Journal of
Biomolecular Research & Therapeutics

Olalekan et al., J Biomol Res Ther 2014, 3:3
DOI: 10.4172/2167-7956.1000114

Research Article Open Access

NMDA R/VDR in Fish Melanocytes; Receptor Targeted Therapeutic Model and Mechanism in Parkinson's disease

Olalekan OM[1*] **and Olurotimi JS**[2]

[1]*Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*

[2]*Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*

[*]**Corresponding author:** Ogundele OM, Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, Tel: +2347031022702; E-mail: ola.ogundele@abuad.edu.ng

Rec date: Feb 26, 2014; **Acc date:** Apr 26, 2014; **Pub date:** Apr 28, 2014

Abstract

The observable trend in the concept of evolution creates a template that advanced cell types are evolved from rudimentary cells over time. This is evident in protein structure and function observed along the evolutionary trend. An important component of cell evolution involves the role of microtubules and other members of the conserved family of the cell cycle/division proteins that have shown consistency from the yeast to the *Homo sapiens* over a billion years. In this study, we used specific imaging technique to compare the structure of melanocytes by manipulating NMDA R and VDR; to foster the study of synaptic denervation and pigment loss observed in PD. This information is important, as careful analysis and guided extrapolation of data can yield results of transnational significance. The outcome from two separate studies shows that both NMDA R and VDR are involved in cellular process formation in a way that can be likened to adrenergic cell process formation. Thus suggesting a possibility of adopting this cell type as a model.

Keywords: Parkinson's disease; Etiology; Drosophila; Ketamine

Abbreviations:

NMDA R: N-Methyl-D-Aspartate Receptor; VDR-Vitamin D Receptor; PD: Parkinson's Disease

Introduction

Parkinson's disease (and other associated movement disorders) is a common condition that is triggered by chemical, environmental, genetic and neurotrophic factors in which dopaminergic neurons are lost and melanin pigment is reduced in the SN [1-3]. If not treated, it can progress rapidly into movement disorders and other cognitive dysfunctions [4]. Since the initial description of this disease and other NDD's, efforts have been directed towards understanding the molecular mechanism of pigment loss and cell death in the SN [5]. Neurodegenerative Diseases (NDDs) are becoming rampant in sub-Saharan Africa due to food based toxicity and thus, there is an urgent need to conduct cell based research using cheap and appropriate models [6,7]. Previous studies have examined the etiology and cellular mechanisms involved NDDs such as Konzo, tropical ataxic neuropathy and movement disorders often associated with the loss of adrenergic pigmented neurons in the Substantia Nigra (SN) [8,9]. Available cellular models often demonstrate cell death due to aging and have been achieved through manipulation and mutation of the PD genes [10-12]. Other models involves the use of primates; through chemotoxin induced Parkinsonism that selectively targets the dopaminergic cells of the SN [13,14]. Most *in vitro* models are non-pigmented and thus cannot demonstrate the role of melanosomes in the selective vulnerability of these cells [15]. Also, the *in vivo* models cannot be observed directly as direct cellular observation is rather invasive [16,17]. Thus, there is a need for the development of *in vitro or ex vivo* cell models capable of showing synaptic denervation and the roles of pigment vesicles in the cause, progression and therapeutic targeting of PD. An important candidate cell for this purpose is thus the melanocyte in the marine species [18,19]. The melanocytes are adrenergic, pigmented, originates from the neural crest; they can also form extensive cellular processes like neurites. In addition the cells are also concentrated in the stream line of the body of the organism where it detects vibration and maintains the relative position of the organism in water [3,18-20].

It has observed that the fish scale melanocytes can be stimulated by adrenergic effector molecules. Considering the flow from monoamines (dopamine) to catecholamine (epinephrine and norepinephrine) this cell type already has two (2) main features of the SN which is the presence of pigmentation and receptors capable of being stimulated by the adrenergic effector molecules [19,21]. From these, it was inferred that these melanocyte populations in the scale of the fish (*Tilapia*) are probably specialized sensors that perform a similar function as those of the SN [3,18]. This function is basically in the determination of the relative position of the organism in space by polymerization and depolymerization of MT-Motor protein assembly to alter the position of melanosomes and thus regulating impulse discharge rate [22-24]. The cause of PD has been broadly described as unclear; in addition loss of adrenergic cells have also been described in the progression of the diseases condition which is characterized by shortening of neuronal projection as a form of synaptic denervation [25]. However, to understand the process involved in this synaptic denervation of adrenergic pigmented neurons, the molecular mechanism needs to be studied at cell and protein level.

Current Models in Parkinson's disease Studies

Parkinson's disease has been known to have several causes. An inherited form of the disease has driven the studies to create new *in vivo* models especially those involving transmission and inheritance of

the PD gene [26-28]. Guo described the use of the drosophila as an efficient tool in the study of PD gene inheritance involving pathogenic PD gene mutations [29-32]. This involves the study of the gene products of the two PD genes (PINK1 and Parkin) both involved in a mitochondria fission/fusion pathway [33,34]. These genes have also been observed in both the fly and humans; they also sub serve the same function of recruiting these mitochondria to the site of final removal. The use of drosophila has been linked with *in vitro* therapeutic targeting [35,36].

