Research in Autism Spectrum Disorders 25 (2016) 137–146

Contents lists available at ScienceDirect

Research in Autism Spectrum Disorders

journal homepage: http://ees.elsevier.com/RASD/default.asp

Difficulties with multi-sensory fear conditioning in individuals with autism spectrum disorder

Patrick S. Powell[a,d,*], Brittany G. Travers[b,c], Laura G. Klinger[d], Mark R. Klinger[d,e]

[a] Department of Psychology & Neuroscience, University of North Carolina—Chapel Hill, CB 7180, Chapel Hill, NC 27599-7180, USA
[b] Occupational Therapy Program in the Department of Kinesiology, University of Wisconsin-Madison, 2000 Observatory Drive, Madison, WI 53706, USA
[c] Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, 1500 Highland Avenue, Madison, WI 53705, USA
[d] TEACCH Autism Program, Department of Psychiatry, University of North Carolina—Chapel Hill, CB# 7180, Chapel Hill, NC 27599-7180, USA
[e] Department of Allied Health Sciences, University of North Carolina—Chapel Hill, 321 S Columbia St., Chapel Hill, NC 27514, USA

ARTICLE INFO

Article history:
Received 9 September 2015
Received in revised form 19 January 2016
Accepted 15 February 2016
Available online 27 February 2016

Keywords:
Autism spectrum disorder
Conditioning
Associative learning
Emotion learning

ABSTRACT

Background: Classical conditioning represents a fundamental aspect of learning, allowing us to infer relationships between coinciding events in our environment. However, recent evidence has suggested this fundamental form of learning may be compromised in individuals with autism spectrum disorder (ASD). The present study utilized galvanic skin responses to examine classical conditioning in individuals with ASD across sensory modalities.
Method: Fifteen individuals diagnosed with ASD and 16 age-, gender-, and IQ-matched individuals with typical development participated in this study. Using a differential fear conditioning paradigm, participants were presented with a series of colors and sounds. A subset of these colors and sounds was paired with an aversive loud noise. Learning the contingency between the color and/or sound and the aversive noise was measured by changes in skin conductance. Following this task, an explicit-knowledge test probed participant's awareness of these contingencies.
Results: Results indicated that individuals with ASD had a general impairment in fear conditioning compared to individuals with typical development. Additionally, participants with ASD who showed greater explicit awareness of the contingencies showed conditioned responses more similar to participants with typical development.
Conclusions: Implications for theories of the neurobiological mechanisms associated with learning and social impairments in ASD are discussed.

1. Introduction

Autism spectrum disorder (ASD) is a developmental disorder with core symptoms consisting of impairments in social communication and the presence of restrictive interests and repetitive behaviors (American Psychiatric Association, 2013). Our understanding of the social and communication impairments of ASD have largely stemmed from deficits in social and

* Corresponding author at: TEACCH Autism Program, 100 Renee Lynne Ct. Carrboro, NC 27510, USA.
E-mail address: pspowell@unc.edu (P.S. Powell).

http://dx.doi.org/10.1016/j.rasd.2016.02.008
1750-9467/

emotional processing. These accounts suggest that difficulties in processing emotional information like faces (Adolphs, Sears, & Piven, 2001; Shultz, 2005) or understanding the mental states of others (e.g., theory of mind, Baron-Cohen, 1997) give rise to atypical social behaviors in ASD. However, some authors have argued that another fundamental feature of the ASD phenotype lies in the ability to relate emotional experiences to environmental stimuli. That is, it may not be simply the experience or perception of emotion that is impaired in ASD (e.g., fear) but rather the ability to learn to associate emotional significance to environmental stimuli (Hobson, 1989; Kanner, 1943; Mundy & Sigman, 1989). One way to investigate this is through the use of classical fear conditioning paradigms.

In a traditional classical fear conditioning paradigm, a relatively neutral stimulus, such as a red light (i.e., the conditioned stimulus, CS), is paired with an aversive stimulus such as a loud sound (i.e., the unconditioned stimulus, UCS). Following several pairings of the CS with the UCS, participants begin to learn that presentation of the CS predicts the presentation of the UCS (e.g., the red light predicts the loud sound). Learning is usually assessed by whether participants show a startle or conditioned response (CR) to the CS in the absence of, or prior to, presentation of the UCS. This response is typically measured through galvanic skin response (GSR) or eye-blink magnitude (Knight, Smith, Stein, & Helmstetter, 1999; Knight, Nguyen, & Bandettini, 2003).

Studies of classical fear conditioning in ASD have indicated patterns of both impaired learning (Gaigg & Bowler, 2007; South, Newton, & Chamberlain, 2012), intact learning (Bernier, Dawson, Panagiotides, & Webb, 2005; South, Larson, White, Dana, & Crowley, 2011), and, in one instance, more rapid learning compared to age and IQ-matched individuals with typical development (see Sears, Finn, & Steinmetz, 1994). However, several methodological differences seem to underlie the conflicting patterns of results. Studies finding intact or more rapid conditioning in ASD have measured eye-blink response to a CS which was reinforced on every trial (i.e., 100% reinforcement schedule) and did not include a neutral stimulus (Bernier et al., 2005; Sears et al., 1994). Eye-blink conditioning is known to rely upon cerebellar and limbic system pathways (Steinmetz, Tracy, & Green, 2001), suggesting these pathways may be intact in ASD.

In contrast, studies of differential classical fear conditioning have measured associative learning by comparing changes in the skin conductance response (SCR) to a CS relative to one or more neutral stimuli. This type of conditioning has been linked to amygdala, hippocampal, and prefrontal brain regions (Jarrell, Gentile, Romanski, McCabe, & Schneiderman, 1987; LaBar, LeDoux, Spencer, & Phelps, 1995; Morris, Friston, & Dolan, 1997; Sehlmeyer et al., 2009). Studies of differential conditioning in ASD have found both impaired (Gaigg & Bowler, 2007; South et al., 2012) and intact associative learning (South et al., 2011). The different results may be attributable to an interaction between task complexity and associated brain regions linked to the fear response. For instance, Gaigg and Bowler suggested that the relative complexity of their paradigm may have contributed to the difficulty of the ASD group associating the CS with the UCS. Importantly, the term "complexity" related to differential fear conditioning is defined as a less obvious association between the CS and UCS, an association that can be obscured by the use of multiple neutral stimuli and/or a partial reinforcement schedule (Gaigg & Bowler, 2007). The complexity of the Gaigg and Bowler (2007) paradigm contrasts with a relatively "simple" differential fear conditioning paradigm that involves learning of a simple one-to-one association (i.e., one CS paired with one UCS). For instance, in a similar study by South et al. (2011), using one CS and one neutral stimulus, they demonstrated comparable associative learning between individuals with ASD and individuals with typical development. Because differential fear conditioning paradigms require greater communication between cortical and subcortical brain regions (Jarrell et al., 1987; Morris et al., 1997), poor connectivity between these brain regions in individuals with ASD (Belmonte et al., 2004; Just, Cherkassky, Keller, & Minshew, 2004; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Minshew & Williams, 2007) may be one explanation why more complex differential conditioning paradigms resulted in poorer learning. That is, learning the association between a visual CS and an auditory UCS when inter-mixed with multiple neutral stimuli may result in poorer learning in ASD for two reasons: (1) learning the association between the CS and UCS requires coordination between visual, auditory, and subcortical brain regions, and (2) discriminating between threatening (i.e., CS) and safe/neutral stimuli requires additional information from pre-frontal brain regions.

