Cerebellar function during development. Eyeblink conditioning, prism adaptation and interval production in children and adults.

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DOCTORAL DISSERTATION

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Cerebellar function during development.
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Abstract

The focus of this thesis is on cerebellar-dependent sensorimotor tasks during development. The performance in classical delay eyeblink conditioning, prism adaptation, and isochronous serial interval production (finger tapping) was explored in typically developing school-aged children, young adolescents, and adults. These three tasks share that the cerebellum plays a critical role when it comes to learning and precision of the motor responses involved. The purpose of the project was to investigate the behavioral relationships between eyeblink conditioning, prism adaptation, and finger tapping. In addition, the performance of eyeblink conditioning was further explored. Included in the analyses were 103 children between 6 and 14 years of age. Comparisons with 45 adults, between 18 and 55 years of age, were made.

The performance on all tasks improved with age during childhood, and there were only weak and unclear associations between the tasks. Further, the duration of the interstimulus interval had effect on the learning during eyeblink conditioning, and there were sex differences in the performance among both children and adults. The findings suggest that there might be different underlying neural mechanisms involved during the three tasks, and that other factors, including cognitive, contribute to the performance. Eyeblink conditioning, prism adaptation, and finger tapping do not seem to reflect one homogeneous cerebellar function and can therefore not replace each other. Together these three tasks may contribute to a more complete picture of cerebellar function, and by extension, cerebellar dysfunction. The project highlights the importance of continuing to explore test parameters and maturational effects on cerebellar-dependent tasks during development. In the future, more knowledge about this and the underlying mechanisms may allow for refined methods investigating cerebellar dysfunction in neurodevelopmental disorders.

Key words Cerebellum, Cerebellar function, Eyeblink conditioning, Prism adaptation, Finger tapping, Development, Children, Adults, Neurodevelopmental disorders, Cognitive disorders.

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LUND
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To the children in my life

Edvin & Iris
Tone & "lilleman"
Alicia, Mio, Lo & Prim

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Abbreviations

ADHD	Attention deficit hyperactivity disorder
ASD	Autism spectrum disorder
CS	Conditional stimulus
CR	Conditioned response
HL	Hearing level
ISI	Interstimulus interval
IQ	Intelligence quotient
RT	Reaction time
SD	Standard deviation
SPL	Sound pressure level
US	Unconditional stimulus
UR	Unconditioned response

List of included papers

I.   **Performance in eyeblink conditioning is age and sex dependent**. Karolina Löwgren, Rasmus Bååth, Anders Rasmussen, Henk-Jan Boele, Sebastiaan Koekkoek, Chris de Zeeuw, and Germund Hesslow. PLoS ONE, 12(5), 2017.

II.   **A longer interstimulus interval yields better learning in adults and young adolescents**. Katarina Kjell, Karolina Löwgren, and Anders Rasmussen. Frontiers in Behavioral Neuroscience, 12:299, 2018.

III.   **Weak correlations between cerebellar tests.** Karolina Löwgren, Rasmus Bååth, and Anders Rasmussen. Manuscript (submitted).

Preface

This PhD project was created as a part of a multidisciplinary and cross-faculty research environment at Lund University, the Linnaeus center Thinking in Time: Cognition, Communication and Learning (CCL) that over the past ten years has been investigating the role of timing in cognitive functions. In my project, I focused mainly on questions regarding tests of cerebellar functioning, with timing properties in the sub-second range from tens to hundreds of milliseconds, where the cerebellum is considered to play a key role.

I have studied cerebellar function mainly at the behavioral level, and with clinical implications. The reason for investigating cerebellar function with methods such as eyeblink conditioning, finger tapping, and prism adaptation, is that they are non-invasive, culturally neutral and can be used in different populations, including individuals with cerebellar pathologies or cognitive disorders. They can be performed in adults, as well as children (eyeblink conditioning even in newborn infants). These tests also capture temporal and spatial precision, whereas for example neuroimaging does not. The tests are relatively simple and easy to participate in, do not require anesthetics, and are quantifiable.

The long-term goal of this research project was to test cerebellar function in children with speech, language, and hearing problems; and to investigate cerebellar contributions to the perception and production of speech and language. During the project, I also collected data for some of these future questions, although it will not be a part of this thesis.

Here I focus solely on exploring cerebellar-based learning and timing in typically developing school-aged children, young adolescents, and adults. Due to the dynamic nature of the brain in general, and the cerebellum in particular, it is essential to know how the performance changes during development before we use these cerebellar-dependent tests to explore cerebellar dysfunction in clinical populations.

Theoretical background

The function of the cerebellum

The anatomy of the cerebellum is relatively uniform and the neurophysiology seemingly regular in different cerebellar regions. Because of the homogenous organization of the cerebellum, it has been suggested that all parts of the cerebellum perform the same neural computations (Eccles, Ito, & Szentagothai, 1967). However, the cerebellum contains subregions and a vast number of independent modules and microzones, where the differences lie within the separate input and output pathways that connect to different networks and brain areas outside the cerebellum. Accordingly, different parts of the cerebellum engage in different movements or other functions.

Eighty percent of the brain's ~86 billion neurons are located in the cerebellum (Azevedo et al., 2009). Input from other brain regions is transmitted to the cerebellum via the climbing fibers from the inferior olive and mossy fibers from the pontine nuclei in the brainstem and the spinal cord (Apps & Garwicz, 2005). These two pathways project to the cerebellar nuclei and the cerebellar cortex. Axons from the cortical Purkinje cells – the only source of output from the cerebellar cortex – project to the cerebellar nuclei. Since Purkinje cells are spontaneously active and GABAergic, they inhibit the cerebellar nuclei. Movements are often triggered by pause responses in the Purkinje cell activity, which disinhibits the nuclei to enable a movement.

The cerebellum has traditionally been considered part of the motor system and is essential for coordination and precision (spatial accuracy and timing) of gross and fine motor responses. The cerebellum computes expected outcomes from behavior and detects deviations between intended and actual movements. Yet, the cerebellum does much more than fine-tune ongoing sensorimotor programs. It is involved in the planning of movements (Gao et al., 2018), and ample evidence shows that the cerebellum plays a part in various cognitive functions (see below). The cerebellum connects to different areas in the cerebral cortex and is involved in a wide variety of brain functions (Benagiano et al., 2018; Buckner, Krienen, Castellanos, Diaz, & Yeo, 2011).

The role of the cerebellum in cognition

The cerebellum is involved in a wide range of behaviors and cognitive processes (Doya, 1999; Ivry & Baldo, 1992; Keele & Ivry, 1990; King, Hernandez-Castillo, Poldrack, Ivry, & Diedrichsen, 2019). During the cognitive evolution of the brain in humans and apes, cerebellar specialization seems to have played an important role. Indeed, the cerebellum underwent a faster size increase than the neocortex (Barton & Venditti, 2014). Neuroimaging shows activation of the cerebellar cortex during language, emotion perception, attentional control, interval discrimination, spatial cognition, and executive functions (Argyropoulos, 2016; Ashida, Cerminara, Edwards, Apps, & Brooks, 2019; De Smet, Paquier, Verhoeven, & Mariën, 2013; King et al., 2019; Mariën & Borgatti, 2018). On a general level, the cerebellum has been suggested to play a critical role whenever supervised learning is required (Doya, 1999). Distinct parts of the cerebellum that connect to separate extra-cerebellar regions may primarily handle different functions, divided into motor, cognitive, or emotional functions (Tiemeier et al., 2010).

Cerebellar learning

The synapses between the different types of neurons in the cerebellum are sensitive to change, which allows for adjustments between input and output signals. Such neuroplasticity has long been thought to be the neural foundation of learning (Hebb, 1949), including cerebellar learning (Ito, Yamaguchi, Nagao, & Yamazaki, 2014). Behavior is programmed and automated by repetition during cerebellar learning. The cerebellum can be involved in both implicit and explicit learning (Desmond & Fiez, 1998). Implicit learning typically refers to procedural learning of motor skills but can also refer to habits and certain cognitive skills (Squire, 2004).

