**ORIGINAL PAPER** 



## Promoting Executive Functioning in Children with Autism Spectrum Disorder Through Mixed Martial Arts Training

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#### Abstract

The present study evaluated the effectiveness of a mixed martial arts (MMA) intervention in improving executive functions (EFs) in a sample with autism spectrum disorder (ASD). School-aged children with ASD were randomly assigned to a MMA intervention group or a waitlist control (WLC) group. The intervention featured a 26-class program over a 13-week period; the WLC group did not participate in any martial arts between pre- and post-test. Results indicated that the MMA group had significantly better EFs at post-test compared to the WLC group. The intervention appeared to be efficacious in meeting its goals of improving the executive functioning of children with ASD. The present study extends the current literature on the malleability of EFs among children with ASD.

**Keywords** Autism spectrum disorder  $\cdot$  Executive functioning  $\cdot$  Martial arts  $\cdot$  Intervention  $\cdot$  Emotion regulation  $\cdot$  Behavior regulation

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by a myriad of deficits including challenges in social communication and the presence of restricted and/or repetitive behaviors (American Psychiatric Association 2013). Although not a core deficit, another challenge faced by most individuals with ASD involves executive functions (EFs; Diamond 2013; Hill 2004; Ozonoff and Jensen 1999), a host of inter-related cognitive processes that drive goal-directed behaviors. The umbrella term of EFs includes three core processes: behavioral inhibition (i.e., controlling and overriding behavioral urges such as maintaining social politeness), working memory (i.e., holding information in mind while mentally manipulating it, such as doing mental arithmetic), and cognitive flexibility (i.e., adjusting to changed demands such as considering ideas from a new perspective) (Diamond and Lee 2011). More complex EFs include verbal and nonverbal fluency (i.e.,

generating ideas and concepts such as listing words that start with a certain letter), planning/problem solving, and reasoning (Anderson 2002; Diamond 2002; Diamond and Lee 2011). The current study introduces a martial-arts based intervention aimed at improving EFs in children with ASD.

Core and complex EFs continue to develop and strengthen throughout early childhood and into middle childhood. Studies examining EFs in middle childhood with typicallydeveloping (TD) children often have tried to help improve EFs through various means such as computerized programs, mindfulness training, and martial arts programs (see Diamond and Lee 2011, for a review). For example, one study using school-based martial arts training with TD children found that fourth- and fifth-grade students benefited more from the intervention than kindergarten and first-grade students, particularly in the areas of inhibitory control and working memory (Lakes and Hoyt 2004). By the time TD children reach adolescence, performance on many standard executive functioning tests is equivalent to an adult-level (Zelazo and Müller 2010; Zelazo et al. 2003).

Restricted and repetitive patterns of behavior (RRBs), which are integral to the diagnostic criteria of ASD (American Psychiatric Association 2013), have also been reported to be highly associated with some, but not all, impairments of EFs (Ozonoff and Schetter 2007; Turner 1999). For example, one study found that RRBs were associated only with

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behavioral inhibition (Boyd et al. 2009), whereas another study found that RRBs were associated with multiple executive processes including behavioral inhibition, working memory, and cognitive flexibility, but not with planning and fluency (Lopez et al. 2005). In another study, partial support was found for the link between RRBs and EFs, but only for specific measures of RRBs (South et al. 2007). Although the examination of executive functioning deficits in ASD does not fully account for the presence of RRBs, evidence suggests that they could partly explain the observed variety of RRBs that characterize ASD (Lopez et al. 2005). In terms of directionality, behavioral gains in RRBs could potentially benefit EFs, which are crucial for academic success (Best et al. 2011), mental and physical well-being, and overall quality of life (for a review, see Diamond 2012).

## Interventions for Improving Executive Functioning

Compared to TD peers, children with ASD demonstrate a delay in the development of EFs and display poorer executive functioning abilities, particularly poorer planning abilities and cognitive flexibility (Hill 2004). Even though executive functioning problems may cause impairment in daily functioning, they are not intractable. Executive functioning intervention studies have found that they are malleable and can be enhanced with practice using a variety of activities (Diamond et al. 2007; Diamond and Lee 2011). For example, some studies have improved executive functioning in children with vulnerable developmental profiles, such as poor working memory in children with low academic performance (Holmes et al. 2009) and poor working memory and behavioral inhibition in children with ADHD (Klingberg et al. 2005). Although children with ASD were not included in these studies, the encouraging results indicate that one or more domains of EFs can be improved in children with developmental disadvantages, which might extend to include ASD.

One way in which executive functioning deficits can be improved is through physical activity. Short bursts of exercise prior to cognitive testing have shown immediate performance improvements in cognitive control, possibly due to increases in neurochemicals in the neural system (Hillman et al. 2009). Sustained and frequent physical activity has been associated with increased neural activity in the frontal region and increased activation in the prefrontal cortex regions, which are responsible for EFs such as working memory and planning (Davis et al. 2011; Kamijo et al. 2011). In a recent study, the intensity and frequency of RRBs (i.e., stereotypic behaviors) in early adolescents with ASD was measured one hour after an exercise session of 10- or 20-min of low- or high-intensity exercise (Schmitz et al. 2017). Stereotypic behaviors were reduced the most by 10-min of low-intensity exercise, whereas the most strenuous condition, 20-min of high-intensity exercise, actually resulted in increased stereotypic behaviors. High-intensity exercise may over-arouse the physiological system beyond sufficient recovery, whereas low- to moderate-intensity exercise are an "optimal" level of stimulation (Schmitz et al. 2017).

A physical activity that may be particularly effective in improving executive functioning is martial arts training. To date, the research findings concerning martial arts training and outcome measures in children and adolescents have been heterogeneous. For example, Vertonghen and Theeboom (2010) reviewed studies linking various styles of martial arts practice (e.g., judo, karate, kickboxing, etc.) with socialpsychological outcomes in TD youth. In terms of targeting EFs, Lakes and Hoyts (2004) used a Taekwondo approach to improve EFs in TD school-aged children. Furthermore, martial arts interventions specifically targeting children with vulnerable developmental profiles have been limited; Lakes et al. (2013) implemented a Taekwondo intervention in TD children vulnerable to executive functioning deficits (children from low socioeconomic backgrounds), and Chan et al. (2013) implemented a Chinese martial arts intervention with children with ASD.

