Table 3
BFMDRS disability subscales in patients with Meige syndrome who underwent bilateral pallidal stimulation.

Disability scale	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean	Range	
Before surgery (range)								
Speech (0–4)	3	2	4	1	1	2.2	1–4	
Writing (0–4)	0	1	3	1	1	1.5	1–3	
Feeding (0–4)	0	0	3	2	1	2	1–3	
Eating and swallowing (0–4)	0	0	3	2	3	2.7	2–3	
Hygiene (0–4)	0	1	3	2	2	2	1–3	
Dressing (0–4)	0	1	3	2	2	2	2–3	
Walking (0–6)	0	0	4	3	2	3	2–4	
Total	3	5	23	13	12	11.2	3–23	
After surgery (range)								p value
Speech (0–4)	0	1	1	0	0	0.4	0–1	0.022
Writing (0–4)	0	0	1	0	0	0.3	0–1	0.04
Feeding (0–4)	0	0	0	1	0	0.3	0–1	0.072
Eating and swallowing (0–4)	0	0	0	0	0	0	0	0.034
Hygiene (0–4)	0	0	0	0	0	0	0	0.013
Dressing (0–4)	0	0	1	0	0	0.3	0–1	0.025
Walking (0–6)	0	0	1	0	1	0.7	0–1	0.046
Total	0	1	4	1	1	1.4	0–4	0.015

Statistical analyses were performed using the Mann–Whitney U test.

4. Discussion

Clinical studies in patients with primary generalized or segmental dystonia have shown the beneficial effects of bilateral GPi-DBS for both motor symptoms and disability caused by dystonia [3]. However, experience with GPi-DBS in other forms of dystonia such as Meige syndrome is limited. Moreover, long-term outcome of patients with Meige syndrome treated with GPi-DBS remain to be elucidated. In this study, we showed that bilateral pallidal stimulation produced a long-lasting suppression of dystonia in 5 patients with primary Meige syndrome. The mean improvement (over 80%) in motor symptoms was comparable, with respect to scores of both BFMDRS motor and disability scales (Table 2), to the results obtained in patients with primary generalized or segmental dystonia [10], and in patients with tardive dystonia [11]. Our results also showed that speech difficulties caused by spasmodic dysphonia and/or oromandibular dystonia in Meige syndrome responded well to pallidal stimulation.

Dystonia is a complex clinical syndrome due to a wide range of etiologies. The pathogenesis of primary Meige syndrome remains unknown. However, it has been suggested that the basal ganglia interconnecting the cortico-striato-pallido-thalamic circuits are involved in models of the pathophysiology of Meige syndrome [5]. The present study provides clinical evidence that dystonia symptoms in primary Meige syndrome could be markedly alleviated by electrostimulation of the GPi, one of the output nuclei of the basal ganglia, and suggests that this movement disorder may result from the basal ganglia dysfunction. Multimodal medical treatments that include botulinum toxin injections are used to treat Meige syndrome, but their therapeutic efficacy has been found to vary across patients and often decreases over time. As reported here, we observed continuous bilateral GPi-DBS to be a safe surgical therapy for producing a sustained and long-term improvement in the dystonia symptoms and functional disabilities of patients with primary Meige syndrome. Recently, an important observation was made that while disease duration can be a good predictor of the outcome of pallidal stimulation in patients with primary dystonias, no particular predictive value should be assigned to age at onset, age at surgery, severity of disease, DYT1 status or the presence of phasic or tonic involuntary movements [12]. The mean duration of disease in our patients with Meige syndrome was greater than 10 years, and a better general outcome of pallidal stimulation might be expected in patients with a shorter duration of this disease. In conclusion, we suggest that patients with disabling dystonia symptoms associated with primary Meige syndrome can be good candidates for treatment with bilateral pallidal stimulation.

Acknowledgements

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Comparison of effects of botulinum toxin subtype A1 and A2 using twitch tension assay and rat grip strength test

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ABSTRACT

Botulinum toxin type A is used as a therapeutic agent for some spastic neurological disorders. Type A organisms have been classified into four subtypes (A1 to A4) based on the amino acid sequence variability of the produced neurotoxin. At present, commercially available preparations of the toxin belong to subtype A1. To date, no study has compared the characteristics of the biological activity of toxins from different subtypes. We compared the efficacy of A1 toxin (LL toxin or neurotoxin: NTX) with that of A2 toxin (NTX) employing the twitch tension assay using the mouse nerve phrenic nerve hemidiaphragm and grip strength test in rats. The inhibitory effects on neuromuscular transmission of A2NTX at pH 7.4 and pH 6.8 were 1.95 and 3.73 times more potent than those of A1LL, respectively. The 50% effective doses for the administered limb, the dose which caused a 50% reduction in grip strength, i.e. ED_{50}, of A1LL, A1NTX, and A2NTX were calculated as 0.087, 0.060, and 0.040 U/head, respectively. These doses for the contralateral limb, i.e. TD_{50}, of A1LL, A1NTX, and A2NTX were calculated as 6.35, 7.54, and 15.62 U/head, respectively. In addition, the time required for A2NTX-injected rats to recover the grip strength of the contralateral limb was 17 days, while that for rats injected with A1LL was 35 days. The results indicated that A2NTX is a more potent neuromuscular blocker than A1 toxins, and suggested that A2NTX will provide a preferential therapeutic agent for neurological disorders.

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1. Introduction

Botulinum toxins act at neuromuscular junctions and induce muscle relaxation by inhibiting acetylcholine release. The toxins cause muscle flaccidity, and have been developed and used as important therapeutic agents for neurological disorders such as blepharospasm, hemifacial spasm, and a variety of dystonias (Jankovic, 2004; Sadick, 2003). The toxins are protein complexes, called progenitor toxins, containing a 150-kDa neurotoxin (NTX) and non-toxic components. Type A progenitor toxins are classified by their molecular weight into three forms: LL toxin, 900 kDa; L toxin, 500 kDa; and M toxin, 300 kDa (Sakaguchi, 1983). The M toxin consists of an NTX and a non-toxic component exhibiting no hemagglutinin (HA) activity (described here as non-toxic non-HA: NTNH), and L and LL toxins are formed by conjugation of the M toxin with HA (Montecucco et al., 1996).

Recently, the amino acid sequences of toxins have been analyzed, and it has been shown that sequence variability occurs in NTX (Willems et al., 1993; Franciosa et al., 2004, 2006). Accordingly, type A organisms have been classified

into four subtypes (A1 to A4) based on the amino acid sequence variability of NTX (Arndt et al., 2006). Botulinum toxin type A products used as treatment for neurologic disorders are LL toxin and NTX, produced by Clostridium botulinum subtype A1 (A1LL and A1NTX) (Dressler and Benecke, 2007). In A1 to A4 toxins, it was reported that A1 and A2 toxins showed no difference in mouse lethal toxicity (Sakaguchi et al., 1990; Tabita et al., 1991). However, there has been no report comparing toxin subtypes regarding their neuromuscular transmission inhibition and muscle relaxation as medicinal effects.

We purified NTX produced by C. botulinum subtype A2 (A2NTX). A2NTX shares 89% amino acid sequence homology with A1NTX (Willems et al., 1993). In this study, we compared the efficacy of A2NTX with that of commercial type A toxin products. The in-vitro assay involved a twitch tension assay using the mouse phrenic nerve hemidiaphragm, employed to compare neuromuscular transmission inhibition between A2NTX and A1LL. The in-vivo assay was a grip strength test involving rats, which was used to compare muscle flaccid paralysis among A2NTX, A1NTX, and A1LL.

2. Materials and methods

2.1. Purification of toxins

Botulinum neurotoxins type A (150 kDa, NTX) were prepared using a modification of a previously reported method (Sakaguchi et al., 1981). C.botulinum type A strains 62A and Chiba-H, which belong to subtypes A1 and A2, respectively, were cultured in PYG medium containing 2% peptone, 0.5% yeast extract, 0.5% glucose, and 0.025% sodium thioglycolate by allowing them to stand at 30 °C for 3 days. M toxin was purified from the culture fluid by acid precipitation, protamine treatment, ion-exchange chromatography, and gel filtration. Each subtype of M toxin was adsorbed onto a DEAE Sepharose column equilibrated with 10 mM phosphate buffer, and eluted with a 0–0.3 M NaCl gradient buffer for the separation of NTX from the non-toxic component. The NTXs were stored at −70 °C until use.

For the control, the commercial progenitor LL toxin (BOTOX®, Allergan Inc., Irvine, US, hereafter A1LL) was used.

2.2. Experimental animals

Female ICR/CD-1 mice (4 weeks of age, about 20 g, Charles River Laboratories Japan, Yokohama, Japan), male ddY mice (5 weeks of age, about 30 g, Charles River Laboratories Japan, Yokohama, Japan), and female S/D rats (8 weeks of age, about 200 g, Charles River Laboratories Japan, Yokohama, Japan) were used for the LD_{50}, twitch tension assay, and grip strength test, respectively. Animals were maintained under controlled light/dark conditions and had free access to food and water. This study was performed in accordance with the guidelines concerning experimental animals established by the Japanese Pharmacological Society, and was approved by the Animal Ethics Committee of our institute.

