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ORIGINAL PAPER

Eyeblink Conditioning: A Non-invasive Biomarker for Neurodevelopmental Disorders

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Abstract Eyeblink conditioning (EBC) is a classical conditioning paradigm typically used to study the underlying neural processes of learning and memory. EBC has a well-defined neural circuitry, is non-invasive, and can be employed in human infants shortly after birth making it an ideal tool to use in both developing and special populations. In addition, abnormalities in the cerebellum, a region of the brain highly involved in EBC, have been implicated in a number of neurodevelopmental disorders including autism spectrum disorders (ASDs). In the current paper, we review studies that have employed EBC as a biomarker for several neurodevelopmental disorders including fetal alcohol syndrome, Down syndrome, fragile X syndrome, attention deficit/hyperactivity disorder, dyslexia, specific language impairment, and schizophrenia. In addition, we discuss the benefits of using such a tool in individuals with ASD.

Keywords Eyeblink conditioning · Neurodevelopmental disorders · Autism spectrum disorder · Associative learning · Cerebellum

Introduction

For over 50 years, eyeblink conditioning (EBC) has been used as a model system to study the underlying neural processes of learning and memory (Christian and Thompson 2003). As well, EBC paradigms have been employed to examine aberrant neural circuitry in a number of neurodevelopmental disorders including fetal alcohol syndrome, genetic disorders (i.e., Down syndrome, fragile X syndrome), attention deficit/hyperactivity disorder, learning disorders (i.e., dyslexia and specific language impairment), schizophrenia, and autism spectrum disorder (ASD). Learning during EBC can be objectively measured and individual differences in the rate of learning have been found to relate to variability in social and cognitive outcomes (Reeb-Sutherland et al. 2012). Thus, presence, rate of learning, and morphology of the response may all serve as potential biomarkers for identifying pathology or risk for negative outcomes. In this paper, we review the literature on EBC in humans and argue for the use of this method to identify biomarkers for neurodevelopmental disorders. As part of this review, we present an in-depth look at how EBC may be helpful in the study of ASD.

Biomarkers are characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention. EBC meets these criteria for a number of reasons. First, it involves well-defined, highly conserved neural circuitry. Extensive work with animals has defined these brain circuits (for review see Christian and Thompson 2003). This animal work has allowed researchers to use EBC and examine the integrity of these brain circuits in humans. Second, some conditioned responses reach adult levels within the first 5 months of age in human infants (Herbert et al. 2003), suggesting that it can be used early in life. A number of studies have examined EBC in newborn or 1-month-old infants (Fifer et al. 2010; Little et al. 1984; Reeb-Sutherland et al. 2011) making it ideal for examining high-risk

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populations prior to the manifestation of a neurodevelopmental disorder. Third, EBC is non-invasive making it ideal for examining underlying neural mechanisms of learning and memory in developing and special populations. Fourth, EBC responses are not related to general intelligence (Cromwell et al. 1961), making it a suitable measure in populations with neurodevelopmental disabilities. Finally, EBC paradigms provide experimental control of stimulus delivery as well as precise measurement of learned responses allowing researchers to manipulate timing of the CS and US so that different underlying neural mechanisms can be activated and examined.

This review is divided into three sections. The first section outlines the different types of EBC paradigms used and the associated dependent measures followed by brief overviews of the underlying neural circuitry and ontogeny of EBC. The second section highlights the various studies that have examined the effects of various neurodevelopmental disorders on EBC performance. The third section focuses on the use of EBC for the study of ASDs.

Eyeblink Conditioning

Delay and Trace Conditioning

EBC is a classical Pavlovian conditioning paradigm in which a once neutral stimulus comes to elicit a learned reflexive response. In a typical EBC paradigm, an individual is presented with a conditioned stimulus (CS), usually a pure tone, which is followed by the presentation of the unconditioned stimulus (US), a mild puff of air to the eye, which elicits a reflexive eyeblink unconditioned response (UR). After several repeated tone-puff pairings, the tone CS comes to elicit an eyeblink conditioned response (CR). The CR represents the learned association between the tone and the puff of air. After several CS–US pairings, the ideal pattern of timing is that of an adaptive CR such that the eyelid is closed and the cornea protected at the time the US is presented.

The two most widely employed EBC paradigms are delay and trace conditioning. During delay conditioning, the tone CS precedes, overlaps, and co-terminates with the presentation of the air puff US (Fig. 1, top). In trace conditioning, there is a brief stimulus-free interval, termed the trace interval, which appears between the offset of the tone CS and onset of the air puff US (Fig. 1, bottom). A number of different dependent measures (Table 1) are obtained from both delay and trace conditioning. These include measures of learning (i.e., rate of acquisition, retention, and extinction of the CR) and morphology of the blink response (i.e., peak amplitude, onset latency, and peak latency of the CR and UR). Each of these dependent measures has been linked to different aspects of the underlying neural circuitry supporting this CR.

Acquisition rate refers to the increase in the number of learned CRs over the course of a conditioning session. This is typically reported as the percentage of CRs (%CR) that occurs within blocks of trials over time. A significant increase in %CR over the course of conditioning determines whether learning has occurred. Repeated measures analysis of %CRs over time, overall average of %CRs, total number of CRs, and trials-to-criterion (i.e., number of trials needed to produce a predetermined number of CRs within a predetermined number of trials over a predetermined number of blocks) have all been used to examine whether differences in learning acquisition exist between two or more groups. *Retention* can be observed over repeated conditioning sessions which can be separated by hours, days, or even years. Retention is determined if improvement in learning acquisition is observed between conditioning sessions. This improvement typically consists of displaying increased %CR during the initial trials of the second conditioning session as well as displaying overall increase in the average %CR across the second conditioning session compared to the last block of trials of the first session. *Extinction* refers to the decrease in the number of CRs across trials of repeated presentations of a CS that is no longer paired with the US. A significant decrease in the %CR across CS-alone trials as well as a significant decrease in average %CR compared to acquisition sessions have been used as determinants of extinction.

In addition to these measures of learning, the morphology of the eyeblink response including *amplitude* and *latency* have been examined as dependent measures. Peak

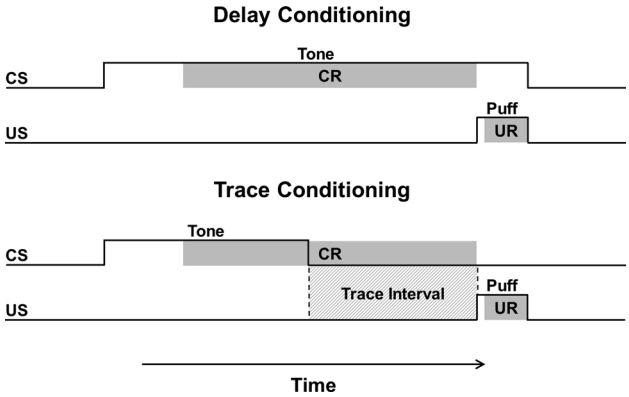


Fig. 1 Experimental design for delay and trace conditioning. During delay conditioning (*top*), presentation of the tone conditioned stimulus (CS) is followed by and coterminates with the presentation of the air puff unconditioned stimulus (US). During trace conditioning (*bottom*), presentation of the tone CS is followed first by a brief stimulus-free trace interval and then the presentation of the air puff US. The conditioned response (CR) occurs ~200–300 ms after the onset of the tone CS and prior to the onset of the air puff US. The unconditioned response (UR) occurs after the onset of the air puff US

Table 1 Definitions of dependent measures used in eyeblink conditioning

Dependent measure	Definition
Learning measures	
Acquisition	Increase in number of CRs during a conditioning session; measured as %CR, mean CRs, # of CRs, trials-to-criterion
Retention	Increase or same level of CRs during the initial trials of a follow-up conditioning session compared to the last trials of the initial conditioning session; measured as %CR, mean CRs, # of CRs, trials-to-criterion
Extinction	Decrease in number of CRs following repeated presentations of the CS alone; measured as %CR, mean CRs, # of CRs, trials-to-criterion
Eyeblink morphology	
Peak amplitude	Highest amplitude value of the EMG waveform associated with the eyeblink response; can be measured for CRs and URs; unit of measure is μV
Peak latency	Time at which the peak amplitude occurs; CR peak latency measured following the presentation of the CS and UR peak latency measured following the presentation of the US; unit of measure is ms
Onset latency	Time at which the rise of the slope of the EMG waveform reaches a predetermined amplitude; CR onset latency measured following the presentation of the CS and UR onset latency measured following the presentation of the US; unit of measure is ms

CS conditioned stimulus, *US* unconditioned stimulus, *CR* conditioned response, *UR* unconditioned response, *EMG* electromyographic, *μV* microvolts, *ms* milliseconds

amplitude of the blink response of both CRs and URs during paired trials and peak amplitude of URs during US-alone trials are common dependent measures. Typically, CR amplitude increases while UR amplitude decreases over the course of training. With respect to latency, both onset and peak latency of URs and CRs have been examined. The UR is measured following the onset of the air puff US while the CR is measured following the onset of the tone CS (Fig. 1). The timing of the CR should adapt after several CS–US pairings in order to optimally protect the eye from the air puff. In typical learning, a decrease in both onset and peak latency over the course of CS–US pairing is observed such that the blink response is beginning prior to the presentation of the US and the eyelid is completely closed by the time the US is presented.

