



Cytosolic non-vesicular dopamine accumulation as the predominant mechanism for developing non-DOPA responsive parkinsonism in late-stage Huntington disease

Rafael Vincent M. Manalo

College of Medicine, University of the Philippines Manila, Ermita, Manila 1000, Philippines



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ABSTRACT

Disturbances in motor movement can have similar clinical presentations, albeit having different pathways and temporal onset. Hypokinetic movements present with rigidity, resting tremors, postural instability and bradykinesia, as seen in parkinsonism, while hyperkinetic movements typically present with chorea, ballismus, tic, athetosis and dystonia. Nonetheless, movement disorders are thought to be a continuum. Long-term therapy of parkinsonism with L-DOPA or dopamine (DA) agonists leads to late-onset dyskinesia – a hyperkinetic movement disorder, while patients with late-stage Huntington disease (HD) often develop non-DOPA responsive parkinsonism. In this paper, it is proposed that late-onset parkinsonism is driven by the overactivity of the nigrostriatal dopaminergic pathway. The excessive synthesis, storage, release, reuptake and degradation of dopamine in the presynaptic terminal and synaptic clefts lead to cellular stress and damage, resulting to progressive neuroapoptosis aggravated by pro-parkinsonism drugs used to treat hyperkinesia. Glutamate excitotoxicity may provide initial stress to neurons during early HD – but as the disease advances, lower glutamate levels are observed, making it less likely to cause the hypokinetic shift on its own. Over time, dopaminergic neurons are depleted and cholinergic influence to striatal GABA release is unopposed, leading to late-onset parkinsonism that is unresponsive to DOPA challenge, due to drastic DA neuron loss previously masked by the dominating choreic presentation. This paper thus provides a mechanism of action to a common clinical sequela and complication of long-term choreic diseases, whose pathophysiologic mechanism is presently lacking.

Introduction

The mechanism behind the progressive transition from hyperkinesia to hypokinesia is presently unknown. One such instance is found in typical Huntington disease (HD) – a progressive disorder of mainly choreiform movements in about 90% of all cases [1]. HD is an autosomal dominant inherited disorder caused by expansion of a glutamine trinucleotide (CAG) repeat in the huntingtin gene of chromosome 4 [2]. Over time, the incidence of developing late-onset non-DOPA responsive parkinsonism among HD patients become more common [3], with some having concurrent rigid-hypokinetic syndrome along with hyperkinetic somatosensory-evoked potentials and blink reflexes in juvenile patients, termed the Westphal variant of HD [1,4]. In almost all cases, there is a pathologic neuronal loss in the striatum and cortex, mostly affecting the GABAergic medium spiny neurons (MSNs) with relative sparing of those producing acetylcholine [5,6]. With this time-dependent neuronal loss dynamics, both typical and juvenile HD can initially present as a choreic disease, followed by a hypokinetic, parkinsonian shift or

concomitant hyperkinesia-hypokinesia. It has been proposed that CAG repeats leading to expression of HD begin from 40 to 80 repeats; however, the CAG repeat size remains to be a poor predictor of the age of onset [7]. In nature, a normal Huntingtin protein forms a polar zipper that mediate neuronal function in vertebrates – without which, significant neuronal dysfunction is observed [8]. Huntingtin interacts with vesicle-associated proteins and contributes to cellular trafficking between the nucleus and cytosol, which is improved upon palmitoylation [9,10]. A mutation, overall, expands the polyglutamine tract in the N-terminal of the Huntingtin protein, which increases its propensity to misfold and aggregate. These aggregates tend to overwhelm the proteasome degradation pathway and co-aggregate with other essential proteins, ultimately affecting protein degradation and neurotransmitter balance probably via the ER stress response [10,11]. However, data on these pathways remain scarce, and since the Huntingtin protein is expressed in all neurons and glial cells, it is difficult to explain the localization of HD to the striatum. To this day, there remains no exhaustive explanation of the biphasic nature of HD: an excellent example of the

E-mail address: rmmanalo3@up.edu.ph.