The practice of "traditional" martial arts is not only physically rigorous, but includes self-discipline, which refers to behavioral, emotional, and cognitive control. Compared to "modern martial arts" that view martial arts as a competitive sport focused only on physical conditioning, a traditional approach to teaching martial arts also emphasizes character development and self-control (Diamond 2012; Trulson 1986). Indeed, whether martial arts can be used to improve executive functioning depends on the approach to the implementation or teaching of the martial arts (i.e., traditional or modern) (Diamond 2012). Thus, a traditional approach to martial arts training may be the better strategy for improving executive functioning deficits in these areas in children with ASD.

In the current study, a traditional approach to martial arts was used to deliver the executive functioning intervention for children with ASD. As commented by Vertonghen and Theeboom (2010), only a few studies have compared specific approaches to martial arts (e.g., traditional vs. modern) and styles (e.g., judo, karate, kickboxing, etc.) with one another. For example, Trulson (1986) compared traditional and modern Taekwondo training in a sample of juvenile delinquents and found lowered delinquency and aggression in the traditional approach group and poorer outcomes in the modern approach group. Reynes and Lorants (2002a, b) compared two styles of martial arts (traditional judo and traditional karate) and found that children who practiced judo reported more aggression than the karate and control groups.

To the best of our knowledge, the current study is the first to select a Mixed Martial Arts (MMA) style of martial arts to implement as an intervention with a sample of child participants. Based on the existing literature, we chose to use a traditional approach rather than a modern approach to teaching the MMA style. MMA grew in part from Jeet Kune Do, a Chinese martial art consisting of consolidated elements and techniques from over 20 different martial arts; the primary goal of Jeet Kune Do is to adapt to different strengths and different contexts (Lee 1975). Compared to other martial arts styles, a traditional teaching of MMA may be especially suitable for children with ASD for several reasons. First, children with ASD are widely diverse with different strengths and weaknesses; an adapted MMA intervention would provide students with a variety of tools that can be tailored to each child's individual abilities. Secondly, adapted MMA would teach students skills that can be generalized to different contexts, thus providing an opportunity to practice applying learned skills across contexts. Third, MMA could reduce stereotypic behaviors given that it is a relatively low-intensity exercise; past research has recommended that low- to moderate-intensity exercise for children with ASD may help with behavioral inhibition of stereotypic behaviors (Schmitz et al. 2017). The varying low- to moderate-intensity of MMA suits this recommendation. Fourth, the MMA style included elements that made it a "traditional" (rather than "modern" approach to teaching) because it emphasized character development and self-control in addition to physical conditioning (Trulson 1986). Finally, the MMA style also involves repeated practice and increasingly challenges EFs demands over the course of training, two features that are recommended by Diamond (2012) for effective executive functioning interventions. Taken together, a traditional MMA style seems to offer a promising martial arts intervention for improving EFs because of its appropriateness for children with ASD, opportunities for low- to moderate-intensity exercise, use of a traditional approach that emphasizes character development and self-control, and incorporation of increasingly cognitively complex curriculum over time. Traditional MMA training that emphasizes these elements has the potential for ecologically valid benefits for children with ASD.

# Martial Arts Interventions and Executive Functioning

To date, only a handful of studies has been conducted examining the use of martial arts to address executive and behavioral functioning in children vulnerable to executive functioning deficits (Bell et al. 2016). Within this sparse literature, we know of only two studies that have implemented a traditional approach to martial arts training to address deficits related to executive functioning in children with ASD; one examined the association indirectly and the other examined it directly.

Bahrami et al. (2012) implemented a traditional Kata (Japanese forms) technique with children and adolescents with ASD. In their study, they indirectly examined EFs by investigating the role of this martial arts training on the malleability of RRBs in terms of stereotypic behaviors (e.g., hand flapping), which were reported by caregivers. Participants who received the Kata training demonstrated reduced stereotypic behaviors both at post-test and at onemonth follow-up compared to the baseline assessment, whereas the no-exercise control group showed no differences (Bahrami et al. 2012). Since this study found lessened stereotypic behaviors following a martial arts intervention in children and adolescents with ASD and given that RRBs could potentially be linked to EFs, a martial arts intervention could yield benefits in EFs as well.

In direct examination of the effect of martial arts training on an aspect of executive functioning. Chan et al. (2013) found improvements in self-control in children with ASD following the practice of Nei Yang Gong (NYG), a traditional Chinese martial arts exercise, compared to a control group that received training in Progressive Muscle Relaxation (PMR). At post-test, the NYG group had significantly higher parent-reported self-control, lower daily behavior problems, and increased EEG brain activity during an EFs task, compared to the PMR group. This multi-method study demonstrated that only 8 hours of martial arts training over the course of 1 month correlated with altered neural activity, improved self-control, and decreased behavioral problems in children and adolescents with ASD. Given the improvements in self-control observed in this study, we examined whether other domains of EFs might be influenced by martial arts training as well. The present study is the first to examine the relationship between multiple EFs and martial arts training in children with ASD.

## **The Current Study**

The goal of the current study was to test the effectiveness of a traditional MMA intervention to improve EFs in children with ASD. The hypothesis was that children with ASD who participated in a MMA intervention that utilized a traditional approach would show improvements in a set of executive functioning abilities compared to children with ASD on a waitlist control group who did not receive the intervention. EFs were assessed with each child in a lab setting, through direct assessments pre- and post- the MMA intervention, and through parent reports of their child's executive functioning abilities in the home context, preceding and following the MMA intervention.

## Methods

## Recruitment

Following approval from the university's institutional review board, children aged 8- to 11-years with a clinical diagnosis of ASD were recruited from a departmental database of participants who consented to be contacted for future studies. Recruitment flyers were also sent to community organizations that serve families with ASD (e.g., local autism treatment centers). Interested families contacted the research team through a secure online survey.

## **Participants**

Thirty-four children (aged 8–11 years, M = 9.34 years, SD = 1.08; 82.4% boys, n = 28) with ASD participated in the study. Participating children had ASD as determined by three sets of criteria: (1) had a parent-reported clinical diagnosis of ASD; (2) met criteria on the parent-reported Lifetime Social Communication Questionnaire (SCQ; Rutter et al. 2003), and (3) met criteria for ASD on the Autism Diagnostic Observation Schedule-Second Edition assessment (ADOS-2; Lord et al. 2012). The overall sample was racially/ethnically diverse: 38.2% (n = 13) Hispanic/Latino, 26.5% (n=9) Asian/Asian American, 23.5% (n=8) Caucasian/White (Non-Hispanic), and 11.8% (n=4) multiracial or other. Using the Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II; Wechsler 2011), the Full-IQ score for total intellectual functioning was equivalent to lowaverage for the sample as a whole, (M = 83.00, SD = 19.96,range 44-122). The verbal comprehension composite score was borderline (between extremely low and low-average) (M = 79.24, SD = 21.27, range 45-115) and the perceptual reasoning composite score was low-average (M = 89.35, SD = 18.98, range 51–124). ADOS-2 scores indicated that autism symptom severity was moderate-to-high (M = 7.00, SD = 1.54, range 4–10).

