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Acupuncture Alleviates Levodopa-Induced Dyskinesia via Melanin-Concentrating Hormone in *Pitx3*-Deficient *aphakia* and 6-Hydroxydopamine-Lesioned Mice

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Abstract

Although L-3,4-dihydroxyphenylalanine (L-DOPA) is currently the most effective medication for treating Parkinson's disease (PD) motor symptoms, its prolonged administration causes several adverse effects, including dyskinesia. To identify the mechanisms underlying the effects of acupuncture on L-DOPA-induced dyskinesia (LID), antidyskinetic effects of acupuncture were investigated in two mouse models of PD. Acupuncture stimulation at GB34 alleviated abnormal involuntary movements (AIMs) in *Pitx3*-deficient *aphakia* mice (*ak/ak*) following L-DOPA administration and these effects were reproduced in 6-hydroxydopamine (6-OHDA)-lesioned mice with LID. A transcriptome analysis of the hypothalamus revealed pro-melanin-concentrating hormone (*Pmch*) gene was highly expressed in acupuncture-treated mouse from *ak/ak* model of LID as well as 6-OHDA model of LID. Acupuncture combined with the administration of MCH receptor antagonist did not have any beneficial effects on dyskinesia in L-DOPA-injected *ak/ak* mice, but the intranasal administration of MCH attenuated LID to the same degree as acupuncture in both *ak/ak* and 6-OHDA mice with LID. A gene expression profile with a hierarchical clustering analysis of the dyskinesia-induced *ak/ak* mouse brain revealed an association between the mechanisms underlying acupuncture and MCH. Additionally, altered striatal responses to L-DOPA injection were observed after prolonged acupuncture and MCH treatments, which suggests that these treatment modalities influenced the compensatory mechanisms of LID. In summary, present study demonstrated that acupuncture decreased LID via hypothalamic MCH using L-DOPA-administered *ak/ak* and 6-OHDA mouse models and that MCH administration resulted in novel antidyskinetic effects in these models. Thus, acupuncture and MCH might be valuable therapeutic candidates for PD patients suffering from LID.

Keywords Acupuncture · Levodopa-induced dyskinesia · Melanin-concentrating hormone · *Pitx3*-deficient *aphakia* mouse · Parkinson's disease

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by various motor symptoms, including rigidity, tremor, and/or slowness of movements, that result from the progressive depletion of dopaminergic neurons in the substantia nigra (SN) [1]. The dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) is predominantly administered for the treatment of PD and initially counteracts the motor symptoms of this disease [2]. However, prolonged L-DOPA administration produces abnormal involuntary movements (AIMs), including chorea and dystonia, that are referred to as L-DOPA-induced dyskinesia (LID) [3]. LID degrades the quality of life of PD patients and may even be more disabling to patients than the disease itself [4]. Therapeutic strategies for LID usually involve modifying the dose and frequency of L-DOPA treatment as well as prescribing other antidyskinetic medications, such as amantadine and clozapine [5]. However, these antidyskinetic agents are also associated with high incidence rates of adverse effects [6, 7] and, thus, novel and safer therapies are needed.

Acupuncture has been effectively used as a treatment for various neurological diseases, including PD [8–10] and stroke [11, 12], in Asian countries for more than 5000 years. Recent clinical trials of acupuncture for PD have shown that it may be an efficacious adjunct therapy with conventional medicine [13–15]. Using a translational approach, our research group previously reported that acupuncture treatment improves motor function in a PD animal model by enhancing dopamine transmission and normalizing post-synaptic proteins such as pDARPP-32 and FosB in the striatum [16]. Acupuncture also exerts synergistic effects with L-DOPA in terms of improving motor function in 6-hydroxydopamine (6-OHDA)-lesioned PD mice, which suggests that acupuncture may be beneficial as a combination therapy with conventional treatments [10]. To further elucidate its role during combination therapy with L-DOPA, our research group demonstrated that acupuncture alleviates AIMs, which is a behavioral method for assessing LID in animals, and that these changes are associated with the normalization of alterations in γ-aminobutyric acid (GABA) content in the SN and FosB expression in the striatum [10]. Taken together, these findings indicate that acupuncture may be an effective antidyskinetic therapy for motor problems induced by L-DOPA. However, the manner in which acupuncture treatments that stimulate peripheral acupoints result in the normalization of striatal alterations in LID models remains unclear.

Acupuncture stimulates specific peripheral tissues called acupoints that transmit signals to the central nervous system and exerts its therapeutic effects via the integration of activities in several brain regions [17, 18]. For example, the hypothalamus is a critical region for the response to acupuncture [19–23]. Despite the fact that the current diagnostic and therapeutic characterizations of PD largely emphasize motor symptomatology, a gradually increasing understanding of non-motor symptom complexes indicates that PD, as a syndrome, involves multiple systems and is not merely due to deficiencies in nigrostriatal dopamine [24, 25]. The hypothalamus is the center for homeostasis and is associated with several non-motor symptoms of PD patients, such as abnormal sleep behaviors [26, 27]. Our group recently demonstrated that acupuncture activates melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus, which increases the release of MCH in the SN, and that this is associated with improvements in motor function and dopaminergic neuroprotection in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced and A53T alpha-synuclein overexpression mouse models of PD [28].

Thus, to extend these previous findings, the present study investigated whether the hypothalamus plays an important role in the antidyskinetic effects of acupuncture on LID models using *Pitx3*-deficient *ak/ak* mice and 6-OHDA-treated mice. A microarray analysis revealed that there was an increase in *pro-MCH* (*Pmch*) levels following acupuncture treatment in LID models that was similar to that observed in PD models [28]. Additionally, to further understand the role of MCH in acupuncture, these effects were assessed after a challenge administration of MCH receptor 1 (MCH1R) antagonist. The present study observed novel antidyskinetic effects after treatment with MCH, which have not been previously demonstrated.

Methods and Materials

Animals

This research was performed mainly in the homozygous *Pitx3*-deficient *aphakia* mice (*ak/ak*) on a C57BL/6 background, the *Pitx3 ak/ak Pmch* −/− mice and corresponding male C57BL/6 mice. *Pitx3 ak/ak* mice derived from the mating of heterozygous mice and *Pitx3 ak/ak Pmch* −/− double knockout mice derived from the mating of homozygous mice (Jackson Laboratory, Bar Harbor, Maine, USA) were determined through genetic analysis of mouse tail-tip DNA via PCR. *Pitx3 ak/ak* mice and *Pmch* −/− mice were initially purchased from the Jackson Laboratory. The *Pitx3 ak/ak* mice, *Pitx3 ak/ak Pmch* −/− mice and the wild-type mice (C57BL/6) used in this research were 3 to 5 or 4 to 6 months old and weighed 24–30 g at the start of each experiment.

The 6-OHDA-lesioning was also applied to 8- to 10-week-old male C57BL/6 mice (Samtako, Gyoung Gi-Do, Republic of Korea) weighing 24–26 g each.

All the mice were kept under standard laboratory conditions with ad libitum feeding on a 12-h/12-h light/dark schedule. All the experiments were approved by the Dongguk

University Animal Care Committee for animal welfare (IACUC-2017-020-1) and were conducted according to the guidelines of the National Institutes of Health and the Korean Academy of Medical Sciences.

Microinjection and Apomorphine Test

The C57BL/6 mice were anesthetized with a mixture of tiletamine and zolazepam (30 mg/kg, i.p., sterilized water, Zoletil 50, Virbac, France) and with xylazine (10 mg/kg, i.p., Rompun, Bayer Korea, Republic of Korea) in the saline. A unilateral injection of either saline or solution of 6-OHDA-HCl (3.0 μg/μl dissolved in saline containing 0.02% ascorbic acid, 2 μl injection for twice, Sigma-Aldrich, MO, USA; H4381) into the right striatum was conducted on each mouse according to the mouse brain atlas: + 1.0 mm of AP, − 2.1 mm of ML, − 3.2 mm of DV and + 0.3 mm of AP, − 2.3 mm of ML, − 3.2 mm of DV. After a stereotaxic surgery, mice were maintained for 14 days in their home cages without any intervention for recovery.

The apomorphine-induced rotations were tested on the 15th day at post-lesion for screening of inducing experimental parkinsonism. A counterclockwise rotation (contralateral to the lesioned right side) induced by a subcutaneous injection of apomorphine hydrochloride hemihydrate (0.8 mg/kg, dissolved in saline, Sigma-Aldrich; A4393) was counted for 5 min. A mouse less than 30 turnings was excluded and only the mice over 30 apomorphine-induced turnings were included in the research.

Drugs and Acupuncture Treatment

The L-DOPA (20 mg/kg or 25 mg/kg, i.p., saline, Levodopa, Sigma-Aldrich; D9628) with the peripheral DOPA decarboxylase inhibitor benserazide hydrochloride (12.5 mg/kg, i.p., saline, Sigma-Aldrich; B7283) were injected once a day according to the previous published studies [29, 30].