2.3. Toxin activity (mouse ip LD_{50}) measurements

The toxin activities of A2NTX, A1NTX, and A1LL were determined employing the mouse intraperitoneal LD_{50} method (Pearce et al., 1994), with one mouse ip $LD_{50} = 1$ unit (U). The mouse ip LD_{50} was determined by employing an assay involving 7 doses at a dilution interval of 1.25 and 20 animals per dose. The chosen evaluation period was the first 96 h after administration, and the LD_{50} was calculated using the probit method.

The specific activities of A2NTX and A1NTX were 93 and 63 U/ng of neurotoxin proteins, respectively. That of A1LL is reported to be about 20 U/ng of the toxin protein complex based on BOTOX® product information (Allergan Inc., 2010).

2.4. Twitch tension assay (in-vitro test)

The twitch tension assay was conducted as previously described, with modification (Sugimoto et al., 1992). Phrenic nerve hemidiaphragm preparations were isolated from ddY mice, and then transferred to oxygenated (95% O_2 + 5% CO_2) Krebs solution of the following composition (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1.24; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26; glucose, 10; pH 7.4. The costal margin of the muscle was fixed and the central tendon was connected to a tension transducer (Nihon Kohden, Tokyo, Japan) by a string. The preparation was suspended in a glass tissue chamber containing 10 mL of Krebs solution. The phrenic nerve was connected to a pair of platinum electrodes and stimulated with supramaximal rectangular pulses of 1 V amplitude and 10 ms duration at a frequency of 0.25 Hz. The tension transducer was connected to an amplifier (Nihon Kohden, Tokyo, Japan), and the muscle tension was recorded on paper using a pen recorder (Nihon Kohden, Tokyo, Japan). In addition, to emulate the possible muscle pH of patients with spasm, these twitch tension experiments were repeated under acidic conditions, with the pH brought to 6.8 using lactic acid (final concentration: 25 mM).

After the twitch tension had become stable, one of the toxins (diluted with 20 mM Tris–HCl containing 150 mM NaCl and 0.02% bovine serum albumin (pH 7.4 or 6.8)) was applied to the bathing solution, and the reduction in twitch tension was recorded. After the experiment, tensions were measured every 4 s from each data record, and these values were averaged over intervals of 1 min. The time required for the twitch tension to decline to $1/e$ of that observed immediately before toxin application was calculated.

2.5. Measurement of grip strength (in-vivo test)

We compared flaccid paralysis on the administered or contralateral side induced by A1LL, A1NTX, and A2NTX. The toxins were diluted two-fold serially to 0.15–2.4 U/mL for the former or at an interval of 40 U serially to 10–240 U/mL for the latter with physiological saline containing 0.5% human serum albumin. Rats were anesthetized by intraperitoneally injecting 40 mg/kg of sodium pentobarbital (Kyoritsu Seiyaku, Tokyo, Japan). After the disappearance of the eyelid reflex, the foreleg of the rat was shaved and 0.1 mL of each toxin concentration was injected into the

foreleg muscle (flexor digitorum muscle). For injection, an insulin syringe (Becton Dickinson, Tokyo, Japan) was used.

The grip strength was measured in the left and right forelegs of each rat using a Grip Strength Meter (Muromachi Kikai, Tokyo, Japan) and a modification of a previously reported method (Meyer et al., 1979). Each rat was fixed horizontally, and then pulled steadily by the root of the tail away from the T bar until its grip was broken. The peak of the grip strength was measured. Each rat was subjected to five such trials, and the average was used as the grip strength. The grip strength was measured at 0 (before administration), 1, 2, 3, 4, 7, and 14 days after injection. It is represented as the gram-force (gf).

To evaluate the duration of the muscle flaccidity-inducing effect of the toxin, the toxins were diluted to 0.12 U/mL with 0.5 w/v% human serum albumin-containing physiological saline and 0.1 mL of each toxin was injected into the muscle of the foreleg. The grip strength was measured until 66 days after injection.

To compare the duration of equivalent muscle flaccidity-inducing effects among the toxins, each toxin was diluted to the dose necessary to cause a 50% reduction in the grip strength on either the administered or contralateral side, and was injected into the muscle of the foreleg. The grip strength was measured until 35 days after injection. The variation range of the grip strength before injection was defined as the mean ± 2 S.D. The duration of the muscle flaccidity-inducing effect of the toxins was defined as the time required for recovery into this variation range of grip strength before injection.

2.6. Statistical analysis

The grip strength is expressed as the mean ± S.E.M., and the time-course is presented graphically. To evaluate the efficacy of the toxins, a regression line for each toxin was calculated for the peak effect versus the dose. Regression lines were used to calculate the doses causing 50% reductions in grip strength, and these values were termed the Effective Dose 50 (ED$_{50}$: administered side) and Toxic Dose 50 (TD$_{50}$: contralateral side), respectively.

The ED$_{50}$ and TD$_{50}$ of each toxin were analyzed using a nonlinear least-squares method employing SAS (SAS Institute Inc., ver. 9.1).

3. Results

3.1. Comparison of neuromuscular blocking activities of the toxins using the twitch tension assay

The inhibitory effects of A2NTX, A1NTX, and A1LL on neuromuscular transmission were determined based on the twitch tension assay. Dose-dependent shortening of the attenuation time of twitch tension was observed in this assay (Fig. 1). We plotted the logarithm of the time for twitch tension to decline to 1/e of that observed immediately before toxin application against the logarithm of toxin activity (LD$_{50}$ doses), and the linearity of the regression line and homogeneity of variance were confirmed by regression analysis. Each toxin in the twitch tension assay could not be compared using identical toxin activities, because the
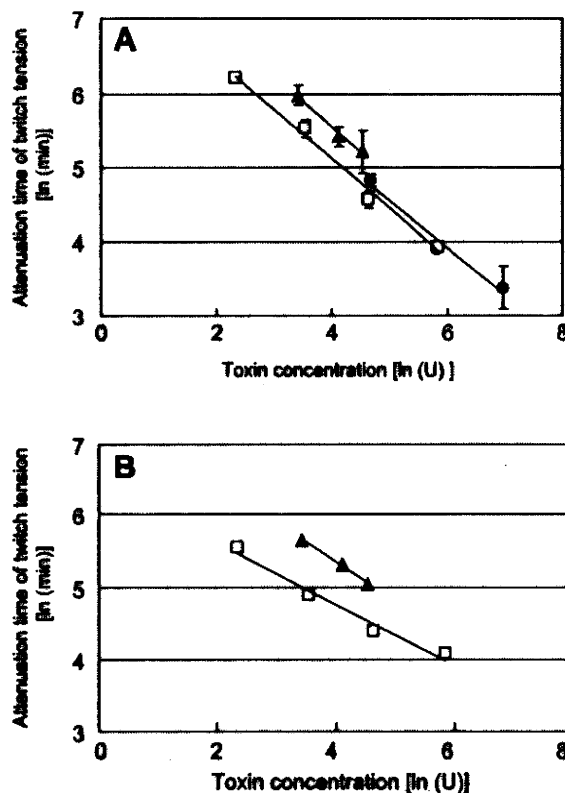

Fig. 1. Dose–response curves of A1LL, A1NTX, and A2NTX for the twitch tension assay using mouse phrenic nerve hemidiaphragm preparations. The response was expressed as the time required for twitch tension to decline to 1/e of that observed immediately before toxin application. (A) pH 7.4 (▲: A1LL, ●: A1NTX, □: A2NTX). (B) pH 6.8 (▲: A1LL, □: A2NTX). Each point is the mean ± S.D. (n = 3–5).

employed doses of each toxin were different. However, comparison of the linear range of the dose–response relationship revealed that A2NTX was 1.21- and 1.95-times more potent than A1NTX and A1LL in its neuromuscular blocking activity, respectively. In addition, under acidic conditions (pH 6.8), the neuromuscular blocking activity of A2NTX was 3.73 times more potent than A1LL.

3.2. Comparison of muscle flaccidity induced by the toxins using the grip strength test

3.2.1. Time-course of grip strength on the administered side

To compare flaccid paralysis on the administered side among A2NTX, A1NTX, and A1LL, the grip strength was measured. It was found that the grip strength decreased with the LD$_{50}$ concentration of toxins (Fig. 2). Grip strengths in the presence of A2NTX, A1NTX, and A1LL decreased until the 2nd, 3rd, and 3 days after toxin injection, respectively, and then recovered.