After completing pre-test study procedures (see Design and Procedures), the total sample of children was randomly assigned to either the MMA intervention group or waitlist control (WLC) group.

#### **Mixed Martial Arts Group**

Fourteen children participated in the mixed martial arts (MMA) group, which consisted of two lab visits separated by the MMA intervention. Characteristics of the MMA group are shown in Table 1. The MMA subsample only included boys. The Full-IQ scores from the WASI-II equated to low-average; the verbal comprehension composite score was borderline (between extremely low and low-average) (range 45–110), and the perceptual reasoning composite scores indicated that autism symptom severity was moderate-to-high (range 6–9).

## Waitlist Control Group

Twenty children participated in the Waitlist Control (WLC) group, which visited the lab twice, separated by a delay equivalent to the length of the intervention; during the delay, children did not participate in any martial arts programs. Characteristics of the WLC group are shown in Table 1. The majority (70%) of the WLC subsample was boys. The Full-scale IQ scores from the WASI-II equated to low-average; the verbal comprehension composite score was borderline (extremely low to low-average) (range 45–115) and the perceptual reasoning composite score was average (range 51–124). ADOS-2 scores indicated that autism symptom severity was primarily moderate-to-high (range 4–10).

The MMA and WLC groups did not differ from one another in terms of demographic and child characteristics, with the exception of child gender (significant) and race/ ethnicity (marginal). Between-group comparisons on these variables are shown in Table 1.

## **Design and Procedures**

Parents of children with ASD who indicated an interest in participating were contacted via telephone or email. A member of the research team scheduled the family's first visit to the university testing space and emailed the parent a link to an online survey on family demographics and background information. Parents were compensated \$10 in cash or gift card for completing the online survey. Parents of participating children agreed not to enroll their children in any other martial arts classes during the study and confirmed that their child had not participated in any martial arts in the preceding year.

The first scheduled visit to the university testing space (two rooms) consisted of diagnostic and behavioral measures. The lead author administered the IQ assessment, the WASI-II (Wechsler 2011). Concurrently, in a separate room, one parent (n=27 mothers) of the child completed

Table 1Demographiccharacteristics of studyparticipants

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	Mixed martial arts (MMA) n = 14 frequency (%)/M (SD)	Waitlist control (WLC) n=20 frequency (%)/M (SD)	Group differences (MMA vs. WLC)
Child characteristics			
Gender			
Female	0 (0%)	6 (30%)	
Male	14 (100%)	14 (70%)	Fisher's exact, $p = .03^*$
Race/ethnicity			
Latinx/Hispanic	4 (28.6%)	9 (45%)	
White/Caucasian	5 (35.7%)	3 (15%)	
Asian/Asian American	5 (35.7%)	4 (20%)	
Multiracial/other	0 (0%)	4 (20%)	LR = 7.07, p = .07†
Child age	9.10 (1.10)	9.52 (1.07)	t(32) = -1.10, p = .28
SCQ total	22.21 (4.58)	20.80 (7.16)	t(32) = .65, p = .52
ADOS-2 comparison score	7.00 (.78)	7.00 (1.92)	t(32) = .00, p = .10
WASI-II full-scale IQ	80.36 (17.34)	84.85 (21.85)	t(32) =64, p = .53
Verbal comprehension	78.36 (20.23)	79.85 (22.47)	t(32) =20, p = .84
Perceptual reasoning	85.57 (16.64)	92.00 (20.44)	t(32) =97, p = .34
Family characteristics			
Income (\$)			
0-49,999	2 (14.3%)	4 (20%)	
50,000–99,999	6 (42.9%)	4 (20%)	
100,000–149,999	4 (28.6%)	7 (35%)	
150,000–199,999	2 (14.3%)	3 (15%)	
200,000-249,999	0 (0%)	0 (0%)	
250,000+	0 (0%)	2 (10%)	LR = 3.82, p = .43
Highest maternal education			
High school	4 (28.6%)	4 (20%)	
Post high school training	2 (14.3%)	2 (10%)	
2-year junior college	3 (21.4%)	2 (10%)	
4-year university	3 (21.4%)	9 (45%)	
Graduate/professional	2 (14.3%)	3 (15%)	LR = 2.48, p = .65

LR Likelihood ratio for cell frequencies < 5

\**p* < .05

questionnaires about their child with a research assistant present. The author then administered the ADOS-2 (Lord et al. 2012) to the child. The parent remained in a separate room for the ADOS-2 unless the child's language ability required the parent to be present during the ADOS-2 administration. After the ADOS-2, the child was administered the Hearts & Flowers test, a computerized executive functioning test.

Following the first laboratory visit, children were randomly assigned to either participate in the MMA or WLC group. At the end of the intervention period, children returned to the university testing space for post-test assessments. All of the participants in the MMA group were tested within 3 days of their last intervention class session. For the post-test assessment, a researcher administered the Hearts & Flowers test to the child while their parent completed questionnaires with a different researcher in a separate room. Each family was paid \$20 in cash or gift card at the end of their first and second visits (\$40 total).

#### **Description of MMA Intervention**

The lead author collaborated with a team of senior martial arts instructors at the local martial arts academy to design the MMA intervention structure and content. The owner of the local martial arts academy funded the program, which was free to the families with ASD. The number of children with ASD in each class session was limited to no more than 12 children with ASD per class.

<sup>†</sup>*p* < .10

#### Intervention Dosage

The intensity of the intervention, or dosage, was modeled on the dosage used by Lakes and Hoyt (2004) in their study involving TD children. The intervention for the present study consisted of 26 class sessions over the course of 13 weeks; this schedule resulted in training taking place two times per week with each class session lasting 45 min, yielding a targeted total of 1170 min of intervention time (similar to the total minutes in Lakes and Hoyt 2004). If children did not miss any of the class sessions and attended twice per week, it would have taken them 91 days to obtain the recommended dosage for this study.

