

Emotion Coregulation in Mother-Child Dyads: A Dynamic Systems Analysis of Children with and without Autism Spectrum Disorder

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Abstract Few studies have investigated patterns of emotion coregulation in families of children with Autism Spectrum Disorder (ASD) or contrasted the ways in which their emotion coregulation patterns differ from families of typically developing (TD) children. To address this gap, we used a dynamic systems approach to compare flexible structure and emotional content of coregulation between mothers and children (3–7 years) with ASD ($n = 47$) and TD children ($n = 26$). Mother-child play interactions in the home were videotaped and emotion-engagement states were coded in micro-level 5-s intervals based on behavioral and affective expressions. Analyses indicated that mother-child dyads in the ASD group spent more time than dyads in the TD group in mismatched emotion-engagement states (e.g., child negative/mother positive), and children with ASD spent more time than TD children engaged exclusively with objects. Mother-child dyads in the TD group stayed longer in mutual positive engagement states. Compared to dyads in the TD group, mother-child dyads in the ASD group exhibited greater flexibility (i.e., a wider range of emotional-engagement states, more frequent

changes in states, and less time in each state). These findings suggest that mothers and their children with ASD do not sustain dyadic positive engagement patterns in a low-stress environment. Findings confirmed the preference of children with ASD for objects over social partners, even when they are at home with their mothers, and elucidated a challenging mother-child interactional style. Results have implications for mother-child interventions aimed at regulating negative emotional states and sustaining positive ones in families raising children with ASD.

Keywords Emotion coregulation · Flexibility · Dynamic systems · Autism spectrum disorder

Early theorists posited that our understanding of typical and atypical child development relies on analyses of the emotion regulation process (Cole et al. 1994). Emotion regulation (i.e., how individuals manage their emotional experiences and expressions) involves automatic and intentional modifications of emotional states (and by extension behavioral responses) to promote adaptive or goal-directed behavior; these transitions between states are commonly referred to as flexibility (Thompson 1994). Negative emotional states tend to decrease attentional control and constrict behavioral responses, whereas positive emotions tend to increase attentional control and expand behavioral responses to environmental demands (Fredrickson et al. 2000). Thus, increased flexibility is generally considered an adaptive characteristic. Specific to early childhood development, internal and external regulation, including flexibility, are critical as the child shifts from depending on caregivers for emotion regulation (i.e., external regulation) to self-regulatory (i.e., internal regulation) processes (Tronick 1989).

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Typically developing (TD) children and those with neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) regulate their behaviors within the context of their emotional and social environments. ASD is a neurodevelopmental disorder that increased markedly in prevalence from 2002 to 2010 (Centers for Disease Control and Prevention (CDC) 2016). Recent estimates, based on a tracking system of 8-year old children living in 11 communities in the U.S., are that 1 in 68 children have been identified with ASD (CDC 2016). Autism Spectrum Disorder, as defined in the revised *The Diagnostic and Statistical Manual of Mental Disorders* (5th Ed.; DSM-5; American Psychiatric Association (APA) 2013) features restricted and repetitive behaviors and persistent deficits in social communication and social interaction across multiple contexts, including deficits in social-emotional reciprocity, nonverbal communication behaviors, and in developing, maintaining and understanding social relationships (American Psychiatric Association 2013). Children with ASD may not use adaptive emotion regulation strategies when presented with a challenging or frustrating situation. Instead, they may react with intense emotional responses and poor emotional control (Mazefsky et al. 2013).

Research over the past two decades on the relationship between emotion regulation and social impairment in children with ASD has focused on emotion recognition, emotional experiences, and emotion regulation strategies (Mazefsky et al. 2012, 2013). Children with ASD tend to be less accurate with their identification of emotions and recognition of facial expressions across the six basic emotions (e.g., happiness, anger) (Lozier et al. 2014). Two studies demonstrated that, compared to TD children, children with ASD tended to use more avoidance and venting strategies and employed less attention shifting and inhibitory control strategies in a frustration-eliciting stimulus (Konstantareas and Stewart 2006; Jahromi et al. 2012). However, in a study where mothers and children were observed across three interaction contexts (free-play, a structured communication context, and a face-to-face interaction), young children with ASD did not differ from TD children in frequency or duration of smiles and frowns, although they showed fewer smiles in direct response to their mothers' smile (Dawson et al. 1990). The latter finding suggests that while children with ASD may experience a similar range of emotions as TD children, their emotional response patterns may differ.

Low flexibility and constricted affect, cognition, and behavior are common characteristics of ASD. Repetitive behaviors are negatively correlated with cognitive flexibility (Lopez et al. 2005; Yerys et al. 2009); children with ASD perform significantly worse than TD children across tasks requiring cognitive flexibility (Yeung et al. 2015). Yet few studies have examined emotional aspects of flexibility in children with ASD. At present, it is unknown whether children with ASD, who are more vulnerable to negative emotional states and rigid behaviors and cognitions, have difficulty switching from

negative to positive emotional states across different social contexts (Mazefsky et al. 2012).

Children with ASD typically display problems with the quality and quantity of joint attention (i.e., shared focus on an object, person, or event with a social partner) (Mundy et al. 1986). They have particular deficits in sharing positive affect during such joint attention (Kasari et al. 1990), thus decreasing shared interests and enjoyment with others (APA 2013). Such deficiencies may have significant repercussions for the development of adaptive skills for bonding, connecting, and enjoying positive interactions with others, starting with their parents during early development.

Coregulation, the interaction of a parent and child dyad while they co-construct affective states (Tronick 1989), is a key component of everyday interaction in families. This dyadic emotional-coregulation system is dynamic, with changing affective arousal and dampening aimed at maintaining an optimal emotional state (Butler and Randall 2013). Children's emotion regulation development arises out of repeated emotional experiences with their caregivers; this dyadic regulation lays the foundation for subsequent self-regulation (Sroufe 1996).

Research contrasting parental responsiveness to preschool-aged children with ASD and TD has been mixed, warranting future inquiry. Some findings indicated similar parenting styles and response frequencies (Adamson et al. 2001; Kasari et al. 1988). Other studies found divergent parental responses (Hirschler-Guttenberg et al. 2015) such as mothers of children with ASD engaging in more physical contact but less social verbal behavior than mothers of TD children (Doussard-Roosevelt et al. 2003).

Despite the important role of coregulation in child development, only recently have studies focused on families raising a child with ASD. Hirschler-Guttenberg et al. (2015) examined how children with ASD regulated both positive and negative emotions during free play with their mothers at home with microcoding of child and mother behavior. Observations indicated that preschoolers with ASD were less socially engaged, more withdrawn, and less compliant than their TD peers. Mother-child-with-ASD dyads also had lower reciprocity than TD dyads. Similarly, Larkin and colleagues (Larkin et al. 2015), using global ratings of engagement and regulation, found that dyads with a child with ASD had reduced coregulation capacity. They spent more time in a rigid interaction state or an instrumental communication state than did dyads without ASD.

