An intranasal herbal medicine improves executive functions and activates the underlying neural network in children with autism

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A B S T R A C T

Our animal and human studies have provided empirical evidence that a patented intranasal herbal medicine alters brain functions and neurophysiology. In particular, it reduces clinical symptoms and immunological anomalies in children with autism spectrum disorders (ASD). The present study explored whether the herbal formula can improve executive functions and the associated neuroelectrophysiological activity in ASD. Thirty children with ASD were evenly assigned to receive a daily intranasal administration of the herbal formula or no treatment. Their executive functions, behavioral problems, and electroencephalographic activity during an executive control task were measured before and after six months of treatment with the herbal formula. After treatment, the experimental group showed significantly improved inhibitory control, mental flexibility, and planning, which coincided with an event-related elevation in the activity of their prefrontal and anterior cingulate cortices (regions that are critical for executive control of behaviors) as well as reduced daily dysexecutive behaviors. In contrast, the control group showed no significant changes in executive functions or neural system activity. These findings support the administration of the intranasal herbal medicine as a possible intervention for improving executive functions in ASD.

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1. Introduction

Executive dysfunction refers to difficulties in response inhibition, mental flexibility, self-monitoring, planning, and/or working memory. Such cognitive deficits are common in people with autism spectrum disorders (ASD) (Kenworthy, Yerys, Anthony, & Wallace, 2008; Sanders, Johnson, Garavan, Gill, & Gallagher, 2008). Executive dysfunction is suggested to be accounted for the typical clinical manifestations of ASD, including uncontrollable behavioral and emotional reactions, repetitive behavior, strong need for sameness, restricted interests, and inappropriate social communication and interaction.

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Executive function is an umbrella term that covers a wide range of cognitive abilities, and its deficiency has pervasive effects on everyday functioning, independent living, and care giving in individuals with ASD.

In view of the pervasive impact of executive dysfunctions in ASD, a growing number of studies have investigated potential interventions for enhancing executive functions in ASD. The majority of interventions have been behavior-based (Goin-Kochel, Myers, & Mackintosh, 2007; National Research Council, 2001). Some of these interventions showed positive outcomes but may be relatively intensive and time-consuming (Ospina et al., 2008; Vismara & Rogers, 2010). Furthermore, some of the methods may be hardly applicable or less effective for individuals with limited intellectual functioning or severe autistic symptoms (Kenworthy et al., 2013; Matson & Smith, 2008). Thus, for many of those cases, especially when uncontrollable emotions or behaviors and severe inattention are involved, pharmacological interventions are considered for their beneficial effects on stabilizing mood and improving attention and behavioral problems (Huffman, Sutcliffe, Tanner, & Feldman, 2011). One study estimated that psychotropic drugs were prescribed to approximately 30% of the ASD cohort, and polypharmacy was observed in one-third of the prescribed individuals (Murray et al., 2013). However, there is still no standard medication guideline for treating ASD, and some individuals are refractory to drug treatment or may have difficulty tolerating or communicating about the side effects of medication (Aman, Van Bourgondien, Osborne, & Sarphare, 1997; Frazier et al., 2011).

While pharmacological treatments are predominantly administered peripherally (e.g., oral administration), there is a growing trend in research of exploring the applicability of intranasal drug delivery to treat central nervous system (CNS) dysfunction. Intranasal treatment, which is a non-invasive means of administration, not only allows drugs to bypass the blood-brain barrier but may also increase the bioavailability of the active drug that reaches the brain compared to oral administration (Chapman et al., 2013). Empirical evidence supports the effectiveness of intranasal administration for the treatment of various brain disorders, including benzodiazepines for epilepsy (Fis¸, Al., 2002; Holsti et al., 2010), stem cells for Parkinson’s disease (Bossolasco et al., 2012; Danieleyan et al., 2011), and insulin for dementia of the Alzheimer’s type (Craft et al., 2012; Schiöth, Frey, Brooks, & Benedict, 2012). Recently, a handful of studies have investigated the effectiveness of the intranasal administration of oxytocin to individuals with ASD in reducing repetitive behaviors, increasing social behaviors and emotion processing, and modulating the brain activity involved in face processing. Although some positive results have been reported (Andari et al., 2010; Domes et al., 2013; Guastella et al., 2010; Hollander et al., 2003, 2007), some studies with larger sample sizes and repeated intranasal administrations failed to find any significant difference between oxytocin and placebo (Anagnostou et al., 2012; Dadds et al., 2013).

