Cognitive Performance After Facial Botulinum Toxin Treatment in a Cohort of Neurologic Patients: An Exploratory Study

Platho-Elwischger, Kirsten; Schmoeger, Michaela; Willinger, Ulrike; Abdel-Aziz, Carmen; Algner, Jennifer; Pretscherer, Sandra; Auff, Eduard; Kranz, Gottfried; Turnbull, Oliver H.; Sycha, Thomas

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Cognition and facial botulinum treatment

Cognitive performance after facial botulinum toxin treatment in a cohort of neurological patients – an exploratory study

Kirsten Platho-Elwischger, MD[1,*], Michaela Schmoeger, Dr.[1], Ulrike Willinger, Univ. Prof.[1], Carmen Abdel-Aziz, Mag. [1] Jennifer Algner, Mag.[1] Sandra Pretscherer, Mag. [1] Eduard Auff, Univ.Prof.[1], Gottfried Kranz, Univ.Prof.[1,**], Oliver Turnbull, Univ.Prof.[2], Thomas Sycha, Univ.Prof.[1]

[1] Department of Neurology Medical University of Vienna, Vienna, Austria.

[2] School of Psychology, Bangor University, Bangor, United Kingdom.

[*] Department of Neurology, Clinic Hietzing, Vienna, Austria.

[**] Rehabilitation Center Rosenhuegel, Vienna, Austria.

Corresponding author:

Michaela Schmoeger

Department of Neurology

Medical University of Vienna

Waehringer Guertel 18-20

1090 Vienna, Austria

E-mail: michaela.schmoeger@meduniwien.ac.at

Phone: +43 1 40400 31200

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Abstract

Objective: To investigate higher cognitive functions after changes of the mimicry by facial botulinum toxin injections, we tested verbal and non-verbal reasoning in patients with blepharospasm or hemifacial spasm before and after their long-term botulinum toxin treatment. **Design:** Explorative, non-randomized, clinical trial. **Setting:** Patients: ambulatory care. Healthy control: general community. **Participants:** Volunteer sample. Patients: 21 patients with blepharospasm or hemifacial spasm – facial botulinum toxin injections. Controls: 30 patients with cervical dystonia – cervical botulinum toxin injections - and 33 healthy subjects. **Intervention:** The two groups receiving injections were tested before and three weeks after their treatment. Healthy subjects received no injections. **Main Outcome Measures:** Verbal and non-verbal reasoning scores. **Results:** The key unexpected finding is that patients who receive facial BTX injections perform significantly worse in non-verbal reasoning tasks, when compared to healthy control ($p=0.022$). There was no significant difference in the baseline reasoning scores and at follow up for verbal reasoning between the three groups. There was no correlation between toxin dose and reasoning scores (verbal: $p=0.132$, non-verbal: $p=0.294$). **Conclusion:** Because of potential confounders, the results do not allow any conclusion on causality yet. Further research is needed to confirm our findings.

Keywords: Cognition, botulinum toxin, facial muscles

Abbreviations

ANCOVA	analyses of covariance
ANOVA	analyses of variance
BEB	blepharospasm
BEB/HS	blepharospasm or hemifacial spasm
BTX	botulinum toxin
CI	confidence interval
CD	cervical dystonia
df	degrees of freedom
HS	hemifacial spasm
IBM	International Business Machines Corporation
rsfMRI	resting state functional MRI
SPSS	Statistical Package for the Social Sciences

There is a well-established link between the mechanics of one's own facial expressions and the ability to perceive the emotions of others. [1] This link includes facial 'mimicry', or the automatic response of analogous muscles when observing the facial expressions of others. One strand of this research is that aesthetic botulinum toxin (BTX) therapy of the corresponding facial muscles leads to a delayed processing of emotions, either positive or negative. [2, 3] This effect is typically explained by the well investigated "facial feedback hypothesis": that emotions are not

only expressed by activation of certain facial muscles, but also that activation of certain facial muscles induces the corresponding emotion.[1] The underlying mechanism remains unclear, but functional MRI data suggest a range of neural circuits, such as emotion-linked activation of the amygdala [4] and insula [5], and facial motor-linked activations of the inferior frontal gyrus. The reports also give further evidence that emotion and cognition may often be closely intertwined.[6, 7] Areas of activation include perception-linked systems such as the primary visual cortex and the inferior temporal cortex, memory-related regions such as the hippocampus, and the orbitofrontal cortex and prefrontal cortex.[7]

BTX has a large spectrum of applications in the rehabilitation of acute and chronic diseases.[8] Amongst others, it is the first line therapy in BEB (blepharospasm) and HS (hemifacial spasm). Here, small doses are injected into facial muscles, such as the orbicularis oculi, corrugator and procerus muscle.[9] The botulinum effect is reversible, and the therapy is typically repeated every three to four months. Since the increasing public awareness of the link between facial palsy and emotion, neurological patients receiving long-term botulinum toxin treatment were concerned about a probable affection of their cognitive function. To date, there has been no systematic study of cognition after BTX induced facial palsy. However, BTX induced plasticity of brain structures, namely the motor cortex in primary dystonia, has been described previously.[10] Thus, the extension to unaffected motor-cortical areas in cervical dystonia, i.e. the hand region, has also been reported.[11] By analogy, we assume, that apart from emotional processing, also cognitive domains could be affected by BTX induced facial palsy. Given the fact that experiencing emotion and understanding emotion in language use the same neural systems[2], this might include networks, which are part of the reading process.[12]

The purpose of the present, exploratory study was to assess cognitive function in neurological patients receiving long-term facial BTX therapy (BEB and HS), before and after BTX treatment. As screening tests, we chose a verbal reasoning task to cover the language domain and added a non-verbal reasoning task to extend the spectrum of cognitive domains. As one form of control, we assessed healthy subjects, who received no injections. We also investigated patients with cervical dystonia, who generally receive higher doses. Their injections are limited to cervical muscles, with no effect on the facial musculature.[13]

Materials and Methods

Participants

Patients with the clinical diagnosis of BEB, HS or CD were recruited from the BTX outpatient clinic for movement disorders at a Clinical Department of Neurology of a University Hospital. All patients included in this study were pre-treated with all common BTX preparations and reported good treatment response. To evaluate a potential correlation of BTX dose and reasoning scores, the equivalent unit ratio of the preparations ona-/inco-BTX : abo-BTX was calculated 1: 3.[14]

Healthy control subjects were recruited amongst patient companions, from geriatric facilities and through public announcements. Participants aged 18 to 85 were eligible. Participants with known neurological or psychiatric comorbidities were excluded.

Procedure

We investigated two groups:

- Patients with blepharospasm or hemifacial spasm (BEB/HS) - BTX treatment of facial muscles;

- patients with cervical dystonia (CD) - BTX treatment of cervical muscles, no treatment of facial muscles;

- healthy subjects – no BTX treatment, to exclude effects of repeated testing.