Emotion coregulation processes in mothers and their children who have behavior problems have been examined in terms of dyadic flexibility. Prior research indicated children with externalizing and internalizing behaviors tend to have rigid interactions with their mothers, exhibiting fewer changes between positive and negative emotional states and longer duration in emotional states compared to children without these behavioral tendencies (Hollenstein et al. 2004). Granic et al. (2007) examined changes before and after cognitive-

behavioral therapy (CBT) with mothers and their children with aggression problems. Dyads where the child improved showed increased flexibility and spent more time in mutual positive emotion states. In contrast, dyads where the child did not improve exhibited decreased flexibility and spent less time in mutual positive states (time spent in mutual negative emotion states did not change in either group). This type of emotional flexibility, however, has not been quantitatively measured in mother-child interactions in samples of children with ASD.

In typical development, parent responses to the behavior of their children and their children's subsequent behavior occur in transactional exchanges (Sameroff 2009). Given that children with ASD often exhibit maladaptive behavioral patterns and a core feature of ASD is impaired interpersonal interaction, it is likely that parent-child transactional exchanges will operate differently than with TD children. Elucidating this variability could advance theoretical understandings of parent-child emotion regulation processes and improve clinical interventions for families with an ASD child and dysregulated families more generally. To our knowledge, no study has investigated micro-level coregulation processes involving both dyadic emotional flexibility and positive and negative emotion in families of children with ASD.

Dynamic Systems Analysis Approach to Studying Emotion Coregulation

Dynamic systems approaches have offered a promising way to investigate coregulatory processes as this method captures the moment-to-moment processes inherent in dyadic interaction (DiDonato et al. 2013; Lunkenheimer et al. 2011). Historically, operationalizing emotional processes within dyadic contexts has been difficult but in the past decade, the systems perspective has provided an exciting means for studying dyads (Hollenstein 2011). The State Space Grid (SSG) offers an innovative methodology to study these processes at a micro-level in real time.

The SSG, which is a configuration of the major possible states of a dynamic system (Thelen and Smith 1994), provides an objective, quantifiable means of capturing the valence of dynamic interactions. State space can be represented as a topographical landscape of interactions, where each individual in the dyad can be coded for a range of behavioral and affective expressions of emotion. Unlike other methods, SSG analyses can distinguish between total time spent in particular affective states from numerous but often fleeting interactions in those states. As a result, SSG analyses can capture the different aspects of emotional content of coregulation such as dyadic ability to initiate and sustain matching emotions (e.g., both members of the dyad display positive emotion) or mismatched emotions (e.g., one member of the dyad shows positive emotion and the other displays negative emotion).

Moreover, the SSG method can be used to distinguish children who have only externalizing problems from children who have comorbid externalizing and internalizing problems (Granic and Lamey 2002).

State space can also capture the flexibility-rigidity continuum in the structure of coregulation. A flexible structure refers to fluid movement from state to state, evidenced by more frequent changes and shorter duration in a given state (Hollenstein 2015). In other words, flexibility involves emotional and behavioral adaptation to a variety of circumstances. For example, Hollenstein and Lewis (2006) examined the relationship between negative emotion and flexibility during positive and negative discussion contexts within mother-adolescent interactions and found that flexibility was lowest when negative emotion was highest. A rigid system designates limited movement across the states (Hollenstein 2007): individuals switch states less often and persist in a given emotional state (Hollenstein 2015). A rigid system tends to have imbalanced negative and positive feedback processes, suggesting the presence of behavior problems or psychopathology (Hollenstein 2015). Therefore, rigidity involves a limited range of responses and constricted ability to adapt and modify psychologically and behaviorally to changing environmental demands. The SSG method has previously been applied to the assessment of rigidity in children with conduct problems (e.g., Hollenstein et al. 2004), although not ASD specifically.

The Current Study

Studies to date have not quantified dyadic flexibility and rigidity and the process of modulating the intensity and timing of both positive and negative emotions in mother-child dyadic interactions when the child has ASD. Yet such inquiries could help elucidate variability in dyadic capabilities, with key implications for interventions aimed at calming negative emotions and sustaining positive ones.

The current study used SSG to conceptualize coregulation and compare and contrast mother-child interactions in families with TD children and with children diagnosed with ASD. We observed mother-child dyadic interactions using a semi-structured play procedure and conducted micro-level comparisons of dyadic flexibility patterns and the positive- or negative-emotional content of coregulation between the dyads. Diagnostic group differences were expected based on characteristics of children with ASD and the greater possibility of their mothers showing features of the broader autism phenotype.

Two hypotheses were tested:

1. The group with dyads of mothers and their children with ASD will display significantly lower dyadic flexibility compared to the group with dyads of mothers and TD children. Specifically, compared to dyads in the TD group, dyads in the ASD group will cover fewer emotion

states, change states less frequently, and spend more time in a particular state.

2. Dyads in the TD group compared to dyads in the ASD group will differ in frequency and time spent in mutual positive engagement, mutual negative engagement, mismatched emotion-engagement (i.e., child positive/mother negative or child negative/mother positive), and object engagement states. Specifically, dyads in the ASD group will share less mutual positive interaction and will have more mutual negative and mismatched interactions. Additionally, with the mother present, children with ASD will more frequently and for a longer duration engage only with objects compared to TD children.

Methods

Participants

The current study analyzed videotapes from a previously conducted study of family interactions. Participants in the original study were 92 families with children aged 3–7 years; 60 families had children diagnosed with an ASD. Eighteen families did not complete the study questionnaires or did not have usable videotapes of parent-child interactions, leaving a final sample of 73 families for the present study (47 mother-child dyads in the ASD group and 26 mother-child dyads in the TD group). The ASD sample was 74.47% boys ($n = 35$) and the TD sample was 65.38% boys ($n = 17$). Mean age for ASD and TD groups of children was 5.27 years and 4.34 years, respectively. See Table 1 for detailed description of the sample including differences between ASD and TD groups.

Eligibility criteria for mothers included: (1) married or currently cohabitating, and (2) English speaking. To meet criteria for ASD, a child must have received a previous clinical diagnosis of ASD from a physician, licensed psychologist, and/or community center that provide services to children with developmental disabilities. Subsequent to the home visits, mothers were contacted by phone and invited to bring their child to a university-based laboratory for the Autism Diagnostic Observation Schedule-2 assessment (ADOS-2; Lord et al. 2012). Forty-five percent ($n = 21$) were reachable and available for ADOS-2 testing; 17 (33%) of children with ASD had their diagnoses confirmed by the ADOS-2. Four children with reported ASD diagnoses did not meet ADOS-2 criteria for ASD and were eliminated from further analyses. ADOS-2 scores were then compared with mothers' reports on the Social Communication Questionnaire (SCQ; Rutter et al. 2003) to confirm that TD children did not display symptoms of ASD. Using the recommended cutpoint of 11 for the SCQ

to maintain the greatest level of confidence in diagnostic categories, one child in the TD group who scored greater than 11 on the SCQ was eliminated from the study. Children with ADOS-2 assessments did not differ statistically from those who did not have them on the following sample descriptors: gender, age, or any family-level variables of SES (mother's education or employment status or household income).