Prism adaptation, eyeblink conditioning, and adaptation of the vestibulo-ocular reflex are three examples of cerebellar-dependent procedural learning. These tasks result in different behaviors but prior research indicates that they have a lot in common when it comes to the neural mechanisms underlying their acquisition. Comparisons of eyeblink conditioning and vestibulo-ocular reflex adaptation suggest that the plasticity distribution is located between the cerebellar cortex and the cerebellar nuclei in both tasks. Further, both tasks require the cerebellar cortex when tuning the timing of the motor responses. According to one account, the cerebellar cortex is thought to transfer and guide the learning in the cerebellar nuclei (Raymond, Lisberger, & Mauk, 1996).

Timing and the cerebellum

Nearly everything we do relies on precisely timed sequences of muscular activity. Several brain regions, including the cerebellum, cerebral cortex, and basal ganglia, are

crucial for time perception and timing of motor output (Paton & Buonomano, 2018). Some biological clocks, such as the cell's circadian rhythm, are well understood (Buonomano, 2017). When it comes to learning that requires accurate timing in the sub-second range, many questions remain unanswered (Paton & Buonomano, 2018). Perception and production of speech and music involve complex temporal patterns, durations, and intervals.

Researchers differentiate between implicit and explicit timing that show discrete neural substrates (Coull & Nobre, 2008). Duration estimation is considered explicit, while temporal expectation, using a temporal pattern to predict an onset or offset (primarily non-temporal) in the near future, is considered implicit (Coull, Cheng, & Meck, 2011). The cerebellum is engaged in both forms of timing (Breska & Ivry, 2016), at least when the intervals are below one second (Coull & Nobre, 2008), and is thought to have a supervising role (Broersen et al., 2016). The brain regions involved depend on the task and temporal context. One idea is that during explicit forms of timing, the cerebellum interacts with the basal ganglia and other brain areas, such as the supplementary motor area, and prefrontal cortex. During implicit forms of learning, on the other hand, the cerebellum interacts more with the parietal and premotor cortex (Coull & Nobre, 2008).

Eyeblink conditioning

In classical pavlovian conditioning, a subject learns an association between a neutral conditional stimulus (CS), and a reflex-eliciting unconditional stimulus (US) (Pavlov, 1927). It is frequently used to study nonverbal learning and memory. In eyeblink conditioning, the CS, typically a tone, is repeatedly paired with a blink-eliciting US, typically an air puff to the cornea. The CS precedes the US in time. Through associative sensorimotor learning, the CS itself will eventually trigger a conditioned response (CR), timed before the US and the unconditioned response (UR), so that the eyelid will be closed before the air puff reaches the cornea (Figures 1 and 2).

The cerebellum has been found to play a critical role in the formation of the acquired CRs, while the cerebellum is not necessary for the URs (Daum et al., 1993; Dimitrova et al., 2002; McCormick, Clark, Lavond, & Thompson, 1982; Thompson, 1986; Thürling et al., 2015; Woodruff-Pak, Papka, & Ivry, 1996; Yeo & Hesslow, 1998). Delay eyeblink conditioning involves brainstem-cerebellar circuits. The CS reaches the cerebellum via mossy fibers originating in the pontine nuclei and enters the cerebellum via the middle cerebellar peduncle. The US reaches the Purkinje cells via the inferior olive and climbing fibers in the inferior peduncle. Both pathways send off collaterals to the cerebellar nuclei (Voogd, Pardoe, & Ruigrok, 2003). Before training, the firing of the Purkinje cells is constant during the CS. After training, Purkinje cells in the

eyeblink zone pause their firing (Jirenhed, Bengtsson, & Hesslow, 2007), which in turn inhibits the neural activity of the nucleus just before the US onset and induces the CR.

Eyeblink conditioning captures the acquisition of CRs and the learning-dependent timing of the CRs. The CR adapts in time with respect to the CS onset (Jirenhed & Hesslow, 2011) and may be delayed by several hundred milliseconds depending on the interstimulus interval (ISI) used. The cerebellar neural firing rate is timed to anticipate the US onset in close temporal proximity (Johansson, Jirenhed, Rasmussen, Zucca, & Hesslow, 2014). The cerebellum is critically involved in sensorimotor timing accuracy at the millisecond level (Wu et al., 2018), but the exact mechanism(s) underlying the acquisition of adaptively timed conditioned eyeblink responses is currently not known in humans. Although the cerebellum is essential, several regions in the cerebral cortex, such as the frontal lobes and cingulate gyri, have also been found to be recruited during eyeblink conditioning (Parker et al., 2012).
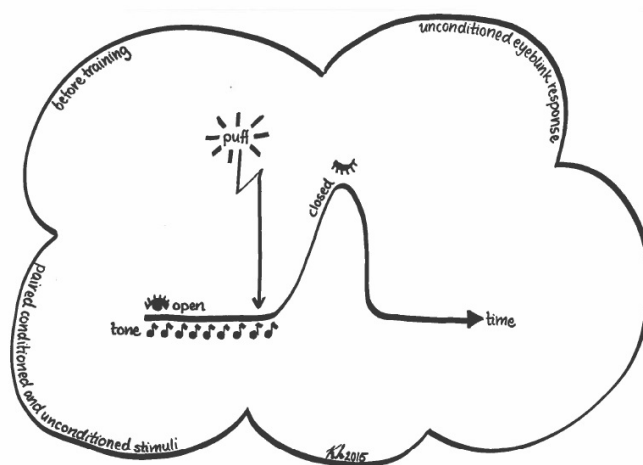
Figure 1. Illustration of an unconditioned blink response (UR) before training.

The eye is closed (amplitude on y-axis and time on x-axis) as a reflex to the air puff to the cornea. The tone is presented before the air puff but does not by itself trigger any blink response.
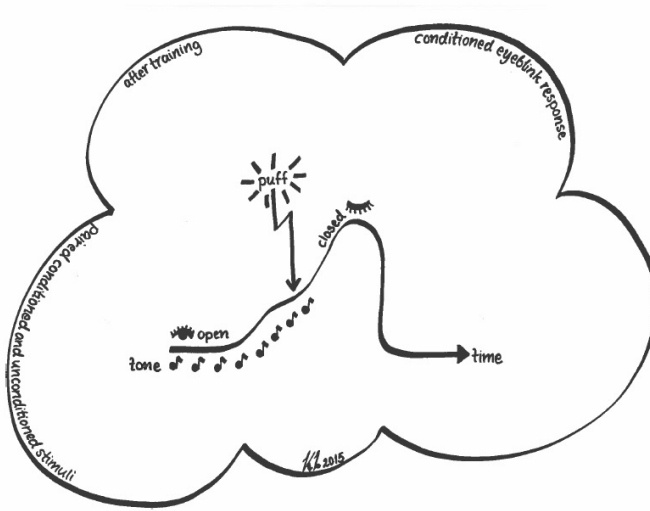
Figure 2. Illustration of a conditioned blink response (CR) after training.

After repeated paired presentations with tones and air puffs, the eye starts to close (amplitude on y-axis and time on x-axis) to the tone, in time before the air puff and unconditioned response (UR).

Prism adaptation

Prism adaptation is a basic form of motor learning. It is a simple task to perform and can be used to study both typical and dysfunctional cerebellar motor learning (Hashimoto et al., 2015). Wedge prisms displace the visual field laterally. Movement errors occur in the direction of the visual displacement when the subject aims for a visual target, usually by pointing. The deviation is gradually compensated for through training when the hand-arm movement recalibrates spatially to the new sensory input. Removing the prisms after training leads to deviations in the opposite direction of the visual field shift. The target is missed by a distance approximately equal to the initial pointing error when using the prisms. The adaptation can be quantified by this after-effect (Norris, Greger, Martin, & Thach, 2001; Panico, Sagliano, Nozzolillo, Trojano, & Rossetti, 2018). This movement adaption, by the visual eye-head system and the proprioceptive hand-head system, is considered dependent on the cerebellum (Luauté

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et al., 2009; Panico et al., 2018). Specifically, cortico-cerebellar networks are also involved, likely when forming cognitive spatial representations during prism adaptation (Chapman et al., 2010; Luauté et al., 2009).

