

Revisit the Effectiveness of Educational Kinesiology on Stress and Anxiety Amelioration in Kindergarteners With Special Needs: A Nonrandomized Trial

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Abstract

Although educational kinesiology is a popular intervention aims to improve brain functioning via physical movements, it lacks supporting scientific evidence. This study explores the effect of educational kinesiology on the changes in stress and anxiety markers in kindergarteners with special needs using psychometrics and biological measures. This open label non-randomized clinical trial was registered retrospectively in the Chinese Clinical Trial Registry (registration number: ChiCTR2000036305, url: <http://www.chictr.org.cn/showproj.aspx?proj=58067>, registration date: 22/08/2020). Thirty-seven kindergarteners with special needs (3.5-6.5 years old) were assigned to either the intervention group, which received one-hour educational kinesiology intervention weekly for a total of 10 weeks, or the wait-list control group. Scores of Parent-rated Preschool Anxiety Scale (PAS-TC), salivary cortisol and oxytocin levels were obtained pre- and post-intervention. After controlling baseline, the changes in oxytocin levels remained significantly different between groups ($F_{1,35} = 5.590, p = 0.020, \eta^2 = 0.145$), but not in cortisol levels ($F_{1,35} = 0.364, p = 0.550, \eta^2 = 0.01$). PAS-TC showed significant improvement in anxiety levels after the intervention in the intervention group ($X^2 = 4.367, p = 0.037, \phi = 0.344, p = 0.037$). Findings from both subjective and objective measures indicate a plausible anti-stress and anxiety effect in kindergarteners with special needs.

Introduction

Depression and anxiety are common mental health problems that significantly affect children in the modern society. In western countries, up to 14% of preschool-age children were found to have clinical levels of depression and anxiety^{1,2}. In a study involved over 1300 primary school children in Hong Kong, about 10% were reported to have major depressive disorders³. From 2012 to 2013, The Child Assessment Service (CAS) of the Department of Health of Hong Kong diagnosed 570 new cases with anxiety disorders / problems, which accounted for 3% of the total referral cases in the Department⁴. A more recent study showed the prevalence rate of children with anxiety disorders in Hong Kong was 27.5%⁵. The prevalence and incidence of having depression and / or anxiety are expected to be even higher in children with learning difficulties and / or neurodevelopmental disorders, given they encounter more daily challenges at learning and living. For instance, the numbers of children with Attention-Deficit and/or Hyperactivity Disorder (ADHD) who are comorbid with another mental disorder were doubled compared to typically developing children⁵. Given the appearance of depression and anxiety symptoms in early childhood were associated with later negative outcomes in middle childhood⁶, it is essential to offer early interventions to children who are at high risk of mental illness, i.e. children with learning difficulties and / or neurodevelopmental disorders. Educational kinesiology, also called Brain Gym, is one potential treatment to tackle this problem. From here after, educational kinesiology will be referred to as Brain Gym.

Paul Dennison and Gail Dennison developed Brain Gym in 1969 as one of the potential treatment/interventions for children⁷. Due to the simplicity and easy-to-follow activities, it is popular among educators and is a training scheme that is particularly suitable for managing stress-induced problems including anxiety. Brain Gym emphasized on facilitating whole-brain learning through different movements, which is a bottom-up approach⁸. In this approach, Dennison and Dennison proposed that the change of body movement could result in the alteration of brain functions⁸. The theoretical framework behind Brain Gym is that due to the imbalanced body-and-mind coordination could hinder an individual to learn⁸. Therefore, Dennison and Dennison developed 26 simple body movements (e.g. Cross Crawl, the Think of an X, the Lazy Eights and the Neck Rolls, etc.) to link up the body with specific brain functions for reducing this imbalance⁸. According to Dennison and Dennison⁸, these movements can be categorized into three dimensions, they are: Laterality, Focus, and Centering. Each of these three dimensions are proposed to target different brain areas and different functioning. For instance, movements in *laterality* dimension enhance the coordination between the left and right hemispheres' functioning, which helps to facilitate one's ability in reading, writing, listening, speaking, and the ability in synchronizing movement and thinking; movements in *focus* dimension synchronize the anterior and posterior portions of the brain, which facilitate comprehension skills, and improve attention; movements in *centering* dimension coordinates the dorsal and ventral parts of the brain, which helps balance rational thoughts and emotions⁸.