Procedure

In total, 73 participants were eligible, agreed to participate, and provided data appropriate for the present analyses. All procedures were approved by the university's Institutional Review Board. Families were recruited from several counties in a large Western U.S. state using flyers distributed at medical offices and autism-related community events, and from a departmental database of families who had agreed to be contacted about participation in ongoing research. Additional participants were recruited through the Interactive Autism Network (IAN), an online database and research registry, e-mail (for IAN registered families meeting study criteria) and the website-based IAN Community Research Opportunities Bulletin Board.

Participating families were mailed a consent form and questionnaire packets and were scheduled for home visits. Extensively trained research assistants conducted the in-home assessments, which consisted of completion of questionnaires and a videotaped parent-child interaction during semi-structured play.

Measures

Demographics and Background Information Mothers provided their age, education, ethnicity, income level, and occupation as well as their children's age, gender, and any medical or behavioral health diagnoses, important medical events, and behavioral intervention history.

Broader Autism Phenotype Mothers completed the Broader Autism Phenotype Questionnaire (BAPQ; Hurley et al. 2007) to assess the possibility they themselves had personality and language characteristics that were phenotypically similar to, but milder than, ASD traits. Mothers rated their level of agreement to a series of statements such as "I like being around other people" and "I can tell when someone is not interested in what I am saying" on a scale ranging from 1 (*very rarely*) to 6 (*very often*). A total score was created by summing the responses to all of the items. Higher summary scores indicate stronger phenotype of symptoms. The BAPQ has demonstrated construct validity (Wainer et al. 2011), as well as strong internal consistency and sensitivity and specificity at about 80% for the total scale score (Hurley et al. 2007). In the current

Table 1 Characteristics of the sample ($N = 73$)

	Diagnostic groups $N = 73^a$		Test of between group differences (ASD vs. TD)	Effect size ^b Cohen's d (95% CI) or Cramér's V
	Autism Spectrum Disorder (ASD) $n = 47$	Typically Developing (TD) $n = 26$		
Child characteristics				
	$n(\%)$	$n(\%)$		
Gender				
Girls	12(25.53%)	9(34.62%)		
Boys	35(74.47%)	17(65.38%)	$\chi^2(1) = 0.67$	$V = 0.10$
	$M(SD)$	$M(SD)$		
Age	5.27(1.42)	4.34(1.12)	$t(70) = -2.85^{**}$	$d = -0.70(-1.19, -0.20)$
Mother characteristics				
	$M(SD)$	$M(SD)$		
Age	36.29(5.34)	35.47(5.89)	$t(70) = -0.61$	$d = -0.15(-0.63, 0.33)$
BAPQ ^c total score	86.48(17.68)	86.81(17.14)	$t(70) = 0.08$	$d = 0.02(-0.46, 0.50)$
	$n(\%)$	$n(\%)$		
Ethnicity				
Caucasian	20(43.48%)	12(46.15%)		
Asian/Asian-American	7(15.22%)	6(23.08%)		
Hispanic	11(23.91%)	2(7.69%)		
Mixed ethnicity	8(17.39%)	6(23.08%)	$\chi^2(3) = 3.29$	$V = 0.21$
Marital status				
Currently married	44(95.65%)	25(96.15%)		
Domestic partnership	2(4.35%)	1(3.85%)	$\chi^2(1) = 0.01$	$V = 0.01$
Employment status				
Full-time	17(36.96%)	12(46.15%)		
Part-time	10(21.74%)	1(3.85%)		
Unemployed/ Not working	19(41.30%)	13(50.0%)	$\chi^2(2) = 4.11$	$V = 0.24$
Education				
High school	5(10.87%)	0(0%)		
Some college	9(19.57%)	1(3.85%)		
Two-year degree	5(10.87%)	1(3.85%)		
Four-year college	15(32.61%)	9(34.62%)		
Master's or advanced credential	8(17.39%)	7(26.92%)		
Doctoral degree	4(8.70%)	8(30.77%)	$\chi^2(5) = 12.37^*$	$V = 0.41$
Income				
0–49,999	4(16.00%)	12(27.91%)		
50,000–99,999	5(20.00%)	13(30.23%)		
100,000–149,999	11(44.00%)	10(23.26%)		
150,000+	5(20.0%)	8(18.60%)	$\chi^2(3) = 3.80$	$V = 0.24$

^a Differences from total N are due to missing data

^b Cohen's d is presented for t-tests; Cramér's V for chi squares

^c BAPQ = Broad Autism Phenotype Questionnaire

* $p < 0.05$; ** $p < 0.01$

study, the internal consistency was excellent for mothers ($\alpha = 0.99$) in the both ASD and TD groups.

Screening and Diagnosis of ASD The Social Communication Questionnaire (SCQ; Rutter et al. 2003), a widely used screening tool for inclusion in research studies on autism, was completed by mothers. The Lifetime SCQ is a 40-item questionnaire in which parents report whether or not certain behaviors had been observed during the child's lifetime. The measure has well-established reliability and validity (Rutter et al. 2003). Scores above 15 suggest that the individual is likely to have ASD; a cutoff of 11 has been used successfully in determinations of sensitivity (Allen et al. 2007).

The Autism Diagnostic Observation Schedule 2 (ADOS-2; Lord et al. 2012), a semi-structured observational procedure for assessing autism spectrum disorder, was used to confirm clinical diagnoses of ASD that parents reported. A trained researcher interacted with the children during play and asked questions of them to assess communication, social interaction, play, and restricted and repetitive behaviors. Standardized scores are compared with cutoff scores to yield a classification of Autism, Autism Spectrum, and Non-spectrum (Lord et al. 2012).

Emotion Coregulation of Dyadic Mother-Child Interaction The Three Boxes procedure (NICHD Early Child Care Research Network 1999; Tamis-LeMonda et al. 2004; Vandell 1979) was used to observe mother-child interaction during play in the home setting. Three numbered plastic boxes with age-appropriate toys were provided ((1) books; (2) cash register, play money, play food; (3) Mega Bloks). Mothers were asked to play with their child as they normally would for 10 min but to open the boxes in the numbered order. No other restrictions were placed on this semi-structured play task. Mother-child interaction was videotaped for later coding.