Despite the inconclusive findings on oxytocin, the intranasal administration method is considered safe and effective (Chapman et al., 2013; Djupesland, 2013; Illum, 2003), and our laboratory has thus studied this delivery route, using an herbal formula as an intervention, to improve brain function. This formula, using natural borneol as one of the active ingredients, was recently patented as a formula for improving brain function and an intervention for brain disorders (patent number ZL 2008 1 0176088.7). In Traditional Chinese Medicine, borneol is a well-known substance for improving the mind and thoughts because of its capacities to direct drugs upward to the head, targeting the brain (Liu, Liang, Chen, Feng, & Zhao, 1994), to facilitate the permeation of drugs across the blood–brain barrier, and to enhance drug distribution in brain tissue (Chen et al., 2010; Yu et al., 2011; Yu, Ruan, Dong, Yu, & Cheng, 2013). In our patent application, we reported that mice receiving seven days of the herbal formula had higher levels (p < 0.001) of norepinephrine in the brain (mean: 46.10 pg/mg of tissue) compared to those receiving saline solution (mean: 22.03 pg/mg of tissue); the mice that received three days of the herbal formula also had higher levels of norepinephrine in the brain (mean: approximately 33 pg/mg of tissue) than did those receiving saline solution (p < 0.001). In sum, the preliminary data suggests that borneol may be an effective agent to improve brain activities.

Additional pilot data from human studies substantiated the effects of the herbal medicine on mice. An experiment on 14 adults with a counterbalanced design showed that the intranasal administration of the herbal formula elicited a significant elevation of electroencephalographic (EEG) activity in the anterior cingulate cortex (ACC) that was localized by low-resolution brain electromagnetic tomography analyses (LORETA), whereas the administration of the saline solution did not result in any change in brain activity (Chan, Cheung, Sze, Leung, & Shi, 2011a). In another double-blinded pilot study, twelve children with ASD were randomly assigned to receive daily intranasal application of the herbal formula or the saline solution for one month. Parents of the children who received the herbal nasal drops reported greater improvement in spontaneous speech output, inhibition of repetitive speech, and initiation of behavior than did the parents whose children applied the saline solution (data published in Chan et al., 2011a). Further empirical support for the therapeutic effect of the herbal nasal drops over a longer term (i.e., for six months) has been indicated in a recent controlled study on children with ASD. Based on parental ratings, significant improvement in social functioning and self-control were found in children given the intranasal treatment but not in the no-treatment control group (data submitted for publication). Interestingly, their reduced problem behaviors were correlated with their altered peripheral blood concentration of specific T lymphocyte subsets, which has been postulated to be a biomarker of the neuropathogenesis of autism and associated with core autistic symptoms (Goines & Van de Water, 2010; Han, Leung, Wong, Lam, Cheung, & Chan, 2011; Han et al., 2013).

Given the emerging evidence for the effectiveness of the herbal nasal drops, the present study aimed to examine the herbal formula as a potential intervention for enhancing executive functions and the associated neuro-electrophysiology of children with ASD. It is anticipated that children in the experimental group will demonstrate a greater improvement in the executive functions that are commonly deficient in ASD, including self-control, flexible thinking, and planning, compared to the control group. Such a treatment-induced improvement in the executive functions is expected to be found in both
cognitive test performance and parental rating in standardized questionnaires. Furthermore, extensive neuroimaging and electrophysiological studies have revealed that a distributed neural network involving the inferior frontal cortex (IFC; BA10/47), dorsolateral prefrontal cortex (DLPFC; BA9/46), pre-supplementary motor area (pSMA; BA6), and ACC (BA24/32) plays a crucial role in mediating the executive control of behavior involving response selection, inhibition, and monitoring (Chambers, Garavan, & Bellgrove, 2009; Kiesel et al., 2010). Cumulative research findings suggest that children and adults with ASD have reduced functional activity in the IFC, DLPFC and ACC compared with normal counterparts, which is associated with the executive control deficits in ASD (Chan et al., 2011d; Kana, Keller, Minshew, & Just, 2007; Philip et al., 2012). Therefore, it is also anticipated that the enhanced executive functions in the experimental group in the present study will be in line with elevated neuro-electrophysiological activity in the prefrontal (PFC) and anterior cingulate cortices during an executive control task as measured using a source localization method (i.e., standardized low-resolution brain electromagnetic tomography analyses, sLORETA).