The majority of the studies highlighted in the current review used a delay conditioning paradigm; only a few used trace conditioning. While all of these studies examined acquisition, some also examined retention and extinction.

Neural Circuitry of Eyeblink Conditioning

The neural circuitry underlying both delay and trace conditioning has been well-defined and is highly conserved across animals and humans (Cheng et al. 2008; Christian and Thompson 2003; Gerwig et al. 2007; Woodruff-Pak and Disterhoft 2008; Woodruff-Pak and Steinmetz 2000a, b). Delay conditioning has been shown to be mediated by brainstem-cerebellar function (Christian and Thompson 2003; Cromwell et al. 1961; Kaufmann et al. 2004; Yung et al. 1996) while trace conditioning requires not only the cerebellum, but also the hippocampus (Kim et al. 1995; Moyer et al. 1990; Solomon et al. 1986). Figure 2 highlights the basic pathways involved in both delay and trace conditioning. For the CS pathway, information about the CS is projected to the pontine nuclei via the auditory nuclei and subsequently projected by mossy fibers of the middle cerebellar peduncle. For the US pathway, information about the US is projected via the trigeminal nucleus to the inferior olive by climbing fibers of the inferior peduncle. Inputs from both the pontine nuclei and inferior olive reach Purkinje cells in the cerebellar cortex which then send information to the deep cerebellar nuclei directly (for details see Christian and Thompson 2003). Appropriately timed activation of inputs to the cerebellar cortex and deep nuclei underlies delay conditioning (Kaufmann et al. 2004; Laasonen et al. 2012; Yung et al. 1996). The CR pathway consists of fibers that project from the interpositus nucleus to the red nucleus. This activity is then relayed to the premotor and motor cortex which then generates an observed eyeblink response (Christian and Thompson 2003). Information about this CR may then be projected to the hippocampus during trace conditioning via cerebellar-thalamic-cortical pathways (Schmajuk and DiCarlo 1991). Disruption of any of the above pathways can lead to impairments in acquisition and retention in delay conditioning and acquisition in trace conditioning while lesioning of the hippocampus leads to disruption in retention of trace conditioning (Christian and Thompson 2003; Kaufmann et al. 2004). In addition, the inferior olivary complex (McCormick et al. 1985), interpositus nuclei (Hardiman et al. 1996; Ramnani and Yeo 1996), and anterior cerebellar cortex (Perrett and Mauk 1995) have all been implicated in extinction of the learned response. With respect to amplitude of the eyeblink response, UR and CR

amplitude have been attributed to the amygdala (Yung et al. 2003) and deep cerebellar nuclei (Berthier and Moore 1990; Sears and Steinmetz 1990), respectively. The anterior lobe of the cerebellum, through Purkinje cells projecting to the interpositus nucleus, has been shown to play a significant role in the timing of the CR (Garcia and Mauk 1998; Garcia et al. 1999; McCormick and Thompson 1984; Perrett et al. 1993).

Development of Eyeblink Conditioning

Initial attempts to classically condition human infants were unsuccessful and led many researchers to conclude that infants were unable to be conditioned until several months after birth (Morgan and Morgan 1944; Rendle-Short 1961; Wenger 1936). However, it is now well established that infants demonstrate learning within minutes after birth (Fitzgerald and Brackbill 1976; Rovee-Collier and Lipsitt 1982) as well as within the womb (DeCasper and Fifer 1980). The EBC paradigm is one of the most reliable and widely employed classical conditioning techniques that can be used to examine the ontogeny of learning and memory mechanisms in human infants (Claflin et al. 2002; Fifer et al. 2010; Herbert et al. 2003; Ivkovich et al. 1999; Ivkovich 2000; Lintz et al. 1967; Little et al. 1984; Naito and Lipsitt 1969; Reeb-Sutherland et al. 2011).

Learning acquisition in EBC emerges gradually over the course of development in both rodents and humans (Stanton 2010). Specifically, in humans, learning in delay EBC paradigms can be observed within the first weeks after birth (Fifer et al. 2010; Little et al. 1984; Reeb-Sutherland et al. 2011), reaching adult levels of acquisition by 5 months of age (Herbert et al. 2003; Hoffman et al. 1985). Although 5-month-old infants display a similar number of conditioned responses as adults, the adaptive timing of the eyeblink response differs with infants showing shorter, more poorly timed conditioned responses compared to adults (Herbert et al. 2003) suggesting that the cerebellar circuitry is still immature during infancy. In contrast to delay conditioning, learning acquisition during trace EBC paradigms does not appear to reach adult levels even well into late childhood (Jacobson et al. 2011; Werden and Ross 1972). Long-term retention of the eyeblink response has been shown to last at least 10 days in infants as young as 20 days of age (Little et al. 1984) and 1.5 years in children who are 12 years of age (Jacobson et al. 2011).

For optimal learning acquisition in delay conditioning, infants younger than 5 months of age require an ISI of approximately 1,000 ms (Little et al. 1984). In contrast, 5-month-old infants can be presented with a short ISI (i.e., 650 ms), an interval that is typically used in adult EBC studies, and display similar learning to adult participants (Herbert et al. 2003). However, when a longer ISI (i.e., 1,250 ms) is used during a delay conditioning paradigm or when a trace conditioning paradigm is used, 5-month-old infants are unable to acquire the learned response (Herbert et al. 2003). These results suggest that the cerebellum is sufficiently developed within the first 6 months of life to maximally form CS-US associations when short interstimulus intervals are used; however, further development

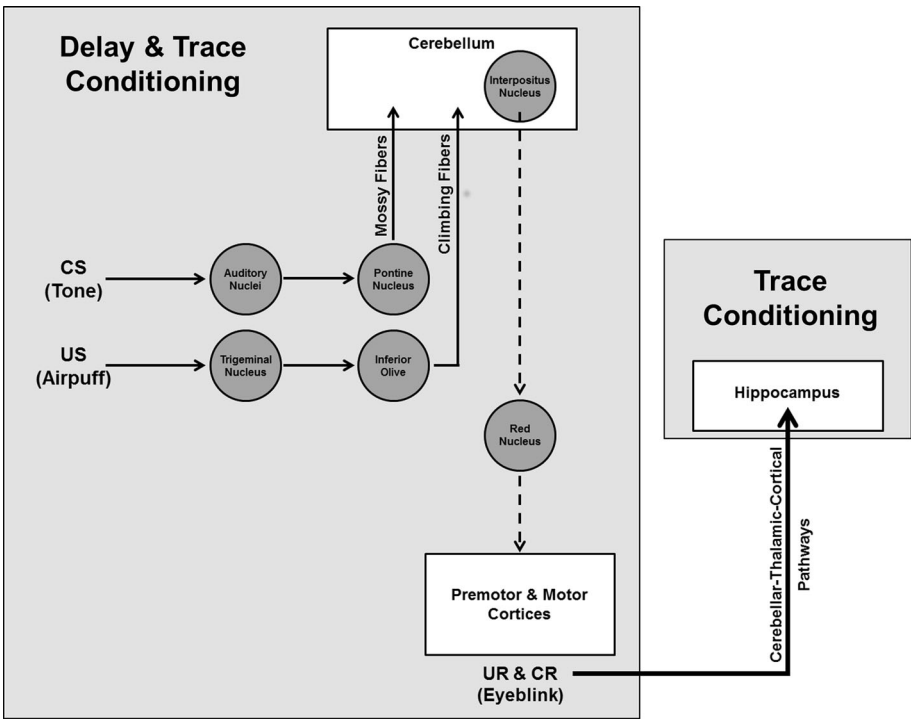


Fig. 2 Simplified diagram of the neural circuitry for delay and trace eyeblink conditioning (adapted from Christian and Thompson 2003). Conditioned stimulus (CS), unconditioned stimulus (US), unconditioned response (UR), conditioned response (CR)

