Pupillary Habituation to Dynamic Audiovisual Media

by Andrea Jara

B.S. in Neuroscience, May 2023, George Washington University

A Thesis submitted to

The Faculty of
The Columbian College of Arts and Sciences
of The George Washington University
in partial fulfillment of the requirements
for the degree of Master of Science

August 31, 2024

Thesis directed by

Gabriela Rosenblau
Assistant Professor of Cognitive Neuroscience

Abstract of Thesis

Pupillary Habituation to Dynamic Audiovisual Media

Our world is filled with noise, light, movement, and even surprise elements that affect how we interact with it. Imagine reading in a library while outside you hear traffic noises. Your ability to tune out these noises dictate how well you will concentrate on your reading. It remains unclear how humans successfully tune out or adapt to multisensory information through habituation. Habituation is crucial for focusing attention on task-relevant information and can be measured in many different physiological domains. My project examines habituation in pupil responses. Pupil responses are a sensitive physiological measure to both arousal and cognitive components of stimuli and relate directly to brain responses. Pupillary habituation is influenced by stimuli complexity and repetition. This study aims to characterize pupillary habituation of young adults (N=33) to audio-visual stimuli of varying complexity. Using eye-tracking, Participants' gaze and pupil responses were recorded in three audiovisual paradigms, the "beeps", "bubblegum", and "elf" paradigms, which differ in audio-visual complexity ranging from low to high complexity. Beeps is simply a series of beeps played 300 times while bubblegum and elf are videos of a bubblegum machine played 50 times and a jack in the box played 25 times respectively. In order to characterize habituation to each of these paradigms, we analyzed changes in pupil diameter (PD) over time. The beeps and elf paradigm were observed to have a moderately negative pattern while the bubblegum paradigm resulted in a moderately positive pattern. Consequently, habituation was only observed during the beeps and elf paradigms

Table of Contents

Abstract of Thesis……..... ii

List of Figures iv

List of Tables.....v

Introduction 1

Methods.....10

Results.....23

Discussion/Conclusion.....30

Work Cited.....36

List of Figures

Figure 1 *Eye-Tracking Experimental Setup*.....12

Figure 2 *Beeps, Bubblegum, and Elf Paradigm Visualizations* 16

Figure 3 *Comparing Early and Late Trials for Bubblegum and Elf*.....25

Figure 4 *Beeps, Bubblegum, and Elf Pupil Responses Across Trials* 28

Figure 5 *Beeps and Bubblegum Pupil Responses Across Bins*.....29

List of Tables

Table 1 *Participant Missing Data Summary*….....19

Introduction

As individuals go about the world, they interact with the environment that is full of sounds, sights, smells, tastes, and textures. Composed of all 5 senses, everyday situations require a human being's ability to acknowledge and use sensory information (Machingura et al., 2019). Sensory processing describes the progression in which individuals perceive, classify, and react to sensory information in the environment (Dunn, 2001). Depending on demographics and individual differences, past research has theorized sensory processing. Dunn (1997) developed the most researched sensory processing model made up of four processing styles. Two different axes, neurological thresholds and behavioral self-regulation strategies combine to comprise each unique style, low registration, sensation seeking, sensation avoiding, and sensory sensitivity (Dunn, 1997). Studying SPPs also helped build knowledge on multisensory processing, an equally important aspect of sensory perception. As people go through their day, they may try to read in a noisy room or look at a map while listening to music. Those are just two examples of everyday situations in which we invoke two of our senses at once, specifically our audio and visual senses. The interactions occur at the cumulation of multiple senses into a singular event making it 'multisensory integration' (Koelewijn et al., 2010). Through bottom-up and top-down processes, singular perceptions are automatically merged in multisensory integration (Parker et al., 2018). Multisensory integration allows individuals to perceive their environment in a more detailed manner and dictate our attention to specific locations (Koelewijn et al., 2010). As past research has collected a plethora of knowledge on sensory input on an isolated level, an interest has grown in the investigation of sensory interaction. Growing interest in the integration

of stimuli gives a realistic picture of events and processes as sensory stimulation infrequently occurs in seclusion. Multisensory processing and SPPs play a large role in overall interactions with an individual's environment and as such contributes to quality of relationships and life in a significant way.

Depending on each individual, multisensory characteristics, like the ability to tune out noise, gear attention toward stimulus, and create sensory expectations differ greatly. Taking into account varying populations and the effects of sensory perception on individuals, it is increasingly important to establish basic multisensory processes such as habituation in adult populations. When considering what methods to use when studying sensory perception and multisensory integration, past research specifies habituation, along with other methods like mere exposure effect, are the preferred methods when investigating human responses to unfamiliar stimuli (Gustafson et al., 2022). Habituation particularly is a theory centered on repeated exposure to a stimulus that typically leads to a drop in physiological reactions to the stimuli (Gustafsson et al., 2022). Defined as recurring experience of a stimulus that typically leads to a drop in biological responses to the stimuli, habituation is a direct measure of sensory processing which has shown promising insight in previous studies (Gustafsson et al., 2022). The non-associative learning in habituation does not require sensory or motor fatigue (Gustafsson et al., 2022). Not only is habituation a basic way bodies learn but is necessary selective attention and reflects nervous system activity in both the sympathetic and parasympathetic systems (Gotham et al., 2018).

Habituation can be measured through many different forms including neurological stimulation or physiological reactions (Muenssinger et al., 2013). Even studies performed

on fetuses have indicated that developing brains can differentiate between repeated and new information (Muenssinger et al., 2013). Galvanic skin responses to access habituation across age groups showed sensitivity differences depending upon age (Eisenstein et al., 1995). There was a significant negative correlation found with age and habituation (Eisenstein, et al., 1995). More specifically, there was an observed pattern of less initial habituation to shock in older participants, meaning that beginning trials show greater reactions to the shock elements in the stimuli (Eisenstein et al., 1995). In other words, initial reactions last longer in older participants than in younger participants during the process of habituation in repeating stimuli. There was a similar pattern of habituation in tone where younger individuals had a stronger initial habituation to tone although the correlation coefficient was not found to be significant (Eisenstein et al.,1995). With the previous findings, habituation has been shown to occur to shocking stimuli in different patterns depending on age. A factor in the difference of habituation speed could be connected to an adult's increased executive control process (Massa et al., 2012).

Though particular patterns of habituation can be observed across children and adults, habituation to engaging multisensory information is underexplored, but theories and previous explorations give the expectation that it occurs through repetition. Habituation creates various measurable reactions, one of which includes eye-centered responses that are not heavily studied but hold promise to reveal key sensory processing. Pupillary movements controlled by the iris are affected by the activation of the autonomic nervous system, and, as habituation is reflected in pupillary characteristics, reflects nervous system activation during multisensory processing (Beatty & Lucero‐Wagoner,

2000). The sympathetic system controls the dilator muscle creating pupil dilation during times at which the sympathetic nervous system is activated such as during stressful or dangerous events (Snowden et al., 2016). Consequently, the parasympathetic nervous system innervates the constrictor muscles, decreasing or constricting the size of the pupil (Snowden et al., 2016).