Brain Gym is especially popular among teachers and educators. Stephenson⁹ performed an internet search study using terms 'Braingym' or 'Brain Gym', and 'School' and found 4,290 website hits. The first 200 sites were visited to check if they are targeting teachers and educators in Australia as audiences. Thirty of which were found explicitly promoting Brain Gym to teachers and educators. All of them included some levels of support to teachers and educators if Brain Gym was chosen to be used in school, and most of them recommended Brain Gym as a form of professional development.

In the past decades, different studies attempted to examine the effectiveness of Brain Gym exercises in various aspects. For instance, a study showed a positive effect of Brain Gym on learning to play musical instruments in a small group of college students⁷. Another study demonstrated positive effects of Brain Gym on reading performance, maths performance, maladaptive behaviors, and adaptive behaviors in 30 at-risk primary school students¹⁰. Likewise, results from another recent study showed that Brain Gym could improve academic performance of children aged 10 – 12 years old¹¹. These studies suggest some positive effects of Brain Gym on learning across different age groups. Nevertheless, scientific evidence that supports the claimed beneficial effects of Brain Gym remains limited, making it hardly become an evidence-based practice.

There is limited research exploring the effectiveness of Brain Gym on the improvements of mental health, and findings from the previous studies were inconsistent. For instance, Azizah and colleagues¹² showed an improvement in perceived levels of psychological distress in elderly people after Brain Gym intervention. Effendy and colleagues¹³ also examined the effects of Brain Gym on anxiety and quality of sleep in 68 elderly people and they found that 8-week of Brain Gym training could improve sleeping quality and reduce anxiety symptoms in the studied cohort. On the other hand, Voss¹⁴ found no effect of Brain Gym on the level of perceived stress in a group of school-age children. It is therefore necessary to readdress the effectiveness of Brain Gym on mental health.

One approach to assess psychological stress is by measuring the level of stress biomarkers in saliva samples, which provides accurate objective data in scientific studies. The use of stress biomarkers can avoid bias generated by the assessor and reporter in the commonly used self-reported method. This approach is well adopted in psychological and clinical studies because of its non-bias and non-invasive nature. Among various stress markers, cortisol and oxytocin are most studied in stress science.

Cortisol

Cortisol is a stress hormone regulated by the hypothalamic-pituitary axis (HPA), which is one of the main components in the stress system¹⁵. A meta-analysis in 2004 reviewed 208 laboratory results showing that psychological stressors increased cortisol levels, especially when the task involved uncontrollability and social evaluation¹⁶. Furthermore, multiple studies' findings support that salivary cortisol is a useful measure of the stress response that can be used to evaluate intervention effectiveness^{17,18,19}. Being widely considered as a consistent measure of stress, studies also examined the change in salivary cortisol in preschoolers in response to stress tasks^{20,21}, which further supports salivary cortisol to be a validated biomarker in measuring stress in children.

Oxytocin

Oxytocin is a neuropeptide produced in the hypothalamus. The primary biological function of oxytocin is for uterine contractions during childbirth and is necessary to produce milk^{22,23}. In addition, oxytocin is regarded as a love hormone due to its effects on social cognition and prosocial behaviors^{24,25,26,27}. More recent research revealed the association between oxytocin and mental health problems. For instance, high oxytocin levels were reported to associate with low anxiety levels in children and adolescents^{28,29}. In agreement, urinary oxytocin levels were found to decrease during a social stress task in children and adolescents³⁰. In clinical subjects with anxiety disorder, salivary oxytocin levels were negatively correlated with anxiety symptoms, suggesting a role of oxytocin in regulating anxiety³¹.

There are currently no studies that investigate the efficacy of Brain Gym exercises on psychological well-being in children with special learning needs by using the combination of psychometrics and physiological approach. This study aims to examine the changes in parent-rated anxiety level and salivary stress markers i.e. cortisol and oxytocin after Brain Gym exercises in kindergarteners with learning difficulties or neurodevelopmental disorders. Results from this study could provide scientific evidence on the effectiveness of Brain Gym in ameliorating emotional instability and its underlying physiology in kindergarteners with special needs.