2. Methods

2.1. Ethics and consent

The study was conducted in accordance with the Helsinki Declaration of the World Medical Association Assembly. The research protocol was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC Ref. No.: CRE-2008-421-T) and the Chinese Clinical Trial Registry (Registration No.: ChiCTR-TRC-12001857). Prior to the study, all children and their parents were briefed on the procedure of the study, and written informed consent was obtained from the parent of each participant.

2.2. Participants

Forty-eight children with ASD, aged between 7 and 17 years old, voluntarily participated in the study. They were recruited from the database of the Neuropsychology Laboratory of the Chinese University of Hong Kong and from two local schools in Hong Kong, through email invitation and a briefing talk conducted at the schools. A clinical psychologist who was blind to the study rationale and group assignment conducted a structured clinical interview of the parents on the developmental and medical history and current functioning of the children; this interview was based on the DSM-IV-TR (American Psychiatric Association, 2000) and the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Couteur, 1994). All but six of the children met the DSM-IV-TR and ADI-R diagnostic criteria of Autistic Disorder, Asperger’s Disorder, or Pervasive Developmental Disorder, Not Otherwise Specified. Six children declined to participate or dropped out due to personal reasons, and six children either with other neurodevelopmental, psychiatric or neurological comorbidities or who had been prescribed psychiatric medication were excluded from the study.

Among the remaining 30 children, 15 children received the six-month intranasal application of the herbal formula and served as the experimental group, while the other 15 children did not receive the treatment and served as the control group. Table 1 shows that the children in the two groups were matched for age, t(28) = 0.29, p = 0.77, gender, χ²(1) = 0.24, p = 0.62, and severity of autistic symptoms as measured by the three ADI-R subscales, with t ranging from −1.43 to 0.74, and p ranging from 0.17 to 0.47. The two groups also demonstrated comparable levels of general intelligence, t(28) = −0.10, p = 0.92.

2.3. Procedures

After obtaining consent from all participants, the baseline assessment was conducted individually on the children and their parents. Trained research assistants assessed the children’s intellectual functioning, executive functions, and EEG activities. During the EEG recording, each child was required to perform a Go/No-Go task while their EEG data were obtained

Table 1
Baseline demographic and clinical characteristics of participants in the control and experimental groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (n = 15)</th>
<th>Experimental group (n = 15)</th>
<th>t/χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD), years</td>
<td>11.60 ± 2.64</td>
<td>11.88 ± 2.70</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>Gender-Male (%)</td>
<td>80.0</td>
<td>86.7</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>IQ (mean ± SD)</td>
<td>85.25 ± 15.98</td>
<td>84.53 ± 23.60</td>
<td>-0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td>2.69</td>
<td>0.26</td>
</tr>
<tr>
<td>Autistic disorder (%)</td>
<td>66.7</td>
<td>40.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDD-NOS (%)</td>
<td>33.3</td>
<td>53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asperger's disorder (%)</td>
<td>0</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity of disorder (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADI-R Social Interaction</td>
<td>19.20 ± 6.73</td>
<td>21.07 ± 7.15</td>
<td>0.74</td>
<td>0.47</td>
</tr>
<tr>
<td>ADI-R Communication</td>
<td>14.20 ± 4.30</td>
<td>11.93 ± 4.42</td>
<td>-1.43</td>
<td>0.17</td>
</tr>
<tr>
<td>ADI-R Stereotyped Behavior</td>
<td>4.73 ± 2.81</td>
<td>3.93 ± 2.91</td>
<td>-0.77</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Notes. ADI-R: Autism Diagnostic Interview-Revised; IQ: intelligence quotient as assessed by the Chinese version of Wechsler Intelligence Scale for Children-Third Edition or the Stanford-Binet Intelligence Scale-Fourth Edition; PDD–NOS: Pervasive Developmental Disorders, Not Otherwise Specified.
using a TruScan measuring set with 19 electrodes positioned across the scalp according to the International 10–20 System (Klem, Lüders, Jasper, & Elger, 1999). With electrode impedances maintained at \( \leq 10 \, k\Omega \), the EEG signals were referenced to linked ears at a 256 sampling rate, with a high-frequency limit band pass of 30 Hz. Meanwhile, their parents were interviewed by a clinical psychologist about the children’s developmental and medical history using a structured clinical interview, and by a trained research assistant about their children’s daily behavioral problems related to executive dysfunction using three standardized questionnaires. The clinical psychologist and research assistants who conducted the assessments were blinded to the rationale of the study and the group assignment.