Interactions were taped for approximately 10 min; emotion coregulation was operationalized by affective, experience-sharing emotion-engagement states. Emotion-engagement states are a combination of mutually exclusive facial expression, vocalization, attention, body posture and behavior that mothers and children display during interactions. Positive, negative, and disengagement states were coded for quality and quantity in terms of low, medium and high levels. The micro-level emotion-engagement coding scheme was developed using an iterative process by the research team until consensus on categories of observational behavior cues was achieved (See Online Supplementary Table 1 and Table 2 for precise descriptions of the coding schemes for children and mothers, respectively).

Child Emotion-Engagement States *Child positive engagement* refers to children's initiating or maintaining social interaction with their mothers by demonstrating neutral or

positive affect (e.g., smiles), showing neutral or positive vocalizations (e.g., exciting vocal tones), focusing sustained attention on mother or a joint activity, displaying positive body posture (e.g., leaning towards mother), or executing positive interaction behavior (e.g., turn-taking). *Child negative engagement* refers to protesting the mother by appearing impatient or angry (e.g., crying), expressing distressed vocalizations (e.g., fussing), displaying negative body posture (e.g., arching back), and refusing or rejecting interaction with the mother (e.g., pushing away). *Child disengagement* refers to withdrawing from the mother or a joint activity by showing flat affect or appearing listless, anxious or sad, while not attending to the mother or a joint activity. Finally, *child-object engagement* refers to children engaging with toys without interacting with their mothers.

Mother Emotion-Engagement States *Mother positive engagement* refers to mothers being sensitive and contingent to children's emotional needs by expressing neutral or positive affect (e.g., a playful facial expression), expressing neutral or positive vocalizations (e.g., a genuinely excited tone), focusing attention on children or a joint activity (e.g., monitoring children's play), and using positive behavior to interact with children (e.g., teaching about a new toy). *Mother negative engagement* refers to venting distress to children by appearing angry, hostile, irritable (e.g., eye rolling), showing negative vocalizations (e.g., a harsh tone), displaying tense body posture (e.g., crossing arms), and controlling or intruding on children's exploration (e.g., grabbing the child's hand). *Mother disengagement* refers to mothers disconnecting from children or a joint activity by appearing bored, flat, uninterested (e.g., by talking to a sibling), displaying lethargy (e.g., slouching), avoiding interaction with children (e.g., ignoring their questions), and not attending to the children or a joint task (e.g., talking to child's sibling).

Reliability of Dyadic Emotion-Engagement States Following extensive training that achieved intercoder reliability of $k > .70$ on pilot cases, four research assistants who were blind to the diagnostic group coded dyadic interactions every 5-s using an observational software (INTERACT 9.07; Mangold 2007). Emotion regulation states of mothers and children were coded independently. Inter-coder reliability for children's and mothers' emotion engagement was 91.07% ($k = 0.82$) and 91.76% ($k = 0.81$), respectively. Discrepant codes were resolved through discussion until consensus was reached.

State Space Grid Measures The coded emotion-engagement data were imported into GridWare 1.1 (Lamey et al. 2004). Emotion-engagement states between mothers and children were plotted in real time in a grid that represented major possible dyadic interaction combinations, with mothers'

Table 2 Comparisons of autism spectrum disorder (ASD) and typically developing (TD) children on key space state grid and region-level variables ($N=73$)^a

	Diagnostic groups $N = 73$				Test of differences between groups (ASD vs. TD)	Effect size Cohen's d (95% CI)
	ASD $n = 47$		TD $n = 26$			
	M	SD	M	SD		
Grid-level variables						
Dispersion	0.79	0.11	0.68	0.14	$t(71) = -3.50^{***}$	-0.86(-1.35, -0.35)
Transition	74.85	17.73	57.31	14.39	$t(71) = -4.32^{***}$	-1.05(-1.56, -0.54)
AMD ^b	9.04	2.56	11.49	3.00	$t(71) = 3.94^{***}$	0.96(0.45, 1.46)
Region-level variables						
Visits						
Mutual positive	14.96	5.01	11.19	5.26	$t(71) = -3.02^{**}$	-0.74(-1.23, -0.24)
Mutual negative	3.53	4.40	1.04	2.60	$t(71) = -2.64^*$	-0.65(-1.13, -0.15)
Child positive/mother negative	8.27	6.01	5.62	5.90	$t(71) = -1.82^\dagger$	-0.45(-0.93, 0.04)
Child negative/mother positive	2.66	3.15	0.81	1.88	$t(71) = -2.74^{**}$	-0.67(-1.16, -0.18)
Child object	10.75	6.24	7.27	4.32	$t(71) = -2.52^*$	-0.62(-1.10, -0.12)
Duration						
Mutual positive	35.42	33.63	61.34	56.95	$t(71) = 2.45^*$	0.60(0.11, 1.09)
Mutual negative	35.04	51.15	9.81	26.81	$t(71) = 2.34^*$	-0.57(-1.06, -0.08)
Child positive/mother negative	80.76	77.37	67.42	79.18	$t(71) = -0.67$	-0.17(-0.65, 0.31)
Child negative/mother positive	16.81	20.70	4.27	10.37	$t(71) = -2.89^{**}$	-0.71(-1.20, -0.21)
Child object	112.30	89.92	75.88	65.30	$t(71) = -1.81^\dagger$	-0.44(-0.93, 0.04)

^a Analyses were conducted using the standardized values for region-level variables; the pattern of results was identical. For ease of interpretation, unstandardized estimates with the raw data are presented