A number of polyphenols have been reported to play important roles in the inhibition of α-sync which might lead to possible prevention of PD resulting from these mutations. The effect of free radicals has also been implicated in aging related Parkinson's disease-often a product of mitochondria dysfunction. Other studies involved dietary supplements of Nordihydroguaiaretic Acid (NDGA) in drosophila models [35,37]. It was discovered that the loss of movement often observed in this model was delayed when NDGA was included in the diet. *In vivo* models includes the use of primates and rodents treated with PD causing chemicals agents like *MTPT* and have been used for studies on how specific drugs affects the progression of PD [38,39]. Novel analogues of MDMA, UWA-101 have been found to improve the therapeutic benefits αin primates being treated with L-Dopa. It was also observed that this UWA -101 was more effective than MDMA as it lacks psycoactivating and cytotoxic effects [40]. Other laboratory models involves the generation of disease specific stem cell lines from patients with incurable diseases [41]. This is often used for drug screenings and design of systems for understanding disease mechanism. Several nervous system cells have been screened and modified into dopaminergic neurons iPSC. Another cell model mimics the mutation of SNCA gene encoding the pro-oxidant α-sync protein in the budding yeast *(S. Cerevisae)*; by studying the increase in cytosolic neutral lipid storage embedded in lipid droplets. The significance of this accumulation was further investigated in a yeast strain which does not possess the machinery to synthesize triglycerides. The outcomes thus show that such strains were more resistant to α-sync toxicity [42].

The major effect of the PD pathogenesis is age related neuronal cell death in the dopaminergic neurons. This has also been found to be the case in mammalian and non-vertebrate models; example is the nematode (*C. Elegans*) [43]. Both genetic and drug screens conducted in *C. Elegans* have aided the identification of small molecules, proteins and discrete biological systems that can impact PD pathology. An example of such system is the identification of the autosomal dominant and idiopathic PD models in *C. Elegans* due to mutations found within the GTPase and kinase domains, both affecting the molecular motor for vesicular movement [44-46]. Previous studies have shown that such mutations are often linked with kinase hyperactivity. In order to understand this further, transgenic *C. Elegans* have been developed that can over express LRRK2, GTPase and Kinase similar to those observed in dopaminergic neurons in PD [47,48]. This system also created reduced locomotor activity, memory dysfunction and reduced dopamine production *in vivo* [47,49].

Selective Vulnerability of Pigment Neurons and Autophagy

Although several cellular and in vivo models described above have taken care of most parts of the disease progression in PD, a major point is the selective vulnerability of the pigment cells due to autophagy and induced oxidative stress. Although the cells of the *C. Elegans* and Drosophila express dopamine, they are however not pigmented as this represents a major limitation of these models. *In vivo* primate models are however priceless as they represents virtually all the aspects of the PD effects including the role of pigments metabolism and neurotransmission in these cells. It is important to note that study of cellular activities through direct observation are invasive and highly restricted in these rodent models, thus it is important to have an *in vitro* models where the cells are adrenergic, expresses major process formation and are also pigmented in order to understand the direct cellular effects of therapeutics and disease causing agents. Although the effect of certain drugs have been described as either increasing locomotor activity or improving dopamine level /L-DOPA uptake, direct cellular observation remains a challenge in these models.

It has been observed that dopaminergic neurons of the SN are selectively degenerate during the cause and progression of PD [50-52]. They also represent the most heavily pigmented population of neurons in the brain [50,53,54]. However, the heavy presence of neuromelanin have long being described as an important factor in the susceptibility of these neurons to aging and autophagy - described in the other *in vitro* models. Previous studies have discussed the unclear nature and role of neuromelanin in this structures, it has been suspected that intra neuronal melanin is neuroprotective through its ability to shield cells from heavy metals and toxins and also excess cathecolamines [2,55]. In contrast melanin released by dying neurons as extraneuronal can trigger inflammation and glia activation [4,50]. Graybiel et al., described the nigrostraital system in induced PD; quantitative analysis however points to selective loss of pigmented cells. Such patterns of loss are important in the study of etiology and clinical symptoms of PD [56].

Neuroprotective Properties of NMDA R Antagonist

Glutamate toxicity has been described as a major cause and facilitator of selective vulnerability in dopaminergic neurons [57-59]. The glutamate receptor, NMDA R is important in the astrocytic-neuronal glutamate-glucose cycling and glucose metabolism in the brain [60-62]. However during development, pharmacological knock down or genetic deletion of NMDA R have been found to greatly impair neuronal development and circuit formation [63,64]. Depending on the state of development, if the cells do migrate, they will not recognize the final destination in the nervous system [65,66]. Thus NMDA R has been implicated in neurite formation, synapse formation, development and neural circuit maturation [67-69]. Experiments involving the use of excitotoxin such as excess glutamate or glutamate analogues capable of persistent potentiation of the NMDA R have been observed to cause degenerative changes in the adult neurons including autophagy [18,70-72]. Thus the role of the glutamate receptor is switched post maturation [73]. In both development and degeneration, the formation of the cytoskeletal core of the axo-dendritic system is important. Through the work of NGF and other kinase receptors like p75 (LNGFR), the pre and post synaptic systems are established on neurite differentiated through cell elongation and process formation during neuronal cell migration in the developing brain layers [65,66,73]. A major effect of glutamate toxicity in the adult is however linked with autophagy which is much more prominent in pigment cells - the vesicles are observed close and clustered around the nucleus leading to cell death by lysosomal fusion [74,75]. The neuronal cytoskeleton, although forms a cellular track for vesicles and organelle moved to and from the synapses, they also keep

organelle in position in these cells [76,77]. Use of depolymerizing agent have shown that loss of the MT assembly will lead to accumulation of these vesicles around the nucleus creating an autophagy scenario in the cell [78,79].

