LRRK2 G2019S Induces Anxiety/Depression-like Behavior before the Onset of Motor Dysfunction with 5-HT_{1A} Receptor Upregulation in Mice

Juhee Lim,¹ Yeojin Bang,¹ Jong-Hyun Choi,¹ Arum Han,² Min-Soo Kwon,² Kwang Hyeon Liu,³ and Hyun Jin Choi¹

¹College of Pharmacy and Institute of Pharmaceutical Sciences, ²Department of Pharmacology, School of Medicine, Cha University, Seongnam, Gyeonggi-do 13488, Republic of Korea, and ³BK21 Plus Kyungpook National University Multi-Omics Based Creative Drug Research Team, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene are the most common genetic cause of Parkinson's disease (PD). The neuropathology of *LRRK2* mutation-related PD, including increased dopaminergic neurodegeneration and Lewy bodies, is indistinguishable from that of idiopathic PD. The subtle nonmotor phenotypes of *LRRK2* mutation-related PD have not been fully evaluated. In the present study, we examined anxiety/depression-like behaviors and accompanying neurochemical changes in differently aged transgenic (Tg) mice expressing human mutant *LRRK2* G2019S. Through multiple behavioral tests, including light–dark test, elevated plus maze, sucrose preference test, forced swimming test, and tail-suspension test, we found that anxiety/depression-like behavior appeared in middle-aged (43–52 weeks) Tg mice before the onset of PD-like motor dysfunction. These behavioral tests were performed using both male and female mice, and there were no sex-related differences in behavioral changes in the middle-aged Tg mice. Along with behavioral changes, serotonin levels also significantly declined in the hippocampus of Tg mice. Additionally, increases in the expression of the 5-HT_{1A} receptor (5-HT_{1A}R) grew more significant with aging and were detected in the hippocampus, amygdala, and dorsal raphe nucleus. *In vitro* study using the serotonergic RN46A and hippocampal HT22 cells showed that 5-HT_{1A}R upregulation was related to enhanced expression of *LRRK2* G2019S and was attenuated by the *LRRK2* inhibitor LRRK2-IN-1. Wild-type *LRRK2* had no significant effect on 5-HT_{1A}R transcription. The present study provides the first *in vivo* and *in vitro* evidence demonstrating abnormal regulation of 5-HT_{1A}R along with the manifestation of anxiety/depression-like, nonmotor symptom in PD related to *LRRK2*.

Key words: 5-HT_{1A} receptor; anxiety; depression; *LRRK2*; Parkinson's disease

Significance Statement

Parkinson's disease (PD), the second most common neurodegenerative disorder, is clinically characterized by motor dysfunctions. In most cases, various nonmotor symptoms present several years before the onset of the classical motor features of PD and severely affect the quality of life of patients. Here, we demonstrate the causative role of *leucine-rich repeat kinase 2* (*LRRK2*), a common PD-linked mutation, in the development of anxiety/depression-like behaviors. We found that age-dependent 5-HT_{1A} receptor upregulation in the hippocampus, amygdala, and dorsal raphe nucleus is accompanied by the expression of the *LRRK2* mutant phenotype. Our findings demonstrating a potential mechanism for nonmotor psychiatric symptoms produced by *LRRK2* mutation suggest that directly targeting the 5-HT_{1A} receptor can improve the therapeutic efficacy of drugs for PD-associated depression.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is diagnosed according to the progressive

loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc). PD is clinically characterized by such motor symptoms as bradykinesia, tremor, rigidity, and postural instability; however, many patients with PD experience nonmotor

Received Nov. 10, 2015; revised Dec. 26, 2017; accepted Dec. 26, 2017.

Author contributions: M.-S.K. and H.J.C. designed research; J.L., Y.B., and A.H. performed research; J.-H.C., K.H.L., and H.J.C. contributed unpublished reagents/analytic tools; J.L., Y.B., M.-S.K., and H.J.C. analyzed data; J.L. and H.J.C. wrote the paper.

The authors declare no competing financial interests.

This work was supported by the GRRC program of Gyeonggi province (GRRC-CHA2017-A02) and the Ministry of

Science, ICT & Future Planning through the National Research Foundation (2015R1D1A1A01059598 and 2015M3A9E1028326) of Korea.

Correspondence should be addressed to Dr. Hyun Jin Choi, College of Pharmacy, Cha University, Seongnam, Gyeonggi-do 13488, Republic of Korea. E-mail: hjchoi3@cha.ac.kr.

DOI:10.1523/JNEUROSCI.4051-15.2017

Copyright © 2018 the authors 0270-6474/18/381611-11\$15.00/0

symptoms, such as chronic fatigue, anxiety/depression, sleep disturbances, autonomic dysfunction, and cognitive decline. In most cases, the nonmotor symptoms precede the classical motor features by years (Chaudhuri et al., 2006). Patients and clinicians alike may not realize that nonmotor symptoms, which are often overlooked without proper treatment, are linked to PD. Moreover, the increased comorbidity of nonmotor symptoms is related to high motoric symptom severity, and some researchers suggest they are a risk factor for PD (Chaudhuri and Odin, 2010; Martínez-Martín and Damián, 2010; Meissner et al., 2011; Kim et al., 2014). Approximately 30–50% of patients with PD also have depression (Perez-Lloret and Rascol, 2012), which is among the most taxing nonmotor symptoms affecting quality of life (Politis and Niccolini, 2015). Despite the relatively high incidence of PD, the pathophysiology of depression and other nonmotor symptoms associated with PD remains unclear. In this regard, the study of nonmotor symptoms in PD animal models with known associated neuropathology is critical to further characterize the mechanisms implicated in these symptoms and to identify both specific early biomarkers and efficient therapeutic strategies.

Mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene are the most common cause of autosomal-dominant PD (Gandhi et al., 2009). *LRRK2* encodes 2527 aa proteins with multiple functional domains: a leucine-rich repeat domain, a Roc (Ras guanine triphosphatase) domain, a C-terminal of the Roc domain, a WD40 (β -transducin repeat) domain, and a tyrosine kinase catalytic domain. G2019S increases LRRK2 kinase activity and is the most common mutation identified. Patients with PD with the G2019S mutation are clinically indistinguishable from those with sporadic PD (Healy et al., 2008). For example, progressive neurodegeneration of DAergic cells is detected in the SNpc of *LRRK2* G2019S transgenic (*LRRK2*^{G2019S} Tg) mice in an age-dependent manner (Chen et al., 2012). Meanwhile, although *LRRK2* transgenic models recapitulate the pathophysiology of motor-related symptoms in PD, the subtle, nonmotor phenotypes have not been fully evaluated.

LRRK2 is highly expressed in several brain regions, including the striatum, hippocampus, amygdala, and cerebral cortex (Taymans et al., 2006). Recent evidence suggests that *LRRK2* mutation is associated not only with DAergic neuronal death but also with modulation of various neurotransmitters. Its effects include (1) neuronal dysfunction of DAergic neurotransmission even in the absence of neuronal loss (Chou et al., 2014); (2) enhanced glutamatergic synaptic activity, which renders neurons more vulnerable to excitotoxicity (Plowey et al., 2014); and (3) elevated serotonin [5-hydroxytryptamine (5-HT)] transporter binding in striatal and cortical regions preceding motor dysfunction (Cheshire et al., 2015). Although the motor symptoms of PD are largely related to late-phase nigrostriatal DAergic neurodegeneration, the pathological processes in nonmotor symptoms of PD can be related to non-DAergic mechanisms (Kish, 2003; Politis and Niccolini, 2015).