Hypotheses

In this study, the following hypotheses were made.

Hypothesis 1: The Brain Gym intervention would reduce the levels of salivary cortisol.

Hypothesis 2: The Brain Gym intervention would increase the levels of salivary oxytocin.

Hypothesis 3: The Brain Gym intervention would reduce the anxiety levels of the participants.

Methodology

Participants

Thirty-seven subjects with special needs (age range = 3.7-6.6 years; 26 males) including confirmed cases of autism spectrum disorder (ASD), ADHD, developmental delay, specific learning difficulty, and at-risk cases of ASD, ADHD, conduct disorder, emotional disorders, muscle development deficits, speech delay, and developmental delay (see Table 1). They were recruited from five local mainstream nursery schools during January 7th, 2019 to January 23rd, 2019 in Hong Kong. Twenty-five quota was set for each group in this pilot study. The final sample size was determined by the number of referred students from the five targeted kindergartens. Each kindergarten served as a blocking factor in the study. Participants' age was matched as much as possible between the two groups to minimize the influence of the effect of age. Kindergarteners who were 1. participating in any intervention program that aims to reduce stress and anxiety; 2. failing to follow instructions; 3. diagnosed with different physical disabilities (e.g. deafness, upper / lower limbs dysfunction, blindness, etc), and 4. having a history of brain injury during and/or prior to our study period would be not be included.

Study Design

This open label non-randomized clinical trial was registered in the Chinese Clinical Trial Registry (registration number: ChiCTR2000036305, url: <http://www.chictr.org.cn/showproj.aspx?proj=58067>, registration date: 22/08/2020) after the completion of the study. No potential adverse effects were anticipated in participating in Brain Gym. A quasi-experimental design was adopted because of practical constraints i.e. time clash with the school scheduled activities. Subject recruitment was done by Hong Kong Sheng Kung Hui Welfare Council Limited. Five kindergartens registered to the programme. The kindergartens helped promote this study and recruit interested families. Participants in three kindergartens that could meet the training schedule were assigned to the experimental group. Participants in the other two kindergartens were in the wait-list control group and were provided with the same intervention after the completion of the project. Participants in both groups shared the same interest in attending Brain Gym intervention. The 10-week Brain Gym intervention program was carried out by the same service provider. Pre-intervention assessments were carried out by the research team within two weeks before the start of the intervention program or the waiting period, which included a parent-rated questionnaire and saliva samples collection. Post-intervention assessments were carried out within two weeks after the end of the last section of the intervention or the waiting period. All assessments were done in a quiet room at the target schools. One-to-one individual assessments were performed. Stickers were given to the participants at the end of each intervention session and after pre- and post-assessment to motivate their engagement. Written consent was obtained from the schools and parents of the participants prior to the experiment. Procedures in this study were approved by the Human Research Ethics Committee at The Education University of Hong Kong (Ref no. 2018-2019-0038). The procedure followed the national checklist of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) statement³⁶.

Brain Gym Intervention

The Brain Gym intervention was carried out by a qualified trainer who had obtained the certificate to practice Brain Gym. The intervention included 8 group-based training sections (3-4 students per group) and 2 family-based training sections for educating the participants' parents the theoretical basis of Brain Gym and guiding them how to practice Brain Gym with their children at home. This added up to 10 sections in total, one session per week, 60 minutes for each section. The intervention was carried out after classes at the kindergartens during January 18th, 2019 to September 24th, 2019. The rundown of the group-based training section is shown in Table 2.

Primary Outcome Measures: Salivary Cortisol and Oxytocin Levels

Saliva (1mL) was collected by instructing the participant to spit out saliva directly into a measuring tube at 2-4pm of the assessment days to avoid influence from circadian rhythm on cortisol levels^{37,38}. Collected saliva samples were processed on the same day and stored at -80°C until further analysis. Cortisol and oxytocin levels were measured in saliva samples using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Enzo Life Sciences (Farmingdale, NY, USA) in duplicate per sample, according to the manufacturer's instructions. The detection range of the cortisol and oxytocin kits was 156–10,000pg/ml and 15.6 – 1,000pg/ml, respectively. To avoid inter-assay variation, the pre- and post-samples of the same participant were analysed in the same ELISA kits.