Autophagy itself is described as an intracellular response to stress often characterized by the presence of autophagosomes [80]. Certain studies have demonstrated the autophagy response of cerebellar granule neuron challenged with NMDA (glutamate analogue). It was shown that fluorescently labeled autophagosomes were accumulated in the cell body and neurite at 3 hours post treatment**.** Lysosomal inhibition studies also reveal that NMDA excitotoxicity diverted the autophagosomes from the usual lysosomal degradation pathway [81]. Another possible path involves the role of cytoskeleton in PD, similar to that of Alzheimer's disease (AZ). High levels of intracellular calcium can disrupt cytoskeleton and NMDA R stimulation can drive an increase in cellular calcium levels which in turns disrupts cytoskeleton [82,83]. Other studies have shown that neuron that contains calcium binding proteins is less susceptible than the neurons that do not have these proteins. This is an indication that NMDA R stimulation can drive cytoskeletal degradation while calcium binding protein prevents the calcium surge; thus protecting the neurons [84-86]. A link between glutamergic and the nigrostraital system have been described through the glutamergic afferent pathways that projects to the nigrostraital system from the glutamergic tracts of the prefrontal cortex and might play a role in release of glutamate leading to degeneration of the nigrostraital system through excitotoxicity [87-89].

Distribution of VDR and the Therapeutic Role of VDRA in Dopaminergic Neurosurvival

Low serum level of Vitamin D is often associated with PD [90]. The final converting enzyme and the VDR receptor are distributed throughout the brain. Studies have shown that vitamin D is important in neurodevelopment, up-regulation of neurotrophic factors, stabilization of mitochondrial function, and antioxidation [90,91]. The VDR gene codes for the VDR and is responsible for calcium regulation, immune response, neuronal functions [92,93]. VDR polymorphism have been linked with PD aetiology and progression [94,95]. Placebo studies have also employed the use of VDRA in dietary supplements [96]. Improved behaviour and cognitive function have been observed in patients receiving Vitamin D supplements. Nissou et al., 2013 have demonstrated that the role of Vitamin D goes beyond cellular mechanisms, over time it has been found to be associated with up regulation of several genes up to 1.9 folds at transcriptome level [97-100]. The active form of Vitamin D is Vitamin D3 and it acts by binding to the VDR. This in effect regulates several cellular machinery at transcriptome level. Most of it effects have been studies extensively in osteoporosis, cancer, inflammation and immune system. Vitamin D analogues have been employed as therapeutic targets of VDR [101-103]. Vitamin D3 compounds are known to influence melanocyte maturation and differentiation and also to up-regulate melanogenesis through pathways activated by specific ligand receptors, such as endothelin receptor and c-kit [104,105]. Studies have shown that although these receptors are highly concentrated in the brain, they are most predominant in the pigmented cell population. Its role in regulating calcium concentration is useful in reduction of calcium ions that might disrupt cytoskeleton, thus helping in the prevention of synaptic denervation [106-109].

Manipulating the *NMDA R1* in *Tilapia* Melanocytes

Considering the super imposed developmental biology of pigment cells in Humans and Fishes; originating from the neural crest in both organisms, these cell types possess certain features of cells of the nervous system (Figure 1) [110]. The most important candidate considered is the N-Methyl-D-Aspartate Receptor (*NMDA R*); that is responsible for neuronal migration, development, process formation and synapse formation at the final site [111]. Our previous findings have shown that these receptors are located on the cell body and cellular projections, similar to what is observable in the mammalian neuronal cells. This was done using a confirmed Human *NMDA R1* antagonist (Ketamine) and an agonist (L-Glutamate) to inhibit and potentiate the receptor in live melanocytes using bright field microscopy [3,18].

Potentiating the *NMDA R* with glutamate caused process formation on the cell body, while inhibiting the receptor *in vitro* facilitated formation of processes having an appearance similar to axo-dendritic process formation pattern in developing neurons (Figure 2A and 2B). Bright field imaging techniques were also used to capture process formation and intercellular structural interactions. At this point, formation of cellular processes does not represents axons but provides an appropriate premise for studying pigmented cellular processes similar to those of axons of pigmented neurons. Extensive process formation and cellular connections were also observed post inhibition of NMDA R using a non-competitive open channel blocker, ketamine (Figure 2A and 2B). It can also be used to combine the study of microtubule-motor protein assembly and autophagy in pigmented adrenergic cells [3,18,63].

VDR-VDRAs

Other studies examined impact of Vitamin D receptor (VDR) and Vitamin D receptor agonists (VDRA) interaction on process formation in this model [112,113]. It was observed that both inhibition of *NMDA R* and VDR stimulation by VDRA facilitated process formation (Figure 3). Certain differences were noted some of which includes; short processes were created by VDR stimulation [114] as compared to longer processes seen in *NMDA R* inhibition [115], the blobbed ends of processes are well seen following VDRA stimulation [3,18]. Extent of branching in VDR shows short projections originating directly from the cell body while in *NMDA R* inhibition, larger processes were observed before rapidly branching to give smaller processes. The cellular process involved in the VDR stimulation suggests a rapidly branching dendritic network facilitating polymerization of the MT system and creating more branches similar to the dendritic nucleation assembly (Figure 3).

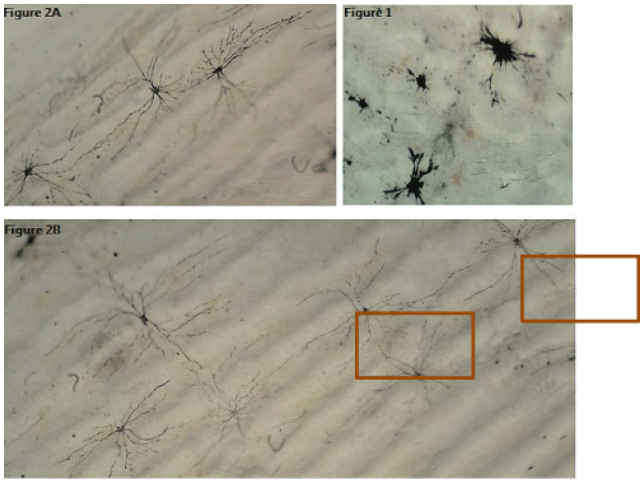

Figure 1 and 2a, 2b: (1) The control melanocyte (2a) the treatment group involving pharmacological knockdown of NMDA R by ketamine, a non-competitive antagonist. The cells showed elongation of cellular processes far higher than those recorded in the control. The cellular process elongation reveals the reverse role of the NMDA R in the embryonic system versus the adult system as these cells are also derived from the neural crest. (2b) Rectangles represent sites of structural intercellular connection.