Following the baseline assessments, the children in the experimental group were instructed to apply the herbal nasal drops at a dose of 10 ml per day, whereas those in the control group did not receive any treatment. After the six-month treatment, the same set of assessments was performed on the children and their parents individually.

### 2.4. Herbal nasal drops

The patented herbal nasal drops (patent number ZL 2008 1 0176088.7) were manufactured by the Hong Kong Institute of Biotechnology Limited, which is wholly controlled by the council of the Chinese University of Hong Kong. The product was manufactured under strict Good Manufacturing Practice (GMP) standards. The product has been tested for heavy metal and toxic elements, microbial examination and pesticides residue. The test results met the product safety guidelines set forth by the Department of Health of the Government of Hong Kong. Its major active ingredients include Borneol and Borax (please refer to the patent record for details). All ingredients in the formula are within the allowed daily dosage as prescribed in the *Chinese Herbal Medicine* published by the Educational Board of the National Drug Administration. Blood samples were collected from each participant in the experimental group at the end of the first and sixth months of intervention to test their liver function and monitor any possible side-effects of the herbal nasal drop. The results of the blood assays indicated that all liver function indices were within normal limits.

### 2.5. Measures

#### 2.5.1. Neuropsychological assessments on executive functions

##### 2.5.1.1. The d2 test of attention (d2) (Brickenkamp & Zillmer, 1998).

The d2 test requires the accurate and speedy cancelation of ‘d’s with two dashes in an array of 14 rows of target and non-target (e.g., ‘d’s without two dashes or ‘p’s) stimuli. A higher concentration performance score and lower total commission errors (i.e., cancelation of non-target stimuli) indicate better response selection and inhibitory control.

##### 2.5.1.2. The second trial of the Children’s Color Trails Test (CCTT) (Williams et al., 1995).

CCTT requires flexibly shifting between colors while connecting numbers from 1 to 15 in ascending order. A shorter completion time and fewer errors (i.e., wrong connection of number or color) indicate more flexible thinking.

##### 2.5.1.3. The Tower of California Test (ToC) (Delis, Kaplan, & Kramer, 1997).

ToC requires planning and formulating efficient strategies to move discs among three vertical pegs to match a target arrangement without rule violation (i.e., no more than one disc can be moved at a time and a larger disc cannot be placed on top of a smaller disc). A higher total achievement score indicates better planning and problem-solving. A lower rule violation ratio (total number of rule violations/total number of items attempted) reflects better inhibitory control.

#### 2.5.2. Parental evaluation of behavioral measures

##### 2.5.2.1. Behavior Rating Inventory of Executive Function (BRIEF) (Gioia, Isquith, Guy, & Kenworthy, 2000).

BRIEF requires parents to rate how often (i.e., “never”, “sometimes” or “often”) their child exhibits dysexecutive behaviors that cause difficulty in daily functioning. The Global Executive Composite was computed as an overall measure of executive functions, with higher scores indicating poorer executive functioning. The Metacognition Index was adopted to reflect the ability to plan and organize materials, as well as working memory and problem-solving. Additionally, the Behavioral Regulation Index was adopted to measure the ability to control emotions and behaviors and to shift the mental set.

##### 2.5.2.2. Hyperactivity Subscales of the Children’s Psychiatric Rating Scale (CPRS) (Fish, 1985) and Conners’ Rating Scales-Revised (CRS-R) (Conners, 1997).

These scales measure the degree of inhibitory control over behavior and emotion. The CPRS rates four self-control aspects on a scale from 0 to 6, and the CRS-R rates 10 disinhibition-related items on a scale from 0 to 3. The total subscale scores were computed separately for comparison, with higher scores indicating poorer inhibitory control.