#### Curriculum

The curriculum for the MMA intervention was adapted from the TD MMA curriculum at the academy, and was

**Fig. 1** Description of MMA intervention: 13-week curriculum and class structure

further developed by the senior martial arts instructors in collaboration with the lead author. The curriculum targets the three core EFs: behavioral inhibition, working memory, and cognitive flexibility. The curriculum was designed to be implemented in thirds, as depicted in Fig. 1. In the first third, participants learned simple two- and three-step combinations of strikes and kicks that were used only on a punching mitt or kicking pad. Behavioral inhibition was used to attend to the instructor's demonstrations. These sessions also practiced working memory, as the children had to remember the different rules that they learned and move different parts of their bodies in accordance with the rules. In the second third of the curriculum, participants extended these skills and learned glove-drills (i.e., "partner drilling," or working directly on a partner's body) and grappling, which had more steps than the first component and also involved more details. The sessions in the second third also emphasized working memory along with behavioral inhibition and

Week Number	Curriculum	Number of steps
(13-weeks total)		in sequence
#1 through 4	Striking (punches/ kicks on punching	2 to 3
	mitts/ kicking pads)	
#5 through 8	Glove drills and grappling (offense/	4 to 9
	defense with a partner)	
#9 through 13	Combination (striking and grappling,	4 to 13
	offense/defense with a partner)	

5 minutes	<ul> <li>Bow-in</li> <li>Line up, stand at attention, bow and pay respects to the instructor</li> <li>Sit in meditation, focus claps</li> </ul>
15 minutes	<ul> <li>Warm up</li> <li>Jogging, footwork, stretching, jumping, etc.</li> </ul>
20 minutes	<ul> <li>Main activity</li> <li>Increasingly complex (over the weeks of the intervention) combinations of strikes (e.g., punches, kicks, elbows, knees) and defenses (e.g., blocks), grappling</li> <li>Practice back-and-forth with TD peer buddies</li> </ul>
5 minutes	<ul> <li>Cool down</li> <li>Social game (e.g., dodgeball)</li> <li>Line up, stand at attention, bow and pay respects to the instructor</li> <li>High-five with all teammates</li> </ul>

cognitive flexibility. Behavioral inhibition was taught when working with a partner so that children did not hit or kick their partner with excessive force as one would when practicing with a mitt or pad. Children also were taught cognitive flexibility when asked to mentally manipulate what was previously learned to accommodate the new information as it was presented. In the last third of the curriculum, participants combined all of their learned skills, which consisted of highly detailed, multi-step techniques, which required the active use of the three core EFs. This increasingly complex curriculum over the course of 13 weeks required the active use of all three core EFs. Because the techniques were difficult, children were expected to become frustrated. Meditation and breathing exercises were practiced at the beginning of the class (see Class Structure), and children were reminded to use their breathing techniques to help regulate their emotions if they became frustrated or overwhelmed throughout the class.

#### **Peer Buddies**

Typically-developing (TD) peers who were martial arts students of the academy also participated in the class as "peer buddies." Peer buddies ranged in age from 8 to 13 years and were unaware of the research study, so they were blind to which participants were in the study. Peer buddies were extensively trained in how to engage with children with ASD. Each peer buddy underwent approximately 4 hours of training over the course of 1 month. The peer buddies volunteered their time to assist in the class and did not receive monetary compensation.

#### Head Instructor and Adult-Facilitators

The head and assistant instructors of the class were senior instructors at the martial arts academy with extensive experience in teaching martial arts to children of wide age ranges and abilities, including children with developmental disabilities such as ASD, cerebral palsy, and Down syndrome. In addition to the head and assistant instructors, undergraduate student facilitators were trained and provided additional support to the children with ASD and peer buddies. Because there were children with ASD in the class who were not enrolled in the research study, instructors were blind to which children were in the research study. The instructors and facilitators volunteered their time and did not receive monetary compensation.

#### **Class Structure**

The 45 min of each class session were allocated into four phases, which are depicted in Fig. 1. Similar to a previous study, during the "Bow-in" phase, students sit in meditation

and are instructed to focus on their breathing and think about questions that promote self-monitoring, namely "Where am I?", "What am I doing?", and "What should I be doing?" (Lakes and Hoyt 2004, p. 289), as well as "What am I working on today?".

## **Measures and Instruments**

#### **Demographics and Background Information**

Parents reported on family demographic information and on child developmental history at pre-test.

## ASD Diagnostic Measures and Child Intellectual Functioning

Diagnostic measures were obtained at pre-test by parentreport and direct assessment of the child at the university testing space, where child intellectual functioning was also assessed.

#### Lifetime Social Communication Questionnaire

The Lifetime Social Communication Questionnaire (SCQ; Rutter et al. 2003) is 40-item parent report tool used by clinicians to quickly screen for ASD. The questionnaire takes 10 min to complete, and parents responded "Yes" or "No" to questions that describe behaviors that are related to social and communication impairments. Responses were summed to yield a total score. Higher scores indicate poorer social communication. SCQ scores of 12 or higher combined with parent report of a clinical diagnosis are consistent with the gold standard of ASD diagnostic assessment (Daniels et al. 2012).

#### Autism Diagnostic Observation Schedule-Second Edition

The Autism Diagnostic Observation Schedule-Second Edition (ADOS-2) is a standardized, semi-structured assessment of ASD consisting of modules appropriate for infants through adulthood (Lord et al. 2012). The assessment takes 40–60 min, during which a trained administrator serves as a social partner during activities designed to elicit behaviors that fall within the social affect and RRBs domains of ASD. Behaviors were coded and converted into algorithm scores, which were then summed to yield a total score that allowed for comparison of a child's autism symptomology to other children with ASD of similar age and language ability. Higher scores indicate more severe autism symptomology. The ADOS-2 is the gold standard for the behavioral assessment of ASD with demonstrated reliability and validity (Lord et al. 2012).

#### Wechsler Abbreviated Scale of Intelligence-Second Edition

The Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II; Wechsler 2011) is a brief measure of intelligence in individuals 6–90 years of age. The WASI-II yields two subscores (verbal comprehension and perceptual reasoning) and a full-scale IQ score. The WASI-II takes 15–30 min to administer and has been used successfully with children and adults with ASD (Minshew et al. 2005). Higher scores indicate greater intellectual functioning.

#### **Child Executive Functioning**

A computerized task and parent-reported questionnaire were used to measure EFs at both pre- and post-test.