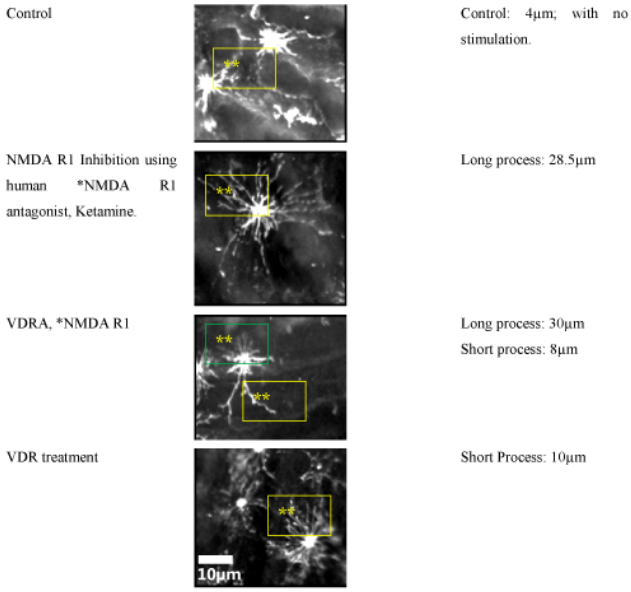

Figure 3: Inverted grey scale cell process measurement for Control cells, Ketamine treatment, VDRA+Ketamine and VDRA Only. Increase in process length was observed in all the treatment categories. Ketamine treatment gave the longest cell process while VDRA treatment induced shorter process formation (Scale bar: 10 µm) (From Ogundele et al., [18])

Conclusion

In this study, we have first described the mechanism involved in neuronal loss in the SN and the general structure of the fish scale melanocyte. Using bright field imaging techniques, live cell imaging was conducted *in vitro* to show the various changes observed in the melanocytes upon manipulation of the *NMDA R* and the VDR. The outcome shows that the fish scale melanocyte contains NMDA R on its membrane just like the human neuronal cells, although it is much more localized on the axon-like processes; while VDR is localized on the cell body and short dendrite-like processes. Upon inhibition of this receptor (using ketamine; a human NMDA R antagonist), the cell projections forms wide array networks of cellular processes following a similar pattern to what is observed in neuronal axon-dendrite formation. Glutamate treatment (NMDA R potentiation) also caused formation of cellular projections but not as extensive as that seen in NMDA R inhibition. These findings therefore creates a premise for the study of pigmented neuronal cells *in vitro* as this cell type (fish scale melanocyte) expresses NMDA R and the role of this receptor in cellular process formation has also been seen to be similar to the pattern observed in neuronal axon-dendrite formation. Inverted gray scale image analysis shows that this cell upon inhibition of NMDA R shows temporary connections between the formed processes, an association similar to the synapse that has been observed in the human neurons. VDR stimulation facilitated more of short process formation radiating directly from the cell body suggesting its role in a cellular process similar to that of the dendritic nucleation assembly in neurons.

Acknowledgements

We acknowledge the Directorate of Research, Afe Babalola University and the Laboratory Staff of the Department of Biological Sciences, Anatomy and Physiology. Also we acknowledge the contributions of Elizabeth Peters of the College of Medicine for assisting in the revision of this work. All other members of my research team are acknowledged for their support and contribution. The Afe Babalola University Sponsored Our Research paper Presentations on *"NMDA R1* as a tool in Neuroprotection and Nerve cell reprogramming" made at the SONA conference in Morocco (June 13-18, 2013).

References

1. Eitan E, Hutchison ER, Mattson MP (2014) Telomere shortening in neurological disorders: an abundance of unanswered questions. Trends Neurosci.
2. Elstner M, Müller SK, Leidolt L, Laub C, Krieg L, et al. (2011) Neuromelanin, neurotransmitter status and brainstem location determine the differential vulnerability of catecholaminergic neurons to mitochondrial DNA deletions. Mol Brain 4: 43.
3. Olalekan Michael, Ajonijebu, Chris D, Adetokunbo OA, Oloruntoba AA, et al. (2013) VDR Potentiation and NMDA R Inhibition Facilitates Axo-Dendritic Process Formation in Melanocyte Model for Pigmented cells in Parkinsonism. Cell and Developmental Biology Cell Dev Biol 2: 127.
4. Zecca L, Wilms H, Geick S, Claasen JH, Brandenburg LO, et al. (2008) Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease. Acta Neuropathol 116: 47-55.
5. Plum LA, DeLuca HF (2010) Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov 9: 941-955.
6. Annese V, Herrero MT, Di Pentima M, Gomez A, Lombardi L, et al. (2014) Metalloproteinase-9 contributes to inflammatory glia activation and nigro-striatal pathway degeneration in both mouse and monkey

models of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism. Brain Struct Funct.