Here, we used *LRRK2*^{G2019S} Tg mice to analyze the nonmotor behavioral changes in a mouse model known to recapitulate PD pathophysiology (Ramonet et al., 2011). We hypothesized that the nonmotor symptoms would be detected before the appearance of motor dysfunction. Toward this goal, we evaluated anxiety/depression-like behaviors of Tg mice and their non-Tg (NTg) littermates in terms of age and identified relevant changes in the putative underlying neurotransmitter systems and signaling pathways. We focused on the hippocampus and amygdala because these regions play an integral role in the regulation of emotion. To better study the neurochemical changes related to

LRRK2 mutation, we evaluated the effect of *LRRK2*^{G2019S} on serotonergic signaling in serotonergic neuronal RN46A cells (White et al., 1994) and hippocampal HT22 cells (Heiser et al., 2002; Hadjighassem et al., 2009).

Materials and Methods

Antibodies and reagents

The primary antibodies used in this study were anti-LRRK2 (NB300-268, Novus Biologicals), anti-5-HT_{1A} receptor (GTx104703, GeneTex), anti-tyrosine hydroxylase (TH; sc-25269, Santa Cruz Biotechnology), and anti-GAPDH (#2118, Cell Signaling Technology). The secondary antibodies (all from Santa Cruz Biotechnology) were horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (sc-2004). The enhanced chemiluminescent HRP substrate (ECL) solutions were obtained from Millipore (#WBKLS0500). All other chemicals were of reagent grade and purchased from Sigma-Aldrich.

Animals and genotyping

All animal care and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) guidelines of Cha University (IACUC 150068). Animals were maintained in a light-controlled room (reversed 12 h light/dark cycle, with the lights turned on at 7:00 A.M.). Food and water were given *ad libitum*.

LRRK2 G2019S mutant (*LRRK2*^{G2019S}) Tg and NTg littermate mice were purchased from The Jackson Laboratory [B6;C3-Tg(PDGFB-LRRK2*G2019S)340Djmo/J] and mated in the university's experimental animal room. In this study, the *LRRK2*^{G2019S} Tg mice have a minimal cytomegalovirus enhancer, human platelet-derived growth factor, and B polypeptide (PDGFB) promoter/enhancer elements to drive the expression of a mutated full-length human *LRRK2* (*LRRK2**G2019S) cDNA (Ramonet et al., 2011). Genotyping was performed via PCR using the following oligonucleotide primers: 5'-ATTACCATGGTTCGAGGTGA-3' (forward) and 5'-CAAGTGTCTGTCAGGAAGGTT-3' (reverse) for *LRRK2*-G2019S; 5'-CTAGGCCACAGAATTGAAAGATCT-3' (forward) and 5'-GTAGGTGGAATTCTAGCATCATCC-3' (reverse) for an internal-positive control.

Cell culture and transient transfection

RN46A cells were purchased from the European Collection of Cell Cultures (Salisbury, UK), and HT22 cells were provided by Professor S.H. Sung (Seoul National University, Korea). The culture conditions for each cell line were as follows: RN46A cells were grown in DMEM/F12 containing 10% FBS, 2 mM glutamine, and 0.25 mg/ml geneticin at 33°C; and HT22 cells were grown in DMEM containing 10% FBS, 100 IU/L penicillin, and 10 μ g/ml streptomycin at 37°C. All cells were incubated in a humidified atmosphere of 95% air and 5% CO₂.

Reporter gene assay

A dual-luciferase reporter assay system (Promega) was used to determine 5-HT_{1A}R promoter activity. RN46A and HT22 cells were incubated on 48-well culture plates for 24 h and then cotransfected with 5-HT_{1A}R luciferase (*firefly*), pRL-TK (*renilla*), and pcDNA3.1, pcDNA3.1-myc-LRRK2, or pcDNA3.1-myc-LRRK2-G2019S plasmid vectors using polyethylenimine (Polysciences), according to the manufacturer's instructions. *Firefly* and *renilla* luciferase activities in the cell lysates were determined using the multimode microplate reader Synergy MX (BioTek). Professor P.R. Albert (University of Ottawa, Canada) kindly provided the 5-HT_{1A}R luciferase plasmid.

Behavioral testing

LRRK2^{G2019S} Tg mice and NTg littermate control mice were divided into three groups according to age as follows: young, 9–19 weeks; middle-aged, 43–52 weeks; or old, 65–83 weeks. We used both male and female mice in this study, and analyzed the experimental data without distinction of sex. All tasks were performed by individuals blinded to the genotype of the animals.

Rotarod test. Locomotor activity of mice was assessed using a rotarod system (Rota Rod-R V2.0, B.S. Technolab). Mice were conditioned on the rod (diameter, 3.5 cm) with an increasing speed from 4 to 40 rpm (accelerated by 1 rpm per 5 s). Motor ability was measured as the time the

Figure 1. Evaluation of age-related motor function and striatal TH levels of *LRRK2*^{G2019S} Tg and NTg mice. **A**, Immunohistochemical staining for LRRK2 in the OB, amygdala, cerebral cortex, dorsal and ventral parts of the hippocampus, substantia nigra, and DRN, using LRRK2 antibody that detects both mouse and human LRRK2. Scale bar, 100 μ m. **B**, **C**, *LRRK2*^{G2019S} Tg and NTg mice were divided into three experimental groups according to age: young (9–19 weeks; NTg, $n = 13$; Tg, $n = 15$), middle-aged (43–52 weeks; NTg, $n = 20$; Tg, $n = 28$), and old (65–83 weeks; NTg, $n = 5$; Tg, $n = 10$). Latency to fall in the accelerating rotarod paradigm (**B**). The striatum was dissected, and TH protein levels were analyzed by Western blot (**C**). α Actin and GAPDH were used as loading controls. All data were quantified by densitometric analysis. Results are represented as mean $\pm$ SEM. ** $p < 0.01$ by Student's t test. DRN, dorsal raphe nucleus; OB, olfactory bulb; SN, substantia nigra.

mouse spent on the rod until it fell off, ≤ 300 s. Before the measurement, mice were familiarized to the rod with a constant speed of 4 rpm for 3 min. The average latency time of three trials was calculated for statistical analyses. Trials were conducted at 20 min intervals.

Light–dark test. The light–dark test was performed as previously described (Han et al., 2014). A cage was divided by a partition with a door leading to the dark (10×30 cm) and light (20×30 cm) chambers. One chamber was brightly illuminated by white diodes (390 lux), whereas the other chamber was dimly lit (2 lux). Each mouse was placed under the passage hole between the two chambers, facing the brighter side, and allowed to move freely between the two chambers for 10 min. The total number of transitions and the time spent in each chamber were recorded.

Elevated plus maze. The maze apparatus comprised two open arms (30×5 cm) and two closed arms (30×5 cm; surrounded by 20-cm-high walls) arranged in a cross. The central platform (5×5 cm) served as the convergence site of the four arms. The apparatus was elevated 30 cm above the floor, and a video camera was attached above the maze to automatically record the trials. Each mouse was placed on the central platform facing an open arm and allowed to move freely for 5 min. An automated computer system connected to the video camera (EthoVision XT9 video tracking system; EthoVision, Version 9, Noldus) scored the total amount of time spent in each arm and the total number of entries into each arm.