^b AMD = Mean duration per visit

[†] $p < 0.10$; * $p < .05$; ** $p < .01$; *** $p < .001$

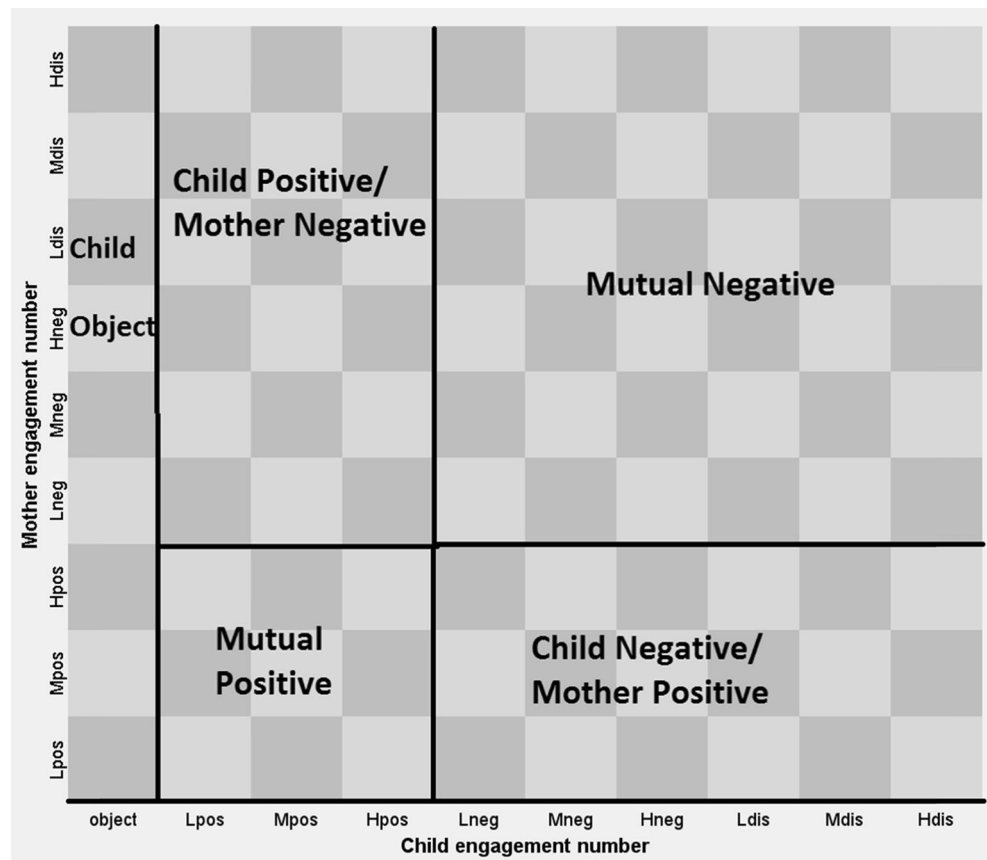
engagement on the y -axis and children's engagement on the x -axis (see Fig. 1). The x -axis (children's emotion engagement) consisted of ten states: one self-object state, three positive states (low, medium, and high), three negative states (low, medium, and high), and three disengagement states (low, medium, and high). The y -axis (mothers' emotion engagement) consisted of nine states, including three positive states (low, medium, and high), three negative states (low, medium, and high) and three disengagement states (low, medium, and high). A ten-by-nine child-by-mother matrix was then created with each of the 90 cells on the grid indexing a possible dyadic state. Emotion engagement was plotted every five seconds. For every change in dyadic location on the x - y plane, a new point is plotted with a trajectory line connecting the old and new point. The coded interactions were then characterized along a number of conceptual parameters for statistical analyses.

The SSG was used to capture variability of emotion intensity and valence in dyadic interactions in terms of *grid-level* and *region-level* variables. *Grid-level parameters of flexibility* characterized the flexible structure of coregulation over the entire grid. Three parameters were constructed: (1)

Dispersion was space covered, calculated as the sum of squared proportional durations across all cells adjusted for the number of cells in the grid with the value from zero (all behavior in one cell) to 1 (behaviors spread out in the grid of behavioral states). Higher values indicated more area covered; (2) *Transitions* were the number of movements between cells on the grid as indicated by the lines, with higher values indicating more frequent changes in dyadic behavioral states; and (3) *Average Mean Duration (AMD)* was the mean duration of each visit to a particular interaction cell. Higher values indicated spending longer times in a particular state. Dynamically flexible interactions had high transitions, high dispersion, and low AMD.

Five *region-level parameters* were identified to capture the emotional content of coregulation between mother and child (see Fig. 1): (1) *Mutual positive*: both mother and child in any positive engagement state; (2) *Mutual negative*: mother and child both in a negative (low, medium, or high) or disengaged (low, medium, or high) state; (3) *Child positive/mother negative*: child in one of the three positive states and mother in either a negative or disengaged engagement state; (4) *Child negative/mother positive*: child in a negative or disengaged

Fig. 1 Five regions in the State Space Grid.
 Note. Object = child engagement with toy/objects. Lpos = low positive engagement. Mpos = medium positive engagement. Hpos = high positive engagement. Lneg = low negative engagement. Mneg = medium negative engagement. Hneg = high negative engagement. Ldis = low disengagement. Mdis = medium disengagement. Hdis = high disengagement



engagement state and mother in a positive state, and (5) *Child object*: child fixated on a play object; mother in any engagement state (positive, negative, or disengaged). For each of the five regions, three parameters – *region-visits*, *region-duration*, *region-ratio* – were constructed to describe dyadic emotional content of coregulation. *Region-visits* measured ability to initiate a dyadic emotional state indicated by the number of times the dyad was observed moving into a defined dyadic state (region) from a previous state (region) on the grid. *Region-duration* measured ability to sustain a dyadic emotional state indicated by the duration of time in each dyadic state (region). A *region-ratio* variable operationalized a similar construct as *region-duration*, except that this variable accounted for slight variations in the length of the experimental procedure; it was calculated by dividing *region-duration* by the total duration spent in the Three Boxes procedure.

Analytic Strategy

Several steps were taken to determine valid scores and reduce measurement discrepancy. The presence of outliers was evaluated; any score greater than three standard deviations above the mean was reduced to that cutpoint (i.e., the value for that case was changed to three standard deviations above the

overall mean for that variable). In total, ten values (seven cases) were reduced.

Bivariate comparisons (*t*-tests for continuous variables and chi-square tests for categorical variables) between ASD and TD groups were conducted for demographic descriptors (gender, child’s age, family household income, mother’s age, employment status, ethnicity, and marital status), SCQ of child, and for grid- and region-level parameter variables. Of note, in bivariate relationships, region-level duration variables were *not* divided by total duration of the interaction for easier interpretation of descriptive statistics. Total recorded time is presented in Table 2.

Next, grid-level variables (total duration of interaction, dispersion, transition, and AMD) and each of the two region-level variables (*region-visits* and *region-duration*) for mutual positive, mutual negative, child positive/mother negative, mother negative/child positive, and child object were tested using multivariate ordinary least squares (OLS) regression analyses in two steps. First, outcome variables were examined using the following predictors: child ASD diagnosis, child age, child gender, mother’s education, and mother’s score on the BAPQ. Second, models were trimmed by eliminating covariates that did not predict outcomes both for parsimony and to conserve power.

Results

Preliminary Analyses of Differences between Groups

Table 1 presents descriptive statistics and bivariate comparisons between ASD and TD groups for key covariates and demographic predictors. Children with ASD were significantly older than TD children. Mothers of children with ASD did not differ from mothers of TD children on age, BAPQ score, marital or employment status, or income (means and standard deviations are presented in Table 1). However, mothers of children with ASD were more highly educated than mothers of TD children.

Emotional coregulation of dyadic interactions was contrasted by diagnostic group (ASD, TD) through bivariate comparisons on SSG grid- and region-level variables (see Table 2, which also presents means and standard deviations for the outcome variables by group). The ASD and TD groups did not differ significantly on total duration of dyadic interaction. On grid-level parameters, dyads in the ASD group exhibited greater flexibility as indicated by higher dispersion, higher transition, and lower AMD compared to dyads in the TD group. The ASD and TD groups differed on measures of region-visits and region-duration. Dyads in the ASD group had significantly more visits to four of the regions (mutual positive, mutual negative, child negative/mother positive, and child object), but they had shorter duration in the mutual-positive region, and longer duration in mutual-negative, child-negative/mother-positive, and child-object regions. Duration of time spent in the child-positive/mother-negative region was not significantly different between dyads in the two diagnostic groups.