All participants performed a baseline assessment of reasoning measures, which was conducted by three investigators. Afterwards, all patients received their regular long-term BTX treatment.

BTX injections were applied by muscle palpation at the known anatomical landmarks without further technical aids. Dose finding and all injection schemes were individualized (see Table1for the list of included muscles).

Table 1. List of included muscles

After three weeks, a different investigator, who was blinded to the baseline test results, repeated the cognitive assessment of each participant.

Cognitive assessment

Verbal reasoning was assessed with the Verbal Analogies subtest of the Intelligence Structure Test 2000-R.[15] Here 20 tasks are presented, each task consisting of three words. In these tasks, a relation exists between the first and the second word, and a similar relation can be applied between the third word, and one of five alternatives.

Nonverbal reasoning was measured using the Matrices subtest of the Intelligence Structure Test 2000-R.[15] This subtest consists of 20 tasks, each showing a two-by-two matrix with three different figures, which are located based on a rule. The task is to detect the rule, and choose the correct missing figure from five alternatives. Overall scores were built by aggregating the correct answers for Verbal Analogies and Matrices.

Standard protocol approval and patient consents

The study was approved by the local ethical committee and has been registered at ClinicalTrials.gov. All participants gave their informed consent for inclusion before they

participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local ethical committee.

Statistical analysis

Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) software (IBM, International Business Machines Corporation, SPSS Statistics, Version 20).[16] The desired number of at least 30 patients was based on the central limit theorem.[17] Equivalence of basic sample characteristics (age, gender, educational level) between patients (i.e. BEB/HS and CD) and healthy controls was analysed using an ANOVA (analyses of variance) and Chi square tests. Furthermore, analyses of variance were conducted to compare nonverbal as well as verbal reasoning scores of patients with CD, BEB/HS and healthy controls at baseline. The analyzed variables were normally distributed (verbal reasoning - baseline: $\chi2=3.10$, df=2, p=0.213; non-verbal reasoning - baseline: $\chi2=2.29$, df=2, p=0.319; verbal reasoning – follow-up: $\chi2=0.615$, df=2, p=0.735; non-verbal reasoning – follow up: $\chi2=1.78$, df=2, p=0.411). Differences in verbal and nonverbal reasoning before and after BTX-treatment, as well as the presence of a potential learning effect, was analysed using two-way mixed ANCOVA (analyses of covariance) with the three groups (CD, BEB/HS and the healthy controls) as the between-subjects factor, time (pre- and post-treatment) as the within-subjects factor, overall treatment time and dose as covariates. Relationships between dose and verbal or nonverbal reasoning was analysed using Pearson correlation. The significance level was set at $p<0.05$. Post hoc power analyses for the used within-between factorial design ($f = 0.25$, $\alpha = 0.05$, sample size = 84, groups 3, measurements = 2) revealed a power of 0.99.

Results

Participants

In the injection groups, a total of 169 patients were screened for participation, 88 patients were eligible and willing to participate. Thirty-seven patients did not complete baseline and were excluded from further analyses (see Table 2 for details of drop out).

Table 2. Details of drop out

Finally, a total of 84 subjects participated in the present study. Fifty-one (60.7%) received BTX injections – 21 (41.2%; BEB: n=14, HS: n=7) patients with BEB/HS and 30 (58.8%) patients with CD - and 33 (39.3%) were healthy subjects. The desired number of at least 30 patients in the BEB/HS group could not be recruited, as some of those patients refused to perform a neuropsychological test, for example when they realized that they would be tested in their cognitive, reading and/or arithmetic competence, or when they realized that some more study-related appointments were necessary. Participants that refused to perform neuropsychological tests were not included in the present study. For clinical details of all participants at baseline assessment, see Table 3.

Table 3. Clinical details of all participants at baseline assessment

The patient group (i.e. BEB/HS and CD) consisted of 30 women (59%) and 21 men (41%). The age of patients ranged from 26 to 78 years (mean=59.7, SD=12.24). 34 of the patients (66.7%) had terminated their education at the end of compulsory schooling. 17 of the patients (33.3%) held a college or university degree.

The healthy controls consisted of 22 women (67%) and 11 men (33%), ranging in age from 29 to 81 years (mean=61.3, SD=11.90). Sixteen of the controls (49%) had compulsory schooling as their highest educational level, with 17 (51%) with college or university degrees.

There were no significant differences between the three groups regarding age ($F(2.81)=1.865$, $p=0.161$), gender ($\chi2=1.454$, $df=2$, $p=0.483$) and educational level ($\chi2=3.085$, $df=2$, $p=0.214$). In addition, the number of subjects per group ($\chi2=2.79$, $df=2$, $p=0.248$), BTX treatment duration ($\chi2=7.57$, $df=3$, $p=0.056$), and the applied BTX preparations ($\chi2=5.84$, $df=3$, $p=0.119$) were equally distributed in all three groups. BTX equivalent dosage was not equally distributed ($\chi2=37,63$, $df=4$, $p<0.0001$), but the dosage did not correlate with verbal and non-verbal reasoning scores (see below).

Baseline assessment of non-verbal and verbal reasoning

There were no statistically significant differences in scores of nonverbal as well as verbal reasoning before BTX-treatment. An analysis of variance showed no significant differences ($F(2,81)=2.14$, $p=0.124$) between CD (mean=7.77, SD=3.52, 95%CI=5.82-9.82), BEB/HS

(mean=6.26, SD=2.72, 95%CI=4.67-7.78) and control subjects (mean=7.83, SD=2.85, 95%CI=6.1-9.52) with respect to scores of nonverbal reasoning at baseline. There were also no statistically significant differences at baseline with respect to scores of verbal reasoning (F(2,80)=2.92, p=0.060) between CD (mean=7.7, SD=3.15, 95%CI=6.03-10.17), BEB/HS (mean=6.32, SD=3.11, 95%CI=4.6-7.85) and control subjects (mean=8.66, SD=3.42, 95%CI=6.5-10.11).

Differences in reasoning scores: pre- and post-treatment

Non-verbal reasoning (Fig 1)

The scores of control subjects improved (mean=9.23, SD=3.62, 95%CI=7.1-11.17), those of CD slightly improved (mean=8.07, SD=3.97, 95%CI=5.86-10.62). and those of BEB/HS slightly decreased (BEB/HS: mean=6.21, SD=3.36, 95%CI=4.25-7.95).

Figure 1. Non-verbal reasoning scores.

A mixed design ANCOVA revealed no significant main effect of time [F(1,74)=0.86, p=0.357, $\eta 2$=0.011], but a significant main effect of group [F(2,74)=3.34, p=0.041, $\eta 2$=0.083] with respect to nonverbal reasoning, indicating no differences between pre-and post-treatment evaluation but differences between the three groups. The covariates dose [F(1,74)=0.12,

p=0.731, η2=0.002] and overall treatment time [F(1,74)=0.22, p=0.641, η2=0.003] showed no significant impact.