Interval production

Serial interval production, so-called finger tapping, is a task that can be used to study sensorimotor interval timing. The subjects produce timed intervals when keeping a tempo to an ongoing or previously ongoing visual or auditory stimulus, often a blinking light or ticking metronome sound. The difficulty of finger tapping can be adjusted by tapping with only one finger, or with several fingers, or both hands. The cerebellum is considered important in such tasks that activates an explicit temporal representation of intervals between repetitive taps (Ivry, Spencer, Zelaznik, & Diedrichsen, 2002). Typically, the anterior cerebellum, together with other brain regions such as sensorimotor cortices, supplementary motor area, premotor cortex, inferior parietal cortices, and basal ganglia are activated during finger tapping (Witt, Laird, & Meyerand, 2008). Active cerebellar areas depend on the type of stimulus, whether the responses are synchronized or self-paced, and task complexity. Timing variability during finger tapping and auditory timing accuracy during interval discrimination have been found to correlate (Keele & Ivry, 1990), suggesting a cerebellar role in different timing processes, not only as a part of the motor system in movement control. It has also been suggested that classical eyeblink conditioning and self-paced isochronous serial interval production share underlying timing mechanisms (Green, Ivry, & Woodruff-Pak, 1999).

Cerebellar dysfunction

The cerebellum develops from the early embryonic period, grows rapidly during the last trimester and onward during pregnancy, and then continues to develop into early adulthood (Tiemeier et al., 2010; Wu, Chen, & Shen, 2011). The long period of cerebellar development makes it extra sensitive to malformations, hypoplasia, and developmental disorders (ten Donkelaar, Lammens, Wesseling, Thijssen, & Renier, 2003). By investigating the problems that occur following damage to the cerebellum, researchers have explored cerebellar function. Lesions can affect the precision of movements and timing in coordination on the ipsilateral side. Though the exact symptoms of cerebellar damage depend on the site of injury, various symptoms have been reported, including: an inability to perform rapid movement alterations, movement tremor, staggering gait, hypotonia, ataxic dysarthria, and nystagmus

(Walker, 2011). Individuals with conditions that affect the cerebellum also show timing deficits, both explicitly and implicitly (Ivry & Keele, 1989; Nobre, Correa, & Coull, 2007). Cerebellar timing deficits have been linked to short sub-second durations, with minimal attention required (Nichelli, Alway, & Grafman, 1996). Cerebellar lesions can also cause behavioral impairments on more cognitive tasks (Baillieux, De Smet, Paquier, De Deyn, & Mariën, 2008).

Like lesions, prematurity can result in cerebellar dysfunction because of the risk for impaired cerebellar development, caused by factors such as cerebellar hemorrhage or growth failure. Individuals born preterm often show both reduced motor skills and cognitive impairments in terms of learning, language, and behavioral problems or show autism-like conditions during development (Limperopoulos et al., 2007).

In addition, structural and functional changes of the cerebellum have been linked to neurodevelopmental disorders, including autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD), developmental language disorder, dyslexia, fragile X syndrome, Down's syndrome, fetal alcohol syndrome, and schizophrenia (Berquin et al., 1998; Castellanos et al., 2001; Courchesne, 1997; Krain & Castellanos, 2006; Moberget et al., 2019). When diagnosing ASD, ADHD, language disorders, and dyslexia, the focus has traditionally been on behavior. The fact that these disorders also are associated with deficits in sensorimotor coordination and attention suggests that tests measuring these abilities could potentially be used for clinical purposes as biomarkers (Coffin, Baroody, Schneider, & O'Neill, 2005; Jacobson, Stanton, & Dodge, 2011; Reeb-Sutherland & Fox, 2015).

Impaired performance on cerebellar-dependent tasks

Cerebellar damage results in deficits on eyeblink conditioning, prism adaptation, and finger tapping (Fernandez-Ruiz et al., 2007; Ivry & Keele, 1989; Woodruff-Pak et al., 1996). Individuals with cerebellar lesions, focal or degenerative cerebellar disease, can blink but show deficits in CRs or do not acquire CRs at all during eyeblink conditioning (Bracha, Zhao, Irwin, & Bloedel, 2000; Gerwig et al., 2005; Yeo, Hardiman, & Glickstein, 1984). Preterm born individuals also show impaired eyeblink conditioning, perhaps explained by abnormal cerebellar development or neuronal loss (Tran et al., 2017). Likewise, individuals with neurodevelopmental disorders, such as ASD, ADHD, fetal alcohol syndrome, and dyslexia show impaired CR acquisition or timing (Reeb-Sutherland & Fox, 2015; Sears, Finn, & Steinmetz, 1994), although the impact varies and the performance is generally not as impaired as in focal cerebellar disorders.

Individuals with cerebellar lesions or degeneration show deficits on finger tapping production (Ivry & Keele, 1989; Schlerf, Spencer, Zelaznik, & Ivry, 2007). Several conditions linked to the cerebellum, including language impairment, dyslexia, and

ASD, are also associated with atypical performance on finger tapping (Colling, Noble, & Goswami, 2017; Corriveau & Goswami, 2009; Morimoto, Hida, Shima, & Okamura, 2018). Likewise, prism adaptation is absent or impaired in individuals with cerebellar lesions (Martin, Keating, Goodkin, & Bastian, 1996). The rate of adaptation has also been shown to be impaired in individuals with dyslexia and developmental coordination disorder (Brookes, Nicolson, & Fawcett, 2007), while the task prior to the adaptation was performed equally well as in the control group.

An easy and non-invasive test of cerebellar function could be useful for researchers as well as clinicians investigating neurodevelopmental disorders with cerebellar dysfunction. Eyeblink conditioning, prism adaptation, and interval production are all linked to cerebellar function, and these tasks could potentially be included in a cerebellar test battery. To be able to interpret the results in different subgroups, it must first be examined how typically developing children and adults perform with certain test parameters. Second, we need more knowledge about the relationships between the tasks and how they potentially overlap. These two questions are in focus in this thesis.

Aim

The main purpose of this project was to explore cerebellar function behaviorally during development. Therefore, the focus in the included papers has been on:

1) Investigating tests that rely on cerebellar functioning in relation to each other, in typically developing children, and in adults.

2) Investigating performance in eyeblink conditioning in school-aged children and young adolescents compared to adults.

Methods

Detailed descriptions of the methods used are given in the individual papers. The main tests and the participants are summarized below.

Classical delay eyeblink conditioning

Eyeblink conditioning is a simple form of associative learning that requires precise timing. A classical delay paradigm was performed to test the acquisition and timing of conditioned responses (CRs) during and after paired presentations of a neutral conditional stimulus (CS) and a blink eliciting unconditional stimulus (US). The human eyeblink conditioning setup used in the experiments in this thesis was developed in collaboration with Professor Chris De Zeeuw, Dr. Bas Koekkoek, and Dr. Henk-Jan Boele at Erasmus MC in Rotterdam, the Netherlands. As the CS, a tone of 1 kHz was presented binaurally at 68 dB SPL. The tone was clearly audible to all participants. The US, an air puff with a pressure of 1 bar and a duration of 15 ms, was aimed towards the subject's left cornea. Before any training, it was always checked that the air puff elicited a blink reflex. To record blink responses, a magnetic sensor was placed on the cheek straight below a magnet that was attached to the left eyelid, close to the eyelash. A blink response caused the magnet on the eyelid to move up and down – causing a change in the magnetic field that the sensor could pick up.

The duration between the CS and US onset, i.e., the interstimulus interval (ISI), was either approximately 300 or 500 ms. The CS and the US co-terminated in time. The intertrial interval varied pseudorandomized between ~10-25 seconds. An eyeblink conditioning session contained 70-100 trials. The protocols varied in the different studies, but in all cases, a majority of the trials (>75 percent) were paired CS and US trials. The remaining trials were probe trials in which the CS was presented alone as well as a couple of trials with the US presented alone to verify the unconditioned response (UR). The CRs usually appeared after a few paired trials and were typically timed before the US. The rate of learning was described by the percentage of CRs along the session. The timing of the CR was analyzed in terms of latency to the CR onset and the CR peak.