The acupuncture treatment was applied 30 min before L-DOPA injection at GB34 acupoint (in a depression anterior and distal to the head of the fibula) or non-acupoint (in the muscle on the hips). A sterilized acupuncture needle (0.18 mm in diameter and 8 mm in length; Haenglim-seoweon Acuneedle Co., Republic of Korea) was inserted bilaterally to depth of 3 mm and was turned bi-directionally for 15 s (at a rate of two spins per seconds; one spin is composed of a 180° clockwise rotation and a 180° counterclockwise rotation) by ungloved fingers.

MCH (0.1 or 0.5 μg/30 μl, i.n., saline, Tocris Biosciences, Bristol, UK; 3806) was administered intranasally to the mice 30 min before L-DOPA injection. Selective MCH1R antagonist (Tc-MCH7c; 10 mg/kg, i.p., dissolved in 2 μl dimethyl sulfoxide and distilled water, Tocris Biosciences; 4365) was injected 30 min before performing the acupuncture treatment.

Experimental Procedure

For the first set of experiments to examine the acupuncture effect on dyskinesia, *Pitx3*-deficient *aphakia* mice (3 to 5 months old, total $n = 19$, ♂15 ♀4) were randomly distributed to three groups: saline group ($n = 5$, ♂4 ♀1), L-DOPA group ($n = 6$, ♂5 ♀1), and L-DOPA + ACU group ($n = 8$, ♂6 ♀2). For 36 days, L-DOPA was injected once a day to L-DOPA group mice and L-DOPA + ACU group mice and saline solution was injected to Saline group mice. Acupuncture treatment on GB34 was administered to L-DOPA + ACU group mice 30 min before L-DOPA injection, 6 days a week. Behavior tests were recorded at days 8, 22, and 36 by measuring the duration of three-paw dyskinesia (Fig. 1a).

The second set of experiments was executed to verify the Pmch upregulation by acupuncture in LID-induced 6-OHDA mice. C57BL/6 mice (8 to 10 weeks old, total $n = 45$, ♂) were randomly assigned to five groups: control group ($n = 8$, ♂), 6-OHDA group ($n = 9$, ♂), 6-OHDA + ACU group ($n = 3$, ♂), L-DOPA group ($n = 8$, ♂), L-DOPA + ACU group ($n = 9$, ♂), L-DOPA + NA group ($n = 8$, ♂). Except the control group, all the mice in 6-OHDA, 6-OHDA + ACU, L-DOPA, L-DOPA + ACU, and L-DOPA + NA groups were administered the 6-OHDA injection. Then, each mouse in L-DOPA, L-DOPA + ACU, and L-DOPA + NA groups was injected L-DOPA intraperitoneally once a day for 10 days, whereas the mice in control, 6-OHDA, and 6-OHDA + ACU groups were injected saline. Thirty minute before the L-DOPA injection, L-DOPA + ACU and L-DOPA + NA group mice were applied the insertion of acupuncture needle on either GB34 or control point bilaterally. The 6-OHDA + ACU group mice also got the acupuncture treatment at GB34 30 min before saline injection. Behavior tests were recorded at day 28 from the 6-OHDA injection (equal to day 10 from the start of L-DOPA injection) by evaluating the AIM assessment score (Fig. 2a).

The third set of experiments to verify the mediating role of MCH in acupuncture LID-reducing effect, *Pitx3*-deficient *aphakia* mice (4 to 6 months old, total $n = 33$, ♂28 ♀5) were randomly categorized to five groups: saline group ($n = 6$, ♂5 ♀1), L-DOPA group ($n = 6$, ♂5 ♀1), L-DOPA + ACU group ($n = 7$, ♂6 ♀1), L-DOPA + NA group ($n = 7$, ♂6 ♀1) and L-DOPA + ACU + TC-MCH7c group ($n = 7$, ♂6 ♀1). L-DOPA injection was applied to L-DOPA group, L-DOPA + ACU group, L-DOPA + NA group, and L-DOPA + ACU + TC-MCH7c group mice once a day for 28 days. The acupuncture treatments on GB34 were carried out in the L-DOPA + ACU and L-DOPA + ACU + TC-MCH7c group mice 6 days a week (L-DOPA + NA group mice were applied on non-acupoints). The L-DOPA + ACU + TC-MCH7c group mice were intraperitoneally injected with Tc-MCH7c, a selective MCH1R antagonist, 30 min before the acupuncture treatment 6 days a week. Behavior tests on LID movement were recorded at days

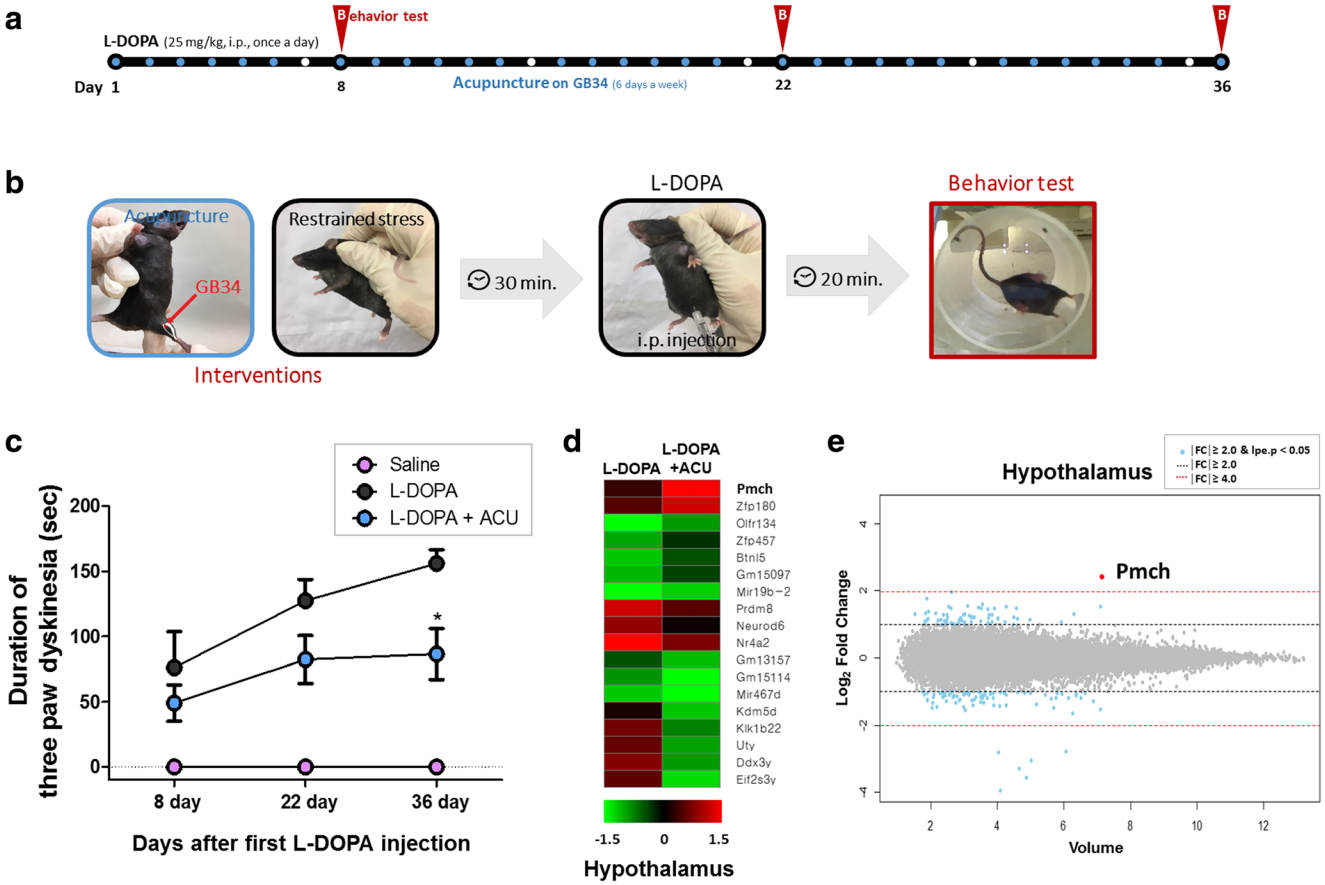

Fig. 1 Effects of acupuncture on L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) and the upregulation of hypothalamic *Pmch* in *Pitx3*-deficient *aphakia* mice. **a** Day schedule of Experiment 1 in *Pitx3*-deficient *aphakia* mice. Beginning on day 1, the mice were injected with saline or L-DOPA and received acupuncture or underwent restraint stress 30 min prior to L-DOPA injection. Black bold lines indicate periods of prolonged administration of L-DOPA once a day with or without acupuncture treatment. Acupuncture treatment was performed 6 days a week and is indicated by blue circles. Behavioral tests of dyskinetic movements were conducted on days 8, 22, and 36. **b** The interventions, including acupuncture on GB34 ($n = 8$) or restraint stress ($n = 6$), were performed 20 min prior to L-DOPA injection. Twenty minutes after the L-DOPA injection, the dyskinetic movements of each mouse were recorded for 4 min. **c** Three-paw dyskinesia tests were conducted on days 8, 22, and 36; acupuncture treatment significantly attenuated dyskinesia on day 36. Black, blue, and purple circles indicate the L-DOPA ($n = 6$), L-DOPA + ACU ($n = 8$) and Saline group ($n = 5$), respectively; a one-way analysis of variance followed by Bonferroni post hoc analyses were conducted. **d** Heat map of candidate gene expression values in the hypothalamus of the L-DOPA and L-DOPA + ACU groups. Candidate genes were obtained via gene expression profiling analyzed with one-way ANOVAs that revealed significant differences between the L-DOPA and L-DOPA + ACU groups. The colors scaled from green to red denote a value of log2 fold change of each gene expression level. **e** Plot of altered genes in the L-DOPA + ACU group compared with the L-DOPA group and significant changes in the expression of the *Pmch* gene; the *x*-axis denotes volume and the *y*-axis denotes a log2 fold change. Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. $^*p < 0.05$ compared to L-DOPA group

5, 12, 19, and 26 by measuring the duration of three-paw dyskinesia (Fig. 3a).