7. Brunden KR, Trojanowski JQ, Smith AB 3rd, Lee VM, Ballatore C (2013) Microtubule-stabilizing agents as potential therapeutics for neurodegenerative disease. Bioorg Med Chem.
8. Alberio T, Bondi H, Colombo F, Alloggio I, Pieroni L, et al. (2014) Mitochondrial proteomics investigation of a cellular model of impaired dopamine homeostasis, an early step in Parkinson's disease pathogenesis. Mol Biosyst.
9. Giordano S, Darley-Usmar V, Zhang J (2013) Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease. Redox Biol 2: 82-90.
10. Kragh CL, Gysbers AM, Rockenstein E, Murphy K, Halliday GM, et al. (2014) Prodegenerative IÎºBÎ± expression in oligodendroglial α-synuclein models of multiple system atrophy. Neurobiol Dis 63: 171-183.
11. de Munter JP, Melamed E, Wolters ECh (2014) Stem cell grafting in parkinsonism--why, how and when. Parkinsonism Relat Disord 20 Suppl 1: S150-153.
12. Chang C, Wu G, Gao P, Yang L, Liu W, et al. (2014) Upregulated Parkin expression protects mitochondrial homeostasis in DJ-1 konckdown cells and cells overexpressing the DJ-1 L166P mutation. Mol Cell Biochem 387: 187-95.
13. Pérez-HJ, Carrillo-SC, García E, Ruiz-Mar G, Pérez-Tamayo R, et al. (2014) Neuroprotective effect of silymarin in a MPTP mouse model of Parkinson's disease. Toxicology 319: 38-43.
14. Picconi B, Calabresi P (2014) Targeting metabotropic glutamate receptors as a new strategy against levodopa-induced dyskinesia in Parkinson's disease? Mov Disord.
15. Falk T, Zhang S, Sherman SJ (2009) Pigment epithelium derived factor (PEDF) is neuroprotective in two in vitro models of Parkinson's disease. Neurosci Lett 458: 49-52.
16. Rawal PV, Almeida L, Smelser LB, Huang H, Guthrie BL, et al. (2014) Shorter Pulse Generator Longevity and More Frequent Stimulator Adjustments With Pallidal DBS for Dystonia Versus Other Movement Disorders. Brain Stimul.
17. Baumann CR, Waldvogel D (2013) [The treatment of Parkinson's disease]. Praxis (Bern 1994) 102: 1529-1535.
18. Ogundele OM, Okunnuga AA, Fabiyi TD, Olajide OJ, Akinrinade ID, et al. (2013) NMDA-R inhibition affects cellular process formation in Tilapia Melanocytes; a model for pigmented adrenergic neurons in process formation and retraction. Metab Brain Dis .
19. Decker AR, McNeill MS, Lambert AM, Overton JD, Chen YC, et al. (2014) Abnormal differentiation of dopaminergic neurons in zebrafish trpm7 mutant larvae impairs development of the motor pattern. Dev Biol 386: 428-439.
20. OstrovskiÄ MA, Dontsov AE (1985) [Physiologic functions of melanin in the body]. Fiziol Cheloveka 11: 670-678.
21. Svensson SP, Adolfsson PI, Grundström N, Karlsson JO (1997) Multiple alpha 2-adrenoceptor signalling pathways mediate pigment aggregation within melanophores. Pigment Cell Res 10: 395-400.
22. Sköld HN, Norström E, Wallin M (2002) Regulatory control of both microtubule- and actin-dependent fish melanosome movement. Pigment Cell Res 15: 357-366.
23. Wu X, Bowers B, Rao K, Wei Q, Hammer JA 3rd (1998) Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function In vivo. J Cell Biol 143: 1899-1918.
24. Drenckhahn D, Wagner HJ (1985) Relation of retinomotor responses and contractile proteins in vertebrate retinas. Eur J Cell Biol 37: 156-168.
25. Rudzinska M, Bukowczan S, Stozek J, Zajdel K, Mirek E, et al. (2013) Causes and consequences of falls in Parkinson disease patients in a prospective study. Neurol Neurochir Pol 47: 423-430.
26. Bozi M, Papadimitriou D, Antonellou R, Moraitou M, Maniati M, et al. (2013) Genetic assessment of familial and early-onset Parkinson's disease in a Greek population. Eur J Neurol.
27. Pankratz N, Beecham GW, DeStefano AL, Dawson TM, Doheny KF, et al. (2012) Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. Ann Neurol 71: 370-384.
28. van Es MA, Schelhaas HJ, van Vught PW, Ticozzi N, Andersen PM, et al. (2011) Angiogenin variants in Parkinson disease and amyotrophic lateral sclerosis. Ann Neurol 70: 964-73.
29. Guo M (2012) Drosophila as a model to study mitochondrial dysfunction in Parkinson's disease. Cold Spring Harb Perspect Med 2.
30. Segura-Aguilar J, Paris I, Muñoz P, Ferrari E, Zecca L, et al. (2014) Protective and toxic roles of dopamine in Parkinson's disease. J Neurochem.
31. Chen Y1, Dorn GW 2nd (2013) PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science 340: 471-475.
32. Hauser DN, Hastings TG (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. Neurobiol Dis 51: 35-42.
33. Zuo L, Motherwell MS (2013) The impact of reactive oxygen species and genetic mitochondrial mutations in Parkinson's disease. Gene 532: 18-23.
34. Venderova K, Kabbach G, Abdel-Messih E, Zhang Y, Parks RJ, et al. (2009) Leucine-Rich Repeat Kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease. Hum Mol Genet 18: 4390-404.
35. Johnston TH, Millar Z, Huot P, Wagg K, Thiele S, et al. (2012) A novel MDMA analogue, UWA-101, that lacks psychoactivity and cytotoxicity, enhances L-DOPA benefit in parkinsonian primates. FASEB J 26: 2154-2163.
36. Wakabayashi K, Tanji K, Odagiri S, Miki Y, Mori F, et al. (2013) The Lewy body in Parkinson's disease and related neurodegenerative disorders. Mol Neurobiol 47: 495-508.
37. Pal R, Miranda M, Narayan M (2011) Nitrosative stress-induced Parkinsonian Lewy-like aggregates prevented through polyphenolic phytochemical analog intervention. Biochem Biophys Res Commun. 404: 324-9.
38. Altun D, Uysal H, Ayar A, Askin H (2011) Removal of the toxic effects of chlormadinon acetate on the development of Drosophila melanogaster via the use of nordihydroguaiaretic acid. Toxicol Ind Health 27: 29-33.
39. Miquel J, Fleming J, Economos AC (1982) Antioxidants, metabolic rate and aging in Drosophila. Arch Gerontol Geriatr 1: 159-165.
40. Siddique YH, Ara G, Jyoti S, Afzal M (2012) The dietary supplementation of nordihydroguaiaretic acid (NDGA) delayed the loss of climbing ability in Drosophila model of Parkinson's disease. J Diet Suppl 9: 1-8.
41. Song S, Jang S, Park J, Bang S, Choi S, et al. (2013) Characterization of PINK1 (PTEN-induced putative kinase 1) mutations associated with Parkinson disease in mammalian cells and Drosophila. J Biol Chem 288: 5660-5672.
42. Sere YY, Regnacq M, Colas J, Berges T (2010) A Saccharomyces cerevisiae strain unable to store neutral lipids is tolerant to oxidative stress induced by a-synuclein. Free Radic Biol Med 49: 1755-64.
43. Harrington AJ, Hamamichi S, Caldwell GA, Caldwell KA (2010) C. elegans as a model organism to investigate molecular pathways involved with Parkinson's disease. Dev Dyn 239: 1282-1295.
44. Muda K, Bertinetti D, Gesellchen F, Hermann JS, von Zweydorf F, et al. (2014) Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. Proc Natl Acad Sci U S A 111: E34-43.
45. Tsika E, Moore DJ (2013) Contribution of GTPase activity to LRRK2-associated Parkinson disease. Small GTPases 4: 164-170.
46. Biosa A, Trancikova A, Civiero L, Glauser L, Bubacco L, et al. (2013) GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. Hum Mol Genet 22: 1140-1156.
47. Vital A, Vital C (2012) Mitochondria and peripheral neuropathies. J Neuropathol Exp Neurol 71: 1036-1046.
48. Manzoni C (2012) LRRK2 and autophagy: a common pathway for disease. Biochem Soc Trans 40: 1147-1151.