#### 2.5.3. Event-related EEG assessment

The event-related EEG signals from each child were collected while they were performing the Go/No-Go task, a computerized executive control test that measures the ability to flexibly select and execute appropriate response to changing stimuli and to inhibit unwanted responses. Details of the Go/No-Go task have been described in two previous
studies (Chan, Sze, Han, & Cheung, 2012a; Chan, Han, et al., 2011). The EEG epochs during the No-Go condition were extracted for analysis using MatLab 7.1, because this condition entails both the executive control processes of selecting to withhold a response and the process of actually inhibiting a response. The epoch limit was set as 50 ms at the start and 900 ms at the end. Artifacts in the epoched data were then pruned by visual inspection and using the rejection method on the EEG Plot. The selected data were exported and then spectrally processed via fast Fourier transformation to compute the power data for the theta (4–7.5 Hz) frequency band using the NeuroGuide software. The theta band was selected because the ACC (one of our ROIs) is a major source generator of neural activity in the theta band (Asada, Fukuda, Tsunoda, Yamaguchi, & Tonoike, 1999) and because brain oscillation at this bandwidth has repeatedly been associated with attention and inhibitory control processes (Chan et al., 2011d; Hermens et al., 2005).

The selected fast Fourier transformed data were analyzed using sLORETA. The sLORETA method is a properly standardized inverse solution that solves the problem of computing the three-dimensional cortical distribution of the electric neuronal source activity from the scalp–EEG measurements to yield images of standardized current density with exact and zero-error localization (Pascual-Marqui, 2002). The sLORETA solution space consists of 6239 voxels (voxel size: 5 mm × 5 mm × 5 mm) and is restricted to cortical gray matter and the hippocampi, as defined by the digitized Montreal Neurological Institute (MNI) 152 template (Fuchs, Kastner, Wagner, Hawes, & Ebersole, 2002). Scalp electrode coordinates on the MNI brain are derived from the international 5% system (Jurcak, Tsuzuki, & Dan, 2007). A number of recent studies have validated the reliability of sLORETA and its applicability and sensitivity in experimental and clinical research (De Ridder, Vanneste, Kovacs, Sunaert, & Dom, 2011; Dumpelmann, Ball, & Schulze-Bonhage, 2012). In the present study, sLORETA was used to localize the source of scalp-EEG activity in terms of the current density in the ROIs, including the IFC (BA10/47), DLPFC (BA9/46), pSM (BA6), and ACC (BA24/32) during the No-Go condition, and the built-in paired t statistics were used to compare the pre-post difference in current density in the ROIs for each group of participants.

2.6. Data analyses

Repeated measures ANOVA followed by post hoc paired t tests were performed to compare the pre-post scores on various neuropsychological measures of executive functions and parental ratings of problem behavior for each group of children. The analyses were conducted using the SPSS software. Given that specific hypotheses were tested, the one-tailed paired t test was adopted, and no adjustment to the alpha level was applied to avoid reducing the power of the tests. The effect sizes and confidence intervals were used to evaluate the extent of pre-post changes for each group. Furthermore, sLORETA analyses using voxel-by-voxel paired-sample t tests for each group of children were performed to compare the pre-post changes in theta current density level in the ROIs during the No-Go condition.

3. Results

3.1. Intranasal herbal medicine improved executive functions

The baseline level of executive functioning was comparable between the experimental and control groups, as measured by the three neuropsychological tests, t range = –1.78 to 2.07, p > 0.05. Repeated measures ANOVAs were used to compare the pre-post performance between groups, with time (pre vs. post) as the within-subjects factor and group (control vs. experimental) as the between-subjects factor. A significant time by group interaction was found for most measures; F range = 4.25–8.07, p < 0.05. All tests showed a significant main effect of time; F range = 5.07–17.13, p < 0.05.

The results of the post hoc paired samples t tests showed that the children in the experimental group demonstrated a significant improvement in all three aspects of executive functions, including response selection and inhibition, mental flexibility, and planning abilities, t range = –5.07 to 2.72, p < 0.05, with medium to large effect sizes from 0.59 to 1.35 (Table 2). These data suggest that after intranasal treatment, children became more flexible in problem solving, more attentive to relevant information and more capable of withholding inappropriate response. The same analyses were repeated for a subgroup of low-functioning children (n = 6), revealing significant improvements across various executive function measures, with t ranges from 2.86 to –4.45, p < 0.05, and suggesting that even low-functioning children with ASD were able to benefit from the herbal treatment.