To clarify the MCH effect in LID, we studied the effects of MCH administration (0.5 μg/30 μl, i.n.) in the fourth set of experiments with *Pitx3 ak/ak* mice and *Pitx3 ak/ak Pmch* −/− mice. *Pitx3 ak/ak* mice (4 to 6 months old, total $n = 16$, ♂13 ♀3) were randomly categorized to three groups: saline group ($n = 5$, ♂4 ♀1), L-DOPA group ($n = 5$, ♂4 ♀1) and L-DOPA + MCH group ($n = 6$, ♂5 ♀1). The *Pitx3 ak/ak Pmch* −/− mice were referred as the Pmch −/− + L-DOPA group (4 to 6 months old, $n = 4$, ♂2 ♀2). L-DOPA injection was applied to L-DOPA group, L-DOPA + MCH group, and Pmch −/− + L-DOPA group mice once a day for 28 days. The MCH treatments were administered in the L-DOPA + MCH group mice 6 days a week. Behavior tests on LID movement were recorded at days 5, 12, 19 and 26 by measuring the duration of three-paw dyskinesia (Fig. 4a).

In the last set of experiments to examine the MCH effect on LID, each C57BL/6 mouse (9-week-old, total $n = 57$, ♂) was allocated to five groups: control group ($n = 10$, ♂), 6-OHDA group ($n = 10$, ♂), L-DOPA group ($n = 12$, ♂), L-DOPA + MCH 0.1 group ($n = 12$, ♂), and L-DOPA + MCH 0.5 group ($n = 13$, ♂). Control group mice and the other group mice were injected with saline and 6-OHDA respectively through stereotaxic surgery. After the resting period, L-DOPA injection once a day to L-DOPA, L-DOPA + MCH 0.1, and L-

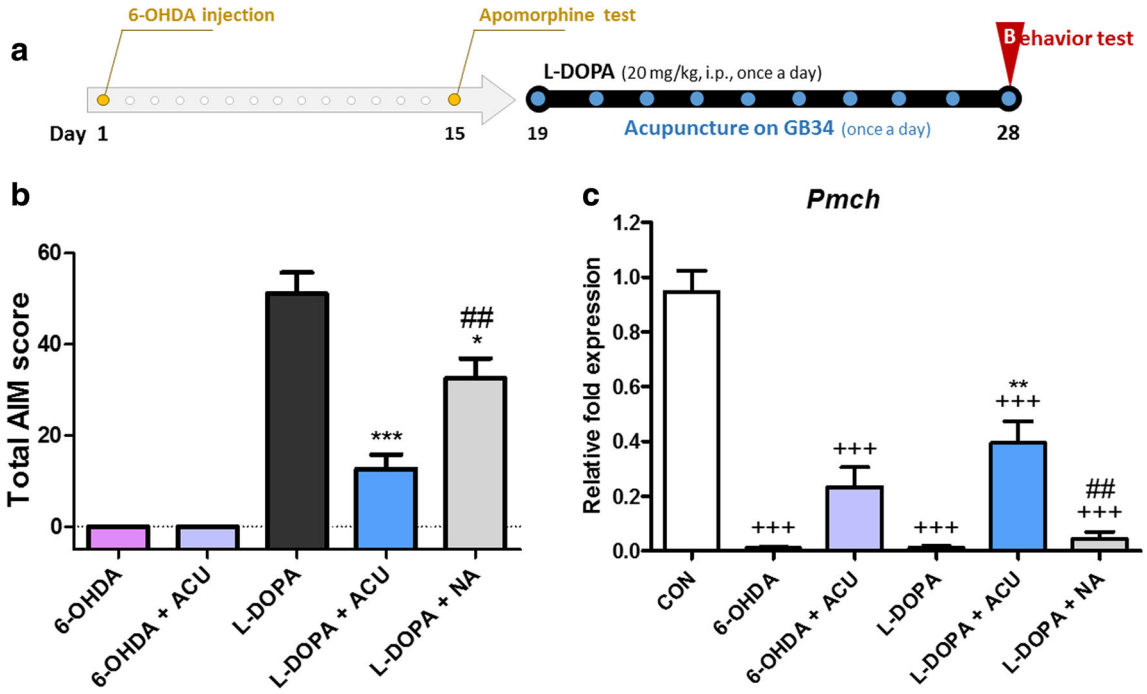

Fig. 2 Acupuncture modulated the mRNA expression levels of *Pmch* in the hypothalamus of 6-hydroxydopamine (6-OHDA)-lesioned mice following chronic L-DOPA administration. **a** Day schedule of experiment 2 in C57BL/6 mice. On day 1, the mice were anesthetized and received an injection of either saline or 6-OHDA into the right striatum. After a recovery period of 14 days, apomorphine tests were carried out on day 15 to validate the Parkinson's disease (PD) model. Mice not exhibiting PD-like symptoms were excluded and dopamine-denervated mice were divided into their respective groups. Following a rest period of 4 days after the apomorphine test, the LID experimental schedule was initiated; either L-DOPA or saline was injected and acupuncture treatment or restraint stress was performed. Black bold lines refer to periods of prolonged administration of L-DOPA with or without acupuncture treatment and blue circles refer to acupuncture treatment performed once a day for 10 days. Behavioral tests were carried out on day 28. **b** Acupuncture treatment produced antidyskinetic effects in dyskinesia-induced 6-OHDA-lesioned mice. Acupuncture stimulation on GB34 ($n = 9$) and non-acupoints ($n = 8$) significantly reduced dyskinesia compared to the L-DOPA group ($n = 8$) but the antidyskinetic effects of non-acupoint stimulation were weaker than those of GB34 treatment. The saline-injected 6-OHDA-lesioned mice ($n = 9$) showed no dyskinetic behavior. **c** Acupuncture treatment upregulated the mRNA expression of hypothalamic *Pmch*; a relative fold change was detected with quantitative polymerase chain reaction (qPCR) analysis (each $n = 3$–4). Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. $^{+++}p < 0.001$ compared to CON group; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to L-DOPA group; $^{\#}p < 0.05$ compared to L-DOPA + ACU group

DOPA + MCH 0.5 group mice and saline injection to control and 6-OHDA group mice began. L-DOPA + MCH 0.1 or L-DOPA + MCH 0.5 group mice were treated with MCH administration of 0.1 μg/30 μl or 0.5 μg/30 μl each 30 min before L-DOPA injection. Behavior tests were recorded at days 22, 25, and 28 from the 6-OHDA injection (equal to days 4, 7, and 10 from the start of L-DOPA injection) by evaluating the AIM assessment score (Fig. 5a).

Behavior Tests

Three-Paw Dyskinesia

The L-DOPA-induced dyskinetic events in *Pitx3 ak/ak* mice were analyzed through quantifying the incidence of abnormal stereotypic paw movements after L-DOPA administration. Each mouse was put in a plastic transparent cylinder (20 cm tall and 12 cm diameter) 20 min after L-DOPA injection and a 4-min video recorded their motor activity. The video-recorded paw movements were analyzed by calculating a total duration of all three-paw dyskinetic events. According to the previous publications [31, 32], when a mouse moves both front paws repeatedly up and down and stands close to the wall of cylinder on its one hind paw, lifting up and down the other hind paw and in turns changing the standing paw, it was evaluated to a three-paw dyskinesia.