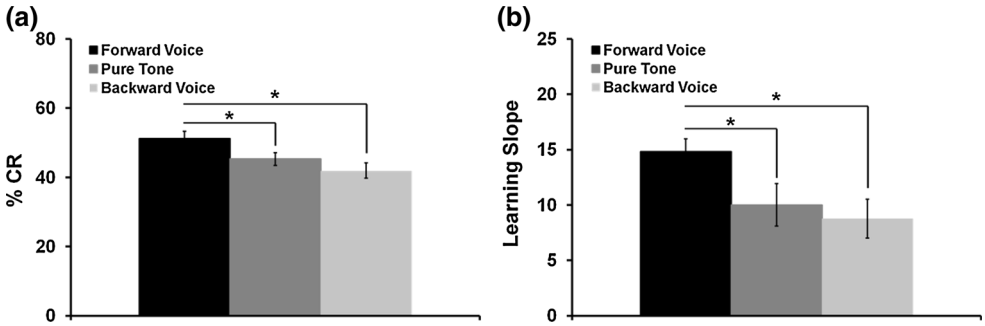


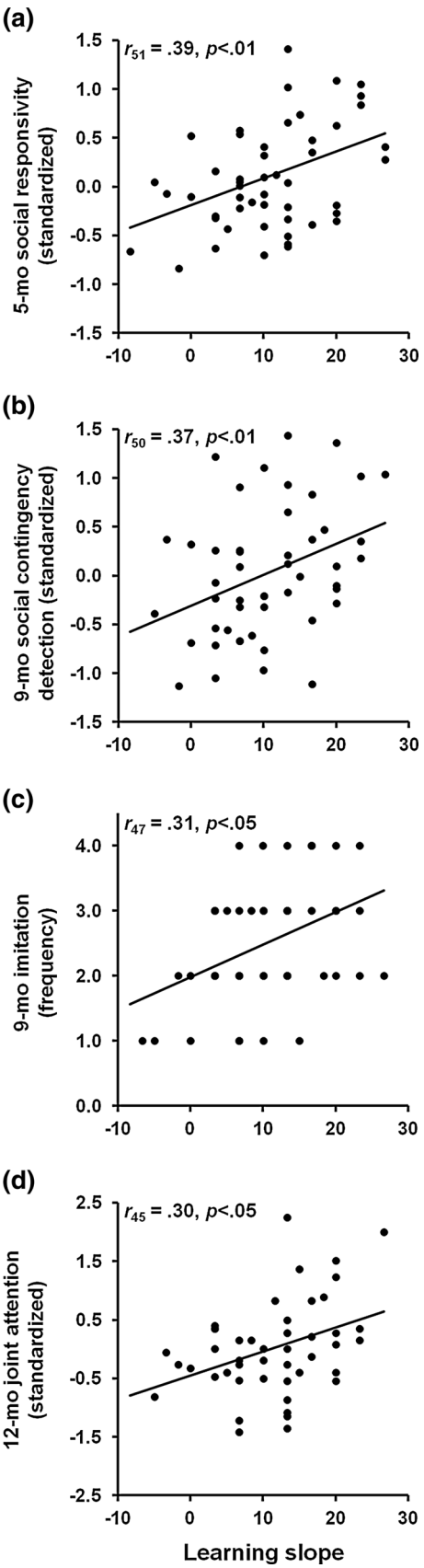
Fig. 3 One-month-old infant eyeblink conditioning is facilitated by social cues. Infants conditioned to a forward female voice (*black bars*) displayed (**a**) a greater percentage of conditioned responses (%CR) and (**b**) a more rapid increase in learning across trials (*Learning Slope*) compared to infants conditioned to a pure tone (*dark gray bars*) or backward female voice (*light gray bars*). *Error bars* represent *Mean* ± *SEM*. $*p < .05$. (From Reeb-Sutherland et al. 2011.)

of the cerebellum is needed in order to demonstrate associative learning during long delay conditioning. As noted above, although children 4–12 years of age demonstrate learning during trace conditioning, they still do not reach asymptotic levels of learning like those observed in adults (Jacobson et al. 2011; Werden and Ross 1972) suggesting that the neural circuitry, particularly the hippocampus, is not fully developed even by 12 years of age. However, to date, no study has systematically examined the ontogeny of long-delay and trace conditioning. Knowing the appropriate ISI length during EBC across development is important not only for creating an optimal conditioning environment for infants and children but also for being able to make appropriate comparisons in learning between typically developing children and those with a neurodevelopmental disorder.

In addition to the ISI playing a crucial role in the acquisition of the conditioned eyeblink response in infants, it has recently been shown that the use of social stimuli can affect learning acquisition during delay EBC in 1-month-old infants (Reeb-Sutherland et al. 2011). In this study, different groups of infants were presented with one of 3 different CSs: pure tone, woman's forward voice saying "hi baby", and backward voice. Although all groups of infants showed significant learning across trials, infants who were presented with the forward voice displayed significantly greater conditioning compared to the infants conditioned to either the tone or the backwards voice (Fig. 3; Reeb-Sutherland et al. 2011). These results suggest that infants learn more readily in the context of ecologically-relevant and salient social stimuli. Being able to examine how social context can influence infants' learning abilities prior to the onset of higher level social cognitive skills may help identify individuals who are at risk for developing a social disorder, such as ASD. Although much is known about the development of EBC during infancy, much less is known about the potential link between this type of associative learning and behavioral or cognitive outcomes. It has been suggested that the infant's abilities to detect and respond to contingencies in the surrounding environment influence the development of social behavior (Hammock and Levitt 2006; Tarabulsy et al. 1996; Watson 1966). Thus associative learning may serve as an important building block for the later development of complex social behaviors. Heterogeneity in associative learning processes like those examined in EBC may predict the development of social behavior. To assess this possibility, heterogeneity in 1-month-olds' learning acquisition during delay EBC was examined in relation to individual differences in social behavior at 5, 9, and 12 months of age (Reeb-Sutherland et al. 2012). In addition, neural activity related to social discrimination was measured at 9 months of age. Faster learning at 1 month was found to be related to increased social positivity at 5 months of age (Fig. 4a), increased social contingency detection (Fig. 4b) and imitation ability (Fig. 4b) at 9 months of age, and increased joint attention at 12 months of age (Fig. 4d). In addition, a significant relation between 1-month learning rate and neural correlates of familiar versus unfamiliar face discrimination at 9 months of age was found, such that infants who showed faster learning at 1 month displayed a greater ability to discriminate between their mother's and an unfamiliar female's face (Fig. 5). Surprisingly, the relations found in the Reeb-Sutherland et al. (2012) study were specific to social behaviors and were not the result of individual differences in general cognitive abilities (Reeb-Sutherland et al. 2012). These results suggest that individual differences in early associative learning may serve as a major building block for the development of social behavior.

Eyeblink Conditioning and Neurodevelopmental Disorders

In this section, we review studies that have examined the effect of various neurodevelopmental disorders on EBC



◀**Fig. 4** Relations between early learning and social behavior during the first year of life. Heterogeneity in 1-month-olds' eyeblink conditioning acquisition was significantly correlated with (**a**) 5-month social responsivity, (**b**) 9-month social contingency detection, (**c**) 9-month imitation, and (**d**) 12-month joint attention. (From Reeb-Sutherland et al. 2012.)