Secondary Outcome Measures: Parent-rated Preschool Anxiety Scale: Traditional Chinese Version (PAS-TC)

Kindergarteners' anxiety level was measured by PAS-TC³⁹. It is a parent-rated questionnaire distributed and collected by the kindergartens. It has 28 items and is rated on a 4-point Likert scale (1 = never to 4 = always)⁴⁰. The total score reflects an overall state of

anxiety; the higher the scores mean the more severe the anxiety symptoms. A score equal to or higher than 34 is considered as showing elevated levels of anxiety symptoms. The Cronbach alpha for the total score in our samples was 0.875, indicating good internal consistency.

Data Analysis

All participants provided data in the pre- and post-intervention time point. We encountered no missing data; thus, all data were used. Non-parametric tests were used for data analyses at group level in this pilot study because of the small sample size. Between-group differences at baseline were examined by Mann-Whitney U test. To test our hypotheses 1 and 2, changes in salivary cortisol and oxytocin levels were calculated by subtracting the pre- from the post-data and the between-group differences in the changed values were analysed by Mann-Whitney U test. Effect sizes (r) were calculated for each Mann-Whitney U test performed⁴¹. In addition, Quade tests were used to examine the between-group differences in the changes in cortisol and oxytocin levels with the effect of their baseline values being controlled⁴². Briefly, the baseline cortisol and oxytocin levels as well as their changed levels were ranked. The ranked baseline levels of cortisol or oxytocin were set as the regressor to predict the ranked changes in cortisol or oxytocin, respectively, using linear regression models. The residuals generated from the linear regression models were then used as the dependent variables in one-way analysis of variance (ANOVA), while group was set as the independent variable. Eta-squared effect sizes were calculated for every Quade's test performed⁴¹. For PAS-TC, those who had a score below the cut-off (score = 34) would be coded as 0, indicating normal in anxiety levels; those who had a score equal to or above the cut-off would be coded as 1, indicating an elevation of anxiety levels. The frequencies of 0 and 1 were counted in each group at baseline and post-intervention. To test our hypothesis 3, the ratio of normal to elevated anxiety subjects was compared across groups at both baseline and post-intervention using Chi-square statistics. To calculate the change in an anxiety state, a second level of coding was performed base on the change of scores of PAS-TC i.e. post-data minus the pre-data for each participant. Participants who had an improvement in an anxiety state (e.g. the change of scores < 0) were coded as 1; participants who had no changes or even deterioration in the anxiety state (e.g. the change of scores \geq 0) were coded as 0. Chi-square statistics were used to test the between-group difference in the ratio between improvement to deterioration or no changes. Phi values were calculated for the effect size. To ensure the Chi-square test results were relevant, each test was checked for no more than 20% of cells with expected frequencies < 5 and no cells have expected frequency < 1⁴³. Statistical analyses were done using SPSS (IBM, version 24.0). A p -value less than 0.05 is considered statistically significant.

Results

The descriptive statistics of the participants are summarized in Table 1. Sixteen participants joined the intervention group, while the remaining twenty-one participants joined the control group. The Consolidated Standards of Reporting Trials (CONSORT) flow diagram³² illustrates the flow of participants from the enrollment to the post-assessment, and the number of participants' data analysed (Figure 1). All 37 of the participants agreed to participate once they were informed with the study details, and all completed all stages of this experiment. Follow-ups were not performed for this pilot study. There was no deviation of the training protocol between planning to execution. No clinical data were retrieved from the school for any participants as the purpose of the current study focuses on the effectiveness of Brain Gym intervention in ameliorating the anxiety for the children with special needs in general, not specifically to any disorders. Three dependent variables were analysed at baseline as well as the changes between pre- and post-intervention, including the PAS-TC, cortisol and oxytocin levels. Summary of the hormonal results are presented in Table 3.

