UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ELEVATED SPONTANEOUS BLINK RATE IN FRAGILE X SYNDROME IMPLICATES ABNORMAL DOPAMINERGIC ACTIVITY IN FXS PATHOPHYSIOLOGY

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

By

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Norman, Oklahoma
2019

ELEVATED SPONTANEOUS BLINK RATE IN FRAGILE X SYNDROME IMPLICATES

ABNORMAL DOPAMINERGIC ACTIVITY IN FXS PATHOPHYSIOLOGY

A THESIS APPROVED FOR THE
DEPARTMENT OF PSYCHOLOGY

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Acknowledgements

First and foremost, I would like to acknowledge Dr. Lauren Ethridge for the incredible depth of patience she's shown me over the last four years, the opportunities and resources she's provided for my development, and all of her hard work, networking prowess, and raw force of will that has positioned our lab as the central point of data analysis for a number of exciting research projects.

Second, I would like to extend my appreciation to Lisa DeStefano and Melody Reese for all their helpful advice, insight, and no small amount of emotional support. Aside from Dr. Ethridge, Lisa and Melody have probably had the greatest impact on my growth as a scientist and have provided excellent examples to model myself after.

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Abstract

Fragile X Syndrome (FXS) is the most common, single-gene cause of heritable Autism Spectrum Disorder (ASD) and intellectual disability (ID). FXS is characterized by sensory hypersensitivity, repetitive behaviors, anxiety, social deficits, and impaired executive function; behavioral impairments also found in ASD. Cortical hyperexcitability has been demonstrated in both FXS and ASD relative to controls, which correlates with the measures of symptom severity. This hyperexcitability results from impaired inhibitory GABA signaling as well as exaggerated excitatory glutamate signaling, and a great deal of research focused on the neurobiology of ASD and FXS is concerned with GABA and glutamate signaling. Despite the clear significance of GABA and glutamate signaling, there are also significant similarities between symptomology of FXS and disorders associated with dopamine (DA) signaling dysfunction such as Schizophrenia (SZ), Parkinson's disease (PD), attention deficit hyperactivity disorder (ADHD), and obsessive-compulsive disorder (OCD). Furthermore, current pharmacological interventions for FXS commonly include drugs that effect DA signaling in some way, indicating a need to methodically evaluate the role of DA signaling in FXS.

Previous research has found that spontaneous eye blink rate (SBR) is elevated in adolescent males with FXS, and task-related changes in SBR correlated with symptom severity measures. SBR is a well-established proxy measure of DA signaling in animal models, which is also thought to be applicable in humans. In order to provide further evidence of a significant role of DA signaling in FXS pathophysiology it will be important to replicate and extend research focusing on a correlation between SBR and symptom measures in FXS to a much larger sample including a wider age range and both genders.

Towards this goal, data from 68 FXS subjects were evaluated to quantify blink rate, and correlations between blink rate dynamics, clinical assessment measures, and EEG data were evaluated in order to evaluate the hypothesis that DA signaling plays a major role in FXS-related behavioral impairments. Consistent with previous research, blink rate is significantly higher in FXS relative to typically developing controls. Significant correlations were found between blink rate and several clinical measures, but the strongest correlations were found between blink rate measures and clinical assessments of sensory processes. Somewhat surprisingly, these correlations show that higher blink rate within the FXS sample is associated with reduced sensory impairment, which suggests a compensatory role of DA signaling associated with sensory processing. Together, these data support the hypothesis that DA signaling is associated with some FXS behavioral impairments and suggest that elevated DA signaling may represent a compensatory response to cortical hyperexcitability.

Chapter 1: Background:

Fragile X Syndrome

Fragile X Syndrome (FXS) is an X-linked developmental disorder characterized by intellectual disability, sensory hypersensitivity, repetitive behaviors, social anxiety, and impaired executive function. FXS is the most common single-gene disorder associated with intellectual disability and Autism Spectrum Disorder (ASD)(Hagerman et al., 2017). FXS results from abnormal suppression of Fragile X Mental Retardation 1 gene (FMR1) expression due to expansion of the CGG triplet repeat region of the 5' UTR of the FMR1 gene. CGG repeat expansion leads to enhanced methylation and subsequent epigenetic silencing of gene expression resulting in reduced levels of Fragile X Mental Retardation Protein (FMRP)(Ashley, Wilkinson, Reines, & Warren, 1993). In healthy individuals the number of CGG repeats falls between 5 and 40 repeats, while presence of 200 or more repeats results in FXS. As an X-linked disorder, FXS is much more prevalent in males, and FXS-related symptoms are usually more severe in males as well. Several psychiatric disorders are also co-morbidities of FXS including: attention deficit hyperactivity disorder (ADHD), anxiety, and obsessive-compulsive disorders (OCD)(Hagerman et al., 2017).

Neurodevelopmental disorders are inherently difficult to study in human populations as non-invasive means of evaluating CNS-related gene effects *in vivo* are relatively rare, expensive, or technically challenging. Even *in vitro* studies are made difficult due to the inaccessibility of the tissues or cell populations of interest. This drove the development of the FMR1 knockout mouse model of FXS, which recapitulate the core behavioral, molecular, and circuit-level pathologies seen in humans (Krueger, Osterweil, Chen, Tye, & Bear, 2011). This model have

proven to be a powerful tool in parsing the effects of FMRP downregulation on the development, maintenance, and baseline activity of neural circuitry associated with FXS-related behavioral impairments such as executive dysfunction and sensory processing impairments (Dickson et al., 2013; Rais, Binder, Razak, & Ethell, 2018).

The neuropathology underlying FXS-related behavioral impairments is complex and incompletely understood (Hagerman et al., 2017). FMRP is expressed ubiquitously throughout the brain across developmental time periods and adulthood. Under normal circumstance it binds a variety of synapse-related mRNA targets associated with synaptic plasticity, stability, and morphology. Considering the role of its mRNA targets and its localization near synaptic terminals FMRP seems to play an important regulatory role at the synapse (Ashley et al., 1993). While the impact of FMRP suppression in FXS has regulatory implications for hundreds of mRNA transcripts, a great deal of the pathophysiology of FXS can be explained by GABA-ergic dysfunction (Heulens, D'Hulst, Braat, Rooms, & Kooy, 2010) or elevated mGluR5 activity (Bear, 2005; Contractor, Klyachko, & Portera-Cailliau, 2015). Dysregulation of GABA- and Glutamate signaling has been studied extensively in the FXS literature, and their significance regarding FXS-related pathologies is not in question. For example, striking research has shown comprehensive phenotype correction in adult FXS mice following administration of a high-specificity mGluR5 inhibitor (Michalon et al., 2012). However, clinical trials evaluating mGluR5 antagonists in humans have failed demonstrating the need for further research in core aspects of FXS neuropathology (Berry-Kravis et al., 2016).

Considering the specific DA-associated behavioral impairments involving executive function, cognitive flexibility, sensory-motor gating associated with FXS, along with direct evidence that auditory evoked potentials are modulated by DAergic activity (Jacob & Nienborg,

2018), it is not unreasonable to hypothesize that central dopaminergic dysfunction also plays a role in the pathophysiology of FXS.

Test batteries have been developed or modified to measure these behavioral impairments, which have been shown to provide valid, reproducible scores across a wide functional range of the FXS population; a non-trivial task in intellectually disabled populations. One relevant example being the Test of Attentional Performance for Children (KiTAP) (Knox et al., 2012), which indexes attention/executive function in terms of response time, flexibility, reaction control (go/nogo), and distractibility. Research in both humans and animal models of FXS has demonstrated that abnormal activity of dopaminergic (DA-ergic) cell populations within the cortico-striatal circuit correlates with impaired executive function and behavioral flexibility (Dickson et al., 2013; Frankland et al., 2004; Groman et al., 2014; Grossberg & Kishnan, 2018; Paul, Venkitaramani, & Cox, 2013). The significance of DA signaling on behavioral impairments associated with FXS relative to other aspects of the disorder's pathophysiology remains unclear.

