RESEARCH ARTICLE

Practically Defined *Off*-State Dyskinesia Following Repeated Intraputamenal Glial Cell Line–Derived Neurotrophic Factor Administration

CME

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ABSTRACT: Background: We recently showed that by employing an enhanced drug-delivery approach, repeated administration of glial cell line–derived neurotrophic factor (GDNF) can produce a spatially distributed increased ^{18}F-DOPA positron emission tomography (PET) uptake, suggesting sprouting of dopaminergic terminals throughout the putamen structure. Despite this, we failed to prove a significant measurable clinical response. Since, however, we have identified a subject demonstrating a temporal relationship between repeated GDNF infusions and dyskinesia arising in the practically defined *off* (prac*off*) state.

Objectives: To describe the development of prac*off* dyskinesia across our study population and consider its utility as an indicator that trophic factor–induced terminal sprouting can affect enhanced endogenous dopamine levels.

Methods: This was a blinded retrospective analysis of videotaped motor assessments at eight weekly study visits. Dyskinesia in the prac*off* and supramaximal *on* state were rated using the Clinical Dyskinesia Rating Scale. Logistic regression was employed to explore the predictors of prac*off* dyskinesia. Generalized estimated equations were used to estimate the cumulative effect of repeated GDNF infusions.

Results: Mild–moderate choreiform dyskinesia in the prac*off* state were seen in 47 assessments in 17 ($n = 41$) subjects. During the 18-month timeframe, each subsequent 8-week period of receiving GDNF increased the risk of demonstrating prac*off* state dyskinesia by 34% (odds ratio [OR], 1.34 (95% confidence interval [CI], 1.20, 1.50); $P < 0.001$). An increasing supramaximal *on* dyskinesia score (OR, 1.17 [95% CI, 1.07, 1.30]; $P = 0.001$) also increased the likelihood of prac*off* dyskinesia at that visit.

Conclusions: We report the first description of increasingly prevalent prac*off*-state dyskinesia developing during the course of a trophic factor study. This may provide a surrogate marker that GDNF can enable recovery of endogenous dopamine release even in advanced Parkinson's disease. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Background

Regenerative therapies in Parkinson's disease (PD) include mesencephalic stem cell transplantation[1] and trophic factors to promote neuronal recovery. We previously reported a randomized controlled trial (RCT) of intermittent intraputamenal administration of glial cell line-derived neurotrophic factor (GDNF) delivered via a

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novel convection-enhanced delivery approach.[2,3] GDNF, a neurotrophic protein for dopaminergic and other neurons,[4] has shown neuroprotective and neurorestorative effects in toxin-based animal studies.[5] Moreover, recent postmortem findings from two patients post–AAV2-neurturin gene therapy (a GDNF analog) demonstrated increased tyrosine hydroxylase staining in treated neurons despite the presence of a-synuclein–positive aggregates.[6,7] Despite clinical benefits in open-label studies,[8,9] however, five double-blind clinical trials of GDNF or neurturin failed to show significant improvement.[2,10-13]

In the latest placebo-controlled study, GDNF infused directly to the putamen every 4 weeks for 40 weeks did not produce significant benefit in the primary endpoint, the practically defined *off* (prac*off*)-state Unified Parkinson's Disease Rating Scale (UPDRS) motor score. Interval positron emission tomography (PET) imaging, however, showed an unprecedentedly widespread and significant increase in putamenal fluorine-18-l-dihydroxyphenylalanine (^{18}F-DOPA) uptake in the GDNF group, which remained unchanged in the placebo group. Given ^{18}F-DOPA uptake is well correlated to numbers of surviving dopamine neurons,[14] the increased uptake observed could suggest that our novel method of administration achieved satisfactory drug delivery across the field of interest, target–receptor engagement, and potentially, sprouting of dopamine terminals throughout the putamen.

That said, increased ^{18}F-DOPA uptake is not synonymous with the regeneration of dopaminergic neurons as ^{18}F-DOPA is taken up and stored in all neurons containing aromatic amino acid decarboxylase. Upregulated enzyme activity, reawakening of hibernating serotonergic neurons,[15] and a phenotypic shift of gamma-aminobutyric acid (GABA) interneurons[16,17] could also be implicated. Furthermore, even if the observed increase in radiotracer signal was related to sprouting of the dopamine terminal plexus, ^{18}F-DOPA PET per se can not determine if a restoration in functional pharmacology has arisen, that is, newly sprouted terminals capable of storing and releasing endogenous dopamine sufficient to affect a clinical response.