Finally, learning in a classical fear conditioning paradigm may also be related to the degree to which individuals are consciously aware of the relation between conditioned and unconditioned stimuli. While it is true that both eye-blink and differential fear conditioning tasks can occur in the presence or absence of explicit awareness (Clark & Squire, 1999; Knight et al., 2003; LaBar et al., 1995; Weike, Schupp, & Hamm, 2007), activation of brain regions associated with explicit processing (e.g., medial temporal lobes; MTL) is less common in eye-blink conditioning tasks (see 'delay conditioning' in Clark, Manns, & Squire, 2002). In contrast, differential fear conditioning is associated with activations in the prefrontal cortex (PFC) and the hippocampus (HC), suggesting that explicit awareness may play a more important role in this type of learning. As a result, differential fear conditioning provides an opportunity to examine the relation between explicit awareness and conditioning in individuals with ASD.

The current study sought to extend previous evidence of impaired classical fear conditioning in ASD using complex presentations. Specifically, the current study used trials with a visual CS (colored square) paired with an auditory UCS (loud tone) and an auditory CS (musical note) paired with an auditory UCS (loud tone), along with two visual and two auditory neutral stimuli. Conditioning was measured as changes in SCRs to stimuli previously paired with the loud sound. Because this was a more complex learning task relative to the simple one CS to one UCS pairing, it was predicted that individuals with ASD would demonstrate poorer classical fear conditioning across modalities (visual CS paired with auditory UCS). That is, individuals with ASD would demonstrate a smaller conditioned response compared to individuals with typical development. However, because less cortical to sub-cortical communication may be required to learn an association within a modality (i.e.,

an auditory CS to auditory UCS pairing), it was predicted that individuals with ASD may demonstrate conditioning for auditory CS–auditory UCS trials that is more comparable to that seen in individuals with typical development. Finally, because previous evidence has indicated that implicit and explicit abilities may be related in ASD (Klinger, Klinger, & Pohlig, 2007), it was predicted that individuals with ASD with greater explicit awareness of the CS–UCS relationship would demonstrate better conditioning compared to individuals with ASD who are unaware of this relationship.

2. Materials and method

2.1. Design

This study used a $2 \times 2 \times 2$ mixed factorial design. There were two diagnostic groups (ASD vs. typical), two types of stimulus modality (visual vs. auditory), and two trial types (conditioned stimulus vs. neutral stimulus). Stimulus modality and trial type were within-subject variables, and diagnostic group (ASD vs. typical) was a between-subjects variable. Each participant's skin conductance response (SCR) was measured and used as the dependent variable in this study.

2.2. Participants

Fifteen adolescents and young adults with high functioning ASD (17–25 years old, 13 male, two female), and 16 adolescents and young adults with typical development (17–23 years old, 14 male, two female) were recruited for this study. Participants with ASD were recruited through the university's ASD Clinic and local service providers. Participants with typical development were recruited through the university's Psychology 101 subject pool, and local schools and churches. All participants reported normal hearing and normal or corrected to normal vision (e.g., contact lenses or eye glasses). Eight individuals with ASD reported antidepressant/anti-anxiety medication use. There were no individuals with typical development on medication.

In order to confirm a previous diagnosis for ASD, participants with ASD received the Autism Diagnostic Observation Schedule–Generic Module 4 (ADOS–G; Lord et al., 2000). All participants met diagnostic cutoff scores. Participants with ASD with a known genetic etiology (e.g., fragile X, Down syndrome) were excluded during study recruitment. Participants with typical development were screened through a self-report background questionnaire that asked if the participant had ever been diagnosed with Autism, Asperger's Disorder, PDD-NOS, a Learning Disability, Intellectual Disability, Cerebral Palsy, or Tourette's/Tic Disorder. No participants with typical development reported the presence of any of these neurological disorders.

Groups were matched on chronological age (ASD: $M = 20.7$ years, SD = 2.5; Typical: $M = 20.2$ years, SD = 1.5), as well as IQ using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999): Verbal IQ (ASD: $M = 106.8$, SD = 10.4; Typical: $M = 109.5$, SD = 9.8), Performance IQ (ASD: $M = 106.6$, SD = 14.8; Typical: $M = 106.4$, SD = 11.3), and Full scale IQ (ASD: $M = 107.4$, SD = 10.7; Typical: $M = 108.81$, SD = 7.6), all p's > .4.

2.3. Apparatus

Skin conductance response was measured using a BioLog 3992 with two 7.5 mm diameter Ag/AgCl surface electrodes attached with an electrolyte of 0.05 M NaCl inert ointment to the medial phalanges of the first and third digits of the right hand. A constant voltage device set at 0.5 V sampled at 12 Hz and BioLog's Downloading and Plotting Software (DPS) was used to record and assess data from electrodermal activity (BioLog, 2010).

2.4. Procedure

Participants were scheduled for a single 2.5 h session. After obtaining appropriate consents, participants completed the WASI and demographic form, and participants with ASD also completed the ADOS-G. Participants were then escorted to a semi-soundproof room for the conditioning task.

Participants were told that the purpose of the experiment was to examine simple learning of visual and auditory stimuli, and they were warned that they would hear some loud sounds. The foghorn sound was initially presented at a moderately loud volume (95 dB) through headphones, and participants were allowed to adjust the volume so that the foghorn sound was as loud as possible without being painful. Participants sat approximately 50 cm away from the computer screen. They were asked to remain as motionless as possible and to pay close attention to the stimuli presented on the screen and over the headphones. Presentation of the stimuli commenced once skin conductance level (SCL) reached a stable baseline activity level. The experimenter was present during the duration of the experiment in order to monitor SCL and participant behavior. The experimenter was positioned approximately three feet to the side of the participant. Only the laptop and electrodes attached to the fingers were in the line of sight of the participant.