Hypothesis 1: Effect of Brain Gym on cortisol levels

The baseline cortisol levels were found significantly higher in the training group (4502.692 ± 651.226 pg/ml) compared with the control group (3042.231 ± 181.683 pg/ml, $U = 65$, $p = 0.002$), with a medium effect size ($r = 0.519$). Significant between-group differences in the change of cortisol levels were observed ($U = 99$, $p = 0.034$), in which a larger change in the intervention group (-1200.519 ± 607.781 pg/ml) than the control group (-118.681 ± 177.892 pg/ml) was observed ($r = 0.348$). However, the group difference became non-significant after controlled for the baseline values ($F_{1,35} = 0.364$, $p = 0.550$, $\eta^2 = 0.01$, Table 3).

Hypothesis 2: Effect of Brain Gym on oxytocin levels

The baseline oxytocin levels were significantly higher in the intervention group (140.626 ± 25.629 pg/ml) compared with the control group (44.619 ± 3.611 pg/ml, $U = 37$, $p < 0.001$) with a medium effect size ($r = 0.66$). The between-group differences in the change of oxytocin levels were significant ($U = 102$, $p = 0.043$, $r = 0.66$), in which the intervention group showed a larger change (78.701 ± 51.885

pg/ml) than the control group (3.186 ± 4.375 pg/ml). The between-group difference in the change of oxytocin levels remained significant after controlled for the effect of baseline oxytocin values ($F_{1,35} = 5.590, p = 0.020, \eta^2 = 0.145$, Table 3).

Hypothesis 3: Effect of Brain Gym on anxiety

No significant between-group differences in the ratio of normal anxiety level to elevated anxiety level were observed at baseline ($\chi^2 = 0.003, p = 0.957$). After training, there was a trend level difference in the ratio of normal anxiety level to elevated anxiety level between the intervention and the control group ($\chi^2 = 3.823, p = 0.051$), in which a larger ratio of normal to elevated anxiety level was observed in the intervention group compared with the control ($\phi = -0.321, p = 0.051$). To further investigate the between group differences in individual improvement of anxiety level, the changed scores were calculated. An improvement in an anxiety state (e.g. the change of scores < 0) was coded as 1; no changes or deterioration in the anxiety state (e.g. the change of scores ≥ 0) were coded as 0. There was a significant difference in the ratio of improvements to deterioration or no change between the two groups ($\chi^2 = 4.367, p = 0.037$) with a medium effect size ($\phi = 0.344, p = 0.037$). In the intervention group, there were fewer cases who showed deterioration / no changes in their anxiety levels measured by PAS-TC ($n=3$) compared with the control group ($n=11$). On the other hand, the number of participants who showed improvement in the anxiety levels after the intervention / waiting period was slightly more in the intervention group ($n=13$) than the control group ($n=10$) (see Table 4).

Discussion

This is the first study that demonstrates significant changes in cortisol and oxytocin levels and improvement in anxiety after Brain Gym in kindergarteners with special needs. Our findings demonstrated that the 10-week Brain Gym intervention can increase salivary oxytocin levels and suppress the deterioration of anxiety levels in kindergarteners with special needs, which robustly support the second hypothesis. We also observed a significant greater reduction in salivary cortisol levels in the intervention group compared with the control. However, such difference became non-significant after controlled for the effect of the baseline cortisol levels, suggesting that the between-group difference at baseline cortisol levels had a significant influence on the result. In this study, we cannot draw a definite conclusion on the first hypothesis due to the small sample size and the inconsistent findings after controlling for the baseline cortisol levels.

Brain Gym has been reported to reduce stress in elderly people¹². Our findings provide preliminary evidence on the stress reduction effect of Brain Gym by demonstrating a greater reduction of salivary cortisol levels after 10-week of Brain Gym intervention than the control in kindergarteners with special needs. The between-group difference in the change of cortisol levels, however, became non-significant after controlled for the effect of baseline cortisol levels. The baseline cortisol levels in the intervention group were significantly higher than the control group, which could possibly be confounded by the influence of each site. Nevertheless, the cortisol levels in both groups are still within the normal range¹⁷. Since we could not perform random group assignment in this study because of the practical constraint i.e. time clash between the intervention schedule and the school timetable, such group difference at baseline cannot be explained by a random distribution in a small size group, and subject bias cannot be avoided. Future studies that adopt a randomized approach with larger sample size are strongly recommended to further confirm our findings.