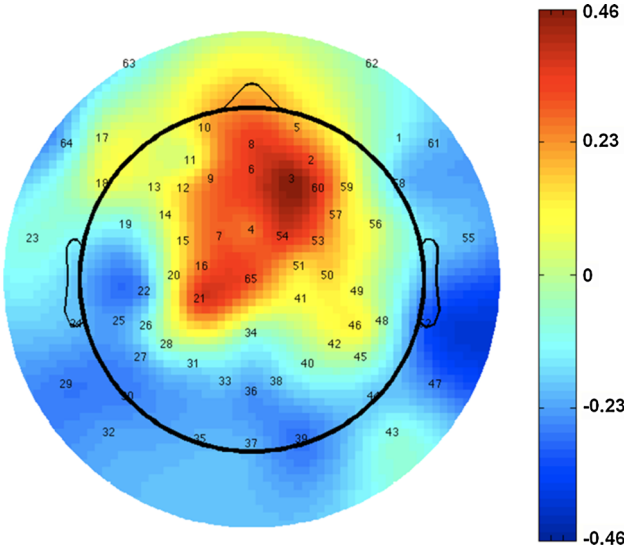


Fig. 5 Relation between early learning and 9-month neural activation of facial discrimination. Infants who conditioned more rapidly at 1 month of age displayed greater discrimination in medial fronto-central activation to mother's versus a stranger's face. (From Reeb-Sutherland et al. 2012.)

performance. The majority of these have utilized the cerebellar-dependent delay paradigm, most probably because of hypothesized aberrant cerebellar development associated with these disorders. It was once believed that the cerebellum was mainly involved in motor function; however, recent evidence suggests that the cerebellum also contributes to a wide range of cognitive, socio-emotional, and communication functions (Bolduc et al. 2011; O'Halloran et al. 2012; Rapoport 2001; Schmahmann et al. 2007). Moreover, studies have identified cerebellar involvement in specific areas of social function including theory of mind (Brunet et al. 2000; Calarge et al. 2003), empathy (Shamay-Tsoory et al. 2005), action understanding (Sokolov et al. 2010, 2012), and emotion recognition (D'Agata et al. 2011) which lends additional support to the possibility that cerebellar dysfunction underlies many of the neurodevelopmental disorders described below, particularly ASD. Results from both delay and trace conditioning (when available) paradigms are reviewed and evidence for the potential use of EBC as a biomarker is highlighted. Table 2 outlines the methodology and dependent measures used in each study. What should be noted is both the variability in the methodology used (# sessions,

Table 2 Study details

Neurodevelopmental disorder study	# Participants (# males)	Age range/mean age	IQ	Conditioning type	# Ses/ # trials	ISI (ms)	Dependent measure	Main results
Fetal alcohol syndrome								
Coffin et al. (2005)	C = 10 (10)	6–12 yr/9.9 yr	106.0	Delay—Acq	1/72	400	% CR	C > P
	P = 10 (6)	6–12 yr/9.5 yr	96.0				TTC	C < P
							Onset lat	C < P
Jacobson et al. (2008)	C = 64 (n/a)	4.3–5.5 yr/5.0 yr	89.7	Delay—Acq	3/50	650	TTC	C < P
	P = 29 (n/a)	4.3–5.5 yr/5.0 yr	82.0					
Jacobson et al. (2011)	C = 34 (14)	8–12 yr/11.3 yr	76.1	Delay—Acq	2–4/50	650	% CR	C > P
	P = 29 (19)	8–12 yr/11.0 yr	62.2				TTC	C < P
							Onset lat	C < P
							Peak lat	C < P
		12.8 yr		Trace—Acq	2/50	1250	% CR	C = P
		12.8 yr					TTC	C < P
							Onset lat	C < P†
							Peak lat	C < P†
Down syndrome								
Ohlrich and Ross (1968)	C = 32 (n/a)	6–8 yr/7.0 yr	n/a	Delay—Acq	3/50	500 & 800	% CR	C > P
	P = 32 (n/a)	10–17 yr/13.3 yr (mental age = 7yr)	n/a					
Woodruff-Pak et al. (1994)	C = 44 (16)	19–64 yr/37.7 yr	111.0	Delay—Acq	1/90	400	% CR	C > P
	P = 44 (22)	19–64 yr/37.9 yr	29.1					
Fragile X syndrome								
Woodruff-Pak et al. (1994)	C = 44 (16)	19–64 yr/37.9 yr	111.0	Delay—Acq	1/90	400	% CR	C = P
	P = 20 (18)	17–77 yr/40.9 yr	28.1					
Koekkoek et al. (2005)	C = 6 (6)	n/a	n/a	Delay—Acq	4/16	500	% CR	C > P
	P = 6 (6)	n/a	n/a				Peak amp	C > P
Smit et al. (2008)	C = 14 (14)	22–45 yr/30.0 yr	n/a	Delay—Acq	1/80	500	% CR	C > P
	P = 11 (11)	21–39 yr/31.0 yr	74.0				Onset lat	C = P
				Delay—Ret	1/80		% CR	C = P
							Onset lat	C = P
				Delay—Ext	1/70		% CR	C > P
							Onset lat	C = P
Tobia and Woodruff-Pak (2009)	C = 20 (15)	17–77 yr/45.8 yr	n/a	Delay—Acq	1/90	400	% CR	C > P
	P = 20 (17)	17–77 yr/45.9 yr	n/a				Onset lat	C < P
							Peak lat	C = P
							Peak amp	C = P
ADHD								
Coffin et al. (2005)	C = 13 (6)	6–12 yr/9.5 yr	106.0	Delay—Acq	1/72	400	% CR	C = P
	P = 16 (13)	6–12 yr /9.8 yr	103.0				TTC	C = P
							Onset lat	C > P†
Frings et al. (2010)	C = 11 (9)	10–15 yr/12.1 yr	110.3	Delay—Acq	2/50	440 & 840	% CR	C = P
	P = 10 (10)	10–15 yr/12.3 yr	101.8				Onset lat	C > P
							Peak lat	C > P
				Delay—Ext	1/5		% CR	C < P
Premature birth								
Herbert et al. (2004)	C = 11 (5)	5 mo ± 10 d	n/a	Delay—Acq	1/50	650	% CR	C = P
	P = 18 (5)	5 mo ± 10 d (corrected for gestational age)	n/a				TTC	C < P
				Delay—Ret	1/50		% CR	C > P
				Delay—Ext	1/50		% CR	C = P

Table 2 continued

Neurodevelopmental disorder study	# Participants (# males)	Age range/mean age	IQ	Conditioning type	# Ses/ # trials	ISI (ms)	Dependent measure	Main results
Dyslexia								
Nicolson et al. (2002)	C = 13 (11)	n/a /20.1 yr	113.3	Delay—Acq	1/60	720	# CRs	C = P
	P = 13 (12)	n/a /19.5 yr	116.1				Peak lat	C = P
							Peak amp	C = P
				Delay—Ext	1/10		# CRs	C = P
Coffin et al. (2005)	C = 13 (6)	6–12 yr/9.5 yr	106.0	Delay—Acq	1/72	400	% CR	C > P
	P = 14 (7)	6–12 yr/9.1 yr	101.0				TTC	C < P
							Onset lat	C < P
Specific language impairment								
Steinmetz and Rice (2010)	C = 16 (11)	9–20 yr/14.2 yr	n/a	Delay—Acq	1/100	350	% CR	C = P
	P = 15 (12)	9–20 yr/14.4 yr	n/a				Onset lat	C = P
							Peak lat	C = P
				Delay—Ext	1/25		% CR	C = P
							Onset lat	C = P
							Peak lat	C = P
Schizophrenia								
Taylor and Spence (1954)	C = 74 (n/a)	n/a	n/a	Delay—Acq	1/80	470	% CR	C < P
	P = 40 (n/a)	n/a	n/a					
O'Connor and Rawnsley (1959)	C = 20 (20)	28–59 yr/39.4 yr	n/a	Delay—Acq	1/48	350	# CRs	C = P
	P = 40 (40)	33–55 yr/44.4 yr	n/a					
Spain (1966)	C = 24 (12)	28–53 yr/40.3 yr	n/a	Delay—Acq	2/50	500	% CR	C < P
	P = 32 (32)	28–53 yr/40.3 yr	n/a					
Sears et al. (2000)	C = 15 (11)	21–41 yr/31.3 yr	n/a	Delay—Acq	1/70	400	% CR	C < P
	P = 15 (11)	20–49 yr/32.8 yr	n/a				TTC	C > P
							Onset lat	C > P
							Peak amp	C < P
				Delay—Ext	1/40		% CR	C = P
Hofer et al. (2001)	C = 20 (17)	22–40 yr/30.9 yr	n/a	Delay—Acq	1/72	720	% CR	C > P
	P = 24 (21)	21–39 yr/30.3 yr	99.8					
Marenco et al. (2003)	C = 10 (n/a)	n/a /41.9 yr	n/a	Delay—Acq	1/77	440	% CR	C = P
	P = 10 (n/a)	n/a /41.8 yr	n/a				Onset lat	C < P
	C = 9 (n/a)	n/a /33.7 yr	n/a	Trace—Acq	1/77	1540	% CR	C = P
	P = 10 (n/a)	n/a /31.8 yr	n/a				Onset lat	C > P
Brown et al. (2005)	C = 13 (7)	23–58 yr/40.2 yr	n/a	Delay—Acq	1/100	350	% CR	C > P
	P = 13 (7)	23–58 yr/42.0 yr	n/a				Onset lat	C > P
							Peak lat	C > P
				Delay—Ext	1/50		% CR	C = P
							Onset lat	C > P
							Peak lat	C > P
Edwards et al. (2008)	C = 7 (4)	n/a /43.5 yr	n/a	Delay—Acq	1/108	350	% CR	C > P
	P = 10 (6)	n/a /40.0 yr	n/a				Peak lat	C = P
							Peak amp	C = P
Bolbecker et al. (2009)	C = 62 (30)	n/a /39.9 yr	n/a	Delay—Acq	1/100	350	% CR	C > P
	P = 62 (39)	n/a /39.8 yr	n/a				Peak lat	C > P
							Peak amp	C = P
				Delay—Ext	1/50		% CR	C > P[†]
							Peak lat	C = P
							Peak amp	C = P