The protocol for the conditioning task was based on the protocol used by Bechara et al. (1995) and Gaigg and Bowler (2007). Each trial consisted of one of three colored squares (e.g., red, yellow, or blue) or one of three instrumental sounds (e.g., violin, guitar, and organ). All colors and instrument sounds were randomly presented during both habituation and extinction trials, and a pseudo-randomization procedure was used for the acquisition phase. The pseudo-randomization procedure evenly distributed the CS–UCS trial pairings throughout the acquisition block while at the same time randomly presented all neutral stimuli. The colored square (CS_{visual}) and the instrument sound ($CS_{auditory}$) were paired with the foghorn

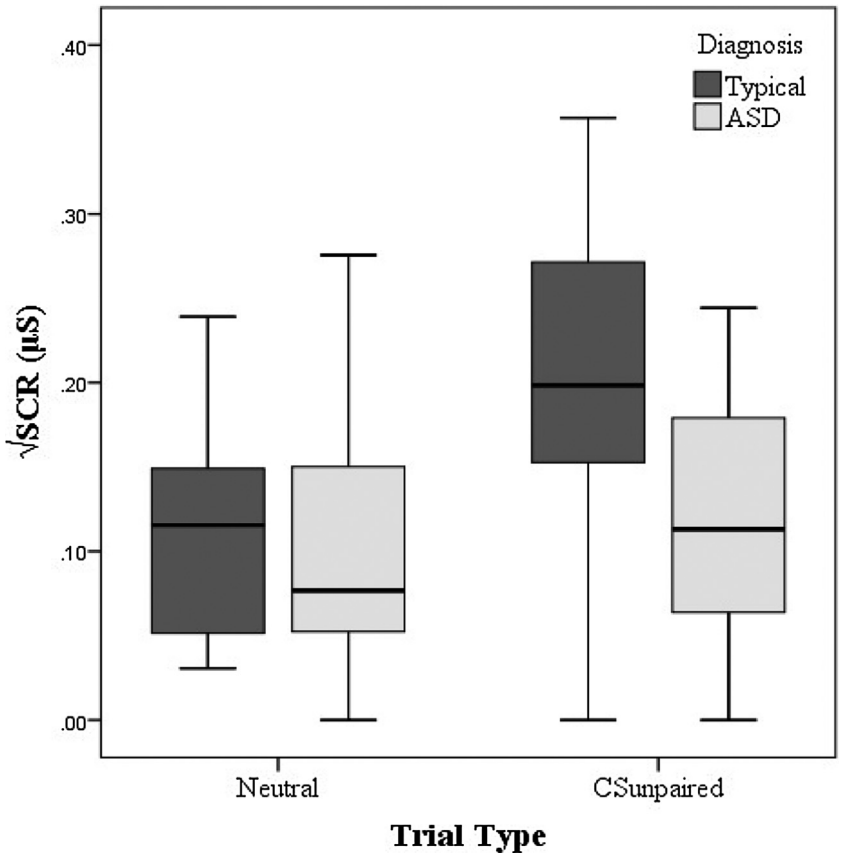

Fig. 1. √SCR (μS) $CS_{unpaired}$ vs. NS for participants with ASD & typical development.

sound (UCS) at 85–100 dB, presented through headphones. All other colored squares and instrument sounds were never paired with the UCS and thus considered neutral stimuli (denoted as NS_{visual} and $NS_{auditory}$ respectively, see Fig. 1). The onset of the foghorn sound occurred 6000 ms after the presentation of the CS. Both the CS_{visual} and the foghorn were simultaneously presented for the duration of the foghorn sound (2000 ms) and co-terminated at the end of the 2000 ms. In the $CS_{auditory}$ trials, the foghorn immediately followed the presentation of the instrument sound due to the inability to distinguish between the instrument sound from the foghorn if presented simultaneously. Each trial lasted a total of 8000 ms. The inter-trial interval was 15 s in order for the participant's SCR to return to baseline prior to each new trial. The color and instrument sound used for the CS was counterbalanced across participants.

The colored squares or musical instrument sounds were presented two times each during the habituation phase for a total of 12 trials. The purpose of the habituation phase was to measure baseline activity to the experimental stimuli before pairing some of the stimuli with the UCS. There were 50 trials during the acquisition phase with the CS_{visual} and $CS_{auditory}$ each presented 13 times. During acquisition, the first 10 occurrences of CS_{visual} and $CS_{auditory}$ were paired with the UCS, and the last three trials of each were presented without the UCS (denoted $CS_{unpaired}$). The purpose of including the $CS_{unpaired}$ trials was to assess the physiological response (i.e., learned association) to the CS without the UCS present. Finally, during the extinction trials participants saw three additional random presentations of the $CS_{unpaired\_visual}$ and $CS_{unpaired\_auditory}$ and one presentation of each of the neutral colors and instrument sounds (NS) (10 trials total). The purpose of the extinction trials was to extinguish any learned response to the visual or auditory CS. At the end of the experiment, participants were given a brief memory test to probe their explicit knowledge of the stimulus pairings. Participants were asked, "What color(s)/instrument(s) was/were paired with the loud noise?"

2.5. Data processing

Data analysis was completed using SPSS IBM 23 statistical software. The current data were sampled at a continuous 12 Hz. Prior to calculation of the SCR we performed a range-correction on the SCL values in order to correct for inter-individual variance (i.e., some participants showed large variability in SCL, while others showed small variability). This range correction was done by selecting each SCL value, subtracting this value from their minimum SCL, and then dividing by the maximum

SCL minus the minimum SCL values for each participant (Appendix A, see Levey, 1980). Following this correction two separate SCR scores were calculated to examine different components of the CR (see Pineles, Orr, & Orr, 2009).

The first SCR score computed is referred to as the first anticipatory response (FAR, see Pineles et al., 2009) and measures the change in SCR prior to the onset of the UCS (first 6 s of trial). FARs were calculated using a peak and valley method. The peak SCL value for each trial was taken within a 6-second window and then subtracted by the SCL value selected at trial onset (valley, see South et al., 2011).

The second SCR score, referred to as the entire interval response (EIR), measured the change in SCR across the entire trial duration. This was done in order to examine the change in SCR following the expected UCS onset (i.e., $CS_{unpaired}$ trials). The EIR was measured by selecting the peak SCL value within an 8-s window and subtracting the minimum SC value at trial onset (see Gaigg & Bowler, 2007).

