled with 2 M NaCl was repeatedly inserted into the hippocampal fields at coordinates (AP − 3.2–3.5; L ± 1.5–3.5; DV +2.8–4.0 mm) for recording spike activity flow of single neurons. High frequency stimulation (HFS) (100 Hz during 1 s) was performed by means of rectangle pulses of 0.05 ms duration and 0.08–0.16 mA amplitude. Electrophysiological recordings and mathematical analysis of spike activity were accomplished using an automated analysis program (V.S. Kamenetski) providing selection of spikes by amplitude discrimination, which pinpoints spikes and excludes artifacts during HFS, allowing not only posttetanic, but also tetanic activity evaluation (Yenkoyan et al. 2011). The timing, frequency and cumulative histograms, as well as a diagram of mean frequency for single neurons and populations of neurons with uniform responses were constructed on the basis of analysis of peristimulus spiking. For statistical evaluation we used t-criteria of Student's t -test, the reliability of differences of interspike intervals before, after and during HFS. To increase reliability of statistical evaluations, we also used the non-parametric method of verification by application of Wilcoxon two-sample test taking into account the asymptotic normality of this criterion and allowing comparison of the calculated values with the table values of the standard normal distribution (at the significance levels 0.05, 0.01, and 0.001). Tetanic potentiation (TP) or tetanic depression (TD) and following posttetanic potentiation (PTP) and posttetanic depression (PTD) were recorded to HFS of ipsilateral EC.

Rotenone rat model and behavior

Cylinder test The effects of curcumin on the rat behavior were studied by cylinder test. Studies were begun 24 h after the injections. The animals were placed in a clear Plexiglas cylinder (20 in cm diameter and 30 cm in height) in order to evaluate motor asymmetry. A mirror was placed to the side of the cylinder at an angle to enable the recording of forelimb movements even when the animal was turned away from the camera. Scoring was done by an experimenter blinded to the condition of the animal using a video cassette recorder with slow-motion and clear stop-frame capabilities. A video camera above the field was connected to a video recorder and a monitor, recording the movement of the rat. During rearing, behavior, the forelimbs will contact the wall of the cylinder. The apparatus was cleaned with 5% ethanol solution before behavioral testing to eliminate possible bias due to odors left by previous rat. Rats were tested only once to prevent habituation to the apparatus. To be classified as a rear, the animal had to raise forelimbs above shoulder level and make contact with the cylinder wall with either one or both forelimbs. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear was scored. Forelimb contacts while rearing are scored with a total of 5 min for each animal. Data of all the results were presented as mean ± SEM. Significant differences between groups were calculated using Student's t-test and $p < 0.05$ was considered statistically significant.

Body weight

Body weight was recorded at the beginning and on alternate days. Rats were weighed weekly from day 1 to day 42 during the procedure.

Results

In vivo extracellular recordings

An electrophysiological analysis in Group I (305 neurons) showed that the tetanic potentiation during HFS (100 Hz) in the hippocampus with TP expressed 2.21 times (M_{TT} = 34.70 / M_{BE} = 15.68 spike/s), TP PTD responses 1.68 times (29.38: 17.52 spike/s), PTP responses 1.2 times (1.31%), TP PTP 1.74 times (Fig. 1). Inhibitory (tetanic depression) during HFS (100 Hz) of EC in neurons with TD PTD and TD PTP responses expressed respectively by 5.02-fold (30.64: 6.10 spike/s) and 3.96-fold (26.53: 6.70 spike/s), TD 5.55 times