Table 2 continued

Neurodevelopmental disorder study	# Participants (# males)	Age range/mean age	IQ	Conditioning type	# Ses/ # trials	ISI (ms)	Dependent measure	Main results
Bolbecker et al. (2011)	C = 55 (26)	n/a /40.9 yr	n/a	Delay—Acq	2/100	250–850	% CR	C > P
	P = 55 (33)	n/a /41.1 yr	n/a				Onset lat	C < P
Forsyth et al. (2012)	C = 18 (10)	n/a /37.9 yr	n/a	Delay—Acq	1/108	350	% CR	C > P
	P = 18 (10)	n/a /37.7 yr	n/a				Peak lat	C > P
							Peak amp	C = P
Autism								
Sears et al. (1994)	C = 11 (n/a)	6–23 yr/12.7 yr	115.4	Delay—Acq	1/90	350	TTC	C > P
	P = 11 (n/a)	7–22 yr/12.2 yr	105.7				Onset lat	C > P
							Peak lat	C > P
							Peak amp	C < P
				Delay—Ext	1/60		Peak amp	C = P
							Habituation	C < P

C controls, *P* patients, *ISI* interstimulus interval, *ms* milliseconds, *yr* years, *mo* months, *d* days, *n/a* not available, *%CR* percentage of conditioned responses, *TTC* trials-to-criterion, *lat* latency, *amp* amplitude, *Ses* sessions, *Acq* acquisition, *Ret* retention, *Ext* extinction

† $p < .10$

trials, interstimulus interval (ISI) length) and in the dependent measures reported. This variability makes it difficult to directly compare findings across studies and may explain some of the inconsistencies reported.

Fetal Alcohol Syndrome

Fetal alcohol syndrome (FAS) is characterized by distinctive craniofacial dysmorphology, small head circumference, growth retardation, and cognitive impairments associated with high levels of alcohol consumption during pregnancy (Hoyme et al. 2005). The examination of EBC in children prenatally exposed to alcohol originated from earlier studies conducted in rodents showing that prenatal alcohol exposure significantly affects cerebellar development (for review, see Green 2004) which subsequently affects performance in cerebellar-dependent EBC in young rats (Stanton and Goodlett 1998) and appears to have a lasting effect well into adulthood (Green et al. 2000). EBC has recently been used to examine children prenatally exposed to alcohol to assess cerebellar damage resulting from this prenatal insult. One of the first studies conducted by Coffin et al. (2005) demonstrated that school-age children with fetal alcohol exposure displayed decreased learning acquisition and longer CR latencies in delay EBC than typically developing children. Specifically, children prenatally exposed to alcohol did not show any evidence of conditioning while typically developing children demonstrated significant learning over the course of the conditioning session.

More recent investigations of the effect of prenatal alcohol exposure on EBC have been conducted in a population of children in South Africa (Foroud 2012; Jacobson et al. 2008, 2011; Spottiswoode et al. 2011). In their initial study, Jacobson et al. (2008) examined individual differences in EBC among children known to have varying levels of prenatal exposure to alcohol based upon both prospective measures of mothers' alcohol consumption during pregnancy as well as diagnostic measures taken. Performance on a delay EBC task was examined in five groups of children: full FAS, partial FAS, heavy exposed-nonsyndromal, non-exposed controls, as well as a small group of non-exposed microencephalic children. Children with any prenatal exposure of alcohol displayed significantly poorer learning acquisition than non-exposed children, with the majority of children displaying no learning after initial training sessions. However, with an additional training session, groups with partial FAS and heavy exposed-nonsyndromal groups displayed some learning while the full FAS group continued to display a lack of conditioning. Conditioning performance in each group was directly related to the amount of prenatal alcohol exposure: the heavy exposed-nonsyndromal group performed the best, showing some learning, the full FAS group performed the worst, and the partial FAS group's performance was in the middle. The progression in conditioning performance among these groups is suggested to reflect increased levels of exposure to alcohol prenatally and increased severity of FAS diagnosis. In addition, these results suggest that conditioning in individuals with prenatal exposure to alcohol is possible with extensive training.

In a follow-up study, both delay and trace conditioning were examined in a group of older children. It was reported that learning acquisition in both delay and trace conditioning was impaired in children with FAS compared to controls although these older children did show some

benefits of having extended sessions of training (Jacobson et al. 2011). In addition, children with FAS also displayed significantly longer CR latencies compared to controls, similar to the findings previously reported by Coffin et al. (2005). Moreover, non-exposed controls demonstrated retention for previous conditioning training after 1.5 years while children with FAS showed no retention of such training (Jacobson et al. 2011). Performance on trace but not delay EBC in children with FAS has been negatively related to cerebellar white matter (Spottiswoode et al. 2011) and has been shown to predict the severity of craniofacial characteristics associated with FAS (Foroud 2012). Given that learning acquisition during EBC is directly related to the amount of prenatal alcohol exposure as well as the severity in exposure-related neural and physiological characteristics, this type of learning may serve as a biomarker for FAS. It should be noted that although children with FAS display little to no conditioning, there are a handful of typically developing children who also do not meet learning criteria after several training sessions suggesting that EBC alone should not be used as a diagnostic test but rather in addition to the examination of characteristics associated with FAS. The addition of EBC performance as an objective measure of the underlying neural circuitry involved in FAS may help to identify those children who may develop cognitive and behavioral deficits related to known prenatal alcohol exposure even when no distinctive FAS dysmorphology is observed. The early identification of such individuals may lead to early intervention to help alleviate some of these exposure-related deficits.

Genetic Disorders

To date, EBC has been examined in the two most common chromosomal-related forms of intellectual disabilities—Down syndrome and fragile X syndrome. Down syndrome is caused by the presence of whole or partial copy of chromosome 21 (LeJeune et al. 1959) resulting in cognitive deficits, physical malformations, and a number of health risks. Classic studies examining the effects of Down syndrome on EBC in children reported that learning acquisition in a delay EBC paradigm is relatively normal compared to typically developing children (Ohlrich and Ross 1968; Ross et al. 1964, 1967). However, some impairments in learning were observed among children with Down syndrome in comparison to control children when a long delay interval was used (Ohlrich and Ross 1968) which may be linked to hippocampal rather than cerebellar dysfunction (Pennington et al. 2003). More recent studies examining delay EBC in adults with Down syndrome have shown that individuals with Down syndrome display impaired learning acquisition in both younger (<35 years of age) and older (>35 years of age) samples compared to age-matched controls (Woodruff-Pak et al. 1994, 1996) with older adults with Down syndrome showing the greatest deficits. Individuals with Down syndrome have an increased onset of Alzheimer's disease after 35 years of age (Chapman and Hesketh 2000; Lai and Williams 1989; Wisniewski et al. 1985); therefore, young and old adults with Down syndrome have also been examined in comparison to elderly adults with Alzheimer's disease. It was found that young adults with Down syndrome condition significantly better than adults with Alzheimer's disease (Woodruff-Pak et al. 1994), but older adults with Down syndrome display no difference in conditioning compared to the Alzheimer's group (Woodruff-Pak et al. 1994, 1996). However, both the older group with Down syndrome and Alzheimer's disease showed retention and improvement in learning acquisition with additional training sessions suggesting that the deficits observed during initial training were not due to cerebellar lesions (Woodruff-Pak et al. 1996). Together, these results suggest that neurobiological substrates involved in EBC (i.e., cerebellum, hippocampus) are affected by Down syndrome and that these brain regions display some deterioration as individuals with Down syndrome reach middle adulthood. As suggested by Woodruff-Pak et al. (1994), EBC may be a useful tool for examining which individuals with Down syndrome may be most susceptible for showing this Alzheimer-like neuropathology.