Eye-tracking precisely measures eye position, eye movement, eye fixation, pupil diameter (PD), and gaze duration. The plethora of information from eye-tracking makes studying perception possible through understanding participant attention (Anderson et al., 2016). From user interaction software design to saccade exploration, eye-tracking has been established as an extensive measure of cognitive processing. Within cognitive neuroscience, the utility of eye-tracking has been well established as a direct measure of habituation (Anderson et al., 2016). The most popular form of eye tracking occurs through video-based analysis based on the center of the pupil (Hutton, 2019). Image processing software carries video-based eye tracking by instantaneously identifying pupil and corneal reflections, both of which act as necessary landmarks (Hutton, 2019). Pupil reflections can exist in one of two forms; “dark pupil” tracking which tracks the comparatively dark circle of a pupil under infrared lighting, or “bright pupil” tracking that illuminates directly through the pupil to have light bounce off the retina (Hutton, 2019). “Dark pupil” tracking is most utilized along with processing algorithms that center the landmark objects like pupil reflection (Anderson et al., 20016). Eye-tracking, though an insightful, non-invasive method, must consider luminance, as dilations and constrictions in the pupil are driven by luminance changes in addition to cognitive load and arousal. Luminance, both locally in the image and global luminance in the

environment should be taken into account when analyzing pupil dilation throughout a trial, as tested effects must be isolated to determine accurate results (Hutton, 2019). Along with luminance, noise presents a variation in gaze and dilation of unknown and unwanted sources (Hutton, 2019). Three main sources of noise include the nature of the ever moving ocular system, environmental disturbances, or through the eye-tracker spatial resolution (Hutton, 2019). Luminance and noise can both be considered limitations, but even so, measures are taken during the analysis for significant results. Utilizing and analyzing habituation through pupillary responses is extremely helpful when studying attention as it measures both immediate and sustained processing of stimuli (Gotham et al., 2018). Due to habituation's deeper ability to identify attentional patterns, eye-tracking studies enables investigation of sensory processing and audio-visual responses. Moreso, habituation highlights biological markers such as PD, gaze direction, gaze duration, all of which can be recorded by eye-tracking measures. Eye-tracking in research is considered more advantageous than behavioral measures as eye-tracking provides more accurate patterns of attention, including length and latency of stimulus engagement, and its non-invasive nature allows individuals who struggle with directions and discomfort to be optimal participants (Hamner et al., 2019). Automatic eye-tracking methods especially use remote cameras to record pupil locations through corneal reflections that have no physical contact between participant and equipment, making it exceptionally suitable for a plethora of large populations such as young adults (Venker et al., 2015). Stimuli, especially auditory and visual stimuli, requires precise, real-time processing measurements in order to provide insight to complex cognitive developments (Venker et al., 2015). This is an improvement to capture

methods like verbal responses or button presses that are captured after processing (Venker et al., 2015).

There is little research on pupillary habituation within the adult population. Still, other studies have explored habituation in neurodivergent populations, comparing neurodivergent infant and child populations to their neurotypical counterparts. In such eye tracking studies, results remain murky as some findings indicate slowed decrease in visual gaze to affective stimuli in ASD children, suggesting less habituation in affective faces (Webb et al., 2010). Contrary research has also found no difference in time spent gazing between the groups, but experienced differences in visual exploration patterns (Bochet et al., 2020). Studies with autistic individuals gives evidence of a link between autistic traits and habituation. Along with observable symptoms, objective sensory experiences in ASD individuals have increasingly become a key trait (Horder et al., 2014). Such a trait has been previously associated with specific sensory scores, suggesting unique sensory experiences (Robertson and Simmons, 2012). To confirm this finding, Horder et al. also investigated the connection when controlling for anxiety and found the ASD-sensory correlation held true (Horder et al., 2014). As well as being heavily associated with ASD, unique sensory processing is associated with broader autistic traits as well (Robertson and Simmons, 2012). Unique sensory processing, especially over-responsively, has been linked to slower habituation though it remains understudied (Green et al., 2019). Reduction in habituation in autistic traits and participants is observed across multiple paradigms (Dwyer et al., 2023). Still, the definition of habituation stays consistent with the process being synonymous to shortening fixation times as one gets more habituated with stimuli and a steady increase

or decrease in PD depending on the stimuli (Bochet et al., 2020). The few habituation studies via eye-tracking conducted on adult populations all revolve around affective stimuli, thus varying results occur. Pupil responses to emotional stimuli in young adults revealed a pupillary modulation that decreased as stimuli were repeated (Sowden et al., 2016). Habituation, no matter what initial response is induced, dictates that the modulation for that particular reaction or emotion reduces over repeated presentations (Snowden et al, 2016). Throughout the study both fear-inducing and neutral stimuli were presented to participants and, in both groups, there was evidence of habituation (Snowden et al., 2016). In particular, the fear and neutral stimuli showed an increase in PD across repeated exposure (Snowden et al., 2016). Similarly, Land and Bradley (2012) presented participants with novel and repeated stimuli (Bradley et al., 2015).. In neutral, violent, and emotional stimuli, the effect of novel stimuli, emotional or otherwise, was an initial increase in pupil diameter. Both experiments stay consistent with the pattern of an increased PD with novel stimuli. Pupil dilations reflect arousal and, as complexity increases, there is a proposed increase on PD with the increase of arousal caused by complexity (Mathôt, 2018). Additionally, through revealing mechanisms underlying pupillary habituation, pupils are expected to show attenuated responses as a function of repetition. Habituation specifies that repetition decreases initial physiological responses (Mathôt, 2018). Currently, investigations have shown specific differences in pupillary reactions across content such as emotional stimuli. Still, there is little research on the habituation process especially during varying levels of complex audiovisual stimuli.

Testing on stimuli, until now, has consisted of images and focused on neutral or emotional faces. This leaves habituation research with a gap of knowledge concerning

pupillary behavior during audio stimuli and more complex visual stimuli. It remains unclear what patterns are observed in naturalistic settings due to the uniformity of stimuli in previous studies. During the current study, I investigated pupillary habituation to audiovisual stimuli in young adults. Said audiovisual stimuli differed in complexity ranging from simple to complex stimuli. The simplest stimulus was a tone while another was a working bubblegum machine, and the most complex was a scene from the movie ”Elf”. The study aimed to identify pupil fluctuations as a function of sensory complexity as well as pupillary habituation mechanism through repetition. Throughout the current study, pupil responses were recorded to examine differences of multisensory integration through habituation represented in PD. Pupil responses differ depending on stimuli type and intensity. As such, this study postulates whether sensory complexity is reflected in pupil fluctuations based on previous research hinting at habituation, stimuli type, and complexity. The first tone presented with no fluctuations is expected to have an increase in pupil dilation followed by a decrease in PD as trials repeat due to its neutrality and simplicity as a stimuli. The stimuli containing an element of surprise is expected to have an initial dilation in PD, and as habituation takes place, the pattern should reveal a slow attenuation of the initial PD. This reaction is anticipated to persist through all varying complexities of stimuli, though in varying degrees reflected in pearson’s correlation coefficients. Higher stimulus complexity should reveal more complex habituation patterns through the appearance of a more gradual habituation compared to steeper, faster habituation in less complex stimuli. Through the current research, I hope to heighten the current knowledge of habituation response patterns and pupil fluctuations, both of which are directly related to sensory processing patterns and multisensory perception.

Sensory processing is important to quality of life as past studies have been able to identify a relationship between sensory processing, motor performance, as well as physical and mental health in adult and child populations (Mary-Benson et al., 2022). Thus, deeply understanding all sensory processes is necessary for the improvement of health throughout large populations. Though Dunn (1997) explores sensory processing that varies across individuals, when sensory processing is especially heightened or reduced, it can cause issues in recording, moderating, and organizing sensory input necessary for daily activities (Machingura et al., 2029). Furthermore, increased understanding of SPPs is necessary to be able to shift and implement intervention strategies when addressing how an individual behaves in reaction to sensory stimuli (Machingura et al., 2019). All of which, when applied, can elevate populations including neurodivergent groups. When considering sensory perception and processing holistically, it becomes meaningful to understand how both could affect quality of life (QOL). More current qualities of QOL include social relationship and environmental interactions as well (May-Benson et al., 2022). As such, sensory processing and multisensory integration have the ability to worsen or improve QOL, making an understanding of the processes and patterns in research important (May-Benson et al., 2022). Furthermore, it sets an important foundation for other areas of study such as autism spectrum disorder populations. Taking into account varying populations and the effects of sensory perception on individuals, it is increasingly important to establish basic sensory processes such as habituation in adult populations.