Abnormal Involuntary Movements (AIM)

The L-DOPA-induced dyskinetic event in 6-OHDA mouse was quantified by measuring the AIM assessment score. The AIM assessment score consists of four different subtypes according to the following topographic distribution: (1) axial dystonia: axial rotation or lateral flexion of the neck and upper body to the side contralateral to the induced 6-OHDA lesion; (2) forelimb dyskinesia: purposeless movements or dystonic posturing of the forelimb contralateral to the lesion; (3) orolingual dyskinesia: tongue

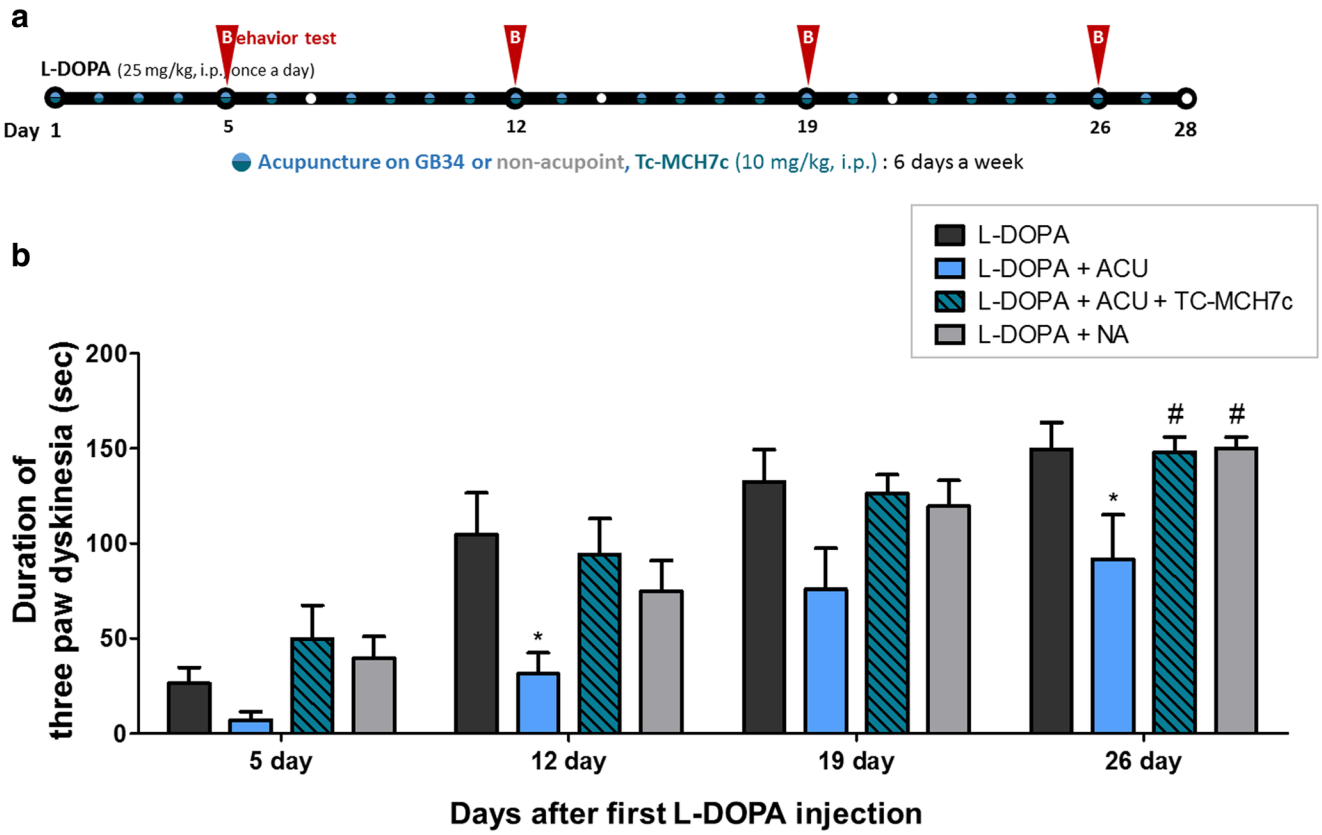

Fig. 3 Acupuncture attenuated dyskinetic movements via alterations of melanin-concentrating hormone (MCH)/MCH receptor 1 (MCH1R) signaling in *Pitx3*-deficient *aphakia* mice with dyskinesia. **a** Day schedule of experiment 3 in *Pitx3*-deficient *aphakia* mice. Beginning on day 1, the mice were injected with L-DOPA once a day and received acupuncture on GB34 or a non-acupoint or underwent restraint stress 6 days a week. Black bold lines refer to periods of prolonged administration of L-DOPA once a day and multi-color circles represent acupuncture on GB34 (L-DOPA + ACU group $n = 7$) or a non-acupoint ($n = 7$), Tc-MCH7c injection ($n = 7$), or restraint stress ($n = 6$) 6 days a week. Behavioral tests assessing dyskinetic movements were performed on days 5, 12, 19, and 26. **b** Three-paw dyskinesia in LID-induced *Pitx3*-deficient *ak/ak* homozygous mice was recorded on days 5, 12, 19, and 26. Acupuncture treatment suppressed abnormal paw movements and Tc-MCH7c blocked the dyskinesia-reducing effects of acupuncture. Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. $^{*}p < 0.05$ compared to L-DOPA group; $^{\#}p < 0.05$ compared to L-DOPA + ACU group

protrusion and jaw movement toward the side contralateral to the lesion; and (4) locomotive dyskinesia: increased locomotion or circling movements away from the lesioned side, sometimes accompanied by grabbing motion of a paw. A severity of each subtype was scored from 0 to 4; 0 = absent, 1 = occasional, 2 = frequent, 3 = continuous but suspended by tactile stimuli, and 4 = continuous, severe and not suspended by tactile stimuli. The sum of each four subtype score was calculated as the total AIM score. The AIM assessment of each mouse was conducted for 1 min, six times every 20 min (beginning at 20 min after L-DOPA administration to 120 min) [33].

Western Blotting

Brain tissues were homogenized in CyQUANT® cell lysis buffer, 20× (diluted 1/20, 200 μl per each sample, Invitrogen, Oregon, USA; C7027). After homogenization, each sample was centrifuged at 12,000 rpm for 20 min at 4 °C and the supernatants were collected. Then the amount of protein was measured using the BCA protein assay and equal amounts of each sample proteins were mixed with SDS-PAGE loading buffer, 5× (diluted 1/5, Biomedic, Republic of Korea; BM657E) and heated at 95 °C for 10 min.

Each protein samples (9 μg) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Western blotting with the following primary antibodies: β-actin (1:15,000 dilution, Sigma-Aldrich) and FosB (1:200 dilution, Cell Signaling Technology, Danvers, MA, USA), followed by the secondary rabbit or mouse (1:10,000 dilution, Pierce Biotechnology, Rockford, IL, USA) antibodies. The bands were visualized using an enhanced chemiluminescence system (West Pico; Pierce Biotechnology), and the band intensity was quantified with the densitometry using image QI software.