3.2.2. Time-course of grip strength of the contralateral foreleg after injection

The safety (diffusion) of each toxin was evaluated by periodically measuring the grip strength of the contralateral
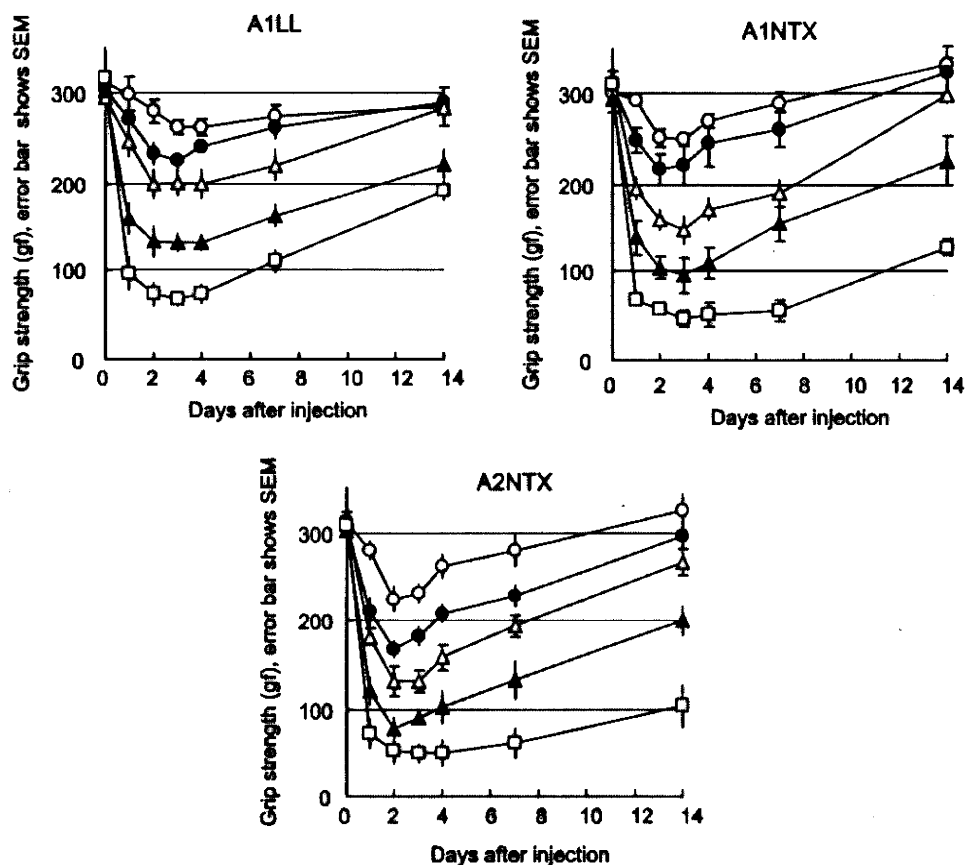
Fig. 2. Time-course of the grip strength on the administered side after toxin injection. Rats received A1LL, A1NTX, or A2NTX injection in the left foreleg (each at ○: 0.015 U, ●: 0.03 U, △: 0.06 U, ▲: 0.12 U, and □: 0.24 U). The grip strength was measured in the left foreleg of each rat at 0 (before administration), 1, 2, 3, 4, 7, and 14 days after injection. Each point is the mean ± S.E.M. ($n = 5$).

foreleg (right front leg) after the intramuscular injection of relatively large amounts of toxin into the left foreleg. It was found that the grip strength decreased according to the LD_{50} concentration of toxins (Fig. 3). The grip strength in the right foreleg, in which toxin was not injected, decreased until the 3rd or 4th day after toxin injection and recovered after the 7th day. The relationship between the grip strength and administered dose was calculated by regression analysis each measurement day, the log LD_{50} was plotted against the grip strength, and the linearity of the regression line and homogeneity of variance were confirmed by regression analysis. A1NTX of 20 and 24 U killed one of five rats, and A1LL of 24 U killed three of five rats. In contrast, all A2NTX-treated rats survived (Data not shown).

3.2.3. ED_{50} and TD_{50}

ED_{50} and TD_{50} were calculated from the peak decrease in the grip strength on the administered and contralateral side, respectively (Table 1). The ED_{50} for A2NTX was 0.040 U, which was lower than that for the other toxins. The ED_{50} values for A1NTX and A1LL were 0.060 and 0.087 U, respectively. The TD_{50} for A2NTX was 15.62 U, a higher dose than that for other toxins. The TD_{50} values of A1NTX and A1LL were 7.54 and 6.35 U, respectively.

3.2.4. Duration of muscle flaccidity induced by the toxins

We compared the duration of the muscle flaccidity-inducing effect of the toxins when the same dosage of each toxin was injected. The effects of A1LL, A1NTX, and A2NTX resolved at 35, 35, and 66 days after injection, respectively (Fig. 4A). A2NTX showed a longer duration of action than A1LL and A1NTX.

We also compared the duration of action of each toxin when adjusting the toxins to show equivalent muscle flaccidity-inducing effects. In spite of the different doses, recovery from all toxins occurred by 14 days after injection on the administered side (Fig. 4B). However, on the contralateral side, recovery occurred at 35, 35, and 17 days for A1LL, A1NTX, and A2NTX, respectively (Fig. 4C).

4. Discussion

We investigated whether the efficacy of botulinum toxin type A differed according to the type A structure or amino acid sequence of the toxin molecule. Our results indicate that A2NTX is more effective than A1LL and A1NTX, which have been used for commercial botulinum toxin type A products.
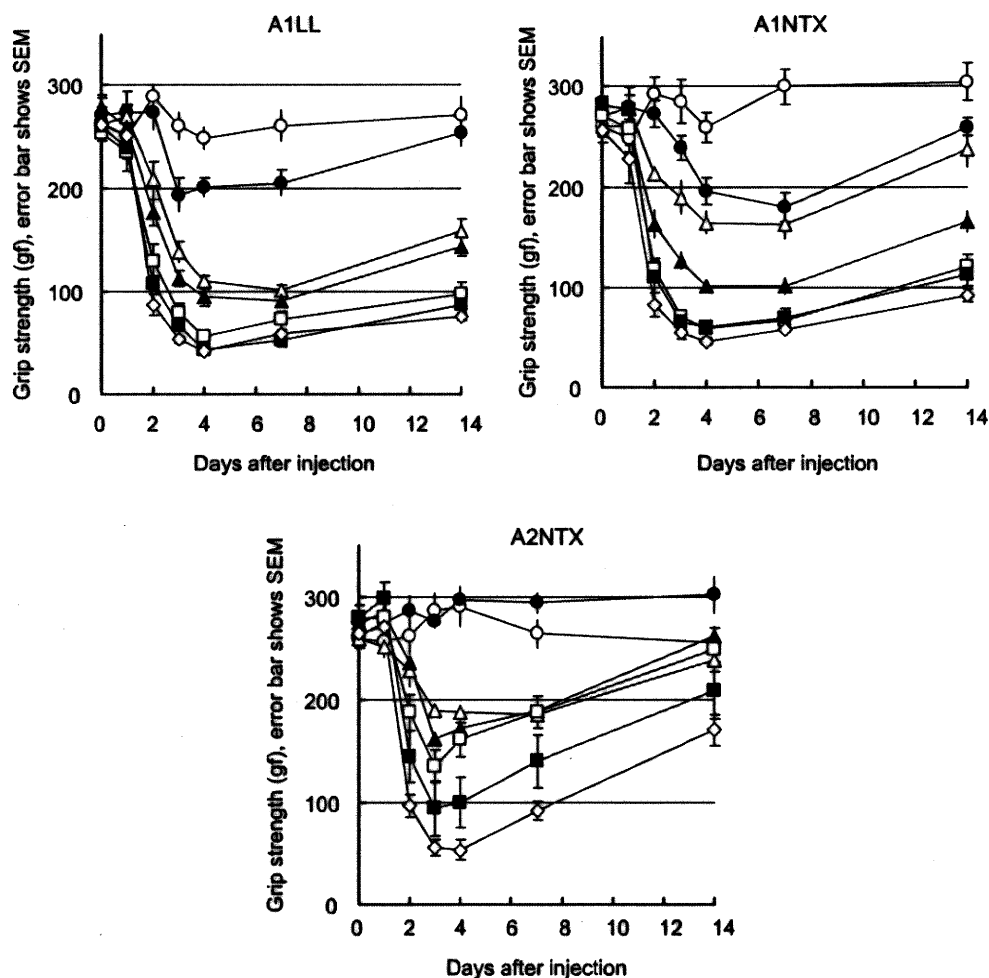
Fig. 3. Time-course of the grip strength of the contralateral foreleg after toxin injection. Rats received A1LL, A1NTX, or A2NTX injection in the left foreleg (each at ○: 1 U, ●: 4 U, △: 8 U, ▲: 12 U, □: 16 U, ■: 20 U, and ◇: 24 U). The grip strength was measured in the right foreleg of each rat at 0 (before administration), 1, 2, 3, 4, 7, and 14 days after injection. Each point is the mean ± S.E.M. ($n = 5$).

In *in-vitro* experiments using the twitch tension assay, A2NTX displayed about a 1.2- and 1.95-times higher inhibitory activity than A1NTX and A1LL, respectively. In a previous report, the inhibitory effect on neuromuscular transmission of the M toxin was compared with that of LL toxin, and M toxin was round to display about a 2-times higher inhibitory activity than LL toxin (Yoneda et al., 2005). The binding of a toxin to its receptor and subsequent endocytosis occur after the dissociation of NTX from the progenitor toxin complex. In this study, the difference in

Table 1
ED_{50} and TD_{50} for A1LL, A1NTX, and A2NTX.

Toxin	ED_{50} (U/head)[a]		TD_{50} (U/head)[b]	
	Value	95% confidence interval	Value	95% confidence interval
A1LL	0.087	0.07–0.10	6.35	5.75–7.24
A1NTX	0.060	0.05–0.07	7.54	6.61–8.51
A2NTX	0.040	0.03–0.05	15.62	13.18–23.44

[a] ED_{50}, dose at which a 50% reduction occurred in efficacy.
[b] TD_{50}, dose at which a 50% reduction occurred in safety.

effectiveness might be thought to indicate that A1LL take long dissociation of HA and NTNH in the *in-vitro* experiment.