Methods

Participants

The study included a group of thirty-three participants (27 females, 6 males, mean age= 20.9 years, SD= 1.7 years) recruited through George Washington University Experiment Management System. Across all thirty-three participants, five participants reported Hispanic ethnicity, seventeen reported their race as White, ten reported Asian, two reported being Native American and more than one race, and two reported African American. Participants were required to be eighteen or older, have normal eyesight and no diagnosed neurological or psychological disorders to participate. As compensation participants were given the option of a 10$ gift card or one credit towards their research requirement. All participants had the opportunity to withdraw at any point during the study with no penalty or consequences as participation throughout the entirety of the study was voluntary. No participants in the current study withdrew from the experiment at any time. An investigation of habituation in young adults is presented in this study.

Missing data

Throughout data collection and file processing, two data sets of paradigms were lost. The beeps paradigm data for one participant and the bubblegum paradigm for another singular participant were both unable to be recovered after initial data collection. Amount of missing data also varies across paradigms for each participant. Based on

exclusion criteria, the beeps paradigm excludes a total of 5 participants that are excluded due to missing data while data from 7 other participants are partially excluded leading to a total of 27 total participants. Similarly the bubblegum paradigm excludes 5 participants entirely and one participant partially for 25 total participants. Elf has 4 participants excluded from missing data and 3 partially excluded totaling 28 participants.

Procedure

The study consisted of two parts: 1) eye tracking experiments to investigate habituation during audiovisual video clips, and 2) questionnaires including a demographic questionnaire and questionnaires to measure autistic traits, specifically, the Adult Autism Spectrum Quotient (AQ) and the Broad Autism Phenotype Questionnaire (BAPQ) (Dorothy et al., 2004; Sasson et al., 2013). As previously discussed, research regarding ASD links autistic traits to specialized sensory processing and habituation, making the measured AQ and BAPQ of each participant integral for further in-depth processing. The AQ measures social skill, attention switching, attention to detail, communication, and imagination in which a score higher than 29 highlights significant traits of autism (Baron-Cohen et al., 2001). Only one participant had an AQ score higher than 29, and the participant was excluded from analyses. Similarly, the BAPQ investigates abnormalities in social settings, language difficulties, and rigid personality or uniform preference (Sasson et al., 2013). Statements such as “I like being around other people” are presented with the scale “very rarely” (1) to “very often” (6) that participants

utilize to assess how they relate to each statement. The study duration was approximately one hour.

Directly following consent and demographics recording, participants were led to the eye tracking room to test their eye dominance and perform the eye tracking portion of the study. Before the positioning of the participant for the duration of the eye tracking videos, participants partook in the dominant eye test which later informed calibration, validation, and analyses of PD data. The dominant eye test consisted of participants focusing on a distant object through a circular opening. Closing one eye at a time, if the object stays centered, the eye that is open was recorded as the dominant eye.

Eye-Tracking experiments

The participants' pupil diameter and gaze direction were recorded simultaneously with an EyeLink 1000 system at a sampling rate of 500 Hz. EyeLink recorded pupil size through pixels (px) where 1000 pixels is 6.3 mm long. Throughout the duration of the habituation section, participants sat on a chair 60 cm in front of a monitor (Figure 1). A chin rest was provided at 60 cm to keep head distances stable during data collection. Breaks were given in between paradigms as well. The intensity of illumination remained constant throughout all the paradigms. Using the eyetracker's online parsing algorithm, saccades and fixations were detected within the recorded data. The data was then digitized and stored on a secure computer disk for further processing and analysis. Post-processing and stimulus presentation was conducted using the PsPM Toolbox in MATLAB (The MathWorks Inc., 2022). PsPM stands for PsychoPhysiological Modeling

and is capable of model-based analysis from PsychoPhysiological cues such as pupil size and respiration (Bagley et al., 2020). PsPM makes the inference of fluctuation as well as quantitative interpretations from observed physiological data possible (Bagley et al., 2020).

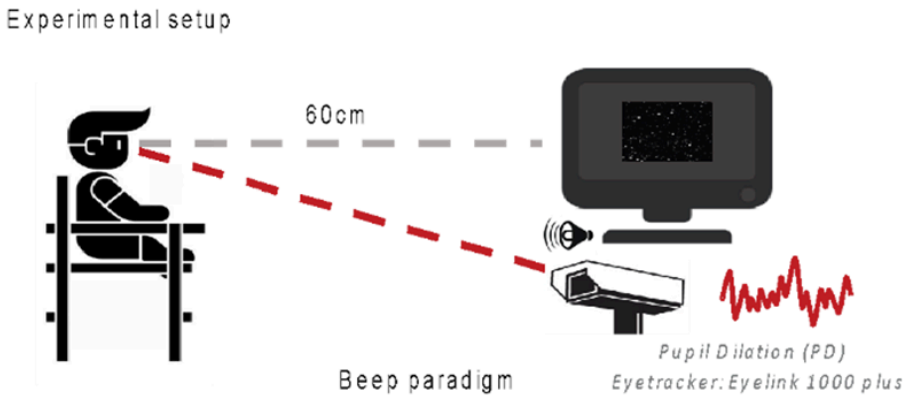


***Figure* 1**. *Eye-Tracking Experimental Setup.* A visual representation of how participants are positioned throughout the duration of the eye tracking portion of the study. Participants are sat 60 cm away from both a screen streaming the stimuli and the EyeLink 1000 camera.

Experimental Design

Eye-Tracking Paradigms

Throughout the eye tracking portion, participants were seated and placed on a chin rest in front of a monitor and camera which tracked the participant's eyes including PD and gaze on stimulus. Luminance was also kept constant across paradigms and participants to avoid fluctuation of pupil size not due to habituation. Using EyeLink,

participants' left and right eyes were calibrated and verified before the start of the audiovisual media trials in order to increase accuracy and precision of data. Accuracy in terms of eye tracking refers to identifying fine differences in eye movements while precision denotes varying error in gaze coordinate signals (Ehinger et al., 2019). During calibration, participants heard and saw a succession of beeps and dots that appeared on varying corners of the screen. When patients fixated on the dot, the participant's eye movements were recorded simultaneously by the stationary eye tracking device. From calibration, validation followed consisting of the same pattern. Beeps and dots appeared on edges or the screen randomly while participants fixated on each dot appearance. Validation of the left and right eye was reported as either poor, good, or great by EyeLink; confirming the quality of accuracy and precision of the participant's eye gazes. Participants went through calibration and validation until good or great was reported on the participant's dominant eye or both eyes. Participants' eyes were re-calibrated and re-validated before each paradigm in order to keep accuracy. Without recalibration and re-validation, accuracy decays through any head movements, minute or otherwise.

Concluding calibration and validation, participants were presented with three paradigms; "beeps", "bubblegum", and "elf". The audiovisual media consisted of three paradigm videos; each were five minutes long and were of three different intensities of audiovisual stimulation.

Beeps Paradigm. The beeps paradigm is composed of a 10 second baseline of an outer space clip followed by five minutes of the same clip while, every second, beeps played in the background. During the 'Beeps' paradigm there is a sequence of tones projected through speakers. Tone frequency is 250 Hz, 73 dB, onset time is 116 ms, ISI is 1 s,

presented in a metronomic sequence. Over the course of about five minutes, the sequence consists of 300 tone bursts (Figure 2).

The following two paradigms, beeps and bubblegum are ‘naturalistic’ paradigms. Naturalistic habituation paradigms involve participants viewing a brief introductory video, for example, an aquarium scene as in elf, followed by 50 or 25 repetitions of a short video comprising a surprise element (SE) (such as the "jack in the box" scene from the movie Elf). These paradigms have been developed as part of a collaboration with researchers at MIT.

Bubblegum Paradigm. The bubblegum paradigm consisted of a brief 10 second introductory period of a children’s cartoon aquarium followed by a repeating clip of an operating bubblegum machine with realistic sounds. The operating bubblegum machine video was repeated 50 times, each clip lasting 4 seconds long for a total time of about 5 minutes (Figure 2).

Elf Paradigm. Elf paradigm also contained a 10 second introductory clip of a realistic busy aquarium tank and a 10 second clip repeated 25 times for a total run time of about 5 minutes taken from the movie elf of a character triggering a jack in the box complete with the movie audio (Figure 2).