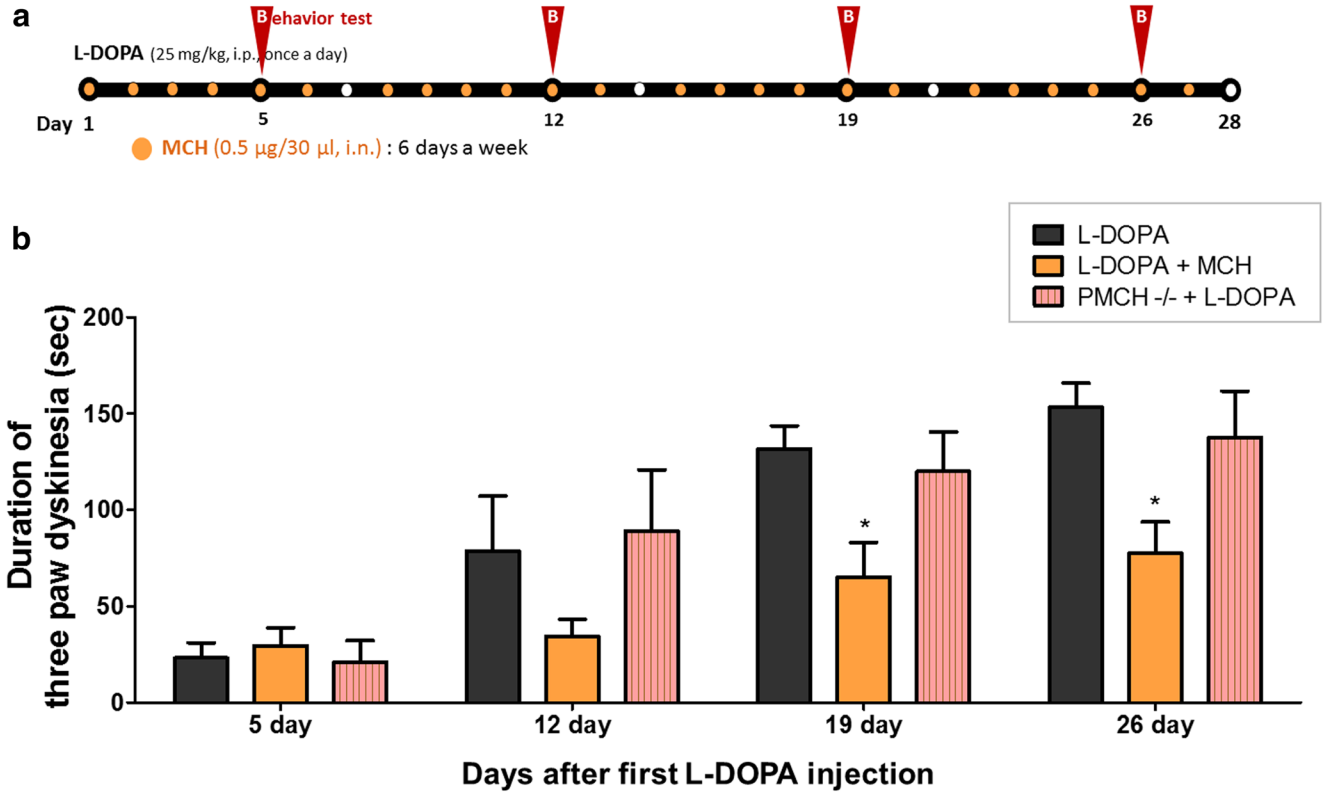

Fig. 4 Novel antidyskinetic effects of MCH in L-DOPA-treated *Pitx3 ak/ak* mice. **a** Day schedule of experiment 4 in *Pitx3*-deficient *aphakia* mice. Beginning on day 1, the mice were injected with L-DOPA once a day and MCH was administered (intranasal [i.n.]) 6 days a week. Black bold lines refer to periods of prolonged administration of L-DOPA once a day and orange circles refer to MCH administration 6 days a week. Behavioral tests of dyskinetic movements were performed on days 5, 12, 19, and 26. **b** Three-paw dyskinesia in LID-induced *Pitx3*-deficient *ak/ak* homozygous mice was recorded on days 5, 12, 19, and 26. MCH administration ($n = 6$) produced a significant antidyskinetic effect compared to L-DOPA group ($n = 5$), similar to that of acupuncture. The degree of dyskinesia induction did not differ in *Pmch* −/− *Pitx3 ak/ak* mice ($n = 4$). Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. *$p < 0.05$ compared to L-DOPA group

Whole Transcript Expression Arrays

Total RNA was isolated from the mouse samples (hypothalamus and striatum each) with the RNeasy MiniKit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. RNA purity and integrity were evaluated by ND-1000 Spectrophotometer (A260/A280 ratio in range of 1.8 to 2.1; NanoDrop, Wilmington, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA). The Affymetrix Whole Transcript Expression array process was executed according to the manufacturer's protocol (GeneChip Whole Transcript PLUS reagent Kit).

Then, 100 ng of each RNA sample was processed using a WT Amplification Kit and WT Terminal Labeling Kit. cDNA was synthesized from each isolated RNA sample (100 ng) by using the Primers Mix and ss-cDNA Master Mix of the GeneChip WT (Whole Transcript) Amplification kit as described by the manufacturer. The synthesized sense cDNA was then fragmented by UDG (uracil DNA glycosylase) and APE 1 (apurinic/apyrimidic endonuclease 1) and each fragment was biotin-labeled with TdT (terminal deoxynucleotidyl transferase) using the GeneChip WT Terminal labeling kit.

Approximately 5.5 μg of fragmented and labeled DNA target was hybridized to the Affymetrix GeneChip Mouse 2.0 ST Array at 45 °C for 16 h. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Affymetrix, Santa Clara, California). Signal values were computed using the Affymetrix® GeneChip™ Command Console software.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

From each mouse brain sample, total RNA was obtained using the RNeasy Mini Kit (QIAGEN). Using a spectrophotometer, the extracted RNA was quantified and only the samples with an A260/A280 of 1.8 or more were taken for reverse transcription with the SuperScript III First-Strand (Invitrogen, Carlsbad, CA; 18,080-044). Real-time PCR was performed using a LightCycler®

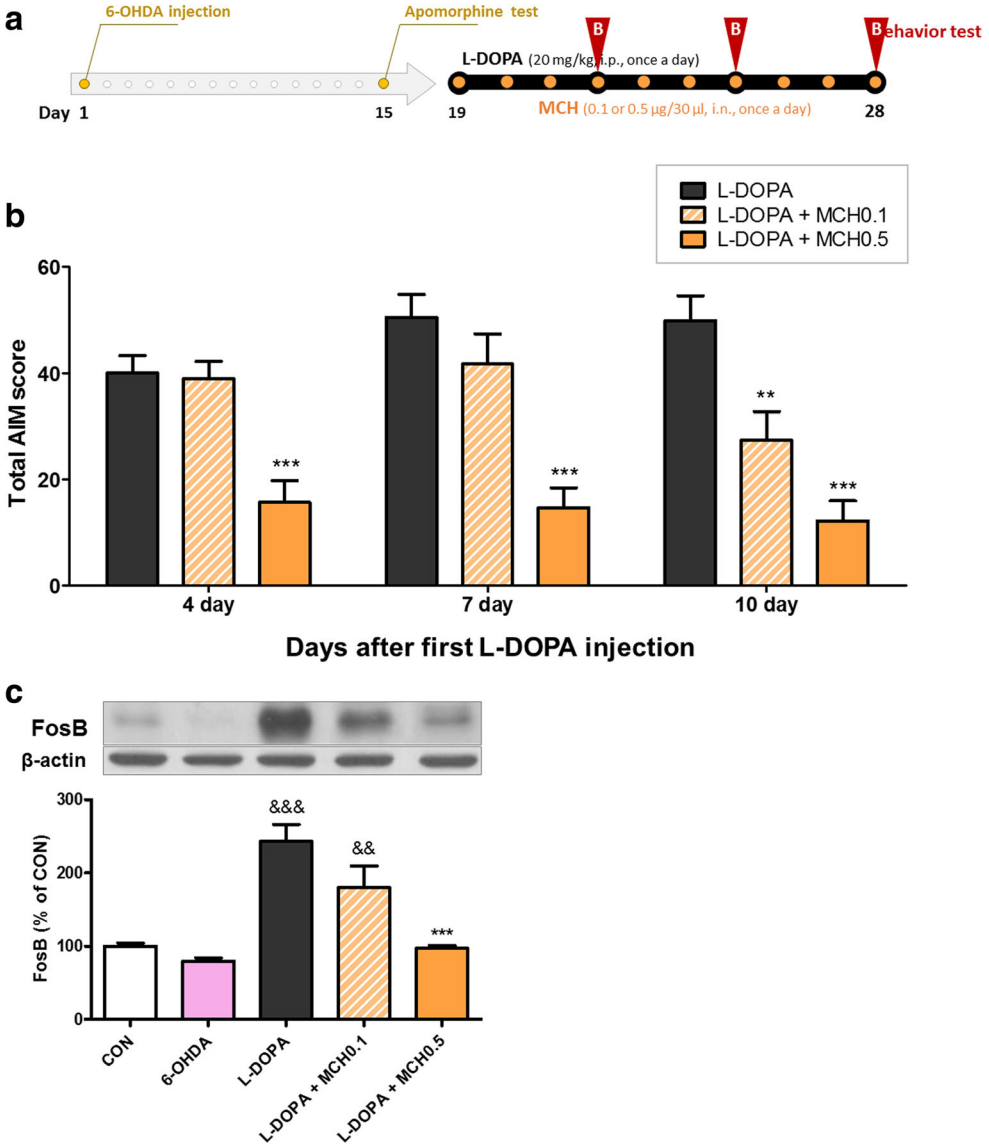

Fig. 5 Verification of the MCH-induced antidyskinetic effects in L-DOPA-treated 6-OHDA mice. **a** Day schedule of experiment 3 in C57BL/6 mice. Beginning on day 1, the mice were anesthetized and received an injection of either saline or 6-OHDA into the right striatum. After a recovery period of 14 days, apomorphine tests were performed on day 15 to validate the PD model. Mice not exhibiting PD-like symptoms were excluded and dopamine-denervated mice were randomly divided into their respective groups. After a rest period of 4 days following the apomorphine test, the LID experimental schedule was initiated. L-DOPA or saline was injected and the i.n. administration of MCH or application of restraint stress were performed. Black bold lines refer to periods of prolonged administration of L-DOPA and orange circles refer to the administration of MCH (0.1 μg/30 μl or 0.5 μg/30 μl once a day for 10 days). Behavioral tests were carried out on days 22, 25, and 28. **b** The administration of 0.5 μg/30 μl MCH ($n = 13$) produced antidyskinetic effects on days 22, 25, and 28 in dyskinesia-induced 6-OHDA mice ($n = 12$), while 0.1 μg/30 μl MCH ($n = 12$) produced the same effects on day 28. **c** Alterations in FosB expression in the striatum; representative bar graph showing the normalized optical densities of FosB contents revealed by Western blot analyses. FosB expression was elevated by the induction of LID and attenuated by MCH treatment. Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. $^{\&\&}p < 0.01$; $^{\&\&\&}p < 0.001$ compared to 6-OHDA group; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to L-DOPA group

Nano Instrument (Roche Molecular Systems, Inc.) with the SYBR Premix Ex Taq (Takara, Republic of Korea; RR420A). 95 °C for 15 min, 94 °C for 15 s, 55 °C or 58 °C (according to primer *Tac2* and *Actb* or *Lcn2*) for 30 s, and 72 °C for 30 s; steps 2–4 were repeated 45 times with the following primers: *Pmch*, 5′-TTC AAA GAA CAC AGG CTC CA-3′ and 5′-GCC AAC ATG GTC GGT AGA CT-3′; *Tac2*, 5′-TGA CTG GCC CTC TCT GAG TT-3′ and 5′-TGC TTT GCA ACC TCG TCT CT-3′; *Lcn2*, 5′-GCC CTG AGT GTC ATG TGT CT-3′ and 5′-GAA CTG ATC GCT CCG GAA GT-3′; *Actb*, 5′-CAT CCG TAA AGA CCT CTA TGC CAA C-3′ and 5′-ATG GAG CCA CCG ATC CAC A-3′ [34]. The results of *Actb* mRNA were used as an internal control probe.