Prism adaptation

Prism adaptation was used to analyze the degree of spatial adaptation of movement when shifting the visual field to the left. The participants pointed rapidly to a target in front using the dominant arm and index finger. The participants had their eyes closed during the movement. After pointing at where they thought the target was located, they opened their eyes to get visual feedback on the location of the movement endpoint. Typically, the participants missed the target after putting on the prism glasses. The error was gradually reduced over a few trials. When the participants removed the prisms again, there were typically an error in the opposite direction – often to the surprise of the participants. The deviation magnitude on the first trial after removing the prisms was used as the primary measure of prism adaptation.

Isochronous serial interval production

Finger tapping was conducted in order to test the ability to produce sub-second timed serial intervals (Madison, 2001). The stimulus was a ~30 ms pulse sound, repeated 15 times with an inter-onset interval of 524 ms, presented at a level of 65 dB (A). The participants tapped with their dominant index finger and palm down. They were instructed to synchronize their taps to the isochronous beat and then maintain the exact rate without any stimuli until they were told to stop after ~70 taps. The session contained one training session followed by four measuring sessions. Both the synchronization phase and the continuation phase (production phase) were analyzed in terms of inter-response intervals and tapping variabilities.

Participants

Participants in three main age spans were included in the papers: 42 school-aged children from 6 to 11 years old (Figure 3); 61 young adolescents from 10 to 14 years old; 45 adults from 18 to 55 years old. Written information about the study and procedure, together with a consent form to sign, and questionnaires about health, habits, and development, were distributed to the participants and their legal guardians prior to testing. The children and adults displayed normal hearing, defined by thresholds better or equal to 20 dB HL on pure tone audiometry.
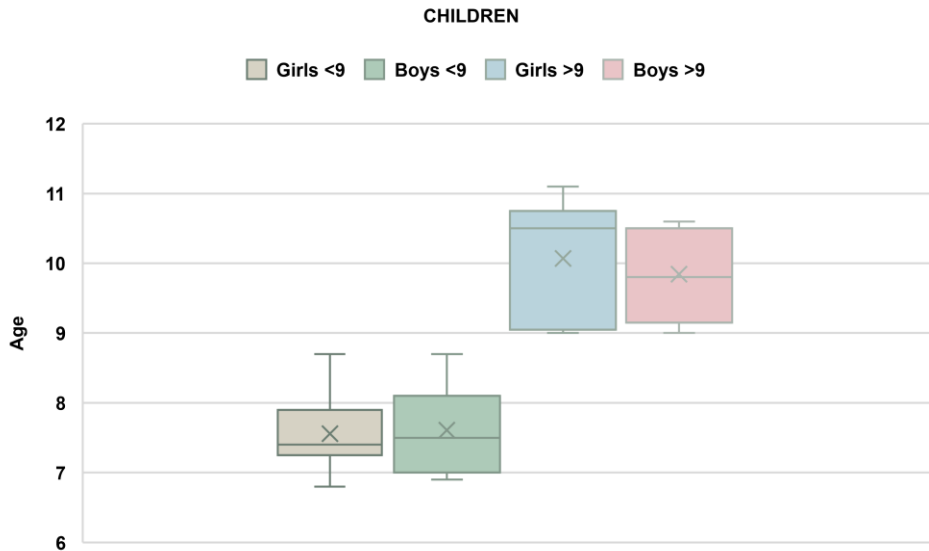
Figure 3. Boxplot of the children's age split by median age (9 years) and sex.

The cross marks state the mean age in each group: 7.5 years (younger girls, n=9), 7.5 years (younger boys, n=11), 10.0 years (older girls, n=13), and 9.8 years (older boys, n=11).

The test batteries that the children completed included: speech and language skills (oral motor skills, diadochokinesis, non-word repetition and discrimination, and past tense inflection of novel verbs), hearing (pure-tone audiometry, temporal auditory resolution), non-verbal intelligence quotient (IQ), motor ability (standard neurological clinical screening tests), and reaction time (auditory simple and dual choice). Several of these tests were carried out only among the children to allow comparing with pathological groups. The participating children were tested on three different occasions with a week in-between, whereas the adults were tested on one single occasion. An overview of the included participants with background descriptives is shown in Table 1.

Table 1. Participant overview.
Description of the participants included in the analyses of the present papers (I, II, and III). The number of participants, and age, are stated in total, divided into females (F) and males (M), and for the five data collections separately. The data includes questions about education, extra school support, eyes, vision, contact lenses at test, medication, and handedness. Displayed are also averages of median reaction time (RT) from a simple auditory reaction time test, and standardized IQ scores assessed by Raven's coloured progressive matrices.

	CHILDREN			ADULTS	
Subjects, n	103 (F:50 M:53)			45 (F:27 M:18)	
Age, years	11 (SD 2) (F:11y M:11y)			28 (SD 8) (F: 28y M:28y)	
	School A[I,III]	**School B[I,III]**	**Adolescents[II]**	**'ISI 300'[II,III]**	**'ISI 500'[I,II,III]**
Subjects, n	23 (F:11 M:12)	19 (F:11 M:8)	61 (F:28 M:33)	19 (F:9 M:10)	26 (F:18 M:8)
Age, years	10 (7-11) (F:9y M:9y)	8 (6-9) (F:8y M:8y)	12 (10-14) (F:12y M:12y)	26 (18-44) (F: 24y M:28y)	29 (20-55) (F:30y M:27y)
Education, %	93^	43^	83^	Students/higher education	
Extra support	0	0	0		
Dominant hand, n	Right:20 Left:3	Right:17 Left:2		Right:17 Left:2	Right:25 Left:1
Eye disease, n	0	0	0	0	0
Vision loss, n	3	3		11	5
Contacts, n	0	0	0	1	4
Medication, n	0	0	0	5¨	5¨
RT mean, ms	295 (SD 72)	367 (SD 82)		193 (SD 18)	189* (SD 23)
IQ mean	109 (67th %ile)	99 (46th %ile)			

^Percentage of pupils with at least one parent with higher education in these schools the year of the data collections, retrieved from: https://www.skolverket.se/skolutveckling/statistik/sok-statistik-om-forskola-skola-och-vuxenutbildning, 2019-10-25.

¨Medication stated: Contraceptive pills, antihistamine, asthma medication (Symbicort, Ventoline), thyroid medication (Euthyrox), and antiepileptics (Lamotrigin actavis).

*N = 25.

Summary of results

Detailed results and statistical methods are described in Paper I, II, and III. A brief summary of the findings is presented below.

The influence of age

Age correlated with the performance of eyeblink conditioning, finger tapping, and prism adaptation (Paper I, II, and III). Older children produced a higher percentage of conditioned responses (CRs), showed greater spatial adaptation to the prisms, and more well-timed interval production. In addition, the adults performed better than the children. However, the children above 9 years of age and the adolescents performed nearly on par with the adults on eyeblink conditioning with a 500 ms interstimulus interval (ISI), whereas the children below 9 years of age did not. The difference between adolescents and adults was more pronounced during eyeblink conditioning with a 300 ms ISI than with a 500 ms ISI. Eyeblink conditioning, finger tapping, and prism adaptation within the adult group were not correlated with age. This suggests that there might be a developmental threshold beyond which maturation does not result in further learning improvements.

Unclear relationships between cerebellar tests

After controlling for age among the children, there were only weak (near zero) or unclear (95% confidence intervals crossing the zero mark) correlations between the performance measures of eyeblink conditioning, finger tapping, and prism adaptation for both the children and adults (Paper III). Splitting the adult group based on ISI during eyeblink conditioning did not clarify the relationships between the different tests. Also, in all the experiments, learning during eyeblink conditioning was associated with great inter-individual variability among both children and adults.

Interstimulus interval affects learning

The learning rate during eyeblink conditioning was increased in the groups tested with the longer 500 ms ISI compared to the groups tested with the shorter 300 ms ISI (Paper II). Adolescents and adults trained with the longer ISI, produced more CRs. The CR production improved the most during the first part of the sessions (Paper I and II) regardless of ISI.

Sex differences in eyeblink conditioning

The results indicated an effect of sex on the performance in eyeblink conditioning among both children and adults (Paper I). Females produced more CRs than males. Girls older than 9 years of age performed similar to women, and males older than 9 years of age performed similar to men, in terms of CR production level. In addition, the CR timing differed between the sexes. Men showed later CR onsets than women and boys showed later CR peaks than girls.