Revisiting the videotaped trial data, we identified a case of a 61-year-old woman receiving serial GDNF infusions every 4 weeks for 112 weeks who developed prac*off*-state dyskinesia over sequential assessments. At each eight-weekly assessment from baseline through to the end of the double-blind phase (week 40, 10 infusions administered), there was no prac*off*-state dyskinesia observed in the video assessments. However, at week 56, following 14 GDNF infusions, mild prac*off*-state dyskinesia was seen. Thereafter, this increased in severity with increasing numbers of infusions before plateauing and was present at each subsequent eight weekly video assessment until week 80 (end of open-label study, 20 infusions post baseline). Following this time point, early trial recruits received yet further GDNF infusions until the last visit of the last enrolled study participant had occured. For the patient described the last assessment was at week 112 and after 28 GDNF infusions post-baseline, and in these additional videoed assessments, prac*off* dyskinesia was seen identical to that seen at week 80. Following stopping GDNF, prac*off* dyskinesia was still observed in a video assessment performed

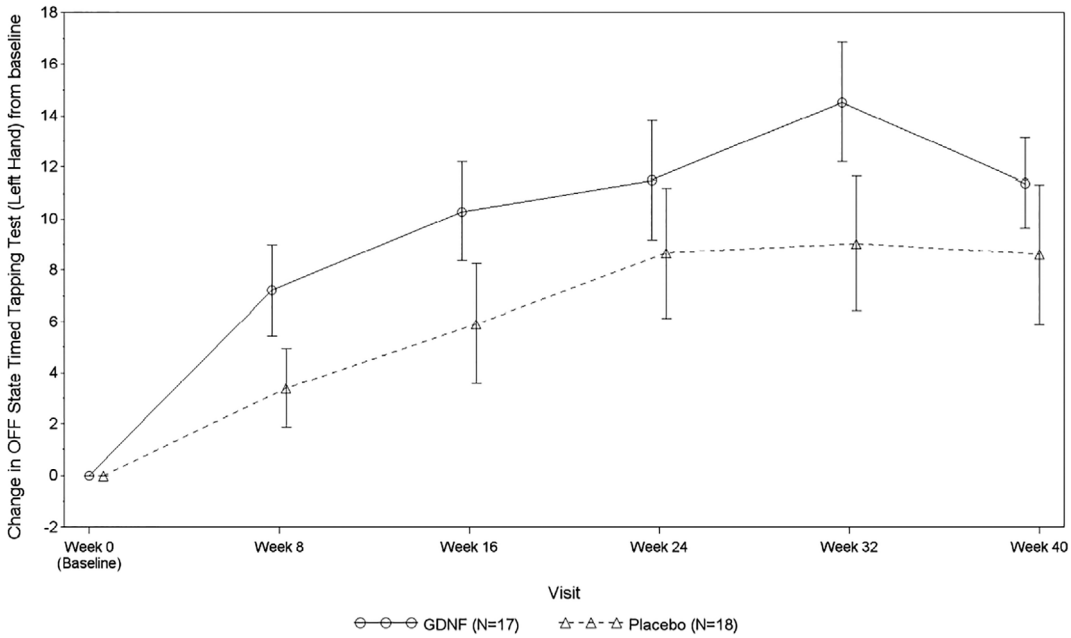

FIG. 1. Practically defined *off*-state timed tapping test for the left hand: change over time in both the glial cell line–derived neurotrophic factor (GDNF) group and the placebo group between week 0 and week 40; there was no significant difference between groups.[2]

2 years post-cessation, although this was less severe. However, prac*off* dyskinesia was no longer present in a video performed 3 years after stopping GDNF. We determined that this could potentially reflect a GDNF dose–response curve (see Video S1).

To help interpretation of the aforementioned case, see Figure 1. The absolute group mean change in the prac*off* state taps performed showed both groups improved over time before hitting a ceiling effect with no difference between the groups. The improvement in the placebo group inferring a placebo and or training and or self-competing response.