Sucrose preference test. The sucrose preference test was performed to assess anhedonic behavior (Han et al., 2014). Mice were housed in individual cages and were given *ad libitum* access to two bottles, with one containing 100 ml of 1% sucrose solution and one containing 100 ml of distilled water. During the test period, the two bottles were randomly changed every 12 h. After 48 h, the consumption volumes (ml) of sucrose solution (V1) and distilled water (V2) were recorded, and the preference of sucrose (%) was calculated as $[V1/(V1 + V2)] \times 100$.

Forced swimming test. Each mouse was gently placed in a glass cylinder (diameter, 18 cm; height, 26 cm) filled with 15 cm of water at room temperature (22 – 25°C). The time spent immobile during 5 min was measured with a stopwatch. At the end of the test, mice were dried with a paper towel and placed in a cage with normal bedding under warm light (Han et al., 2014).

Tail-suspension test. Each mouse was suspended in the air using adhesive tape attached to the tail and fixed to a wire 30 cm above the surface of a wooden box. The time spent immobile during 5 min was measured with a stopwatch (Han et al., 2014).

Western blot analysis

For Western blot analysis of mouse brain regions, mice were killed and the brain tissues were quickly dissected. Striatal and hippocampal tissues were prepared using a micropunch (inner diameter, 2 mm) from 2 mm slice (bregma 0 to 2 mm for striatum; bregma -2 mm to -4 mm for

Figure 2. Age-related anxiety/depression-like behaviors in *LRRK2*^{G2019S} Tg mice. *LRRK2*^{G2019S} Tg and NTg mice were divided into three experimental groups according to age: young (9–19 weeks), middle-aged (43–52 weeks), and old (65–83 weeks). **A**, Light–dark test. The time spent in each chamber of the light–dark box (left) and total number of transitions (right) were calculated for a 10 min session. The number of animals in each group was as follows: 9–19 weeks: NTg, *n* = 13; Tg, *n* = 14; 43–52 weeks: NTg, *n* = 9; Tg, *n* = 11; 65–83 weeks: NTg, *n* = 5; Tg, *n* = 9. **B**, EPM. The percentage of entries into the open (left) and closed arms (right) over 5 min was recorded. The number of animals in each group was as follows: 9–19 weeks: NTg, *n* = 13; Tg, *n* = 14; 43–52 weeks: NTg, *n* = 9; Tg, *n* = 9; 65–83 weeks: NTg, *n* = 4; Tg, *n* = 6. **C**, Sucrose preference test. The preference of sucrose (%) was calculated as described in Materials and Methods. The number of animals in each group was as follows: 9–19 weeks: NTg, *n* = 6; Tg, *n* = 6; 43–52 weeks: NTg, *n* = 7; Tg, *n* = 7; 65–83 weeks: NTg, *n* = 3; Tg, *n* = 4. **D**, **E**, Time spent immobile during 5 min was measured in FST (**D**) and TST (**E**). The number of animals in each group was as follows: 9–19 weeks: NTg, *n* = 12; Tg, *n* = 15; 43–52 weeks: NTg, *n* = 9; Tg, *n* = 9. All data were represented as mean ± SEM. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 by Student's *t* test.

hippocampus). They were then homogenized and lysed in immunoprecipitation lysis/RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, and 50 mM Tris-Cl, pH 7.5) containing a phosphatase inhibitor and protease inhibitor mixture (Roche) for 30 min in ice. Tissue lysates were centrifuged at 21,130 × *g* for 30 min at 4°C, and the supernatant proteins were quantified using Bradford's reagent (Bio-Rad). Equal amounts of protein (10–30 μg) were separated using 8%–12% SDS polyacrylamide gels and transferred onto polyvinylidene difluoride nitrocellulose membranes (Millipore). Membranes were first incubated with specific primary antibodies for 16–18 h, then with HRP-conjugated secondary antibodies for 1 h. Specific proteins were visualized using ECL detection kits (Millipore) and analyzed via a luminescent image analyzer (LAS-4000, Fujifilm). Each brain sample was obtained from 3–4 brain tissues, and Western blot analysis for each sample was performed in duplicate. Densitometric analysis was performed for each band obtained from all replicated experiments using ImageJ software (National Institutes of Health).

Immunohistochemistry

LRRK2^{G2019S} Tg mice and NTg littermate control mice were anesthetized and transcardially perfused with PBS and ice-cold 4% paraformaldehyde. Brain tissue samples for immunohistochemistry (IHC) were prepared as paraffin-embedded tissue. Sections of the paraffin-embedded tissue were placed onto glass slides and incubated with primary antibodies 5-HT_{1A}R (1:100; GeneTex) or LRRK2 (1:100; GeneTex) at 4°C overnight. Immunohistochemical staining was performed using EnVision system-HRP rabbit (DAKO) and DAB Quanto kit (Thermo Fisher Scientific) as the chromogen and counterstained with hematoxylin. To ensure consistency in labeling and imaging across different sections, all sections for a given experiment were immunostained at the same time; moreover, identical microscope settings were maintained for all sections (Leica). The intensity of 5-HT_{1A}R immunostaining was quantified using Fiji/ImageJ (National Institutes of Health) and Multi Gauge (Fujifilm) software. The boundaries of the 5-HT_{1A}R-positive cells were marked using digital separation of DAB-stained images, and the mean staining intensities (expressed as intensity/unit area) were measured. Four to eight images were analyzed for each section, and the results

were expressed relative to the staining intensity for the respective NTg section.

Quantification of 5-HT, dopamine, and their metabolites via high-pressure liquid chromatography and electrochemical detection. For high-pressure liquid chromatography and electrochemical detection (HPLC-ECD) analysis, the brain was removed, and the cerebral cortex and hypothalamus tissues were quickly dissected. Striatal and hippocampal tissues were prepared using a micropunch (inner diameter, 2 mm) from 2 mm slice (bregma 0 to 2 mm for striatum; bregma 0 to −2 mm for hippocampus). The 5-HT, dopamine (DA), and their major metabolites, namely, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), respectively, were measured in several brain regions (hippocampus, striatum, and cerebral cortex) via HPLC-ECD. Brain tissue blocks were rapidly dissected in ice and homogenized with ice-cold 0.4 M perchloric acid then incubated on ice for 1 h. After centrifugation at 21,130 × *g* for 30 min at 4°C, the supernatant was filtered using an appropriate column (#Sc1000-1Kt, SigmaPrep spin column). Filtered supernatants were directly injected onto the Nova-Pak C18 reversed-phase column. The mobile phase consisted of 0.1 M sodium phosphate monobasic, 0.1 mM EDTA, 1 mM sodium octyl sulfate, 0.003% trimethylamine, and 10% methanol at pH 3.7. The external standards for each analysis were DA (#H8502, Sigma-Aldrich), HVA (#H850217, Sigma-Aldrich), 5-HT (sc-298707, Santa Cruz Biotechnology), and 5-HIAA (#H8876, Sigma-Aldrich) in HPLC-grade water with 0.4 M perchloric acid. The flow rate was maintained at 1 ml/min. Chromatographic peak analysis was accompanied by identification of unknown peaks in a sample matched according to retention times.

Statistical analysis

All numerical results are represented as mean ± SEM. Statistical significance was determined with two-tailed, unpaired Student's *t* tests of the means for single comparisons. One-way ANOVA with *post hoc* Bonferroni test was performed using GraphPad Prism version 7.0 software to determine the statistical significance for multiple comparisons. A *p* value of <0.05 was considered statistically significant.