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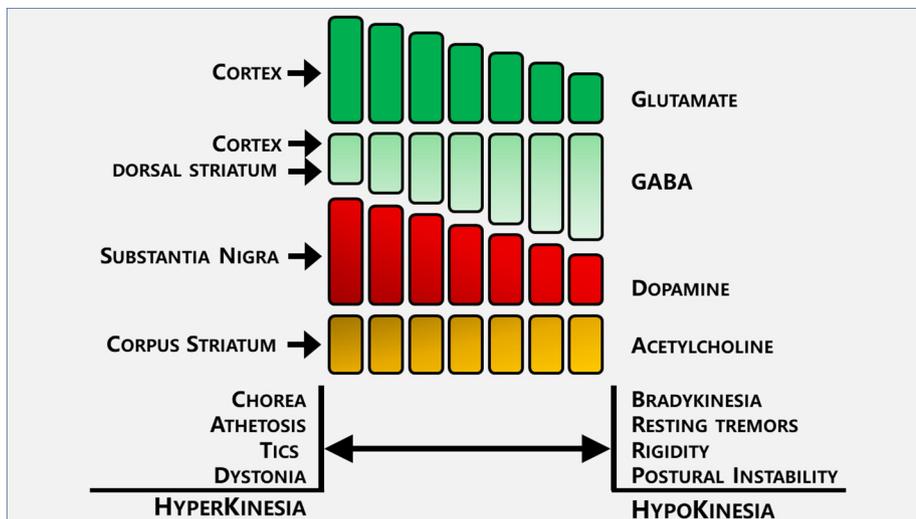


Fig. 1. Neurotransmitter balance forms a movement continuum. Movement disorders are often progressive, and patients present with a continuum of movements over time rather than becoming fixed at a classification. Increased glutamate-mediated excitation or GABA-mediated inhibition of the substantia nigra by the cortex or the dorsal striatum (globus pallidus and putamen) respectively leads to excessive and unopposed synthesis and release of dopamine to the striatum, which overcomes acetylcholine release to favor hyperkinetic responses. As neurotransmitter balance is perturbed, less glutamate or more GABA lead to less dopamine release from the substantia nigra, which will now favor a hypokinetic presentation. At midline of this continuum, a patient may present with both hyperkinetic and hypokinetic features, as in the Westphal variant of Huntington disease. In this example, acetylcholine release by the corpus striatum is relatively spared.

continuum of movement disorders.

Hypothesis

Movement disorders are a continuum

The bridge between hyperkinesia and hypokinesia can be exemplified by the pharmacologic approaches to well-known disorders. For instance, in Parkinson disease (PD), the loss of dopaminergic stimulation and unopposed cholinergic influence to the corpus striatum from the substantia nigra leads to increased GABA release to the globus pallidus (Fig. 1). This will uninhibit the subthalamic nucleus and activate the pars reticulata (Fig. 2), leading to bradykinesia as a common presentation in the indirect pathway [12]. To treat this symptomatically, dopamine stores are replenished by means of levodopa combined usually with carbidopa (eg. Sinemet) or an additional COMT inhibitor (eg. Stalevo) [13]. Alternatively, an antimuscarinic agent (i.e. trihexyphenidyl, procyclidine, benztropine mesylate) is used to taper down cholinergic stimulation (Fig. 2), thus leveling off the dopamine-acetylcholine balance in the substantia nigra and restoring neurotransmitter homeostasis [14]. However, a usual complication of long-term

dopaminergic treatment is dyskinesia, commonly presenting as choreoathetosis of the face and distal extremities [15]. Further, mental disturbances begin to develop over time, including but not limited to confusion, psychosis, depression, nightmares, and certain disorders of impulse control [16]. These disturbances, as well as the hyperkinetic complications, are interestingly treated by atypical antipsychotics (Fig. 3), serotonin modulators, or drug discontinuation [13] – all of which converging to a common pathway of attenuating the activation of the dopamine D2 receptor family (D3, D4, mainly D2).

In a hyperkinetic movement disorder such as Huntington disease, overactivity of the dopaminergic pathway in the nigrostriatal tracts lead to hyperkinetic movements such as chorea [6]. This dominance of the dopaminergic pathway, which is probably enhanced by genetic variations predisposing to more dopamine production or less acetylcholine, leads to marked reduction of GABA in the basal ganglia of patients with HD, in addition to reduced enzyme activity that make GABA (glutamic acid decarboxylase) and acetylcholine (choline acetyltransferase), respectively [17,18]. Treatment with tetrabenazine, which blocks striatal VMAT2, reduces the severity of chorea. Meanwhile, postsynaptic dopamine receptor blockers such as phenothiazines and atypical antipsychotics such as olanzapine, may help in