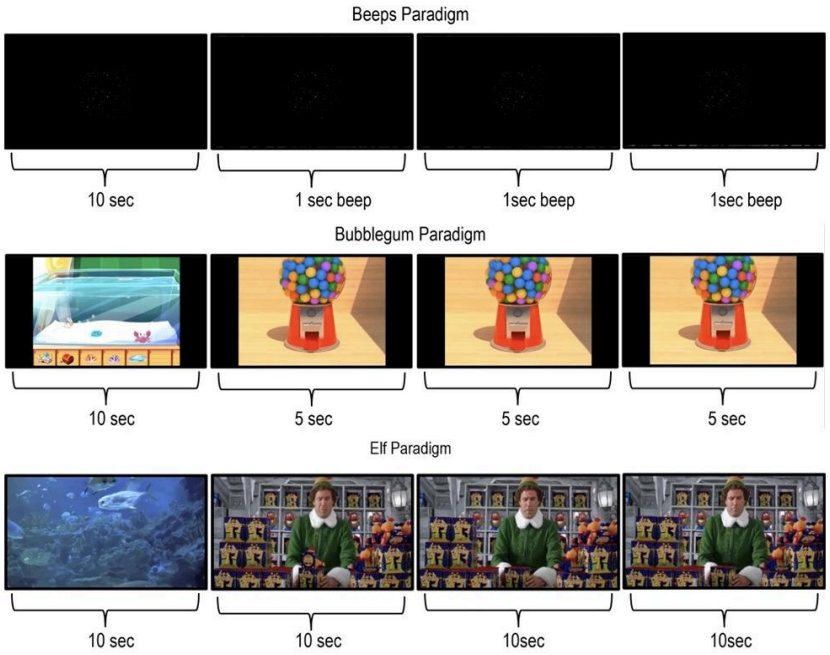


***Figure* 2**. *Beeps, Bubblegum, and Elf Paradigm Visualizations.* Temporal representations and stills of the beeps, bubblegum, and elf paradigm including the 10 second introductory clip followed by the same repeating video clip. During the beeps clip, 300 beeps are triggered with an ITI of 0.9 seconds.The bubblegum clip is repeated 50 times for a total of 50 trials with an ITI of 1 to 4 seconds. The elf clip is repeated 25 times for a total of 25 trials with an ITI of 1 to 10 seconds.

Autistic Trait Measures

After the completion of the audiovisual media paradigms in a randomized order for each participant, participants filled out two measures that assess autistic traits, the AQ and BAPQ.The BAPQ and AQ confirmed that participants had autistic traits in the subclinical range.

The AQ was administered as an assessment of autistic traits in individuals older than 16 with a normal IQ through statements such as “I am willing to take risks.”

Participants rated how strongly they agreed on a “definitely agree” to “definitely disagree” scale. On average, participants had an AQ score of 16.93 (Standard deviation, SD= 5.46). The AQ measures social skill, attention switching, attention to detail, communication, and imagination in which a score higher than 29 highlights significant traits of autism (Baron-Cohen et al., 2001). Only one participant had an AQ score higher than 29, and the participant was excluded from analyses. Similarly, the BAPQ investigates abnormalities in social settings, language difficulties, and rigid personality or uniform preference (Sasson et al., 2013). Statements such as “I like being around other people” are presented with the scale “very rarely” (1) to “very often” (6) that participants utilize to assess how they relate to each statement. Among all participants, the BAPQ overall mean is 2.92, indicating an average score among participants (SD=0.58). Scores between the values of 2.75 and 3.5 are considered average and any score less than 2.75 or more than 3.5 are above the cut off score indicating significant autistic traits present. Nine participants total had a score below or above the cut off and out of the nine total, only three participants had a score more than one standard deviation over or under the cut off.

Data Analysis

Preprocessing of pupil data

Following data collection, eye tracking data was processed to .asc and .edf files to be further preprocessed and subsequently analyzed in PSPM using MatLab. Preprocessing consisted of importing, trimming, plotting, and excluding data. Importing files from .asc to PSPM included setting a saccade discard factor of .05 and a distance

unit of millimeters. Saccade eye movements are quick shifts within the visual field that change the fixation point and thus must be accounted for during analyses as individuals have little visual perception during saccades. A saccade factor of .05 dictates that for 50 milliseconds of data surrounding a saccade identified by PSPM, the code is marked as not a number (NAN). The factor of .05 was chosen to minimize missing data while still managing to control noise. Higher saccade factors range from .2 to .25 and, though they adjust the data to mediate peaks, missing data increases to more than 60%. Thus, for more accurate analyses of recorded pupil diameter while keeping sufficient data, .05 saccade factor was implemented across all participants and paradigms. When eye-tracking data was first recorded by the EyeLink, trials were identified by markers that signify the start of all video clips. Including the introductory video clip, beeps had a total of 302 markers, bubblegum contained 52, and elf consisted of 27 total markers. All paradigms were trimmed from directly after the start of the first trial until the trial length after the last "marker" in order to not cut off any trial data. For beeps, bubblegum, and elf respectively, this included 1, 5, and 10 seconds of data after the last marker. The beeps paradigm had a total of 300 trials and was trimmed to include markers two to 302 to capture the beginning and end of all 300 trials as well as the baseline. In the same manner, bubblegum and elf were composed of 50 and 25 trials respectively. Thus, bubblegum was trimmed from marker two to 52, while elf was trimmed from two to 27. Directly after importing and trimming the raw data, the first exclusion criteria was applied.

The first exclusion criteria was identified through missing data. If missing data exceeded total participant average missing data plus one standard deviation, participant data was not included in further analysis. When calculated, average missing data was about 28% and standard deviation was calculated as 10.4. Rounded, participants were excluded if missing data surpassed 38%. beeps excluded five participants due to missing data while bubblegum excluded six and elf excluded four. Participants who had differing missing data percentages in the left and right eye were included if one eye had less than 38% missing data. For such participants, further analyses were completed using only the data of the applicable eye. Five, three, and three participants in beeps, bubblegum, and elf respectively had only one eye of viable data. In total there were ten to seven participants that were either partially or completely excluded for missing data depending on the paradigm. Beeps contained a total of 27 participants, bubblegum included 25, and elf had a total of 28 viable participants. Average missing data across all paradigms ranged from 27.7% to 30.52% (Table 1). Data from included participants is then linearly interpolated to connect NAN values and fill in missing data with projected values. Linear interpolation uses a linear model to connect two data points with NAN values in-between points. After interpolation, all missing data points are filled including NAN values from saccades and NAN values due to unrecorded data.

Table 1. *Participant Missing Data Summary.* Percentage of average and standard deviation (SD) missing data for the left and right pupil as well as the percentage and SD of left and right gaze outside of the stimuli.

Participant ID:	**Left Pupil Missing Data**		**Right Pupil Missing Data**		**Left Gaze Out of Stimuli**		**Right Gaze Out of Stimuli**	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**

Beeps	**27.70%**	**11.45**	**30.08%**	**14.41**	**2.32%**	**6.17**	**1.87%**	**6.53**
Bubblegum	**29.08%**	**11.01**	**29.90%**	**11.94**	**2.98%**	**4.46**	**2.06%**	**1.90**
Elf	**28.42%**	**10.35**	**30.52%**	**13.98**	**3.78%**	**5.41**	**4.32%**	**6.75**

Directly following interpolation, further exclusion was determined through the gaze position of both eyes on the stimuli. At the time gazes were plotted and measured, participants with more than 30% of gazes outside of the stimuli were excluded due to inaccurate focus on the audiovisual stimuli. Over all paradigms and participants, only one participant was found to have only their right eye gaze outside of the stimuli area more than 30% of the time for the beeps and elf paradigm (Table 1). On average gaze outside the stimuli averaged 1.87% to 4.32% across the paradigms (Table 1). After both exclusion criteria, beeps consisted of 27 total participants with six participants having viable data from only one eye. Bubblegum consisted of 25 participants, two of which had data from one viable eye and elf had 28 total participants with four of the 28 having one viable eye.