Statistical Analysis

All data analysis and statistical parameter calculation were conducted using the SPSS Statistics (version 23.0, International Business Machines Corp.), the R 3.0.2 (www.r-project.org) and ClueGo and CluePedia Cytoscape apps. Behavioral data were expressed as the mean ± standard error of the mean (SEM). Data analysis was performed by using one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc tests. A criterion for statistical significance was $p < 0.05$.

Results

Effects of Acupuncture Treatment on LID in *Pitx3 ak/ak* Mice

The present study employed an LID model using *ak/ak* mice because the bilateral dyskinetic symptoms and bilateral degeneration of dopaminergic neurons in these mice provide a useful model for mimicking human PD patients. Before the start of experiments, we conducted a preliminary test to evaluate LID development in *ak/ak* mice ($n = 15$). The degree of dyskinetic movements varied until the third week from the first L-DOPA injection and at 4-weeks, the LID behaviors of each mouse appeared to be in a constant level. Then, we tested the acupuncture effect on LID behaviors (Fig. 1a, b) and found that acupuncture on GB34 alleviates dyskinetic events in *ak/ak* mice (Fig. 1c). One-way ANOVA showed a significant effect of groups in dyskinetic behaviors (F (2, 16) = 21.523, $p < 0.05$). That chronic L-DOPA (25 mg/kg) injections administered to *ak/ak* mice ($n = 6$) induced three-paw dyskinetic movements compared to saline-injected *ak/ak* mice ($n = 4$) ($p < 0.05$), whereas acupuncture treatment at GB34 ($n = 8$) resulted in behavioral improvements ($p < 0.05$), consistent with previous studies [10] were found through Bonferroni post hoc test. More specifically, the L-DOPA + ACU group exhibited significant reductions in dyskinetic movements compared with the L-DOPA group (Fig. 1c and Supplementary material).

Identification of Hypothalamic Genes Related to the Antidyskinetic Effects of Acupuncture

Based on previous findings [19, 25, 28], it was hypothesized that the hypothalamus would be a crucial mediator of the effects of acupuncture treatment in the LID model. Thus, an mRNA microarray analysis of the hypothalamus of mice in the L-DOPA and L-DOPA + ACU groups was performed to identify potential hypothalamic biochemical factors that mediated the antidyskinetic effects of acupuncture. We found that the acupuncture treatment resulted in the changes of global gene expression in hypothalamus compared to L-DOPA injection (Fig. 1d, e). We identified six downregulated genes and two upregulated genes ($|\text{fold change}| \geq 4$) in acupuncture-treated hypothalamus (Fig. 1e). Of the various hypothalamic genes regulated by acupuncture, we observed that the most upregulated gene was the *Pmch*, which showed the highest volume among the significantly regulated genes by acupuncture implying an obvious quantitative change. Since it has been reported that MCH, generated from *Pmch* gene, acts as a neurotransmitter or neuromodulator in a broad array of neuronal functions in central nervous system, we hypothesized that *Pmch* might be a key factor for the antidyskinetic effects of acupuncture (Fig. 1d, e).

Validation of the Antidyskinetic Effects of Acupuncture and the Upregulation of *Pmch* mRNA in 6-OHDA Mice with LID

To validate the above-mentioned findings, we investigated whether the improvements in LID and the upregulation of the hypothalamic level of *Pmch* mRNA induced by acupuncture in *Pitx3 ak/ak* mice could be reproduced in 6-OHDA mice. The 6-OHDA injection was conducted to C57BL/6 male mice and the apomorphine test confirmed the dopaminergic depletion (success rate was about 77.2%). By using the successfully lesioned mice ($n = 37$), the acupuncture effects on LID behaviors were studied (Fig. 2a). One-way ANOVA revealed a significant difference in groups for AIM scores (F (2, 22) = 22.79, $p < 0.001$) and Bonferroni post hoc tests showed that the L-DOPA + ACU group ($n = 9$) exhibited a significant reduction in total AIM score compared to L-DOPA group ($n = 8$) ($p < 0.001$), which is an assessment of dyskinetic movements in 6-OHDA PD models, at day 28 (i.e., day 10 from the beginning of L-DOPA injections). Acupuncture stimulation at non-acupoints ($n = 8$) also decreased dyskinetic movements ($p < 0.01$) but the scores following this stimulation were significantly higher than those of the L-DOPA + ACU group ($p < 0.05$) (Fig. 2b). The qRT-PCR analysis was performed with mRNA extracted from the hypothalamus of the 6-OHDA mice (one-way ANOVA, F (5, 16) = 44.18, $p < 0.001$) and, consistent with the data from the *Pitx3 ak/ak* mouse model, the relative mRNA expression level of *Pmch* was higher in the L-DOPA + ACU group than both in the L-DOPA group ($p < 0.01$) and in the L-DOPA + NA group ($p < 0.01$) (Fig. 2c).

Mediating Role of MCH on the Antidyskinetic Effects of Acupuncture in *Pitx3*-Deficient *ak/ak* Mice

To verify the involvement of the MCH/MCH1R pathway in the antidyskinetic effects of acupuncture treatment, three-paw dyskinetic behavior resulting from chronic L-DOPA injections was assessed in *Pitx3*-deficient *ak/ak* mice following

the administration of the MCH1R antagonist Tc-MCH7c. Prolonged L-DOPA injections induced abnormal three-paw movements and the quantified dyskinesia periodically increased from days 5 to 26. One-way ANOVA showed a significant effect of group for LID development (F (3, 25) = 4.208, $p < 0.05$) and Bonferroni post test revealed that acupuncture treatment ($n = 7$) alleviated these abnormal paw movements to a significant degree at day 26 (versus L-DOPA group ($n = 6$), $p < 0.05$), whereas the administration of Tc-MCH7c ($n = 7$) reversed the antidyskinetic effects of acupuncture treatment ($p < 0.05$) (Fig. 3b).

Modulating Effects of MCH on LID in *Pitx3*-Deficient *ak/ak* Mice

To determine the involvement of the MCH system in LID induction or reduction, three-paw dyskinetic behavior resulting from chronic L-DOPA injections was assessed in *Pitx3*-deficient *ak/ak* mice treated with intranasal MCH and *Pmch*-deficient transgenic mice. Prolonged L-DOPA injections induced three-paw dyskinetic movements and the quantified dyskinetic behaviors periodically increased from days 5 to 26. A significant effect of groups was found with one-way ANOVA (F (2, 15) = 6.257, $p < 0.05$) and post test showed the i.n. administration of MCH ($n = 6$) reduced the occurrence of dyskinetic events ($p < 0.05$), similar to acupuncture treatment. The knockout of *Pmch* in the *Pitx3 ak/ak* mice ($n = 4$) had no influence on the induction of abnormal paw movements (Fig. 4b).

Effects of MCH Treatment on LID in 6-OHDA-Lesioned Mice

To confirm the role that MCH plays in the attenuation of LID, we checked it in 6-OHDA-lesioned mice after 6-OHDA injections and following apomorphine tests (success rate was about 78.3%) (Fig. 5a). MCH agents (0.1 μg/30 μl or 0.5 μg/30 μl) were intranasally administered to L-DOPA-injected 6-OHDA mice and one-way ANOVA revealed a significant effect of groups for AIM scores (F (2, 34) = 17.020, $p < 0.001$). Significant antidyskinetic effects were observed in both the L-DOPA + MCH 0.1 group ($n = 12$) and L-DOPA + MCH 0.5 group ($n = 13$) but the 0.5 μg/30 μl dose lowered total AIM scores faster and to a greater extent than the 0.1 μg/30 μl dose (Fig. 5b). More specifically, the dyskinetic behaviors of the L-DOPA + MCH 0.5 group decreased ($p < 0.001$) to a level similar to that of the acupuncture-treated mice shown in the second set of experiments at the same time (Figs. 2b and 5b). The FosB contents of the dopamine-denervated striatal areas of each group were measured and analyzed with one-way ANOVA (F (4, 15) = 16.264, $p < 0.001$). It was revealed that LID induced an upregulation of FosB (L-DOPA group versus 6-OHDA group, $p < 0.001$), which is consistent with previous findings [10, 35]. On the other hand, MCH treatment (0.5 μg/30 μl) reduced FosB expression (L-DOPA + MCH 0.5, $p < 0.001$ vs. L-DOPA) as observed in dyskinetic behaviors (Fig. 5b, c).