Pairwise comparisons showed a significant difference between healthy controls and BEB/HS (p=0.022), with the lowest mean difference in non-verbal abilities regarding pre- and post-treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD (p=0.794) and between the two patient groups (p=0.197).

The interaction effect of time and group was not significant [F(2,74)=1.23, p=0.297, η2=0.032] indicating no differences in the mean change in nonverbal reasoning scores between patient groups and healthy controls and no learning effect occurred regarding non-verbal reasoning. There were also no significant interaction effects of time and dose [F(1,74)=0.11, p=0.743, η2=0.001] as well as time and overall treatment time [F(1,74)=0.15, p=0.696, η2=0.002].

Verbal reasoning (Fig 2)

The scores of CD improved (mean=8.77, SD=4.01, 95%CI=7.13-11.76), those of controls (mean=8.34, SD=3.38, 95%CI=5.74-9.8) and BEB/HS (mean=6.0, SD=3.38, 95%CI=3.98-7.63) slightly decreased.

Fig 2. Verbal reasoning scores.

With respect to verbal reasoning a mixed design ANCOVA revealed no significant main effect of time [$F(1,73)=0.37$, $p=0.546$, $\eta2=0.005$] but a significant main effect of group [$F(2,73)=3.37$, $p=0.040$, $\eta2=0.084$], indicating no differences between pre-and post- treatment evaluation but differences between the three groups. The covariates dose [$F(1,73)=0.07$, $p=0.793$, $\eta2=0.001$] and overall treatment time [$F(1,73)=0.85$, $p=0.359$, $\eta2=0.012$] did not have a significant impact.

Pairwise comparisons showed a borderline significance suggesting a noticeable difference between healthy controls and BEB/HS ($p=0.051$) as well as borderline significant differences between the two patient groups ($p=0.067$), with the lowest mean difference in verbal abilities regarding pre- and post- treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD ($p=0.669$).

The interaction effect of time and group was not significant [$F(2,73)=1.12$, $p=0.331$, $\eta2=0.030$], indicating that the mean changes in verbal reasoning scores did not differ between both patient groups and healthy controls and no learning effect occurred regarding verbal reasoning. Furthermore, there are no significant interactions between time and dose [$F(2,73)=0.13$, $p=0.718$, $\eta2=0.002$] as well as overall treatment time [$F(2,73)=0.02$, $p=0.893$, $\eta2=0.000$].

<u>Relationship between BTX dose und verbal or nonverbal reasoning</u>

There was no significant correlation between BTX dose and verbal reasoning (Pearson correlation, $r(49)=0.22$, $p=0.132$) as well as nonverbal reasoning scores (Pearson correlation, $r(49)=0.153$, $p=0.294$) after treatment.

Discussion

The main finding of this exploratory study was a significant difference of non-verbal reasoning scores in the patient group who received BTX treatment of their facial muscles (BEB/HS), when compared to healthy controls. In passing, we note that there was also a noticeable difference of the post-treatment verbal reasoning scores, but they failed to reach significance ($p=0.051$).

These preliminary results are in several respects surprising, given that the treatment might have been predicted to *address* potential physical impairments, and thus *improve* test scores. For example, an improvement of the visual sustained attention span might have been expected, after clinical improvement of the BEB symptoms (as has been described previously).[18] A related issue would be an expected improvement in the performance of written tests, given that patients report difficulties reading, which also tends to improve after their botulinum treatment. Indeed, when the effect of the BTX treatment wears off, patients tend to again complain about those difficulties. This was also reflected in the higher number of patients who refused to participate in this study, as a result of these impairments. Cognitive impairment after facial BTX injections in the treatment of neurological disorders has never been reported.[19, 20] However, discrete impairment of cognitive performance has been described as a non-motor syndrome of BEB.[21] Two-thirds of patients in the facial injection group suffered from BEB. We cannot exclude that the pathophysiological alterations due to the non-motor syndrome might be a potential confounding factor. However, cognitive disturbances as non-motor symptoms have also been

described in cervical dystonia.[22] Therefore, one might expect a similar performance. But in the case of verbal scores, the cervical injection cohort *improved* with a borderline significance (p=0.067), when compared to the facial injection group.

There is little data referring to neural correlates of the two specific aspects of reasoning, which were investigated in the present study.[23] Verbal analytic reasoning has been correlated with rsfMRI (resting state fMRI) data and has been related with brain regions for integration (i.e. the angular and supramarginal gyrus), hypothesis testing, cognitive control (i.e. inferior frontal gyrus) and response selection (i.e. dorsal anterior cingulate cortex). Non-verbal reasoning scores were non-significantly associated with the left occipital- and right anterior temporal lobe, and right frontoinsular cortex, respectively.[23]

One account might be a more direct link between facial muscles and these cognitive networks. In this context, the role of the corrugator muscle in several emotional and non-emotional facial expressions has been reported.[24] Amongst others, the corrugator muscle is activated during concentration, and plays an important role in communication and interaction, including when accompanying or emphasizing elements of speech.[25] A recent investigation measured motor activity of grip strength during verbal processing, and found a context sensitive increase during processing of "action words".[26]

An alternative approach might be that the BTX induced facial palsy leads to a delay in emotion-related responses, which is well-established.[2] There is an increasing awareness of the ways that emotion might interact with cognitive processes – such as perception, attention, memory and decision making. [7] However, emotion-linked domains like memory -i.e. emotion based learning

[27], or planning and decision-making[7] were not covered by the present reasoning tasks. Further investigations might profit from additional objective tests of these cognitive domains.

Regardless of cause, our unexpected findings illustrate the importance of careful monitoring during a regular rehabilitative treatment of chronic diseases, even though this treatment is well-established. In addition, further research might consider tests of the cognitive performance during the rehabilitation of facial palsies of other origin, e.g., Bell's palsy.

Notably, we found no indications for a dose dependent effect on the reasoning scores. It is also notable that performance of the control patients with cervical muscle injections (CD) did not differ from healthy controls at any time point, even though those patients received high cumulative doses (Table 3). This might be a relevant issue for other BTX applications with high cumulative doses in neurological rehabilitation, namely spasticity. These data also support the assumption that there is no direct effect of BTX due to a questionable retrograde transport or systemic distribution.[28, 29]

Study limitations

At this point, - and as the major limitation of this study- these preliminary results do not allow a causality of facial palsy and cognitive performance; thus, the interpretation of the present data remains highly speculative and cannot be generalized. The data needs to be confirmed by trials involving a larger sample size and additional control groups to adjust for confounding factors, such as a selection bias.