Figure 3. Measurement of 5-HT, DA, and their metabolites in various brain regions of *LRRK2*^{G2019S} Tg and NTg mice. *LRRK2*^{G2019S} Tg and NTg mice were divided into two experimental groups: young (9–19 weeks; NTg, *n* = 8; Tg, *n* = 8) and middle-aged (43–52 weeks; NTg, *n* = 8; Tg, *n* = 8). **A, B**, Five-HT and its main metabolite 5-HIAA (**A**) and DA and its metabolite HVA (**B**) were measured using HPLC-ECD, as described in Materials and Methods. The amounts of neurotransmitters are represented as pmol/mg tissue. Data are expressed as mean ± SEM. N.D., Not detected. **p* < 0.05, and ****p* < 0.001 by one-way ANOVA with *post hoc* Bonferroni test. DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; HVA, homovanillic acid.

Results

LRRK2^{G2019S} Tg mice show age-dependent changes in locomotor activity with downregulation of TH proteins in the striatum

In this study, we used *LRRK2*^{G2019S} Tg mice, which drive the expression of a mutated full-length human *LRRK2* (*LRRK2**G2019S) cDNA (Ramonet et al., 2011). The expression pattern of human G2019S-*LRRK2* mRNA in the *LRRK2*^{G2019S} Tg mouse brain is reported to be broadly similar to endogenous *LRRK2* mRNA; the highest expression was in the olfactory bulb (OB), cerebral cortex, hippocampus, striatum, and amygdala. Here, we additionally compared the expression pattern of *LRRK2* protein in the brains of young (16 weeks) *LRRK2*^{G2019S} Tg mice with that of NTg mice via IHC using an *LRRK2* antibody that detects both mouse and human *LRRK2*. A similar expression pattern of *LRRK2* protein was detected in the OB, amygdala, cerebral cortex, dorsal and ventral parts of the hippocampus, substantia nigra, and dorsal raphe nucleus (DRN) of *LRRK2*^{G2019S} Tg and NTg mice (Fig. 1A).

Because age-dependent motor dysfunction is considered the characteristic hallmark of patients with PD, we first examined motor disabilities in *LRRK2*^{G2019S} Tg mice according to age. The body weight of both NTg and Tg mice increased with age, and no significant differences were noted between NTg and Tg mice in the same age group (data not shown). Motor coordination/balance based on rotarod performance (Fig. 1B) was not different between the NTg and Tg mice in the young (9–19 weeks) and middle-aged (43–52 weeks) groups (latency to fall, *t*₍₂₆₎ = 0.5956, *p* = 0.5566 in the young group and *t*₍₄₄₎ = 0.3840, *p* = 0.7028 in the middle-aged group). However, in the old group (65–83

weeks), NTg mice stayed on the rotarod significantly longer than Tg mice (143.7 ± 28.76 s vs 69.93 ± 9.650 s, *t*₍₁₃₎ = 3.077, *p* = 0.0088), suggesting age-dependent impairment in motor function in the *LRRK2*^{G2019S} Tg mice.

The pathophysiological hallmark of motor symptoms in PD is the imbalance between the direct and indirect striatal pathways, which is caused by diminished DA afferents to the striatum (Escande et al., 2016). To verify whether the *LRRK2* mutation causes loss of DA afferents in the striatum with age, we examined the expression of the DAergic marker TH. In the striatum, significantly decreased TH protein levels were detected in old *LRRK2*^{G2019S} Tg mice relative to old NTg mice (Fig. 1C; *t*₍₆₎ = 5.900, *p* = 0.0011), but this decrease was not seen in the young and middle-aged groups (*t*₍₆₎ = 1.403, *p* = 0.1984 and *t*₍₆₎ = 0.6493, *p* = 0.5402, respectively).

LRRK2^{G2019S} Tg mice display anxiety/depression-like behaviors before motor dysfunction

Next, we evaluated whether nonmotor symptoms, particularly anxiety-like and depression-like behaviors, appeared in *LRRK2*^{G2019S} Tg mice and determined whether any of these symptoms occurred before the onset of PD-like motor dysfunction or downregulation of TH immunoreactivity in the striatum. Anxiety/depression-like behaviors were examined through multiple behavioral tests, namely the light–dark test, elevated plus maze (EPM), sucrose preference test, forced swimming test (FST), and tail-suspension test (TST).

To assess anxiety-related behaviors, we performed the light–dark test, which is based on the innate characteristics of a preference for dark places and a decrease in spontaneous exploratory behavior with an increase in anxiety among rodents. In the light–dark test, *LRRK2*^{G2019S} Tg mice in the young group did not show behavioral changes as assessed through the duration in the dark chamber or the number of transitions between light and dark compartments (Fig. 2A). However, Tg mice of the middle-aged group made fewer transitions compared with their NTg counterparts ($t_{(18)} = 2.299$, $p = 0.0337$). The elevated anxiety-like behavior of the Tg mice increased with age, such that the old Tg mice demonstrated an even greater immobility time in the dark compartment ($t_{(12)} = 6.690$, $p < 0.0001$) and fewer transitions between the two compartments ($t_{(12)} = 6.896$, $p < 0.0001$), compared with their NTg counterparts.

We also evaluated anxiety-related behavior with the EPM. A decrease in open-arm activity (duration and/or entries) reflects anxiety-like behavior. As shown in Figure 2B, *LRRK2*^{G2019S} Tg mice in the young group did not show anxiety-like behavior. However, compared with the NTg mice of the middle-aged group, their Tg counterparts showed a significant decrease in the percentage of open-arm entries ($t_{(16)} = 2.541$, $p = 0.0218$) and a significant increase in the percentage of closed-arm entries ($t_{(16)} = 2.385$, $p = 0.0432$). The same statistically significant differences were found between NTg and Tg mice in the old group (open-arm entries, $t_{(8)} = 2.709$, $p = 0.0267$; closed-arm entries, $t_{(8)} = 2.582$, $p = 0.0325$).

The sucrose preference test is a standard tool used to assess anhedonia. *LRRK2*^{G2019S} Tg mice in both the middle-aged and old groups exhibited a significant reduction in sucrose preference compared with their respective NTg counterparts (Fig. 2C; $t_{(12)} = 2.687$, $p = 0.0198$ and $t_{(5)} = 3.094$, $p = 0.0270$ in the middle-aged and old groups, respectively). This represents a decrease in the ability to experience pleasure, which is among the core symptoms of depression. As with the light–dark test and EPM, no significant differences were noted between the Tg and NTg mice in the young group.

Next, we evaluated depression-related behavior in *LRRK2*^{G2019S} Tg mice using the FST and TST, in which immobility appears to correlate with hopelessness-related depressive behavior. Because impaired motor function, as seen in the Tg mice of the old group (Fig. 1B), affects the time spent immobile in the experimental conditions of the FST and TST, the old group was excluded in these two experiments. In the FST, significant increases in the time spent immobile were noted in the Tg mice compared with the NTg mice as early as in the young group (Fig. 2D; $t_{(25)} = 4.374$, $p = 0.0002$ and $t_{(16)} = 2.972$, $p = 0.009$ in young and middle-aged groups, respectively). In the TST, Tg mice in the middle-aged group showed significant increases in immobility time compared with the NTg mice (Fig. 2E; $t_{(16)} = 2.786$, $p = 0.0132$). These results indicated that behavioral despair in the Tg mice becomes more pronounced with aging.

When we evaluated behavioral changes for each behavioral test in male mice only, female mice only, and both sexes without distinction, we found no sex-related differences in behavioral changes and an increased tendency to display anxiety/depression-like behavior in the middle-aged *LRRK2*^{G2019S} Tg mice compared with their NTg counterparts.