General discussion

Summary – first aim and findings

When investigating the relationships between the performance in eyeblink conditioning, prism adaptation, and interval finger tapping (aim 1), the statistical analyses revealed only weak or unclear relationships between the performance measures among the 86 participants. These three tests are all supposed to engage the cerebellum and measure its functioning. The hypothesis was, therefore, that the performances would correlate to some extent, but the associations are surprisingly weak and theoretically problematic to interpret. Whether any common underlying cerebellar function or shared neural mechanisms could be captured with these behavioral tasks was not established. The measures, however, reflect that the performance improves with age during development from early school age into adulthood.

Summary – second aim and findings

When investigating the performance in eyeblink conditioning (aim 2) in the 148 participants, age, sex, and the interstimulus interval (ISI), emerged as important parameters with effects on learning and timing patterns. Also, in all eyeblink conditioning experiments, a large inter-individual variability was observed. Whereas some individuals gradually acquired conditioned responses (CRs), some subjects produced more than fifty percent CRs in the first block of ten trials, and some subjects did not produce any CRs whatsoever.

Maturity and performance

It might seem obvious that maturity is reflected in the learning and precision of these cerebellar-dependent tasks and associations between them. However, there is relatively little research on how the performance of cerebellar learning changes during childhood. What has been more thoroughly studied previously is a decline in performance on

eyeblink conditioning in older adults (Finkbiner & Woodruff-Pak, 1991; Woodruff-Pak & Thompson, 1988).

Neuroplasticity, which enables learning, is generally greater in children than in adults (Voss, Thomas, Cisneros-Franco, & de Villers-Sidani, 2017). Neural connections are more open to environmental influence during sensitive periods in childhood (Meltzoff, Kuhl, Movellan, & Sejnowski, 2009). While sensitive periods for sensory processing occurs early on during development, cortical maturation develops throughout adolescence (Meltzoff et al., 2009). Neuroplasticity is, however, associated with substantial inter-individual variability throughout life (Voss et al., 2017). Even if younger individuals could be flexible when learning specific sensorimotor skills, they might not learn as fast as older individuals, or even reach the same level of performance. Learning mechanisms may be more basic in infancy and increase in complexity with maturation (Meltzoff et al., 2009). The myelination of axons that develops throughout adolescence is associated with the maturation of the brain and plays a vital role in motor, sensory and cognitive functions (Nagy, Westerberg, & Klingberg, 2004; Pujol et al., 2004). Further, older individuals often exhibit superior precision and timing of movements, which could also facilitate the learning of new skills.

Newborn infants can acquire conditioned reflexes during eyeblink conditioning, even during sleep (Fifer et al., 2010), but show slower learning than adults. Infants are able to reach the same level of CRs as adults with additional training (Herbert, Eckerman, & Stanton, 2003; Hoffman, Cohen, & Devido, 1985). During finger tapping, cerebellar activity has been found to differ between children and adults, probably reflecting children's less automatic behavior (De Guio, Jacobson, Molteno, Jacobson, & Meintjes, 2012). Sensorimotor learning and timing involve a variety of motor networks and brain mechanisms that evolve during development. In prism adaptation, slower re-calibration after adaptation has been attributed to a less mature visuo-motor system in children compared to adolescents (Adams et al., 2018). In the present investigations, the children's latencies of the unconditioned responses (URs) that are not cerebellar-dependent, correlated negatively with age among the children, and were also consistently slower compared to the adult's.

This indicates that there are maturational phenomena attributed both to the development of the cerebellum and to other functions involved in these tasks. Cognitive contributions to the test situations, such as awareness, understanding of the situation, and volition, probably also increase from infancy to school age, and continues to change in older age. Also, the way children use positive and negative feedback to change behavior have been found to change during development (van Duijvenvoorde, Zanolie, Serge, Raijmakers, & Crone, 2008).

The age of the participants is best to explain maturity among the measures used in the present investigations. Of course, individuals do not mature at the exact same rate, nor do different functions and subregions. There are also sex differences to consider when

it comes to development. Cerebellar volume peaks in early adolescence in females and a few years later in males. That also means that cerebellar development peaks later than cerebral development (Tiemeier et al., 2010). In Paper I, sex differences in eyeblink conditioning were found both among the children and among the adults. A part of this disparity might be attributed to maturity, at least among the children. Other aspects related to genetic, environmental, or social aspects could also contribute to the sex differences of the performance.

Children with neurodevelopmental disorders and cerebellar pathology are frequently investigated with eyeblink conditioning and finger tapping in research, using protocols similar to the ones in the present papers. Their performance is often compared to small age matched control groups, and group differences are linked to cerebellar dysfunction. The age effect in the present investigations indicates that this is a factor that needs to be taken into account when interpreting the performance on eyeblink conditioning, finger tapping, and prism adaptation during childhood development. The course of maturation, cerebellar or other, and how it affects the acquisition of new skills, could differ between individuals with typical development and individuals with cognitive disorders. More research is required to answer these questions.

Temporal parameters

Paper II shows that the ISI during eyeblink conditioning affects acquisition. Moreover, it shows that the difference in the learning rate between young adolescents and adults depends on the ISI used. Adolescents performed worse than adults with the 300 ms ISI, while adolescents and adults performed more equally with the 500 ms ISI. Both adolescents and adults performed better with the longer ISI. In Paper I it is described that children above 9 years of age performed almost as well as adults with the 500 ms ISI. Infants seem to be especially sensitive to the ISI. Usually, longer ISIs are applied during delay eyeblink conditioning in infants, even as long as 1500 ms (Little, Lipsitt, & Rovee-Collier, 1984). Another study suggests 650 ms as an appropriate ISI for one-year-olds (Goodman, Anstice, Stevens, Thompson, & Wouldes, 2018). The evidence in this thesis suggests that it is essential to consider the ISI when studying eyeblink conditioning, even when testing older children or adolescents. The younger school-aged children did not reach the same level of performance as older school-aged children and might have benefitted from a somewhat longer ISI, above 500 ms. The results in paper II indicate that a 300 ms ISI is too short for young adolescents. Moreover, one study found that young adolescents with ADHD produced earlier peaks than controls only with a long 840 ms ISI and not with a short 440 ms (Frings et al., 2010; Reeb-Sutherland & Fox, 2015). Both groups acquired more CRs with the longer of the two ISIs, and the difference in CR incidence was greater between the groups following training with the long ISI.

Adults also performed better with the longer ISI in the present investigations. Consistent with the evidence presented here, previous research shows that learning is affected negatively when using an ISI below 250 ms (Ebel & Prokasy, 1963; Kimble, 1947; McAllister, 1953). It is, however, not entirely clear what the optimal ISI would be for adults. One study suggests that 250 ms produces the best learning (McAllister 1953), while other studies suggest that an ISI of 350-500 ms produces the best learning (Ebel & Prokasy, 1963; Kimble, 1947; Steinmetz et al., 2011). Taken together, to ensure that poor learning is not due to the ISI it is probably wise to use an ISI longer than 300 ms.

The ability for sensorimotor interval synchronization develops gradually from a few years of age (Repp, 2005). Further, the spontaneous motor tempo in finger tapping tasks has been found to slow down with age during development, and also throughout adulthood. On average, younger children produce preferred inter-response intervals below 500 ms and young adults above 500 ms (McAuley, Jones, Holub, Johnston, & Miller, 2006; Provasi & Bobin-Bègue, 2003). Since temporal properties in eyeblink conditioning and finger tapping at least partially reflect maturity, it is important to take this into account when comparing performances on timing. This also means that it is potentially problematic to compare the performance in groups of different ages and to choose whether the temporal parameters should be kept constant or separate.

The cerebellar contribution

Even though age was not statistically associated with the performance in eyeblink conditioning, finger tapping, and prism adaptation among the adults, the relationships between the tasks were not clarified in this group either. The shorter ISI during eyeblink conditioning did not contribute to more certain associations between the tests. All 95% confidence intervals of the correlations still crossed the zero mark, and none of the correlations were statistically significant. One previous study (Woodruff-Pak et al., 1996) found that the clock component of the tapping variability correlated with CR percentage in their control subjects. The same computations to the finger tapping data were also made in the present investigations, but again without significant correlation to CR percentages during the eyeblink conditioning session.