The aforementioned case suggests a possible temporal relationship between the development and then resolution of prac*off*-state dyskinesia as GDNF infusions are repeatedly delivered and then halted. This led us to consider the potential utility of prac*off* dyskinesia as a marker of recovering dopamine release from newly sprouted dopaminergic terminals. In this retrospective analysis of study video data, we aim to do the following:

1. Describe across the whole study population the development of prac*off* dyskinesia through a blinded retrospective analysis of study videos, something not reported in previous trials of GDNF.[8,13,18]
2. Consider their underlying mechanism and whether their evolution supports heightened dopamine release arising from recovering dopaminergic neurons through the examination of their relationship with other disease-related factors and or parameters of GDNF exposure.

Methods

Original Study Design

This is a retrospective analysis of data from our single-center RCT, followed by an open-label study, to assess the efficacy of GDNF directly infused into the putamen on an intermittent administration basis.[2,3] A total of 41 study particapants with idiopathic PD underwent implantation of a novel delivery system. Patients were randomly assigned to GDNF or placebo infusions (diluent, artificial cerebrospinal fluid) on a four-weekly basis for 40 weeks.[3] An open-label extension subsequently ran from weeks 40 to 80 where all participants received GDNF.[4] All patients were on stable levodopa (L-dopa) and other dopaminergic or dopamimetic therapy for at least 2 months prior to study inclusion. Medication was kept stable during the study but could be modified if required.

Study Data Available

Every 8 weeks (4 weeks after the last infusion and immediately before the next), patients underwent videotaped assessment of motor outcome measures including UPDRS (Part III),[19] alternating timed taps,[20] and timed walks. Assessments were conducted in both the prac*off* state (morning assessment following withholding of long-acting PD medication for 24 hours before and all other PD medication from 6 PM the evening before) and then in the *on* state following a supramaximal L-dopa challenge (supra*on* state; at least 1.5× the usual morning L-dopa dose). Other original data were also extracted (see Appendix S1).

Video Review, Rating Scale, and Data Extraction

All available videos from the recorded eight-weekly assessments in both the prac*off* and supra*on* states were retrospectively reviewed. Hyperkinetic (choreiform) dyskinesia was rated using the Clinical Dyskinesia Rating Scale (CDRS)[21] by one rater (K.L.) blinded to treatment allocation and post-training by A.W. The CDRS was selected as it was previously used in studies of *off*-state dyskinesia following mesencephalic fetal cell grafts.[22] Given the retrospective nature of our analysis, we also required a scale appropriate to the content of the video footage recorded. The scale gives a dyskinesia severity score (0, absent; 1, mild; 2, moderate; 3, severe; 4, extreme) for each body area (face, neck, trunk, and each limb).[19] The sum gives the global CDRS score (range, 0–28). For a frequency analysis, the global CDRS score was generated into a binary variable (DYS+/DYS-) for the presence/absence of dyskinesia depending on if the global dyskinesia score was ≥2. This cutoff was decided prospectively given the difficulty in distinguishing "normal" overspill hyperkinetic motion during movement tasks (eg, alternating timed tapping) versus subtle dyskinesia in any body region, the purpose being to reduce type 1 error. For additional information on the variables recorded and variable management, see Appendix S1.

Analysis

All analysis was conducted on Stata version 17.0.[23] Dyskinesia severity in the *on* and *off* states were compared using the nonparametric Wilcoxon signed-rank test. Logistic regression (binary outcome, dyskinesia present or absent) was used to explore the predictors of prac*off* dyskinesia. Candidate predictors were determined a priori (see Table 1). To account for the nonindependent nature of the data (repeated observations within individuals), we used clustered sandwich estimators to adjust standard errors. Variables that were measured at each study visit (e.g., UPDRS Part III, supra*on* CDRS) were analyzed as predictors for the presence of prac*off* dyskinesia at the same visit. Nonchanging variables (e.g., sex, disease duration) were analyzed using the baseline value across all visits. L-dopa equivalent doses (LEDD) at weeks 0, 40, and 80 were used in a

time-varying manner in which the week 0 value was used for each visit until week 40, when this value was used until week 80.