Inspection of the SCR data revealed substantial positive skew. Thus, SCRs were square-root transformed to normalize distribution of the data (Gaigg & Bowler, 2007). Shapiro–Wilk tests on the square-root transformed SCR scores indicated a normal distribution for both unpaired CS and neutral trials ($W = .971$, $p = .56$; $W = .953$, $p = .19$, respectively).

3. Results

3.1. Baseline measures

To ensure similar baseline SCR values between diagnostic groups, we examined electrodermal activity to ensure group equivalency. Individuals with ASD demonstrated statistically equivalent baseline SCR values ($M = 9.56$ μS, SD = 4.08 μS) compared to individuals with typical development ($M = 11.99$ μS, SD = 5.11 μS), $t(29) = 1.46$, $p = .16$. Also, because participants adjusted the volume of the UCS to be as loud as tolerable with a maximum allowed of 100 dB, we compared the adjusted decibel levels of the two diagnostic groups. The ASD group adjusted the intensity (dB level) of the UCS slightly but significantly lower ($M = 90.21$ dB, SD = 5.30) than individuals with typical development ($M = 93.41$ dB, SD = 2.17), $t(29) = 2.21$, $p = .04$, Cohen's $d = .79$. Measurement of decibel level was not available for one participant with ASD.

3.2. SCR amplitude to the UCS

Because individuals with ASD preferred a slightly lower decibel setting to the UCS compared to individuals with typical development, we compared the SCR to the UCS across the diagnostic groups to test whether the groups showed similar SCR reactivity.[1] This comparison was necessary to ensure that any group differences in conditioning were not affected by these differences in volume of the stimuli and differences in sensitivity to those volumes. Both diagnostic groups exhibited strong SCRs to the UCS relative to the neutral stimuli (Typical: $M = 1.40$ μS; SD = .74, $t(15) = 7.03$, $p < .001$; ASD: $M = 1.15$ μS; SD = .66, $t(14) = 6.19$, $p < .001$). There was no significant difference between diagnostic groups response to the UCS, $t(29) = 1.03$, $p = .31$, Cohen's $d = .37$.

3.3. SCR amplitude to CS and NS trials

Initially, we examined first anticipatory responses (FARs) across all CS trials (i.e., CS_{paired} and $CS_{unpaired}$ trials). This analysis showed a non-significant difference between CS and NS trials, $F(1,29) = .12$, $p = .73$. The pattern of results showed higher than expected FARs for NS trials. Given that conditioned responses could not occur during NS trials (because they are never paired with the UCS), it was determined that the elevated FARs were likely due to an initial orienting response to stimulus onset (for both CS and NS trials) making it difficult to detect conditioned responses. Therefore, analyses were conducted only on the $CS_{unpaired}$ trials that occurred near the end of the conditioning task. It was expected that these trials would be more reflective of actual conditioned responses for two reasons, (1) by the end of the conditioning task orienting responses to conditioned stimuli would be diminished due to greater familiarity with them, and (2) any learned associations between CS and UCS should be stronger after the larger number of repeated pairings. Preliminary analyses indicated similar findings across visual and auditory trials with no significant main effects or interactions of sensory modality. Therefore, analyses were collapsed across modality. We examined both FAR scores and EIR scores to the $CS_{unpaired}$ trials and NS trials. The same pattern of significance was seen for analyses of FAR and EIR scores. We have presented the EIR scores here because the slow potentiation of the SCR caused some individual's peak responses to occur after the six seconds in the FAR but within the 8 s of the EIR.

Table 1 shows the mean change in SCRs to CS and NS trials when the UCS was not presented. As can be seen, individuals with typical development demonstrated a significant increase in SCR across both the visual and auditory CS trials compared to the NS trials. In contrast, individuals with ASD demonstrated a smaller, non-significant increase in SCR to CS relative to NS trials.

As with our previous analysis of FAR scores, we collapsed across visual and auditory trials because similar rates of conditioning were found for both modalities with no significant main effects or interactions. Following this, we conducted a 2 (Typical vs. ASD) by 2 ($CS_{unpaired}$ vs. NS) repeated measures ANOVA. This analysis showed a main effect of trial type, F

[1] Reanalysis of results with dB level as a covariate did not change the results. Thus, all reported analyses did not include dB level as a covariate.

Table 1
EIR [$\sqrt{}$SCR (μS)] means and difference scores (CS – NS).

	Visual			Auditory			Overall learning		
	$CS_{unpaired}$	NS	Difference	$CS_{unpaired}$	NS	Difference	$CS_{unpaired}$	NS	Difference
Typical	.183	.096	+.087*	.214	.134	+.080*	.198	.115	+.083**
ASD	.124	.097	+.027	.146	.130	+.016	.134	.113	+.021

Visual $CS_{unpaired}$ = colored square presented without UCS; visual NS = colored squares never presented with UCS; auditory $CS_{unpaired}$ = instrument sound presented without UCS; auditory NS = instrument sound never presented with UCS.
* $p \leq .05$.
** $p \leq .001$.

(1,29) = 15.62, $p < .001$, $\eta_p^2 = .35$, and a significant diagnosis by trial type interaction, $F(1,29) = 11.59$, $p = .002$, $\eta_p^2 = .29$. Follow-up comparisons of $CS_{unpaired}$ to NS trials indicated that individuals with typical development demonstrated a significant increase in SCR to $CS_{unpaired}$ relative to NS trials, $t(15) = 5.12$, $p < .001$, $\eta_p^2 = .53$. In contrast, individuals with ASD showed no difference in SCR between $CS_{unpaired}$ and NS trials, $t(14) = .40$, $p = .70$, $\eta_p^2 = .01$ (Fig. 1).

We also compared the magnitude of conditioning for those participants with ASD who reported antidepressant/anti-anxiety medication use to those who did not report medication use. Comparisons revealed no significant differences between those with and without medication, $F(1,13) = .77$, $p = .77$, $\eta_p^2 = .007$.

3.4. Explicit memory test

Next, the relation between explicit awareness and conditioning was examined. Thirty-one percent of individuals with typical development ($n = 5$) showed explicit awareness of stimulus pairings by correctly identifying both CSs as being paired with the foghorn and not identifying other stimuli as being associated with the foghorn. Forty percent of individuals with ASD ($n = 6$) showed explicit awareness of stimulus pairings, correctly identifying both CSs and no NSs as being paired with the foghorn. Rate of explicit awareness was not significantly different between diagnostic groups, $\chi^2(1, N = 31) = 0.26$, $p = .61$.