There is a possibility that areas of muscle damage in patients with spasm are acidic due to persistent muscle contraction. To reproduce this condition in *in-vitro* experiments, the effects of each toxin were determined under acidic conditions (pH 6.8 by the addition of lactic acid). Under these conditions, the neuromuscular blocking activity of A2NTX was 3.73 times more potent than that of A1LL, with a greater difference than at pH 7.4. In this study, the difference in effectiveness might be thought to indicate that the dissociation rate of A1LL at pH 6.8 is slower than at pH 7.4. This result suggests that that therapeutic effect of A2NTX is greater than that of A1LL in spasm patients.
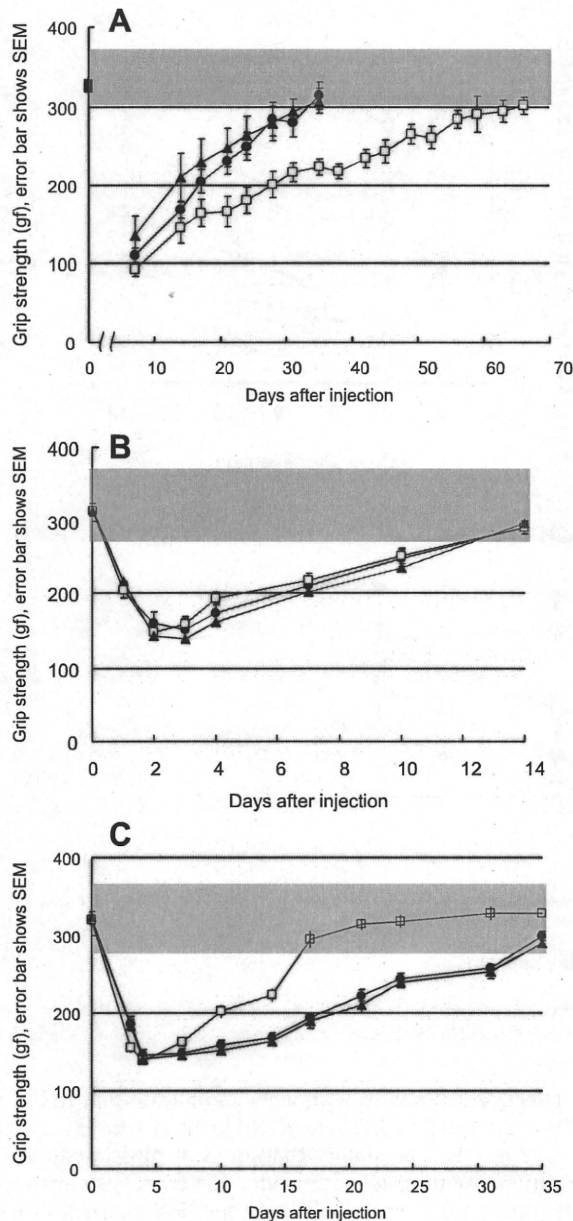
To evaluate whether the *in-vitro* inhibitory effects of the toxins on neuromuscular transmission are correlated with their effects on muscle flaccidity, we conducted an investigation using the rat grip strength test. When we compared the ED_{50} values of the toxins (muscle flaccidity effect), we found that A2NTX was 2.1-times more effective than A1LL. This was virtually the same relationship between the toxins as seen in the twitch tension assay. These results indicate

Fig. 4. The duration of the muscle flaccidity-inducing effects of the toxins (▲: A1LL, ●: A1NTX, □: A2NTX). The grey area shows the variation range (mean ± 2 S.D.) before injection. (A) Time-course of the grip strength on the administered side after toxin injection. Rats received 0.012 U of A1LL, A1NTX, or A2NTX. Each point is the mean ± S.E.M. ($n = 5$). (B) Time-course of the grip strength on the administered side after toxin injection. Rats received ED_{50} levels of A1LL, A1NTX, or A2NTX. Each point is the mean ± S.E.M. ($n = 5$). (C) Time-course of the grip strength on the contralateral side after injection. Rats received SD_{50} levels of A1LL, A1NTX, or A2NTX. Each point is the mean ± S.E.M. ($n = 5$).

that A2NTX is effective at half the usual dose of the conventional botulinum toxin type A product (A1LL).

When we compared the duration of the muscle flaccidity-inducing effect of the toxins, we found that a half-dose of A2NTX and one dose of A1LL were equivalent regarding the duration of the muscle-relaxing effect. When equivalent doses of the toxins were injected, A2NTX

produced an effect with about a 2-times longer duration than that of A1LL. This result suggests that A2NTX might cause a lower incidence of adverse effects than the conventional botulinum toxin product (A1LL). Furthermore, as frequent administration might not be necessary, its injection might be unlikely to induce neutralization antibody production.

The diffusion of each toxin was evaluated by measuring the grip strength in the contralateral foreleg (toxin-untreated). A higher dose of A2NTX than that of A1 toxins (A1LL and A1NTX) was required to decrease the grip strength on the contralateral side. A2NTX diffused less effectively than A1 toxins. The difference in diffusion among these toxins may be due to the variation in the amino acid sequence of NTX. When it was given at a high dose, which caused reductions in grip strength on the contralateral side, recovery following the injection of A2NTX was more rapid than for A1 toxins. This result suggests that A2NTX is safer than A1 toxins. Thus, A2NTX has the potential to become a safer drug for human use than A1 toxins.

We measured the time-course of the grip strength on the administered side, and found that A2NTX acted more rapidly than A1LL or A1NTX. The conventional botulinum toxin type A products have been reported to require about 1 week until the effect is observed (Truong and Jost, 2006). These slow-acting agents contribute to patient distress. A2NTX provides a possible solution to the problem because it acts more quickly than the conventional toxin preparation. The muscle flaccidity-inducing effect of A2NTX peaked at 2 days, whereas that of A1LL and A1NTX peaked at 3–4 days. The difference in the onset of effect between A2NTX and A1 toxins may be due to the variation in the amino acid sequence of NTX.

In summary, in this study, we found the following: 1) The effects of neuromuscular transmission inhibition and muscle relaxation are more effective for A2NTX than A1LL and A1NTX. 2) A2NTX shows a lower-level diffusion than A1LL and A1NTX, requiring a higher dose to reduce the grip strength on the contralateral side.

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Conflict of interest statement

None.

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Letters to the Editor Related to New Topics

Assessment of Impairment or Monitoring Change in Friedreich Ataxia

Singh et al.[1] reported an experimental study highlighting the oral motor and motor speech deficits present in individuals with Friedreich ataxia (FA). In the abstract and discussion sections of their article, they suggest that this information provides evidence for the use of their assessment protocol for monitoring change in patient functioning.

We believe that the conclusion cannot be supported on the basis of the analytical techniques presented. Importantly, the assessment tools used in the study are either not designed for use in repeated assessment protocols and/or their stability and reproducibility has not been evaluated in an empirical context. Diadochokinetic tasks (or PATA examinations) are notoriously variant in nature, difficult to assess,[2] and their stability over different test–retest intervals is unknown. The "oral motor examination" derived from the Boston Diagnostic Aphasia Examination is unsuitable for objectively monitoring change in oral motor function as it suffers from several psychometric limitations. No measures of test–retest reliability are provided; it is designed for diagnostic decision making and for making an estimate of severity of impairment; thus, its validity as a marker of change is questionable; it has limited intra/inter-rater reliability[3]; and grading of performance is difficult to quantify as it is based on a finite rating scale that relies on clinical expertise and subjective judgment. Perhaps, these reasons account for the authors not conducting reliability investigations on this aspect of their assessment protocol. The Assessment of Intelligibility of Dysarthric Speech (ASSIDS) also provides a useful index of the severity of dysarthric speech by quantifying both single-word and sentence intelligibility, however, perhaps incorporating the other metrics typically used within the ASSIDS [e.g., speaking rate (words per min) and rate of intelligible speech (number of intelligible words per min)] may have yielded more tangible quantitative findings. Finally, it is not surprising that findings from the picture description task (cookie theft) were poorly correlated with measures of disease severity. The gross measure of quantity (i.e., total number of intelligible words) is intrinsically linked to the number of words produced in a sample. Given that the number of words produced by participants is dependent on their expressive language skill and descriptive vocabulary and that no set word limit was applied, monitoring change in patient function using this method may be inappropriate.

Sophisticated methods exist for the application of standardized speech and oral motor assessments where the data generated in response to various challenges are compared to established normative data. Similarly, patterns of strengths and weaknesses on performance measures can be interpreted and aid the process of differential diagnosis by skilled clinicians. Data provided by Singh et al.[1] fulfill this purpose; however, there is not the same sophistication for assessment of speech in monitoring change in patient function. In related fields (e.g., cognitive testing), it is argued that the assessment of behavior for supporting classifications of CNS impairment has a different practical, methodological, and statistical framework for the assessment of behavior to guide decisions about change in the CNS.[4] Singh et al.[1] acknowledge the need for testing that can be "reproduced without excessive technical expertise," yet they fail to recognize that several other criteria need to be met before an assessment protocol is appropriate for monitoring change. Tasks designed to monitor change should be brief, easy to complete, suitably motivating, and preferably have alternate forms (all of which are designed to limit the impact of practice/familiarity). In addition, monitoring intrasubject variation requires a different statistical framework that examines change from baseline rather than differences from controls or normative data.[5] This requires that assessments satisfy assumptions of normality or can be corrected to normal and that they utilize continuous variables that are not restricted by range, floor, or ceiling effects.