After confirming patient inclusion and gaze accuracy, PD was plotted against time and paradigm trials. Before plotting, data sets that included both left and right eye measurements were linearly regressed to ensure the left and right pupil diameter values were highly correlated to each other. For those who had a correlation coefficient .9 or higher, the average pupil size of both eyes was analyzed across trials. Left and right eye data were not aggregated for participant data sets with a correlation coefficient of less than .9. In these cases, only the dominant eye data were analyzed . For the beeps

paradigm, all participants were reported to have a correlation coefficient above .9.s. Within the bubblegum paradigm, only one participant was found to have a correlation coefficient of less than .9, and in the elf paradigm four participants had a a coefficient of less than .9.

Testing pupillary habituation over number of repetitions

Comparing Early and Late Trials. The first and last 25% of trials applicable to the bubblegum and elf paradigm were averaged. A paired samples t-test was performed to evaluate whether the late trial PD was greater than or less than the early trials PD.

Data were plotted across a function of trials. Full trials were plotted for early and late trial comparisons. Beeps paradigm was not plotted in this manner due to the clip which did not consist of a surprise element. The first 25% of trials recorded during bubblegum and elf paradigms were averaged across participants and plotted over a function of time in seconds along the x-axis. The same was done for the last 25% of trials in both paradigms. As calculated, the first and last 12 trials in bubblegum and the first and last 6 trials in elf were averaged and graphed across the trial length. In total two figures comparing PDduring early and late trials were completed. *PD Across Bins and Trials.* Pearson's correlation coefficients between pupil averages in each of the three paradigms and repetition time were computed to test for habituation over time. Averaged pupil diameters were also plotted over time to visualize the effect. Pupil diameters were then plotted per trial or per “bin”. “Bins” grouped the data of six repetitions for beeps and five repetitions

for bubblegum and treated each group of ‘bin’ as one point during analyses and plotting. As a result of the limited number of trials in elf, no bins were made for the paradigm. In addition to data binning, PDs were plotted over sliding averages of repetitions. Sliding average refers to the method that averages results within reference ranges including combining surrounding repetitions to one data point. Sliding averages help smooth unexpected peaks and valleys to make continuous patterns easier to identify. Beeps, bubblegum, and elf PD were averaged across participants and trials to create a graph that plots average PD across all trials. Due to the large number of trials in beeps paradigm, sliding averages were set at 6 at a time meaning data from six surrounding trials were combined in addition to the data from each individual trial. Thus all 300 trials were retained while also smoothing the data progression. Bubblegum and elf sliding averages used 3 trials at a time for 50 and 25 trials respectively. and bins of each paradigm

Results

Pupil diameter averages

Beeps

The data utilized for trials over bins differ slightly from the raw data due to the exclusion of the first 6 trials in the PD across trials plot. Exclusion of the first six trials was completed due to the value's unprecedentedly low values. At that time, the recorded values of PD would not have accurately reflected the rest of the trial data. Likewise, the average PD for the paradigm was 1998.38 px with a range of 1896.02 to 2164.04 px. In order to preserve participants, outlier data was kept in analyses as long as the majority of the data set was within a normal PD range.

Bubblegum

The bubblegum paradigm, with PD plotted across trials and bins, is assessed to investigate habituation patterns present. During the PD across trials and bins, standard deviation and average were 34.25 and 1098.80 px respectively with a range of 1006.35 to 1168.05 px.

Elf

The average PD during trials was 1089.86 px with a range of 1049.77 to 1138.02 px. In order to preserve participants, outlier data was kept in analyses as long as the majority of the data set was within a normal PD range.

Comparing Early and Late Trials

When considering paradigms, examining in-trial differences can give valuable insight to habituation. Specifically, measuring PD during early and late trials within each paradigm offers detailed pupil patterns and how they differ as repetition of stimuli continues. Averaged participant full trial data graphed across the four and ten second trial lengths shows significant change in pupil fluctuations after video repetition (Figure 3). Bubblegum trials occurring during the first 25% of the paradigm were averaged across participants and plotted as a function of time (Figure 3). The last 25% of trials were also averaged across participants to show within-trial habituation differences.After conducting t-tests on the early and late trials of PD during both the bubblegum and elf paradigm, there is a significant increase in pupil diameters. Bubblegum averages showed and increase from early to late trial repetitions ($M=1558.99$, $SD=26.33$) is significantly greater than in the early trial PD ($M=958.45$, $SD=27.49$), $t(12)=-54.60$, $p=2.05e-25$. The t- value is not in the 95% region of acceptance indicating the difference between the average PD is large when testing whether the late trial PD average is greater than the early trial PD average. As in bubblegum, elf trials occurring during the first and last 25% of the paradigm were averaged across all participants and time (Figure 3). Comparing the trials, there is an overall elevation in PD from an average of approximately 1000 to 1500

(Figure 3). In the same manner, the elf paradigm t-test showed a significant difference in PDduring early and late trials. Average PD for early trials (M=1008.24, SD=22.99) are significantly less than PD in later trials (M=1434.3, SD= 19.8), t(6)=-34.388, p=7.8e-12. The t-value in this case is not in the 95% region of acceptance confirming that the difference between average PD is large when testing is the PD average during late trials

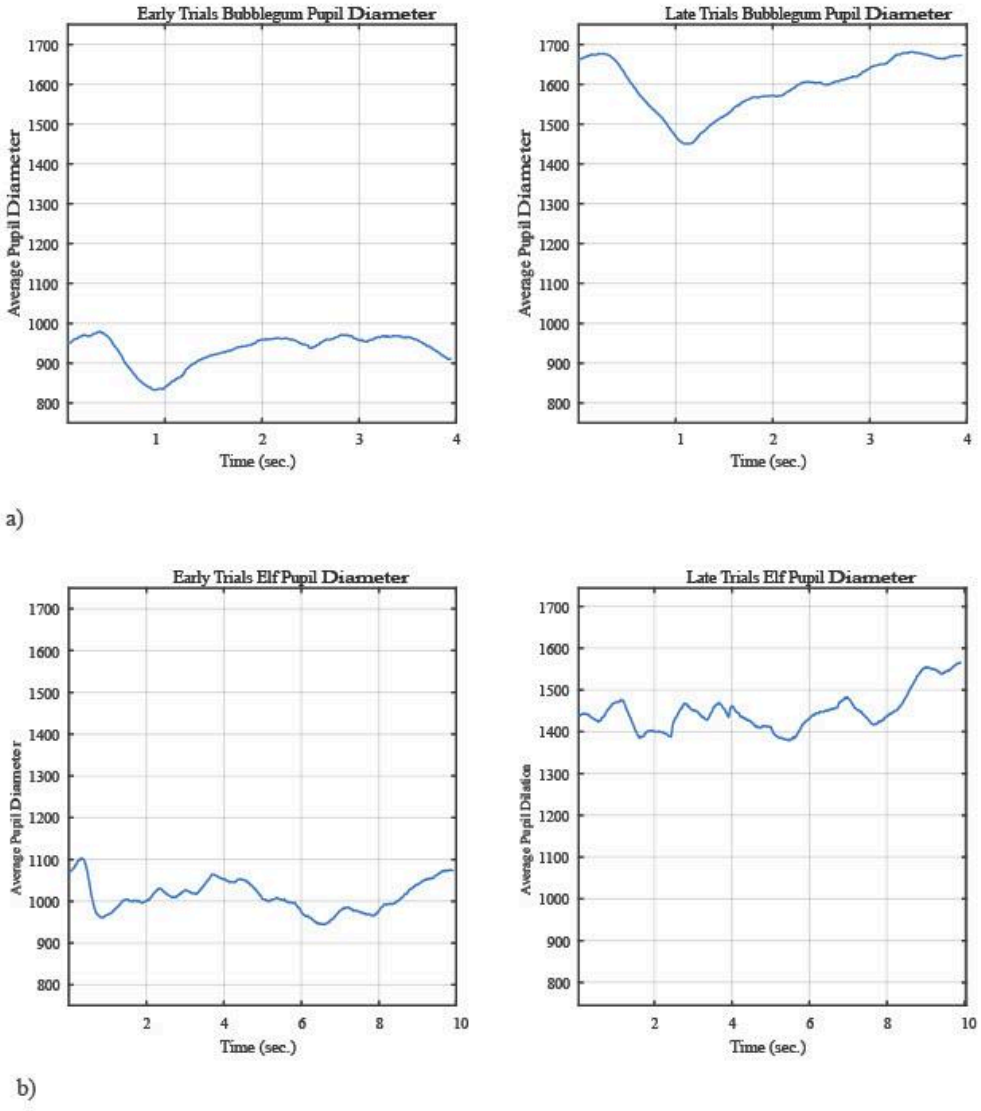


Figure 3. *Comparing Early and Late Trials for Bubblegum and Elf.* First and last 25% of trials plotted for a)bubblegum (top) and b)elf (bottom) paradigms. Pupil diameter was