Oxytocin has been associated with stress and anxiety. It was highly correlated with cortisol during a social stress task, and its levels in saliva were negatively associated with anxiety and insecurity²⁸. Similar findings that negative association between higher salivary oxytocin levels and lower anxiety levels were reported in hospitalized children²⁹. In youths with anxiety disorder, salivary oxytocin levels were also found to negatively correlate with anxiety symptoms³¹. These findings suggest the anti-anxiety role of oxytocin. A previous study demonstrated that 8-week Brain Gym intervention significantly decreased anxiety levels in 68 elderly people¹². We observed significant group difference in the change of oxytocin levels with or without controlled for the effect of baseline oxytocin levels, in which greater increases in oxytocin levels were observed in the intervention group compared with the control. Our findings of increased salivary oxytocin levels and improvement in anxiety level after 10-week Brain Gym intervention supports the potential anti-anxiety effect of Brain Gym in kindergarteners with special needs.

In terms of the subjective measure, there was a significant difference in the ratio of improvements to deterioration or no change of the PAS-TC scores between the groups. Although the numbers of improvements were similar for both groups, our findings showed that Brain Gym reduced the deterioration of anxious and stressed emotions during early childhood. The trend level between-group difference in the ratio of normal to elevated anxiety levels further indicates the plausible anti-anxiety effect of Brain Gym, which partially supports our

third hypothesis. Taken together, we speculate that through the alteration of HPA system and oxytocin pathway, anti-anxiety effects may be achieved after the Brain Gym intervention¹⁵.

The strength of the current study is the application of both psychometrics and standardized and validated biological markers in describing stress and anxiety. Previous studies examining the effectiveness of Brain Gym adopted only subjective measures^{12, 13, 14}, in which subjective bias is inevitable.

Limitations

There are several limitations in this pilot study. First, a quasi-experimental design was adopted because of the practical constraint. Subject bias and influence from the experiment sites cannot be avoided, which limits the interpretation of our findings. Second, we included subjects with special needs in general but not focused on any specific disorders. The types of disorder and/or the severities however can be potential variables affecting the training effect. Future studies are recommended to focus on a specific type of special need or disorder to control for the influence. Third, the follow-up phrase was not planned into this pilot study due to the practical constraint in the target kindergartens, which led to the failure of examining the carry-over effect of the Brain Gym intervention. Future studies should include this phrase as a part of the study. Fourth, active controls i.e. aerobic exercise was not included in this study. Physical activities have been shown to be effective in reducing stress and ameliorating stress-induced anxiety or depression^{33, 34}. A meta-analysis revealed that physical exercises such as aerobic exercise, jogging or walking could reduce cortisol levels in adults with stress-induced mental health problem i.e. depression³⁵. Future studies should include an active control group that provides physical activities training to compare their efficacy with Brain Gym. Last, the sample size was relatively small in this study, the generalizability was therefore limited. In addition, the small sample size could result in a higher false positive and false negative rate. Although our findings in the change of oxytocin levels after training were robust, future studies with a larger sample and adopt a randomized controlled setting are strongly recommended to confirm our findings and investigate other potential changes.

Conclusion

In conclusion, our findings indicate potential anti-anxiety effects of Brain Gym in kindergarteners with special needs. Whether Brain Gym can be considered as an evidence-based practice requires more good quality empirical studies to further evaluate its effectiveness.

Abbreviations

ADHD - Attention-Deficit and/or Hyperactivity Disorder

ANOVA - Analysis of Variance

ASD - Autism Spectrum Disorder

CAS - The Child Assessment Service

CONSORT - The Consolidated Standards of Reporting Trials

ELISA - Enzyme-linked Immunosorbent Assay

HPA - The Hypothalamic-pituitary Axis

PACE - Positive, Active, Clear and Energetic

PAS-TC - Parent-rated Preschool Anxiety Scale: Traditional Chinese Version

SD - Standard Deviation

TREND - The Transparent Reporting of Evaluations with Nonrandomized Designs

Declarations

Ethics approval and consent to participate

Procedures in this study were approved by the Human Research Ethics Committee at The Education University of Hong Kong (Ref no. 2018-2019-0038). The procedure followed the national checklist of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) statement. This open label non-randomized clinical trial was registered in the Chinese Clinical Trial Registry (registration number: ChiCTR2000036305, url: <http://www.chictr.org.cn/showproj.aspx?proj=58067>, registration date: 22/08/2020) after the completion of the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.P.L.T.: Collection of data, Data analysis, and interpretation, Manuscript writing. W.K.W.L.: Conceptualized and designed the research, Provision of the study materials and participants, Collection and assembly of data, Data analysis and interpretation, Manuscript writing.