To better capture the longitudinal nature of the data and to delineate the effect of GDNF versus placebo from the effect of repetitive infusions per se or simply time, we used generalized estimating equations (GEEs) to model the effect of cumulative GDNF infusions (for a more detailed description and rationale, see Appendix S1).

Results

Describing *Off*-State Dyskinesia

Hyperkinetic dyskinesia in the prac*off* state was seen at 41 of 445 assessments (n) during the 80-week period and in 16 study participants of 41 (N). A total of 6 visits had missing data. Of the subjects who demonstrated prac*off* dyskinesia, the median number of assessments in which prac*off* dyskinesia was present was 2 (interquartile range [IQR], 1–4; minimum, 1; maximum, 7). Prac*off* choreiform dyskinesia was most often seen in the neck (n = 36 assessments, 88%) and trunk (n = 18, 44%) and less commonly in the arms (n = 10, 24%), legs (n = 8, 20%), and face (n = 7, 17%). It was primarily present on action (n = 37, 90%). Prac*off* dyskinesia was of lower severity (median global CDRS score, 3 [IQR, 2–4; range, 2–8]) compared with supra*on* dyskinesia (median global CDRS score, 8 [IQR, 5–11; range, 2–20]; Wilcoxon signed-rank test, $z = 17.07$; $P < 0.001$). Supra*on* dyskinesia was seen at baseline in 27 subjects (66%) and across the whole study period in 320 of 445 assessments (72%) in 35 participants. In study visits where prac*off* dyskinesia was seen, supra*on* dyskinesia was always present in the paired assessment. There was concordance between the body part affected in prac*off* and supra*on* dyskinesia at each time point (range, 85.7%–100%), suggesting that if prac*off* dyskinesia develops, it is more commonly seen in the same distribution as supra*on* dyskinesia, although there were situations where prac*off* dyskinesia arose in a body region where supra*on* dyskinesia was not observed.

Only one subject showed prac*off* dyskinesia at baseline (week 0), demonstrating that choreiform prac*off*

TABLE 1 Univariable logistic regression for predictors of practically defined *off*-state dyskinesia at each study visit

Candidate predictors of pracOFF dyskinesia	Number of Assessments		OR (95% CI)	*P* value
	DYS−, N = 404	DYS+, N = 41		
Practically defined *off*-state UPDRS Part III score at that visit	27.70 (10.57)	28.93 (14.99)	1.01 (0.96, 1.06)	0.714
Supramaximal *on*-state UPDRS Part III score at that visit	15.94 (6.96)	14.64 (5.53)	0.97 (0.90, 1.05)	0.442
Supramaximal *on*-state global CDRS score at that visit	4 (0–9)	11 (7–12)	1.19 (1.08, 1.31)	0.001
Super responder[a] in double-blind study—>10-point improvement in Part IIII UPDRS score			1.46 (0.54, 4.01)	0.453
No	175 (74%)	5 (45%)		
Yes	60 (26%)	6 (55%)		
Δ ^{18}F-DOPA putamenal uptake, OR per SD	0.08 (0–0.28)	0.115(0–0.26)	0.87 (0.56, 1.36)	0.555
LEDD, mg, OR per 100 mg	990 (800–1207)	1119 (891–1291)	0.97 (0.84, 1.11)	0.651
Disease duration, years, OR per SD of log(disease years)	8 (5–11)	9 (8–18)	1.54 (0.90, 2.63)	0.114
Sex			1.02 (0.36, 2.86)	0.968
Male[b]	217 (54%)	25 (53%)		
Female	187 (46%)	22 (47%)		
Age, years	54 (7.98)	57 (8.55)	1.03 (0.96, 1.11)	0.349

Note: Data are expressed as mean (SD) for practically defined *off*-state UPDRS Part III score, supramaximal *on*-state UPDRS Part III score, and age. Median (interquartile range) are shown for supramaximal *on*-state global CDRS score, change in ^{18}F-DOPA putamenal uptake, LEDD, and disease duration. Frequency (percentage) are provided for super responder status and sex.

[a]Super responder status was those subjects with an absolute improvement of ≥10 points at week 40 in the practically defined *off*-state motor UPDRS score (see Whone and colleagues[2]).

[b]Male is reference category.