Fragile X syndrome is the most widespread heritable form of intellectual disability among boys (De Vries et al. 1997) and is characterized by cognitive, socio-emotional, and motor deficits as well as abnormalities in craniofacial structure (Hagerman and Hagerman 2002). The disorder is caused by a mutation of the X chromosome. Specifically, fragile X is caused by a hyperexpansion of the trinucleotide CGG repeat in the fragile X mental retardation gene, FMR1, leading to suppression of the transcription of the fragile X mental retardation protein (FMRP; Verkerk et al. 1991).

It has been suggested that one of the areas of the brain specifically targeted by this mutation is the cerebellum (Gothelf et al. 2008; Hessl et al. 2004; Hinds et al. 1993; Koekkoek et al. 2005). Several recent studies have employed delay EBC to examine whether this mutation affects the functionality of the cerebellum (Koekkoek et al. 2005; Smit et al. 2008; Tobia and Woodruff-Pak 2009; Woodruff-Pak et al. 1994). In the majority of these studies, it was reported that individuals with fragile X syndrome have impaired learning acquisition (Koekkoek et al. 2005; Smit et al. 2008; Tobia and Woodruff-Pak 2009; but see Woodruff-Pak et al. 1994 for report of no difference), longer onset latency (Tobia and Woodruff-Pak 2009), and greater extinction (Smit et al. 2008) compared to control

participants. However, individuals with fragile X syndrome displayed similar retention (6–12 months after initial acquisition) in conditioning as controls (Smit et al. 2008; Tobia and Woodruff-Pak 2009). This effect appeared to also be influenced by participant's age with younger adults with fragile X syndrome (<45 years of age) performing significantly better than older patients (>45 years of age) (Tobia and Woodruff-Pak 2009). These results suggest that some of these deficits observed in fragile X including phenotypic cognitive and behavioral deficits may involve the cerebellum (Gothelf et al. 2008; Hessl et al. 2004; Hinds et al. 1993; Koekkoek et al. 2005). However, since the studies that have been conducted thus far have only examined group differences in EBC, it is difficult to assess whether these learning differences are directly related to cognitive and behavioral cerebellar-linked deficits in individuals with fragile X. Future studies should examine whether a correlation exists between EBC performance and cognitive and behavioral abilities. Such a relation would indicate the possibility that EBC could serve as a biomarker for severity of fragile X syndrome.

Premature Birth

Being born prematurely is a common risk factor for intellectual disabilities and other cognitive and motor impairments (Aylward 2002) and can have long-lasting effects on the development of the brain including the cerebellum (Allin et al. 2001; de Kieviet et al. 2012; Hart et al. 2008; Limperopoulos et al. 2007). Surprisingly, only one study to date has examined the effects of premature birth on EBC. Herbert et al. (2004) examined both learning acquisition and retention in delay EBC in 5-month-old infants. Preterm infants demonstrated similar learning acquisition across trials as full-term infants; however, when trials-to-criterion were examined, it took the pre-term infants significantly more trials to reach criterion in comparison to full-term infants (Herbert et al. 2004). Furthermore, preterm infants displayed impairment in retention of the conditioned response, but no difference from controls during extinction (Herbert et al. 2004). Most interestingly, individual differences in measures of trials-to-criterion were examined and it was reported that a greater number of preterm compared to full-term infants failed to reach criterion even after two sessions. In addition, the majority of these non-learners remained unable to reach criterion even after a third conditioning session (Herbert et al. 2004). Furthermore, there was a significant negative correlation between head size at birth and trials-to-criterion during acquisition suggesting that head size may predict the severity of cerebellar damage leading to poor subsequent EBC performance. This finding further suggests that EBC performance may be a potential biomarker for later cognitive and/or motor abilities. However, this question still remains given that this is the only study that has examined EBC in premature infants. Additional studies to better characterize EBC in this special population should be conducted. In addition, it would beneficial to conduct longitudinal assessments to examine the relation between conditioning performance and cognitive and motor abilities in individuals born prematurely to determine if EBC may be a useful biomarker.

Attention Deficit/Hyperactivity Disorder

Attention deficit/hyperactivity disorder (ADHD) is a neurobiological disorder that manifests in early childhood and is characterized by three clinical features—inattention, motor hyperactivity, and impulsivity. Like several of the disorders described above, cerebellar dysfunction has also been identified in individuals with ADHD (Berquin et al. 1998; Krain and Castellanos 2006; Rapoport 2001). To date, two studies have indirectly examined cerebellar function in children with ADHD using delay EBC (Coffin et al. 2005; Frings et al. 2010). In the first study, it was reported that typically developing children and children with ADHD display similar performance in conditioning, but children with ADHD tended to produce non-adaptive blink latencies, displaying shorter onset latencies than controls, but this difference did not reach significance (Coffin et al. 2005).

To further explore this potential timing deficit in children with ADHD, Frings et al. (2010) recently manipulated the ISI presenting children with both a short interval (440 ms) and a long interval (840 ms). Children with ADHD did not differ from the control group in their acquisition performance (Frings et al. 2010). When onset and peak latency to blink were examined, it was found that children with ADHD displayed significantly shorter latencies than controls, but this effect was found only for the long interval condition. These results suggest that timing deficits in children with ADHD only become evident when a longer ISI is used, which may explain the null effects previously reported by Coffin et al. (2005) who used only a short ISI (400 ms). When extinction of the learned response was examined, children with ADHD displayed similar extinction during the short-interval paradigm, but impaired extinction in the long-interval paradigm compared to controls (Frings et al. 2010). These timing deficits observed in children with ADHD suggest cerebellar dysfunction. It should be noted that all children in the Frings et al. (2010) study were medicated with methylphenidate which may affect CR acquisition and timing. It has been previously reported that ADHD-associated cerebellar abnormalities are still apparent even after controlling for the use of stimulant medication (Castellanos et al. 2002),

making it unlikely that EBC in children with ADHD is affected by medication. Regardless, future studies examining EBC in children with ADHD may want to examine such effects in non-medicated children.

Dyslexia and Specific Language Impairment

Dyslexia is a learning disability in which children exhibit difficulty in accurately and fluently reading and comprehending simple written sentences at age-appropriate levels (Shaywitz and Shaywitz 2003). One region of the brain thought to play a major role in the development of dyslexia is the cerebellum (Linkersdorfer et al. 2012; Nicolson et al. 1999, 2001). Parallel to this aberrant cerebellar development is impairment in EBC (Coffin et al. 2005; Nicolson et al. 2002). In a sample of mostly adults, it was reported that learning acquisition in delay conditioning was similar between individuals with dyslexia versus those without dyslexia (Nicolson et al. 2002). However, individuals with dyslexia displayed non-adaptive timing in their blink responses over the course of the experiment such that their blink response latency did not change from the initial learning trials to the last learning trials. In comparison, control participants showed a significant change in onset of their blink response such that their response adapted to get closer in time to the presentation of the US (Nicolson et al. 2002). In contrast to these initial findings in adults, a separate study examining EBC in children reported that children with dyslexia displayed a complete lack of conditioning (similar to that shown in children with FAS; Jacobson et al. 2008, 2011) compared to typically developing children (Coffin et al. 2005). Similar to findings reported in adults with dyslexia, children with dyslexia also displayed longer blink latencies compared to controls suggesting that their CRs did not adapt to the presentation of the US over the course of the experiment (Coffin et al. 2005). Together with the results from the adult study, these data suggest that cerebellar abnormalities are present in both children and adults with dyslexia and that these abnormalities appear to be more severe in children at a time in which they are acquiring the skills to read. In addition, it should be noted that heterogeneity in conditioning was observed in the adult population such that some participants displayed both impaired learning acquisition and timing while others displayed impaired learning but not timing or impaired timing but not learning (Nicolson et al. 2002). It would be interesting to observe whether such heterogeneity exists within the child population and to determine whether this heterogeneity is related to individual differences in severity of reading difficulties.