Here, we did not perform sham-injections as placebo control. One reason for this were ethical considerations, as BTX is the first line therapy and very effective in dystonia.[9, 13] Furthermore, unblinding appears highly probable. We suggest the additional investigation of subjects receiving cosmetic facial BTX injections.

These appear to be conventional clinical samples, with normal baseline intelligence measurements of the patient and control groups. Novelty does not seem to be a relevant confounding variable, as there were no BTX "naive" patients included, and both patient groups have a mean treatment duration of almost six years, as part of a regular cycle of treatments.

We did not perform a follow up evaluation to clarify if the reduced performance is temporary and completely reversible. Therefore, future research should address the sustainability of the results after the paralysis of facial muscles wears off.

Conclusions

The unexpected key finding is that patients who receive facial BTX injections appear to perform significantly worse in non-verbal (and as a trend, verbal) reasoning tasks. However, these are preliminary results of an exploratory study with potential confounders. Thus, at this point, BTX induced facial palsy and cognitive performance cannot be related, and therefore the interpretation of these results remains highly speculative. It is clear that the findings

394 should be backed by further controlled investigations and illustrate the importance of careful
395 monitoring during a well-established, rehabilitative treatment of chronic diseases.
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398 **References**
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Figure legends

Fig 1. Non-verbal reasoning scores. Estimated marginal mean scores and 95% CI of non-verbal reasoning tests absolved by patients and controls at baseline and three weeks after BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm; BoNT: botulinum neurotoxin; HS: hemifacial spasm.

475 **Figure 2. Verbal reasoning scores.** Estimated marginal mean scores and 95% CI of
476 verbal reasoning tests absolved by patients and controls at baseline and three weeks after
477 BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm;
478 BoNT: botulinum neurotoxin; HS: hemifacial spasm.

Cognitive performance after facial botulinum toxin treatment in a cohort of neurological patients – an exploratory study

Abstract

Objective: To investigate higher cognitive functions after changes of the mimicry by facial botulinum toxin injections, we tested verbal and non-verbal reasoning in patients with blepharospasm or hemifacial spasm before and after their long-term botulinum toxin treatment. **Design:** Explorative, non-randomized, clinical trial. **Setting:** Patients: ambulatory care. Healthy control: general community. **Participants:** Volunteer sample. Patients: 21 ~~of 38~~ ~~eligible~~ patients with blepharospasm or hemifacial spasm – facial botulinum toxin injections. Controls: 30 ~~of 50 eligible~~ patients with cervical dystonia – cervical botulinum toxin injections - and 33 healthy subjects. **Intervention:** The two groups receiving injections were tested before and three weeks after their treatment. Healthy subjects received no injections. **Main Outcome Measures:** Verbal and non-verbal reasoning scores. **Results:** The key unexpected finding is that patients who receive facial BTX injections perform significantly worse in non-verbal reasoning tasks, when compared to healthy control (p=0.022). There was no significant difference in the baseline reasoning scores and at follow up for verbal reasoning between the three groups. There was no correlation between toxin dose and reasoning scores (verbal: p=0.132, non-verbal: p=0.294). **Conclusion:** Because of potential confounders, the results do not allow any ~~robust~~ conclusion on causality yet. Further research is needed to confirm our findings.

Keywords: Cognition, botulinum toxin, facial muscles

Abbreviations

ANCOVA	analyses of covariance
ANOVA	analyses of variance
BEB	blepharospasm
BEB/HS	blepharospasm or hemifacial spasm
BTX	botulinum toxin
CI	confidence interval
CD	cervical dystonia
df	degrees of freedom
HS	hemifacial spasm
IBM	International Business Machines Corporation
rsfMRI	resting state functional MRI
SPSS	Statistical Package for the Social Sciences

There is a well-established link between the mechanics of one's own facial expressions and the ability to perceive the emotions of others. [1] This link includes facial 'mimicry', or the automatic response of analogous muscles when observing the facial expressions of others. One strand of this research is that aesthetic botulinum toxin (BTX) therapy of the corresponding facial muscles leads to a delayed processing of emotions, either positive or negative. [2, 3] This effect is

typically explained by the well investigated "facial feedback hypothesis": that emotions are not only expressed by activation of certain facial muscles, but also that activation of certain facial muscles induces the corresponding emotion.[1] The underlying mechanism remains unclear, but functional MRI data suggest a range of neural circuits, such as emotion-linked activation of the amygdala [4] and insula [5], and facial motor-linked activations of the inferior frontal gyrus. The reports also give further evidence that emotion and cognition may often be closely intertwined.[6, 7] Areas of activation include perception-linked systems such as the primary visual cortex and the inferior temporal cortex, memory-related regions such as the hippocampus, and the orbitofrontal cortex and prefrontal cortex.[7]

BTX has a large spectrum of applications in the rehabilitation of acute and chronic diseases.[8] Amongst others, it is the first line therapy in BEB (blepharospasm) and HS (hemifacial spasm). Here, small doses are injected into facial muscles, such as the orbicularis oculi, corrugator and procerus muscle.[9] The botulinum effect is reversible, and the therapy is typically repeated every three to four months. Since the increasing public awareness of the link between facial palsy and emotion, neurological patients receiving long-term botulinum toxin treatment were concerned about a probable affection of their cognitive function. To date, there has been no systematic study ~~of the effect~~ of cognition after BTX induced facial palsy. However, BTX induced plasticity of brain structures, namely the motor cortex in primary dystonia, has been described previously.[10] Thus, the extension to unaffected motor-cortical areas in cervical dystonia, i.e. the hand region, has also been reported.[11] By analogy, we assume, that apart from emotional processing, also cognitive domains could be affected by BTX induced facial palsy. Given the fact that experiencing emotion and understanding emotion in language use the same neural systems[2], this might include networks, which are part of the reading process.[12]

The purpose of the present, exploratory study was to assess cognitive function in neurological patients receiving long-term facial BTX therapy (BEB and HS), before and after BTX treatment. As screening tests, we chose a verbal reasoning task to cover the language domain and added a non-verbal reasoning task to extend the spectrum of cognitive domains. As one form of control, we ~~also~~ assessed healthy subjects, who received no injections. We also investigated patients with cervical dystonia, who generally receive higher doses. Their injections are limited to cervical muscles, with no effect on the facial musculature.[13]

Materials and Methods

Participants

Patients with the clinical diagnosis of BEB, HS or CD were recruited from the BTX outpatient clinic for movement disorders at a Clinical Department of Neurology of a University Hospital. All patients included in this study were pre-treated with all common BTX preparations and reported good treatment response. To evaluate a potential correlation of BTX dose and reasoning scores, the equivalent unit ratio of the preparations ona-/inco-BTX : abo-BTX was calculated 1: 3.[14] ~~Co-medication has been routinely assessed and documented by the treating neurologist in the patient's file and constant during study period.~~

Healthy control subjects were recruited amongst patient companions, from geriatric facilities and through public announcements. Participants aged 18 to 85 were eligible. Participants with known neurological or psychiatric comorbidities were excluded.