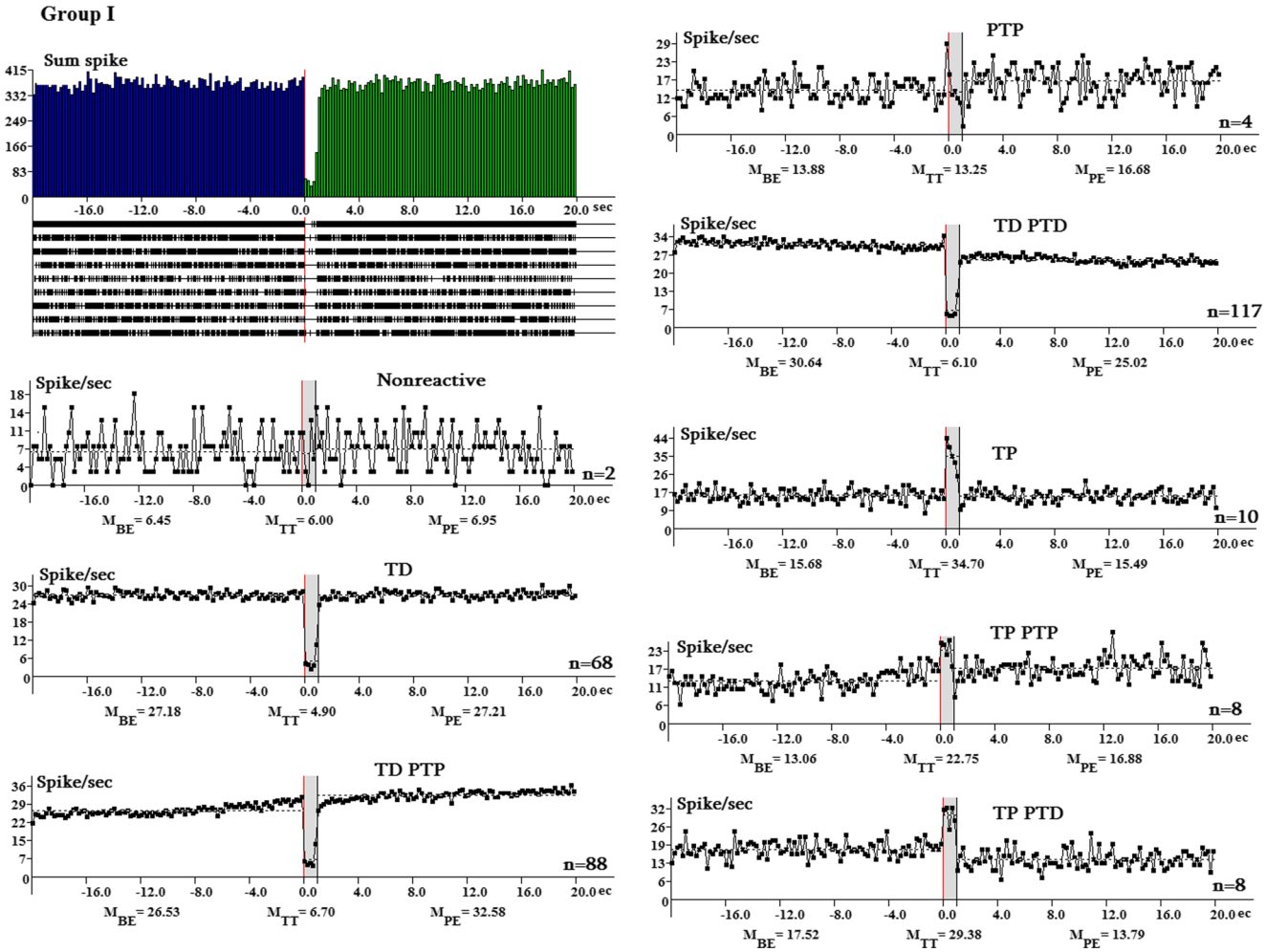

Fig. 1 Experimental Group I (Rotenone), mean frequency of spikes diagram for hippocampal neurons exhibiting TP PTP, TP PTD, TD PTD, TD PTP, TD, TP, PTP responses, and non reactive neurons with indication of numeric values of spiking mean frequency (spikes / sec) in real time for 20 s before HFS (M BE), 20 s post HFS (M PE) and at HFS (M HFS). n – The number of neurons (frequency spike activity which averaged in the diagram) exhibiting the given type of responses

(Fig 1), nonreactive neurons (0.66%). The share of hippocampal neurons with TD PTD (38.36%) and TD PTP (28.85%) responses is predominant. In general, during HFS (M_{TT}) in Group I the nonreactive neurons show the lowest share (Fig 5).

In norm group (Group V) tetanic potentiation during HFS in hippocampal neurons with TP PTP expressed 3.94 times (32: 8.11 spike/s), tetanic depression during HFS in neurons with TD PTD responses – 8.92 times (6.53: 0.74 spike/s), TD PTP responses – 8.38 times (5.78: 0.69) (Fig 5). In norm group the share of neurons exhibiting TD PTD responses were dominant (42.04%).

In Group II (338 neurons) tetanic potentiation during HFS in hippocampal neurons with TP expressed 1.38 times (28.18: 20.8 spike/s), tetanic depression during HFS in neurons with TD PTD responses – 3.64 times (27.08: 7.44 spike/s), TD PTP responses – 4.22 times (21.94: 5.19), TD-4.66 times (22.65: 4.86). In general, during HFS (M_{TT}) in Group II the TP responses show the lowest share (3.55%) (Fig 2).

In Group III (272 neurons) tetanic potentiation during HFS in hippocampal neurons with TP PTP expressed 1.52 times (19.75: 12.98 spike/s), tetanic depression during HFS in neurons with TD PTD responses – 3.9 times (33.29: 8.52 spike/s), TD PTP responses – 3.72 times (34.66: 9.32), TD-3.59 times (39.67: 11.04). In general, during HFS (M HFS) in Group III the TD PTP responses show the highest share (60.3%) (Fig 3).

In Group IV (Fig 4) tetanic potentiation during HFS in hippocampal neurons with TP expressed 1.59 times (M_{TT} = 19.75 / M_{BE} = 12.41spike/s), tetanic depression during HFS in neurons with TD PTD responses – 4.49 times (M_{BE} = 35.23 / M_{TT} = 7.84 spike/s), TD PTP responses – 3.28 times (M_{BE} = 37.55 / M_{TT} = 11.45), TP PTD responses – 2.89 times (M_{TT} = 115.14 / M_{BE} = 39.78), TD-4.08 times (M_{BE} = 30.48 / M_{TT} = 7.48). In general, during HFS (M HFS) in Group IV the TP and TP PTD responses show the lowest share (2.4% and 4.2%). TD PTD responses show the highest share (47.3%, Fig 5).