#### **Hearts & Flowers Test**

The Hearts & Flowers test is a computerized task that directly measures three core EFs: behavioral inhibition, working memory, and cognitive flexibility (Davidson et al. 2006; Diamond et al. 2007). The 8-minute test can be used to measure EFs in children as young as 4-years-old and through adulthood (Davidson et al. 2006). In the present study, the Hearts & Flowers test was given at both pre- and post-test using a touch-screen laptop computer. The Hearts & Flowers test has previously been used in a sample of children with developmental challenges (e.g., Down syndrome; Edgin et al. 2010), and with children with ASD (Calderon et al. 2014). In addition, numerous studies with TD children have implemented the Hearts & Flowers test in a pre- and post-test design (e.g., Blair and Raver 2014; Schonert-Reichl et al. 2015). Because the delay between the pre- and post-test lab visits was relatively long (a minimum of 3 months apart), learning effects were not seen as a threat to validity in the current study. Three blocks were presented in the following order: congruent (trials with same-side stimulus), incongruent (trials with opposite-side stimulus), and mixed block (trials with same- and opposite-side stimulus) (for description of the blocks, see Wright and Diamond 2014).

Executive functioning performance was measured for two dependent variables, *accuracy* and *response time* (in milliseconds, *ms*). Per the scoring instructions, trials with response times less than 250 *ms* were excluded from the analyses for being too fast (Wright and Diamond 2014). Accuracy was then computed by dividing the total number of correct responses over total number of responses that were greater than 250 *ms*. Next, trials with incorrect responses were excluded from the response times. Median response time was calculated for correct responses only; median values were used in order to reduce the effects of outliers (Davidson et al. 2006).

## Behavior Rating Inventory of Executive Function-2nd edition

The Behavior Rating Inventory of Executive Function (BRIEF-2) is an 86-item parent rating scale designed to assess executive functioning and self-regulation in children and adolescents ages 5-18 years (Gioia et al. 2000). The BRIEF-2 takes 10-15 min to complete (Gioia et al. 2000). The BRIEF-2 was designed to allow for comparison of changes in ratings of executive functioning over time, enabling researchers to monitor participant response to treatment and intervention (Gioia et al. 2000). The BRIEF-2 has consistently shown sensitivity to change as an outcome measure (see Isquith et al. 2014, for a review). To date, numerous clinical trials and treatment studies have used the BRIEF-2, including one testing the effectiveness of an executive functioning intervention for children with ASD (Kenworthy et al. 2014). Parents were given the BRIEF-2 at pre- and post-test and reported on children's functioning on three indexes of EFs: Behavior Regulation Index (BRI; scales: inhibit, self-monitor), Emotion Regulation Index (ERI; scales: shift, emotional control), and Cognitive Regulation Index (CRI; scales: initiate, working memory, plan/ organize, task-monitor, organization of materials). The three indexes were combined according to the scoring instructions to yield a *Global Executive Composite* (GEC) score. Higher scores indicate poorer executive functioning (Gioia et al. 2000).

## Results

#### **Plan of Analysis**

Mixed-effects regression models were conducted to examine the relationship between group status (MMA group vs. WLC group) and time (pre- vs. post-test scores) on the target variables: Hearts & Flowers test (i.e., accuracy and response times) and parent-reported BRIEF-2 (i.e., Behavior Regulation Index, Emotion Regulation Index, Cognitive Regulation Index, and Global Executive Composite), using Stata-15 (StataCorp., 2017). Overall intervention group and time effects were tested by examining the regression coefficients in the mixed-effect regression models.

Cohen's d-index change scores were calculated to compare the effect sizes across outcome variables. The d-index change score has been used in previous intervention research (e.g., Sheridan et al. 2012). In the present study, it was used to calculate the effect sizes for the interaction effects of time (pre- vs. post-test) by group (MMA vs. WLC) on outcome measures. Positive d-index change scores indicate the MMA group had the higher scores; negative d-index change scores indicate the WLC group had the higher scores. Cohen (1988) **Table 2** Means and SDs formajor study variables byexperimental group and time

	$\frac{\text{Mixed martial arts (MMA)}}{M(SD)}$		Waitlist control (WLC) $n=20$ $M(SD)$		
	Pre: time 1	Post: time 2	Pre: time 1	Post: time 2	
Computer-based executive funct	ion: Hearts & Flow	ers test			
Accuracy (% correct)					
Congruent block	91.57 (13.08)	96.86 (9.48)	93.83 (12.51)	89.35 (15.79)	
Incongruent block	68.04 (36.13)	79.56 (24.84)	74.81 (25.12)	83.27 (21.52)	
Mixed block	61.85 (19.98)	72.01 (17.55)	62.60 (15.21)	63.17 (15.88)	
Response time ( <i>ms</i> )					
Congruent block	622.25 (217.21)	555.43 (121.65)	584.68 (236.45)	575.73 (190.27)	
Incongruent block	647.89 (156.00)	588.61 (172.89)	598.63 (168.77)	622.45 (161.06)	
Mixed block	629.32 (173.55)	642.39 (137.32)	666.53 (120.62)	669.65 (136.14)	
Parent-reported executive func	tion: Behavior Rati	ng Inventory (BRI	EF-2)		
Behavior regulation index	71.36 (9.03)	67.79 (8.70)	72.35 (9.48)	73.15 (8.42)	
Emotion regulation index	69.21 (9.36)	66.21 (8.91)	73.15 (8.96)	75.55 (8.47)	
Cognitive regulation index	69.21 (7.71)	67.29 (7.79)	68.90 (8.86)	69.10 (7.87)	
Global executive composite	74.93 (8.87)	71.71 (8.54)	74.70 (9.34)	75.85 (7.81)	

ms milliseconds

classic definitions of effect sizes as small (d=.2), medium (d=.5), and large (d=.8) were used to interpret the magnitude of d-index change scores on the outcome measures.

Power analysis was conducted using GPower software. Power was set at 95% (Cohen 1988). With the current total sample size of 34, the present study had enough power to detect large effects of statistical significance but lacked sufficient power to find moderate or small effects.

## **Preliminary Analyses**

#### **Demographic Variables**

Following data screening, demographic variables were examined for group differences at pre-test (see Table 1). Only child gender significantly differed between the MMA and WLC group. Because both groups were majority boys and to preserve power, we did not control for gender in the analyses. Means and standard deviations for major study variables by group and time are presented in Table 2.

#### Pretreatment Comparability

Children were randomly assigned to group. The two groups were compared for selection effects (differences on pre-test scores). As expected, none of the pre-test scores on main study variables were significantly different between the groups (p values from .15 to .94).