Multivariate Analyses of Flexibility and Emotional Content of Coregulation

Tables 3 and 4 present multivariate ordinary least squares regression analyses for grid- and region-level variables, respectively. Maternal education and child age were significant predictors of outcome variables and were included as covariates in final multivariate models. Notably, higher education was negatively correlated with dispersion and AMD, and positively correlated with number of transitions. The pattern of results in adjusted models for both grid- and region-level outcomes was consistent with those in bivariate models. As in bivariate analyses, when controlling for covariates, dyads in the ASD group exhibited higher dispersion, higher transition, and lower AMD, indicating overall greater flexibility (see Table 3). As illustrated in Table 4, dyads in the ASD group displayed more frequent visits to three of the five regions (mutual positive, child negative/mother positive, and child object). Dyads in the ASD group also spent less time in the mutual-positive region and more time in the child-negative/mother-positive and child-object regions.

Discussion

The present study sought to compare emotion coregulation processes in mother-child dyads when children have ASD compared to dyads with TD children. It provided an innovative way to conceptualize and measure coregulation by using a dynamic systems approach to capture momentary mother-child interactions and to compare the flexible structure and emotional content of coregulation in mother-child dyads. We hypothesized that mother-child dyads in the ASD group would show a lower level of dyadic flexibility, a lower degree of shared positive engagement, higher mutual negative engagement, higher mismatched interaction, and higher object engagement when compared to the interactions of dyads in the TD group. The higher dyadic flexibility and greater frequency of initiating mutual positive emotion revealed in ASD dyads relative to TD dyads ran counter to our initial hypotheses. This unexpected finding illustrates a potential strength in the emotional content of dyadic interaction between mothers and their children with ASD (i.e., the ability to engage in positive emotional interactions) while highlighting a potential deficiency in their ability to sustain these positive exchanges or parlay them into adaptive behavior. This finding, revealed by the novel application of the SSG, may be quite useful in designing new interventions that can more effectively capitalize on strengths and target weaknesses.

Dyadic Flexibility of Mother-Child Interactions

Flexibility was conceptualized by the SSG in terms of displaying a range of emotional expressions, the capacity for switching between emotions, and the tendency to perseverate in a particular emotional state (Granic et al. 2007; Hollenstein et al. 2004). These patterns of dyadic emotional coregulation were examined using the SSG within the context of this theoretical and practical framework. Unexpectedly, compared to dyads in the TD group, dyads in the ASD group showed greater flexibility in the structure of their emotion coregulation. Moreover, our results indicated that dyads in the ASD group were able to engage in positive dyadic interaction, although they did not regularly sustain these positive states. Initially, these results may seem inconsistent with the characterization of ASD as a disorder of rigidity rather than flexibility. Two factors may explain the counterintuitive findings: (1) the context of the emotional quality and intensity of the Three Boxes procedure for mother-child interaction, and (2) the context of the mother-child relationship.

Prior SSG studies tended to conceptualize flexibility of dyadic interaction in contexts of positive-negative-positive sequences (Granic and Lamey 2002, 2007; Hollenstein et al. 2004). The Three Boxes procedure utilized in our study would typically be implemented as a task primarily meant to elicit positive emotions. As such, adaptive responses would involve

Table 3 Multivariate ordinary least squares regression analyses of autism spectrum disorder (ASD) diagnostic group, key covariates, and state space grid parameters ($N = 73$)^a

	Dispersion β (95% CI)	Transition β (95% CI)	AMD ^b β (95% CI)
ASD ^c diagnostic group ^d	0.86(0.33, 1.38)**	1.20(0.71, 1.69)***	-1.15(-1.63, -0.67)***
Child age	-0.19(-0.42, 0.04)	-0.29(-0.51, -0.07)*	0.39(0.18, 0.61)***
Mother's education	-0.07(-0.31, 0.17)	0.07(-0.15, 0.30)	-0.02(-0.24, 0.20)
Constant	-0.56(-0.96, -0.16)**	-0.77(-1.14, -0.39)***	0.74(0.37, 1.10)***
Model statistics	$F(3, 68) = 4.99, p = 0.004,$ Adjusted $R^2 = 0.14$	$F(3, 68) = 8.92, p < 0.001,$ Adjusted $R^2 = 0.25$	$F(3, 68) = 10.30, p < 0.001,$ Adjusted $R^2 = 0.28$

^a Differences from total N are due to missing data

^b AMD = Mean duration per visit

^c ASD = Autism spectrum disorder

^d ASD diagnostic group, 0 = ASD negative, 1 = ASD positive

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

high levels of positive emotion and interaction in general. In the current study, the greater flexibility exhibited in dyads with children with ASD indicated that these dyads accessed a wider range of engagement states, both positive and negative, during a task where negative states are relatively unexpected. In other words, highly flexible patterns of coregulation in the dyads with children with ASD imply impairments in the capacity to sustain positive-engagement states in the low-stress context of the family home, with mother present, during semi-structured play with appealing toys. This interpretation is congruent with prior studies of emotion range and regulation in children with ASD. In a mild frustration context, children with ASD used a greater range of emotion regulation strategies but were less effective compared to TD children (Konstantareas and Stewart 2006). Children with ASD also displayed a greater variety of affect expressions than TD children during a child-experimenter interaction (Yirmiya et al. 1989). Thus, the wide range of emotion states and strategies may not necessarily imply adaptive flexibility for children with ASD but rather reveal problems with sustaining positive interaction even in a low-stress context.

Greater structural flexibility in children with ASD must also be considered within the context of the mother-child relationship. Prior research on restricted interests and cognitive perseveration focused primarily on the individual child with ASD (Mostert-Kerckhoffs et al. 2015; Warreyn et al. 2007), whereas the current study emphasized the dyad. During the Three Boxes procedure, mothers facilitated their children's exploration of a variety of toys. The highly flexible interaction pattern observed in the ASD group may signify that mothers of children with ASD were either making an effort to extend the restricted interests and cognitive perseveration that characterize children with ASD, or were using a multifaceted approach to manage their emotionally labile

children with ASD. In other words, mothers of children with ASD appeared able to implement effective parenting strategies that facilitated their children's ability to shift into positive emotional states. The resulting increased capacity for flexible coregulation may provide opportunities to increase the time spent in positive states, improving overall coregulation in children with ASD and their mothers.

Indeed, our results are consistent with recent studies in which the social and emotional development of children with ASD appears different when analyzed within the context of interactions with primary caregivers (Hirschler-Guttenberg et al. 2015; Jahromi et al. 2012; Warreyn et al. 2005). For example, during the observation of spontaneous play, mothers of children with and without ASD were asked to respond briefly to their children's demands and also asked to restrict further interactions (Warreyn et al. 2005). Results of this study indicated that children with ASD showed less joint attention with their mothers but did not have clinically significant impairments in social referencing compared to TD children. In fact, children with ASD initiated more social referencing with their mothers than did TD children perhaps because of mothers' responses to experiment instructions to restrict their interactions, which reduced the level of social stimulation and could have led the children to initiate more. In our study, mothers were encouraged to play with their children.