Abbreviations: DYS−, absence of practically defined *off*-state dyskinesia; DYS+, presence of practically defined *off*-state dyskinesia; OR, odds ratio; CI, confidence interval; UPDRS, Unified Parkinson's Disease Rating Scale; CDRS, Clinical Dyskinesia Rating Scale; SD, standard deviation; LEDD, levodopa equivalent dose; Δ ^{18}F-DOPA, change in fluorine-18-l-dihydroxyphenylalanine uptake

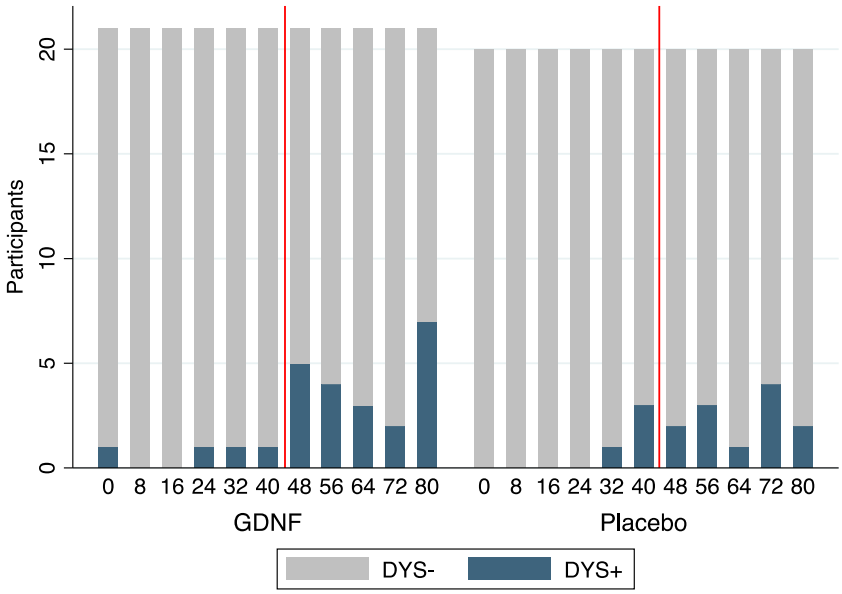

FIG. 2. Bar chart showing the number of participants at each study visit (0–80 weeks) with (DYS+, Clinical Dyskinesia Rating Scale [CDRS] score ≥2) and without (DYS-, CDRS score <2) practically defined *off*-state dyskinesia. The red reference line represents the move from the double-blind randomized trial to the open-label extension study with all participants receiving glial cell line–derived neurotrophic factor (GDNF). [Color figure can be viewed at wileyonlinelibrary.com]

dyskinesia is not a usual consequence of chronic L-dopa therapy. Across both groups, there were less cases of prac*off* dyskinesia in the first 40 weeks (assessments = 8 [n], subjects = 6 [N]) compared with 40 to 80 weeks (n = 33, N = 14; $P < 0.001$) (Fig. 2). Incidence of prac*off* dyskinesia was uncommon in both treatment arms in weeks 0 to 40 (GDNF: n = 4, N = 3; placebo: n = 3, N = 1); however, prac*off* dyskinesia was seen on three assessments before week-32 in the GDNF group, but on 0 assessments prior to week 32 in the placebo group. In weeks 40 to 80, 21 assessments in nine subjects in the GDNF/GDNF group and 12 assessments in five participants in the placebo/GDNF group showed prac*off* dyskinesia. The increase in the daily LEDD from baseline to week 80 was 59 ± 194 mg in the GDNF/GDNF group and 289 ± 365 mg in the placebo/GDNF group (least squares mean difference: −233 mg; 95% confidence interval [CI]: −419, −47; $P = 0.02$).

Predictors of Prac*off*-State Dyskinesia at Each Visit

In the univariable logistic models, supra*on* global CDRS score at each visit was predictive of prac*off* dyskinesia at the same visit (see Table 1). Each point increase in the global CDRS score in the supra*on* state at each visit increased the likelihood of having prac*off* dyskinesia at that assessment visit by 19% (odds ratio [OR], 1.19 [95% CI: 1.08, 1.31]; $P = 0.001$). The UPDRS Part III score in both the supra*on* and prac*off* condition, however, did not predict the presence of prac*off* dyskinesia at the same study visit. Although noted to be greater in subjects showing visits associated with prac*off* dyskinesia, change in ^{18}F-DOPA uptake between week 0 and week 40 also did not predict prac*off* dyskinesia. Likewise, LEDD, years since diagnosis, sex, and age were not found to be predictors.