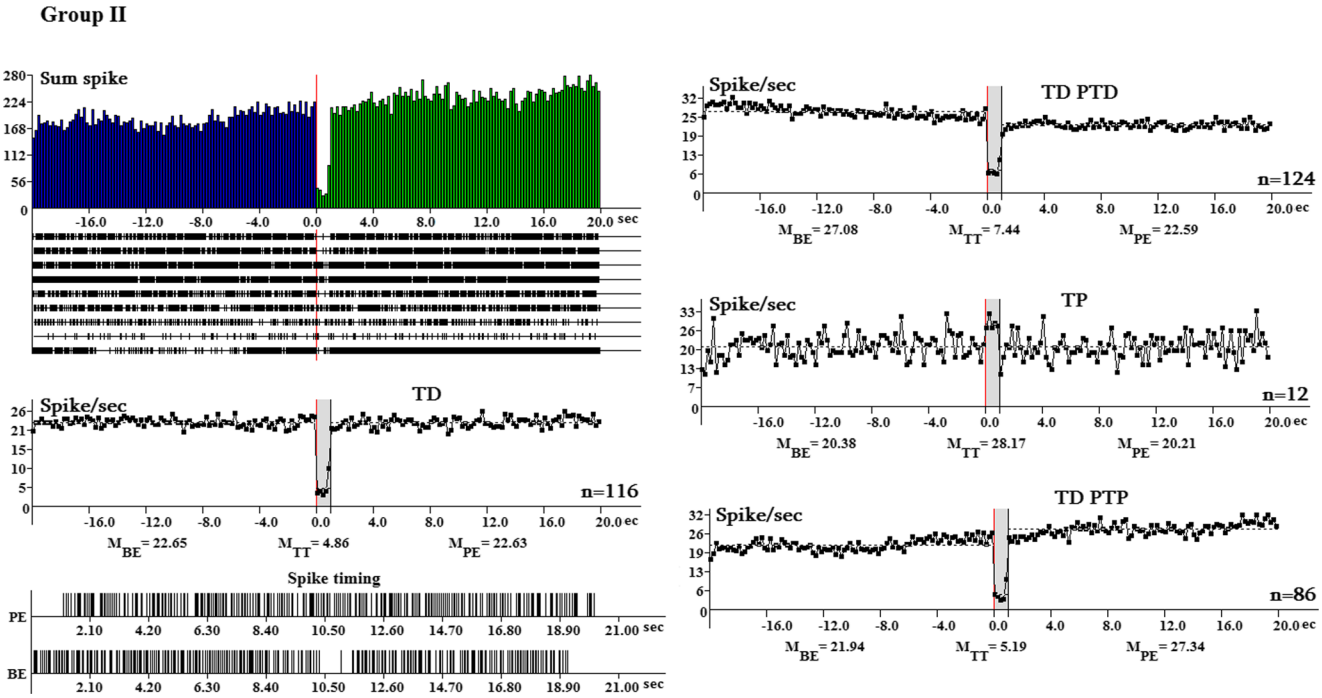

Fig. 2 Experimental Group II (Sunflower oil), mean frequency of spikes diagram for hippocampal neurons (rotenone group) exhibiting TD, TD PTD, TD PTP, TP responses with indication of numeric values of spiking mean frequency (spikes / sec) in real time for 20 s before HFS (M BE), 20 s post HFS (M PE) and at HFS (M HFS). n – the number of neurons (frequency spike activity which averaged in the diagram) exhibiting the given type of responses

The tetanic potentiation with TP PTP responses were observed in Group I and Group III (Fig. 1, Fig 3). Tetanic depression in neurons with TD PTD responses was also more expressed in Group I (5.02 times, Fig. 1) compared with those in Group IV (4.49 fold (Fig 4).

The equity ratio of these neurons was 38.36% and 47.3% (Fig. 1, Fig 4). In Group I a more expressed tetanic

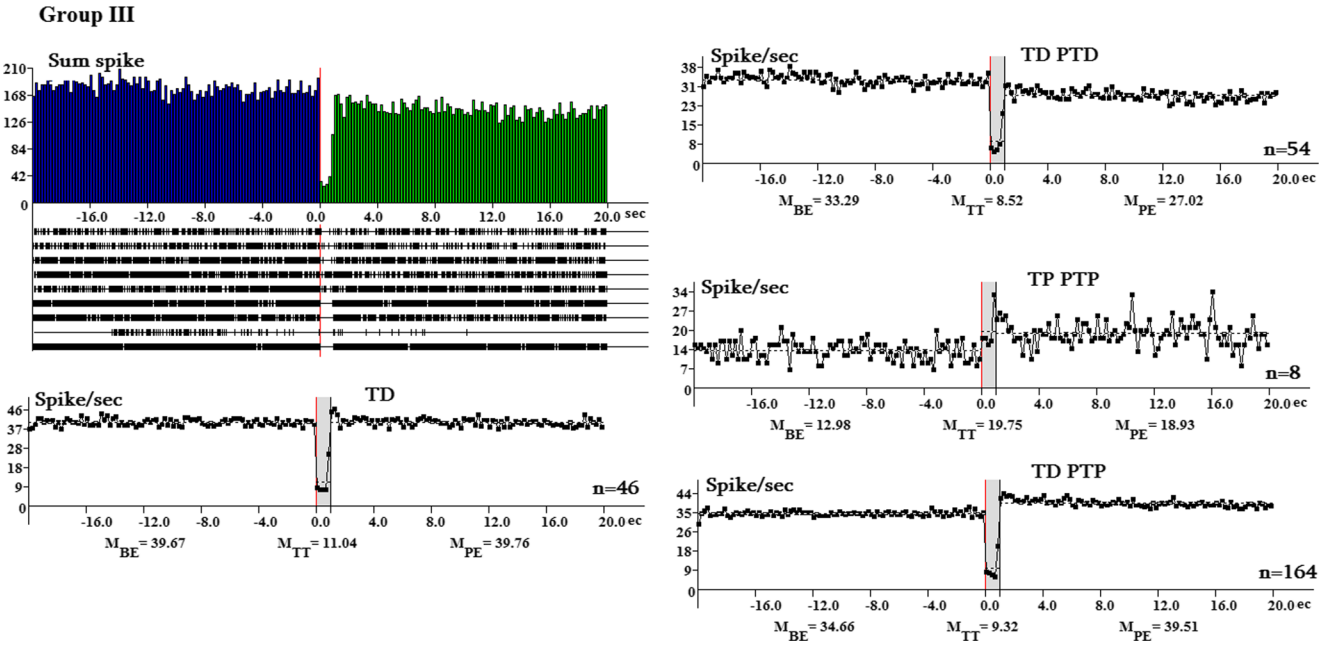

Fig. 3 Experimental Group III (Rotenone + DMSO), mean frequency of spikes diagram for hippocampal neurons exhibiting TP PTP, TD PTD, TD PTP and TD responses with indication of numeric values of spiking mean frequency (spikes / sec) in real time for 20 s before HFS (M BE), 20 s post HFS (M PE) and at HFS (M HFS). n – the number of neurons (frequency spike activity which averaged in the diagram) exhibiting the given type of responses