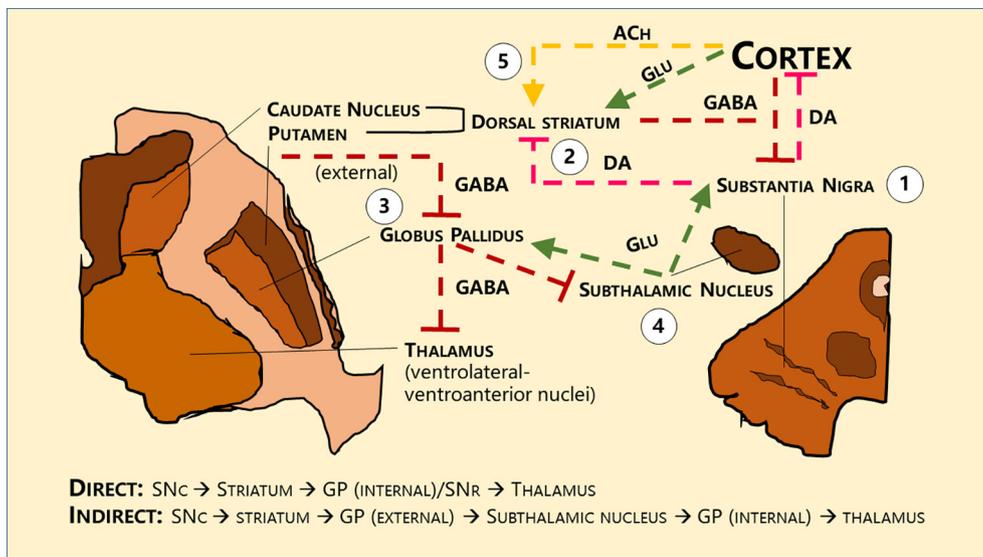


Fig. 2. The cortex, basal ganglia, and thalamus cooperate to execute movement. When dopamine release from the substantia nigra is increased (1), there is increased inhibition of the striatum (2), which allows the globus pallidus (external) to negatively influence the subthalamic nucleus and the ventrolateral-ventroanterior nuclei of the thalamus because the influence of GABA is relieved (3). This results to hyperkinetic movement disorders seen in choreic diseases like Huntington disease. Likewise, when dopamine release is decreased, the striatum actively inhibits the globus pallidus by way of GABA, resulting to overactive release of glutamate by the subthalamic nucleus (4). This leads to hypokinetic movement disorders seen in parkinsonism. Meanwhile, unopposed acetylcholine influence in the striatum favors GABA release into the globus pallidus (5), which favors parkinsonism and is a known target for symptomatic treatment by way of antimuscarinic agents.

Snc = substantia nigra pars compacta; GP = globus pallidus; SNr = substantia nigra pars reticulata; Ach = acetylcholine; GABA = gamma aminobutyric acid; Glu = glutamate; DA = dopamine.