In contrast, children in the control group showed less improvement. Although the control group demonstrated some improvement in two test measures (completion time in CCTT and total achievement score to ToC; t = 2.39 and –2.20, effect size = 0.64 and 0.59, respectively, p < 0.05), the extent of the improvement observed in the experimental group was four times greater than that observed in the control group (CCTT: –33.24 in the experimental group vs. –7.29 in the control group; ToC: 4.38 in the experimental group vs. 1.36 in the control group) and the differences were statistically significant, t = –2.06 and 2.18, respectively, p < 0.05.

In summary, the children who received the herbal formula demonstrated significant improvements in inhibitory control, flexibility, and planning compared with their control counterparts. These findings indicate that the intranasal application of the herbal formula may have some positive effects on the executive functions of children with ASD.
Table 2
Mean performance in executive functioning of the control and experimental groups at pre- and post-six-month period.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 15)</th>
<th>Experimental group (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>E.S.</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td></td>
</tr>
<tr>
<td>Response selection and inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d2: concentration performance</td>
<td>131.07 (45.96)</td>
<td>0.46</td>
</tr>
<tr>
<td>d2: commission*</td>
<td>9.43 (17.91)</td>
<td>0.10</td>
</tr>
<tr>
<td>ToC: RVR*</td>
<td>1.01 (0.97)</td>
<td>0.47</td>
</tr>
<tr>
<td>Mental flexibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCTT: error</td>
<td>0.50 (0.76)</td>
<td>0.27</td>
</tr>
<tr>
<td>CCTT: time (in seconds)**</td>
<td>52.00 (21.00)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Planning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToC: TAS</td>
<td>9.00 (4.66)</td>
<td>10.36 (5.43)</td>
</tr>
</tbody>
</table>

Note. d2 – the d2 Test of Attention; CCTT: error – total errors in Trial 2 of the Children’s Color Trails Test; CCTT: time – total completion time in Trial 2 of the CCTT; ToC: RVR – rule violation ratio of the Tower of California Test; ToC: TAS – total achievement score of the ToC. E.S. – effect size; C.I. – upper and lower limits of the 95% confidence interval of the difference. Standard deviations are in parenthesis.

* Lower value indicates better performance.
** p < 0.05.
*** p < 0.01.
* Medium effect size.
** Large effect size.

Fig. 1. Activation of the neural network after intranasal administration. Neuro-electrophysiological activity changes at the end of the six-month intervention as indicated by the graphical representation of the sLORETA paired t-statistics results. The experimental group demonstrated significantly elevated theta current density (shown in red) in the anterior cingulate cortex, inferior frontal cortex, dorsolateral prefrontal cortex, and pre-supplementary motor area during the No-Go condition after the six-month intervention (max. t = 9.4, p < 0.05), whereas the control group did not show any significant change. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

3.2. Intranasal herbal medicine enhanced prefrontal and anterior cingulate cortices activity

The neural mechanism underlying the enhancement in executive control of behaviors was examined by localizing EEG theta source activities in the ROIs (IFC: BA10/47; DLPFC: BA9/46; pSMA: BA6; ACC: BA24/32) during a Go/No-Go task using sLORETA voxel-by-voxel paired t statistics for each group. After six months of treatment, the experimental group showed significantly elevated theta current density power across all ROIs during the No-Go condition, t maximum = 9.4, p < 0.05 (lower image in Fig. 1). In contrast, the control group did not show a significant change in current density at any coordinate within the ROIs after six months, t maximum = 1.65, p > 0.05 (upper image in Fig. 1). Table 3 presents the corresponding
number of voxels with a significantly increased current density and their distribution across different ROIs. In the experimental group, a total of 509 voxels within the ROIs showed above-threshold increments in the current density power after intervention. An inspection of the distribution of significant voxels in each hemisphere revealed that the ACC was more bilaterally involved, and that the IFC, DLPFC, and pSMA were more right-lateralized. As revealed by the maximum t value, the IFC (BA10/47) and ACC (BA32) showed the greatest pre-post difference. However, in the control group, there was no significant change in the current density power for any voxel within the ROIs.