Effect of GDNF on Prac*Off*-State Dyskinesia

In the GEE model, receiving placebo at each subsequent 8-week visit ("time without GDNF") altered the risk of showing prac*off*-state dyskinesia by 24% (OR, 1.24 [95% CI: 0.98, 1.59]; $P = 0.079$), although the P value does not show strong evidence against the null hypothesis. However, each subsequent 8-week period receiving GDNF ("time on GDNF") increased the risk of prac*off*-state dyskinesia by 34% (OR, 1.34 [95% CI: 1.20, 1.50]; $P < 0.001$), with very strong evidence against the null hypothesis. This "time on GDNF" slope is a combination of any risk attributed to factors other than GDNF (so the slope we observed on placebo) plus any risk attributed to taking GDNF. The difference in slopes between the GDNF and placebo (risk attributed to GDNF alone) gives an OR of 1.08 ([95% CI: 0.82, 1.42]; $P = 0.60$). This suggests that the majority of risk of developing prac*off*-state dyskinesia for patients on GDNF is not attributed to receiving GDNF alone for 80 weeks (OR of 1.08 vs. 1.24) but could be to a combination of factors, including repeated GDNF infusions (see Fig. 3).

Discussion

To our knowledge, we report the first description of hyperkinetic pracoff-state dyskinesia developing during the course of a trophic factor study in PD. Video-evidenced pracoff dyskinesia of mild to moderate severity was observed in some subjects, with an increasing incidence as the study progressed. By week 80, 34% of subjects had shown choreiform dyskinesia at ≥1 study visit despite withdrawal of immediate-release dopaminergic agents for 12 hours and long-acting formulations for 24 hours prior to assessments. The incidence of pracoff-state dyskinesia increased throughout the 80-week assessment period in both the GDNF-GDNF and the placebo-GDNF groups. Our GEE analysis showed that for each cumulative GDNF infusion received, the likelihood of developing choreiform dyskinesia in the pracoff state increased by 34% every 8 weeks. Supraon-state dyskinesia severity at a study assessment predicted the presence of pracoff dyskinesia at the same assessment, but none of the other factors we investigated did.

Understanding the relevance of the aforementioned effect of time is complex as increasing visit number is a marker of several potential interplaying factors:

1. The cumulative effect of increasing number of times GDNF administered.
2. The longer time elapsed for GDNF to effect neurorestoration and recover functional pharmacology.
3. The cumulative effect of infusions per se in terms of repeated tissue disruption.

Ethical considerations limited the duration of the double-blind portion of the study to 9 months, and this may be too short a period to allow a significant differential effect between GDNF and placebo. That noted, the relationship with time for 80 weeks in the GDNF-GDNF group, as well as the temporal relationship between pracoff dyskinesia and receiving and stopping GDNF in our illustrated case, is potentially consistent with GDNF producing a restoration in pharmacology sufficient to generate a clinical response. Given GDNF is hypothesized to work via promoting neurorestoration, rather than through a symptomatic effect, it would fit that observations of pracoff dyskinesia increased over months. Furthermore, we recognize that our study population was small owing to the novelty of the drug-delivery system employed and that this coupled with the low frequency of pracoff dyskinesia limit the ability to draw firm conclusions.