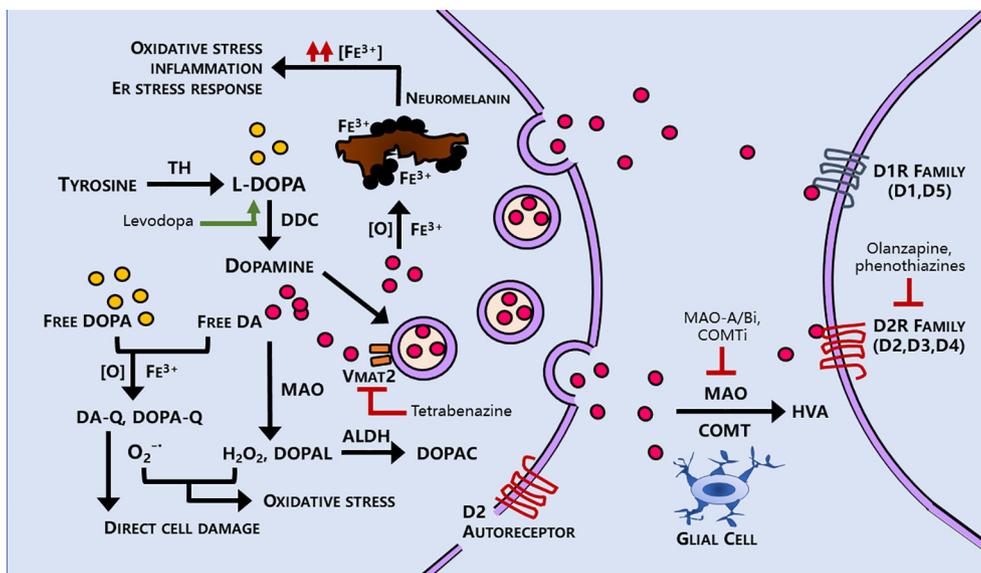


Fig. 3. Dopamine is degraded into toxic metabolites and accumulate to form neuromelanin, which can both result to neuroapoptosis. By the action of tyrosine hydroxylase, tyrosine is converted to L-DOPA, which is then catalyzed by DOPA decarboxylase into dopamine. By the action of VMAT2, dopamine is then stored in synaptic vesicles for eventual release, which can be inhibited by tetrabenazine. This pathway is favored by administration of exogenous L-DOPA. When excessive dopamine is produced, non-vesicular dopamine and L-DOPA accumulate in the cytosol, which are readily deaminated by monoamine oxidase into H₂O₂ and DOPAL. Alternatively, they are oxidized in an Fe³⁺-dependent manner to orthoquinones (DA-Q, DOPA-Q), releasing superoxide anions in the process. The H₂O₂ and O₂⁻ generated increase oxidative stress, which may then damage the cell. Meanwhile, the formed orthoquinones can alter cellular structures and cause direct

neurodegeneration. The other free dopamine molecules accumulate and are oxidized to form neuromelanin, which can initially be neuroprotective by sequestering metal ions. In iron overload, neuromelanin can act as a reservoir of oxidative stress and inflammation, possibly activating the ER stress response and causing neuroapoptosis. The released dopamine can either interact with D1 or D2 receptor families, with the latter inhibited by phenothiazines and atypical antipsychotics (i.e. olanzapine) and can be helpful in tapering off mental disturbances. Synaptic cleft dopamine can either be reuptaken or processed by glial cells to homovanillic acid, which can be inhibited by MAO-A/MAO-B or COMT inhibitors.

symptomatically treating HD (Fig. 3). Interestingly, these drugs are pro-parkinsonism, consistent with our previous finding that olanzapine attenuates the dopaminergic neuroprotection of caffeine [19,20]. While it is intuitive that drugs for hyperkinetic disorders are hypokinetic and vice-versa, the case in point rests on the common mechanism involving dopamine and D2 receptor families [21–23]. These suggest that hyperkinesia and hypokinesia are a continuum – rather than being fixed to a certain category, these movement disorders occur according to the balance of neurotransmitters in the brain, and can possibly move from one classification to another, due possibly to disease progression or as an adverse effect of a drug (Fig. 1).

Long-term dopamine overproduction leads to neuroapoptosis

In the brain, dopamine is synthesized as one of three neurotransmitters (with epinephrine and norepinephrine) diverging from the common amino acid precursor tyrosine. By the action of tyrosine hydroxylase, tyrosine is converted to L-DOPA, which is the rate-limiting step in catecholamine biosynthesis [24]. Lastly, L-DOPA is then converted to dopamine through the actions of the enzyme DOPA decarboxylase. Dopamine is then imported into synaptic vesicles via the vesicular monoamine transporter 2 (VMAT2), where they are protected from oxidation in the cytosol due to the acidic pH inside the vesicles [25,26] (Fig. 3).

In dopamine overproduction, it is probable that the amount of dopamine produced in the cytosol far exceeds the number of vesicles formed. This leads to free cytosolic dopamine, which is then processed by monoamine oxidase (MAO) via oxidative deamination into hydrogen peroxide (H₂O₂) and 3,4-dihydroxyphenylacetaldehyde (DOPAL). Since both dopamine and L-DOPA are reactive via their catechol moiety, they are easily converted via spontaneous or iron (Fe³⁺) catalysis to orthoquinones that are electron poor. These generate superoxide radical anions (O₂⁻) in the process of electron exchange with oxygen inside the neurons [27]. Meanwhile, the orthoquinones DOPA-quinone and dopamine-quinone may react with nucleophiles and cellular components – altering intracellular integrity and causing degeneration [28,29]. Some of the cytosolic, non-vesicular dopamine are synthesized into neuromelanin – a metal-sequestering and possibly antioxidant pigment found in neurons with excessive catecholamines – which may act as a