Dopaminergic Activity and Cognitive Processes

Dopamine has several, well-established roles regarding cognition, which are primarily driven by activity within the striatum. Phasic, stimulus-related DA release plays a role in coding prediction error (Hollerman & Schultz, 1998) while tonic DA levels act to enhance signal-to-noise ratio of neural activity by suppressing activity of neurons with low membrane potential and enhancing activity of neurons with high membrane potentials (Frank, 2005). Of note is the seemingly opposing functions of D1 and D2 receptors in the PFC. Activity within a D1-rich neuronal pathway within the basal ganglia codes a "go" signal to allow cognitive representations in the cortex to be updated, which is opposed by a D2-rich pathway that encodes a "no go" signal

to repress competing representations. Importantly, DA has an excitatory effect on D1-expressing neurons and an inhibitory effect on D2-expressing neurons (Maia and Frank, 2011). Together this means that DA release from striatal projections (in response to positive prediction error for example) will facilitate activity in the "go" pathway while suppressing activity of the "no go" pathway, which results in a "go" signal allowing cortical representations to be updated. Alternatively, reduced DA levels (such as dips in striatal DA release due to negative prediction error) leads to disinhibition of the D2-expressing "no go" pathway which acts to enhance the stability of cortical representations. Consistent with this model of DA's action as a modulator of cognitive flexibility/stability is supporting research demonstrating an inverted-u-shape association between DA levels and performance on cognitive tasks (Cools & D'Esposito, 2011).

DA's modulatory influence across the cortex has wide-spread, non-linear effects on cortical activity, which are largely driven by DA altering the activity of inhibitory interneurons responsible to organizing the oscillatory activity of the cortex. A variety of studies have been conducted in animal models to establish an association between DA signaling dynamics and aspects of cognitive performance. For the purpose of this research, behavioral flexibility is a relevant example. Dopamine transporter (DAT) knock out in the orbitofrontalcortex (OFC) and dorsomedial striatum (DMS) of a transgenic mouse model lead to DA depletion, which causes reversal learning deficits and overall reduction in activity within those brain regions. Treatment with targeted DA antagonists demonstrated that DAT, outside of other DA effects, modulates behavioral flexibility (Cybulska-Klosowicz, Dabrowska, Niedzielec, Zakrzewska, & Rozycka, 2017). D2, but not D1, agonism improves cognitive performance in non-human primates (Marino & Levy, 2019). D2Rs have been shown to play a critical role in reversal learning and are involved in behavioral inhibition mechanisms more broadly (Linden, James, McDaniel, &

Jentsch, 2018). Activity of D2-expressing neurons within the nucleus accumbens (NAc) of healthy mice is involved in suppression of previously-correct behavioral strategies allowing transfer of behavior to new strategies (Macpherson et al., 2016). In the same study, optogenetic activation of D1R-expressing medium spiny neurons (D1R-MSNs) of the anterior dorsomedial striatum (aDMS) impaired flexibility in reversal learning tasks while suppression enhanced reversal. Another study using positron emission tomography (PET) measures of D2R expression levels in non-human primates found a correlation between D2R levels and performance on task evaluating reversal learning and cognitive flexibility (Groman et al., 2014).

Research in FXS as well as its animal models has demonstrated that impaired reversal learning and behavioral flexibility is a characteristic phenotype associated with the disorder (Van der Molen et al., 2012; Ventura, Pascucci, Catania, Musumeci, & Puglisi-Allegra, 2004). Additionally, repetitive behaviors associated with ASD and FXS have also been shown to be impacted by D1- and D2R activity within the striatum (Grossberg & Kishnan, 2018). Together, these results suggest a specific role of DA signaling in an FXS-related behavioral impairment.

Despite DA's canonical role in cognitive processes, it is also involved in modulating cortical activity within and between functionally defined cortical regions associated with sociability and sensory processing (Deliano et al., 2018; Kim et al., 2014; Radwan, Dvorak, & Fenton, 2016) (Kehagia, Murray, & Robbins, 2010). Considering that sensory processing impairments constitute another core aspect of FXS pathology, DA dysfunction may play a significant role in this context as well.

Dopaminergic Modulation of Sensory Processing

In conjunction with other monoaminergic signaling pathways, DA has been demonstrated to play an important role in a variety of sensory processes (Jacob & Nienborg, 2018), but, for the purposes of the present study, auditory processing is particularly relevant. DA signaling has been found to influence neuronal activity in response to auditory stimulation with heterogenous effects in different brain areas associated with processing auditory signals. For example, auditory evoked responses in the inferior colliculus (IC) are modulated by endogenous and exogenous DA signaling. While the effects of DA release in the IC are heterogenous at the neuronal level, as a whole, the IC showed decreased neuronal firing probability and firing rate in response to an auditory stimulus (Hoyt, Perkel, & Portfors, 2019). Additionally, D1- and D2-like receptors in cochlear afferents of healthy mice exert a protective inhibitory influence in response to high intensity sound stimuli or hypoxia (Valdes-Baizabal, Soto, & Vega, 2015). Other research in non-human primates has found that electrical stimulation of the dopaminergic ventral midbrain decreases spontaneous firing of auditory evoked potentials in the auditory cortex as well as having bidirectional effects on the power of auditory evoked potentials (Huang, Mylius, Scheich, & Brosch, 2016). While these studies only represent a sub-set of relevant research focusing on the importance of DA signaling on sensory processes, they provide evidence that, in addition to directly effecting the circuit-level activity of the auditory cortex, DA plays an important role in modulating the brain's response to auditory stimuli more broadly. Considering this, it is not unreasonable to hypothesize that DA signaling dysfunction in FXS may be associated with measures of sensory hypersensitivity as well as EEG measures indexing E/I ratio and phase locking through a similar mechanism as that linking DA signaling, modulation of PFC oscillatory activity, and behavioral flexibility.

Spontaneous Blink Rate and Central Dopamine Activity

Relative to healthy comparison groups, differences in spontaneous eyeblink rates (SBR) exist in populations with Parkinson's Disease (PD) and Schizophrenia (SZ) (Chan et al., 2010; Karson, 1983; Levy-Gigi et al., 2019; Waltz, 2017), diseases associated with abnormal central dopaminergic activity. Furthermore, the direction of the change in DA-ergic function (hyper- vs. hypo-) positively correlates with changes in SBR (Jongkees & Colzato, 2016; Waltz, 2017). Importantly, changes in SBR in these populations correlate with measures of disease symptom severity for behavioral impairments that overlap with some of those seen in FXS (Chan et al., 2010; Jongkees & Colzato, 2016; Levy-Gigi et al., 2019; McCutcheon, Abi-Dargham, & Howes, 2019). Evaluation of blink rate in animals demonstrates similar strong correlations between SBR, central DA activity, and behavior (Desai, Neumeyer, Bergman, & Paronis, 2007; Elsworth et al., 1991; Groman et al., 2014; Jutkiewicz & Bergman, 2004; Kleven & Koek, 1996; Linden et al., 2018). These studies evaluated changes in SBR in response to dopamine agonists/antagonists in animal models and clearly demonstrated a strong positive correlation between DA receptor agonism and increased SBR while DA receptor antagonists reduced SBR in a dose-dependent manner (Desai et al., 2007; Jutkiewicz & Bergman, 2004; Kleven & Koek, 1996). Further research using agonists selective for specific DA receptor types found that broad activation of the DA-ergic cells by simultaneous activation of all DRs did not elevate SBR, but selective agonism did. Dopamine Receptor 1 (D1)-specific agonists elevate SBR in mice and non-human primates, and pre-treatment with D1-specific antagonists abolishes the SBR increase due to D1 agonist treatment (Groman et al., 2014; Jutkiewicz & Bergman, 2004). Similar results were found for D2-specific agonism/antagonism. One interesting finding is that D1- and D2-mediated SBR enhancement seems to be independent of one another. Pretreatment with D2-antagonists does not