Singh et al.[1] rightly state that "long examination times are not ideal for use in FA because of the highly fatigable nature of the patient population" and that some of their assessments are limited by the use of discrete rather than continuous variables. Given the potential implications of conclusions based on assessments that fail to acknowledge the different methodological requirements for monitoring change in patient functioning, it is important that appropriately conservative analyses are undertaken in studies addressing this issue.

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Refractory Head Movements Secondary to Sandifer Syndrome Treated with Enteral Feeding

Video

Sandifer syndrome is a paroxysmal movement disorder characterized by abnormal movements of the head, neck, and trunk in association with gastroesophageal reflux disease (GERD).[1] Most cases are neurodevelopmentally normal children with symptom onset in early childhood, although some cases have been reported in adults. Infants often have retrocollis and opisthotonic posturing, whereas older children have the side-to-side head movements.[1,2] The movements usually respond to anti-reflux medications and/or fundoplication.[3] We present a case of Sandifer syndrome caused by GERD and delayed gastric emptying that was refractory to fundoplication and improved with enteral feeding. To our knowledge, this is the first published case of Sandifer syndrome being treated with enteral feeding.

A four-year-old boy presented with a 3 month history of abnormal side-to-side head movements (see Video Segment 1). The movements lasted less than 1 min and occurred many times per day. Apart from the movements, his neurological exam was unremarkable. An EEG, head CT, and MRI were unremarkable. No Kayser-Fleischer rings were noted via slit lamp, and a 24-hour urinary copper was normal. With the hypothesis that the movements were tics, clonidine was initiated with no effect.

Eventually, an association was noted between the movements and gastrointestinal symptoms such as abdominal pain and regurgitation. The child was started on lansoprazole empirically, titrated to a maximum of 45 mg/day with no effect. An upper gastrointestinal endoscopy and a gastric emptying study revealed reflux esophagitis and delayed gastric emptying. An esophageal pH probe demonstrated acid reflux with regurgitation and long periods of pH less than 4. Thus, the movements were thought to be a manifestation of

Sandifer syndrome. Treatment with domperidone (5 mg, TID) appeared to make the movements worse.

Laparoscopic fundoplication was performed to treat his GERD, as medical therapy failed and was followed by laparoscopic pyloroplasty to improve gastric emptying. However, the movements persisted after both surgeries.

Eventually a naso-jejunal (NJ) feeding tube was inserted which was associated with significant improvement in the patient's movements (see Video Segment 2). Attempts to wean the patient back to feeding by mouth resulted in the return of the movements. As his symptoms improved, the NJ tube was switched to naso-gastric feeding tube, which was well tolerated. A gastrostomy tube was inserted and, after follow-up 3 years after his initial presentation, the patient continues to be predominantly enterally fed with some oral intake.

The first symptoms of Sandifer syndrome often resemble torticollis or dystonia, thus early evaluation usually focuses on neurological etiologies.[1] EEG, CT, and MRI studies are all usually normal. Tics, unlike the movements in Sandifer syndrome, are suppressible, can wax and wane, and can be associated with an urge to perform the movements. Sandifer movements are often precipitated by meals, unlike other movement disorders. A psychogenic etiology in our case would be extremely unlikely considering the young age of the patient.

Why some children with GERD present with abnormal movements and others do not remains unresolved. Research supports the theory that the movements are learned behaviors used by children to reduce reflux. One study showed that head tilting results in an increase in esophageal motility and decreased reflux.[4] Another found that abnormal movements only occurred when the pH of the esophagus was less than.[3] Finally, most reports of Sandifer syndrome describe near complete cessation of abnormal movements with fundoplication, performed to reinforce the function of the lower esophageal sphincter and reduce reflux.[3]

In our patient fundoplication failed to stop the abnormal movements, nor did pyloroplasty, done to improve the patient's delayed gastric emptying. It was not until an NJ tube was inserted for enteral feeding that a decrease in abnormal movements was observed. Enteral feeding is an established treatment for children with refractory GERD.[5] Although previous case studies have investigated gastric emptying in patients with Sandifer syndrome, only one found delayed emptying and neither required enteral feeding.[6,7] It is still unclear as to when our patient will be able to tolerate full oral feeding again although it is possible that the symptoms will improve on their own with time.

This case report demonstrates that even when associated with GERD, other features such as delayed gastric emptying may also result in the abnormal posturing characteristic of Sandifer syndrome. If initial treatments aimed at reducing reflux are unsuccessful, physicians may need to explore other options such as enteral feeding.

LEGENDS TO THE VIDEO

Segment 1. Patient's abnormal head movements at presentation.

Segment 2. Patient demonstrating cessation of abnormal head movements after initiation of enteral feeding.

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Chylomicron Retention Disease: Dystonia as a New Clinical Feature

Video

Chylomicron retention disease (CRD) is a rare autosomal recessive disorder characterized by malabsorption, failure to thrive (FTT), developmental difficulties, mental retardation, abnormal vibration sense, and hyporeflexia.[1] Movement disorder has been reported only once.[2] Laboratory findings

include fat-soluble vitamins deficiency, low cholesterol, and selective absence of chylomicrons. The diagnosis is made by electron microscopy of jejunal biopsy specimens demonstrating accumulation of lipid droplets in enterocytes. The majority of cases are caused by mutations of the *SAR1B* gene on chromosome 5.[3] We report 3 cases with biopsy proven CRD presenting dystonia as a new clinical feature.

The first patient was seen at age 49 for tremor in the context of CRD. Her family history is significant for consanguinity, an affected sister and the infantile death of 3 male siblings with prior history of FTT and developmental delay. She presented in early childhood with development delay, malabsorption, and FTT. Later, she was found to have learning difficulties and low average intellectual quotient. She developed a sensory polyneuropathy and a progressive cerebellar syndrome. She was diagnosed with CRD by small bowel biopsy at the age of 21 and started on fat-soluble vitamins. At the end of adolescence, she developed a slowly progressive dystonic tremor of the upper extremities, neck, and voice. The tremor was irregular in amplitude and frequency, with a sensory trick and zero position. A treatment trial with propranolol was performed with limited success.

The second patient was seen at age 50, also for tremor in the context of a known CRD. She is the sister of the first patient. Her clinical presentation, laboratory and jejunal biopsy results were very similar. She was less severely affected and developed a dystonic tremor of the head and upper extremities only in her early thirties.

The third patient was seen at age 47 for tremor in the context of CRD. His family history was significant for consanguinity. He presented in early childhood with malabsorption, slowly progressive cerebellar syndrome, and predominantly sensory polyneuropathy. He was diagnosed with CRD by jejunal biopsy at the age of 6. At the end of adolescence, he developed a slowly progressive and disabling dystonic tremor of the left more than right upper extremities (Supporting Information video 1a), head, and voice. Because of suboptimal response to several medications, a deep brain stimulator (DBS) was implanted in the right ventrolateral thalamus. Following surgery, both the proximal and distal components of his tremor were significantly reduced (Supporting Information video 1b) and the patient regained use of his left hand.

The pathophysiology of CRD is not well understood. The clinical manifestations may be secondary to malabsorption, which leads to deficiency in important vitamins and nutrients. Deficiency in vitamin E is the most likely cause of the cerebellar ataxia and sensory neuropathy, as seen in other conditions such as AVED (Ataxia with isolated Vitamin E Deficiency) and abetalipoproteinemia.[4] Dystonia has been reported as a clinical feature of AVED[5] but not of abetalipoproteinemia, suggesting that the pathophysiology of dystonia involves probably more than the vitamin E deficiency alone. We hypothesize that a dysfunction of the cortico-striato-pallido-thalamo-cortical circuits is implicated in the pathophysiology, as in other forms of secondary dystonia, and as supported by the improvement seen with stimulation of the ventrolateral thalamus.

Levy et al. postulated that CRD was secondary to a defect in the formation and secretion of chylomicrons, resulting from a defect in glycolysation.[6] In 2003, Jones et al.[3] identified mutations in the *SAR1B* gene in 10 cases. This gene encodes for a protein belonging to a family of small GTPases called the

Sar1-ADP-ribosylation factor family.[7] This family of proteins is responsible for the intracellular trafficking of proteins in coat protein (COP)-coated vesicles. SAR1B is ubiquitously expressed; its expression has been demonstrated in several tissues including the brain. The relation between the defective gene and the clinical manifestations is not well understood.

This article is the first to report dystonia (dystonic tremor) as a clinical manifestation of CRD. Similarly to cases with essential and resting tremors, ventrolateral thalamus stimulation was effective and well tolerated.

Legends to the Video

Video 1a. Patient 3: important dystonic tremor involving more the left than the right upper extremity.

Video 1b. Same patient, after implantation of a deep brain stimulator in his right ventrolateral thalamus.