Following this analysis, the relation between explicit awareness and conditioning was examined. A 2 (ASD vs. Typical) by 2 (aware vs. unaware) univariate ANOVA was conducted using a difference score as the dependent variable. The difference

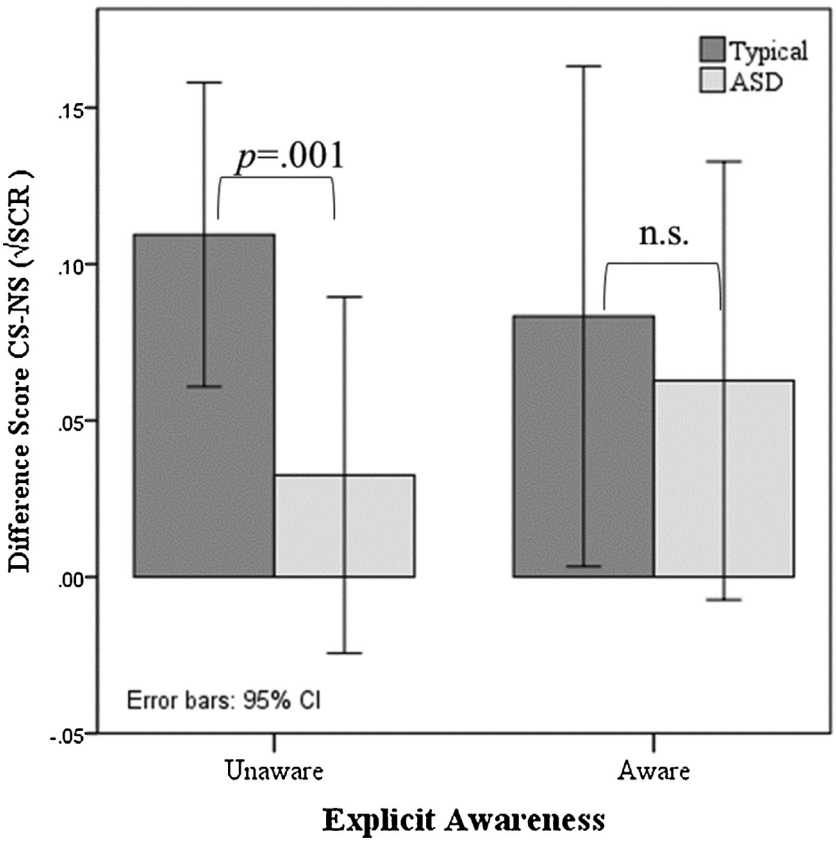

Fig. 2. SCR as a function of explicit awareness.

score was calculated by subtracting the response to NS trials from $CS_{unpaired}$ trials (CS-NS). This analysis showed a large main effect of diagnosis, $F(1,27) = 9.02$, $p = .006$, $\eta_p^2 = .25$, but no main effect of awareness $F(1,27) = 1.56$, $p = .22$, $\eta_p^2 = .06$. However, there was a medium effect for the diagnosis by awareness interaction term, $F(1,27) = 2.89$, $p = .10$, $\eta_p^2 = .097$. Because this non-significant interaction may be due to a relatively small sample size, we compared participants who were explicitly aware of the CS–UCS relationship to those who were not aware within each diagnostic group. Individuals with typical development showed a similar increase to the CS compared to the NS when they were explicitly aware (.093 $\sqrt{}$SCR mean difference) and not aware (.105 $\sqrt{}$SCR mean difference) of the CS–UCS relationship, $t(14) = .283$, $p = .78$. In contrast, individuals with ASD who were explicitly aware showed a greater difference between the $CS_{unpaired}$ and NS (.056 $\sqrt{}$SCR mean difference) than individuals who were not aware (−.025 $\sqrt{}$SCR mean difference), $t(13) = 2.44$, $p = .03$, Cohen's $d = 1.24$ (see Fig. 2). These findings suggest that awareness did not affect the magnitude of the CR in individuals with typical development, but significantly impacted the CR observed in individuals with ASD.

4. Discussion

In the current study we examined classical fear conditioning in individuals with ASD compared to age- and IQ matched individuals with typical development to determine whether individuals with ASD displayed atypical conditioning across visual and auditory modalities. Across modalities, there was clear evidence of learning in individuals with typical development, while there was limited evidence of learning in individuals with ASD. Importantly, both groups showed significant increases in SCR to the aversive sound (1.28 $\sqrt{}$SCR increase for participants across diagnostic groups). This suggests that both diagnostic groups were equally reactive to the aversive stimulus used in this experiment eliminating the possibility that decreased conditioning in individuals with ASD was the result of diminished physiological reactivity to the aversive sound.

This current study provides evidence for atypical classical fear conditioning in individuals with ASD when the task was relatively complex, consisting of two conditioned stimuli and multiple neutral stimuli. Unlike previous conditioning studies in ASD which have used either visual or auditory stimuli, the current study's use of both visual and auditory stimuli made the relationship between the CS–UCS pairings more difficult to explicitly detect. Only 35% of participants accurately identified the relations between CSs and UCS. For typically developing participants, the ability to accurately identify the relations between CSs and UCS was not related to the magnitude of conditioning. However, individuals with ASD who accurately identified the CS–UCS relationship demonstrated significantly better conditioning than individuals with ASD who did not accurately identify the CS–UCS relationship. The finding that explicit awareness had little impact on learning in individuals with typical development is in line with previous studies showing that conditioning can occur in the presence or absence of conscious awareness. That is, individuals with typical development seemed to show learning of the CS–UCS relationship by either implicit or explicit processes. However, the pattern of performance in ASD suggests that there may be differences in explicit and implicit learning with poorer learning using implicit processes. This is consistent with a growing literature demonstrating impaired implicit learning in ASD (Gastgeb, Rump, Best, Minshew, & Strauss, 2009; Klinger & Dawson, 2001; Mostofsky, Goldberg, Landa, & Denckla, 2000; Gidley Larson & Mostofsky, 2008; Schipul & Just, 2015), and a relationship between implicit task performance and explicit learning abilities in persons with ASD; a relationship that did not exist in individuals with typical development (Gastgeb, Dundas, Minshew, & Strauss, 2012; Klinger et al., 2007; Vladusich, Olu-Lafe, Kim, Tager-Flusberg, & Grossberg, 2010). Taken together, one interpretation of the present findings is that individuals with ASD who demonstrate conditioning may not do so using implicit processes, but instead rely on more explicit processes in order to learn. However, this interpretation is somewhat speculative due mixed evidence regarding implicit learning in ASD (see Eigsti & Mayo, 2011) and due to the fact that the use of implicit processes involved in the current conditioning task can only be inferred when a participant demonstrated conditioning in the absence of explicit awareness. As a result, it is difficult to estimate the degree to which conditioning was the product of more implicit or more explicit processes in this study. Future research ought to more directly manipulate whether explicit or implicit learning processes are used to more directly compare their role in learning in ASD.