Procedure

We investigated two groups:

- Patients with blepharospasm or hemifacial spasm (BEB/HS) - BTX treatment of facial muscles;

- patients with cervical dystonia (CD) - BTX treatment of cervical muscles, no treatment of facial muscles;

- healthy subjects – no BTX treatment, to exclude effects of repeated testing.

All participants performed a baseline assessment of reasoning measures, which was conducted by three investigators. Afterwards, all patients received their regular long-term BTX treatment.

BTX injections were applied by muscle palpation at the known anatomical landmarks without further technical aids. Dose finding and all injection schemes were individualized (see Table1for the list of included muscles).

Table 1. List of included muscles

~~of BEB and HS involved orbicularis oculi, palpebral part of the orbicularis oculi, corrugator supercilii, levator anguli oris, depressor anguli oris and platysma muscle. Injection scheme of CD involved sternocleidomastoid, splenius capitis, semispinalis capitis, trapezius and levator scapulae muscle.~~

After three weeks, a different investigator, who was blinded to the baseline test results, repeated the cognitive assessment of each participant.

Cognitive assessment

Verbal reasoning was assessed with the Verbal Analogies subtest of the Intelligence Structure Test 2000-R.[15] Here 20 tasks are presented, each task consisting of three words. In these tasks, a relation exists between the first and the second word, and a similar relation can be applied between the third word, and one of five alternatives.

Nonverbal reasoning was measured using the Matrices subtest of the Intelligence Structure Test 2000-R.[15] This subtest consists of 20 tasks, each showing a two-by-two matrix with three different figures, which are located based on a rule. The task is to detect the rule, and choose the correct missing figure from five alternatives. Overall scores were built by aggregating the correct answers for Verbal Analogies and Matrices.

149

150 Standard protocol approval and patient consents

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153 The study was approved by the local ethical committee and has been registered at
154 ClinicalTrials.gov. All participants gave their informed consent for inclusion before they
155 participated in the study. The study was conducted in accordance with the Declaration of
156 Helsinki, and the protocol was approved by the local ethical committee.

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159 Statistical analysis

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162 Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences)
163 software (IBM, International Business Machines Corporation, SPSS Statistics, Version 20).[16]
164 The desired number of at least 30 patients was based on the central limit theorem.[17] Equivalence
165 of basic sample characteristics (age, gender, educational level) between patients (i.e. BEB/HS
166 and CD) and healthy controls was analysed using an ANOVA (analyses of variance) and Chi
167 square tests. Furthermore, analyses of variance were conducted to compare nonverbal as well
168 as verbal reasoning scores of patients with CD, BEB/HS and healthy controls at baseline. The
169 analyzed variables were normally distributed (verbal reasoning - baseline: $\chi 2=3.10$, df=2,
170 p=0.213; non-verbal reasoning - baseline: $\chi 2=2.29$, df=2, p=0.319; verbal reasoning – follow-
171 up: $\chi 2=0.615$, df=2, p=0.735; non-verbal reasoning – follow up: $\chi 2=1.78$, df=2, p=0.411).
172 Differences in verbal and nonverbal reasoning before and after BTX-treatment, as well as the
173 presence of a potential learning effect, was analysed using two-way mixed ANCOVA (analyses

of covariance) with the three groups (CD, BEB/HS and the healthy controls) as the between-subjects factor, time (pre- and post-treatment) as the within-subjects factor, overall treatment time and dose as covariates. Relationships between dose and verbal or nonverbal reasoning was analysed using Pearson correlation. The significance level was set at $p<0.05$. Post hoc power analyses for the used within-between factorial design ($f = 0.25$, $\alpha = 0.05$, sample size = 84, groups 3, measurements = 2) revealed a power of 0.99.

Results

Participants

In the injection groups, a total of 169 patients were screened for participation, 88 patients were eligible and willing to participate. Thirty-seven patients did not complete baseline and were excluded from further analyses (see Table 2 for details of drop out). ~~BEB: n=13/27, 48.1%; HS: n=4/11, 36.4%; CD: n=20/50, 40%~~

Table 2. Details of drop out

~~Physical handicap (BEB:n=3/13, 23.1%; CD:n=1/20, 5%), fear of a bad test performance (BEB:n=1/13, 7.7%), lack of time (CDn=3/20, 15%), language barrier (BEB:n=1/13, 7.7%; CD:n=1/20, 5%) or no specific reason (BEB:n=8/13, 61.5%; hemifacial spasm:n=2/4, 50%; CD:n=14/20, 70%) have been reported as reasons for drop out.~~

Finally, a total of 84 subjects participated in the present study. Fifty-one (60.7%) received BTX injections – 21 (41.2%; BEB: n=14, HS: n=7) patients with BEB/HS and 30 (58.8%) patients with CD - and 33 (39.3%) were healthy subjects. The desired number of at least 30 patients in the BEB/HS group could not be recruited, as some of those patients refused to perform a neuropsychological test, for example when they realized that they would be tested in their cognitive, reading and/or arithmetic competence, or when they realized that some more study-related appointments were necessary. Participants that refused to perform neuropsychological tests were not included in the present study. For clinical details of all participants at baseline assessment, see Table 3.

Table 3. Clinical details of all participants at baseline assessment

The patient group (i.e. BEB/HS and CD) consisted of 30 women (59%) and 21 men (41%). The age of patients ranged from 26 to 78 years (mean=59.7, SD=12.24). 34 of the patients (66.7%) had terminated their education at the end of compulsory schooling. 17 of the patients (33.3%) held a college or university degree.

The healthy controls consisted of 22 women (67%) and 11 men (33%), ranging in age from 29 to 81 years (mean=61.3, SD=11.90). Sixteen of the controls (49%) had compulsory schooling as their highest educational level, with 17 (51%) with college or university degrees.

There were no significant differences between the three groups regarding age ($F(2.81)=1.865$, $p=0.161$), gender ($\chi2=1.454$, $df=2$, $p=0.483$) and educational level ($\chi2=3.085$, $df=2$, $p=0.214$). In addition, the number of subjects per group ($\chi2=2.79$, $df=2$, $p=0.248$), BTX treatment duration ($\chi2=7.57$, $df=3$, $p=0.056$), and the applied BTX preparations ($\chi2=5.84$, $df=3$, $p=0.119$) were

equally distributed in all three groups. BTX equivalent dosage was not equally distributed ($\chi 2=37,63$, df=4, $p<0.0001$), but the dosage did not correlate with verbal and non-verbal reasoning scores (see below).