reservoir of oxidative stress when metals accumulate in them excessively [27,30] (Fig. 3). Indeed, neuromelanin is found in the substantia nigra of patients with PD, and is purported to contribute to its pathophysiology [31,32]. Hence, it is evident that mere dopamine overproduction can lead to neurodegeneration and neuroapoptosis, with subsequent development or worsening of hypokinetic manifestations. A study by Han et al showed that retina from day 7 postnatal rats presented with definite signs of neuroapoptosis when incubated with ethanol, which significantly decreased when dopamine was co-incubated [33]. While this finding seems to disagree with dopamine-induced neuroapoptosis, several points regarding the type of neurons, the stage of development, and the quantity of dopamine must be discussed. The predominant neurotransmitter in the mammalian retina is glutamate, which is used by the photoreceptor and bipolar cells [34,35]. These neurons are postsynaptic to dopaminergic neurons, and probably express greater levels of dopamine D1 receptors (D1R) at early development. Consistent with this anatomic variation, ethanol, which has been shown to decrease D1R levels in the retina, can cause neuroapoptosis as a direct toxicant or by reducing D1R function [36]. Since the study focused on neurodevelopment, exposure to ethanol will likely stunt neuronal activity and lead to eventual cell death [37]. Consistently, dopamine will protect retinal neurons by activating the cAMP/PKA pathway and promoting neurogenesis, which can be a direct consequence of D1R or adenosine A2A receptor (A2AR) agonism [33]. In other areas of the brain for which dopaminergic neurons are predominantly found, such as the ventral tegmental area (VTA), ethanol is expected to have the opposite effect. In fact, several studies have shown that ethanol exposure to the VTA promotes dopamine synthesis and release, which may provide a mechanism for the reward circuitry in alcohol addiction [38]. Hence, exogenous dopamine administration to this area of the brain will instead increase dopamine levels, leading to the neurodegenerative mechanisms described above. To this end, the protective or degenerative effects of dopamine has partly to do with the region of the nervous system involved, the stage of development, and its quantity. At excessive dopamine levels and at regions predominantly dopaminergic, neuroapoptosis is more likely than neuroprotection.

Late-onset non-DOPA responsive parkinsonism is a function of dopamine overproduction

Previous reports have shown early increases in dopamine in the nigrostriatal pathway followed by an eventual decrease, increased tyrosine hydroxylase activity, or increased dopamine receptors [39,40]. Interestingly, mouse and rat models of HD have shown progressive decline in striatal dopamine levels, despite initially increased dopamine [41–43], followed by progressive loss of striatal dopamine D1 and D2 receptors [6,44]. These point to a spontaneous shift of processes from that favoring hyperkinesia to that of a rigid, hypokinetic clinical presentation, which is unlikely due to adverse drug events alone.

An alternative mechanism is glutamate excitotoxicity, in which glutamate – the excitatory neurotransmitter of the corticostriatal projection – provides major input in activating the striatal pathway and when in excess, or via exposure to an exogenous NMDA receptor agonist, can lead to selective neurodegeneration of the MSNs in rodent striatum [45,46]. This is because an excessive stimulation of NMDA receptors by glutamate or its agonist leads to sustained Ca^{2+} influx and membrane depolarization – leading to Ca^{2+} overload that possibly activates the ER overload response and causes subsequent mitochondrial failure [47,48]. However, most recent evidence points to concomitant decline in both dopamine and glutamate functions as HD develops, suggesting that glutamate excitotoxicity may not completely explain progressive dopaminergic neuronal loss in late-stage HD [49]. With the dopamine hypothesis, unopposed dopamine production from both decreasing GABA-mediated inhibition and continuous glutamate-mediated excitation can lead to accumulation of cytosolic non-vesicular L-DOPA and dopamine, which can produce damaging radicals and metal accumulation from eventual formation of neuromelanin (Figs. 2 and 3). This provides consistency in explaining the development of late-onset parkinsonism in advanced HD, despite the observed decrease in corticostriatal glutamate signaling.