The current findings also can be understood by considering the role of task complexity. Minshew and colleagues' have posited the complex information processing hypothesis (Minshew & Goldstein, 2001; Minshew, Goldstein, & Siegel, 1997; Williams, Goldstein, & Minshew, 2006), suggesting that complex learning tasks require a high degree of information integration across multiple cognitive processes, and tend to be more difficult for individuals with ASD. On the other hand, simpler tasks that require fewer processes may be easier to learn (Travers et al., 2013). This notion is in line with the extant literature on classical fear conditioning in ASD. Three classical fear conditioning studies that used more complex associations (Gaigg & Bowler, 2007; South et al., 2012, and the present study) all have shown impaired conditioning relative to individuals with typical development, while the three studies that used simpler associations all have shown similar conditioning between individuals with ASD and individuals with typical development (Sears et al., 1994; Bernier et al., 2005; South et al., 2011). Thus, the present findings may be explained by the degree of complexity in the current conditioning task compared to conditioning tasks that use only one CS and one UCS.

Finally, it should be noted that learning was measured in the current study, by comparing the six unpaired CS trials to the NS trials. Thus, the current findings should not be taken as across the board evidence of impaired classical conditioning in ASD. Our first examination of the CR prior to the UCS (i.e., FAR analyses) for both CS_{paired} and $CS_{unpaired}$ trials did not demonstrate clear evidence of conditioning in either the ASD or typical groups. As previously mentioned, it was unclear whether FARs reflected an initial orienting response or a true conditioned response in CS trials considering that we also

observed an unexpected elevation of the FAR in NS trials. As a result, we chose to examine $CS_{unpaired}$ trials within an 8-second window (i.e., EIR) to analyze changes in SCR when the UCS was no longer paired with the CS. However, this examination of the unpaired CS trials may reflect an atypical pattern of extinction that is not observed in individuals with typical development. For instance, it is possible that individuals with ASD demonstrate an increase in SCR to the first $CS_{unpaired}$ trial and then a rapid decline in the CR with subsequent presentations (i.e., faster extinction rate). This would, of course, result in an overall reduction in the CR when collapsed across trials. However, an examination of trial order for $CS_{unpaired}$ trials provided no evidence of a faster extinction rate in ASD. Instead, this analysis showed a significantly smaller CR in individuals with ASD compared to individuals with typical development across trials. Therefore, the present findings support a pattern of atypical acquisition rather than atypical extinction in individuals with ASD.

4.1. Basic learning in ASD

Although the present findings may not be generalizable to learning across all domains in ASD, fear conditioning itself can provide insight into the integrity of basic learning mechanisms in ASD. First, the acquisition of fear is contingent upon the degree to which a threatening or fearful stimulus elicits a fear response. Specifically, response to a fearful stimulus activates the amygdala which then sends information throughout the body via the sympathetic nervous system (SNS) resulting in increases in heart rate, pupil dilation, respiration, and perspiration (e.g., GSR). As mentioned above, the current study showed no difference in the startle response (i.e., GSR increase to the UCS) between individuals with ASD and individuals with typical development, suggesting that the mechanism underlying the emotional response to fearful or threatening auditory stimuli is not different in ASD. In addition to fear, previous evidence has demonstrated no difference between individuals with ASD and individuals with typical development when comparing the GSR to more positive or rewarding stimuli (Neuhaus, Bernier, & Beauchaine, 2015). Evidence that the emotional response to threatening and rewarding information is relatively intact in ASD suggests those brain regions associated with emotional response, such as the amygdala, function well (e.g., Lanteaume et al., 2007). However, these findings also seem to suggest these areas associated with a fear-related response, such as the amygdala and the SNS, may have difficulty coordinating with other brain regions such as auditory and visual cortical areas in persons with ASD. This is consistent with the broader literature on classical conditioning suggesting that the response to a threatening stimulus is primarily associated with amygdala activation, yet the ability to associate this response to an environmental stimulus involves coordination between the amygdala and cortical brain regions (Jarrell et al., 1987; Morris et al., 1997). Therefore, atypical conditioning in ASD may arise from poor connectivity between the amygdala and associated cortical brain regions rather than a basic impairment in amygdala functioning. However, the current study by itself cannot speak to a general impairment in emotion-related associative learning in ASD because it only measured one kind of emotion-related learning. Caution must be taken when considering whether fear-conditioning involves mechanisms common to other forms of emotion learning. Instead, evidence from studies of fear conditioning in ASD (including the present study) suggests that fear conditioning in ASD may be disrupted by increases in task complexity and coordination between associated brain regions. In future research, it will be important to examine classical conditioning using paradigms that are less dependent on emotionally threatening stimuli. If a general deficit in emotion-related associative learning is present or if learning is disrupted when greater task demands are placed on individuals with ASD, it should be possible to observe this impairment in studies using emotionally rewarding reinforcements as well as within subject manipulations of task complexity.

4.2. Implications for the characterization of ASD symptomatology

This study did not specifically address anxiety-related symptomatology, however, eight individuals with ASD did report some antidepressant/anti-anxiety medication use (no individuals with typical development reported any type of medication use). Because medication use for depression or anxiety may be indicative of clinically significant anxiety, we compared conditioning between those with and without medication. This analysis showed no differences in conditioning. Analyses indicated that antidepressant/anti-anxiety medication use was not related to conditioning in the participants with ASD. Nevertheless, given the high prevalence of anxiety in ASD, it is important for future studies of fear conditioning to assess anxiety-related symptomatology and examine whether the presence of clinically significant anxiety contributes to atypical fear learning in ASD. For instance, an examination of the broader literature on anxiety and fear conditioning by Lissek et al. (2005) showed that clinically significant anxiety in a non-ASD population was associated with larger conditioned responses when presented with only one CS and no NS. However, when presented with a discrimination task (e.g., comparing CRs to CS and NS trials), individuals with anxiety tended to respond equally to both the conditioned and neutral stimuli. Thus, co-morbid anxiety in ASD may not disrupt the response to a specific CS, rather it may disrupt the ability to discriminate between safe (e.g., NS) and threatening stimuli (e.g., CS). However, the present findings did not show evidence of heightened CRs to both CS and NS trials in individuals with ASD. The CRs to both the CS and NS in ASD resembled the response to the NS observed in individuals with typical development, suggesting diminished responding in ASD regardless of trial type. Future studies of fear conditioning that compare those with and without co-morbid anxiety in ASD may be important for understanding whether decreased discrimination of threatening and non-threatening stimuli is specific to those with co-morbid anxiety or a more general deficit in ASD.