#### Intervention Sessions and Fidelity Checks

Sessions were assessed for fidelity of program intervention, or the adherence to the intervention protocol in terms of frequency, duration, dosage, and content (Carroll et al. 2007). Frequency, duration, and dosage were assessed for each participant. Content of the intervention was assessed for each class session and consisted of the number of peer buddies, the gender composition of peer buddies (i.e., number of boy peer buddies), and the numbers of instructors, adult facilitators, and children with ASD in attendance. Content fidelity also included the percentage of class sessions requiring a one-to-one facilitator to support lower-functioning children through the class session. Implementation fidelity for adherence to the protocol was computed by taking the ratio of the observed protocol variable over the intended protocol variable. The averages for intervention implementation (observed and intended) and for implementation fidelity adherence (percentage of observed over intended implementation) are shown in Table 3. Implementation fidelity adherence ranged from 73 to 100%.

## Mixed-Effects Regression Models on Executive Functioning

Hypothesis 1, to examine direct assessments of executive functioning following a MMA intervention, was examined using a linear mixed-effects model. Group status (MMA group vs. WLC group), Time (pre- vs. post-test), and their interaction were posited as the predictors of performance on the Hearts & Flowers test (accuracy and response times on

Table 3 Intervention implementation fidelity for adherence to protocol (n = 14)

	Intervention implementation		Imple- mentation fidelity
	Observed M(SD)	Intended	%
Frequency (total number of sessions) <sup>a</sup>	22.86 (2.51)	26	87.90
Duration (total days to complete intervention) <sup>a</sup>	124.64 (27.90)	91	73
Dosage (total minutes of intervention) <sup>a</sup>	995.22 (257.14)	1170	85.06
Content of intervention			
Number of peer buddies <sup>b</sup>	2.86 (2.22)	3	95.33
Number of boy peer buddies <sup>b</sup>	1.88 (1.75)	2	94
Number of instructors <sup>b</sup>	1.74 (.52)	2	87
Number of adult facilitators <sup>b</sup>	1.94 (1.03)	2	97
Number of children with ASD <sup>b,c</sup>	5.03 (2.07)	12	100
Percentage of class sessions requiring one-to-one adult facilitator <sup>b,d</sup>	40.79%	33%	80.90

<sup>a</sup>Total values per participant

<sup>b</sup>Total values per class session

<sup>c</sup>We intended for a target number of *no more than* 12 children with ASD per class. Given that all of the class sessions had less than 12 children with ASD per class, implementation fidelity was 100% <sup>d</sup>One-to-one adult facilitator to support lower-functioning children

Table 4 Multilevel between-subjects effects of martial arts intervention on post-test executive functioning scores (MMA: n = 14, WLC: n = 20)

Variable	Coefficient <sup>a</sup>	SE	Effect size $d_{change}^{b}$		
Computer-based executive function: Hearts & Flowers test <sup>c</sup>					
Accuracy (% correct)					
Congruent block	9.77*	4.10	.83		
Incongruent block	3.07	6.89	2.17		
Mixed block	9.58**	3.20	1.01		
Response time (ms)					
Congruent block	- 57.87	63.55	31		
Incongruent block	-83.11	54.97	56		
Mixed block	9.95	56.01	.06		
Parent-reported Executive Function: Behavior Rating Inventory (BRIEF-2) <sup>c</sup>					
Behavior regulation index	-4.37*	2.19	67		
Emotion regulation index	-5.40**	2.08	88		
Cognitive regulation index	-2.13	1.65	46		
Global executive composite	-4.36*	1.84	81		

ms milliseconds

<sup>a</sup>WLC group as the reference group

 ${}^{b}d_{change}$  = Cohen's d-index of change scores,  $d_{change}$  = (mean treatment group change score- mean control group change score)/sd<sub>weighted</sub>

<sup>c</sup>Based on 34 participants with 68 observations

\*p < .05

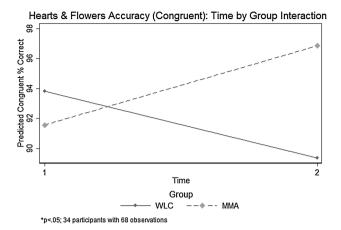
\*\*p<.01

the congruent, incongruent, and mixed blocks). In terms of

accuracy in the congruent block, no significant main effect of time was found (b = -.45, p = .84). However, a significant interaction between group and predicted accuracy in the congruent block over time was found. The MMA group had an accuracy score that was 9.77% higher than the WLC group at post-test controlling for pre-test scores, with a large effect size (Table 4; Fig. 2). In terms of accuracy in the incongruent block, a significant main effect of time (b=9.72,p = .00), but no significant interaction of group and time was found (large effect size). Finally, a significant main effect of time was found for accuracy in the mixed block (b = 4.52, p = .01). In addition, a significant interaction between group and predicted accuracy in the mixed block over time was also found. The MMA group had an accuracy score that was 9.58% higher than the WLC group at post-test controlling for pre-test scores, with a large effect size (Table 4; Fig. 2).

For response time across all three blocks, no significant main effects of time (congruent: b = -32.78, p = .30; incongruent: b = -10.40, p = .71; mixed: b = 7.22, p = .79) were found. No significant group by time interactions were found; effect sizes ranged from small-to-medium. Hypothesis 1, that direct assessment of children's executive functioning would be higher at post-test for the MMA group compared to the WLC, was partially-supported for accuracy, but not for response times, on the Hearts & Flowers test.

Hypothesis 2, to examine parent-reported executive functioning following a MMA intervention, was examined using a linear mixed-effects model. Group status (MMA group vs. WLC group), Time (pre- vs. post-test), and their interaction were posited as the predictors of the parent report of



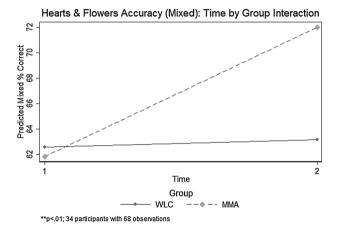
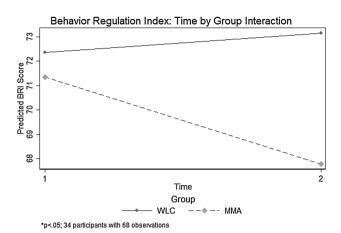
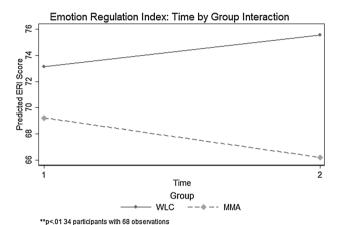


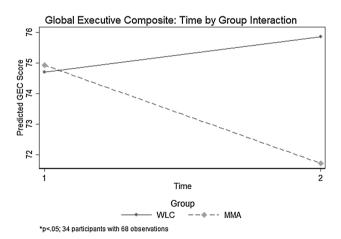
Fig. 2 Time by group interactions on Hearts & Flowers accuracy: congruent and mixed blocks

executive functioning on the BRIEF-2. In terms of parentreported behavior regulation (BRI), results indicated no main effect of time (b = -1.00, p = .30). However, there was a statistically significant interaction between group and predicted behavior regulation over time, such that the MMA group had 4.37 lower BRI score at post-test, as compared to the WLC group and controlling for pre-test scores, with a medium-to-large effect size (Table 4; Fig. 3). In terms of parent-reported emotion regulation (ERI), results also indicated no significant main effect of time (b = .18, p = .88) and a significant interaction between group and predicted emotion regulation over time, such that the MMA group had 5.40 lower ERI score at post-test, as compared to the WLC group and controlling for pre-test scores, with a large effect size (Table 4; Fig. 3).