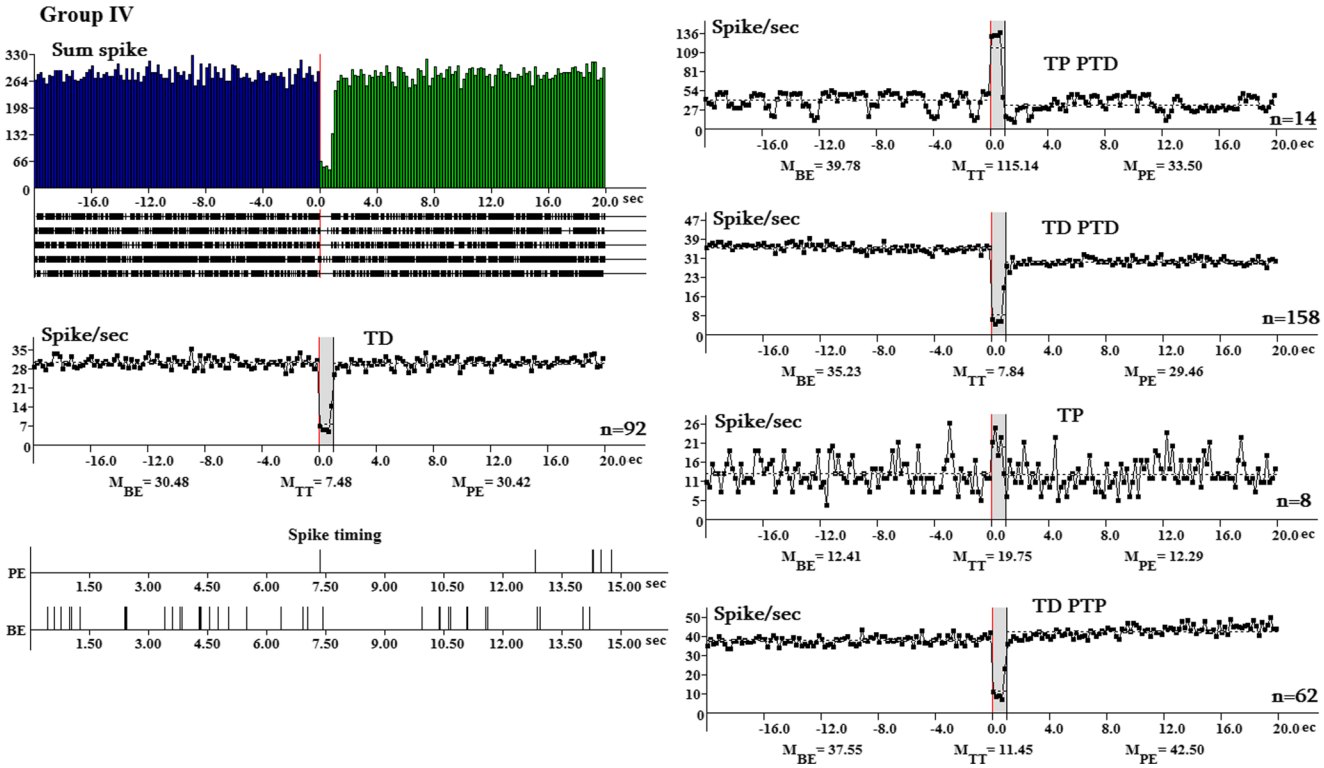

Fig. 4 Electrophysiological evaluation of curcumin effect on hippocampal neuronal activity. Experimental Group IV (Rotenone + Curcumin), mean frequency of spikes diagram for hippocampal neurons exhibiting TP, TD PTD, TD PTP, TD and TP PTD responses with indication of numeric values of spiking mean frequency (spikes / sec) in real time for 20 s before HFS (M BE), 20 s post HFS (M PE) and at HFS (M HFS). n – the number of neurons (frequency spike activity which averaged in the diagram) exhibiting the given type of responses

potentiation with TP responses (2.21 times) compared with Group II (1.38 times) and Group IV (1.59 times) was detected. Only in Group I neurons with PTP responses were detected (1.2 times (M_{PE} = 16.68 / M_{BE} = 13.06 spike/s) (Fig 1). TP PTD responses in Group IV were expressed significantly more – 2.89 times (M_{TT} = 115.14 / M_{BE} = 39.78 spike/s) (Fig 4) compared with Group I (1.68 times). TD was also significantly decreased in Group III (3.59 times, Fig 3) and