The present results are consistent with our previous findings of a significantly increased ACC current density after the application of the herbal nasal drops (Chan et al., 2011a). Furthermore, the elevated current density in the IFC, DLPFC, and sPMR, which coincided with the enhanced ACC current density, is consistent with the distributed functional network that has been proposed to mediate response selection and inhibition (Chambers et al., 2010; Kiesel et al., 2010). Such a treatment-specific alteration in neural oscillation within the anterior and medial brain regions provides a possible explanation for the enhanced executive functions in the experimental group.

3.3. Intranasal herbal medicine improved daily executive behaviors

In light of the subjects’ positive changes in executive function test performance and EEG activity after herbal nasal administration, it would be important to investigate whether the improvements observed under experimental conditions could be generalized to everyday behaviors. Therefore, a further pre-post comparison was performed on the parents’ evaluations of their child’s executive behaviors in everyday life based on three standardized questionnaires.

While there was no between-group difference in parental ratings before the intervention, t range = –0.24 to 0.16, p > 0.05, after the six-month intervention, parents of children in the experimental group, but not those in the control group, reported fewer behavioral problems related to executive dysfunctions. Repeated measures ANOVAs showed a marginally significant group by time interaction effect on the Hyperactivity subscale of the CRS-R, F = 3.97, p = 0.06, and a significant or marginally significant main effect of time on the Hyperactivity subscale of the CPRS, F = 7.55, p = 0.01, the Global Executive Composite and Metacognition Index of the BRIEF, F = 3.14 and 4.04, p = 0.09 and 0.05, respectively. Post hoc paired t tests revealed that the experimental group reported reduced behavioral problems related to overall executive functions, response inhibition and flexibility, and planning, t = 1.29–2.71, p = 0.01–0.03, with medium effect sizes (0.54–0.70) (Table 4). In contrast, the control group did not show such improvements after six months, t = –0.56 to 1.12, p = 0.14–0.40, with small effect sizes (0.07–0.30). The maximal extent of improvement in the experimental group was 39%, which is more than a double that of the control group, 15%. These findings suggest that the therapeutic effect of the herbal nasal administration can be generalized to daily executive behaviors, implying that the herbal nasal drops could be an intervention for executive dysfunction in ASD.

4. Discussion

The study revealed enhancements in both the experimental test results and the real-life behaviors related to executive functions in children with ASD after the intranasal application of the herbal medicine for six months. Given that the control group, which did not receive the herbal treatment, did not show as much improvement in executive functions, the positive outcomes found in the experimental group could be attributed to the herbal nasal drops, rather than natural developmental growth. The therapeutic effects of the herbal formula are consistent with our pilot data, which revealed a greater improvement in some frontal lobe functions, including initiation and inhibition of speech and actions, and some pro-social
behaviors after one month (data published in Chan et al., 2011a) and six months of treatment (data submitted for publication). Although the specific mechanism for the herbal formula’s effectiveness remains unknown, it could be related to the medical principle underlying the development of the herbal formula. The formula was developed using a basic principle, “healing by opening the bodily orifices,” of the traditional Shaolin Chan-based medicine, namely Dejian Mind–Body Intervention (DBMI). In the DBMI model, the intranasal application of the herbal formula is one of the methods to open the orifices, while diet modification and practicing Nei Gong (a form of mind–body exercise) are the others (can refer to Chan, 2010, 2013 for more details). Because they are governed by the same principle, treatments applying the other methods have shown similar positive outcomes (Chan, Sze, & Shi, 2008; Chan, Sze, Cheung, Lam, & Shi, 2009; Chan, Cheung, Sze, Leung, & Shi, 2011b; Chan, Cheung, Tsui, Sze, & Shi, 2011c; Chan, Sze, Cheung, Han, & Leung, 2011e; Chan, Sze, Han, & Cheung, 2012a; Chan et al., 2012b, 2012c; Chan, Han, Sze, Wong, & Cheung, 2013; Chan, Sze, Siu, Lau, & Cheung, 2013; Chan, Han, & Cheung, 2014; Chan, Sze, Woo, & Yu, 2014; Yu, Woo, Chan, & Sze, in press). However, the extent of improvement in the present study using herbal nasal drops is greater than those observed in the studies using other methods. Specifically, the present results showed a consistent 40–68% enhancement in inhibition, flexibility, and planning, whereas the previous findings showed a 17–42% improvement on the same test measures (Chan et al., 2012a, 2013b). Such a greater degree of treatment efficacy in the present study could be due to a longer treatment period (i.e., six months vs. one to two months), and/or the specific robust effect of the herbal nasal drops that have the capacity to trigger neurophysiological alterations through the direct nose–brain pathway.