The pracoff dyskinesia we report is more representative of the L-dopa–induced dyskinesia seen at peak dose, which is often choreiform involving the face, neck, and trunk, rather than the dystonic posturing that typically occurs in the *off* state.[24] Furthermore, the diphasic dyskinesia seen in PD, as the patient tails-off from the *on* to the *off* state, are usually rapid alternating movements involving the lower limbs, which are distinct from those we report.[25] Residual exogenous L-dopa administered the day before could be implicated given that the true washout period of all dopaminergic drugs exceeds the practically defined washout period, and L-dopa can have a persistent effect more than 2 weeks after withdrawal.[25] However, although some ongoing benefit on bradykinesia and rigidity may be expected, observing choreiform dyskinesia in the pracoff state phenotypically identical to peak-dose dyskinesia would not be expected. Furthermore, residual exogenous L-dopa would not explain the pattern of increasing incidence of pracoff dyskinesia throughout the study period. As an alternative explanation to inadequate washout, we recognize that the hyperkinetic pracoff dyskinesia we describe could be the result of GDNF acting to extend the duration of the medication *on* state rather than reflect restoration of endogenous dopamine release. However, as we are reporting the presence of choreiform pracoff dyskinesia 24 hours after stopping long-acting dopaminergic agents and 12 hours after holding shorter acting medication, the latter explanation instead of the former seems more likely to us given the markedly long time period since the last L-dopa dose. L-dopa equivalent doses increased for 80 weeks by ± only 59 ± 194 mg in the GDNF-GDNF group versus 289 ± 365 mg in the placebo-GDNF group (mean difference: −233 mg; 95% CI: −419, −47; $P = 0.02$), which also would not explain the increased incidence of pracoff dyskinesia in the GDNF-GDNF

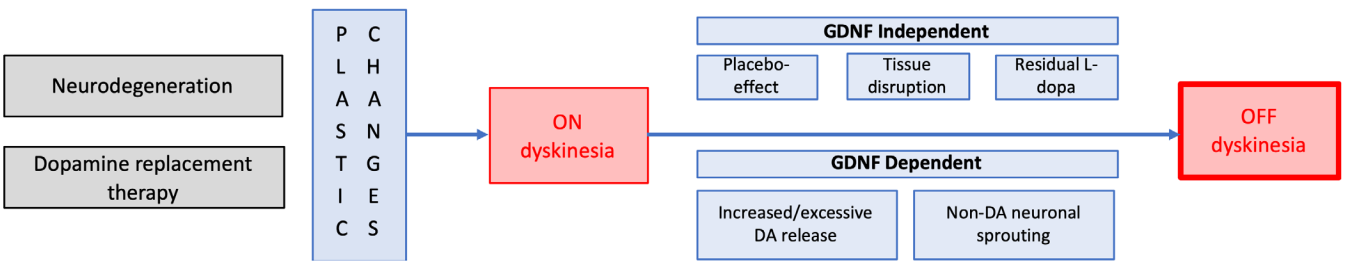

FIG. 3. Possible mechanisms producing practically defined *off*-state dyskinesia in an intraputamenal repeated-infusion study testing glial cell line–derived neurotrophic factor (GDNF). DA, dopamine; L-dopa, levodopa. [Color figure can be viewed at wileyonlinelibrary.com]

group over 80 weeks. A final consideration is whether prac*off*-state dyskinesia is a marker of poor compliance with the study protocol with subjects taking their morning L-dopa medication rather than holding. Given such high compliance with other study aspects, including a 99.1% attendance at drug-infusion visits and all participants completing the trial, we feel this is unlikely. Likewise, the UPDRS Part III prac*off* score at each visit was not a predictor of prac*off* dyskinesia, further revoking the poor-compliance argument.

Albeit rare, the development of prac*off* dyskinesia in the placebo group prior to the switch at week 40 to receive GDNF is puzzling. Similarly puzzling is the development of prac*off* dyskinesia from the outset of the extension study in some placebo-GDNF patients immediately after switching to GDNF. One possible explanation for these findings, and indeed the increased development of prac*off* dyskinesia with time across both groups, is the potential for repetitive putamenal infusions per se to produce tissue damage leading to stimulation of the nigrostriatal dopamine system and/or promotion of endogenous growth factor release.[26-28] The short duration of the randomized phase did not allow for any differential effect of the infusions themselves from the effect of infusate contents (active or placebo) to be determined. Against an acute tissue disruption response stimulating dopamine release, it needs to be appreciated that assessments were performed 4 weeks after the last infusion. It is worth noting that the potential effect of tissue damage on the development of *off* dyskinesia in previous fetal graft studies could not be assessed as the control group received sham surgery comprising burr holes but no penetration of the dura.[20,29] If in our study, however, if a restorative effect had been mediated through repeated tissue damage causing heightened endogenous growth factor expression, we would have expected to see an improvement in ^{18}F-DOPA uptake in our placebo group, which we did not.[2]