Late-onset L-DOPA resistant parkinsonism is characteristic of choreic masking

Late-onset disease presentation suggests either a time-dependent expression of certain genes, or a progressive aggravation of an initial cellular occurrence. Of the two, it is simpler and easier to rationalize a progressive nature of events, which is characteristic of HD. The combined reduced GABA inhibition from the MSNs, increased glutamate excitation from the neocortex, and increased cytosolic dopamine accumulation and metabolism all lead to neuroapoptosis in the striatum of the brain. Hence, striatal DAergic neurons progressively decrease over time, which may begin the development of a hypokinetic disorder (Fig. 2). However, because neurons in the striatum continue to overproduce dopamine unopposed, the hypokinetic consequence of neuroapoptosis remains masked by the choreic presentation of HD. Only when the death of DAergic neurons far exceeds the dopamine overproduction of the remaining neurons will parkinsonism begin to present, which may explain its late-onset feature (Figs. 1 and 2). Since this balance entails drastic neuronal death to overcome, a substantial amount of neurons will have been lost by the time parkinsonian features occur, and symptomatic treatment by L-DOPA will no longer work because there are not enough neurons to synthesize dopamine from it – making the parkinsonian feature also L-DOPA resistant. This hypothesis is supported by the Westphal variant of HD, where both hypokinetic and hyperkinetic features co-exist upon doing a targeted physical examination and laboratory upwork [1,4]. This suggests that hyperkinesia and hypokinesia are concurrently occurring at the molecular level, which is consistent with the hypothesis of DAergic neuronal death masked by choreiform movements. The difference between typical, late-onset HD and the Westphal variant might probably be due to the excitability of DAergic neurons in the juvenile striatum and its inherent capacity for neurogenesis – persistent excitation or lack of inhibition

can easily develop into cell death and parkinsonism if the DAergic neurons are highly excitable, but since neurogenesis is marked in this age group [50–51], there is continuous replenishing of DAergic neurons and the hyperkinesia-hypokinesia clinical presentation is maintained [52,53].

Conclusions

In this paper, it is proposed that movement disorders are a continuum, requiring neither external influence nor time-bound genetic expression to shift from kinetic extremes. In the typical cases of Huntington disease (HD) – a progressive movement disorder mainly of the choreic type – there is a shift from hyperkinetic movements to hypokinetic parkinsonian-like manifestations in the late stages, which is proposed to arise from the death of DAergic neurons in the striatum of the brain. Because both glutamatergic and DAergic neurons degenerate as HD progresses, this paper proposes that it is the accumulation of cytosolic, non-vesicular dopamine in the DAergic neurons that form the predominant mechanism for neuroapoptosis, which is then supported by the weakening glutamate influence and the reduced GABA-mediated inhibition of dopamine release. L-DOPA and dopamine are easily oxidized in either a spontaneous or Fe^{3+} -dependent manner into orthoquinones with resulting superoxide radicals, and these form neuromelanin which eventually become a reservoir of toxic metals. Meanwhile, the unopposed synthesis of dopamine and stimulatory effects of glutamate favor continuous membrane depolarization and massive Ca^{2+} influx, leading to Ca^{2+} overload and the activation of the ER stress response, which may result to mitochondrial failure and neurodegeneration. When the death of these DAergic neurons becomes sufficient to overcome the excessive dopamine release from nearby neurons, the features of parkinsonism will begin to surface clinically. However, because this entails drastic neuronal death, this shift is often seen as late-onset and has a rational resistance to L-DOPA medication. Thus, late-onset non-DOPA responsive parkinsonism in advanced HD is predominantly driven by cytosolic, non-vesicular dopamine accumulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests nor personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

RVM is the sole contributor of ideas in the article, and is the author of the written hypothesis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.109377>.

References

- [1] Ciammola A, Sassone J, Poletti B, Mencacci N, Benti R, Silani V. Atypical parkinsonism revealing a late onset, rigid and akinetic form of Huntington's disease. *Case Rep Neurol Med* 2011;696953. <https://doi.org/10.1155/2011/696953>.
- [2] Jimenez-Sanchez M, Licitra F, Underwood BR, Rubinsztein DC. Huntington's disease: mechanisms of pathogenesis and therapeutic strategies. *Cold Spring Harb Perspect Med* 2017;7:a024240.
- [3] Reuter I, Hu MTM, Andrews TC, Brooks DJ, Clough C, Chaudhuri KR. Late onset levodopa responsive Huntington's disease with minimal chorea masquerading as