The lack of strong correlations between the performance on the tasks could reflect that the cerebellar involvement is distributed to different cerebellar subdivisions. This resonates with the fact that different motor processes and cognitive tasks activate distinct functional subregions in the cerebellar cortex and that the neurophysiological properties within the cerebellum differs (King et al., 2019; Stoodley, Desmond, & Schmahmann, 2013; Zhou et al., 2014). The different tasks engage different anatomical cerebellar sites and hemispheres, and connections to separate extra-

cerebellar networks (De Guio et al., 2012; Redding & Wallace, 2006; Tsujimoto et al., 2019). When testing behavior, other processes and individual characteristics are naturally also involved, and can affect the strength of the correlation. In addition, different forms of learning depend on different anatomical sites, functional networks and mechanisms (Squire, 2004). Eyeblink conditioning, prism adaptation, and finger tapping all recruit and are likely monitored by the cerebellum (Doya, 1999) but the nature of the learning and timing processes differs. Delay eyeblink conditioning and prism adaptation are both primarily forms of procedural learning, essentially unconscious, and involves implicit memory (Christian & Thompson, 2003; Desmond & Fiez, 1998; Nobre et al., 2007). The timing during finger tapping is, on the other hand, considered more explicit, involving declarative learning and memory.

The mix of circuits and functions involved during the tasks could mean that it is realistic to find only vague relationships between the tasks. The initial expectation in this project was that the results would point out certain associations and directions between the cerebellar-dependent tasks, even if not strong. On a population level, it is not likely that performance measures like these would not correlate at all, even if the tasks are not tightly related. However, the cerebellar contributions are simply not reflected in any straightforward way in these results, perhaps because of separate dominating processes and mechanisms. There is a possibility that the three tasks measure different independent parts of cerebellar functioning and combined could show an extensive picture of cerebellar function.

Methodology

The findings in this thesis suggest that there could be different underlying cerebellar mechanisms in eyeblink conditioning, prism adaptation, and finger tapping; that maturity and other cognitive processes contribute to the outcome and might overshadow the common cerebellar function; or, that the measurements or comparisons should have been conducted differently. Perhaps all these factors contribute. Along the way, many methodological questions arose. Below follows a discussion of the reliability and validity of the test methods, and the challenges faced during the progress of the project.

As previously mentioned, the inter-individual variability was high during the eyeblink conditioning, and the average level of CR production was quite low, as often reported in human eyeblink conditioning. In all age spans, some individuals did not produce any CRs, not even towards the end of the training. Other individuals reached a moderate or high level of CRs within the first ten trials and maintained that level throughout the training. The inconsistent learning patterns might reflect normal variance, but it is uncertain whether the same function was tested in all individuals or

not. It is problematic to exclude individuals that do not display particularly high learning rates because of this uncertainty. In animal studies, the learning rate is more predictable and consistent. Then again, animals are usually trained in longer training sessions and on several consecutive days. Moreover, the unconditional stimulus (US) is often significantly stronger, and there is evidence suggesting that a stronger US results in faster learning (Gormezano & Moore, 1962), although this conclusion has been questioned (Burstein, 1967). In humans, the cognitive system seems to be involved in eyeblink conditioning to a greater extent (Gormezano & Moore, 1962; Rasmussen & Jirenhed, 2017). The mechanisms could differ between species and are perhaps more predominantly cerebellar in certain animals.

A large proportion of the learning took place at the beginning of the session and then levelled out, as has also been reported in other human studies (Frings et al., 2010; Tran et al., 2017) and in studies with prolonged training over several sessions and days (Cheng et al., 2014). The incidence and timing of the unconditioned responses (URs) did not change during the session in the present investigations. The CR production, on the other hand, did, and the CR timing varied more. Two raters analyzed the raw data on separate occasions, and the inter-rater reliability of the eyeblinks was high. It is possible that the incidence and timing of the CRs would have improved with additional training. Even if almost no measurable learning occurs in the first session, for some individuals, CRs can appear early on during the second session (Hardiman, Hsu, & Bishop, 2013). How measures of such later learning would correlate to the other cerebellar-based tasks is yet to be investigated. The cerebellum is at least engaged during early trials (Thürling et al., 2015). Studies investigating individuals with cerebellar pathology often show differences in CR incidence compared to control groups early on during the acquisition (Tran et al., 2017).

More CRs were displayed with the longer of the two ISIs. Why this is the case and what it means is not obvious. As discussed above, the shorter interval might be less suitable for young adolescents and adults. It is, however, also possible that both groups tested with the shorter ISI were poorer learners. Once a subject has been conditioned in an eyeblink conditioning paradigm, they cannot go back to the naive state. Therefore, the same individuals were not tested with both ISIs. The difference in performance could also mean that using different ISIs recruit distinct neural mechanisms. Non-human animals are typically trained with shorter ISIs than humans. Perhaps a shorter ISI results in more pure cerebellar learning whereas longer ISIs might be more sensitive to cognitive contributions. The fact that subjects are able to voluntarily produce precisely timed blink responses that are indistinguishable from CRs suggests that we cannot rule out such influences (Rasmussen & Jirenhed, 2017). On the other hand, there is strong evidence that the cerebellum is also active during training with a longer ISI (Thürling et al., 2015).

The test instructions are important in eyeblink conditioning. The learning rate is reduced if the participants are unaware of the temporal relationship between the CS and US (Weidemann, Satkunarajah, & Lovibond, 2016). Even if all subjects receive the same instructions, there are likely subjective differences in the perception of the experiment which could contribute to the rate of learning and inconsistent learning patterns across subjects. Cognitive contributions are probably affecting the outcome in humans, even if the basic learning paradigm is procedural and implicit. Indeed, in all behavioral tasks, personal traits or factors related to the test situation are possible contributors to the performance and variance. During the prism adaptation, some individuals got embarrassed when they realised that their pointing was off, some got nervous, and some seemed competitive.

Both unconscious and conscious processes are directly involved in prism adaptation and finger tapping. Two error correction processes have been identified as contributors during sensorimotor synchronization, one automatic subconscious, and one conscious (Repp, 2005). Beyond the cerebellar contribution, the ability to participate, which is different in children compared to adults, also influences performance on finger tapping. The four longer continuation trials (70 taps) after the shorter training trial (31 taps), led to more tapping variability and often increased tempo towards the end of each trial, among the children. On a general level, individuals who tap to relatively fast isochronous intervals (as in the present investigations) generate a faster tempo compared to the target tempo (Repp, 2005). The fact that young children tend to increase the tempo along the trial is likely related to preferred tempo, which is faster than adults' preferred tempo, and approached as the self-paced tapping proceeds (McAuley et al., 2006). Fewer taps could have been more suitable for the children. In contrast, tapping variability among adults is generally decreased during training (Madison, Karampela, Ullén, & Holm, 2013). This decrease has been linked to motor processes rather than to cognitive processes during interval tapping.

In order to reduce the contribution of direct conscious error correction to prism adaptation, the participants were instructed to close their eyes when pointing at the target. This, however, affects the ability to reach the target and most likely the variance. The standard deviation compared to the mean deviation was proportionally higher among children than among adults. Even if it only took a few trials for each participant to adapt to the prisms, the adults might have adapted more than the children since they were tested with five more trials during the adaptation to the prisms. However, the older half of the group of children, above 7.5 years (n=10), deviated from the target to the same extent as the adults. Moreover, there was no significant difference between the tenth trial and the fifth trial of adaptation among the adults. In both the adult and the child group, there were significant differences between the first two to three trials. The main adaptation to the prisms seems to have occurred during the first few trials, which has also been reported in a previous study (Brookes et al., 2007).

The ability to time intervals improves with age during development (McAuley et al., 2006; Repp, 2005), which the results in the experiments presented in this thesis support. This was shown both in terms of slower tapping tempo closer to target and less tapping variability the older the age, during the self-paced continuation phase. Both children and adults were able to synchronize their responses to the ongoing beat with close to millisecond precision. The children younger than 9 years of age performed an average tempo that was only a few milliseconds faster during the synchronization phase than the children above 9 years and adults did. The difference in tempo and variability was greater between the groups during the self-paced interval production. Adults still performed significantly better than children older than 9 years on all tapping measures, except regarding the mean synchronization tempo. The same error-correction possibilities are not available in this phase as in the synchronization phase (Repp, 2005) and seem to lead to a greater disadvantage for the children.