Similarities in Global Gene Expression in the Striatum Following MCH and Acupuncture Treatments

To further the current understanding of the MCH mechanisms underlying the effects of acupuncture, a transcriptome analysis was performed in the striatum of *Pitx3*-deficient *ak/ak* mice (saline, L-DOPA, L-DOPA + ACU, and L-DOPA + MCH groups). A battery of changes in global gene expression in the striatum of *aphakia* mice was revealed by analyses of the saline, L-DOPA, L-DOPA + ACU, and L-DOPA + MCH groups. Remarkably, the striatum samples of the L-DOPA + ACU, and L-DOPA + MCH groups merged into one cluster (Fig. 6a). Furthermore, the genes significantly modulated by acupuncture relative to the L-DOPA group were compared with the genes modulated by MCH using ClueGo and CluePedia, which are apps of Cytoscape, and candidate genes for the study of LID-reducing mechanisms were identified (Fig. 6b). Subsequently, the expression levels of *Tac2* and *Lcn2* mRNA were shown to be significantly upregulated in the L-DOPA + ACU (Tac2, $p < 0.05$; Lcn2, $p < 0.05$) and L-DOPA + MCH groups (Tac2, $p < 0.01$; Lcn2, $p < 0.001$) by qPCR, which is consistent with the results of the transcriptome analysis and expression levels of *Cartpt* mRNA were also increased but failed to reach statistical significance (Fig. 6c).

Discussion

The present study found that acupuncture produced antidyskinetic effects against LID in both *Pitx3*-deficient *ak/ak* and 6-OHDA-induced PD mice. Additionally, the *Pmch* gene was shown to be upregulated in the hypothalamus by acupuncture treatment in the LID models and the administration of MCH had a novel antidyskinetic effect in both 6-OHDA and *Pitx3 ak/ak* mice. On the other hand, an MCH1R antagonist prevented the LID-reducing effects of acupuncture. To the best of our knowledge, this is the first study to demonstrate the antidyskinetic role of MCH in a model of PD, as well as the mediation of hypothalamic MCH in the antidyskinetic effects of acupuncture.

Acupuncture has potential as an adjunct therapy for PD with conventional treatments such as L-DOPA. Acupuncture treatment not only improved dopaminergic transmission in the striatum in an MPTP-induced mice PD model [16] but also synergistically enhanced the efficacy of low-dose L-DOPA in a 6-OHDA-induced mice PD model [10]. The previous study showed that acupuncture treatment results in antidyskinetic effects by rebalancing GABA contents in the SN [10].

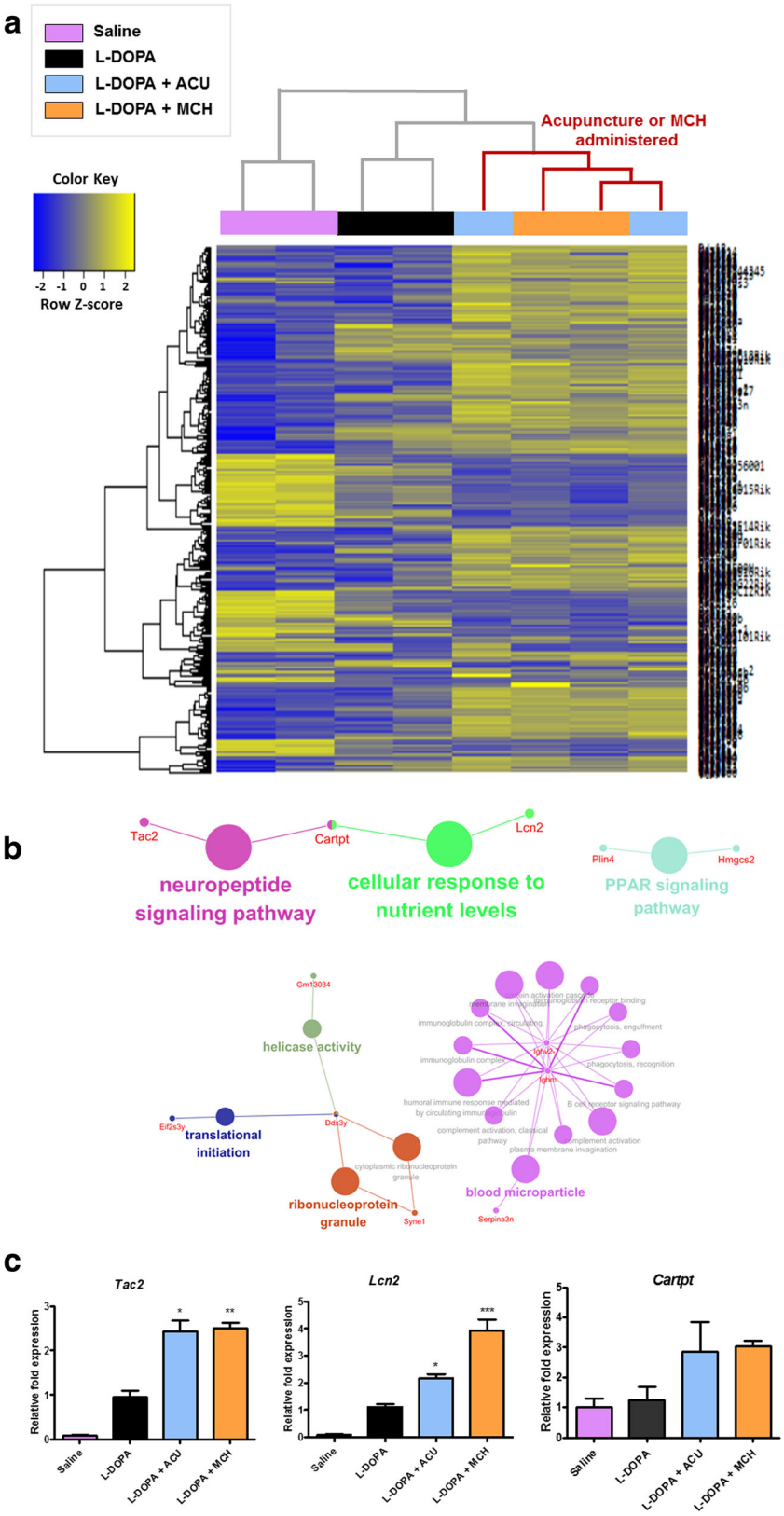

Fig. 6 Transcriptome analyses of genes associated with the antidyskinetic effects of acupuncture and MCH in the striatum. **a** Hierarchical clustering of the microarray data performed using the R 3.0.2 with lowest adjusted *p* value from local pooled error test. A hierarchical clustering analysis revealed that the L-DOPA + ACU and L-DOPA + MCH groups were classified into the same cluster. **b** The grouped networks visualized by ClueGo and CluePedia using Gene Ontology categories. Of the common genes regulated by both L-DOPA + ACU and L-DOPA + MCH groups, the networks indicated that the neuropeptide signaling pathway and cellular response to nutrient level plays major roles. **c** Confirmatory qPCR analyses of *Tac2*, *Lcn2*, and *Cartpt* in the striatum. Data were expressed as the mean ± SEM and statistical analysis was performed by using one-way ANOVA followed by the Bonferroni post hoc tests. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to L-DOPA group

Although acupuncture affects various regions of the brain [17, 18], the hypothalamus is considered to be one of the most important ones [19–23]. Thus, the present study aimed to identify genes in the hypothalamus that mediate the antidyskinetic effects of acupuncture by performing a gene expression profiling analysis. Microarray analysis revealed

that the expression levels of *Pmch* in the hypothalamus were significantly upregulated following acupuncture treatment in *ak/ak* mice with LID. The increased expression of *Pmch* was also observed in an LID model of 6-OHDA-induced PD mice, while further research is needed whether *Pmch* genes was upregulated specifically by acupuncture on GB34, because those changes by other acupoint or non-acupoint have yet investigated in this LID models. Interestingly, the previous study of our team demonstrated that acupuncture increased *Pmch* gene expression in the hypothalamus and led to the activation of hypothalamic MCH neurons which, in turn, release MCH in the SN, activating post-synaptic MCH1R and resulting in dopaminergic neuroprotection [28]. In the present study, we also compared the effects of acupuncture under conditions with or without the MCH1R antagonist Tc-MCH7c (10 mg/kg, i.p.) to determine whether the effects of acupuncture are specifically mediated by MCH1R. The antidyskinetic effects of acupuncture were substantially attenuated following pretreatment with the MCH1R antagonist. These data support the idea that the effects of acupuncture are dependent on MCH1R and are not unspecific effects.

MCH is a hypothalamic peptide consisting of a cyclic 19-amino acid structure that is synthesized exclusively in the lateral hypothalamus and zona incerta. The lateral hypothalamus is a target brain region stimulated by acupuncture treatment [19–23]. Takeshige et al. [22] reported that the stimulation of an acupoint activates specific brain regions, including the lateral hypothalamus, through afferent pathways. Choi et al. [19] found that electroacupuncture regulates natural killer cell activity by modulating activity in the lateral hypothalamus, which suggests that this region is important for the effects of acupuncture. MCH neurons project widely throughout the central nervous system, which implies that they may play a role as a neurotransmitter and/or neuromodulator in the regulation of a variety of physiological functions [36–38]. MCH operates as a neuropeptide in the regulation of the sleep-wake cycle, feeding behaviors, and learning and memory formation [37, 39, 40]. Additionally, patients with PD exhibit a massive loss of MCH-expressing neurons in the hypothalamus, which may lead to deficiencies in sleep regulation, energy homeostasis, and the control of autonomic function [41, 42]. However, the role of MCH in LID has yet been elucidated.