- Parkinson plus syndrome. *J Neurol Neurosurg Pysch* 2000;68:238–41.
- [4] Töpper R, Schwarcz M, Lange HW, Hefter H, Noth J. Neurophysiological abnormalities in the Westphal variant of Huntington's disease. *Mov Disord* 1998;13:920–8.
- [5] Cicchetti F, Prensa L, Wu Y, Parent A. Chemical anatomy of striatal interneurons in normal individuals and in patients with Huntington's disease. *Brain Res Brain Res Rev* 2000;34:80–101.
- [6] Raymond LA, André VM, Cepeda C, Gladding CM, Milnerwood AJ, Levine MS. Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. *Neurosci* 2011;198:252–73.
- [7] Myers RH. Huntington's disease genetics. *NeuroRx* 2004;1:255–62.
- [8] Clabough EB, Zeitlin SO. Deletion of the triple repeat encoding polyglutamine within the mouse Huntington's gene results in subtle behavior/motor phenotypes in vivo and elevated levels of ATP with cellular senescence in vitro. *Hum Mol Genet* 2006;15:607–23.
- [9] Yanai A, Huang K, Kang R, Singaraja RR, Arstikaitis P, et al. Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat Neurosci* 2006;9:824–31.
- [10] Schulte J, Littleton JT. The biological function of the Huntingtin protein and its relevance to Huntington's disease pathology. *Curr Trends Neurol* 2011;5:65–78.
- [11] Hatters DM. Protein misfolding inside cells: the case of huntingtin and Huntington's disease. *IUBMB Life* 2008;60:724–8.
- [12] Magrinelli F, Picelli A, Tocco P, Federico A, Roncari L, et al. Pathophysiology of motor dysfunction in Parkinson's disease as the rationale for drug treatment and rehabilitation. *Parkinson Dis* 2016;2016:9832839.
- [13] Connolly BS, Lang AE. Pharmacological treatment of Parkinson's disease: a review. *JAMA* 2014;311:1670–83.
- [14] Brocks DR. Anticholinergic drugs used in Parkinson's disease: an overlooked class of drugs from a pharmacokinetic perspective. *J Pharm Pharm Sci* 1999;2:39–46.
- [15] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. *Postgrad Med J* 2007;83:384–8.
- [16] Grover S, Somaiya M, Kumar S, Avasthi A. Psychiatric aspects of Parkinson's disease. *J Neurosci Rural Pract* 2015;6:65–76.
- [17] Bird ED, Mackay AV, Rayner CN, Iversen LL. Reduced glutamic acid decarboxylase activity of post-mortem brain in Huntington's chorea. *Lancet* 1973;1:1090–2.
- [18] D'Souza GX, Waldvogel HJ. Targeting the cholinergic system to develop a novel therapy for Huntington's disease. *J Huntington Dis* 2016;5:333–42.
- [19] Videnovic A. Treatment of Huntington disease. *Curr Treat Options Neurol* 2013;15:424–38.
- [20] Manalo RVM, Medina PMB. Caffeine protects dopaminergic neurons from dopamine-induced neurodegeneration via synergistic adenosine-dopamine D2-like receptor interactions in transgenic *Caenorhabditis elegans*. *Front Neurosci* 2018;12:137.
- [21] Chen JY, Wang EA, Cepeda C, Levine MS. Dopamine imbalance in Huntington's disease: a mechanism for the lack of behavioral flexibility. *Front Neurosci* 2013;7:114.
- [22] Ledonne A, Mercuri NB. Current concepts on the pathophysiological relevance of dopaminergic receptors. *Front Cell Neurosci* 2017;11:27.
- [23] Tozzi A, Tantucci M, Marchi S, Mazzocchetti P, Morari M, et al. Dopamine D2 receptor-mediated neuroprotection in a G2019S Lrrk2 genetic model of Parkinson's disease. *Cell Death Dis* 2018;9:204.
- [24] Daubner SC, Le T, Wang S. Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch Biochem Biophys* 2011;508:1–12.
- [25] Miesenböck G, De Angelis DA, Rothman JE. Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. *Nature* 1998;394:192–5.
- [26] Chaudhry FA, Edwards RH, Fonnun F. Vesicular neurotransmitter transporters as targets for endogenous and exogenous toxic substances. *Annu Rev Pharmacol Toxicol* 2008;48:277–301.
- [27] Sulzer D, Bogulavsky J, Larsen KE, Behr G, Karatekin E, Kleinman MH, et al. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc Natl Acad Sci* 2000;97:11869–74.