Emotional Content of Coregulation

Regression models controlling for covariates indicated that, compared to the TD group, dyads in the ASD group shared a higher degree of frequency and duration of time spent in object engagement and one type of mismatched interaction (child negative/mother positive). The first finding is consistent

Table 4 Multivariate ordinary least squares regression analysis of autism spectrum disorder (ASD) diagnostic group, key covariates and state space grid region-level parameters ($N = 73$)^a

Region visits variables ^b		Mutual negative	Child positive/Mother negative	Child negative/Mother positive	Child object
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
ASD ^c diagnostic group ^d	0.64(0.09, 1.18)*	0.49(-0.06, 1.04)	0.41(-0.16, 0.98)	0.56(0.04, 1.09)*	0.70(0.14, 1.25)*
Child age	-0.18(-0.42, 0.06)	-0.07(0.31, 0.17)	0.02(-0.23, 0.27)	-0.13(-0.36, 0.10)	-0.17(-0.42, 0.07)
Mother's education	-0.14(-0.38, 0.10)	-0.15(-0.40, 0.10)	-0.02(-0.28, 0.24)	-0.13(-0.37, 0.11)	0.04(-0.22, 0.29)
Constant	-0.41(-0.82, -0.01)†	-0.32(-0.74, 0.10)	-0.25(-0.68, 0.18)	-0.38(-0.78, -0.02)	-0.44(-0.87, -0.02)*
Model Statistics	$F(3, 68) = 3.61, p = 0.018,$ Adjusted $R^2 = 0.10$	$F(3, 68) = 2.45, p = 0.06,$ Adjusted $R^2 = 0.06$	$F(3, 68) = 1.05, p = 0.376,$ Adjusted $R^2 = 0.002$	$F(3, 68) = 3.14, p = 0.031,$ Adjusted $R^2 = 0.08$	$F(3, 68) = 2.43, p = 0.072,$ Adjusted $R^2 = 0.06$
Region ratio variables (duration) ^e		Mutual negative	Child positive/Mother negative	Child negative/Mother positive	Child object
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
ASD ^c diagnostic group ^d	-0.72(-1.27, -0.17)*	0.42(-0.13, 0.97)	0.10(-0.47, 0.68)	0.61(0.07, 1.14)*	0.66(0.12, 1.21)*
Child's age	0.18(-0.06, 0.42)	-0.08(-0.32, 0.17)	0.10(-0.16, 0.35)	-0.11(-0.35, 0.12)	-0.31(-0.55, 0.07)*
Mother's education	0.03(-0.22, 0.29)	-0.13(-0.39, 0.12)	0.01(-0.25, 0.28)	-0.12(-0.36, 0.12)	0.06(0.20, 0.31)
Constant	0.47(0.05, 0.88)*	-0.28(-0.71, 0.14)	-0.06(-0.50, 0.38)	-0.40(-0.81, -0.001)†	-0.43(-0.85, 0.01)*
Model Statistics	$F(3, 68) = 3.10, p = 0.033,$ Adjusted $R^2 = 0.08$	$F(3, 68) = 1.83, p = 0.149,$ Adjusted $R^2 = 0.03$	$F(3, 68) = 0.33, p = 0.80,$ Adjusted $R^2 = -0.03$	$F(3, 68) = 3.17, p = 0.03,$ Adjusted $R^2 = 0.08$	$F(3, 68) = 3.20, p = 0.029,$ Adjusted $R^2 = 0.08$

^a Differences from total N are due to missing data

^b Region visits variables = number of visits to a particular region divided by the total duration of the interaction

^c ASD = Autism spectrum disorder

^d ASD diagnostic group, 0 = ASD negative, 1 = ASD positive

^e Region ratio variables = proportion of time spent within a particular region divided by the total duration of the interaction

† $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

with work indicating that during early development in children with ASD, social attention reduces while attention to exploration of objects increases (Bhat et al. 2010; Koterba et al. 2014). The second finding aligns with prior work demonstrating that, compared to TD peers, children with ASD responded to their mothers' approach behavior with more withdrawal behavior such as ignoring or shifting attention (Doussard-Roosevelt et al. 2003), complied less with their mothers' instructions (Konstantareas and Stewart 2006), and displayed deficits in positive affect during joint attention activities (Kasari et al. 1990). This result also is congruent with recent findings that, although mothers of children with ASD reported increased stress compared to mothers of TD children, many mothers were able to manage their expression of distress during interaction with their children (Tomeny 2016). Several plausible explanations exist for this finding: mothers of children with ASD may have had more training (e.g., therapy) and more daily experiences with managing behaviorally and emotionally challenged children, or it may simply be illustrative of the difficulty that children with ASD have in sustaining positive states. We did not find differences between ASD and TD groups on mismatched interaction where the children were positive and their mothers negative. Future research should explore these issues further, particularly the distinctions between dyads in the ASD and TD groups.

Our second hypothesis, that dyads in the ASD group would have fewer mutual positive and more mutual negative interactions, was partially supported. As expected, ASD and TD groups differed both in frequency and duration of mutual positive engagement. Children with ASD had greater frequency but shorter duration of mutual-positive engagement, likely due to the fact that they were moving in and out of various emotional states more frequently. This result suggests that dyads with children with ASD may have difficulty sustaining positive emotional states, a finding compatible with that of Granic et al. (2007). They reported that children with intervention-resistant externalizing problems could not maintain positive affective expression even in a positive situation, compared to children whose externalizing symptoms improved post-intervention. Our findings also highlight the importance of measuring both frequency and duration of emotion regulation behavior in children with ASD. Both constructs (frequency and duration of behavior) may have different – yet equally important – clinical implications for initiating and sustaining positive engagement.