Next, the present study aimed to determine whether the acupuncture-induced release of MCH played an essential role in the modulation of LID. Thus, the antidyskinetic effects of MCH in an LID model using *Pitx3 ak/ak* mice were assessed by directly introducing MCH to the brain via i.n. administration. This method allows peptides to enter the brain rapidly and directly because they can bypass the blood–brain barrier [43]. The present results indicated that MCH treatment (i.n.) significantly alleviated AIMs in *Pitx3 ak/ak* mice as well as in an LID model of 6-OHDA-induced PD mice. The present finding that MCH and acupuncture exert antidyskinetic effects in addition to their previously identified antiparkinsonian effects [28] suggests that these therapeutic modalities may be effective strategies for PD patients because they inhibit the progress of the disease as well as reduce the adverse effects of L-DOPA. To clarify whether depletion of the MCH gene was associated with the induction of LID, the dyskinetic phenomena of *Pmch* and *Pitx3* double knockout mice were assessed but no significant differences were found. Thus, MCH may not be directly involved in the development of LID. Further research is necessary to identify the exact role of MCH in the development of LID.

Several studies have reported links between the MCH system and the mesolimbic dopamine pathway in terms of the modulation of hedonic properties. Depending on the conditions, dopamine-associated responses may increase following either MCH administration or MCH deficiencies. For example, MCH deficiencies enhance locomotor responses to repeated amphetamine administration, which is related to dopamine release and reuptake in the mesolimbic pathway [44]. On the other hand, the intracerebroventricular (i.c.v.) administration of MCH heightens cocaine-induced locomotor activities that are based on a buildup of dopamine in the synapse [45]. Our research group reported that the administration of MCH in the nigrostriatal pathway improves impaired motor function induced by dopamine depletion in a PD model [28] and it also alleviate abnormal behaviors induced by exogenously increased dopamine (LID). These findings suggest that MCH plays a modulating role in the homeostasis of dopaminergic activity. A variety of studies have demonstrated an interaction between the MCH and dopaminergic systems but the detailed mechanisms underlying the manner in which MCH influences the dopaminergic system remain to be further elucidated.

To better understand how acupuncture and MCH regulate LID, changes in gene expression were assessed with transcriptome analyses of the striatum. A hierarchical clustering analysis revealed that the L-DOPA + ACU and L-DOPA + MCH groups were classified in the same cluster, which supports the notion that MCH mediates the effects of acupuncture on LID. Of the common genes regulated by both acupuncture and MCH, the grouped networks visualized via ClueGo indicated that LID-regulating mechanisms in the striatum were associated with the cellular response to extracellular stimuli (neuropeptide signaling pathway, cellular response to nutrient levels, and blood microparticles) and functions related to gene expression (translational initiation, ribonucleoprotein granule, helicase activity, and the PPAR signaling pathway); more specifically, the *Tac2*, *Lcn2*, and *Cartpt* genes were highly linked. *Tac2* encodes the gene neurokinin B (NKB), which is a tachykinin neuropeptide that preferentially binds to neurokinin-3 receptors (NK3Rs) [46]. NKB/NK3R signaling is involved in the regulation of dopaminergic transmission [47, 48] and Zhang et al. [49] demonstrated that the mRNA expression of NKB increases following L-DOPA injections

into the striatal region of 6-OHDA-lesioned rats. The first speculation of these authors was that elevations in NKB are related to the induction of LID. However, the injection of an NK3R antagonist potentiated abnormal L-DOPA-induced contralateral rotations, which suggests that the L-DOPA-induced release of NKB might be a rescue mechanism. *Lcn2* encodes a lipocalin-2 (LCN2) protein, which is involved in cellular iron transport [50]. LCN2 is a multifunctional protein related to neuroinflammation [50–52]. In terms of its association with PD, LCN2-deficient mice exhibit attenuations in dopaminergic cell death and motor dysfunction induced by MPTP and 6-OHDA [51]. However, recent findings suggest that LCN2 functions as a "help me signal" following stroke-induced neuronal injury to promote the differentiation of microglia and astrocytes into phenotypes beneficial for recovery [52]. In the case of sepsis, a lack of LCN2 results in the elevation of inflammatory molecules and the exacerbation of behavioral phenotypes; thus, LCN2 may play a compensatory role during abnormal changes in the brain [53]. These discrepancies could be interpreted as a context-dependent role for LCN2 under conditions of neuroinflammation [50]. Based on these previous reports, it is postulated that the acupuncture- and MCH-induced upregulation of *Tac2* and *Lcn2* expression in the striatum might contribute to reducing the adverse effects of L-DOPA as a type of compensatory mechanism. However, further studies would be necessary to elucidate the exact roles of NKB/NK3R and LCN2 in LID. Though the increase of *Cartpt* expression failed to reach statistical significance, a network analysis suggested that *Cartpt* might also play a role in this mechanism. It encodes cocaine- and amphetamine-regulated transcript (CART), which has been well documented to affect the reward and reinforcement of a drug via modulation of the mesolimbic dopamine systems [54]. Recently, CART has drawn attention due to its role in neurodegenerative diseases because it may afford neuroprotective effects [55, 56]. In PD, pretreatment with CART (i.p.) prevents MPTP-induced dopaminergic neuronal cell loss and motor dysfunction [55, 56]. Furthermore, Upadhya et al. [57] reported that CART treatment (i.c.v.) attenuates contralateral rotations followed by apomorphine or L-DOPA administration and CART treatment per se induces ipsilateral rotations in unilateral 6-OHDA mice, which suggests a modulatory role of CART on dopamine systems in PD and LID models. Taken together, these results suggest that the antidyskinetic effects of acupuncture and MCH might be caused by d the enhancement of compensatory responses against abnormal changes induced by L-DOPA administration in the striatum. However, the present study only suggests this possibility and, thus, the direct roles of the NKB/NK3R and/or LCN2 systems in the modulation of LID require further examination.

The present study has several issues and limitations that need to be addressed. First, acupuncture stimulation is a type of peripheral stimulation that induces central modulatory effects. Previous studies have shown that acupuncture increases extracellular signal-regulated kinase (ERK) phosphorylation in local skin tissues of acupoint GB34 and that the central modulatory effects of acupuncture are reduced by the local blockage of phosphor-ERK [58], which indicates the role of acupuncture in peripheral and central crosstalk. However, the present study could not identify a signaling link between local acupuncture stimulation and the lateral hypothalamus. If the modulatory role of this peripheral (acupuncture)-central connection is revealed, then the associated beneficial effects thereof can be expanded for patients with neurological diseases experiencing adverse effects. The present study also could not elucidate a direct link between hypothalamic MCH and changes in the striatum. Emerging studies are using novel techniques such as optogenetics, live imaging, and tracing in evaluating pathways and the techniques can help clarify this connection. Additionally, the precise brain regions responsive to MCH after i.n. administration should be further clarified. It has also been reported that many brain regions, including the basal ganglia (striatum and SN reticulata) [59, 60] and areas outside the basal ganglia (motor and somatosensory cortices, bed nucleus of the stria terminalis, dorsal hippocampus, zona incerta, lateral habenula, and/or frontal cortex) [61–63] are affected by LID. Because intranasally administered MCH can spread to whole brain regions, we cannot rule out the fact that brain areas other than the striatum might be responsive to this treatment. Additionally, it is well-known that MCH regulates homeostasis and metabolism and, thus, it is possible that MCH may cause abnormal feeding behaviors. However, the present results showed that the i.n. administration of MCH for 36 days did not induce a greater degree of weight gain than the administration of saline. Although the peripheral actions of MCH are not well understood, MCH1Rs are located in peripheral tissues and MCH knockout mice exhibit lower levels of intestinal inflammation [64], reductions in intestinal tumorigenesis [65], and an increased mortality against intestinal pathogens [66]. Because small amounts of intranasally administered MCH can enter the bloodstream, the peripheral function of MCH should also be considered [66]. Thus, the development of MCH as a pharmacological treatment for LID will require long-term monitoring to rule out the occurrence of possible adverse events.

Conclusions

In summary, the present study found that hypothalamic *Pmch* expression was upregulated by acupuncture treatment and that the administration of an MCH antagonist reversed the antidyskinetic effects of acupuncture in both the *Pitx3 ak/ak* and 6-OHDA-induced PD mice models of LID. Additionally, the present study was the first to report that MCH exhibited

antidyskinetic effects in these models. Thus, the effects of acupuncture and MCH may be beneficial for overcoming LID in patients with PD.

Authors' contributions HJ Park designed experiment and wrote, edited, and revised the manuscript. J Kim and SH Jeon designed experiment and revised the manuscript. YK Kim, AR Lee, and H Park performed experiments. S Ahn and J Yoo analyzed and interpreted the data. YK Kim, AR Lee, and HJ Park contributed materials and method tools. YK Kim wrote the draft. All authors had input into the manuscript and have approved the manuscript for publication.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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