block D1 agonist-mediated SBR enhancement and vice versa. Additionally, co-treatment with D1- and D2-agonists attenuated the elevation of SBR suggesting an inhibitory interaction between the two receptor systems (Desai et al., 2007). Importantly, in wildtype mice, D1 receptor activity within the prefrontal cortex (PFC) induces long-lasting enhancement of inhibitory post-synaptic currents (iPSCs) of GABAergic interneurons. D1-mediated iPSC enhancement is absent in FMR1-KO mice, but cAMP mediated iPSC enhancement is unaffected suggesting a specific D1-related deficit in inhibitory regulation of PFC neurons (Paul et al., 2013). Additionally, repetitive behaviors found in FXS and ASD are impacted by the activity of D1- and D2Rs associated with the direct- and indirect- pathways of the basal ganglia (Grossberg & Kishnan, 2018).

Taken together this research demonstrates the utility of SBR as a simple, non-invasive measure of central DA-ergic activity in animal models which, in conjunction with behavioral testing, can be used to produce testable hypotheses linking DA-ergic activity of particular brain regions and neuronal sub-populations to the specific aspects of complex behavioral traits known to be impaired in FXS animal models as well as humans.

Spontaneous Blink Rate and Central Dopamine Activity in Humans

In addition to a wealth of data linking cognitive behaviors to DA signaling and activity of specific DA receptors in animal models, similar lines of research have been carried out in humans as well. In healthy adults DA signaling has been shown to correlate with aspects of executive function including performance in tasks measuring cognitive flexibility, inhibition of previously learned responses, and accuracy in updating (Zhang et al., 2015). Striatal DA signaling dynamics have been shown to relate to working memory demands during task performance. (Rac-Lubashevsky, Slagter, & Kessler, 2017). A wealth of research focused on the

role DA signaling in cognition exists, but, for the purpose the current study, the utility of using measures of blink rate as an index of central DA signaling is particularly significant. For example, SBR can be used to predict measures of cognitive flexibility known to be modulated by DA signaling in healthy adults (Muller et al., 2007). Furthermore, differences in blink rate dynamics between tasks evaluating different dimensions of executive function have been used to identify the differential effects of central DA activity in regard to shifting, inhibition, and updating aspects of executive function (Zhang et al., 2015).

There is a great deal of interest in using blink rate as a non-invasive, low-cost measure of central dopaminergic activity as a clinical tool due to the relative ease that it could be implemented by the medical community. Additionally, if valid, it would provide an invaluable research tool in the context of very young or intellectually disabled subjects that would not tolerate other means of evaluating central DA such as PET. Similar approaches utilizing eye tracking/pupillometry have been demonstrated to be viable in research focusing on individuals with FXS (Farzin, Scaggs, Hervey, Berry-Kravis, & Hessl, 2011). SBR has been evaluated in a variety of disease states as well as event-related changes in blink rate (erBR) during administration of psychological tests targeting specific aspects of cognition, learning & memory, and sensory processing(Chan et al., 2010; Rac-Lubashevsky et al., 2017; Siegle, Ichikawa, & Steinhauer, 2008; Slagter, Georgopoulou, & Frank, 2015; Zhang et al., 2015) with significant correlations found within each.

It is important to note, despite the clear and consistent correlations between central DA activity and SBR in animal models, this relationship is much less clear in humans. While the preponderance of evidence is in favor of SBR's utility as a proxy measure of DA activity in humans (Jongkees & Colzato, 2016), equivocal or negative findings from a significant portion of

experimentally-rigorous studies(Dang et al., 2017; Sescousse et al., 2018; van der Post, de Waal, de Kam, Cohen, & van Gerven, 2004) demonstrate that care must be taken when using SBR to index central DA activity in healthy adults. Despite its variability across the healthy adult population, SBR has been shown to be a highly valid proxy measure of DA activity in certain healthy subpopulations. For example, blink rate variations due to a variety of stimulus paradigms in infants are highly consistent and correlate strongly with salience and other stimulus properties known to be coded by DA-ergic activity within the brain (Amodeo, Jacobs-Brichford, McMurray, & Roitman, 2017). Furthermore, the ventral striatum has been shown to be hyper-responsive in adolescents relative to other age groups. SBR and erBR in adolescents were strongly positively correlated with reward-maximization behaviors in a risky decision-making task, a correlation which was absent in adults (Barkley-Levenson & Galvan, 2017). Interestingly, correlations of SBR and DA activity are much stronger and consistent in populations with diseases affecting central DA function (Jongkees & Colzato, 2016).

Blink rate is impacted by a variety of external as well as internal factors that are not related to dopamine with anxiety, gender, and age having the greatest impact on SBR in healthy populations(Jongkees & Colzato, 2016). Furthermore, SBR appears to be highly variable among health adults, which complicates comparative studies trying to identify disease-specific blink rate abnormalities relative to healthy controls. The equivocal findings seen across studies correlating SBR to central DA activity could be explained by the significant variability among the control groups used for the studies. Indeed, research aimed at identifying a broadly applicable SBR baseline for the human population has identified a group of individuals that blink at abnormally high rates even though they are nominally healthy (Doughty & Naase, 2006) suggesting that classification of comparison groups into low- and high-SBR groups may be an important

consideration for comparative studies between SBR of healthy and diseased populations. While the utility of SBR for evaluation of central DA activity in healthy adults has not been conclusively established, there is compelling evidence suggesting that SBR is related to activity of the D2 receptor system (Groman et al., 2014). Considering the "no go" signal associated with D2 activity in the striatum, elevated SBR (indexing higher DA levels) would inhibit the D2R pathway resulting in increased cognitive flexibility; this prediction has been experimentally verified (Muller et al., 2007; Tharp & Pickering, 2011).

While correlational and theory-driven research suggests that SBR is a valid index of central DA, the neural circuitry through which DA influences SBR remains unclear. Currently, the best candidate seems to be the spinal trigeminal complex due to its role in the spontaneous blink generator circuit (Kaminer, Powers, Horn, Hui, & Evinger, 2011). Importantly, activity within basal ganglia can modulate excitability of- and inputs to the trigeminal complex via the inferior colliculus and nucleus raphe magnus. One mechanistic explanation has been proposed that DA inhibits the trigeminal complex through its effects on the nucleus raphe magnus, which has the net result of increasing SBR (Kaminer et al., 2011).

Spontaneous Blink Rate and Central Dopamine Activity in FXS

To date, relatively little research has been devoted to identification of a possible role of central DA-ergic dysfunction in the pathophysiology of FXS despite growing evidence of significant differences in SBR between adolescent males with FXS and healthy comparisons (Roberts, Symons, Johnson, Hatton, & Boccia, 2005). The changes in SBR between a passive and cognitive task in this sample correlated with measures of FXS-related behavioral impairments known to involve DA-ergic signaling (Grossberg & Kishnan, 2018; Hagerman et al., 2017). Taken together, this body of research suggests that central DA-ergic activity may play

a relatively unexplored role in aspects of FXS behavioral impairment, which may be evaluated in a human sample using non-invasive techniques of EEG and blink rate analysis in conjunction with behavioral testing.