Finally, the current findings point to a deficit in emotion-related associative learning within the context of a classical fear conditioning task, but it is unknown whether broader deficits in emotion-related associative learning are present in ASD. A large body of research has shown that socially rewarding stimuli (e.g., eye contact) do not elicit the same positive emotional response in individuals with ASD as seen in individuals with typical development. This has led to suggestions that impoverished emotional responses may lead to many of the observed social and communication impairments in ASD (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Dawson & Bernier, 2007; Dichter et al., 2012; Schmitz et al., 2008) If ASD is characterized by a general deficit in emotional responding, then atypical fear conditioning in ASD ought be related to a diminished fear response to the aversive stimulus. However, the current study demonstrated that the emotional response to the aversive stimulus (UCS) was not impaired in ASD demonstrating that fear conditioning could be impaired when emotional responding was intact. This result suggests that emotional responding does not underlie the observed impairments in learning. Future research should examine both positive (e.g., reward learning) and negative associative learning (e.g., fear conditioning) to more fully test the relation between emotional responding and emotion-related learning in ASD. Given the simplicity of many associative learning paradigms it may serve as a suitable methodology for difficult to test populations such as infants or individuals with moderate intellectual disability. That being said, the inclusion of only adolescents and young adults with ASD with average or above average IQ in the current study does limit generalizability. However, evidence of impaired classical fear conditioning in this higher IQ and older sample (a group usually thought of as having substantially better learning abilities than others with ASD) makes it reasonable to speculate that the same type of impairment may be present in lower-functioning or younger samples with ASD. Future studies of infant populations are important to understanding whether difficulties in emotion-related learning are related to the aberrant development of social and communication abilities in ASD.

Acknowledgements

The authors would like to thank the following individuals for the data collection efforts, Megan Crisler, Seth Madison and Casey O'Quinn. The authors acknowledge that no external funding was received to support the current study.

Appendix A.

Range correction formula.

$$SCL_i' = \frac{SCL_i - SCL_{min}}{SCL_{max} - SCL_{min}}$$

where SCL_i is the uncorrected SCL value, SCL_i' is the range corrected SCL value at the time point i, while SCL_{max} is the highest possible SCL value, and SCL_{min} is the lowest possible value for each participant.

References

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *Journal of Cognitive Neuroscience*, *13*(2), 232–240.
American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders*, 5th ed. . http://dx.doi.org/10.1176/appi.books.9780890425596.910646.
Baron-Cohen, S. (1997). *Mindblindness: an essay on autism and theory of mind*. Cambridge, Massachusetts: MIT Press.
Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science*, *269*, 1115–1118.
Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., & Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *Journal of Neuroscience*, *24*, 9228–9231.
Bernier, R., Dawson, G., Panagiotides, H., & Webb, S. (2005). Individuals with autism spectrum disorder show normal responses to a fear potential startle paradigm. *Journal of Autism and Developmental Disorders*, *35*, 575–583.
BioLog (1990–2010). UFI Biolog (3992 SCL). UFI Instruments Pty. Ltd. http://ufiservingscience.com.
Chevallier, C., Kohls, G., Troiani, V., Brodkin, E. S., & Schultz, R. T. (2012). The social motivation theory of autism. *Trends in Cognitive Science*, *16*, 231–239.
Clark, R. E., & Squire, L. R. (1999). Human eyeblink classical conditioning: effects of manipulating awareness of the stimulus contingencies. *Psychological Science*, *10*, 14–18.
Clark, R. E., Manns, J. R., & Squire, L. R. (2002). Classical conditioning, awareness, and brain systems. *Trends in Cognitive Sciences*, *6*, 524–530.
Dawson, G., & Bernier, R. (2007). Development of social brain circuitry in autism, In D. Coch, G. Dawson, & K. Fischer (Eds.), *Human behavior and the developing brain: atypical development* (pp. 28–55).2nd ed. New York: Guilford Press.
Dichter, G. S., Felder, J. N., Green, S. R., Rittenberg, A. M., Sasson, N. J., & Bodfish, J. W. (2012). Reward circuitry function in autism spectrum disorders. *Social Cognitive and Affective Neuroscience*, *7*, 160–172.
Eigsti, I. M., & Mayo, J. (2011). Implicit learning in ASD. In D. Fein (Ed.), *The neuropsychology of autism* (pp. 267–279).New York: Cambridge University Press.
Gaigg, S. B., & Bowler, D. M. (2007). Differential fear conditioning in Asperger's syndrome: implications for an amygdala theory of autism. *Neuropsychologia*, *45*, 2125–2134.
Gastgeb, H., Rump, K. M., Best, C. A., Minshew, N. J., & Strauss, M. S. (2009). Prototype formation in autism: can individuals with autism abstract facial prototypes? *Autism Research*, *2*(5), 279–284. http://dx.doi.org/10.1002/aur.93.
Gastgeb, H., Dundas, E. M., Minshew, N. J., & Strauss, M. S. (2012). Category formation in autism: can individuals with autism form categories and prototypes of dot patterns? *Journal of Autism and Developmental Disorders*, *42*(8), 1694–1704. http://dx.doi.org/10.1007/s10803-011-1411-x.
Gidley Larson, J. C., & Mostofsky, S. H. (2008). Evidence that the pattern of visuomotor sequence learning is altered in children with autism. *Autism Research*, *1*, 341–353.
Hobson, R. P. (1989). Beyond cognition: a theory of autism. In G. Dawson (Ed.), *Autism: nature, diagnosis & treatment* (pp. 22–48).New York: The Guilford Press.