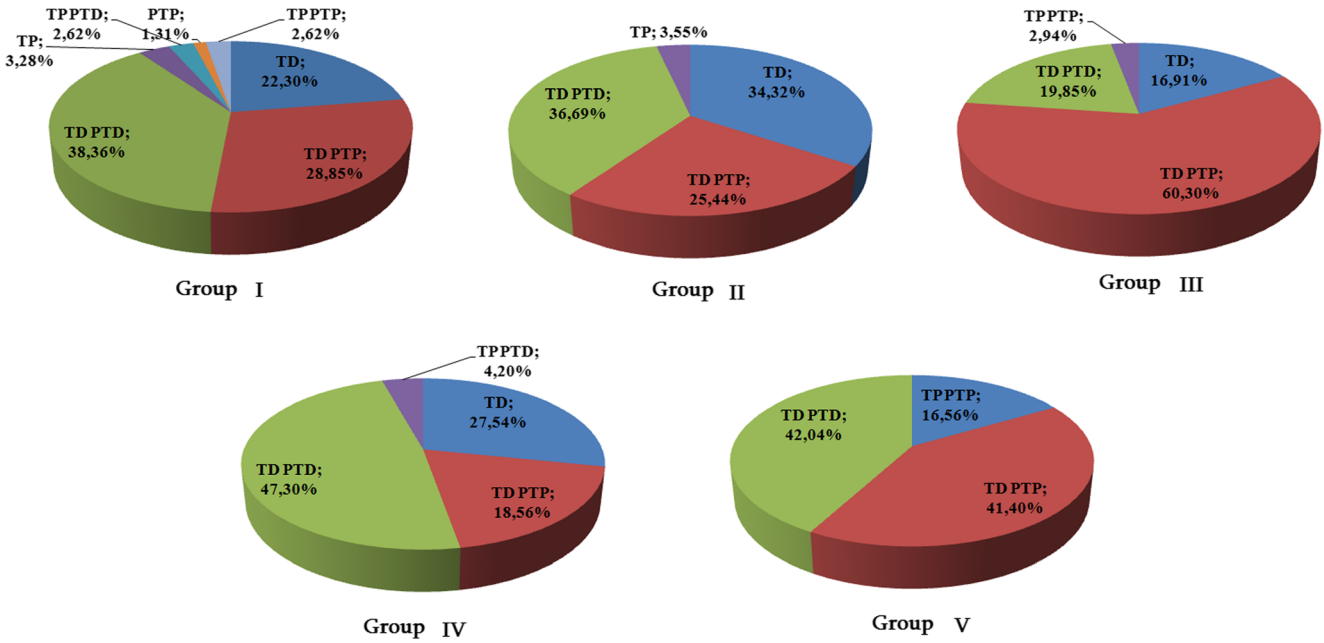

Fig. 5 A percentage distribution of types of responses in hippocampal neurons

Group IV (4.07 times) compared with Group I (5.55 times). In Group IV 334 hippocampal neurons were recorded, of which 92 units (27.54%) were TD, 62 units (18.56%) TD-PTP, 14 units TP-PTD (4.2%), 158 units (47.8%) TD-PTD and 8 units (2.4%) TP (Fig 5).

Cylinder test

24 h after the last day of treatment, the rats were evaluated with behavioral test. The cylinder test was performed according to the method described previously (Darbinyan et al. 2017). The advantages of this test have been reviewed by Schallert et al. (Schallert and Tillerson 1999). Four groups of rats were tested. In the current study both forelimbs were averaged together because rotenone produces bilateral symptoms. Because rotenone affects motor behavior bilaterally, activity was measured by counting the number of rears made by each animal in a 5-min period without recording specific limb use. In the present study, Rotenone dose (2.5 mg/kg/day) for up to 21 days produced behavioral features (Fig. 6). Betarbet et al. (2) reported that much lower dose application of rotenone (2–3 mg/kg/day) produced selective nigrostriatal degeneration. None of the vehicle-treated (Group II, Sunflower oil, 1 ml/kg) exhibited Parkinson's-like symptoms. At each timepoint, 3 trials for each forelimb were recorded and the average was issued. Any animal that showed severe signs of illness during the experiment was prematurely euthanized. All animals survived the length of the experiment.

Rearing activity for animals treated with vehicle (Sunflower oil, 1 ml/kg) and 2.5 mg/kg/day of rotenone, DMSO and curcumin tested at baseline, at 1st, 2nd, 3rd, 4th, 5th, 6th weeks. Values are expressed as mean ± standard error of the mean. When quantifying the forelimb deficits using a forelimb score (a score of 0 indicates no forelimb function and 20 indicates no deficit) rats in the Group I receiving Rotenone (3–6 weeks) only performed somewhat worse than Group II rats (Fig 6); however this difference was found to be statistically significant (The p-value is 0.0001, $p < 0.05$). Group I and Group III were compared to the rats receiving the curcumin (after 3 weeks, Group IV) and significant increase in the performance was found (The p-value is 0.0003, $p < 0.05$) (Fig 6). Postural instability may underlie many of the behavioral deficits observed in common animal models of PD. In the Parkinson rat, there is a decreased reliance on the impaired forelimb for movements involving a response to weight shift. The animals preferentially initiate movement with the non-impaired forelimb, particularly for lateral movements during vertical exploration of surfaces (Schallert et al. 2000). Intraperitoneal Rotenone (2.5 mg/kg) produced consistent rearing deficit throughout the experiment. Although PD is characterized by movement disorders in later stages of the disease, it is now appreciated that there also may be cognitive impairment, including dementia and behavioral changes (Kehagia et al. 2010). Behavioral assessments were carried out before the start of the treatment, then regularly at an interval of 42 days post treatment and final behavioral quantification was done after 24 h of last dose. Animals of all groups were subjected to cylinder test on day 42 of drug treatment. The present results showed that rotenone-treated rats (Group I, Group III) had a low rearing frequency in cylinder test as compared to the Group II. Administration of Curcumin succeeded in normalizing the rearing frequency (Fig 6). The scores of the cylinder test in the Group IV were ameliorated over time at 4, 5, and 6 wk. after rotenone injections, compared with those in the Group I. Curcumin (200 mg/kg) treated groups showed significant increase in number of rearing when compared with disease control group as shown in Fig 6.