In terms of parent-reported cognitive regulation (CRI), no significant main effect of time or significant interaction between group and predicted CRI (small-to-medium effect size) over time was found. Finally, in terms of parent-reported global executive functioning (GEC), no significant main effect of time was found (b = -.65, p = .51).







**Fig. 3** Time by group interactions on parent-reported BRIEF-2: behavior regulation index, emotion regulation index, and global executive composite

However, a significant interaction between group and predicted global executive functioning over time was found. The MMA group had 4.36 lower GEC score at post-test, as compared to the WLC group and controlling for pre-test scores, with a large effect size (Table 4; Fig. 3). Hypothesis 2, that parent-reported executive functioning would be higher at post-test for the MMA group compared to the WLC, was supported for the behavior and emotion indexes, and for global EF.

## Discussion

Intervention studies have found that executive functions (EFs) are malleable and can be enhanced with effortful practice. The goal of the current study was to examine the effectiveness of a MMA intervention for improving EFs in children with ASD. The current study is novel in its examination of both direct and parent-reported assessments of executive functioning in children with ASD, its study of multiple components of EFs, and its consideration of both improvements in executive functioning and lessening of executive deficits. The study is noteworthy for its rigorous implementation of a MMA intervention in collaboration with a community-based martial arts studio. Additionally, the sample was economically and racially/ethnically diverse, and diverse in terms of child intellectual functioning and autism symptom severity.

The MMA intervention consisted of an average of 26 sessions over 13 weeks. Child-based and parental assessments were conducted before and after the intervention. Support was found for hypothesis 1 regarding intervention group and direct assessment of executive functioning. Overall, results indicated that a traditional approach to MMA training increased executive functioning in 8- to 11-year-old children with ASD compared to children in a waitlist control group. Specifically, as assessed on the Hearts & Flowers test, all three core domains of executive functioning improved. Because the accuracy findings with the congruent block (i.e., working memory only) and mixed block (i.e., all three EFs: working memory, behavioral inhibition, and cognitive flexibility) were significant, but the incongruent block (i.e., both working memory and behavioral inhibition) was not, the results suggest that behavioral inhibition was the least improved of the three EFs.

Regarding hypothesis 2, the findings from the parentreported BRIEF-2 suggest that the EFs acquired in the context of the MMA intervention were generalized to contexts observable by parents. Of the three subscales, emotion regulation had the largest effect size. Lakes and Hoyts (2004) found that self-regulation, which included emotion and behavior regulation, improved after martial arts training in TD children. Cognitive regulation was not found to be significant; this was unexpected. The MMA intervention challenged and strengthened the behavior and emotion regulation domains, but likely did not provide sufficient opportunities to challenge cognitive regulation, which included more complex EFs subcomponents such as planning and organizing. Possibly, the present intervention successfully targeted core but not complex EFs. Future research should examine which specific components of cognitive regulation, if any, can be feasibly targeted by martial arts training.

Sample size constrained the ability to sacrifice degrees of freedom in order to control for child gender, which differed between the MMA intervention and waitlist control (WLC) groups. Despite random assignment, the MMA group was composed entirely of boys, whereas the WLC group had six girls. Additional analyses were conducted removing the six girls from the WLC group, so that there were 14 boys in each of the MMA and WLC groups (total n = 28). The results omitting girls were robust and mostly consistent with the reported results that included girls; the exception was the behavior regulation index on the BRIEF-2, which became marginal. Although all significant findings were maintained with a boys-only sample, future research should aim to equalize the number of girls and boys in each group and further explore the possibility of gender differences.

The present study's findings that a traditional approach to martial arts training challenged and strengthened the behavior and emotion regulation domains of EF, are consistent with and expand on previous research linking martial arts with decreased stereotypic behaviors among children with ASD (Bahrami et al. 2012) and improved self-control findings (Chan et al. 2013). The findings from the direct assessment of children's executive functioning suggest that cognitive flexibility and working memory are also challenged during martial arts training. Because EFs are multiple interrelated functions, and different facets may be differentially associated with other aspects of child functioning, this study highlights the importance of including multiple components of EFs among children with ASD. The suggested mechanism behind the effectiveness of the MMA intervention was not only the variety of techniques, but also the physical activity incorporated into each class and the increasing complexity over the course of the intervention. Importantly, the current study used a traditional approach to teach the MMA style. The traditional approach's emphasis on character development and self-control over physical growth and competition is consistent with the approaches used in previous martial arts intervention studies that also yielded a number of participant benefits (Bahrami et al. 2012; Chan et al. 2013; Lakes and Hoyt 2004; Trulson 1986). Further research is needed to specify which elements of the intervention are key to the noted improvements. Future research also is needed to compare outcomes for specific martial arts styles (e.g., judo, karate, kickboxing, etc.) that also use a traditional approach to teaching, examining their potentially efficacious use to improve EFs in children with ASD.

A number of autism intervention studies have used a benchmark of 80% or greater as having "high treatment fidelity" (e.g., Mandell et al. 2013; Stahmer et al. 2015; see

Reichow 2011 for fidelity guidelines for autism field trials). Based on this criterion, all but one of the fidelity components (duration of the intervention, 73%) in the current study had high treatment fidelity; participants missed more sessions than was intended, and as a result, children took more days to obtain the dosage than was intended at design.

#### **Study Limitations**

The main limitation of the present study was the sample size, which limited power. As noted above, the gender distribution in the two study groups was not equal. The overall preponderance of boys in the sample was not surprising given that ASD is four times more common among males than females (Baio et al. 2018), and because martial arts is stereotypically an activity that tends to attract more boys than girls. Future research should attempt to appeal to girls and enroll more girls in the intervention, which would increase the gender diversity of the martial arts class. Subsequent analyses with a larger sample size should also control for child intellectual functioning, which was heterogeneous in the present study.