Challenges

Looking at the results, it is evident that there were quite a few challenges during this project regarding methodology and questions concerning the interpretation of the results. Overall, as discussed above, there is usually substantial individual variance in these types of tasks, which involves cognition and requires conscious participation, and all the contributing factors to the performance are not known. Above that, since learning will necessarily affect the brain one can never test the same individual twice under identical circumstances.

During the first few data collections, the test procedure was still being developed, which means that there were details that did not match for all participants, in particular for the eyeblink conditioning protocols, but also the arrangement of the prism adaptation. Other than the criteria for the sessions to be endurable in different age groups, there were also different time limits to take into account for the children at the different schools, as well as for the adults. Prism adaptation was added to the test battery at the second school and later tested with an increased number of trials for the adults. The relatively poor eyeblink conditioning results of the children at the first school led to a prolonged test session with several additional trials at the second school. However, the result did not improve. The number of trials was reduced again when performing the procedure on the adults, to fit all tests into one single occasion, rather than on three occasions as for the children. The test equipment was also developed along the way. Therefore, when testing the adolescents during the last data collection of the five, a different system was used to deliver the stimuli and record the data.

At the beginning of the project, the expectation was that the majority of the participants would reach a high CR percentage and that it hence would be easy to measure the timing of the CRs when the asymptote of the learning was reached. When the results

revealed that many of the participants almost did not produce any CRs, the first impression was that something could be wrong with the measurement, procedure, or data analysis. Much time during the project was spent on improving and checking the equipment, reanalyzing the data, and changing the ISI. Eventually, it appeared that the results did not seem to improve, and that other researchers in the field of human eyeblink conditioning are experiencing the same kind of results. Due to the changes between data collections, it can be questioned whether the results were comparable between the groups. Most of the procedures were still similar, with parts of the protocols being identical. The same test instructions, stimuli settings and adjustments, background conditions, and so forth were used. There was no evidence suggesting that the variations in the protocol had any considerable impact on the results. Also, since a large proportion of the overall learning occurred early on during the testing, that specific part of the session was used as a basis for comparisons between groups. Of course, this scenario is not optimal. Although cautions have been applied, and details are described in the individual papers, it cannot be guaranteed that these dissimilarities have not had any impact on the results.

During the statistical analysis, numerous variables were taken into consideration to best present the outcome of the project. Extracted from each performance were a vast number of measures, such as different variability measures, velocities, asynchronies, amplitudes, motor components, trial types, time properties, learning criteria, etc. Many times, it was hard not to get overwhelmed by all of the data and the variety of options. The performance measures were chosen through principal component and factor analyses to best represent the different aspects of the tasks in the hypothesis testing. Eventually, it became clear that the findings were essentially the same, regardless of the statistical computations.

Implications

The overall purpose of the included papers was to explore cerebellar function during development. The results reflect uncertainty about the extent to which cerebellar function is captured by eyeblink conditioning, prism adaptation, and finger tapping and whether the performances rely on the same neural mechanisms. The results indicate that these methods and measures cannot replace each other in order to describe general cerebellar function, which could have been beneficial for clinical purposes. More research is needed to explore the underlying cerebellar mechanisms, extra-cerebellar contributions, and if the three methods combined can contribute to a more nuanced picture of cerebellar involvement in neurodevelopmental disorders.

The results highlight a few important aspects to consider when using these test methods. One aspect is age, and by extension maturity, the effect of which is clearly

expressed in this sample and has not been regularly accounted for in some studies of these methods up until this point. Delay eyeblink conditioning has previously been suggested useful when studying typical neurodevelopment in infancy (Ivkovich, Krasnegor, Eckerman, & Stanton, 2002). The findings in the included papers suggest that this might be applied up to early adolescence, although more research is needed to clarify this. Moreover, the maturation thresholds for the different tests likely differ, perhaps partly because cerebellar sub-regions develop at different rates during development in childhood (Tiemeier et al., 2010). In the present investigations the children above 9 years of age produced as many conditioned responses as the adults with the longer ISI during eyeblink conditioning. The performance of children above 7.5 years performed on par with the adults on prism adaptation. In contrast, the children above 9 years did not reach the adult level of performance during serial interval production. In addition, sex differences might be reflected in the outcome of eyeblink conditioning. Further, the results in this thesis suggest that temporal test parameters should be considered carefully and altered with respect to the purpose of the research question in focus. Comparisons between studies and clinical populations seem complex and cautions must be applied to not confuse cerebellar dysfunction with immaturity.

Future directions

Generally, small groups of participants are tested in this field of research. Even if the samples in this thesis are relatively large in comparison, testing more participants in the future will hopefully shed light on the questions raised here. How does the distribution of the test scores look like in a typical population? What characterizes individuals that do not perform as well as their peers? Does the absence of learning on eyeblink conditioning mean that these individuals perform below average on other learning tasks, such as working memory, motor learning, and language? What is their level of maturity? Can the performances be standardized in different age groups?

Further, to sort out what we actually test with the different tasks might call for separate test protocols in different subgroups during development. This since learning mechanisms, cerebellar sub-regions and extra-cerebellar networks changes depending on the nature of the tasks as well as with the subject's level of maturity. Appropriate temporal parameters and training duration could then be considered. It might give an answer to the question whether age differences during performance level out with more extensive training. Depending on the purpose and context, either similar or different training parameters could be more appropriate when making comparisons between groups. In addition, the extent to which the numerous studies on eyeblink conditioning are in fact measuring the same function or if comparisons between studies are limited, needs to be further clarified. There are also questions to investigate regarding how the

performance is influenced by other contributing factors, including genetics, personality traits, experiences, environment, and cognitive processes.

It is also essential to explore if individuals with neurodevelopmental disorders improve their performance with age and with different training parameters. Is legitimate to compare groups diagnosed with neurodevelopmental disorders to age matched controls or should they instead be compared to younger subjects to capture a core cerebellar contribution more efficiently? What do the norms look like in populations with different disorders? What is the clinical accuracy for the different tests regarding impaired performance?

If some of the questions above are answered, these test methods could potentially be used combined as a measure of cerebellar function, and by extension cerebellar dysfunction. In the long run, it might enable us to clarify some of the underlying causes and cerebellar contributions to cognitive disorders.

Sammanfattning på svenska

Introduktion

Uppgifter som att lära sig blinka i tid, att anpassa sitt rörelsemönster till en förskjutning av synfältet och att hålla takten med god precision har alla en gemensam nämnare: lillhjärnan. I det här doktorandprojektet har jag fokuserat på att med hjälp av dessa typer av uppgifter undersöka hur lillhjärnan fungerar hos barn i skolåldern och hos vuxna. I projektet deltog barn mellan 6 och 14 år samt vuxna mellan 18 och 55 år.

En av lillhjärnans huvuduppgifter är att samordna rörelser av olika slag med hjälp av sinnesintryck. Lillhjärnan hjälper till att finjustera så att precisionen eller timingen blir så bra som möjligt för sitt ändamål och gör detta genom att ändra nervsignalerna ut till de områden i hjärnan som sedan styr kroppens muskler. Ett tidsmässigt rörelsefel på bara några bråkdelar av en sekund kan vara tillräckligt för att orsaka en bilolycka, kan göra att man missar att fånga en boll eller kan förstöra ett musikstycke. Mänskligt tal kan bli mer eller mindre obegripligt om olika språkljud artikuleras för tidigt eller för sent. Om det uppstår en skada på lillhjärnan kan förmågan att utföra smidiga och väl koordinerade rörelser gå förlorad.

På senare tid har forskare insett att skador på lillhjärnan inte bara påverkar motoriken utan kan också påverka kognitiva förmågor. Lillhjärnans funktion verkar inbegripa mer än vad man tidigare trott och det finns mycket kvar att kartlägga kring det. Diagnosgrupper som ADHD, autismspektrumstörningar, språkstörningar och dyslexi, har också har kopplats till lillhjärnan. Lillhjärnan kan vara mindre till storleken eller vara mindre välutvecklad. Den motoriska förmågan kan vara nedsatt och förmågan att uppfatta tid eller tajma rörelser kan vara påverkad. Med tanke på hur vanliga dessa diagnoser är skulle det vara värdefullt om man med ett enkelt och mätbart test skulle kunna få insyn i bakomliggande mekanismer vid denna typ av problematik.