Five-HT levels are reduced in the hippocampus of middle-aged *LRRK2*^{G2019S} Tg mice

Because emotional symptoms without motor dysfunction were significantly detected in the middle-aged group, we further

evaluated relevant neurochemical changes in the young and middle-aged groups. Research has shown that monoaminergic dysfunction correlates with both depression and the efficacy of antidepressants (Celada et al., 2004; Jans et al., 2007). Thus, we first quantified 5-HT and its main metabolite, 5-HIAA, in various brain regions (the hippocampus, striatum, and cerebral cortex) of *LRRK2*^{G2019S} Tg and NTg mice in the young and middle-aged groups using the HPLC-ECD system. The brain regions were selected based on the relevance to anxiety/depression-like behaviors and the ease of sampling for HPLC analysis.

No significant differences in the levels of 5-HT and 5-HIAA in the hippocampus were noted between *LRRK2*^{G2019S} Tg and NTg mice in the young group (Fig. 3A). However, significantly lower amounts of 5-HT were detected in the hippocampus of Tg mice compared with that of the NTg mice in the middle-aged group ($F_{(5,42)} = 13.91$, $p = 0.0245$, ANOVA). Meanwhile, no significant differences were noted in the 5-HT or 5-HIAA levels in the striatum and cerebral cortex of Tg and NTg mice in any age group.

The amounts of DA and its metabolite HVA were also measured in the same brain regions of *LRRK2*^{G2019S} Tg and NTg mice in the young and middle-aged groups. As shown in Figure 3B, significantly lower amounts of DA were detected in the striatum of the Tg mice relative to that in the NTg mice in both young and middle-aged groups ($F_{(5,42)} = 154.6$, $p < 0.0001$; $F_{(5,42)} = 55.66$, $p < 0.0001$ in the young and middle-aged groups, respectively), and this decrease was more pronounced with age. The amount of HVA in the striatum of middle-aged Tg mice was also significantly decreased compared with that of NTg mice ($F_{(5,42)} = 128.2$, $p < 0.0001$). Meanwhile, no significant differences in the amounts of DA and HVA were found between the Tg and NTg mice in any other brain regions, in any age group.

Five-HT_{1A}R immunoreactivity in the hippocampus, amygdala, and DRN increases with age in *LRRK2*^{G2019S} Tg mice

The 5-HT_{1A} somatodendritic autoreceptor negatively regulates the serotonergic system by inhibiting the firing of raphe serotonergic neurons (Piñeyro and Blier, 1999), whereas postsynaptic 5-HT_{1A}Rs in the limbic and cortical brain regions mediate serotonergic neurotransmission (Yamamura et al., 2011). Therefore, changes in 5-HT_{1A}R expression are directly linked to the regulation of serotonergic signaling. We first compared 5-HT_{1A}R-immunoreactive cells in the dorsal and ventral parts of the hippocampus between *LRRK2*^{G2019S} Tg and NTg mice. Five-HT_{1A}R immunoreactivity in the dorsal (Fig. 4A) and ventral (Fig. 4B) parts of the hippocampus was higher in Tg mice than in the NTg mice in the middle-aged group. The relative intensity of 5-HT_{1A}R-positive cells in the dorsal and ventral hippocampal subareas was significantly higher in Tg mice than in the NTg mice in the middle-aged group (Fig. 4A: $t_{(6)} = 8.740$, $p = 0.0001$, $t_{(6)} = 4.234$, $p = 0.0017$, and $t_{(10)} = 5.340$, $p = 0.0003$ for dentate gyrus, CA1, and CA3 regions, respectively; Fig. 4B: $t_{(6)} = 5.879$, $p = 0.002$, $t_{(6)} = 10.69$, $p < 0.0001$, and $t_{(6)} = 2.684$, $p = 0.0229$ for dentate gyrus, CA1, and CA3 regions, respectively). Meanwhile, no significant differences were noted for the corresponding regions in the young group except the CA1 region of ventral hippocampus ($t_{(6)} = 3.143$, $p = 0.0200$). Results of the Western blot analysis also indicated a significant increase in 5-HT_{1A}R positivity in the hippocampus of Tg mice compared with that of NTg mice in the middle-aged group, but no significant difference was noted in the young group (Fig. 4C: $t_{(4)} = 0.2062$, $p = 0.8467$ and $t_{(10)} = 5.037$, $p = 0.0005$ for young and middle-aged groups, respectively).

Figure 5. Age-related 5-HT_{1A}R immunoreactivity in the amygdala of *LRRK2*^{G2019S} Tg and NTg mice. **A**, 5-HT_{1A}R expression in the amygdala is examined using IHC. **B**, Representative images of 5-HT_{1A}R-positive cells in the amygdala of *LRRK2*^{G2019S} Tg and NTg mice in young and middle-aged groups have been obtained using bright-field microscopy (Scale bar, 50 μ m). The relative intensity of 5-HT_{1A}R is calculated as described in Materials and Methods and expressed as a mean $\pm$ SEM. *** p < 0.001 by Student's *t* test.

We verified whether the same changes in 5-HT_{1A}R were observed in the amygdala, which is among the key brain regions regulating anxiety/depressive behaviors and expressing 5-HT_{1A}R (Morrison and Cooper, 2012). As shown in Figure 5, the relative intensity of 5-HT_{1A}R-positive cells in the amygdala was significantly higher in *LRRK2*^{G2019S} Tg mice than in the NTg mice in the overall cohort (Fig. 5B: $t_{(14)} = 7.607$, $p < 0.0001$). No significant difference was noted in the young group ($t_{(14)} = 1.411$, $p = 0.1800$).

To verify whether presynaptic 5-HT_{1A}R expression increases with age in the brain of Tg mice, we performed IHC for 5-HT_{1A}R in the DRN, which is a major source of ascending serotonergic innervation to the forebrain and limbic regions (Michélsen et al., 2008). Five-HT_{1A}R in the DRN is located at somatodendritic sites and provides negative feedback regulation of firing rates (Andrade et al., 2015). Significant upregulation of 5-HT_{1A}R was observed in the DRN of *LRRK2*^{G2019S} Tg mice in the middle-aged group (Fig. 6B: $t_{(10)} = 10.61$, $p < 0.0001$). Interestingly, a significant increase in the immunoreactivity of 5-HT_{1A}R was also detected in the DRN of Tg mice in the young group (Fig. 6A: $t_{(16)} = 4.838$, $p = 0.0002$).

LRRK2^{G2019S} overexpression increases 5-HT_{1A}R transcription in RN46A and HT22 cells

To verify whether *LRRK2*^{G2019S} directly upregulates 5-HT_{1A}R in serotonergic cells, we used RN46A and HT22 cells and evaluated the effect of *LRRK2*^{G2019S} overexpression on 5-HT_{1A}R transcription. RN46A cells are serotonergic raphe-derived neuronal cell lines expressing 5-HT_{1A}R (Storring et al., 1999). HT22 cells are immortalized mouse hippocampal cell lines that express 5-HT_{1A}R similar to postsynaptic 5-HT_{1A}R (Fricker et al., 2005; Xu et al., 2011). *LRRK2*^{G2019S} overexpression substantially increased 5-HT_{1A}R transcriptional activity in both RN46A (Fig. 7A: $F_{(2,61)} = 14.42$, $p < 0.0001$) and HT22 cells (Fig. 7B: $F_{(2,42)} = 14.42$, $p < 0.0001$). Interestingly, overexpression of wild-type (WT) *LRRK2* did not induce 5-HT_{1A}R transcriptional

Figure 6. Age-related 5-HT_{1A}R immunoreactivity in the DRN of *LRRK2*^{G2019S} Tg and NTg mice. **A**, 5-HT_{1A}R expression in the DRN is examined by IHC. **B**, Representative images of 5-HT_{1A}R-positive cells in the DRN of *LRRK2*^{G2019S} Tg and NTg mice in young and middle-aged groups have been obtained using bright-field microscopy (Scale bar, 50 μ m). The relative intensity of 5-HT_{1A}R is calculated as described in Materials and Methods and expressed as a mean $\pm$ SEM. *** p < 0.001 by Student's *t* test.