These findings are relevant through the lens of the imbalance between excitatory and inhibitory signaling within the cortex that is associated with behavioral impairments seen in FXS. An association between SBR and D1R activity, in the context of impaired behavioral flexibility is interesting, but a correlation between SBR and measures of cortical hyperexcitability would provide a more compelling correlational link between a behavior, symptom measures, and underlying biology. Considering the compelling electrophysiological data suggesting cortical hyperexcitability, as indexed by elevated gamma power, correlates with measures of sensory hypersensitivity (Ethridge et al., 2019; Ethridge et al., 2016; Lovelace, Ethell, Binder, & Razak, 2018; Wang et al., 2017), a correlation between SBR and these types of EEG measures in relation to cognitive performance may be informative. Additionally, similar correlations with sensory processing measures would provide evidence of a relatively unexplored role of DA signaling in FXS.

Chapter 2: Overview of Experiment

Purpose:

There is relatively little research exploring the role for DA-ergic dysfunction in FXS-related pathology despite a growing body of evidence supporting this hypothesis. Existing research identifying correlations linking SBR and degree of behavioral impairment in FXS have focused on SBR differences among adolescent males with FXS and healthy controls. The primary goal of this research is to provide further support for the hypothesis that DA-ergic dysfunction is a core FXS-related pathology by extending findings of a correlation between symptom severity measures and SBR across a broader age range, including both genders in an FXS sample. The secondary goal is to identify further significant correlations between SBR, clinical measures, and electrophysiological recordings of brain activity as measured by EEG to generate data-driven hypotheses regarding the impact of DA-ergic activity on specific aspects of FXS-related neuropathology. These techniques are non-invasive, well-tolerated by low-functioning/intellectually disabled populations, and translate well between animal models and humans, perfectly situating this approach for evaluation of central DA-ergic activity in a variety of difficult-to-study neurodevelopmental disorders.

Objectives:

The first phase of this research is to replicate previous findings of a correlation between FXS symptom severity and elevated SBR in adolescent males with FXS relative to healthy controls and extend this analysis to a larger age range across genders. Similar correlations between symptom severity and elevated SBR across the FXS population would further support the hypothesis that abnormal DA-ergic activity is a core component of FXS-related pathology.

The second phase of this research is to evaluate the FXS cohort based on factors known to affect SBR such as gender, age, co-morbidity of DA-related disorders, and treatment with stimulant/anti-psychotic medications to establish appropriate stratification protocols. Assuming significant factor effects, stratification of the FXS sample based on these factors and comparison of SBR correlations between sub-groups would further refine hypotheses regarding central DA-ergic function in the context of different FXS subpopulations. Additionally, SBR variability in the healthy comparison group will be evaluated in order to produce an appropriate comparison group. A bimodal distribution of SBR across healthy controls would justify stratification of healthy individuals into High vs. Low SBR groups for these comparisons.

Lastly correlations will be made between EEG data, clinical measures, and blink rate measures with the goal of associating DA activity, measures of symptom severity, and direct measurements of CNS activity. This approach would provide evidence directly linking a behavioral measure (ostensibly indexing DA-ergic activity), specific behavioral impairments, and underlying biological differences resulting in altered brain activity. Significant differences among EEG data for FXS sub-groups would provide useful insight for future studies regarding appropriate stratification of FXS samples. This approach, which is feasible in very young or intellectually disabled populations, has the potential to provide a wide range of specific hypotheses regarding DA's role across domains of FXS-associated behavioral deficits. These hypotheses can then be rigorously tested in animal models, which may provide novel therapeutic targets for further translational studies.

Chapter 3: Method

Data Collection

The data used in this research consists of baseline measurements taken from an ongoing study of FXS-related sensory hypersensitivity (Ethridge et al., 2019) in which EEG recordings from FXS and typically developing control (TDC) samples were made during resting and passive auditory habituation and frequency matching tasks. Additionally, clinical assessments were made using several caregiver-report surveys, as well as cognitive testing protocols (see below for description of EEG protocols and clinical assessments).

Participants:

Baseline data from 125 subjects were included in this study, consisting of 68 individuals with full mutation FXS [Mean age = 21.7, standard deviation (SD) = 10.7; age range 6-53; 32 females] and 57 age- and sex-matched typically developing controls (Mean age =26.5, SD = 14.9; age range 6-63; 28 female). TDCs had no prior diagnosis or treatment for neuropsychiatric illness as reported in clinical history interviews. Exclusion criteria for the FXS sample included history of seizures and treatment with anticonvulsant medications or benzodiazepines due to their known EEG effects. Samples vary slightly across tasks due to non-compliance and data quality issues. Refer to **Table 1** for sample characteristics.

Procedure:

Clinical Evaluation: The following clinical assessment measures were utilized to evaluate symptom severity in the FXS sample: The Child Sensory Profile (CSP; (Brown, Tollefson, Dunn, Cromwell, & Filion, 2001)), the Social and Communication Questionnaire (SCQ; Rutter et al., 2003), Anxiety Depression and Mood Scale (ADAMS, Esbensen et al. 2003), and the

Aberrant Behavior Checklist-Community (ABC-C;(Sansone et al., 2012)). Additionally, the Woodcock-Johnson III Tests of Cognitive Abilities Auditory Attention subscale (McGrew and Woodcock, 2001), the Vineland Adaptive Behavior Scales (Sparrow et al., 2005), and the computerized Test of Attentional Performance for Children (kiTAP; (Knox et al., 2012)) were administered. IQ was measured in both FXS and TDC using the Stanford-Binet Intelligence Scale 5th Ed. Abbreviated IQ (Roid, 2003), and the TDC sample completed the SCQ, ADAMS, ABC-C, and KiTAP.

EEG Recording: Continuous EEG data was recorded at 1000Hz, filtered from 0.01 – 100Hz, referenced to Cz, and amplified 10,000X via a saline-based, 128-channel Electrical Geodesics system (EGI, Eugene, Oregon). Sensor placement of 128-channel EGI Hydrocel nets approximates the International 10/10 system (Russell, Jeffrey Eriksen, Poolman, Luu, & Tucker, 2005). Participants were seated throughout the recording and watched a silent movie during testing to improve behavioral compliance. Stimuli were delivered at 65db through headphones in each auditory task.

Resting EEG: Three minutes of EEG data were collected for each participant while watching a movie with no additional stimulation.

Auditory Habituation Task: The stimulus associated with this task consisted of 150 stimulus trains composed of four 50ms bursts of white noise with a 500ms inter-stimulus interval. Stimulus trains were separated by 4000ms inter-trial intervals. Auditory habituation, in this context, refers to a reduced amplitude of subsequent stimulus-related N1 ERP components relative to the N1 amplitude for the initial stimulus of the train.

Chirp Task: The auditory chirp stimulus is characterized by a white noise carrier wave, which is amplitude modulated by a linearly increasing frequency from 0 to 100Hz over 2000ms. 200 chirp stimuli were presented over the course of the task, separated by randomly-jittered, 1500-2000ms inter-trial intervals.