49. Mills RD, Mulhern TD, Liu F, Culvenor JG, Cheng HC (2014) Prediction of the Repeat Domain Structures and Impact of Parkinsonism-Associated Variations on Structure and Function of all Functional Domains of Leucine-Rich Repeat Kinase 2 (LRRK2). Hum Mutat 35: 395-412.
50. Zucca FA, Basso E, Cupaioli FA, Ferrari E, Sulzer D, et al. (2014) Neuromelanin of the human substantia nigra: an update. Neurotox Res 25: 13-23.
51. Li J1, Yang J, Zhao P, Li S, Zhang R, et al. (2012) Neuromelanin enhances the toxicity of Î±-synuclein in SK-N-SH cells. J Neural Transm 119: 685-691.
52. Rabey JM, Hefti F (1990) Neuromelanin synthesis in rat and human substantia nigra. J Neural Transm Park Dis Dement Sect 2: 1-14.
53. Hirsch EC, Graybiel AM, Agid Y (1989) Selective vulnerability of pigmented dopaminergic neurons in Parkinson's disease. Acta Neurol Scand Suppl 126: 19-22.
54. Hirsch E, Graybiel AM, Agid YA (1988) Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature 334: 345-348.
55. Fedorow H, Tribl F, Halliday G, Gerlach M, Riederer P, et al. (2005) Neuromelanin in human dopamine neurons: comparison with peripheral melanins and relevance to Parkinson's disease. Prog Neurobiol 75: 109-124.
56. Graybiel AM, Hirsch EC, Agid Y (1990) The nigrostriatal system in Parkinson's disease. Adv Neurol 53: 17-29.
57. Oster S, Radad K, Scheller D, Hesse M, Balanzew W, et al. (2014) Rotigotine protects against glutamate toxicity in primary dopaminergic cell culture. Eur J Pharmacol 724: 31-42.
58. Sun XR, Chen L, Chen WF, Xue Y, Yung WH (2013) Electrophysiological and behavioral effects of group III metabotropic glutamate receptors on pallidal neurons in normal and parkinsonian rats. Synapse 67: 831-838.
59. Xiong N, Long X, Xiong J, Jia M, Chen C, et al. (2012) Mitochondrial complex I inhibitor rotenone-induced toxicity and its potential mechanisms in Parkinson's disease models. Crit Rev Toxicol 42: 613-632.
60. Bagga P, Chugani AN, Varadarajan KS, Patel AB (2013) In vivo NMR studies of regional cerebral energetics in MPTP model of Parkinson's disease: recovery of cerebral metabolism with acute levodopa treatment. J Neurochem 127: 365-377.
61. Mandybur GT, Miyagi Y, Yin W, Perkins E, Zhang JH (2003) Cytotoxicity of ventricular cerebrospinal fluid from Parkinson patients: correlation with clinical profiles and neurochemistry. Neurol Res 25: 104-111.
62. Feigin A, Kaplitt MG, Tang C, Lin T, Mattis P, et al. (2007) Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. Proc Natl Acad Sci U S A 104: 19559-19564.
63. Yansong W, Wei W, Dongguo L, Mi L, Peipei W, et al. (2014) IGF-1 Alleviates NMDA-Induced Excitotoxicity in Cultured Hippocampal Neurons Against Autophagy via the NR2B/PI3K-AKT-mTOR Pathway. J Cell Physiol.
64. Dunfield D, Haas K (2009) Metaplasticity governs natural experience-driven plasticity of nascent embryonic brain circuits. Neuron 64: 240-250.
65. Namba T, Ming GL, Song H, Waga C, Enomoto A, et al. (2011) NMDA receptor regulates migration of newly generated neurons in the adult hippocampus via Disrupted-In-Schizophrenia 1 (DISC1). J Neurochem 118: 34-44.
66. Volbracht C, van Beek J, Zhu C, Blomgren K, Leist M (2006) Neuroprotective properties of memantine in different in vitro and in vivo models of excitotoxicity. Eur J Neurosci 23: 2611-2622.
67. Han Z, Yang JL, Jiang SX, Hou ST, Zheng RY (2013) Fast, non-competitive and reversible inhibition of NMDA-activated currents by 2-BFI confers neuroprotection. PLoS One 8: e64894.
68. Jablonski AM, Kalb RG (2013) GluA1 promotes the activity-dependent development of motor circuitry in the developing segmental spinal cord. Ann N Y Acad Sci 1279: 54-59.