Jarrell, T. W., Gentile, C. G., Romanski, L. M., McCabe, P. M., & Schneiderman, N. (1987). Involvement of cortical and thalamic auditory regions in retention of differential bradycardia conditioning to acoustic conditioned stimuli in rabbits. *Brain Research*, *412*, 285–494.
Just, M. A., Cherkassky, V. L., Keller, T. A., & Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain*, *127*, 1811–1821. http://dx.doi.org/10.1093/brain/awh199.
Just, M. A., Cherkassky, V. L., Keller, T. A., Kana, R. K., & Minshew, N. J. (2007). Functional and anatomical cortical underconnectivity in autism: evidence from an fMRI study of executive function task and corpus callosum morphometry. *Cerebral Cortex*, *17*(4), 951–961. http://dx.doi.org/10.1093/cercor/bhl006.
Kana, R. K., Keller, T. A., Cherkassky, V. L., Minshew, N. J., & Just, M. A. (2006). Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain*, *129*, 2484–2493. http://dx.doi.org/10.1093/brain/aw1164.
Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child*, *2*, 217–250.
Klinger, L., & Dawson, G. (2001). Prototype formation in autism. *Development and Psychopathology*, *13*, 111–124.
Klinger, L. G., Klinger, M. R., & Pohlig, R. A. (2007). Implicit learning impairments in autism spectrum disorders: implications for treatment. In J. M. Perez, P. M. Gonzalez, M. L. Comi, & C. Nieto (Eds.), *New developments in autism*London: Jessica Kinglsey.
Knight, D. C., Smith, C. N., Stein, E. A., & Helmstetter, F. J. (1999). Functional MRI of human Pavlovian fear condition: patterns of activation as a function of learning. *NeuroReport*, *10*, 3665–3670.
Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2003). Expression of conditional fear with and without awareness. *Proceedings of the National Academy of Sciences*, *100*, 15280–15283. http://dx.doi.org/10.1073/pnas.2535780100.
LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *The Journal of Neuroscience*, *15*, 6846–6855.
Lanteaume, L., Khalfa, S., Régis, J., Marquis, P., Chauvel, P., & Bartolomei, F. (2007). Emotion induction after direct intracerebral stimulations of human amygdala. *Cerebral Cortex*, *17*(6), 1307–1313. http://dx.doi.org/10.1093/cercor/bhl041.
Levey, A. B. (1980). Measurement units in psychophysiology. In I. Martin, & P. H. Venables (Eds.), *Techniques in Psychophysiology* (pp. 597–628).New York: Wiley.
Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., et al. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy*, *43*(11), 1391–1424. http://dx.doi.org/10.1016/j.brat.2004.10.007.
Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., et al. (2000). The Autism Diagnostic Observation Schedule—Generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorder*, *30*, 1573–3432.
Minshew, N. J., & Goldstein, G. (2001). The pattern of intact and impaired memory functions in autism. *Journal of Child Psychology and Psychiatry*, *42*(8), 1095–1101. http://dx.doi.org/10.1111/1469-7610.00808.
Minshew, N. J., & Williams, D. L. (2007). The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Archives of Neurology*, *64*, 945–950.
Minshew, N. J., Goldstein, G., & Siegel, D. J. (1997). Neuropsychologic functioning in autism: profile of a complex information processing disorder. *Journal of the International Neuropsychological Society*, *3*, 303–316.
Morris, J. S., Friston, K. J., & Dolan, R. J. (1997). Neural responses to salient visual stimuli. *Proceedings of the Royal Society London, Series B: Biological Sciences*, *264*, 769–775.
Mostofsky, S. H., Goldberg, M. C., Landa, R. J., & Denckla, M. B. (2000). Evidence for a deficit in procedural learning in children and adolescents with autism: implications for cerebellar contributions. *International Neuropsychological Society*, *6*, 752–759.
Mundy, P., & Sigman, M. (1989). Specifying the nature of the social impairment in autism. In G. Dawson (Ed.), *Autism: nature diagnosis and treatment* (pp. 3–21).New York: The Guildford Press.
Neuhaus, E., Bernier, R. A., & Beauchaine, T. P. (2015). Electrodermal response to reward and non-reward among children with autism. *Autism Research*. http://dx.doi.org/10.1002/aur.1451.
Pineles, S. L., Orr, M. R., & Orr, S. P. (2009). An alternative scoring method for skin conductance responding in a differential fear conditioning paradigm with a long-duration conditioned stimulus. *Psychophysiology*, *46*, 984–995. http://dx.doi.org/10.1111/j.1469-8986.2009.00852.x.
Schipul, S. E., & Just, M. A. (2015). Diminished neural adaptation during implicit learning in autism. *NeuroImage*, *125*, 332–341. http://dx.doi.org/10.1016/j.neuroimage.2015.10.039.
Schmitz, N., Rubia, K., van Amelsvoort, T., Daly, E., Smith, A., & Murphy, D. G. M. (2008). Neural correlates of reward in autism. *British Journal of Psychiatry*, *192*, 19–24.
Sears, L. L., Finn, P. R., & Steinmetz, J. E. (1994). Abnormal classical eye-blink conditioning in autism. *Journal of Autism and Developmental Disorders*, *24*(6), 737–751.
Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., et al. (2009). Human fear conditioning and extinction in neuroimaging: a systematic review. *PLoS One*, *4*(6), e5865.
South, M., Larson, M. J., White, S. E., Dana, J., & Crowley, M. J. (2011). Better fear conditioning is associated with reduced symptom severity in autism spectrum disorders. *Autism Research*, *4*, 412–421.
South, M., Newton, T., & Chamberlain, P. D. (2012). Delayed reversal learning and association with repetitive behavior in autism spectrum disorders. *Autism Research*, *5*, 398–406. http://dx.doi.org/10.1002/aur.1255.
Steinmetz, J. E., Tracy, J. A., & Green, J. T. (2001). Classical eyeblink conditioning: clinical models and application. *Integrative Physiological and Behavioral Sciences*, *36*, 220–238.
Travers, B. G., Powell, P. S., Mussey, J. L., Klinger, L. G., Crisler, M. E., & Klinger, M. R. (2013). Spatial and identity cues differentially affect implicit contextual cueing in adolescents and adults with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *43*(10), 2393–2404. http://dx.doi.org/10.1007/s10803-013-1787-x.
Vladusich, T., Olu-Lafe, O., Kim, D.-S., Tager-Flusberg, H., & Grossberg, S. (2010). Prototypical category learning in high-functioning autism. *Autism Research*, *3*, 1–11.
Wechsler, D. (1999). *Wechsler abbreviated scale of intelligence*. San Antonio, TX: Psychological Corporation.
Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. *Psychophysiology*, *44*, 170–180. http://dx.doi.org/10.1111/j.1469-8986.2006.00469.x.
Williams, D. L., Goldstein, G., & Minshew, N. J. (2006). The profile of memory function in children with autism. *Neuropsychology*, *20*(1), 21–29. http://dx.doi.org/10.1037/0894-4105.20.1.21.