EEG Data Processing: Raw data were evaluated offline, bad channels were identified and interpolated (5% of sensors or less per subject, 2 or fewer contiguous sensors) via spherical spline interpolation in BESA 6.1 (MEGIS Software, Grafelfing, Germany). Data were high- and low-pass filtered from 0.5 to 120Hz with 12 and 24 db/octave roll-offs respectively (zero phase; 60 Hz notch filter). Segments of data with significant movement artifacts were removed to improve performance of independent component analysis (ICA; Infomax) implemented in EEGlab 14.1.1 (Delorme & Makeig, 2004) through Matlab (The Mathworks, Natick, MA). Following ICA, components associated with eye-, cardiac-, and muscle-related artifacts were removed by researchers blind to participant group. For both auditory tasks, data was epoched into 3250ms trials (-500ms pre-stimulus, 2750ms post-stimulus). Data were averaged across trials and base-line corrected using the 500ms pre-stimulus period for ERP analyses. ERPs for the habituation task were low-pass filtered at 40Hz; chirp ERPs and single-trial power data were low-pass filtered at 120Hz. Number of retained trials for both chirp and hab was greater in the TDC group relative to FXS, so valid trial count was included as a covariate for all analyses. Resting EEG data has yet to be evaluated and will not be included in the EEG correlation analyses. 23 sensors across the fronto-central scalp were chosen to average across *a priori* for all subsequent analyses based on a spatial distribution of sensors consistent with previous literature aimed at measuring activity in the auditory cortex **(Fig. 6)**. Single-trial power (STP) and inter-trial coherence (ITC) measures for un-baseline-corrected, epoched, single-trial data were

obtained using Morlet wavelets with 1Hz frequency step utilizing a cycle length of 1 cycle at the lowest frequency that linearly increased to 30 cycles at the highest frequency. STP and ITC measure frequency-specific response amplitude and phase-locking of neural activity to the auditory stimuli respectively.

Characterizing Blinks: The first three minutes of Raw EEG data from resting, habituation, and chirp tasks were used for identification of blinks using the Brain Electrical Source Analysis 6.1 (BESA) software package. EEG data was filtered to 0.5 (12dB/octave slope; zero phase) to 50Hz (24 dB/octave slope; zero-phase) with a 60Hz notch filter. Virtual electrooculograms for horizontal and vertical eye movement components were plotted alongside channel data. Blinks were identified by comparison of EEG and vEOG waveforms, alongside topographical analysis of electrical activity on the scalp **(Figure 5.)** and blinks were marked at their peak amplitude for downstream analyses of stimulus-related effects on blink rate.

Blink Counts: Total blink counts for each subject were independently coded by two trained researchers, and Inter-rater reliability was calculated as the total percentage of subjects with at least 90% agreement between raters (IRR = 92%). No subjects had <80% agreement among blink counts, which was the threshold for omission from further analysis.

Blink Measures: Matlab R2017a (The Mathworks, Natick, MA) was used to calculate spontaneous blink rate (SBR), event-related blink rate (erBR), blink latency following stimulus onset, and blink variability (StDev). Event-related blinks refer to any blinks that occur within 500ms epochs following stimulus offset. Event-related eyeblink rate (erBR) was calculated by dividing the total number of event-related blinks by the summation of all stimulus-related epochs. Stimulus-related blink latencies for each subject were calculated by averaging the latencies between stimulus onset and the first event-related blink across trials. Spontaneous blink

rate (SBR) was calculated as the difference between total blink count and event-related blink counts divided by the difference of total time and summed stimulus-related epoch duration. Lastly, data was segmented into 3 second bins, and the standard deviation was calculated based on the number of blinks in each bin to provide a measure of blink variability for each subject.

EEG Results

Refer to (Ethridge et al., 2019) for a full account of the results of the EEG analysis described above. The EEG measures utilized in the research discussed here will be briefly outlined below.

Habituation Task EEG Results

N1: The initial N1 component of the ERP associated with the habituation task had a significantly higher amplitude in FXS compared to control; however, habituation of N1 amplitude for subsequent repetitions within stimulus trains was not different between FXS and TDC samples (percent change in amplitude between initial and subsequent N1s was not different between groups). There were also no significant differences between groups regarding N1 latency following stimulus onset.

P2: Similar to N1, initial P2 ERP components had significantly higher amplitude in the FXS sample, which was significantly reduced across stimulus repetitions indicating habituation had occurred. The percent change in P2 amplitude was not different between FXS and TDC. However, P2 latency was significantly shorter in FXS relative to controls.

Single Trial Power: Analysis of time frequency plots of STP identified 3 time-frequency clusters that differ significantly between FXS and TDC samples. Theta band (3-7Hz) power was significantly higher in the FXS sample (no significant effects of trial number or sex). Alpha (8-12Hz) power was not significantly different between groups. However, there was a significant

group*sex interaction with females with FXS showing higher alpha power than TDC females. A marginal effect of group on gamma (31-70Hz) power was found, suggesting higher gamma power in FXS.

Chirp Task EEG Results

Single Trial Power: Analysis of time frequency plots of ITC and STP identified 4 time-frequency clusters significantly different between FXS and TDC. FXS showed significantly stronger alpha band phase-locking (ITC) to stimulus onset relative to controls. A significant main effect of group was found for ITC to the chirp stimulus while it was in the low gamma oscillatory range (31-57Hz) suggesting that the TDC sample was better able to modulate neural oscillations in the low-gamma band to match those of the stimulus. Furthermore, a group*sex interaction indicated FXS females were more like male and female controls than FXS males. Gender and group effects were found for theta (3-7Hz) power, which indicated higher theta power in FXS. Females with FXS showed higher theta power than TDC females, but theta power for males did not differ between groups. A main effect of group on gamma power (31-70Hz) was found suggesting higher gamma power in FXS than TDCs.

Gamma power and Phase-locking: Elevated single trial power in the gamma band significantly correlated with decreased phase-locking to the chirp stimulus in the gamma frequency range in the TDC sample. A similar effect was found in FXS, but the correlation was not significant

Analysis

Blink Rate Analysis: All statistical tests were carried out using SPSS version 25. Differences between SBR, erBR, blink latency, and blink variability for FXS and control samples were evaluated by ANOVAs with factors of group and sex, including age as a covariate. Task effects

were evaluated using mixed-effects ANOVAs with within-subjects factor of task and between-subjects factors of group, and sex, with age as a covariate. Similarly, stimulus effects were evaluated for each task using mixed-effects ANOVAs with within-subject factor of stimulus (spontaneous vs. event-related blink rate) and between-subjects factors of group and sex, including age as a covariate.

Clinical Correlations: Blink measures found to be significantly different between FXS and control groups were then correlated with clinical and EEG measures. Spearman's correlations were calculated, and significant correlations between blink measures and clinical or EEG measures were identified within the FXS group. The Benjamini-Hochberg procedure was used to correct for false discover rate due to the large number of comparisons. Additionally, correlations will be separated by gender due to the interaction between gender and cognitive ability in FXS Significant correlations can be found in Appendix B **(Tables 4-8)**.

Chapter 4: Results

Group Differences in Blink Rate Measures

Blink Rate Distributions: Evaluation of resting SBR distribution between FXS and TDC groups did not demonstrate any significant differences. SBR did not appear to be bimodally distributed in the TDC group so the entire sample was included in further analyses **(Figure 1 & 1a)**.

Rest: Mean spontaneous blink rate was 0.321 blinks/sec (.21) in the FXS sample, which was significantly higher than mean SBR of 0.234 blinks/sec (.15) in controls ($F_{1,115}$ = 6.332, p = 0.013). No significant effects were found for sex ($F_{1,115}$ = .062, p =.804) or age ($F_{1,115}$ = 0.227, p = .635). Blink variability (StDev) did not vary significantly between groups ($F_{1,115}$ = 2.646, p = .107), sexes ($F_{1,115}$ = 0.000, p = .997), or ages ($F_{1,115}$ = 0.111, p = .739).