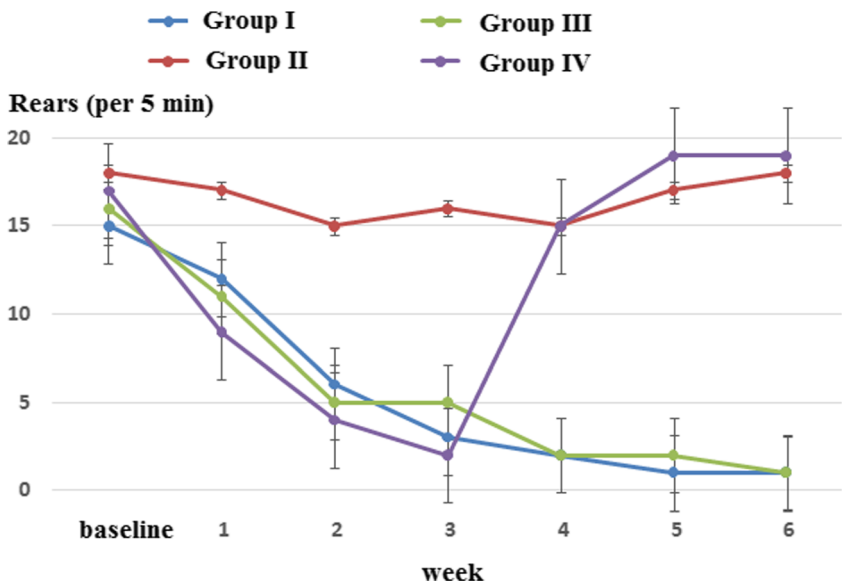

Fig. 6 Cylinder test. Values are given as Mean ± SEM for $n = 6$ in each group, comparison were made between a) Group I and Group II b) Group I with Group IV, c) Group III with Group IV, $P < 0.05$

Effects of curcumin on body weight

All animals were weighed on the days of injection and monitored weekly. Before the experiment, the average body weight was 201.67 ± 1.2 g (Group I), 209.33 ± 1.54 g (Group II), 216.33 ± 1.23 g (Group III) and 194.67 ± 3.32 g (Group IV). At the end of 3 weeks, the body weight of Group I, Group III and Group IV decreased (191.50 ± 1.98 g, 208.50 ± 1.18 g, 182.50 ± 1.61 g, respectively). After 6 weeks the Group IV showed an increase in body weight (203.50 ± 0.62 g). The body weight of rats in other groups decreased by 185.33 ± 0.21 g (Group I), 202.50 ± 0.43 g (Group III) compared to 211.67 ± 0.88 g (Group II) and (Group IV) rats (Fig 7). Gastrointestinal dysfunction is most frequently occurred symptom of PD with other symptoms, such as early satiety and weight loss, constipation during rotenone toxicity (Patel et al. 2005). The reduced body weight of animals after rotenone exposure could be related to delay in gastric emptying (Donzanti and Yamamoto 1988). Treatment with curcumin attenuated the reduction in body weight.

Discussion

Several animal models have been used to mimetic and elucidate the pathogenesis of PD, using rotenone, MPTP and 6-hydroxydopamine (Blesa et al. 2010). Synaptic dystrophy characterizes the early stages of PD neuropathology (Janezic et al. 2013). In addition, PD mice present an impaired synaptic plasticity in the hippocampus, which might correlate with cognitive deficits (Sweet et al. 2015). We show here that treatment of hippocampal neurons with the pesticide rotenone inhibits their neuronal activity and causes impairment of the neuronal integrity. To further characterize the responses of hippocampal neurons to rotenone, we performed *in vivo* recordings from these cells. Different types of post-stimulus effects such as excitatory (TP, PTP, TP PTP) and inhibitory responses (TD, PTD, TD PTD) were recorded in hippocampal neurons upon high frequency stimulation of EC (100 Hz during 1 s). In this study, we showed that neurons showed significant changes in their responses in CA1 and CA3 regions of hippocampus after rotenone treatment. We found that 2.5 mg/ml rotenone (following 6 weeks) significantly increased neuronal excitability (TP-2.1 times) (Fig 1) compared with Rotenone 3 weeks (1.72 times) and Group II (1.38 times). TP PTP responses remained stable (1.74 times). PTP responses were reduced at 6 weeks in the Group I (1.2 times) compared to the 3 weeks rotenone-treated group (1.39 times) (35). Only in Group I we found nonreactive neurons (0.64%). We suggest that rotenone causes electrophysiological alterations in hippocampus-EC neuronal pathways probably reflecting neuronal death. It was found in Group I (Fig 1) that the expression of TD PTD (5.02 times) and TD PTP (3.96 times) responses is a lower compared with those in Group V (TD PTD 8.92 times, TD PTP 8.38 times). It was shown that rotenone-induced microtubule-depolymerizing activity causes toxicity because it disrupts the microtubule-based transport of neurotransmitters vesicles (Eisenhofer et al. 2004). This result in vesicle accumulation in the soma, which leads to increased oxidative stress due to oxidation of neurotransmitters leaked from the vesicles (Ren et al. 2005). Rotenone-treated neurons with disorganized centrosomes exhibited neurite retraction and microtubules destabilization, and astrocytes showed disturbances of mitotic spindles (Diaz-Corrales et al. 2005). Rotenone may exert its inhibitory effect on axon formation by stabilizing growth cone actin filaments (Arimura and Kaibuchi 2007). Plasticity is a crucial feature in the brain, which accommodates neuronal structure and function to patterns of electrical activity. Activity of L-type calcium channels is linked to neuronal survival and death via regulating calcium