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Long-Term Suppression of Meige Syndrome After Pallidal Stimulation: A 10-Year Follow-Up Study

Video ⊙

Meige syndrome is an adult-onset, idiopathic movement disorder that manifests as blepharospasm, facial and oromandibular dystonia, and frequently cervical dystonia.[1] This syndrome is often refractory to medication, and some patients do not adequately respond to botulinum toxin therapy. There is now an increased interest in the use of globus pallidus internus (GPi) deep brain stimulation (DBS) for medically refractory, generalized or segmental dystonia.[2] However, little is known about its effects in the treatment of other types of dystonias such as focal dystonia. In addition, the use of GPi-DBS for the treatment of Meige syndrome has rarely been reported.[3–7] Here, we report a long-term outcome of the patient in whom we first showed a striking impact of bilateral GPi-DBS on dystonia symptoms characteristic of Meige syndrome 10 years ago.[3]

The patient was a 71-year-old woman with no history of exposure to neuroleptics and no family history of dystonia. She experienced a gradual onset and exacerbation of blephar-

FIG. 1. Long-term effects of bilateral pallidal stimulation on Meige syndrome. A: Preoperative state. B: Postoperative state at the time of the 10-year follow-up: with bilateral GPi-DBS. C: Postoperative state at the time of 10-year follow-up: without bilateral GPi-DBS.

ospasm and oromandibular dystonia at age 43. At age 51, she was diagnosed with Meige syndrome at another hospital, and subsequently underwent two-stage operations for Vo complex (Voa + Vop) thalamotomy on the right and left sides. However, bilateral thalamotomy was not beneficial to this patient. In addition, multiple sequential pharmacological trials produced unsatisfactory results.

At the age of 61 years, she underwent bilateral GPi-DBS. Before the surgery (Fig. 1A and video segment 1), she manifested marked facial grimacing with excessive blinking and sustained forceful eye closure, and severe cervical dystonia characterized by turning and tilting of the head in the right and posterior directions. She also exhibited trunk bending toward the right and a mild dystonic tremor in the right arm. Her preoperative scores for the Burke-Fahn-Marsden Dystonia Movement Scale (BFMDRS) Movement and Disability Scales were 35 and 23, respectively (Table 1). Magnetic resonance images did not show any obvious abnormalities, except for the presence of previous surgical lesions in the thalamic nuclei on the right and left sides.[3] Under general anesthesia with propofol, quadripolar DBS electrodes (Model 3387; Medtronic, Minneapolis, MN) were implanted into the bilateral GPi. Furthermore, after confirming the beneficial effects of GPi-DBS, we implanted a receiver for the external transmitter (Mattrix Transmitter Model 3272; Medtronic). The postoperative course was uneventful. Optimal therapeutic results were obtained when the system was operated using the maximum pulse width of 500 μs, an amplitude of 3.6 V, and a frequency of 60 Hz.[3] Bilateral pallidal stimulation immediately improved the symptoms of mobile dystonia such as blepharospasm, facial grimacing, and phasic head movement. With continuous stimulation, the patient's fixed postural

dystonias, such as cervical retrocollis and trunk bending, were gradually ameliorated and almost completely disappeared within a few months after the initiation of GPi-DBS (Table 1). The differential responses of the phasic and fixed dystonias to treatment with GPi-DBS in our patient supported the general thought that phasic hyperkinetic movements are ameliorated more rapidly than fixed tonic postures are after treatment with GPi-DBS.[2]

To avoid the inconvenience and troublesomeness caused by the use of external batteries, implantable pulse generators (IPGs; Itrel 3, Medtronic) were used instead of the Mattrix transmitter systems when the patient was 63 years old. In many trials wherein monopolar stimulation at an amplitude of less than 3.7 V was applied using IPGs, the results were unsatisfactory. Optimal benefits were derived when bipolar stimulation was applied using a pulse width of 450 μs, frequency of 60 Hz, and pulse amplitude of 3.9 and 3.6 V on the right and left sides, respectively (Table 1). After bilateral GPi-DBS, the patient's BFMDRS movement and disability scores were 5 and 4, respectively. Because continuous stimulation at an amplitude of 3.9 V shortened the battery life on the right side, the IPGs had to be replaced within 2 years. Bilateral pallidal stimulation resulted in sustained suppression of Meige syndrome until the time of the 10-year follow-up (Fig. 1B and video segment 2). Furthermore, we noted that dystonic symptoms, similar to those observed at the preoperative stage (Fig. 1C and video segment 3), were reproduced in this patient immediately after the IPGs were switched off.

GPi-DBS has emerged as the treatment of choice for patients with disabling dystonias; however, very little is known about its long-term effects in patients with different subtypes of dystonia. This study showed that in a patient with Meige syndrome, bilateral GPi-DBS resulted in a sustained, long-term improvement in both the movement and disability scores measured using the BFMDRS: these scores had improved by more than 80% at the time of the 10-year follow-up. We propose that bilateral GPi-DBS is an effective and a safe procedure that has long-lasting benefits in patients with Meige syndrome.

Legends to the Video

Segment 1. Preoperative state.
Segment 2. Postoperative state at the time of the 10-year follow-up: with bilateral GPi-DBS.

TABLE 1. *Deep brain stimulation status and impact on dystonia rating scale scores*

| | | DBS programming parameters (contacts/voltage/PW/freq) | | BFMDRS | |
| | | | | | |
Evaluation stage	DBS device	Right brain	Left brain	Movement score	Disability score
Preoperative state				35	23
Postoperatve 1 wk	Extrel with mattrix	1(−)3(+)/3.6 V 500 μS/60 Hz	1(−)3(+)/3.6 V 500 μS/60 Hz	7 (ON)	6 (ON)
Postoperative 3 mo	Extrel with mattrix	1(−)3(+)/3.6 V 500 μS/60 Hz	1(−)3(+)/3.6 V 500 μS/60 Hz	6 (ON)	4 (ON)
Postoperative 3 yr	Itrel 3	1(−)3(+)/3.9 V 450 μS/60 Hz	1(−)3(+)/3.6 V 450 μS/60 Hz	5 (ON)	4 (ON)
Postoperative 10 yr	Itrel 3	1(−)3(+)/3.9 V 450 μS/60 Hz	1(−)3(+)/3.6 V 450 μS/60 Hz	5 (ON) 36 (OFF)	4 (ON) 24 (OFF)

PW, pulse width (μS); freq, frequency (Hz); BFMDRS, Burk-Fahn-Marsden dystonia rating scale; wk, weeks; ON, on stimulation; mo, months; yr, years; OFF, off stimulation.

Segment 3. Postoperative state at the time of the 10-year follow-up: without bilateral GPi-DBS.

Author Roles: Nobuhiro Inoue was involved in conception of research project; surgery and programming of DBS; and review and critique of manuscript. Shinji Nagahiro was involved in organization of research project; review and critique of manuscript. Ryuji Kaji was involved in organization of research project; review and critique of manuscript. Satoshi Goto was involved in conception, organization, and execution of research project; surgery and programming of DBS; writing of the first draft, review and critique of manuscript.

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Retraining and Transcranial Direct Current Stimulation in Musician's Dystonia — A Case Report

Focal dystonia in musicians (MD) is a task-specific movement disorder, which presents itself as a loss of motor control during instrumental playing.[1] Deficient inhibition at different levels of the CNS is involved in the pathophysiology.[2] MD is difficult to treat and retraining strategies aiming to establish non-dystonic movements have contradictory outcomes.[1,3] As acquisition of new motor skills is accompanied by changes of neuronal activity and excitability, transcranial direct current stimulation (tDCS) might be a tool to assist retraining. Anodal tDCS enhances, whereas cathodal tDCS reduces cortical excitability.[4,5] Hereby, anodal tDCS has been shown to facilitate motor learning, whereas cathodal tDCS improves performance in overlearned tasks.[4,5] Daily repeated application prolongs effects.[6] The aim of the study was to investigate whether repeated tDCS improves retraining effects in a pianist with MD either by anodal or by cathodal tDCS.

The patient was a male professional pianist aged 43. He had been suffering from a finger flexion dystonia of the right hand for 15 years. Other neurological disorders were excluded, and he was not under pharmacological treatment. Motor learning consisted of a retraining on the piano (20 min per day) based on following principles: (1) finger movements were limited to a tempo and force at which dystonic movements would not occur; (2) compensatory movements (e.g., of adjacent fingers) were avoided as far as possible. During retraining, the patient received tDCS. The study was placebo controlled and double blinded. Three treatment conditions were applied for 5 days consecutively with 6 weeks between conditions: 20 min retraining plus anodal tDCS, plus cathodal tDCS, or plus placebo stimulation. The stimulating electrode was placed over the left primary motor cortex (C3 according to the international 10–20 system) and the reference electrode over the right supraorbital area. Current strength was 2 mA for the active conditions and 0.2 mA (fading out after 20 seconds) for placebo. tDCS was induced through sponge electrodes (surface 35 cm^2) and delivered by a constant-current stimulator (eldith GmbH, Germany). Stimulation conditions were randomly assigned: 1. placebo, 2. anodal, and 3. cathodal tDCS. Motor control was assessed by MIDI-based scale analysis, a reliable and valid quantification of motor control in pianists with MD.[7] The patient played 10 C-major scales with the affected hand in a metronome-paced tempo over
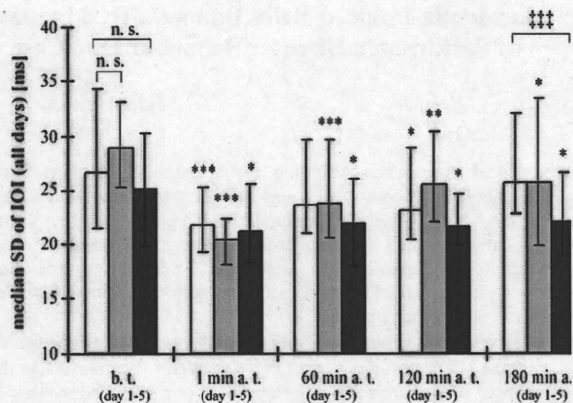
FIG. 1. Results of tDCS effects on retraining in a pianist with musician's dystonia. Bars show motor performance as the median SD of inter-onset intervals (IOI). High values indicate poor motor control and vice versa. Treatment condition is displayed as open bars for retraining and placebo tDCS, as light gray bars for retraining and anodal tDCS, as dark gray bars for retraining and cathodal tDCS. Whiskers depict the 25th and the 75th percentiles of data. b.t.: before treatment; a.t.: after treatment. The median SD of IOI of all performance tests of 5 days is displayed for each treatment condition and time. Asterisks depict motor performance after treatment vs. before treatment: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Mann-Whitney-U, Bonferroni-Holm-corrected). Intertreatment comparisons between active and placebo conditions: $‡‡‡P < 0.001$ for cathodal vs. placebo (Mann-Whitney-U, Bonferroni-Holm-corrected).

two octaves (desired inter-onset interval 125 ms). Standard deviation (SD) of inter-onset intervals (IOI) was used as target parameter.[7] Motor control was assessed before and after treatment (1 min, 60 min, 120 min, and 180 min after end of treatment). Mann-Whitney-U tests were applied to analyze performance differences ($\alpha = 0.05$). Correction of multiple comparisons was done according to Bonferroni-Holm.