Chirp: A significant effect of group on blink variability (StDev) was found for the auditory chirp task ($F_{1,104}$ = 9.163, p = 0.003) with significantly higher blink variability in the FXS group (Mean StDev = 0.860; (.29)) relative to TDC (Mean StDev = 0.710; (.19)). Additionally, a significant effect of group on SBR was found ($F_{1,104}$ = 3.842, p = 0.042) with significantly higher SBR in the FXS group (0.33 blinks/sec) (.19) relative to controls (0.25 blinks/sec) (.18). A marginally significant effect of group on erBR ($F_{1,104}$ = 3.842, p = 0.053) was found in the same direction (higher erBR in FXS). No significant effects for sex or the group*sex interaction were found for either erBR or SBR.

Hab: A significant effect of group on blink variability (StDev) was observed ($F_{1,104}$ = 10.032, p = 0.002) with significantly higher blink variability in the FXS group (Mean StDev = 0.883; (.28)) relative to TDC (Mean StDev = 0.730; (.19)). A significant effect of group on erBR was found ($F_{1,104}$ = 6.581, p = 0.012) with the FXS sample exhibiting significantly greater event-

related blink rate (0.330 blinks/sec; (.19)) relative to controls (0.240 blinks/sec; (.17)). A marginally significant effect of group on SBR was found ($F_{1,104}$ = 3.664, p = .058) in the same direction (higher SBR in FXS). No significant effects of sex or sex*group were observed.

Stimulus Effects on Blink Rate

Chirp: Significant effects were found for stimulus ($F_{1,104}$ = 3.930, p = 0.050) and the stimulus*sex*group interaction ($F_{1,104}$ = 5.487, p = 0.021). Aside from a significant between-subject effect of group ($F_{1,104}$ = 4.161, p = 0.044), no significant between-subject effects were found. Within subjects, erBR was lower relative to SBR (0.26 and 0.29 blinks/sec respectively). The difference between SBR and erBR is more pronounced in females relative to males (0.042 blinks/sec increase in females relative to 0.024 blinks/sec increase in males). Viewed as a percent change between erBR and SBR, females show a 15.9% increase in BR while males show a 9.5% increase. Considering the significant stimulus*sex*group interaction, FXS and TDC females both showed elevated SBR relative to erBR (FXS change = 19.5%, TDC change = 11.1%), as did TDC males (16.7%). However, FXS males had highly similar erBR and SBR (4.1% change). Refer to appendix C for blink rate comparisons across tasks.

Hab: No significant within-subject effects were found among data in the hab task. A marginally significant within-subject stimulus*sex interaction ($F_{1,104}$ = 3.592, p = 0.061) was found, but no significant stimulus*group*sex interaction ($F_{1,104}$ = 0.088, p = 0.768). Despite the stimulus*sex interaction only being marginally significant (p = 0.061) , similar to results seen in the chirp task, erBR and SBR were similar in males (0.285 and 0.288 blinks/sec), but erBR was lower than SBR in females (0.281 and 0.308 blinks/sec**).** A significant between-subject effect of group ($F_{1,104}$ = 5.105, p = 0.026) was found, but no significant effects of sex ($F_{1,104}$ = 0.050, p = 0.823) or group*sex interaction ($F_{1,104}$ = .527, p = 0.470).

Medication Effects on Blink Rate

No significant effect of DA medication on BR in FXS was found ($F_{1,52}$ = 0.018, p = 0.894). However, there was a marginally significant effect of the sex*medication interaction on BR ($F_{1,52}$ = 4.004, p = 0.051). Unmedicated females with FXS had higher BRs relative to medicated females (0.342 and 0.242 blinks/sec respectively). The opposite was true for the male FXS sample with unmedicated males having lower BRs relative to medicated males (0.264 and 0.378 blinks/sec respectively). There were an insufficient number of TDC subjects taking DA medications to allow for comparison of medication effects across groups.

Exploratory Clinical Correlations

Significant correlations surviving correction for multiple comparisons are listed below. Refer to appendix B for a full list of significant spearman's correlations broken down by gender and task (p* < 0.05, p** < 0.01).

IQ Correlations: FXS male SBR during the chirp task correlates with deviation IQ (-.508**) and verbal Z-score (-.548**). Blink variability in FXS males during chirp also correlates with IQ (-.493**) and verbal z-score (-.591**). No significant correlations for IQ measures were found for BR measures in the overall FXS sample, FXS females, or TDCs **(Tables 4, 4a, 4b)**.

Sensory Profile Correlations: SBR during the resting task for the overall FXS sample correlates with CSP registration (-.652**). FXS male resting SBR also correlates with CSP registration (-.740**), but there is not a significant correlation between CSP scales and BR measures from FXS females or TDCs **(Tables 4, 4a, 4b).**

Vineland Correlations: FXS male resting SBR correlates with vineland composite scores (-.515**). FXS male blink variability during the rest task also correlates with Vineland composite-

(-.505**), communication- (-.505**), and daily living - (-.473**) scores. FXS male erBR during the habituation task correlates with Vineland coping- (.502**), communication- (-.531**), and composite- (-.501**) scores. Blink variability of FXS males during the habituation task also correlates with Vineland composite scores (-.504**). No significant correlations for BR measures and Vineland scores were found in FXS female and TDC samples **(Tables 5, 5a, 5b)**.

Cognitive Flexibility Correlations: Resting SBR in FXS males correlates with number of errors during a distractor task on the kiTAP (.501**). Errors during a go-no-go task correlate with SBR (.583**) and erBR (.596**) during habituation in FXS males. Similarly, number of errors during the distractor task of the kiTAP correlates with erBR (.543**) in FXS males during habituation. No significant correlations were found for kiTAP performance and BR measures for FXS female and TDC samples **(Tables 7, 7a, 7b)**.

EEG Correlations: Relative to the FXS sample, many significant correlations for EEG and blink measures can be found in the TDC sample during rest and habituation tasks **(Table 8c)**. Blink variability in FXS males during habituation correlates with EEG measures of Alpha (.618**) and Theta (.707**) power measured during the same task. FXS female SBR during the rest task correlates with ERP onset ITC during the habituation task (0.845**) **(Tables 8, 8a, 8b, 8c)**.

Chapter 5: Discussion

This study succeeded in its primary goal in that it replicated previous findings of significantly elevated spontaneous blink rate in an FXS sample relative to typically developing controls. Importantly, this study demonstrated that this difference was true for a relatively large FXS sample including both genders, which suggests elevated SBR is a behavioral trait that manifests across the FXS population. It is worth mentioning that the primary factors known to impact blink rate in healthy adults (age and gender) did not significantly impact blink rate measures in the overall FXS sample according to the analyses conducted in this study. Gender effects only became apparent upon deeper investigation of blink rate dynamics between tasks and stimulus conditions. Generally, FXS females appeared more similar to TDCs, which mirrors results from behavioral and biological research into gender differences in FXS symptom presentation.

The results of our exploratory correlations between blink rate, clinical measures, and EEG data are somewhat surprising. The most well-established correlations between specific behaviors, DA signaling, and SBR are focused on cognitive measures. In the context of FXS, which exhibits reduced D1R expression in the PFC, elevated SBR, and impaired reversal learning, a theoretically sound hypothesis could be proposed linking differences in SBR to impairments in performance on tests of cognitive flexibility. Data exists demonstrating such a correlation in animal studies, but no significant correlation between blink rate measures and cognitive flexibility scores survived correction for multiple comparisons in the overall FXS sample. Despite a lack of correlation across the entire FXS sample, blink rate measures in FXS males significantly correlate with number of errors in distractor and go-no-go tasks on the kiTAP. These correlations were not seen in the FXS female sample suggesting that DA-mediated

cognitive flexibility is impaired in males, but not females, with FXS. It should be noted that BR measures taken during the chirp task did not significantly correlation with kiTAP performance, which indicates that, in the context of auditory processing, the habituation task may be better situated to evoke DA-signaling that is reflected in BR changes. Another consideration is the fact that the association between BR measures and clinical measures of cognitive function in FXS reported by (Roberts et al., 2005) were apparent only when comparing BR during a cognitive task and at rest. Similarly, D1R-mediated BR effects in mice were only apparent during cognitively demanding tasks (Dickson et al., 2013). Since all EEG tasks in this study were passive, that could account for the relative lack of correlations between blink rate and clinical measures of cognitive function.