Figure 7. Five-HT_{1A}R transcriptional activity in RN46A and HT22 cells overexpressing LRRK2. **A–D**, RN46A (**A**, **C**) and HT22 (**B**, **D**) cells were cotransfected with 5-HT_{1A}R Luciferase plasmid and pRL-TK, and pcDNA3.1, pcDNA3.1-myc-LRRK2, or pcDNA3.1-myc-LRRK2–G2019S plasmids. **A**, **B**, After 48 h transfection, 5-HT_{1A}R transcriptional activity was measured. **C**, **D**, After 24 h transfection, the media was replaced with fresh media, including LRRK2-IN-1 (3 μ M), for additional 24 h, and then 5-HT_{1A}R transcriptional activity was measured. All data were quantified by densitometric analysis. Results are represented as mean $\pm$ SEM ($n = 6–8$ per group). * p < 0.05, ** p < 0.01, and *** p < 0.001 by one-way ANOVA with *post hoc* Bonferroni test; $n = 3$ independent experiments.

activation. When pretreated with the LRRK2 kinase inhibitor LRRK-IN-1 (Weygant et al., 2014), *LRRK2*^{G2019S}-induced transcriptional activation of 5-HT_{1A}R was significantly attenuated in both cell types (Fig. 7C: $F_{(5,56)} = 14.42$, $p < 0.0001$; Fig. 7D: $F_{(5,56)} = 8.458$, $p = 0.0182$).

Discussion

A battery of behavioral tests in this study consistently showed that anxiety/depression-like behaviors appeared in the middle-aged group (43–52 weeks) of *LRRK2*^{G2019S} Tg mice before the onset of motor dysfunction, which did not appear until old age (65–83 weeks). The behavioral tests in this study were performed using both male and female mice, and no sex-related differences in behavioral changes in the middle-aged *LRRK2*^{G2019S} Tg mice were noted. Significant decreases in 5-HT were detected in the hippocampus of *LRRK2*^{G2019S} Tg mice (Fig. 3A), suggesting a concomitant dysfunction in serotonergic neurotransmission, while no significant changes were noted in the striatum and cerebral cortex. Interestingly, the hippocampus, which is part of the limbic system, interacts with the amygdala, and the connection between the hippocampus and amygdala plays a crucial role in the regulation of emotional responses (Phelps, 2004). The cerebral cortex and striatum are also known to receive serotonergic signaling and contribute to emotional reaction (Hare et al., 2005; Etkin et al., 2011). Although all these regions strongly express LRRK2 (Taymans et al., 2006; Ramonet et al., 2011), a significant decrease in 5-HT was observed only in the hippocampus.

Studies have demonstrated that the dorsal hippocampus is primarily involved in cognitive functions, whereas the ventral hippocampus regulates emotional and motivated behaviors (Fanselow and Dong, 2010). However, other studies also showed that the dorsal hippocampus can be an integral brain region for the action of antidepressants, suggesting its relation to emotional responses (Kim et al., 2012b). In the *LRRK2*^{G2019S} Tg mice, 5-HT_{1A}R was upregulated in the dorsal and ventral hippocampus (Fig. 4), amygdala (Fig. 5), and DRN (Fig. 6) with increasing age. Evidence supports the contribution of 5-HT receptors to the pathogenesis of depression (Samuels et al., 2016). Five-HT_{1A}R is the most abundant and widely distributed 5-HT receptor subtype and the most likely candidate implicated in stress responses and depressive symptoms (Stiedl et al., 2015). Five-HT_{1A}R-mediated signaling is largely inhibitory, and functions by coupling to Gi/Go proteins, which inhibit adenylyl cyclase and increase potassium channel conductance (Raymond et al., 1999). Activation of presynaptic 5-HT_{1A}R (autoreceptors) in the DRN negatively regulates 5-HT neuronal activity, while postsynaptic 5-HT_{1A}R mediates 5-HT transmission in other brain regions, including the hippocampus. In this study, both seem highly likely to be upregulated by the mutant *LRRK2* gene, given the increased expression of 5-HT_{1A}Rs in both the hippocampus and amygdala (mainly postsynaptic; Figs. 4, 5) and DRN (mainly presynaptic; Fig. 6). This theory is corroborated by our *in vitro* data showing that *LRRK2*^{G2019S} overexpression significantly increases 5-HT_{1A}R promoter activity not only in the RN46A cells of serotonergic raphe-derived neuronal cell lines, but also in hippocampal HT22 cells (Fig. 7). One interesting IHC finding was that 5-HT_{1A}Rs expression exhibited the highest increase in the DRN region of *LRRK2*^{G2019S} Tg mice, despite the relatively decreased expression of LRRK2 in this region (Taymans et al., 2006). Although we could not identify why 5-HT_{1A}R upregulation was more significant in the DRN, different regulatory mechanisms for 5-HT_{1A}R autoreceptors and postsynaptic 5-HT_{1A}R could have played a role. First, several key positive/negative regulators, such as raphe-specific enhancer Pet-1, exist in the DRN and are implicated in 5-HT_{1A}R autoreceptor expression (Jacobsen et al., 2011; Albert, 2012). Second, glucocorticoid receptors, which negatively regu-

late 5-HT_{1A}R expression, are enriched in the hippocampus compared with the raphe (López et al., 1998; Albert, 2012). The specific mechanism by which *LRRK2* mutations regulate 5-HT_{1A}R expression will be investigated in our next study.

The negative correlation between 5-HT_{1A}R autoreceptor binding and 5-HT synthesis (Frey et al., 2008) in the raphe nuclei may be responsible for the decreased amounts of 5-HT in the hippocampus of *LRRK2*^{G2019S} Tg mice. In addition, 5-HT_{1A}R negatively regulates adenylyl cyclase; thus, the high density of postsynaptic 5-HT_{1A}R in the hippocampus and amygdala can inhibit protein kinase A/CREB-mediated intracellular events, such as BDNF synthesis (Lin et al., 2014). Because BDNF promotes serotonergic phenotype-specific markers by activating tyrosine TrkB (tropomyosin receptor kinase B; Galter and Unsicker, 2000), decreased BDNF also can regulate 5-HT neurotransmission. Because the increased 5-HT derived from chronic desensitization of presynaptic 5-HT_{1A}Rs is necessary to maximize the therapeutic effect of 5-HT-targeting antidepressants, such as fluoxetine (Albert et al., 2011), abnormal regulation of 5-HT_{1A}Rs by *LRRK2*^{G2019S} might decrease patient sensitivity to the therapeutic effects of such antidepressants. We cannot eliminate the alternative hypothesis that the primary effect of *LRRK2*^{G2019S} is a decrease in 5-HT transmission, which leads to the homeostatic upregulation of 5-HT_{1A}Rs.