Det finns olika metoder för att mäta lillhjärnans funktion. Vid hjärn-avbildningsmetoder kan man upptäcka om lillhjärnan är aktiv i specifika uppgifter. Precisionen eller timingen i utförandet mäts dock inte på detta sätt. De metoder som använts i detta avhandlingsarbete fångar däremot rörelsers temporala eller spatiala egenskaper. Sådan rörelseprecision anpassas och samordnas genom feedback från sinnesintryck. Mätningarna kallas för blinkbetingning, prisma-adaptation och rytmproduktion (finger tapping). I tidigare forskning har en välfungerande lillhjärna

visat sig spela en avgörande roll för prestationen på dessa tre test. Förutom blinkbetingning, prisma-adaptation och rytmproduktion kontrollerades deltagarnas hörsel, reaktionsförmåga och ytterligare bakgrundsfaktorer med en enkät. Barnens tal, språk, motorik och icke-verbala intelligens undersöktes också. Material från 103 barn och 45 vuxna analyserades och inkluderades i de tre delstudierna till avhandlingen.

Blinkbetingning

Med blinkbetingning undersöks den associativa inlärningen av betingade blinkningar och timingen av dessa blinkningar. Inlärningen sker successivt och timingen anpassas till intervallet mellan de två stimuli som används. Grundprincipen bygger på den klassiska princip om betingning som Pavlov tillämpade för länge sedan. Det började vattnas i munnen på hans hundar redan till ljudet av en klockringning som de brukade höra strax innan de skulle få mat. Skillnaden är att det i blinkbetingning istället undersöks hur djur eller människor lär sig blinka till en ton som de får höra precis innan en luftpuff träffar ögat. Luftpuffen gör att ögonlocken stänger sig som en ren skyddsmekanism. Från början har en ton ingenting med en blinkning att göra men om tonen presenteras vid ett antal tillfällen precis innan en luftpuff så kommer tonen till slut att trigga en blinkning. Detta sker även om luftpuffen sedan uteblir. Just de betingade blinkningarna har visat sig vara beroende av lillhjärnans funktion. Blinkbetingning har studerats noggrant på nervcellsnivå hos djur. Lillhjärnan hjälper till att samordna informationen från tonen som hörs med irritationen som luftpuffen orsakar för att sedan generera betingade blinkningar med hjälp av de motoriska banorna som går ut till musklerna kring ögat och ögonlocket. Syftet är att de inlärda betingade blinkningarna ska vara så vältajmade som möjligt för att hindra ögat från att vara öppet när luftpuffen kommer. Om intervallet mellan tonens igångsättning och luftpuffen är långt så dröjer blinkningen mer än om intervallet är kort. Detta sker för att ögat ska vara maximalt stängt och inte ska ha öppnats igen när luftpuffen kommer mot ögat. När lillhjärnan är påverkad kan blinkningarna komma för tidigt eller för sent, utebli helt eller variera mycket från gång till gång, så att de inte lika effektivt kan skydda ögat mot luftpuffen.

Prisma-adaptation

Prisma-adaptation är en annan metod där lillhjärnans funktion är avgörande. Synintrycken förskjuts åt ett håll med hjälp av prismaglas framför ögonen. Samordningen mellan motoriken och de nya synintrycken sker när en person försöker peka mot en punkt rakt framför sig. Armen hamnar långt ut mot det håll som prismorna förskjuter bilden åt. Det tar några gånger för rörelsen att successivt anpassas i rätt riktning. När precisionen förflyttats till punkten rakt framför och prismorna tas av igen hänger rörelsen på nytt inte med. Pekningen drar då istället långt ut åt sidan i

motsatt riktning, oftast utan att deltagaren är beredd på det. Det blir då ett mått på hur mycket rörelsen anpassats. När lillhjärnan inte fungerar normalt påverkas adaptationen eller uteblir helt.

Rytmproduktion

Vid rytmproduktion är uppgiften att hålla ett konstant tempo genom att trycka eller trumma pekfingret i takt till en metronom. Deltagarna får även fortsätta med att försöka hålla samma takt efter att ljudet från metronomen slutat. För att hålla tempot behöver lillhjärnan hjälpa till med att övervaka fingermotoriken i relation till takten som hörs eller nyss hördes. Det kan vara svårt normalt sett för vissa men när lillhjärnan är skadad eller påverkad så blir detta påtagligt och variationen i takten kan bli stor.

Syfte

Det har bedrivits en hel del forskning med de här metoderna men det framgår ännu inte hur de hör ihop och inte heller hur barn i olika åldrar klarar dessa test. Syftet med de tre delstudierna i avhandlingen var därför att undersöka det. Till grund låg antagandet om att lillhjärnan spelar en justerande roll vid dessa tre testmetoder och att eftersom olika områden i lillhjärnan verkar bearbeta information relativt likartat så borde prestationerna som mäts vara associerade med varandra och bygga på överlappande mekanismer.

Slutsatser

Resultaten visade att det enbart fanns mycket svaga eller oklara samband mellan blinkbetingning, prisma-adaptation och rytmproduktion, vilket kan tyda på att de bakomliggande mekanismerna i lillhjärnan är separata. Det kan inte bekräftas om de tre testmetoderna testar samma egenskap. De kan därför inte ersätta varandra vid undersökning av lillhjärnans funktion. Tillsammans skulle de kunna bidra till en mer komplett bild av hur lillhjärnan fungerar och vad som inte fungerar i olika patientgrupper men mer forskning behövs för att klargöra detta.

Det framgick tydligt i undersökningen att prestationerna förbättrades med åldern för barnen och att de vuxna presterade bättre än barnen. Inlärningen i blinkbetingningen var snabbare, anpassningen till prismorna var större och timingen i rytmproduktionen var bättre hos de äldre deltagarna. Relationen mellan ålder och testprestation skulle kunna bero på lillhjärnans områdens olika mognadsförlopp, som också kan skilja sig åt

mellan könen. Den kan också spegla annan generell eller specifik mognad hos barnen. Tidpunkten då barnen börjar nå upp till de vuxnas prestationsnivå verkar skilja sig åt åldersmässigt för de olika uppgifterna. Andra individuella faktorer kan också bidra till att överskugga samvariansen mellan testerna.

Tre faktorer visade sig vara extra betydelsefulla för inlärningsprocessen vid blinkbetingning: ålder, kön och intervallet mellan tonens start och luftpuffen. De äldre barnen fick resultat som var likvärdiga de vuxnas, medan de yngre barnen presterade sämre. Flickor och kvinnor lärde sig snabbare än pojkar och män. När intervallet mellan tonen och luftpuffen var längre var resultatet bättre för både barn och vuxna. Vid det kortare intervallet var inlärningen av de betingade blinkningarna extra lång hos barnen. En stor del av inlärningen skedde tidigt under testningen oavsett intervall, vilket är vanligt vid blinkbetingning på människor. Resultaten varierade också mycket från deltagare till deltagare. Vissa lärde sig inte alls att producera betingade blinkningar medan andra lärde sig snabbt efter bara några få exponeringar för toner och luftpuffar. Processerna bakom blinkbetingning verkar vara mer komplexa än vad man tidigare trott gällande människor. Faktorerna som i avhandlingsarbetet visats påverka resultaten gällande barn med typisk utveckling, kan också behövas ta hänsyn till när man använder och tolkar blinkbetingning i patientgrupper med påverkan på lillhjärnan.

Framtida studier

Det finns inom både forskning och klinisk verksamhet ett värde av mer kunskap kring hur grupper med normal utveckling och patientgrupper med utvecklingsmässig påverkan presterar på blinkbetingning, prisma-adaptation och rytmproduktion i olika åldrar. Så småningom skulle denna typ av forskning kunna leda fram till bättre testmetodik för att kvantifiera och identifiera diagnoser med påverkan på lillhjärnans funktion.

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