Specific language impairment (SLI) is diagnosed when a child has delayed or disordered language development that cannot be attributed to another cause (e.g., hearing loss, intellectual disability, ASD). SLI overlaps significantly with reading impairments such as those displayed by individuals with dyslexia (Catts 2004). Furthermore, there has been some research suggesting that abnormal development of the cerebellum may underlie the development of SLI (Hill 2001). However, when cerebellar-mediated learning via delay EBC was examined, no difference in conditioning acquisition, extinction, or latencies was found between adolescents or adults with SLI compared to control participants (Steinmetz and Rice 2010). These findings suggest that although there is overlap between SLI and reading impairments such as those observed in individuals with dyslexia, the underlying neural circuitry involving the cerebellum differs between the two impairments.

Schizophrenia

It has recently been suggested that schizophrenia is a neurodevelopmental disorder (Insel 2010; Lewis and Levitt 2002). The manifestation of schizophrenia presents with a broad range of symptoms including positive (e.g., hallucinations and delusions), negative (e.g., blunted affect), cognitive (e.g., impaired memory and executive function), and motor symptoms (APA 2000). The first studies examining delay EBC in patients with schizophrenia were conducted over 50 years ago (O'Connor and Rawnsley 1959; Spain 1966; Taylor and Spence 1954). The findings from these studies were relatively inconsistent: some studies reported no difference in learning acquisition (O'Connor and Rawnsley 1959) while other studies reported increased acquisition (Spain 1966; Taylor and Spence 1954) among individuals with schizophrenia compared to control participants. Somewhat surprisingly, the study of EBC and schizophrenia was discontinued for approximately 3 decades and has only recently seen resurgence within the past 10 years. This resurgence is likely due to increased interest in the cerebellum's involvement in the development of schizophrenia. More specifically, it has been suggested that schizophrenia is the result of disrupted development in the cortico-cerebellar-thalamic-cortical circuit (CCTCC) resulting in impairment in cognitive functions (i.e., cognitive dysmetria model, Andreasen et al. 1996, 1998; Andreasen and Pierson 2008). Since the cerebellum plays such a critical role in the production of EBC, it may then be used to indirectly assess cerebellar function in individuals with schizophrenia.

Several recent studies have examined delay EBC in individuals with schizophrenia compared to healthy controls (Bolbecker et al. 2009, 2011; Brown et al. 2005; Edwards et al. 2008; Forsyth et al. 2012; Hofer et al. 2001; Marenco et al. 2003; Sears et al. 2000). Similar to the findings reported in classic studies, the results from these

more recent studies have been inconsistent. Although some studies have reported facilitated learning acquisition (Sears et al. 2000) or no learning differences (Marenco et al. 2003) as a result of schizophrenia, a majority of studies reported that patients with schizophrenia display impaired learning acquisition compared to healthy controls (Bolbecker et al. 2009, 2011; Brown et al. 2005; Edwards et al. 2008; Forsyth et al. 2012; Hofer et al. 2001). Inconsistencies in learning acquisition may be due to the use of different methodologies (see Table 1) and patient medication usage. However, it should be noted that individuals with schizophrenia continue to show learning impairment independent of medication (Bolbecker et al. 2009) and when large samples of patients have been observed (Table 1; Bolbecker et al. 2009, 2011), lending greater confidence that learning impairment is a function of the aberrant neurobiology associated with the disorder.

Inconsistencies in timing of the CR between controls and individuals with schizophrenia have also been reported. Some studies show that individuals with schizophrenia display shorter, non-adaptive CR latencies compared to controls (Bolbecker et al. 2009; Brown et al. 2005; Edwards et al. 2008; Forsyth et al. 2012; Sears et al. 2000). In contrast, other studies found longer latencies in patients compared to controls (Bolbecker et al. 2011; Marenco et al. 2003). In the few studies that examined extinction of the delay EBC response, no differences in extinction rates were found between patients with schizophrenia and controls (Bolbecker et al. 2009; Brown et al. 2005; Marenco et al. 2003). To date, only one study has examined the effect of having schizophrenia on trace EBC performance (Marenco et al. 2003). In this study, individuals with schizophrenia and controls did not differ in the rate of learning acquisition suggesting that schizophrenia does not affect the hippocampus. However, individuals with schizophrenia displayed significantly shorter CR latencies compared to controls, a finding that has also been reported when delay conditioning paradigms have been employed (Bolbecker et al. 2009; Brown et al. 2005; Edwards et al. 2008; Forsyth et al. 2012; Sears et al. 2000).

Interestingly, a number of the above studies examined individual differences in EBC performance in relation to measures of cognition (Bolbecker et al. 2009; Forsyth et al. 2012) as well as structural measures of the cerebellum (Edwards et al. 2008). A significant positive relation between mean levels of conditioning (i.e., %CR) and processing speed as measured by the Digit Symbol Coding subscale of the Wechsler Adult Intelligence Scale (3rd Edition) was found among participants with schizophrenia, but no relation was found among healthy controls (Forsyth et al. 2012). However, when examining full-scale IQ in relation to conditioning performance, a significant positive relation was observed in healthy controls but not participants with schizophrenia (Bolbecker et al. 2009). Parallel to these findings (Bolbecker et al. 2009), it was found that cerebellar volume, specifically the volume of the anterior cerebellar lobes, was positively related to mean CR peak latency in healthy controls while no relation was found in participants with schizophrenia (Edwards et al. 2008). Specifically, larger anterior cerebellar volume predicted better adaptive timing in conditioned responses, but only among the control participants. These results suggest a potential role for cerebellar structural abnormalities in individuals with schizophrenia in the contribution of both cognition and EBC providing further evidence for Andreasen's proposed cognitive dysmetria model of schizophrenia (Andreasen et al. 1996, 1998; Andreasen and Pierson 2008).

Given that the majority of recent studies demonstrate impairments in EBC, it may be used as a potential biomarker for individuals at high risk for schizophrenia. For example, prospective studies examining risk factors (i.e., family history of schizophrenia, display of attenuated psychosis) in the development of schizophrenia have shown that the number of risk factors that one has is directly related to the likelihood that an individual will be diagnosed with schizophrenia within 3 years (Yung et al. 2003). However, not all individuals displaying these risk factors develop schizophrenia. Including additional measures in these prospective studies that tap into the underlying neural circuitry involved in schizophrenia, such as EBC, may help to better distinguish those who develop schizophrenia from those who do not so that treatment may be implemented earlier.

Summary

In summary, the above findings suggest that EBC may be used as a reliable biomarker that can be employed to indirectly examine underlying neural mechanisms involved in several neurodevelopmental disorders, primarily disorders known to involve cerebellar deficits, although some studies suggest hippocampal deficits as well. It should be noted that an aberrant EBC response by itself lacks specificity to any single disorder. This lack of specificity may be related to a common underlying mechanism of these disorders such as the timing of insult to the brain, thus affecting cerebellar development. Although aberrant EBC is not specific to any one disorder, it may be useful as a biomarker not as tool to discriminate between different neurodevelopmental disorders, but rather as a marker of severity of the disorder possibly related to the severity of the early insult. To examine this further, individual difference studies should be conducted to determine the relation between EBC performance and cognitive and behavioral markers of disorder severity. In addition, future

studies should take advantage of the fact that the EBC can be applied in developing populations and can therefore be administered in both infants and children to potentially examine both risk as well as disorder severity.

Autism Spectrum Disorder and Eyeblink Conditioning as a Biomarker for Risk

In this section, we focus on the examination of EBC in individuals with ASD and make an argument for why this paradigm may be a useful tool in both populations of children already diagnosed with ASD as well as those who are at the greatest risk for developing ASD (i.e., infant siblings of children with ASD).