To verify whether *LRRK2*^{G2019S} is directly involved in the regulation of 5-HT_{1A}R transcription, we transfected RN46A and HT22 cells with *LRRK2*^{G2019S} and evaluated the transcriptional activity of 5-HT_{1A}R (Fig. 7). Both cell types overexpressing *LRRK2*^{G2019S}, but not WT *LRRK2*, showed a significant increase in 5-HT_{1A}R transcription, which was significantly attenuated by the *LRRK2* inhibitor LRRK-IN-1. The basal expression of 5-HT_{1A}R in neurons is regulated by a proximal promoter and an upstream repressor region (Ou et al., 2000; Lemonde et al., 2004). The upstream minimal promoter is highly conserved, where nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) mediates induction of 5-HT_{1A}R transcription, and glucocorticoid receptor binding inhibits transcription (Albert et al., 2011). In addition, a series of repressors are located upstream from the minimal promoter and silence 5-HT_{1A}R expression in non-neuronal and neuronal cells (Albert et al., 2011). Although we did not evaluate relevant upstream signaling events mediating *LRRK2*^{G2019S}-induced activation of 5-HT_{1A}R promoter activity in the current study, one candidate that can mediate this effect is NF- κ B signaling. Studies have demonstrated that *LRRK2* activated NF- κ B-dependent transcription (Kim et al., 2012a) and *LRRK2*^{G2019S} induced NF- κ B activation in a manner comparable to WT (Gardet et al., 2010), suggesting the possible contribution of NF- κ B activation to the *LRRK2*^{G2019S}. Collectively, the results of the present study show that (1) anxiety/depression-like behaviors in *LRRK2*^{G2019S} Tg mice appear before the onset of the characteristic motor dysfunction of PD; (2) a significant decline of 5-HT is detected in the hippocampus of the Tg mice; (3) 5-HT_{1A}R expression is increased in the hippocampus, amygdala, and DRN of the Tg mice; and (4) *LRRK2*^{G2019S} activates 5-HT_{1A}R transcription. To the best of our knowledge, these data are the first *in vivo* and *in vitro* evidence that *LRRK2*^{G2019S} causes abnormal regulation of 5-HT_{1A}Rs, which might be involved in the pathogenesis of anxiety/depression-like nonmotor symptoms in PD with *LRRK2*^{G2019S}. Interestingly, the present study also shows that directly targeting 5-HT_{1A}Rs can improve the therapeutic efficacy of antianxiety medications and antidepressants for PD.

References

- Albert PR (2012) Transcriptional regulation of the 5-HT_{1A} receptor: implications for mental illness. *Philos Trans R Soc Lond B Biol Sci* 367:2402–2415. [CrossRef Medline](#)
- Albert PR, Le Francois B, Millar AM (2011) Transcriptional dysregulation of 5-HT_{1A} autoreceptors in mental illness. *Mol Brain* 4:21. [CrossRef Medline](#)
- Andrade R, Huereca D, Lyons JG, Andrade EM, McGregor KM (2015) 5-HT_{1A} receptor-mediated autoinhibition and the control of serotonergic cell firing. *ACS Chem Neurosci* 6:1110–1115. [CrossRef Medline](#)
- Celada P, Puig M, Amargós-Bosch M, Adell A, Artigas F (2004) The therapeutic role of 5-HT_{1A} and 5-HT_{2A} receptors in depression. *J Psychiatry Neurosci* 29:252–265. [Medline](#)
- Chaudhuri KR, Odin P (2010) The challenge of non-motor symptoms in Parkinson's disease. *Prog Brain Res* 184:325–341. [CrossRef Medline](#)
- Chaudhuri KR, Healy DG, Schapira AH, Schapira AH (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol* 5:235–245. [CrossRef Medline](#)
- Chen CY, Weng YH, Chien KY, Lin KJ, Yeh TH, Cheng YP, Lu CS, Wang HL (2012) (G2019S) LRRK2 activates MKK4-JNK pathway and causes degeneration of SN dopaminergic neurons in a transgenic mouse model of PD. *Cell Death Differ* 19:1623–1633. [CrossRef Medline](#)
- Cheshire P, Aytton S, Bertram KL, Ling H, Li A, McLean C, Halliday GM, O'Sullivan SS, Revesz T, Finkelstein DI, Storey E, Williams DR (2015) Serotonergic markers in Parkinson's disease and levodopa-induced dyskinesias. *Mov Disord* 30:796–804. [CrossRef Medline](#)
- Chou JS, Chen CY, Chen YL, Weng YH, Yeh TH, Lu CS, Chang YM, Wang HL (2014) (G2019S) LRRK2 causes early-phase dysfunction of SNpc dopaminergic neurons and impairment of corticostriatal long-term depression in the PD transgenic mouse. *Neurobiol Dis* 68:190–199. [CrossRef Medline](#)
- Escande MV, Taravini IR, Zold CL, Belforte JE, Murer MG (2016) Loss of homeostasis in the direct pathway in a mouse model of asymptomatic Parkinson's disease. *J Neurosci* 36:5686–5698. [CrossRef Medline](#)
- Etkin A, Egner T, Kalisch R (2011) Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 15:85–93. [CrossRef Medline](#)
- Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65:7–19. [CrossRef Medline](#)
- Frey BN, Rosa-Neto P, Lubarsky S, Diksic M (2008) Correlation between serotonin synthesis and 5-HT_{1A} receptor binding in the living human brain: a combined alpha-[¹¹C]MT and [¹⁸F]MPPF positron emission tomography study. *Neuroimage* 42:850–857. [CrossRef Medline](#)
- Fricker AD, Rios C, Devi LA, Gomes I (2005) Serotonin receptor activation leads to neurite outgrowth and neuronal survival. *Brain Res Mol Brain Res* 138:228–235. [CrossRef Medline](#)
- Galter D, Unsicker K (2000) Sequential activation of the 5-HT_{1A} serotonin receptor and TrkB induces the serotonergic neuronal phenotype. *Mol Cell Neurosci* 15:446–455. [CrossRef Medline](#)
- Gandhi PN, Chen SG, Wilson-Delfosse AL (2009) Leucine-rich repeat kinase 2 (LRRK2): a key player in the pathogenesis of Parkinson's disease. *J Neurosci Res* 87:1283–1295. [CrossRef Medline](#)
- Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C, Korzenik JR, Rioux JD, Daly MJ, Xavier RJ, Podolsky DK (2010) LRRK2 is involved in the IFN- γ response and host response to pathogens. *J Immunol* 185:5577–5585. [CrossRef Medline](#)
- Hadjigasssem MR, Austin MC, Szewczyk B, Daigle M, Stockmeier CA, Albert PR (2009) Human Freud-2/CC2D1B: a novel repressor of postsynaptic serotonin-1A receptor expression. *Biol Psychiatry* 66:214–222. [CrossRef Medline](#)
- Han A, Sung YB, Chung SY, Kwon MS (2014) Possible additional antidepressant-like mechanism of sodium butyrate: targeting the hippocampus. *Neuropharmacology* 81:292–302. [CrossRef Medline](#)
- Hare TA, Tottenham N, Davidson MC, Glover GH, Casey BJ (2005) Contributions of amygdala and striatal activity in emotion regulation. *Biol Psychiatry* 57:624–632. [CrossRef Medline](#)
- Healy DG, Wood NW, Schapira AH (2008) Test for LRRK2 mutations in patients with Parkinson's disease. *Pract Neurol* 8:381–385. [CrossRef Medline](#)
- Heiser P, Hausmann C, Frey J, Geller F, Becker R, Wesemann W, Krieg JC, Remschmidt H, Vedder H (2002) Serotonergic effects of clozapine and its metabolites in hippocampal HT22 cells. *Psychiatry Res* 112:221–229. [CrossRef Medline](#)
- Jacobsen KX, Czesak M, Deria M, Le François B, Albert PR (2011) Region-specific regulation of 5-HT_{1A} receptor expression by Pet-1-dependent mechanisms in vivo. *J Neurochem* 116:1066–1076. [CrossRef Medline](#)
- Jans LA, Riedel WJ, Markus CR, Blokland A (2007) Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. *Mol Psychiatry* 12:522–543. [CrossRef Medline](#)
- Kim B, Yang MS, Choi D, Kim JH, Kim HS, Seol W, Choi S, Jou I, Kim EY, Joe EH (2012a) Impaired inflammatory responses in murine Lrrk2-knockdown brain microglia. *PLoS One* 7:e34693. [CrossRef Medline](#)
- Kim CS, Chang PY, Johnston D (2012b) Enhancement of dorsal hippocampal activity by knockdown of HCN1 channels leads to anxiolytic- and antidepressant-like behaviors. *Neuron* 75:503–516. [CrossRef Medline](#)
- Kim S, Park JM, Moon J, Choi HJ (2014) Alpha-synuclein interferes with cAMP/PKA-dependent upregulation of dopamine beta-hydroxylase and is associated with abnormal adaptive responses to immobilization stress. *Exp Neurol* 252:63–74. [CrossRef Medline](#)
- Kish SJ (2003) Biochemistry of Parkinson's disease: is a brain serotonergic deficiency a characteristic of idiopathic Parkinson's disease? *Adv Neurol* 91:39–49. [Medline](#)
- Lemond S, Rogaeva A, Albert PR (2004) Cell type-dependent recruitment of trichostatin A-sensitive repression of the human 5-HT_{1A} receptor gene. *J Neurochem* 88:857–868. [CrossRef Medline](#)
- Lin P, Wang C, Xu B, Gao S, Guo J, Zhao X, Huang H, Zhang J, Chen X, Wang Q, Zhou W (2014) The VGF-derived peptide TLQP62 produces antidepressant-like effects in mice via the BDNF/TrkB/CREB signaling pathway. *Pharmacol Biochem Behav* 120:140–148. [CrossRef Medline](#)
- López JF, Chalmers DT, Little KY, Watson SJ (1998) A.E. Bennett Research Award. Regulation of serotonin_{1A}, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry* 43:547–573. [CrossRef Medline](#)
- Martínez-Martín P, Damián J (2010) Parkinson disease: depression and anxiety in Parkinson disease. *Nat Rev Neurol* 6:243–245. [CrossRef Medline](#)
- Meissner WG, Frasier M, Gasser T, Goetz CG, Lozano A, Piccini P, Obeso JA, Rascol O, Schapira A, Voon V, Weiner DM, Tison F, Bezard E (2011) Priorities in Parkinson's disease research. *Nat Rev Drug Discov* 10:377–393. [CrossRef Medline](#)
- Michelsen KA, Prickaerts J, Steinbusch HW (2008) The dorsal raphe nucleus and serotonin: implications for neuroplasticity linked to major depression and Alzheimer's disease. *Prog Brain Res* 172:233–264. [CrossRef Medline](#)
- Morrison KE, Cooper MA (2012) A role for 5-HT_{1A} receptors in the basolateral amygdala in the development of conditioned defeat in Syrian hamsters. *Pharmacol Biochem Behav* 100:592–600. [CrossRef Medline](#)
- Ou XM, Jafar-Nejad H, Storrington JM, Meng JH, Lemond S, Albert PR (2000) Novel dual repressor elements for neuronal cell-specific transcription of the rat 5-HT_{1A} receptor gene. *J Biol Chem* 275:8161–8168. [CrossRef Medline](#)
- Perez-Lloret S, Rascol O (2012) Parkinson disease: serotonin reuptake inhibitors for depression in PD. *Nat Rev Neurol* 8:365–366. [CrossRef Medline](#)
- Phelps EA (2004) Human emotion and memory: interactions of the amygdala and hippocampal complex. *Curr Opin Neurobiol* 14:198–202. [CrossRef Medline](#)
- Piñeyro G, Blier P (1999) Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol Rev* 51:533–591. [Medline](#)
- Plowey ED, Johnson JW, Steer E, Zhu W, Eisenberg DA, Valentino NM, Liu YJ, Chu CT (2014) Mutant LRRK2 enhances glutamatergic synapse activity and evokes excitotoxic dendrite degeneration. *Biochim Biophys Acta* 1842:1596–1603. [CrossRef Medline](#)
- Politis M, Niccolini F (2015) Serotonin in Parkinson's disease. *Behav Brain Res* 277:136–145. [CrossRef Medline](#)
- Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, et al. (2011) Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One* 6:e18568. [CrossRef Medline](#)
- Raymond JR, Mukhin YV, Gettys TW, Garnovskaya MN (1999) The recom-