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Tables

Table 1: Group differences on changes in salivary cortisol and oxytocin levels

Dependent Variables	Groups	N	Mann-Whitney U				Quade Test*						
			Baseline Mean \pm SE (pg/mL)	U	<i>p</i>	<i>r</i>	Mean of Change \pm SE (pg/mL)	U	<i>p</i>	<i>r</i>	F	<i>p</i>	eta ²
Cortisol	Control	21	3042.231 \pm 181.683	65	0.002	0.519	-118.681 \pm 177.892	99	0.034	0.348	0.364	0.550	0.010
	Training	16	4502.692 \pm 651.226				-1200.519 \pm 607.781						
Oxytocin	Control	21	44.619 \pm 3.611	37	<0.001	0.660	3.186 \pm 4.375	102	0.043	0.333	5.590	0.020	0.145
	Training	16	140.626 \pm 25.629				78.701 \pm 51.885						

*Quade Test: Between group differences in the change of cortisol / oxytocin levels, the effect of baseline levels was controlled. Statistically significant differences were bold.

Table 2: Chi-square tests for the PAS-TC

Conditions		Control (N=21)	Training (N=16)	<i>X</i> ²	<i>p</i>	ϕ
Baseline	Normal : Elevation of anxiety	9:12	7:9	0.003	0.957	-0.009
Post-intervention	Normal : Elevation of anxiety	9:12	12:4	3.823	0.051	-0.321
Change	Improvements: Deterioration /no change	10:11	13:3	4.367	0.037	0.344

Note: 0 cells (0.0%) have expected count < 5, and no cell has expected frequency < 1 for all tests.

Table 3: Descriptive statistic of participants

Variables	Control (N=21)	Training (N=16)	<i>t</i> / <i>X</i> ²	<i>p</i>
Age (mean \pm SD)	5.325 \pm 0.805	5.109 \pm 0.564	0.025	0.98
Gender (Male: Female)	15:6	11:5	0.031	0.86
Special Needs (%)				
Developmental Delay	4 (10.811)	1 (2.703)		
ASD	2 (5.405)	2 (5.405)		
ADHD	0 (0)	1 (2.703)		
Specific learning difficulty	0 (0)	4 (10.811)		
At-risk cases*	10 (27.027)	13 (35.135)		

Note: ADHD (Attention Deficits and/or Hyperactivity Disorder), ASD (Autism Spectrum Disorder), SD (Standard Deviation).

*At-risk cases were referred by the target kindergartens including suspected ADHD, ASD, conduct disorder, emotional disorders, muscle development deficits, speech delay, and developmental delay. They were not diagnosed at the time of this study.

Table 4: Procedures of Brain Gym training

Timings	Activities	Resources
5 mins	Welcome & Greeting	Hello Song
10 mins	Brain Gym PACE (Positive, Active, Clear and Energetic) movements: <ul style="list-style-type: none"> - Sipping water - Brain Buttons - The Cross Crawl - Hook-ups 	PACE poster, water, energy ball and small equipment for learning cross crawl (self-design)
5 mins	Set session goal (for specific body part)	/
20 mins	Brain Gym movements include*: <ul style="list-style-type: none"> - Literality <ul style="list-style-type: none"> o The Double Doodle o Alphabet 8s o Belly Breathing o The Cross Crawl o Lazy 8s - Centering <ul style="list-style-type: none"> o Sipping Water o The Thinking Cap o Brain Buttons o The Positive Points o Hook-ups (Part I & II) - Focus <ul style="list-style-type: none"> o The Owl o The Calf Pump 	Finger dolls, laser pointer and blank drawing cards
10 mins	Brain Gym movement: Lazy 8s. Doing a Lazy 8s with various fun ways and tools (Claim-down period)	Worksheets, crayons
5 mins	Wrap-up, appreciation and singing goodbye song	Stickers

*Each session included eight to ten movements from the list.