The most consistent, significant correlations identified in this study for the entire FXS sample were between blink rate and clinical measures of sensory processing. Specifically, higher resting SBR leads to reduced sensory registration, avoidance, and sensitivity. While research has demonstrated that central DA activity can modulate auditory evoked responses in the auditory cortex (Huang et al., 2016), there is less known about the receptor-level interactions relative to the well-established D1R vs. D2R dynamics within the PFC and striatum associated with cognitive flexibility. Of particular interest is the fact that higher blink rate in the FXS sample correlate with reduced sensitivity in sensory processing scores. This contrasts our initial hypothesis that elevated SBR reflects exaggerated DA signaling, which would correlate with greater impairment in behavioral measures. Indeed, the correlations between SBR and sensory measures suggest a compensatory role of elevated DA signaling in the context of sensory processing. Similar BR dynamics between sexes in the FXS sample suggest that this correlation is not simply due to the fact that female BR is greater on average relative to males.

A growing body of evidence exists supporting the hypothesis that the cortical hyperexcitability associated with FXS and ASD underlies sensory hypersensitivities characteristic of these disorders (Cea-Del Rio & Huntsman, 2014; Lee, Lee, & Kim, 2017; Nelson & Valakh, 2015). At the local circuit level, gamma band (31-70Hz) oscillatory activity is primarily driven by activity of inhibitory, GABA-ergic interneurons on pyramidal neurons of the cortex (Gibson, Bartley, Hays, & Huber, 2008). Given the well-established deficits in GABA-ergic signaling and exaggerated glutamatergic signaling in FXS and ASD, gamma band spectral power as measured by EEG provides a non-invasive means of characterizing cortical hyperexcitability (Ethridge et al., 2016). Converging evidence from electrophysiological studies of auditory hypersensitivity in both humans and animal models of FXS along with cellular and molecular data suggest that gamma power is significantly elevated in FXS relative to healthy controls and that gamma power correlates with worse scores on measures of sensory processing and sensitivity (Ethridge et al., 2019; Goswami, Cavalier, Sridhar, Huber, & Gibson, 2019; Lovelace et al., 2018). With this in mind along with the modulatory effect DA signaling has on auditory evoked responses, a possible explanation of the seemingly compensatory effect of exaggerated DA signaling could be that DA acts to modulate the circuit level activity of the auditory cortex to reduce resting gamma power with the result of improving signal to noise ratio for auditory signals, which could account for reduced sensory processing impairments. This, however, does not seem to be the case as no significant correlation was found between blink rate measures and EEG measures of gamma power within the FXS sample.

Another possible explanation of the beneficial effects of elevated DA levels focuses on research in mice showing that treatment with DA receptor agonists/antagonists modulates the phase-locking of auditory evoked potentials in the auditory cortex to stimulus characteristics

(Huang et al., 2016). Inter-trial coherence measures index phase-locking of neural activity to the stimulus and is thought to represent the ability of the cortex to reorganize its ongoing activity in response to a stimulus. ITC measures have been shown to be reduced in FXS and ASD samples relative to healthy controls, and this reduction correlates with sensory processing impairments (Ethridge et al., 2016; Lovelace et al., 2018; Wang et al., 2017). Elevated DA signaling could improve phase-locking of auditory responses to the stimulus (as indexed by greater ITC) resulting in reduced sensory processing impairments. Again, the correlational data from this study does not support this hypothesis as no significant correlations were found between blink rate and EEG measures of ITC for either auditory task.

Analyses of stimulus effects (blink rate during trials relative to between trials) demonstrated a clear lack of stimulus effect in the habituation task ($p = 0.179$) as compared to the significant effect found for the chirp task ($p = 0.050$), which suggests that the chirp stimulus may be better able to elicit DA effects reflected in BR differences relative to the habituation task. However, this interpretation is complicated by the fact that the majority of significant correlations for clinical and EEG measures are associated with BR measures in the habituation and resting tasks. In order to interpret these results, it is important to understand the different neural processes driven by the two stimuli, which were engineered to elicit distinct reactions from the auditory cortex: habituation (reduced ERP amplitude indicating inhibition of the auditory response) and frequency following (reorganizing the phase of ongoing neural activity to synchronize with stimulus frequency). The chirp stimulus was designed to drive gamma-related neural oscillations while the habituation stimulus was not. The lack of correlation between BR during chirp, clinical measures, and gamma power in any task suggests that DA signaling

abnormalities in FXS are not related to re-organizing ongoing neural oscillations in the gamma band.

The lack of significant correlations between blink rate and EEG measures for either task in the overall FXS sample is contrasted by the strong correlation between BR and EEG measures for the habituation task in the TDC sample. Significant correlations between BR measures and ERP ITC, N1 latency, and percent habituation were apparent for the TDC sample. Interestingly, there were no such correlations between BR and EEG measures taken during the chirp task. These results demonstrate that the habituation task is more suited to engage aspects of DA signaling that impact BR, which correlate with relevant EEG measures associated with auditory processes.

While this correlation between the BR difference and task-specific EEG measures support the hypothesis that DA signaling (as indexed by BR) is actively modulating the responses within the auditory cortex in TDCs, the lack of a similar correlation in the FXS sample does not stand as evidence against that hypothesis. Considering the differential dynamics of DA-mediated modulation of circuit-level activity associated with different neural processes, it could be the case that significant DA-mediated effects are occurring in parts of the auditory pathway outside of the auditory cortex itself. If there is some stimulus effect on DA signaling that acts upon upstream aspects of auditory processing, a correlation between blink rate differences and differences in neural activity within the auditory cortex may not be immediately apparent.

An interesting result that came out of these analyses was the fact that males showed very little difference between SBR and erBR (0.285 and 0.288 blinks/sec) in the habituation task. BR dynamics were similar for FXS females and TDCs (decreased erBR relative to SBR), but FXS male SBR and erBR scores were virtually identical (0.293 and 0.305 blinks/sec).This difference

between FXS males and other groups was not sufficient to produce a significant group*sex*stimulus interaction in the habituation task ($p = 0.768$). However, there was a significant effect for the group*sex*stimulus interaction in the chirp task ($p = 0.021$) with FXS males showing no difference between erBR and SBR while FXS females and TDC males and females had reduced erBR relative to SBR. This dynamic of greater similarity between FXS females and TDCs of both genders mirrors results from EEG data in the chirp task indicating that FXS females' ITC between neural activity and the chirp stimulus in the low gamma band (31-57Hz) was more like TDCs while FXS males were significantly different. While this similarity is evocative, attempts to associate DA signaling to sensory processing measures via BR dynamics is frustrated by the lack of correlation between BR and EEG measures in the FXS sample. Despite this, these results stand as a potential justification for the use of BR measures as a hypothesis generating tool in the context of FXS.