69. Cserép C, Szabadits E, Szőnyi A, Watanabe M, Freund TF, et al (2012) NMDA receptors in GABAergic synapses during postnatal development. PLoS One 7: e37753.
70. Kumari S, Mehta SL, Li PA (2012) Glutamate induces mitochondrial dynamic imbalance and autophagy activation: preventive effects of selenium. PLoS One 7: e39382.
71. Fabrizi C, Somma F, Pompili E, Biagioni F, Lenzi P, et al. (2012) Role of autophagy inhibitors and inducers in modulating the toxicity of trimethyltin in neuronal cell cultures. J Neural Transm 119: 1295-1305.
72. Herrando-Grabulosa M, Casas C, Aguilera J (2013) The C-terminal domain of tetanus toxin protects motoneurons against acute excitotoxic damage on spinal cord organotypic cultures. J Neurochem 124: 36-44.
73. Luchkina NV, Sallert M, Clarke VR, Taira T, Lauri SE (2013) Mechanisms underlying induction of LTP-associated changes in short-term dynamics of transmission at immature synapses. Neuropharmacology 67: 494-502.
74. Kulbe JR, Mulcahy Levy JM, Coultrap SJ, Thorburn A, Bayern KU (2014) Excitotoxic glutamate insults block autophagic flux in hippocampal neurons. Brain Res 1542: 12-19.
75. An T, Shi P, Duan W, Zhang S, Yuan P, et al. (2014) Oxidative Stress and Autophagic Alteration in Brainstem of SOD1-G93A Mouse Model of ALS. Mol Neurobiol.
76. Paus T, Pesaresi M, French L (2014) White matter as a transport system. Neuroscience. 2014 Feb 7. pii: S0306-4522(14)00076-1.
77. Chia PH, Li P, Shen K (2013) Cell biology in neuroscience: cellular and molecular mechanisms underlying presynapse formation. J Cell Biol 203: 11-22.
78. Hu DJ, Baffet AD, Nayak T, Akhmanova A, Doye V, et al. (2013) Dynein recruitment to nuclear pores activates apical nuclear migration and mitotic entry in brain progenitor cells. Cell 154: 1300-1313.
79. Moughamian AJ, Osborn GE, Lazarus JE, Maday S, Holzbaur EL (2013) Ordered recruitment of dynactin to the microtubule plus-end is required for efficient initiation of retrograde axonal transport. J Neurosci 33: 13190-13203.
80. Schwarz TL (2013) Mitochondrial trafficking in neurons. Cold Spring Harb Perspect Biol 5.
81. Shankar Sadasivan, Zhiqun Zhang, Stephen F Larner, Ming C Liu, Wenrong Zheng, et al. (2010) Acute NMDA toxicity in cultured rat cerebellar granule neurons is accompanied by autophagy induction and late onset autophagic cell death phenotype. BMC Neuroscience 11: 21.
82. Law BM, Spain VA, Leinster VH, Chia R, Beilina A, et al. (2014) A direct interaction between leucine-rich repeat kinase 2 and specific ß-tubulin isoforms regulates tubulin acetylation. J Biol Chem 289: 895-908.
83. Esteves AR, Gozes I, Cardoso SM (2014) The rescue of microtubule-dependent traffic recovers mitochondrial function in Parkinson's disease. Biochim Biophys Acta 1842: 7-21.
84. Selvakumar GP, Janakiraman U, Essa MM, Thenmozhi AJ, Manivasagam T (2014) Escin attenuates behavioral impairments, oxidative stress and inflammation in a chronic MPTP/probenecid mouse model of Parkinson?s disease. Brain Res.
85. Samantaray S, Knaryan VH, Shields DC, Banik NL (2013) Critical role of calpain in spinal cord degeneration in Parkinson's disease. J Neurochem 127: 880-890.
86. Hurley MJ, Brandon B, Gentleman SM, Dexter DT (2013) Parkinson's disease is associated with altered expression of CaV1 channels and calcium-binding proteins. Brain 136: 2077-2097.
87. Loopuijt LD, Schmidt WJ (1998) The role of NMDA receptors in the slow neuronal degeneration of Parkinson's disease. Amino Acids 14: 17-23.
88. Brothers HM, Bardou I, Hopp SC, Marchalant Y, Kaercher RM, et al. (2013) Time-Dependent Compensatory Responses to Chronic Neuroinflammation in Hippocampus and Brainstem: The Potential Role of Glutamate Neurotransmission. J Alzheimers Dis Parkinsonism 3: 110.