Figures

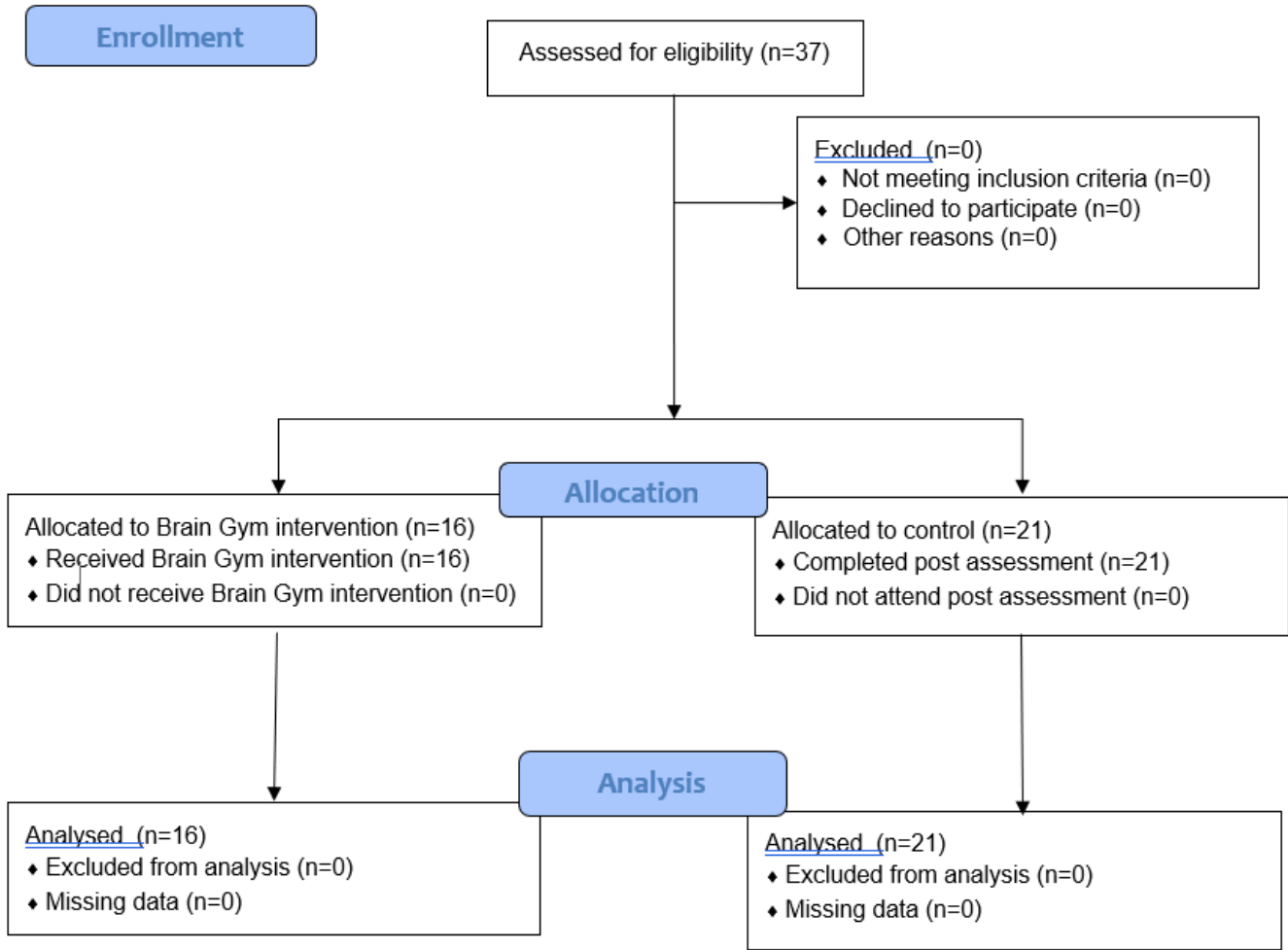


Figure 1

The consolidated standards of reporting trials (CONSORT) diagram for the flow of the study.