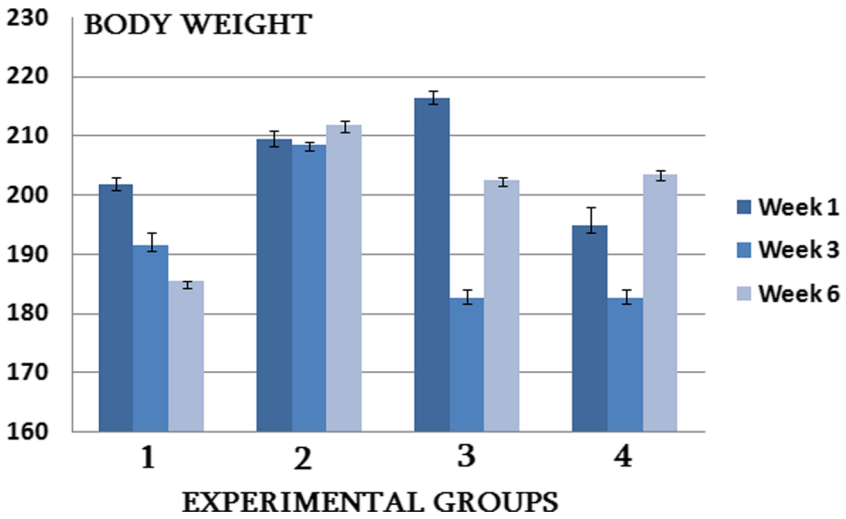

Fig. 7 Body weight of the experimental rats (Group I, Group II, Group III and Group IV). Values are expressed as Mean ± SEM (n = 6 animals per group), $P < 0.05$

and its signaling events to the nucleus. Glutamate elicits synaptic responses via metabotropic mGLU and ionotropic NMDA receptors leading to increased cellular excitation and induce the release of Ca^{+2} from intracellular stores. On the other hand, activation of ionotropic receptors increases cellular permeability to Ca^{+2} (Weil and Norman 2008). Glutamate is the most abundant excitatory amino acid transmitter in the CNS (Roberts et al. 1981). Glutamate-mediated excitotoxicity has been hypothesized to play a major role in various neurodegenerative diseases, such as Parkinson's disease (Gubellini et al. 2006). The inhibition of mitochondrial complex I and oxidative phosphorylation with rotenone may lead to an increase in the rate of the tricarboxylic acid cycle, the second major mitochondrial pathway for maintenance of cellular energy supply (Moussa et al. 2007). Therefore, the increase in glutamate and glutamine pools may be indicative of changes in the glutamate-glutamine cycle, which is a major source of glutamate production and detoxification in the cell (Danbolt 2000); and this increase in glutamate level may result in excitotoxic death of neurons (Danbolt 2001). However, glutamate excitotoxicity involves over-activation of NMDA2B and mGLUR5 receptors may lead to release of Ca^{+2} from the intracellular stores, which causes over activation of enzymes like protein kinases leading to the degradation of proteins and membranes, thus enhancing cellular damage (Hu et al. 2015). Different NR2 subunit composition confers distinct electrophysiological and pharmacological properties to the receptors, differently contributing to excitotoxic neuronal cell damage. In various studies, activation of either synaptic or extrasynaptic NR2A-containing NMDArs has been found to promote protection against NMDA-induced neuronal damage, or at least to be less involved in NMDA induced toxicity (Zhou and Baudry 2006), while activation of NR2B-containing NMDArs results in increased neuronal apoptosis (Liu et al. 2007). Rotenone is extremely lipophilic, which makes it penetrate all cell types and move freely across cellular membranes independent of any reuptake transport mechanism (Talpade et al. 2000). Rotenone is known to depolymerize microtubules, the components of the cell skeleton that are involved in vesicular transportation (Marshall and Himes 1978). The CA1 hippocampal area is a very vulnerable brain region affected by adverse conditions such as ischemia and anoxia (Sims and Pulsinelli 1987). Information flow in hippocampus is mostly unidirectional. Tightly packed cell-layers are paths for propagating signals. The main output region in hippocampus is CA1 pyramidal cell region (Zola-Morgan et al. 1986). Saybasili et al. (2001) reported that rotenone was found to generate a higher level of superoxide generation in rat hippocampal slices compared to rat striatal slices, showing that this structure is more vulnerable to oxidative stress (Saybasili et al. 2001). It is well-known that the hippocampus is highly sensitive to oxidative stress, which makes hippocampal synaptic transmission highly dependent on mitochondrial products such as ATP and ROS (Keating 2008). It was shown that rotenone treatment may decrease the level of presynaptic release of glutamate. The inhibitory effect of rotenone on neuronal responses is presynaptic rather than on the postsynaptic site (Ren and Feng 2007). It has been reported that rotenone decreased field potentials (fEPSP) by 17% in CA1 region of hippocampus (Wu and Johnson 2009).

Effects of curcumin on hippocampal electrical activity and behavior

To gain more insight into curcumin protective effects, we examined the activity of hippocampal neurons combining behavioral and *in vivo* electrophysiological methods. The effects of curcumin were investigated by using extracellular recording from 334 neurons. Rats were given curcumin (200 mg/kg in DMSO) intraperitoneally for 21 days. Our initial results using electrophysiological and morphological (Nissl staining) techniques demonstrated that rotenone affects electrical functionality and the survival of the cells (Darbinyan et al. 2016). Our recent study has shown the modulatory effects of a single injection of curcumin (200 mg/kg, IP) on intact rats' hippocampal neuronal activity during high frequency stimulation of ipsilateral entorhinal cortex (Darbinyan 2016). It was shown that acute exposure to rotenone impairs hippocampal synaptic transmission dependent upon concentration and exposure period. In addition, a recent report has shown that acute exposure of rat hippocampal slices to 0.1–1 μ M rotenone for 30 min dramatically reduced the amplitude of evoked population spikes. Results showed that rotenone impaired high frequency stimulation-induced LTP (Kim do et al. 2010). For instance, studies have shown that brief exposure (1 h) of cultured hippocampal slices to 1–2 μ M rotenone induced CA1 neuron death (Xu et al. 2003), and chronic exposure (24 h) of cultured hippocampal slices to 200–300 nM rotenone significantly worsened cellular degeneration (Schuh et al. 2008). Extensive animal model studies have been conducted about the sustained protective role of different natural bioactive compounds against dopaminergic neuron loss in PD. Phytobioactive compounds from various medicinal plants show neuroprotective effects in various animal models (Son et al. 2012). Curcumin possesses many biological activities against neurodegenerative diseases. Here we confirmed and further characterized its protective effect in rotenone-induced neurotoxicity. To better estimate the toxic action of rotenone in hippocampus, we compared the effects of rotenone with curcumin effects. Potentials were recorded in the CA1 and CA3 regions after the activation of EC. In addition, we described its protective ability in hippocampus. By measuring electrical currents in hippocampal neurons, we could confirm that curcumin affected excitotoxic neuronal activity. These results point to NMDAr as a target for curcumin