Treatment results were assessed by the median SD of IOI of respective time points of all days (Fig. 1). Baseline motor control did not differ between conditions. In the placebo condition, motor control was improved 1 min ($P < 0.001$) and 120 min ($P < 0.05$) after treatment as compared to before treatment. In the anodal condition, motor control was improved 1 min ($P < 0.001$), 60 min ($P < 0.001$), 120 min ($P < 0.01$), and 180 min ($P < 0.05$) after treatment. In the cathodal condition, motor control was improved 1 min, 60 min, 120 min, and 180 min after treatment (each $P < 0.05$). Intertreatment comparisons revealed a better performance outcome in the cathodal condition compared to placebo 180 min after treatment ($P < 0.001$).

We observed a beneficial effect of retraining on fine motor control in the reported patient, which was enhanced by cathodal tDCS. Improved motor control after treatment was found in all three conditions and was most pronounced immediately after retraining (Fig. 1). The intertreatment comparison suggests that cathodal tDCS may prolong retraining effects. In contrast, anodal tDCS did not enhance retraining effects beyond placebo stimulation. Inhibitory mechanisms related to movement preparation and execution are crucial for fine motor control and disturbed in patients with MD.[1] Thus, the inhibitory effect of cathodal tDCS might have facilitated physiological inhibition in this patient. A similar mechanism

with improvement of visuomotor performance was seen after cathodal tDCS of V5 in healthy subjects, probably due to an increased signal-to-noise ratio.[5] As a limitation, cumulative retraining effects in the cathodal stimulation week (3rd week) might have influenced the outcome. In summary, retraining seems to be a promising tool with therapeutic potential. Repeated cathodal tDCS might facilitate retraining-based treatment. Studies on large numbers of patients are required to identify optimal retraining strategies and to clarify effects of repeated tDCS on retraining in patients with MD.

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Levodopa-Induced Belly Dancer's Dyskinesias in Parkinson's Disease: Report of One Case

Video

Focal dyskinesias affecting the abdominal wall were first described by Iliceto et al.[1] and named "belly dancer's dyskinesias." So far, only a few cases have been reported, following an abdominal surgical procedure or local trauma,[1,2] prolonged antidopaminergic treatment,[3] central pontine and extra-pontine myelinolysis,[4] or as a result of spinal myoclonus[5,6] and spinal tumors.[7]

Herein, we report a 72-year-old woman with Parkinson's disease (PD) who developed belly dancer's dyskinesias in the context of dopaminergic therapy. To our knowledge, this is the first report of Levodopa (L-dopa)-induced belly dancer's dyskinesias in PD. Patient's consent was provided for the writing of this manuscript and video filming.

When she came to our attention, the patient had a six-year history of PD whose onset was characterized by bradykinesia and rigidity in the left upper limb. She was initially started on Ropinirole 0.5 mg twice a day, and L-dopa/Carbidopa 100/25 mg a day was then added to improve her motor performances. Six months prior to admission to our department, L-dopa was increased up to 200 mg a day in a controlled release formulation, and she was switched to Pramipexole 0.18 mg twice a day due to an unsatisfactory control of motor symptoms.

On admission, the examination showed continuous, not suppressible writhing movements of the abdominal wall which got worse while standing, causing a circular displacement of the umbilicus. They were not affected by respiration or breathholding and ceased at night without being associated with local pain or abdominal discomfort. They started around 30 minutes after each L-dopa intake lasting about 3 hours. The neurological examination showed a short-step, shuffling gait with reduced arm swings bilaterally, moderate bradykinesia and rigidity in upper and lower limbs more marked on the left side, and cogwheel phenomenon in both elbows.

A magnetic resonance imaging of the spinal cord showed no structural abnormalities, and a computed tomography scan of the brain only revealed diffuse subcortical hypodense small lesions consistent with chronic vascular damage.

To investigate the neurophysiological features of the abdominal dyskinesias, an EEG-EMG recording was performed, and the back averaging technique did not demonstrate any time-locked correlate. Needle electromyography of both recti abdominis and external oblique muscles showed spontaneous bilateral synchronous bursts lasting 220 to 400 millisecond that occurred at variable intervals of 0.5 or 1 second.

To demonstrate the chronological correlation between L-dopa intake and the onset of abdominal dyskinesias, its administration was suspended for two consecutive days, with a complete cessation of abdominal movements. Pramipexole was increased up to 0.7 mg three times a day, and the patient

was discharged with no abdominal dyskinesias. However, she resumed a single dose of L-dopa/Carbidopa 100/25 mg and developed end of dose deterioration and again abdominal dyskinesias with some mild distal dyskinesias in the lower limbs.

On readmission, an acute L-dopa challenge confirmed the onset of abdominal dyskinesias around 90 minutes after the L-dopa intake with duration of 3 hours. UPDRS III was 27 in the "off" phase, 20 one hour after L-dopa intake and 14 two hours later.

She was started on L-dopa/Carbidopa/Entacapone (50/12.5/200 mg) three times a day, Rasagiline 1 mg per day, and Pramipexole was maintained at the same dose. This therapy significantly reduced but did not entirely suppress the abdominal dyskinesias.

Peak dose and, less frequently, diphasic dyskinesias are a well-known motor complication of long-term L-dopa treatment, consisting of involuntary choreiform movements or dystonic postures usually involving neck, trunk, and upper limbs. Abdominal dyskinesias following L-dopa treatment have only been described in a patient affected by Multiple System Atrophy so far.

In our patient, belly dancer's dyskinesias were secondary to L-dopa exposure and intriguingly one case has been reported after chronic antidopaminergic treatment (Clebopride),[3] suggesting that a nonphysiological stimulation of postsynaptic dopaminergic receptors due to the use of exogenous L-dopa or antidopaminergic drugs may play a role in the genesis of abdominal dyskinesias. Interestingly, no cases of belly dancer's dyskinesias after chronic neuroleptic use have been reported so far.

In cases with no structural abnormalities of the spinal cord, the pathophysiology of belly dancer's dyskinesias has been explained with a dysfunction of inhibitory spinal interneurons or structural reorganization of local neuronal circuits.[1,2] In our case, given the clear temporal correlation between L-dopa intake and the onset of dyskinesias, these mechanisms are unlikely to have played a significant role.

LEGENDS TO THE VIDEO

Abdominal dyskinesias (belly dancer's dyskinesias) after acute L-dopa challenge. A partial spreading to the trunk and the lower limbs and some mild distal dyskinesias in the left foot are also visible.

Author Roles: Miryam Carecchio: Conception, Organization, Execution of Research project; Writing of the first draft of Manuscript; Acquisition of data, analysis and interpretation of data of Other contributions. Alessandra Collini: Conception, Organization of Research project; Acquisition of data of Other contributions. Cristoforo Comi: Review and critique of Manuscript; analysis and interpretation of data of Other contributions. Roberto Cantello: Review and critique of Manuscript. Kailash P. Bhatia: Review and critique of Manuscript. Francesco Monaco: Review and critique of Manuscript.

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Parkinson's Disease Rehabilitation: A Pilot Study with 1 Year Follow Up

There is emerging evidence of the positive acute effects of intensive rehabilitation treatments to improve motor aspects of patients with Parkinson's disease (PD).[1] However, whether the effects of rehabilitation persist over time or not remains an open question.

In this pilot study, we tested in 20 PD patients whether the effects of our rehabilitation protocol are maintained over a 12 months follow up period, and investigated whether a second rehabilitation cycle administered after 1 year has the same efficacy as the first treatment. Inclusion criteria were: Hoehn-Yahr Stage 3, ability to walk without physical assistance and mini-mental state examination score ≥26.

All patients reported in the last year a deterioration of motor performance despite increase of Levodopa (L-dopa) dosages (average increase 90.18 mg/die).

The patients underwent an intensive 4-week cycle of physiotherapy that entailed 2 daily sessions, 5 days a week, in which standard physical therapy techniques were associated with treadmill training and auditory and visual cues.[1]

Clinical evaluation at baseline and at the end of the rehabilitation treatment was based on the Unified Parkinson's Disease Rating Scale II and III Section (UPDRS II and III), Berg Balance Scale (BBS), Timed "Up and Go" test (TUG), Comfortable Gait Speed (CGS), and Fast Gait Speed (FGS).