89. Nair AG, Gutierrez-Arenas O, Eriksson O, Jauhiainen A, Blackwell KT, et al. (2014) Modeling intracellular signaling underlying striatal function in health and disease. Prog Mol Biol Transl Sci 123: 277-304.
90. Petersen MS, Bech S, Christiansen DH, Schmedes AV, Halling J (2014) The role of vitamin D levels and vitamin D receptor polymorphism on Parkinson's disease in the Faroe Islands. Neurosci Lett 561: 74-79.
91. Peterson AL, Murchison C, Zabetian C, Leverenz JB, Watson GS, et al. (2013) Memory, mood, and vitamin d in persons with Parkinson's disease. J Parkinsons Dis 3: 547-555.
92. Li YC, Chen Y, Liu W, Thadhani R (2013) MicroRNA-mediated mechanism of vitamin D regulation of innate immune response. J Steroid Biochem Mol Biol.
93. Dimitrov V, Salehi-Tabar R, An BS, White JH (2013) Non-classical mechanisms of transcriptional regulation by the vitamin D receptor: Insights into calcium homeostasis, immune system regulation and cancer chemoprevention. J Steroid Biochem Mol Biol.
94. Török R, Török N, Szalardy L, Plangar I, Szolnoki Z, et al. (2013) Association of vitamin D receptor gene polymorphisms and Parkinson's disease in Hungarians. Neurosci Lett 551: 70-74.
95. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Noya M, et al. (2013) Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease. Am J Clin Nutr 97: 1004-1013.
96. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Noya M, et al. (2013) The transcriptomic response of mixed neuron-glial cell cultures to 1,25-dihydroxyvitamin d3 includes genes limiting the progression of neurodegenerative diseases. J Alzheimers Dis. 35: 553-564.
97. Nissou MF, Brocard J, El Atifi M, Guttin A, Andrieux A, et al. (2013) The transcriptomic response of mixed neuron-glial cell cultures to 1,25-dihydroxyvitamin d3 includes genes limiting the progression of neurodegenerative diseases. J Alzheimers Dis 35: 553-564.
98. Nakahashi O, Yamamoto H, Tanaka S, Kozai M, Takei Y, et al. (2014) Short-term dietary phosphate restriction up-regulates ileal fibroblast growth factor 15 gene expression in mice. J Clin Biochem Nutr 54: 102-108.
99. Rezende LR, Delgado EF, Júnior AR, Gasparin G, Jorge EC, et al. (2013) Expression of 1alpha-HYD and 24-HYD in bovine kidney mediated by vitamin D3 supplementation. Genet Mol Res 12: 6611-6618.
100. Hummel DM, Fetahu IS, Gröschel C, Manhardt T, Kállay E (2013) Role of proinflammatory cytokines on expression of vitamin D metabolism and target genes in colon cancer cells. J Steroid Biochem Mol Biol.
101. Jacobsen R, Abrahamsen B, Bauerek M, Holst C, Jensen CB, et al. (2013) The influence of early exposure to vitamin D for development of diseases later in life. BMC Public Health 13: 515.
102. Plum S, Helling S, Theiss C, Leite RE, May C, et al. (2013) Combined enrichment of neuromelanin granules and synaptosomes from human substantia nigra pars compacta tissue for proteomic analysis. J Proteomics 94: 202-206.
103. Baeke F, van Etten E, Gysemans C, Overbergh L, Mathieu C (2008) Vitamin D signaling in immune-mediated disorders: Evolving insights and therapeutic opportunities. Mol Aspects Med 29: 376-387.
104. AlGhamdi K, Kumar A, Moussa N (2013) The role of vitamin D in melanogenesis with an emphasis on vitiligo. Indian J Dermatol Venereol Leprol 79: 750-758.
105. Lee CH, Wu SB, Hong CH, Yu HS, Wei YH (2013) Molecular Mechanisms of UV-Induced Apoptosis and Its Effects on Skin Residential Cells: The Implication in UV-Based Phototherapy. Int J Mol Sci 14: 6414-6435.
106. Lacour JP (1992) [Culture of human melanocytes. Its contribution to the knowledge of melanocyte physiology]. Pathol Biol (Paris) 40: 114-120.
107. Liu Y, Li YW, Tang YL, Liu X, Jiang JH, et al. (2013) Vitamin D: preventive and therapeutic potential in Parkinson's disease. Curr Drug Metab 14: 989-993.
108. van den Bos F, Speelman AD, Samson M, Munneke M, Bloem BR, et al. (2013) Parkinson's disease and osteoporosis. Age Ageing 42: 156-162.
109. Newmark HL, Newmark J (2007) Vitamin D and Parkinson's disease--a hypothesis. Mov Disord 22: 461-468.
110. Marques RC, Dórea JG, Leão RS, Dos Santos VG, Bueno L, et.al (2012) Role of methylmercury exposure (from fish consumption) on growth and neurodevelopment of children under 5 years of age living in a transitioning (tin-mining) area of the western Amazon, Brazil. Arch Environ Contam Toxicol 62: 341-50.
111. McGrath JJ, Féron FP, Burne TH, Mackay-Sim A, Eyles DW (2004) Vitamin D3-implications for brain development. J Steroid Biochem Mol Biol 89-90: 557-60.
112. Maskos U, McKay RD (2003) Neural cells without functional N-Methyl-D-Aspartate (NMDA) receptors contribute extensively to normal postnatal brain development in efficiently generated chimaeric NMDA R1 -/- <--> +/+ mice. Dev Biol 262: 119-36.
113. Taniura H, Ito M, Sanada N, Kuramoto N, Ohno Y, et al. (2006) Chronic vitamin D3 treatment protects against neurotoxicity by glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons. J Neurosci Res 83: 1179-1189.
114. da Costa NM (2013) Diversity of thalamorecipient spine morphology in cat visual cortex and its implication for synaptic plasticity. J Comp Neurol 521: 2058-2066.
115. Slominski AT, Janjetovic Z, Kim TK, Wright AC, Grese LN, et al. (2012) Novel vitamin D hydroxyderivatives inhibit melanoma growth and show differential effects on normal melanocytes. Anticancer Res 32: 3733-3742.