neuroprotection. Interestingly, as reported in Fig. 4 Curcumin (Group IV) significantly decreased neuronal excitability (TP-1.59 times) (Fig 4) compared with Group I (2.21 times). Changes of TD PTP responses were lower in the Group IV (3.28 times) versus the Group I (3.96 times), Group II (4.22 times) and the Group III (3.72 times). Significantly higer TP PTD responses were observed in the curcumin group (2.89 times) compared with the Group I (1.68 times). These data showed that curcumin prevented rotenone-induced excitatory TP PTP and PTP expressions (Group I-TP PTP and PTP, Group III-only TP PTP). According to equity ratio of types of responses (Fig 4) the neurons with TD-PTD (47.3%) were maximally presented (Group IV). Treatment with curcumin preserved inhibitory TD PTD (4.49 times) and TD (4.07 times) responses in the hippocampus compared with Group I (5.02 and 5.5 times), Group II (3.64 and 4.66 times) and Group III (3.72 and 3.59 times, respectively). Cellular mechanisms that directly impact synaptic function, likely constitute effective future interventional targets in PD therapy. Currently, the effects of curcumin on LTD are not fully understood. It has been reported that hippocampal field potential LTD was prevented by curcumin (Choi et al. 2017). The number of spontaneous rears made during 5 min in the cylinder was measured for each animal. At week three, rats reared significantly less than Group II. Rotenone (2.5 mg/kg/day) effectively disrupted motor behavior. It has been shown that nondopaminergic striatal neurons and the globus pallidus can be damaged by rotenone administration (Hoglinger et al. 2003). Rotenone produced loss of striatal dopamine terminals, nigral pathology (a-synuclein- and ubiquitin-positive intracellular inclusions and ~45% cell loss) and a clear parkinsonian phenotype characterized by postural instability and reduced paw reaching in the cylinder test (Cannon et al. 2009). Alterations to other neurotransmitter systems and damage to basal ganglia structures may contribute to the observed motor impairments. We herein report that curcumin (200 mg/kg, administered for 3 weeks) have a specific effect in suppressing the behavioral features associated with PD induced by rotenone. Curcumin treatment improved behavioral recovery within the 3 weeks (at the end of 5 rd week which improved further at the end of 6 th week). Thus, Curcumin alleviated the deficits behavior in rats as the rearing frequencies of animals were enhanced (Fig 6).

Curcumin significantly prevented rotenone-induced impairment of hippocampal synaptic plasticity, which is likely mediated via dysfunction of mitochondrial complex I. It was shown that Curcumin is able to protect hippocampal neurons against NMDA-induced cell death, confirming its anti-excitotoxic property and induced an increase in NMDAr subunit type 2A (NR2A) level, with kinetics closely correlated to time-course of neuroprotection and decrease in [Ca(2+)](i) (Matteucci et al. 2011). Curcumin's protective effects appear to be mediated by inhibition of apoptosis, as there was a dose-dependent reduction in apoptosis and a parallel decrease in caspase3 levels (Qualls et al. 2014). Curcumin attenuated Aβ-induced elevation of the ratio of cellular glutamate/γ-aminobutyric acid (GABA) with a concentration-dependent manner (Huang et al. 2015).

It was shown that chronic curcumin treatment resulted in increased BDNF expression in the hippocampus. BDNF has been directly implicated in cell survival and neurogenesis (First et al. 2011). Curcumin interaction with various neurotransmitters, e.g.,serotonin and dopamine (Kulkarni and Akula 2012), GABAergic system (Gilhotra and Dhingra 2010), and glutamatergic system have been reported. In-vitro studies indicate a protective effect of curcumin against NMDA-mediated toxicity, suggesting inhibitory effects of curcumin on NMDA receptors. It was found that Curcumin protects hippocampal neurons against NMDA-induced apoptosis (Matteucci et al. 2005). The effects of curcumin also have been tested in rotenone based cell and Drosophila PD models. Curcumin also rescued rotenone-induced locomotor impairment and early mortality and restrained dopaminergic neuronal degeneration in Drosophila via reducing mitochondrial ROS levels (Liu et al. 2013). Previous studies have shown the cytoprotection of curcumin against 6-hydroxydopamine (6-OHDA)-induced neuronal death and the neuroprotective effects of curcumin were attributed to the modulation of nuclear factor-kappaB translocation (Wang et al. 2009). Thus, Curcumin protects hippocampal neurons against rotenone-induced cell death.

Acknowledgements This article was extracted as part of the Ph.D. thesis of Lilit Darbinyan. We thank Neuroendocrine Relationships Lab for technical support.

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Compliance with ethical standards

Funding The authors declare that they obtained no funding for this study.

Availability of data and material The datasets supporting the conclusions of this article are included within the article.

Competing interests The authors declare that they have no competing interests.

Consent for publication Not applicable.

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