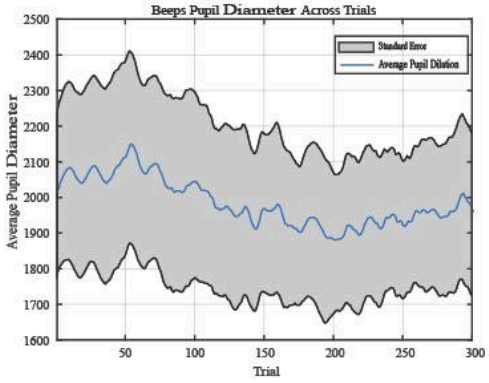
averaged across participants during the first and last twelve trials for bubblegum. Diameter during elf was also averaged across participants during the first and last six trials. One point along the x axis represents one second of time during the paradigm trial. In total the bubblegum trial lasted 4 seconds while the elf trial lasted 10 seconds. The y-axis represents the average pupil size in pixels for all participants during the trial.

Pupillary changes over number of repetitions

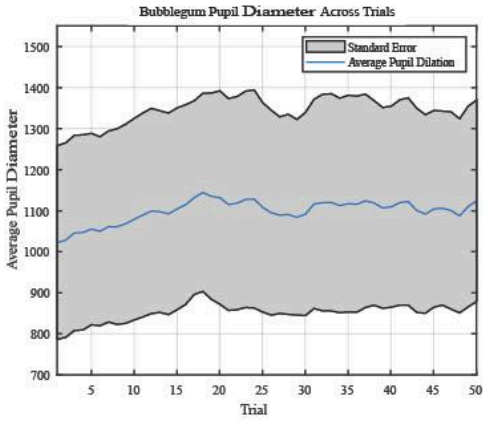
Habituation, throughout the study, has been observed and measured through pupil diameter changes across repetitions in three different paradigms. Defined by attenuation of physiological reactions, pupillary reactions based on the initial stimulus decreases over time. Plotted graphs allow for visual representation of PD across repetition while correlation analysis addresses the presence, significance, and pattern of habituation that occurs in each paradigm (Figure 4). Due to exclusion criteria, the number of participant data present in each paradigm differs slightly. The beeps paradigm has 27 total data sets while bubblegum has 25 and elf has 28 total participant data sets. Considerably the degrees of freedom for beeps, bubblegum, and elf is 25,23,and 26 respectively which were utilized within the Pearson's correlation. The Beeps paradigm pupil diameter across trials was found to be moderately negatively correlated, $r(23)=-.64$, $p=.001$ (Figure 4, Figure 5). This significant correlation dictates there is an expected drop in PD as trials and time increases (Figure 4, Figure 5). As repetition of stimulus continues, the stimulus triggers a diameter that becomes less and less intense with the passage of time. Thus, the model exemplifies habituation through a decrease in pupil diameter (Figure 4, Figure 5). In a similar manner, the elf paradigm sees a moderate negative correlation for PD across

trials, r(26)=-.62, p<.001 (Figure 4). Thus, with an increase in trials, there is an expected decrease in PD (Figure 4,). As pupil sizes decrease over time, the PD shows a reduction in strength of the initial diameter. As stimulus repeats, the pupil decreases in diameter through the process of habituation (Figure 4). In regards to the bubblegum paradigm, pearson's correlation suggests a moderate positive correlation between PD and trials, r(25)=.49, p=.0095 (Figure 4, Figure 5). In other words, there is an expected increase in PD as trials increase (Figure 4, Figure 5).

a)



b)



c)

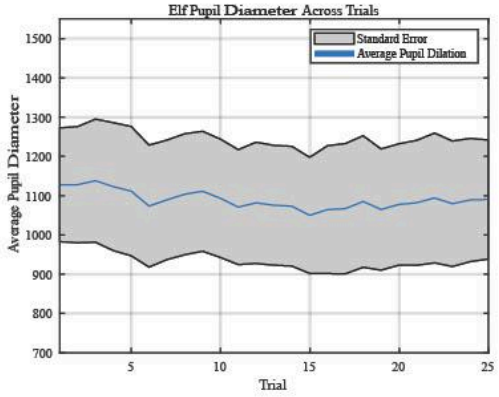


Figure 4. *Beeps, Bubblegum, and Elf Pupil Diameter Across Trials.* A) beeps (top), b) bubblegum (middle), and c) elf (bottom) paradigm average pupil diameter across trials. Trials consisted of eight, three, and three sliding averages respectively. Shaded areas indicate calculated data set standard error while plotted blue lines signify average pupil diameter across participants. The y-axis shows pupil size in pixels and the x-axis represents the number of trials.

Bins

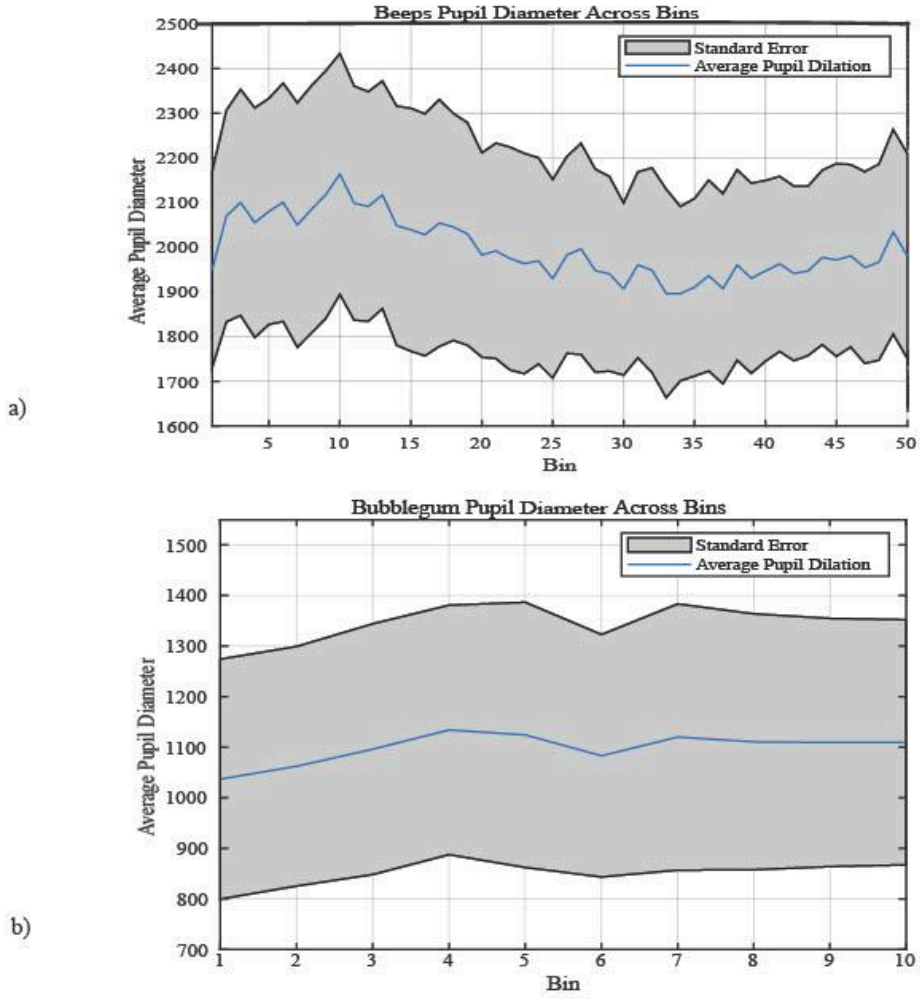


Figure 5. *Beeps and Bubblegum Pupil Responses Across Bins.* A) Beeps (top) and b) bubblegum (bottom) pupil diameter is plotted across bins. Bins consisted of an average of six and five trials respectively. Shaded areas indicate calculated data set standard error while plotted blue lines signify average pupil diameter across participants. The Y axis shows pupil size in pixels and the x axis represents the number of bins.