Participating in the study was time intensive; families were asked to attend the MMA class two times per week for a minimum of 3 months. Busy family lives made it difficult for some of the children to complete the classes within the anticipated window. Families raising children with ASD tend to center their lives around the child with ASD; time demands for child care and therapy (including transporting the child to appointments, juggling different types of therapies, etc.) impact the entire family unit (Myers et al. 2009). Future research could offer more MMA classes throughout the week and weekend to accommodate to families' schedules. Longitudinal followup of study participants would be useful to see whether the significant executive functioning benefits endure.

Further research should include a comparison group of children with ASD enrolled in non-martial arts sports (e.g., basketball). Different sports certainly still have the potential to be cognitively complex and challenge EFs, but children would need to be able to work in pairs in the group setting. Because martial arts typically involve dyads rather than interaction in a group, addressing executive functioning challenges in children with ASD is more feasible in martial arts than in sports. In addition to isolating which parts of the martial arts intervention accounted for the observed improvements, future research could address whether these components can be mapped onto sports and other activities. For example, Diamond and Lee (2011) reviewed a variety of activities, such as mindfulness and yoga, as possible alternative activities that pick up on the complexity of martial arts and its meditative elements. In the present study, the meditation and breathing exercises were not separated out from the MMA routines and rather, were practiced at the beginning of class to cultivate mindfulness. Flook et al.

(2010) reported that mindfulness training improved EFs in TD children. Future research should isolate these components to determine if mindfulness training uniquely contributes to better EFs, or if it interacts with the martial arts training to help improve EFs.

Parents in the study were not blind to the condition their child was assigned, so reporter bias is possible on the parentreport measure. Parents may have been inclined to report improvement due to their knowledge of their child's participation. However, pre- and post-test assessments were at minimum 3 months apart, which may have lessened the likelihood that parents remembered their previous responses. In addition, the direct child assessment of executive functioning, the Hearts & Flowers test, yielded significant findings, corroborating the parent reports of improved EFs. A possible limitation of the Hearts & Flowers test could be learning effects that occurred between pre- and post-test; however, in the current study, there was sufficient delay between administrations to mitigate that concern given the similar delay used in Schonert-Reichl et al. (2015). Moreover, if learning indeed took place, it should have occurred in both the MMA and WLC groups, yet the MMA group showed significantly better performance on accuracy in both the congruent and mixed blocks at post-test. Nonetheless, future studies might include multiple direct child assessments of executive functioning (e.g., Flanker task, Wisconsin Card Sorting Task, etc.) and multiple informants (e.g., caregivers, teachers) to replicate and further corroborate our findings.

Finally, a concern voiced by parents when martial arts is suggested as an activity is the possible increase of aggressive behaviors in children with ASD. We did not measure aggressive behaviors in our study. However, the research examining martial arts and aggressive behaviors in TD children does not support this direction of effects. Vertonghen and Theeboom (2010) reviewed studies that examined the social-psychological outcomes of martial arts practice. They highlighted nine studies conducted between 1991 and 2009 that examined the link between martial arts practice and aggressive behaviors (e.g., verbal aggression, hostility, violent and antisocial behavior) in cross-sectional and longitudinal designs of youth under age 18. They concluded that, in general, when martial arts practice used a traditional rather than modern approach to teaching (e.g., Trulson 1986), it was linked to lowered aggression and hostility; positive outcomes, such as higher self-esteem, better cognitive and affective self-regulation, and more prosocial behaviors were also found. Taken together, the research to date on the social-psychological outcomes of martial arts practice has found that longer duration of martial arts training, especially when a traditional approach is used, is associated with lower levels of aggression and positive outcomes in youth (see Vertonghen and Theeboom 2010 for a review). However, this literature continues to be mixed and further work is needed. The link between martial arts and aggression in children could vary depending on the style of the martial art (e.g., judo, karate, kickboxing, etc.). For example, Reynes and Lorants (2002a, b) compared a traditional approach to judo versus a traditional approach to karate and found that children who practiced judo reported more aggression than the karate and control groups. Finally, the link between martial arts and aggression in children could also vary depending on individual child characteristics (e.g., temperamental traits) and characteristics of the family (e.g., cultural values, parenting attitudes and practices).

## **Study Strengths**

The current study used both parent-report and direct child measures of executive functioning. Additional strengths of the study include random assignment to group, and within the MMA classes, blindness of the instructors and TD peer buddies to study participants. Another strength of the intervention program was that the families did not pay for the classes. The MMA classes were hosted by the academy owner, and the instructors who volunteered to teach the classes were not financially compensated. Instead of screening for only high-functioning children, we included lower-functioning children as well, as measured by intellectual functioning on the WASI-II (Wechsler 2011) and autism symptom severity on the ADOS-2 assessment (Lord et al. 2012). Future research with a larger sample should examine if the intervention differentially affects children with ASD of varying functioning levels, and the magnitude of these possible effects. In one respect, higher-functioning children may benefit more from the executive functioning scaffolding because they have better verbal comprehension, but on the other hand, lower-functioning children may have more room for improvement.

## **Conclusions and Significance**

A MMA intervention program was implemented with school-aged children with ASD, and improvements were found on both parent-reported and direct child measures. The next steps would be to further adapt the program based on the findings and replicate it with the suggested improvements to study design. The theoretical and applied implications of the present study are expected to have relevance for the field of autism intervention research and for the autism community. On a theoretical level, the present study supplements the current literature on the malleability of EFs among children with ASD. On an applied level, the present study is the first to our knowledge to use a multi-method approach to examine executive functioning outcomes of a MMA intervention in a sample of children with ASD. Although there are numerous recreational and therapy programs available to children with ASD, few are empirically-based with data on program efficacy vis-a-vis child outcomes; this missing step is crucial for evidence-based practice (Epp 2008; Reichow and Volkmar 2010; Reichow et al. 2008). The practice of MMA is a feasible way to improve executive functioning in school-aged children with ASD and targeting EFs has implications for improvements in externalizing behaviors and academic success. Furthermore, a traditional approach to MMA training may confer benefits that extend beyond the cognitive and behavioral domains to include improvements in mental and physical health, goal directedness that may appeal to those with ASD, socialization with peers, and overall quality of life.

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#### **Compliance with Ethical Standards**

**Conflict of interest** Janice N. Phung and Wendy A. Goldberg declare that they have no conflict of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent** Informed consent was obtained from all individual participants or their parent/guardian.

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