ASD is a neurodevelopmental disorder characterized by impairments in social interactions, communication, cognition, and motor and sensory function and the display of restricted and/or repetitive patterns of behavior, interests, or activities. The severity of such a phenotype occurs along a broad continuum and is typically not able to be reliably diagnosed until 2–3 years of age. Parallel to the broad range of impairments observed in children with ASD, abnormalities in various brain regions have been implicated in the development of ASD (for review, see Amaral et al. 2008; Courchesne et al. 2005, 2007; Eigsti and Shapiro 2003). One region of particular interest in the context of EBC and ASDs is the cerebellum. Although it is not the only region that appears aberrant in individuals with ASD, there are several studies that consistently provide evidence of abnormal morphology in the cerebellum (for review, see Courchesne 1997; Stanfield et al. 2008). In addition, as mentioned earlier, the cerebellum has recently been shown to be involved in a wide range of motor, cognitive, socio-emotional, and communication functions (Bolduc et al. 2011; O'Halloran et al. 2012; Rapoport 2001; Schmahmann et al. 2007), all functions that are impaired in individuals with ASD. Furthermore, specific areas of social function that are known to involve the cerebellum include theory of mind (Brunet et al. 2000; Calarge et al. 2003), empathy (Shamay-Tsoory et al. 2005), action understanding (Sokolov et al. 2010, 2012), and emotion recognition (D'Agata et al. 2011), all of which are impaired in individuals with ASD (Adolphs et al. 2001; Gleichgerrcht et al. 2012; Klin et al. 2009; Senju et al. 2009). Given that individuals with ASD display cerebellar abnormalities and that the cerebellum has been shown to be involved in the range of behaviors associated with ASD, particularly social behavior, employing EBC in this special population may give some insight into both risk as well as severity of the disorder.

Delay EBC has been examined in high-functioning individuals with ASD and controls (Sears et al. 1994). In contrast to EBC performance in other neurodevelopmental disorders, individuals with ASD actually displayed acquisition and extinction enhancement compared to control participants, and this difference was more apparent in children with ASD compared to adults (Sears et al. 1994). When onset and peak latency of the CR were examined, it was found that participants with ASD displayed significantly shorter latencies than controls suggestive of a non-adaptive blink response pattern. In addition, compared to control participants, individuals with ASD also displayed a greater number of double peaks in their blink pattern meaning that those with ASD blinked soon after the presentation of the CS, opened their eyes just prior to the presentation of the US, and then blinked again once the US was presented. This is considered an aberrant eyeblink response because the normal pattern is to adapt the blink response in order to close the eyelid to protect the cornea when the US is presented. These results led Sears and colleagues (1994) to conclude that both the abnormally rapid learning acquisition and blink latency are indicative of aberrant cerebellar function among individuals with ASD.

There are some limitations to Sears et al.'s (1994) study. For example, the etiology associated with ASD is highly heterogeneous and these differences may differentially affect the dependent measures obtained during EBC. In addition, the sample size was relatively small (11 participants/group) and the age range was relatively large (6–23 years) making the findings even more difficult to interpret when considering both the heterogeneity of ASD as well as development of EBC performance. As well, although there were no differences in IQ between controls and individuals with ASD, the individuals with ASD were high functioning (IQ mean = 105.7) making the findings difficult to generalize to ASD populations with low IQ. Finally, although Sears et al.'s (1994) study is an important first step in understanding how ASD affects EBC and its related neural circuitry, it does not address this issue of heterogeneity that is observed in ASD.

Heterogeneity of social behavior in humans is substantial, ranging from socially reclusive to outgoing. Contingent social interaction is a key element in the development of adaptive social behaviors (Gergely and Watson 1996; Rochat and Striano 1999; Stern 2000; Tarabulsy et al. 1996; Trevarthen 1979). Human infants learn to associate complex multisensory stimuli with affective and social meaning, enabling the expression of socially appropriate behaviors in complex contexts. Therefore, we have recently suggested that associative learning mechanisms, such as classical conditioning, may serve as an important building block for the development of social behavior (Reeb-Sutherland et al. 2012). This relation can have important implications for examining the heterogeneity observed in social behavior in individuals with ASD.

Two recent studies from our laboratory provide some evidence for the role of associative learning via EBC in the development of social behavior and further suggest the utility of EBC for the study of ASD. First, we have shown that learning acquisition in the delay EBC paradigm is enhanced in typically developing 1-month-old infants when an ecologically-relevant socially CS (woman's voice) versus a pure tone is used suggesting that EBC is sensitive to social stimuli (Reeb-Sutherland et al. 2011). Furthermore, we have shown that individual differences in typically developing 1-month-olds learning during EBC is related to both behavioral and neural correlates of social behavior during the first year of life (Reeb-Sutherland et al. 2012). If this is so, then it may be beneficial to examine EBC as a tool that may be able to detect risk for the development of ASD in at-risk populations (i.e., infant siblings of children with ASD) prior to the onset of ASD. Currently, studies examining infant sibs have had little success reliably identifying behavioral markers during infancy that predict the later manifestation of ASD (Rogers 2009), suggesting that new tools, particularly ones that combine both behavioral and neurobiological assessments, should be assessed in this infant population. We believe that EBC may be one of these tools.

In addition to examining EBC as a potential biomarker of ASD risk in infant sibs, further examination of EBC in children already diagnosed with ASD is also important. It has been shown that young children (18–30 months) with ASD who experienced 2 years of intensive behavioral intervention with the Early Start Denver Model (ESDM) displayed improvements in cognition, communication, and an ASD diagnosis compared to community samples of children with ASD (Dawson et al. 2010). ESDM and other similar intensive intervention programs use teaching strategies based on applied behavior analysis (ABA). ABA employs associative learning principles, in order to change children's behavior through punishment and reinforcement strategies (i.e., operant conditioning) based upon the individual child's behavioral responses to specific punishers and reinforcers. When this intensive associative learning-based therapy was administered during toddlerhood, children with ASD displayed normalized patterns of brain responses which were also shown to be directly related to changes in social behavior during later childhood (Dawson et al. 2012) suggesting that the brain of a child with ASD is relatively plastic during the first years of life. EBC paradigms may be utilized in a similar manner (i.e., pre- and post-intervention assessment) to determine whether learning acquisition and eyeblink response latency patterns in children with ASD can be normalized as a result of this type of early intervention. If changes are observed in EBC outcomes, one will be able to draw more definitive conclusions as to the location in the brain where some intervention-induced changes are occurring, specifically the cerebellum. In addition, because ABA intervention employs associative learning principles via operant conditioning and EBC is a form of associative learning (i.e., classical conditioning), it may be that individual differences observed in EBC during the initial assessment prior to intervention may be able to predict those children who will be better responders to ABA intervention and show the greatest improvements in social behavior. Additional examination of timing of ABA intervention and maintenance of such treatment on neurobiology associated with ASD including EBC is needed.

Although we feel that there is great value to be gained from examining EBC in both individuals with ASD and infant siblings, it is unclear what dependent measures would be the most valuable in discriminating between typically developing children and infant siblings or individuals with ASD. For example, our study shows that faster learning acquisition is related to greater social abilities; however, individuals with ASD demonstrate enhanced learning acquisition compared to controls (Sears et al. 1994) suggesting that this measure may not necessarily reflect their social abilities. Because aberrant blink latency appears to distinguish individuals with ASD from controls (Sears et al. 1994), this may be a better predictor variable of heterogeneity of social behavior in an ASD population. In addition, using the non-social CS to relate to later social behavior as was used in our study (Reeb-Sutherland et al. 2012) may not be ideal to examine similar relations in individuals with ASD or infant siblings. Using a social CS (voice) rather than a non-social CS (tone) may prove to be more fruitful when examining these special populations. Therefore, there is a great deal of research still needed to establish EBC as a successful biomarker for ASD.

Conclusions

EBC paradigms have been extensively employed to examine the underlying neural processes of learning and memory. EBC has a well-defined neural circuitry, is non-invasive, and can be employed shortly after birth, making it an ideal tool to use in both developing and special populations. The well-defined neural circuitry underlying delay and trace EBC primarily includes the cerebellum and hippocampus, respectively. A number of neurodevelopmental disorders including ASD have implicated atypical cerebellar development ultimately leading to the aberrant production of motor, cognitive, language, and social function observed in many of these disorders. We reviewed several studies that have used EBC to examine possible aberrant cerebellar or hippocampal function in a number of neurodevelopmental disorders including fetal alcohol

syndrome, genetic disorders, attention deficit/hyperactivity disorder, specific disabilities, schizophrenia, and ASD. In the majority of these studies, individuals with a neurodevelopmental disorder differed from control participants. Because these differences are so prevalent, we have suggested that EBC paradigms should be further examined as a biomarker for risk and severity. Indeed, early detection has proven to be an important avenue for early intervention with risk populations, and early intervention appears to have greatest success for remediation. Thus, application of EBC methods to at-risk infant populations may be an important new avenue for providing early treatment for a range of developmental problems including ASD.

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