All patients were readmitted 1 year later, and underwent the same rehabilitation protocol and the same clinical evaluations. During this 1 year period, patients were managed by their own neurologists, with no indications from our Hospital. There was no patient attrition and compliance was good for all patients.

The time course of each clinical variable considered was assessed by repeated measurements analysis of variance with four repeated measurements: first admission, first discharge, second admission (after 1 year) and second discharge.

The demographic and clinical characteristics of studied patients are reported in Table 1. The performance of patients improved significantly by the end of the first rehabilitation cycle for all variables ($p < 0.0001$). At the second admission (after 11.8 ± 1.5 months) most parameters had returned to val-

ues similar to those of the first admission, but all improved again at the end of the second rehabilitation program.

L-dopa equivalent dosage at second admission was slightly reduced (588 ± 308 mg/die versus 633 ± 291 mg/die, $p = 0.12$).

The importance of these results can be appreciated considering the chronic-degenerative nature of PD. A recent study showed that, despite optimal treatment, UPDRS III score worsened after one year in 26% of PD patients and L-dopa dose had to be increased in 52%.[2] Also our patients reported a deterioration of motor performance in the year preceding enrolment, despite a documented significant increase of drugs dosage, which was >75 mg/die in 55% of them.

In this study, we have demonstrated that the beneficial effects of our intensive rehabilitation treatment persist over a 12 months follow up period, reducing the need for increasing L-dopa doses.

Both peripheral and central mechanisms are likely to be involved in the improvement of our patients. With regards to the first mechanism, exercise training is associated with pulmonary, cardiovascular, and skeletal muscle metabolic adaptations that have sustained beneficial effects on patients. Exercise training might increase muscle oxidative capacity, normalize skeletal muscle metabolism, and reduce oxidative stress. Moreover exercise reduces all peripheral risk factors, improving cardiovascular health, cholesterol levels, insulin sensitivity, and inflammation.[3]

The hypothesis of an involvement of central mechanisms is supported by data from clinical studies suggesting that high intensity exercise may be important in promoting activity-dependent neuroplasticity in the basal ganglia. Animal experiments provided evidence that exercise has a neuroprotective effect against neurodegenerative diseases.[4] A direct effect of exercise on the level of several growth factors has been shown,[5] and a beneficial effect of exercise on Parkinsonism in animal model was demonstrated.[6] Both central and peripheral effects of exercise improve brain health modulating growth factor signaling: exercise increase growth factor levels and reduce pro-inflammatory conditions, which impair

TABLE 1. *Demographic and clinical characteristics of the patients*

	First admission	First discharge	Second admission	Second discharge
Age (years)	71 ± 8			
Male/female	8/12			
Duration of disease (years)	7.8 ± 2.7			
L-Dopa equivalent (mg/die)	633 ± 291		588 ± 308	
UPDRS III score	22.8 ± 6.8	15.9 ± 5.5	22.0 ± 5.2	16.9 ± 3.8
UPDRS II score	14.6 ± 5.1	9.7 ± 5.1	13.9 ± 5.4	11.2 ± 5.0
BBS score	46.2 ± 6.9	51.0 ± 6.4	45.8 ± 7.5	50.8 ± 5.1
TUG score (s)	12.4 ± 2.3	9.7 ± 2.4	12.2 ± 2.5	9.5 ± 2.0
CGS score (s)	12.1 ± 2.3	10.2 ± 1.7	11.7 ± 2.3	9.5 ± 1.5
FGS score (s)	9.4 ± 1.7	7.8 ± 1.2	8.9 ± 1.4	7.5 ± 1.4

Demographic and clinical characteristics of the patients, at first admission, at first discharge after 4 weeks of in hospital rehabilitation programme, at second admission after about one year and at second discharge after another 4 weeks rehabilitation programme. Data were available for all of the participants at all of the time points.

growth factor signalling.[7] However, further studies are needed to assess whether intensive treatment such as ours might determine significant and long-lasting changes in dopaminergic transmission.

In conclusion, our preliminary results suggest that the natural worsening of symptoms associated with PD can be effectively contrasted by a properly designed intensive rehabilitation protocol. If these findings will be confirmed in larger studies with proper experimental design, the association of periodic cycles of intensive rehabilitation with pharmacological treatment should be considered as a valid option to delay the increase in drugs dosage and the beginning of related adverse effects.

Author Roles: Giuseppe Frazzitta: Conception, Organization, and Execution of Research project; Design of Statistical analysis; Writing of first draft and Review and Critique of Manuscript. Gabriella Bertotti: Conception, Organization, and Execution of Research project; Writing of first draft of Manuscript. Davide Uccellini: Organization and Execution of Research project. Roberto Maestri: Conception of Research project; Design and Execution of Statistical analysis; Writing of first draft and Review and Critique of Manuscript.

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OLFACTORY TYPE G-PROTEIN α SUBUNIT IN STRIOSOME-MATRIX DOPAMINE SYSTEMS IN ADULT MICE

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Abstract—There is a growing body of evidence that striosome-matrix dopamine systems are tightly linked with motor and behavioral brain functions and disorders. In this study, we used an immunohistochemical method to show differential expression of the olfactory type G-protein α subunit (Gαolf) that involves in the coupling of dopamine D1 receptor with adenylyl cyclase in the striatal compartments of adult mice, and observed heightened density of Gαolf labeling in the striosomes relative to the matrix compartment. Our findings suggest that Gαolf could be one of the key molecules for controlling differential responses of the striosome and matrix compartments to dopamine D1 receptor signaling in the striatum of adult mice. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: striatum, compartmentalization, dopamine D1 receptor, DARPP-32, c-Fos, G-protein.

The mammalian striatum comprises a unique mosaic organization composed of two functional subdivisions: the striosome and matrix compartments (Graybiel and Ragsdale, 1978). These compartments are defined according to the intensity of histochemical staining for a wide variety of neurotransmitter-related substances including the dopamine-signaling molecules (Graybiel, 1990; Gerfen, 1992). Accumulating evidence has suggested that differential involvement of the striosome-matrix dopamine systems is associated with movement and behavioral disorders (for recent reviews, see Graybiel, 2008; Goto et al., 2010), and psychostimulant addiction (Capper-Loup et al., 2002; Granado et al., 2008).

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Abbreviations: DAB, diaminobenzidine; DARPP-32, the dopamine and cAMP-regulated phosphoprotein of 32 kDa; Gαolf, the olfactory type G-protein α subunit; Gαs, the isoform of stimulatory G-protein α subunit; IgG, immunoglobulin G; MOR, μ-opiate receptor; PB, phosphate buffer; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TH, tyrosine hydroxylase.

Dopamine receptor signaling plays a key role in motor and behavioral control of brain function. Among five known subtypes of dopamine receptors, the dopamine D1 receptor (D1R) that stimulates cAMP production is most abundant and widespread in the brain. It is known that in the rodent striatum, D1R couples to adenylyl cyclase via the olfactory type G-protein α subunit (Gαolf), and not via the isoform of stimulatory G-protein α subunit (Gαs; Zhuang et al., 2000; Corvol et al., 2001). By using an immunohistochemical technique, we here show a striking pattern of Gαolf distribution in the striatum of adult mice, and heightened expression of the protein in the striosomes relative to the matrix compartment. Our results suggest that in the adult mouse striatum, striosomal enrichment of the Gαolf protein is attributable to the predominant responsiveness of the striosomes to D1R signaling.

EXPERIMENTAL PROCEDURES

All procedures involving the use of animals and analysis of brain anatomy were approved by the Institutional Care and Use Committees of Tokushima University.

Animals and tissue preparation

The adult mice were administered an i.p. injection of a lethal dose of pentobarbital and were perfused transcardially with 0.9% saline in 0.01 M phosphate buffered saline (PBS; pH 7.4) and cold 0.1 M phosphate buffer (PB; pH 7.4) containing 4% paraformaldehyde. The brains were removed, post-fixed overnight in the same fixative at 4 °C, and stored in 0.1 M PB containing gradient (10–30%) sucrose at 4 °C for cryoprotection. Sections with 30 μm-thickness were cut on a cryostat and stored in PBS/0.05% NaN$_3$ until use. In a c-Fos induction experiment, adult mice were given an i.p. injection of apomorphine (10 mg/kg) 2 h prior to perfusion.

Western blot analysis

The brains from deeply anesthetized adult mice were homogenized in 0.05 M Tris–HCl (pH 7.2) containing 0.025 M KCl, 0.005 M MgCl$_2$, and 0.32 M sucrose. The protein lysates were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and separated proteins were then transferred onto a polyvinylidene difluoride membrane. The membranes were incubated with an antibody to Gαolf (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:5000) and Can Get Signal (Toyobo Inc., Osaka, Japan), and then with horseradish peroxidase-conjugated anti-rabbit IgG. Bound antibodies were detected by chemiluminescence staining (ECL plus kit, GE Healthcare, Buckingham, UK).

Immunohistochemistry and digital imaging

Immunohistochemical staining was performed on free-floating sections as described in a previous report (Sato et al., 2008). Rabbit polyclonal antibody to tyrosine hydroxylase (TH; 1:100,000) (Sato et al., 2008), rat monoclonal antibody to D1R (Sigma-Aldrich, St