Another important finding was the treatment-specific alteration in electrophysiological activity of a neural network that mediates the executive control of behaviors. The present study replicated the immediate effect of intranasal application of the herbal nasal drops that was observed previously in a group of normal adults, specifically an increase in EEG theta oscillation localized to the ACC and theta cordance (an index correlated with cerebral perfusion) in the frontal brain region (Chan et al., 2011a). It should be noted that the extent of elevation in brain activity in the present study is much greater than that observed in a previous study on normal individuals, e.g., the maximum t values in ACC activity were 2–3 times higher in the present study (t = 4.79 and 7.79) compared to those in the previous study (t = 2.65). The greater effect in the present study is reasonable because it involves patients with ASD (who are known to have functional abnormalities in ACC, and hence greater room for enhancement) and a longer treatment duration (i.e., six months compared to the single dose in the previous study). Cumulative empirical evidence has suggested that suppressed activity in the prefrontal and/or anterior cingulate cortices in individuals with ASD is associated with an impairment of the executive functions that manifests in daily life, including emotional processing, inhibitory control, and stimulus-response monitoring (Chan et al., 2011d; Kana et al., 2007; Philip et al., 2012). Particularly, the IFC in the right hemisphere has been suggested to be a key region for response inhibition in normal individuals (Aron, Robbins, & Poldrack, 2004) and has repeatedly been reported to show abnormal activation and reduced functional connectivity with other inhibition-related brain regions in the ASD population (Kana et al., 2007; Lee et al., 2009). In our present study, children who applied the herbal nasal drops demonstrated the greatest degree of post-intervention elevation in the current density at the IFC. The greatest degree of change in the IFC is reflected by the highest t value which indicates the most significant pre-post difference and a three to four times greater number of voxels with significant differences at the IFC, compared to the results obtained for the ACC, DLPFC and sPMR. The present findings suggest the possibility of applying the herbal nasal drops as a complementary treatment for patients with functional abnormalities in the PFC and ACC.

The potential benefits of the herbal nasal drops are consistent with the treatment effects of some conventional behavioral interventions and with oxytocin treatment for enhancing executive functions and related behavioral and emotional problems in ASD (Andari et al., 2010; Guastella et al., 2010; Osypka et al., 2008; Vismara & Rogers, 2010). In view of the limited therapeutic effects of conventional behavioral therapy for low-functioning children and that of oxytocin (previous
studies have reported a performance improvement of approximately 10%), the effects of the herbal nasal drops for children with ASD reported in this study may shed some light on the possibility of applying the nasal drops as a complementary intervention for individuals with ASD, specifically those with limited intelligence or more severe autistic symptoms.

Despite the encouraging results of the present study, the sample size is relatively small. Further studies with larger sample size are warranted to investigate whether the magnitude of the therapeutic effects of the herbal nasal drops can be replicated. Additionally, it is worth conducting randomized controlled trials on the herbal formula with placebo treatment as a control to rule out a possible placebo effect underlying the positive treatment outcomes. Given that executive function deficits are common in patients with various brain disorders (e.g., attention-deficit/hyperactivity disorder, traumatic brain injury, dementia), it may be worthwhile to extend our investigation of this intervention to other clinical populations. In light of our clinical observations and pilot data on the potential benefits of the herbal nasal drops to children with more severe autistic symptoms and/or mental retardation, a treatment study with those specific populations with larger samples will also be considered.

5. Conclusion

This study provides support for the long-term therapeutic effects of a patented herbal formula on a wide range of executive functions that are measured by neuropsychological tests and daily behavioral checklists, which are in line with the increased current density power in the ACC and PFC. Such encouraging findings provide added support for the effectiveness of the herbal formula for ASD and shed some light on its potential clinical applicability for improving executive functions and altering the neural processing patterns of people suffering from frontal lobe dysfunctions.

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