The dyads in the ASD group did not have significantly more mutual negative engagement compared to the TD group. This finding is not without precedent: prior research on expression of negative emotions in ASD children has been mixed. Jahromi et al. (2012) indicated that children with ASD had shorter durations of positive/neutral vocalizations but longer durations of negative vocalizations. Yirmiya et al. (1989), however, found that children with ASD showed more

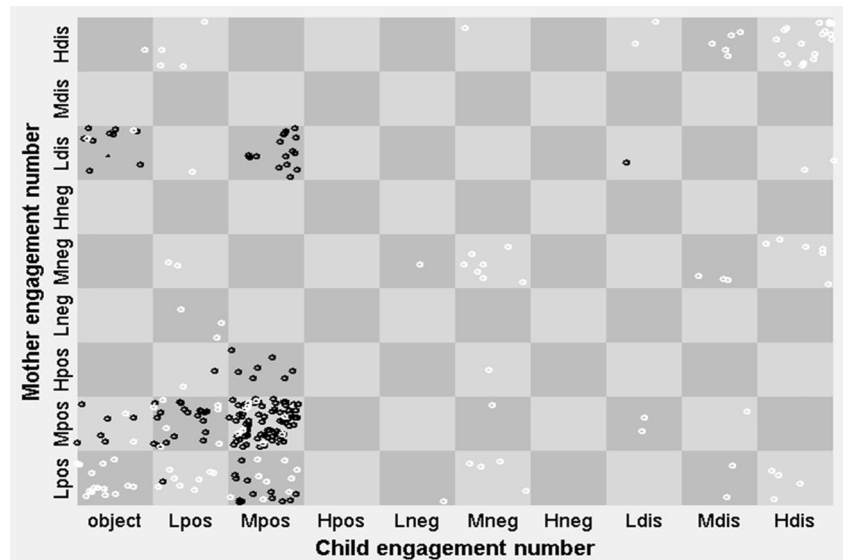
negative affect blends (e.g., anger and fear simultaneously), but did not demonstrate more discrete negative affect in comparison to TD children. The discrepant findings around displays of negative emotion may be related to differences in measurement of emotion across studies. Future research needs to clarify the apparent similarities and differences in mutual negative interactions between mother-child dyads in ASD and TD groups by using a variety of indicators for negative emotion such as facial expression, vocalizations, and behaviors.

To our knowledge, this is the first study to use the SSG method to operationalize emotion coregulation processes in children with ASD and their mothers and to contrast emotion coregulation between ASD and TD groups. The SSG yielded micro-analytic indicators of different aspects of dyadic interaction. It allowed us to make the distinction between total time spent in contingent positive interactions from numerous but often fleeting interactions. Our study elucidated the connection between structure and content in emotion coregulation. Both flexible structure and the emotional content of coregulation were congruent in that flexibility signaled more frequent movement in and out of states in the ASD group compared to the TD group; dyads with children with ASD moved more frequently in and out of mutual positive, child negative/mother positive, and child-object dyadic engagement states. Our finding supports the observation that unstable structures of dyadic interactions observed in dyads with children with ASD (recall Fig. 2) also signal dysregulated affect. Children with ASD may be more vulnerable to affect dysregulation while interacting with their mothers in a low-stress context such as the family home. Results also suggest that dyads with children with ASD had difficulty *sustaining* dyadic positive engagement but not *initiating* positive interaction. This finding suggests that it may be crucial to facilitate, in families of children with ASD, the acquisition of the skills to maintain positive interaction.

Limitations and Future Directions

Our study is not without limitations. Generalizability is limited because mothers in our sample were all married or cohabitating. Mothers were aware that they were being videotaped and research personnel were present, which might have caused participant reactivity. Mothers may have been less likely to display negative affect or more likely to exaggerate positive affect toward their children. However, a review of direct observational techniques (Gardner 2000) suggests that the presence of an observer does not necessarily distort the nature of interactions and the home setting is more representative of everyday behavior than lab settings. To minimize self-consciousness due to the video recorder, a sensitizing

Fig. 2 Comparisons of Emotion Coregulation between ASD Dyads and TD Dyads.
Note. White = a trajectory of an ASD Dyad. Black = a trajectory of a TD Dyad. Object = child engagement with toy/objects. Lpos = low positive engagement. Mpos = medium positive engagement. Hpos = high positive engagement. Lneg = low negative engagement. Mneg = medium negative engagement. Hneg = high negative engagement. Ldis = low disengagement. Mdis = medium disengagement. Hdis = high disengagement



session could have been added before the actual play sessions began (Haidet et al. 2009).

Although the home environment provided a naturalistic context for the study, interaction was assessed based on one 10-min semi-structured play session. Prior research indicates emotional availability in mothers towards their children with ASD varies across free play, structured play, and social play contexts (Dolev et al. 2009). Additionally, our study was cross-sectional. Longitudinal designs may tease out age effects on developmental pathways to emotion regulation and clarify whether impaired emotion coregulation is a mechanism for psychiatric comorbidity with anxiety and depression in children with ASD (Mazefsky et al. 2013). Future research could expand flexibility as described in Hollenstein's Flex3 model (Hollenstein 2015) to include not only micro-real-time measurement but also context-to-context reactive flexibility and developmental changes over months and years.

The flexible structure and emotional content of coregulation identified in this study only applies to mother-child interaction. Recent research indicated that negative emotion coregulation patterns differed between mother-child dyads and father-child dyads (Hirschler-Guttenberg et al. 2015); future research should use the SSG to examine these processes with fathers and their children with ASD. Although we were able to assess a unique population using microcoding techniques, the sample size may have limited power for testing other theoretically-relevant predictors.

Implications

We provide evidence that could be used to inform the development of ASD interventions that capitalize on emotion coregulation strategies. Beginning in childhood, individuals with ASD typically show persistent impairments in social

communication and social interaction across multiple contexts along with restrictive and repetitive behaviors (APA 2013). While emotion dysregulation is not a core feature of ASD in DSM-5 (APA 2013), our findings highlight that children with ASD may exhibit higher levels of such dysregulation. This observation should be considered when designing and implementing individual and family interventions. Simultaneously, our findings indicate that dyads with children with ASD are able to engage in positive interactions although do not necessarily sustain these states. Interventions that capitalize on this sustaining capacity should be implemented. Since emotion regulation plays a crucial role in the development of adaptive skills and is integral in the development of effective social interaction (Dodge and Garber 1991; Lemerise and Arsenio 2000), interventions that target parental processes that promote emotional regulation (e.g., Gulsrud et al. 2010) might enhance social development in this population. Using characteristics of varying coregulation strategies as clinical markers may improve monitoring of child development and the efficacy of targeted interventions.

In sum, we utilized SSG to operationalize change capacity as a core of dyadic flexibility by integrating two individual emotional states into one dyadic state. Our study implements a novel application of an exacting methodology to study momentary shifts in the valence of dyadic interaction in children with ASD. Our inquiry refines conceptual notions of flexibility in children with psychopathology, demonstrating that children with ASD exhibit flexibility, but not necessarily stickiness (i.e., the ability to maintain a particular state). This finding that has a number of key implications for clinical interventions. Results suggest that children with ASD may benefit from engagement with mothers who facilitate their children's capacity to expand their rigid and repetitive emotional repertoire into increased positive states. Moreover, a useful avenue

for future research would be to develop and test ways mothers with children with ASD can increase time spent in positive engagement states that already occur. Our study provides key insights into emotion coregulation in children with ASD that can inform theories regarding interaction patterns and clinical interventions to help children with ASD and their families adapt to their unique interactional challenges.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that we 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 participants in the study; parents provided written consent and children provided verbal assent.

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