Discussion/Conclusion:

This research was focused on developing a deeper understanding of habituation during audiovisual stimuli. To properly engage in the investigation, PD were recorded across a repetition of trials from three differing complexities of audiovisual stimuli. Early and late trials gave insight on within trial habituation differences to contrasting audiovisual stimuli. PD across trials and bins for each paradigm informed the effect of stimuli repetition to PD, a vital part of habituation. Overall, results show habituation through attenuation of pupil diameter in the beeps and elf paradigm. In both paradigms, there is a negative change in PD, but the same pattern is not seen in the bubblegum paradigm. Instead, the bubblegum paradigm has a positive association of PD as trials increase. The correlation could be rooted in the particular stimuli's ability to activate the parasympathetic nervous system causing an initial constriction of the pupil leading to the positive correlation. Though, in the current study only PD, and not constriction, was measured off of a baseline presented before the stimulus trials. Thus, confirmation of habituation during the bubblegum paradigm becomes impractical and infeasible.

Beeps. During the beeps paradigm the negative PD was as projected showing an initial increase in PD followed by a consistent decrease of PD ($r(31)= -.64$). This is reflected in the negative association and visualization of average PD across trials and bins. Habituation is present through the decreasing PD as stimuli repeats. In past research, neutral stimuli elicited an initial positive PD and literature dictates a decrease in pupil diameter is predicted (Bala et al., 2020). Though the pattern is similar between current and past data, the stimuli used in other research differs. In the present case, stimuli

included the integration of multiple senses at once with both audio and visual stimuli, yet, habituation is still detected through the consistent decrease of PD across time.

Bubblegum. Bubblegum paradigm displayed a moderate positive correlation for PD and trials (r(29)=.43). Though the correlation was found significant, the pattern in terms of habituation needs to be clarified. Overall, there is no diameter decrease over time and thus no habituation due to lack of evidence. The pupil pattern mimics a pattern of an average pupillary response not undergoing habituation, especially considering the duration of the paradigm is five minutes long. Therefore, the conclusion drawn from the results is one of no habituation. In Land and Bradley (2012), repeated stimuli produced greater pupil dilations such that, when compared, the novel items reflected a smaller amount of dilation (Bradley et al., 2015). This was attributed to recognition where, regardless of content, enhanced pupil diameter will occur in repeated recognized stimuli. If so, the increasing pattern in bubblegum could be due to the initial novel stimulus that increases diameter to a lesser extent than recognition of stimuli occurring in later trials. Though unable to conclude habituation from the positive correlation in the current study, habituation which displays a positive pattern of PD is not unfeasible as there is past research that has seen increased pupil change with recognized stimuli..

Elf. A PD pattern was observed for the elf paradigm where a moderately negative correlation which reflects habituation present at the repetition of stimuli, r(31)=-.62. With an initial strong pupil diameter, the gradualism of PD exemplifies the attenuation process in habituation. Thus through the presence of a negative correlation, habituation is confirmed and observed in the current paradigm. The significant negative correlation

coefficient emphasizes the existence of a PD pattern and subsequent habituation. Previous research using a surprise affective visual stimuli showed an increase in PD which is unlike the pattern found (Mathot, 2018). Other studies have supported the habituation pattern found when considering novel and more fearful stimuli (Ueda et al., 2021). The specific stimuli should be considered for the significant negative pattern. The video could have affected the PD seen in participants considering the audiovisual complexity or their familiarity to the clip.

The study aimed to grasp habituation patterns during audiovisual stimuli by recording and analyzing PD across time in three paradigms. The paradigms differed in complexity of audiovisual stimuli, adding to the novelty of the research. We found pupillary habituation to simpler auditory beeps and to highly complex audio-visual movie segments . As repetition of stimuli increased, PD was observed to decrease from initial observations. In a medium complexity condition, consisting of a cartoon with a surprise element we did not find the same habituation pattern. This could be due to the artificial nature of the stimulus. Future studies with larger samples are needed to conclusively determine differences between this paradigm and the simpler and more complex one used in this study. ,

Limitations

In this study participants per condition ranged from 25 to 28 individuals and to include as many participants as possible, outlier participants were included if partially consisting of outlier data. Thus, it's necessary to acknowledge the constraint of available participants

and data points. Also, though the recruitment information for participants was put in a pool consisting of both males and females, there were only a total of 6 male participants recruited compared to their female counterparts of 27 individuals. In other instances, an uneven number of males and females, methods such as bootstrapping would be implemented to infer results to a population through sampling replacements during analyses. For the purposes of this study, bootstrapping was not applied due to increased complexity of the analyses and research restraints. Also, the bubblegum and elf paradigms contained a "surprise element". Though little to no young adults had probably seen the bubblegum clip before, the clip from the Elf movie was much more likely to be observed beforehand. As it is a popular movie, especially among the generation that the participants are a part of, it is possible that the participants had recognized the scene before the "surprise element" and were prepared for the element more than in the bubblegum paradigm. This could have affected the initial habituation pattern in an unknown way.

Future Studies

Through further analyses of PD across time when controlling for other variables, the habituation research captured in the current study can be applied to other areas of research. Further examination of PD using the methods outlined in this study can be pursued by integrating BAP-Q and AQ scores to the correlation of PD over time and comparing mathematical models across paradigms. Habituation has been established to be reflective of neural and nervous system activation. As such, developing knowledge of

habituation in the young adult population serves as a good foundation from which research can draw upon. Specifically, a novel area of interest is using habituation as a possible way to aid ASD diagnosis. In recent years, there has been a surge in research within the autism spectrum disorder (ASD) community. The diagnostic criterion for ASD now includes sensory sensitivity as a significant trait, with individuals experiencing hyper- and hypo-reactivity to sensory input (American Psychiatric Association, 2000). Sensory issues in ASD individuals can include auditory, visual, and physical perceptions, and there is a positive relationship between ASD traits and sensory sensitivity (Landon, 2016). Sensory perception also impacts multisensory integration, which affects emotion recognition and social interactions (Kawakami, 2021). Understanding sensory issues in ASD populations is crucial for enhancing relationships and quality of life. Previous research has identified differences in perception and deficits in multisensory integration in ASD individuals, and theories such as the Weak Central Coherence theory and the Enhanced Perceptual Functioning theory have been proposed to explain these differences (Plaisted et al., 2003). Habituation, a theory centered on repeated exposure to a stimulus, is a preferred method for studying perception differences in ASD individuals (Gustafson et al., 2022). Eye-tracking, which measures patterns of attention and physiological reactions, is advantageous for studying sensory processing and audio-visual responses in ASD individuals (Falck-Ytter & Greenback, 2013). Eye-tracking has also been considered for early diagnosis of ASD, as it can demonstrate learning and development (Bouchet et al., 2021). Previous eye-tracking research on babies has shown mixed results, but there is a need for more reliable physiological methods for early diagnosis (Jones et al., 2008). Diminished habituation in children with ASD can lead to "stimulus overload"

and social impairments (Bouchet et al., 2021). Eye-tracking studies have shown differences in habituation patterns between ASD and neurotypical children, but there is limited data on pupillary habituation (Wedd et al., 2010). However, there is a lack of research focusing on habituation and eye-tracking in ASD individuals. Thus, investigating habituation in neurotypical young adults helps set a background from which ASD habituation research can later compare and build upon.