Baseline assessment of non-verbal and verbal reasoning

There were no statistically significant differences in scores of nonverbal as well as verbal reasoning before BTX-treatment. An analysis of variance showed no significant differences ($F(2,81)=2.14$, $p=0.124$) between CD (mean=7.77, SD=3.52, 95%CI=5.82-9.82), BEB/HS (mean=6.26, SD=2.72, 95%CI=4.67-7.78) and control subjects (mean=7.83, SD=2.85, 95%CI=6.1-9.52) with respect to scores of nonverbal reasoning at baseline. There were also no statistically significant differences at baseline with respect to scores of verbal reasoning ($F(2,80)=2.92$, $p=0.060$) between CD (mean=7.7, SD=3.15, 95%CI=6.03-10.17), BEB/HS (mean=6.32, SD=3.11, 95%CI=4.6-7.85) and control subjects (mean=8.66, SD=3.42, 95%CI=6.5-10.11).

Differences in reasoning scores: pre- and post-treatment

Non-verbal reasoning (Fig 1)

The scores of control subjects improved (mean=9.23, SD=3.62, 95%CI=7.1-11.17), those of CD slightly improved (mean=8.07, SD=3.97, 95%CI=5.86-10.62). and those of BEB/HS slightly decreased (BEB/HS: mean=6.21, SD=3.36, 95%CI=4.25-7.95).

Figure 1. Non-verbal reasoning scores.

A mixed design ANCOVA revealed no significant main effect of time [$F(1,74)=0.86$, $p=0.357$, $\eta2=0.011$], but a significant main effect of group [$F(2,74)=3.34$, $p=0.041$, $\eta2=0.083$] with respect to nonverbal reasoning, indicating no differences between pre-and post-treatment evaluation but differences between the three groups. The covariates dose [$F(1,74)=0.12$, $p=0.731$, $\eta2=0.002$] and overall treatment time [$F(1,74)=0.22$, $p=0.641$, $\eta2=0.003$] showed no significant impact.

Pairwise comparisons showed a significant difference between healthy controls and BEB/HS ($p=0.022$), with the lowest mean difference in non-verbal abilities regarding pre- and post-treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD ($p=0.794$) and between the two patient groups ($p=0.197$).

The interaction effect of time and group was not significant [$F(2,74)=1.23$, $p=0.297$, $\eta2=0.032$] indicating no differences in the mean change in nonverbal reasoning scores between patient groups and healthy controls and no learning effect occurred regarding non-verbal reasoning. There were also no significant interaction effects of time and dose [$F(1,74)=0.11$, $p=0.743$, $\eta2=0.001$] as well as time and overall treatment time [$F(1,74)=0.15$, $p=0.696$, $\eta2=0.002$].

Verbal reasoning (Fig 2)

The scores of CD improved (mean=8.77, SD=4.01, 95%CI=7.13-11.76), those of controls (mean=8.34, SD=3.38, 95%CI=5.74-9.8) and BEB/HS (mean=6.0, SD=3.38, 95%CI=3.98-7.63) slightly decreased.

Fig 2. Verbal reasoning scores.

With respect to verbal reasoning a mixed design ANCOVA revealed no significant main effect of time [$F(1,73)=0.37$, $p=0.546$, $\eta 2=0.005$] but a significant main effect of group [$F(2,73)=3.37$, $p=0.040$, $\eta 2=0.084$], indicating no differences between pre-and post- treatment evaluation but differences between the three groups. The covariates dose [$F(1,73)=0.07$, $p=0.793$, $\eta 2=0.001$] and overall treatment time [$F(1,73)=0.85$, $p=0.359$, $\eta 2=0.012$] did not have a significant impact.

Pairwise comparisons showed a borderline significance suggesting a noticeable difference between healthy controls and BEB/HS ($p=0.051$) as well as borderline significant differences between the two patient groups ($p=0.067$), with the lowest mean difference in verbal abilities regarding pre- and post- treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD ($p=0.669$).

The interaction effect of time and group was not significant [$F(2,73)=1.12$, $p=0.331$, $\eta 2=0.030$], indicating that the mean changes in verbal reasoning scores did not differ between both patient groups and healthy controls and no learning effect occurred regarding verbal reasoning.

Furthermore, there are no significant interactions between time and dose [$F(2,73)=0.13$, $p=0.718$, $\eta 2=0.002$] as well as overall treatment time [$F(2,73)=0.02$, $p=0.893$, $\eta 2=0.000$].

Relationship between BTX dose und verbal or nonverbal reasoning

There was no significant correlation between BTX dose and verbal reasoning (Pearson correlation, $r(49)=0.22$, $p=0.132$) as well as nonverbal reasoning scores (Pearson correlation, $r(49)=0.153$, $p=0.294$) after treatment.

Discussion

The main finding of this exploratory study was a significant difference of non-verbal reasoning scores in the patient group who received BTX treatment of their facial muscles (BEB/HS), when compared to healthy controls. In passing, we note that there was also a noticeable difference of the post-treatment verbal reasoning scores, but they failed to reach significance ($p=0.051$).

These preliminary results are in several respects surprising, given that the treatment might have been predicted to *address* potential physical impairments, and thus *improve* test scores. For example, an improvement of the visual sustained attention span might have been expected, after clinical improvement of the BEB symptoms (as has been described previously).[18] A related

issue would be an expected improvement in the performance of written tests, given that patients report difficulties reading, which also tends to improve after their botulinum treatment. Indeed, when the effect of the BTX treatment wears off, patients tend to again complain about those difficulties. This was also reflected in the higher number of patients who refused to participate in this study, as a result of these impairments. Cognitive impairment after facial BTX injections in the treatment of neurological disorders has never been reported.[19, 20] However, discrete impairment of cognitive performance has been described as a non-motor syndrome of BEB.[21] Two-thirds of patients in the facial injection group suffered from BEB. We cannot exclude that the pathophysiological alterations due to the non-motor syndrome might be a potential confounding factor. However, cognitive disturbances as non-motor symptoms have also been described in cervical dystonia.[22] Therefore, one might expect a similar performance. But in the case of verbal scores, the cervical injection cohort *improved* with a borderline significance ($p=0.067$), when compared to the facial injection group.

There is little data referring to neural correlates of the two specific aspects of reasoning, which were investigated in the present study.[23] Verbal analytic reasoning has been correlated with rsfMRI (resting state fMRI) data and has been related with brain regions for integration (i.e. the angular and supramarginal gyrus), hypothesis testing, cognitive control (i.e. inferior frontal gyrus) and response selection (i.e. dorsal anterior cingulate cortex). Non-verbal reasoning scores were non-significantly associated with the left occipital- and right anterior temporal lobe, and right frontoinsular cortex, respectively.[23]

One account might be a more direct link between facial muscles and these cognitive networks. In this context, the role of the corrugator muscle in several emotional and non-emotional facial expressions has been reported.[24] Amongst others, the corrugator muscle is activated during

concentration, and plays an important role in communication and interaction, including when accompanying or emphasizing elements of speech.[25] A recent investigation measured motor activity of grip strength during verbal processing, and found a context sensitive increase during processing of "action words".[26]

An alternative approach might be that the BTX induced facial palsy leads to a delay in emotion-related responses, which is well-established.[2] There is an increasing awareness of the ways that emotion might interact with cognitive processes – such as perception, attention, memory and decision making. [7] However, emotion-linked domains like memory -i.e. emotion based learning [27], or planning and decision-making[7] were not covered by the present reasoning tasks. Further investigations might profit from additional objective tests of these cognitive domains.