Stereotypic and choreiform dyskinesia in the *off* state involving the head and neck that were mostly mild were also reported following implantation of human fetal mesencephalic grafts in PD (GID).[20,27,30] Despite similarities in their nature and distribution, GID developed 6 months[28] and 3 years[29] following grafting, later than the dyskinesia we report. We did not see a similar subgroup of severe GID that persisted despite reduction or withdrawal of dopaminergic therapy and continued over years.[31] Relevant to our hypothesis, GID was thought to be caused by excess dopamine release from grafts,[32] but this was later questioned.[33] Grafted fetal tissue was seen to have a significant serotonergic component that has been postulated to explain the dyskinesia observed. Indeed, heightened serotonin release may have arisen in our population as GDNF promotes sprouting of all monoaminergic fiber types and not just dopamine neurons.[4] The above said, one important difference between graft-induced and GDNF-induced *off* dyskinesia is that in grafted patients any enhanced dopamine levels would have arisen from implanted exogenous tissue rather than from rejuvenated endogenous neurons.

The association we observed between the severity of supra*on* state and developing prac*off*-state dyskinesia suggests a dependence of prac*off* dyskinesia on similar plastic mechanisms to L-dopa–induced dyskinesia.[34] Increased endogenous dopamine release from newly sprouted neurons may act on already "primed" circuits. This is supported by the presence of supra*on* dyskinesia from the beginning of the study period followed by increasing incidence of prac*off* dyskinesia over time. Although GDNF itself can also induce plastic changes at the dopaminergic synapse, these are presynaptic in origin, and GDNF does not increase postsynaptic D2 receptor density.[35] That prac*off*-state dyskinesia was not seen in 25 of 41 subjects could mean that GDNF did not increase dopaminergic tone in more than half of the study participants and therefore perhaps explains the negative clinical outcomes in the primary study.[2] That said, no firm conclusion can be drawn as we only saw prac*off* dyskinesia arising in those who also demonstrated supra*on* dyskinesia, and 14 participants did not show supra*on* dyskinesia at baseline, and six participants did not show supra*on* dyskinesia at any visit. In clinical practice, some patients with advanced disease never develop peak-dose choreiform dyskinesia and seem to be relatively protected from this phenomenon despite high L-dopa doses. Hence, it could be the case that a lack of dyskinetic response does not mandate against a restoration in endogenous dopamine levels in certain individuals.

We did not find a correlation between increased ^{18}F-DOPA uptake over 9 months in the GDNF versus placebo group and the emergence and intensity of prac*off* state hyperkinetic dyskinesia. Had we, this would have strengthened our ability to draw a firmer conclusion. That we did not, however, does not lend support either way. ^{18}F-DOPA imaging was only acquired at baseline and at the end of the double-blind portion of the study and not at the end of the open-label extension phase.[2,3] Most participants who developed prac*off*-state dyskinesia did so in the second 9 months. The combination of the small numbers that did in the first 9 months and the inherent noise in test–retest ^{18}F-DOPA imaging in advanced PD means that this correlation was not sufficiently powered.

Another potential cause of *off* dyskinesia may include a placebo effect. We recognize that the magnitude of the placebo response was marked during our double-blind study on clinician-observed motor outcomes.[13] However, although scales employed in trials to assess antidyskinetic agents are prone to a placebo

effect,[36] the emergence of dyskinesia over time in the prac*off* state as a placebo effect seems unlikely given that participants would not have anticipated such a response.

In conclusion, we identified a participant from a trial assessing intermittent intraputamenal GDNF infusions in PD who demonstrated a temporal relationship between evolving and then remitting choreiform dyskinesia arising in the prac*off* state after repeated and then halted GDNF infusions. This led us to consider that, although prac*off* dyskinesia is an adverse event, evaluating this phenomenon may provide some evidence into whether GDNF can affect an alteration in functional pharmacology in vivo. Hence, we performed a retrospective analysis of video data from 41 participants captured during the aforementioned trial. We report the first description of increasingly prevalent prac*off*-state dyskinesia developing during the course of a trophic factor study. Our generalized estimated equation model shows that each subsequent GDNF infusion increases the likelihood of developing such involuntary movements. We are able to suggest possible mechanisms why this developed (see Fig. 3), which are both GDNF independent and GDNF dependent, including that GDNF can enable recovery of endogenous dopamine expression even in advanced PD. ■

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.