Work Cited

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Washington, DC: American Psychiatric Association; (2013)

Anderson, B. B., Jenkins, J. L., Vance, A., Kirwan, C. B., & Eargle, D. (2016). Your memory is working against you: How eye tracking and memory explain habituation to security warnings. *Decision Support Systems, 92,* 3–13. https://doi.org/10.1016/j.dss.2016.09.010

Bagley BA, Bordelon B, Moseley B, Wessel R. Pre-Synaptic Pool Modification (PSPM): A supervised learning procedure for recurrent spiking neural networks. PLoS One. 2020 Feb 24;15(2):e0229083. doi: 10.1371/journal.pone.0229083. PMID: 32092107; PMCID: PMC7039446.

Bala ADS, Whitchurch EA, Takahashi TT. Human Auditory Detection and Discrimination Measured with the Pupil Dilation Response. J Assoc Res Otolaryngol. 2020 Feb;21(1):43-59. doi: 10.1007/s10162-019-00739-x. Epub 2019 Dec 2. PMID: 31792632; PMCID: PMC7062948.

Bradley MM, Lang PJ. Memory, emotion, and pupil diameter: Repetition of natural scenes. Psychophysiology. 2015 Sep;52(9):1186-93. doi: 10.1111/psyp.12442. Epub 2015 May 5. PMID: 25943211.

Bochet A, Franchini M, Kojovic N, Glaser B, Schaer M. Emotional vs. Neutral Face Exploration and Habituation: An Eye-Tracking Study of Preschoolers With

Autism Spectrum Disorders. Front Psychiatry. 2021 Jan 13;11:568997. doi: 10.3389/fpsyt.2020.568997. PMID: 33519540; PMCID: PMC7838366.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The autism-spectrum quotient (AQ): Evidence from asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. Journal of autism and developmental disorders, 31(1), 5-17.

Dorothy VM Bishop, et al. Using Self-Report to Identify the Broad Phenotype in Parents of Children with Autistic Spectrum Disorders: A Study using the Autism-Spectrum Quotient. 45(8): 1431-1436. Journal of Child Psychology and Psychiatry (2004).

Dunn, Winnie PhD, OTR, FAOTA. Supporting Children to Participate Successfully in Everyday Life by Using Sensory Processing Knowledge. Infants & Young Children 20(2):p 84-101, April 2007. | DOI: 10.1097/01.IYC.0000264477.05076.5d

Dwyer P, Williams ZJ, Vukusic S, Saron CD, Rivera SM. Habituation of auditory responses in young autistic and neurotypical children. Autism Res. 2023 Oct;16(10):1903-1923. doi: 10.1002/aur.3022. Epub 2023 Sep 9. PMID: 37688470; PMCID: PMC10651062.

Gustafsson E, Francoeur C, Blanchette I, Sirois S. Visual exploration in adults: Habituation, mere exposure, or optimal level of arousal? Learn Behav. 2022 Jun;50(2):233-241. doi: 10.3758/s13420-021-00484-3. Epub 2021 Sep 20. PMID: 34545534; PMCID: PMC9233633.

Horder J, Wilson CE, Mendez MA, Murphy DG. Autistic traits and abnormal sensory experiences in adults. J Autism Dev Disord. 2014 Jun;44(6):1461-9. doi: 10.1007/s10803-013-2012-7. PMID: 24305777; PMCID: PMC4022987.

Kafkas A, Montaldi D. How do memory systems detect and respond to novelty? Neurosci Lett. 2018 Jul 27;680:60-68. doi: 10.1016/j.neulet.2018.01.053. Epub 2018 Feb 3. PMID: 29408218; PMCID: PMC6565889.

Kawakami S, Otsuka S. Multisensory Processing in Autism Spectrum Disorders. In: Grabrucker AM, editor. Autism Spectrum Disorders [Internet]. Brisbane (AU): Exon Publications; 2021 Aug 20. Chapter 4. PMID: 34495620.

Koelewijn, T., Bronkhorst, A., & Theeuwes, J. (2010). Attention and the multiple stages of multisensory integration: A review of audiovisual studies. *Acta Psychologica*, *134*(3), 372–384. https://doi.org/10.1016/j.actpsy.2010.03.010

Landon, J., Shepherd, D., & Lodhia, V. (2016). A qualitative study of noise sensitivity in adults with autism spectrum disorder. *Research in Autism Spectrum Disorders*, *32*, 43–52. https://doi.org/10.1016/j.rasd.2016.08.005

Machingura, Tawanda & Kaur, Gurjeet & Lloyd, Chris & Mickan, Sharon & Shum, David & Rathbone, Evelyne & Green, Heather. (2019). An exploration of sensory processing patterns and their association with demographic factors in healthy adults. Irish Journal of Occupational Therapy. ahead-of-print. 10.1108/IJOT-12-2018-0025.

Massa, J., & O'Desky, I. H. (2012). Impaired Visual Habituation in Adults With ADHD. Journal of Attention Disorders, 16(7), 553-561. https://doi.org/10.1177/1087054711423621

Mathôt S. Pupillometry: Psychology, Physiology, and Function. J Cogn. 2018 Feb 21;1(1):16. doi: 10.5334/joc.18. PMID: 31517190; PMCID: PMC6634360.

May-Benson TA, Teasdale A, Easterbrooks-Dick O. Relationship Between Childhood Sensory Processing Differences and Quality of Life as Adults. Front Psychol. 2022 Jul 27;13:886833. doi: 10.3389/fpsyg.2022.886833. PMID: 35967700; PMCID: PMC9363864.

Mennemeier M. S., Chatterjee A., Watson R. T., Wertman E., Carter L. P., Heilman K. (1994). Contributions of the parietal and frontal lobes to sustained attention and habituation. *Neuropsychologia*, 32, 703-716.

Robertson AE, Simmons DR. The relationship between sensory sensitivity and autistic traits in the general population. J Autism Dev Disord. 2013 Apr;43(4):775-84. doi: 10.1007/s10803-012-1608-7. PMID: 22832890.

Sasson NJ, Lam KS, Childress D, Parlier M, Daniels JL, Piven J. The broad autism phenotype questionnaire: prevalence and diagnostic classification. Autism Res. 2013 Apr;6(2):134-43. doi: 10.1002/aur.1272. Epub 2013 Feb 20. PMID: 23427091; PMCID: PMC3661685.

Snowden RJ, O'Farrell KR, Burley D, Erichsen JT, Newton NV, Gray NS. The pupil's response to affective pictures: Role of image duration, habituation, and viewing mode. Psychophysiology. 2016 Aug;53(8):1217-23. doi: 10.1111/psyp.12668. Epub 2016 May 13. PMID: 27172997; PMCID: PMC5031225.

The MathWorks Inc. (2022). PsychoPhysiological Modeling, Natick, Massachusetts: The MathWorks Inc. https://www.mathworks.com

Ueda K, Sekoguchi T, Yanagisawa H. How predictability affects habituation to novelty. PLoS One. 2021 Jun 1;16(6):e0237278. doi: 10.1371/journal.pone.0237278. PMID: 34061853; PMCID: PMC8168884.

Webb SJ, Jones EJH, Merkle K, Namkung J, Toth K, Greenson J, et al.. Toddlers with elevated autism symptoms show slowed habituation to faces. *Child Neuropsychol*. (2010) 16:255–78. 10.1080/09297041003601454

Zekveld AA, Koelewijn T, Kramer SE. The Pupil diameter Response to Auditory Stimuli: Current State of Knowledge. Trends Hear. 2018 Jan-Dec;22:2331216518777174. doi: 10.1177/2331216518777174. PMID: 30249172; PMCID: PMC6156203.