Regardless of cause, our unexpected findings illustrate the importance of careful monitoring during a regular rehabilitative treatment of chronic diseases, even though this treatment is well-established. In addition, further research might consider tests of the cognitive performance during the rehabilitation of facial palsies of other origin, e.g., Bell's palsy.

Notably, we found no indications for a dose dependent effect on the reasoning scores. It is also notable that performance of the control patients with cervical muscle injections (CD) did not differ from healthy controls at any time point, even though those patients received high cumulative doses (Table 3). This might be a relevant issue for other BTX applications with high cumulative doses in neurological rehabilitation, namely spasticity. These data also support the assumption that there is no direct effect of BTX due to a questionable retrograde transport or systemic distribution.[28, 29]

Study limitations

At this point, - and as the major limitation of this study- these preliminary results do not allow a causality of facial palsy and cognitive performance; thus, the interpretation of the present data remains highly speculative and cannot be generalized. The data needs to be confirmed by trials involving a larger sample size and additional control groups to adjust for confounding factors, such as a selection bias.

Here, we did not perform sham-injections as placebo control. One reason for this were ethical considerations, as BTX is the first line therapy and very effective in dystonia.[9, 13] Furthermore, unblinding appears highly probable. We suggest the additional investigation of subjects receiving cosmetic facial BTX injections.

These appear to be conventional clinical samples, with normal baseline intelligence measurements of the patient and control groups. Novelty does not seem to be a relevant confounding variable, as there were no BTX "naive" patients included, and both patient groups have a mean treatment duration of almost six years, as part of a regular cycle of treatments.

We did not perform a follow up evaluation to clarify if the reduced performance is temporary and completely reversible. Therefore, future research should address the sustainability of the results after the paralysis of facial muscles wears off.

Conclusions

The unexpected key finding is that patients who receive facial BTX injections appear to perform significantly worse in non-verbal (and as a trend, verbal) reasoning tasks. However, these are preliminary results of an exploratory study with potential confounders. Thus, at this point, BTX induced facial palsy and cognitive performance cannot be related, and therefore the interpretation of these results remains highly speculative. It is clear that the findings should be backed by further controlled investigations and illustrate the importance of careful monitoring during a well-established, rehabilitative treatment of chronic diseases.

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Figure legends

Fig 1. Non-verbal reasoning scores. Estimated marginal mean scores and 95% CI of non-verbal reasoning tests absolved by patients and controls at baseline and three weeks after BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm; BoNT: botulinum neurotoxin; HS: hemifacial spasm.

Figure 2. Verbal reasoning scores. Estimated marginal mean scores and 95% CI of verbal reasoning tests absolved by patients and controls at baseline and three weeks after BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm; BoNT: botulinum neurotoxin; HS: hemifacial spasm.