- [28] Sulzer D, Zecca L. Intraneuronal dopamine-quinone synthesis: a review. *Neurotox Res* 2000;2000(1):181–95.
- [29] Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Commun Signal* 2013;11:34.
- [30] Double KL. Functional effects of neuromelanin and synthetic melanin in model systems. *J Neural Transm* 2006;113:751–6.
- [31] Zecca L, Zucca FA, Albertini A, Rizzio E, Fariello RG. A proposed dual role of neuromelanin in the pathogenesis of Parkinson's disease. *Neurology* 2006;67:S8–11.
- [32] Isaias IU, Trujillo P, Summers P, Marotta G, Mainardi L, et al. Neuromelanin imaging and dopaminergic loss in Parkinson's disease. *Front Aging Neurosci* 2016;8:196.
- [33] Han J, Gao L, Dong J, Wang Y, Zhang M, Zheng J. Dopamine attenuates ethanol-induced neuroapoptosis in the developing rat retina via the cAMP/PKA pathway. *Mol Med Rep* 2017;16:1982–90.
- [34] Wu SM, Maple BR. Amino acid neurotransmitters in the retina: a functional overview. *Vision Res* 1998;38:1371–84.
- [35] Farmen RH, Schuchardt TJ, Vrbancak JJ, Linn DM, Iwamoto K, Linn CL. Determination of neurotransmitter levels in rodent retina. *Invest Ophthalmol Visual Sci* 2011;52:5629.
- [36] Sarc L, Lipnik-Stangelj M. Comparison of ethanol and acetaldehyde toxicity in rat astrocytes in primary culture. *Arh Hig Rada Toksikol* 2009;60:297–305.
- [37] Lotfullina N, Khazipov R. Ethanol and the developing brain: inhibition of neuronal activity and neuroapoptosis. *Neuroscientist* 2018;24:130–41.
- [38] Morikawa H, Morrisett RA. Ethanol action on dopaminergic neurons in the ventral tegmental area: interaction with intrinsic ion channels and neurotransmitter inputs. *Int Rev Neurobiol* 2010;91:235–88.
- [39] Bolam JP, Smith Y. The GABA and substance P input to dopaminergic neurons in the substantia nigra of the rat. *Brain Res* 1990;529:57–78.
- [40] Spokes EGS. The neurochemistry of Huntington's chorea. *TINS* 1981;4:115–8.
- [41] Hickey MA, Reynolds GP, Morton AJ. The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. *J Neurochem* 2002;81:46–59.
- [42] Johnson MA, Rajan V, Miller CE, Wightman RM. Dopamine release is severely compromised in the R6/2 mouse model of Huntington's disease. *J Neurochem* 2006;97:737–46.
- [43] Jahanshahi A, Vlaming R, Kaya AH, Lim LW, Janssen ML, et al. Hyperdopaminergic status in experimental Huntington disease. *J Neuropathol Exp Neurol* 2010;69:910–7.
- [44] van Oostrom JC, Dekker M, Willemsen AT, de Jong BM, Roos RA, Leenders KL. Changes in striatal dopamine D2 receptor binding in pre-clinical Huntington's disease. *Eur J Neurol* 2009;16:226–31.
- [45] Schwarcz R, Coyle JT. Striatal lesions with kainic acid: neurochemical characteristics. *Brain Res* 1977;127:235–49.
- [46] Fonnun F, Storm-Mathisen J, Divac I. Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. *Neuroscience* 1981;6:863–73.
- [47] Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689–95.
- [48] Manalo RVM, Medina PMB. The endoplasmic reticulum stress response in disease pathogenesis and pathophysiology. *Egypt J Med Hum Genet* 2018;19:59–68.
- [49] André VM, Cepeda C, Levine MS. Dopamine and glutamate in Huntington's disease: a balancing act. *CNS Neurosci Ther* 2010;16:163–78.
- [50] He J, Crews FT. Neurogenesis decreases during brain maturation from adolescence to adulthood. *Pharmacol Biochem Behav* 2007;86:327–33.
- [51] Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, et al. Neurogenesis in the striatum of the adult human brain. *Cell* 2014;156:1072–83.
- [52] Sturman DA, Moghaddam B. Striatum processes reward differently in adolescents versus adults. *Proc Natl Acad Sci* 2012;109:1719–24.
- [53] Hoops D, Flores C. Making dopamine connections in adolescence. *Trends Neurosci* 2017;40:709–19.