- binant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br J Pharmacol* 127:1751–1764. [CrossRef Medline](#)
- Samuels BA, Mendez-David I, Faye C, David SA, Pierz KA, Gardier AM, Hen R, David DJ (2016) Serotonin 1A and serotonin 4 receptors: essential mediators of the neurogenic and behavioral actions of antidepressants. *Neuroscientist* 22:26–45. [CrossRef Medline](#)
- Stiedl O, Pappa E, Konradsson-Geuken Å, Ögren SO (2015) The role of the serotonin receptor subtypes 5-HT_{1A} and 5-HT₇ and its interaction in emotional learning and memory. *Front Pharmacol* 6:162. [Medline](#)
- Storring JM, Charest A, Cheng P, Albert PR (1999) TATA-driven transcriptional initiation and regulation of the rat serotonin 5-HT_{1A} receptor gene. *J Neurochem* 72:2238–2247. [Medline](#)
- Taymans JM, Van den Haute C, Baekelandt V (2006) Distribution of PINK1 and LRRK2 in rat and mouse brain. *J Neurochem* 98:951–961. [CrossRef Medline](#)
- Weygant N, Qu D, Berry WL, May R, Chandrakesan P, Owen DB, Sureban SM, Ali N, Janknecht R, Houchen CW (2014) Small molecule kinase inhibitor LRRK2-IN-1 demonstrates potent activity against colorectal and pancreatic cancer through inhibition of doublecortin-like kinase 1. *Mol Cancer* 13:103. [CrossRef Medline](#)
- White LA, Eaton MJ, Castro MC, Klose KJ, Globus MY, Shaw G, Whittemore SR (1994) Distinct regulatory pathways control neurofilament expression and neurotransmitter synthesis in immortalized serotonergic neurons. *J Neurosci* 14:6744–6753. [Medline](#)
- Xu Y, Zhang C, Wang R, Govindarajan SS, Barish PA, Vernon MM, Fu C, Acharya AP, Chen L, Boykin E, Yu J, Pan J, O'Donnell JM, Ogle WO (2011) Corticosterone-induced morphological changes of hippocampal and amygdaloid cell lines are dependent on 5-HT₇ receptor related signal pathway. *Neuroscience* 182:71–81. [CrossRef Medline](#)
- Yamamura S, Abe M, Nakagawa M, Ochi S, Ueno S, Okada M (2011) Different actions for acute and chronic administration of mirtazapine on serotonergic transmission associated with raphe nuclei and their innervation cortical regions. *Neuropharmacology* 60:550–560. [CrossRef Medline](#)