Figure 1

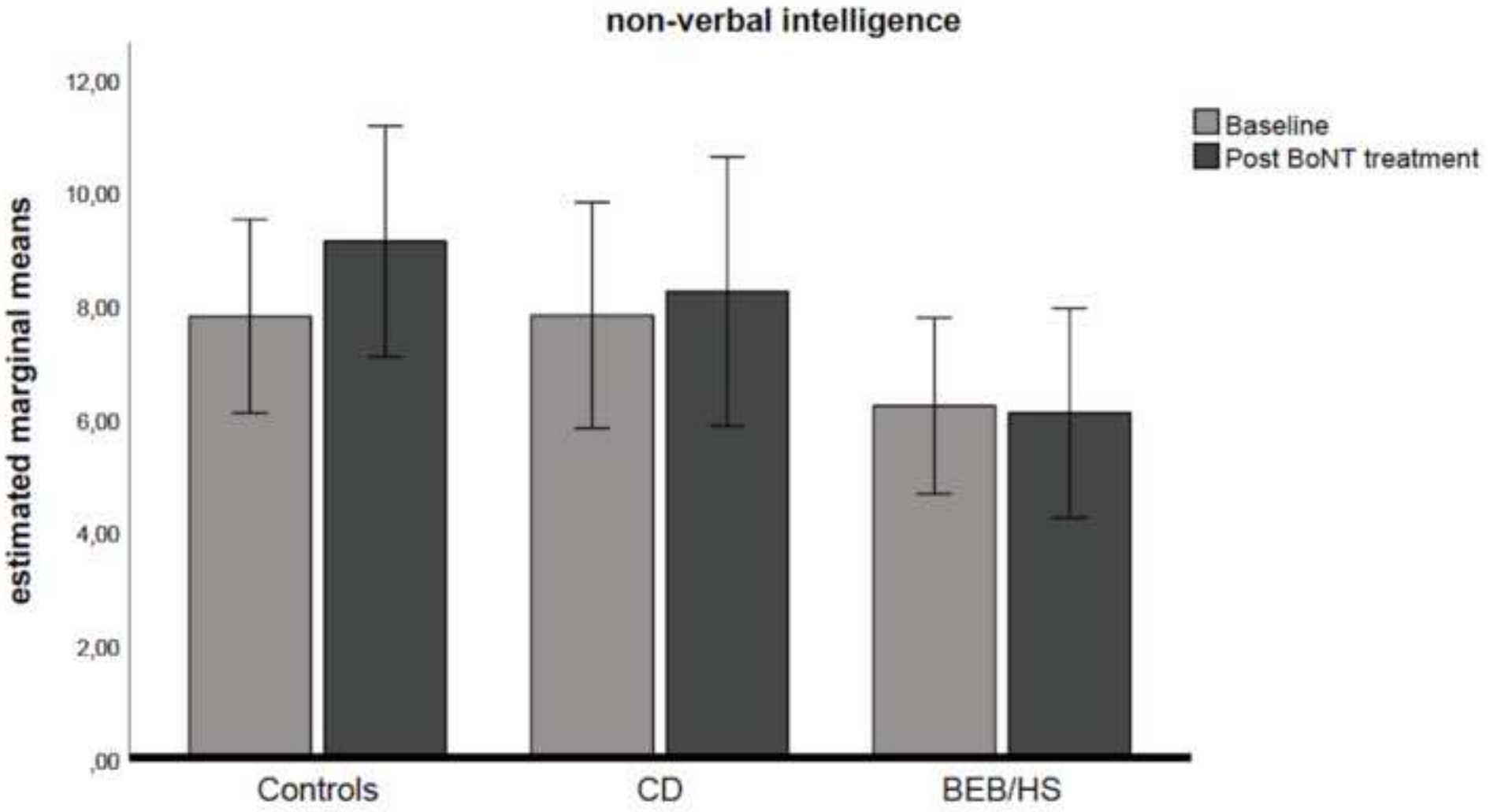

Figure 2

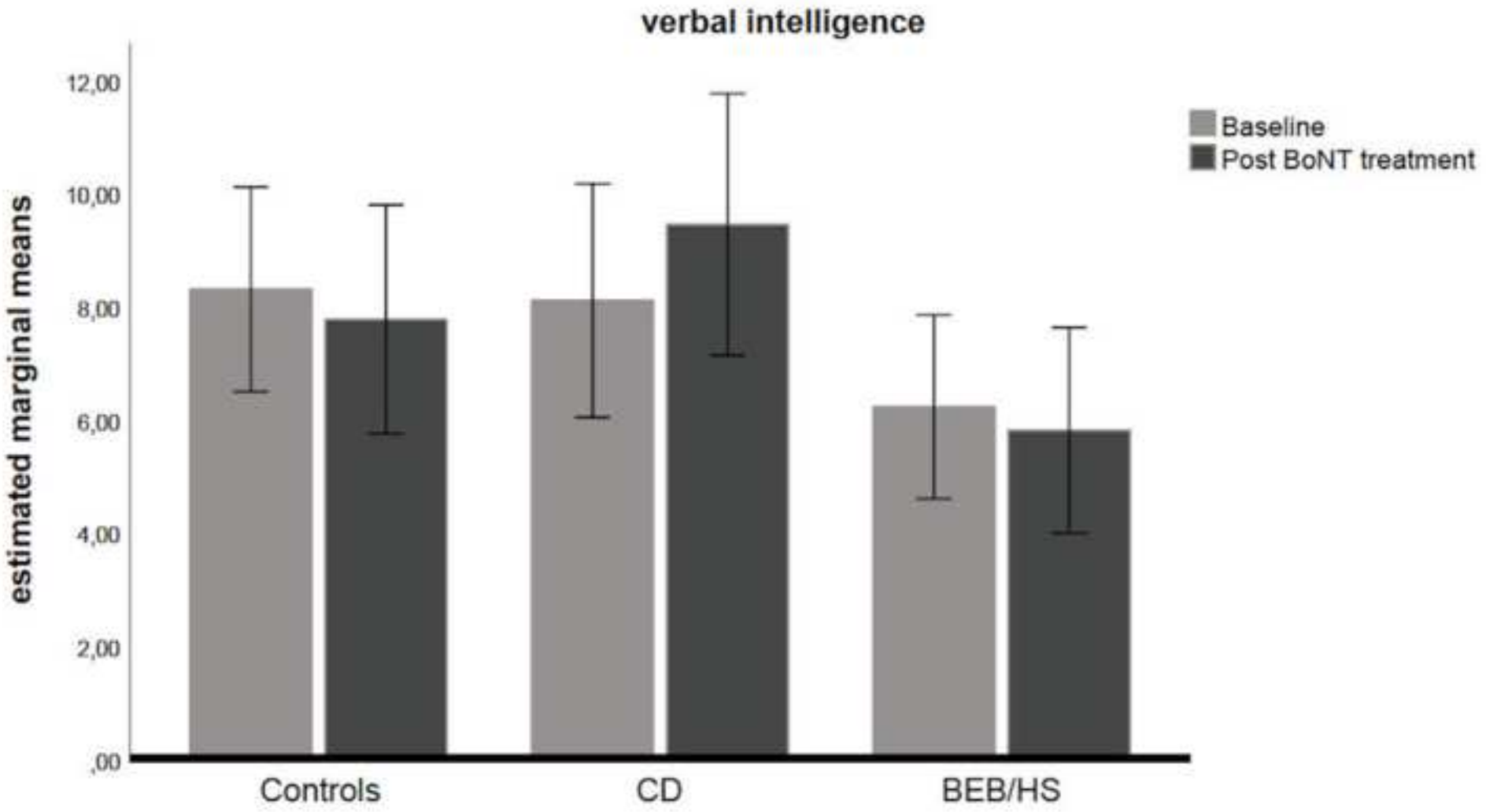

Table 1. List of included muscles

BEB/HS	**CD**
Orbicularis oculi	Sternocleidomastoid
Palpebral part of the orbicularis oculi	Splenius capitis
Corrugator supercilii	Semispinalis capitis
Levator anguli oris	Trapezius
Depressor anguli oris	Levator scapulae
Platysma	

Table 2. Details of drop out

	BEB	HS	CD
Total number of drop out	13/27 (48.1)*	4/11 (36.4)*	20/50 (40)*
Physical handicap	3 (23.1)†	-	1 (5) †
Fear of a bad test performance	1 (7.7) †	-	-
Lack of time	-	-	3 (15) †
Language barrier	1 (7.7) †	-	1 (5) †
No specific reason	8 (61.5) †	2 (50) †	14 (70)†

*Total number of drop out /number of eligible patients (% of number of eligible patients).
†Number of patients (% of total number of drop out)

Table 3. Clinical details of all participants at baseline assessment.

	BEB/HS	**CD**	**HC**
Number of subjects	21 (25.0)*	30 (35.7)*	33 (39.3)*
Age	63.4a (11.9)†	57.1a (12)†	61.3a (11.9)†
Male	7 (33.3)*	14 (46.7)*	11 (33.3)*
Female	14 (66.7)*	16 (53.3)*	22 (66.7)*
BTX treatment duration†	5.8a (5.96)†	8a (6.7)†	-
< 3 years	11 (52.4)*	6 (20)*	
3 to 5 years	7 (33.3)*	11 (36.7)*	
6 to 8 years	1 (4.8)*	2 (6.7)*	
> 8 years	2 (9.5)*	11 (36.7)*	
BTX preparation applied			-
Abo-BTX A‡	7 (33.3)*	19 (63.4)*	
Inco-BTX A§	5 (23.8)*	4 (13.3)*	
Ona-BTX A‖	9 (42.9)*	6 (20.0)*	
Rima-BTX B¶	-	1 (3.3)*	
BTX equivalent dosage	55.2MU (37.2)†	236.5MU (94.3)†	-
< 100	17 (81.0)*	-	
100 to 200	4 (19.0)*	17 (56.7)*	
201 to 300	-	7 (23.3)*	
301 to 400	-	5 (16.7)*	
> 400	-	1 (3.3)*	

*Number of subjects (%), †Mean (SD), ‡Dysport® (Ipsen), §Xeomin® (Merz), ‖Botox® (Allergan), ¶NeuroBloc® (USWorldMed); Abbreviations: a=years, MU=Mouse Units

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	3-4;5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8, Table2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	8-9, Table3
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9-12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14;15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	n.a.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.