In addition to supporting the hypothesis that significant differences exist between DA signaling between FXS and healthy populations, utilizing this approach identified a possible compensatory relationship between elevated DA signaling and sensory processing impairments in FXS, which, to the author's knowledge, represents a novel finding related to FXS neuropathology. Considering the inhibitory influence DA play at the circuit level in both cognitive and sensory processes, along with the correlation between hyperexcitability of auditory circuitry and sensory hypersensitivity in FXS, DA may be acting to dampen circuit activity resulting in reduced sensory processing impairment. Further research evaluating the role of DA modulation of phase-locking, habituation, and ITC in relation to sensory hypersensitivity in FXS samples would be informative.

This study was unable to achieve its tertiary goal of detecting correlations linking blink rate, clinical measures, and EEG data representing underlying biological activity within the FXS sample, which would provide a compelling hypothetical link between DA activity and sensory hypersensitivity with specific predictions about DA's impact on network level activity. However, a biomarker's utility is, to an extent, independent of a mechanist understanding of how it relates to disease pathology. If nothing else, this study demonstrates the utility of BR analyses as a tool for hypothesis generation and opens new avenues of research aimed at reproducing and validating BR as a biomarker of sensory hypersensitivity in FXS. Furthermore, the differential effects of the chirp and habituation stimuli on blink rates of FXS males relative to other subjects is exactly the kind of data necessary to identify appropriate stratification protocols for FXS samples, as well as informing decisions about task and stimulus characteristics for future research involving BR and auditory processing measures. Lastly, comparison of the remarkably strong correlations for BR and EEG measures during the habituation task for TDC and the lack of significant correlations for the same measures in the FXS group is intriguing. Assuming BR indexes central DA, these results suggest that DA signaling is involved in key aspects of auditory habituation in TDCs. The fact that these correlations are largely absent in the FXS population may suggest DA-mediated modulation of the auditory cortex is impaired or dysregulated in FXS. Why this would correlate with reduced sensory hypersensitivity is not immediately clear.

Another important point is that, while the most consistently significant correlations indicated a negative relationship between blink rate and measures of sensory impairment in the overall FXS sample, significant correlations were found between multiple measures of adaptive behaviors including social, language, coping, personal skills in FXS males. While elevated BR in FXS males correlated with worse scores on scales measuring communication, a significant

positive correlation between coping skills and BR was found, suggesting that elevated DA signaling in FXS has complex, bi-directional effects in multiple domains including social behaviors as well as cognitive and sensory processes. Given the relative weakness of the correlation of BR and social behaviors, the known importance of task on DA signaling (as measured by BR), and the fact that the tasks utilized in this study focused on sensory processes, making any specific claims about an association between blink rate measures and DA-mediated social impairments would be premature. A further investigation of blink rate dynamics utilizing tasks focusing on social cognition, attention, and motivation in conjunction with EEG measures would be insightful, especially considering the significant role that DA signaling plays in modulating neural circuitry associated with these processes.

An important note worth mentioning here, is that EEG data represents the cumulative activity of neuronal activity across levels of cortical network organization. Within a dataset a virtually infinite number of analytical techniques can be applied to identify signals associated with different levels of network organization embedded within the overall EEG signal. The lack of correlation between blink rate and the EEG measures used in this study may simply reflect a lack of resolution or specificity for the analytic approach used here to measure DA-related influences on network activity. Another approach to analysis of EEG data focuses on metastability of network activity in which changes in the complexity of the EEG signal reflect dynamic changes in the degree of organization of network activity. Signals with greater complexity (higher entropy) reflect less-organized, stochastic activity inherent in neural systems, while reduced signal complexity (lower entropy) reflects synchronization and organization of network activity. Detectable difference in entropy measures obtained using EEG have been found within subjects that are associated with a variety of factors including arousal, engagement,

task performance, as well as correlations between entropy measures and behavioral measures of cognitive flexibility (Frohlich, Irimia, & Jeste, 2015). Importantly, detectable differences in resting state set-shifting between states of high- and low- entropy can differentiate healthy from atypical populations such as ASD or PD (Cruz, Mallet, Magill, Brown, & Averbeck, 2009), as well as being able to predict an ASD diagnosis later in life with a high degree of accuracy in a sample of infants at high risk of developing ASD (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011). The fact that entropy measures are sensitive to task effects, differ significantly between healthy and atypical samples, correlate with behavioral and psychological measurements, and can be used to reliably predict future diagnoses make this analytic approach particularly relevant in the context of research focusing on identifying a correlation between blink rate and EEG measures (Jeste, Frohlich, & Loo, 2015). Considering the important role DA plays in modulating excitatory and inhibitory signals thought to underlie changes in network activity, it is not unreasonable to expect that DA-related changes in EEG signals may be more apparent using measures of entropy and metastability. Furthermore, novel analytic approaches such as this can be applied to existing data, which is particularly relevant for research focusing on neurodevelopmental disorders or other atypical populations where recruitment of subjects can be a significant hurdle. Given the relative dearth of studies of FXS with large sample sizes, applying novel analytic techniques to existing EEG datasets from such studies is a well-reasoned approach that can identify novel differences in neural activity across levels of biological organization of the brain in a cost-effective, time-efficient manner, which, in conjunction with blink rate analyses, has a great deal of potential for disentangling the complex, non-linear relationship between DA signaling, cortical network dynamics, and behavior.

Summary

This study provides evidence that spontaneous blink rate is elevated in the FXS population relative to typically developing controls. This was true for a relatively large sample including both genders across a wide age range, suggesting that increased SBR is a behavioral difference that can be generalized to FXS as a whole. This is significant given the correlation between SBR differences and symptom severity in a number of disorders known to involve DA signaling impairments, the association between DA signaling and behavioral impairments in FXS and the prevalence of DAergic drug prescription to the FXS population. Further evaluating the hypothesis that elevated SBR in FXS represents a core deficit in DA signaling, correlations between SBR and clinical measure were calculated, and a negative relationship between SBR and symptom severity was observed for several measures of sensory processing, social impairments, and affect. This surprising result of a seemingly compensatory role of DA signaling in FXS is suggestive and demonstrates the utility of SBR analysis as a hypothesis generating tool.

The finding of differential task and stimulus effects on BR based on gender and group is interesting, and, even without a complete mechanistic explanation for these findings, these results are valuable for future studies involving BR comparison among FXS and TDC groups in terms of stratification protocols and task/stimulus selection to maximize the likelihood of detecting a difference between groups.

While no significant correlations between available EEG measures and FXS BR were identified, novel analytic approaches to EEG data analysis have the potential to parse DA signaling with a higher degree of resolution. Given the important modulatory influence DA exerts on circuit level activity in a variety of contexts across the cortex. The expectation of a

correlation between BR and EEG measure of cortical activity is not unreasonable. Indeed, EEG studies using measures of signal variability, metastability, and entropy have successfully measured changes in organization of cortical activity due to the action of DA (Cruz et al., 2009; Darbin et al., 2016; Shafiei et al., 2019), and, as such, a promising future line of work regarding the research discuss here would employ similar techniques evaluating metastability of EEG signals.

The results of this study support the hypothesis that differences exist between DA signaling in FXS and healthy populations, and that this increase in DA signaling represents a compensatory response to sensory processing impairments in FXS. Valuable information regarding task, sex, and group differences in BR dynamics will inform future research focusing on these topics. Taken together, these results demonstrate the utility of the SBR analysis as a hypothesis generating tool and suggest that BR may be a useful monitoring biomarker for studies focusing on improving sensory hypersensitivity in FXS.

Limitations and Concerns

A number of important considerations should be discussed regarding the interpretation of the data presented here. First, the Child Sensory Profile was the source of clinical measures of sensory processing impairment, but, of the total FXS sample of 68, CSP data only exists for 28 subjects. Evaluation of sample characteristics of those 28 subjects reveals no significant difference in age range relative to the entire FXS sample, however, IQ and non-verbal Z-scores were significantly different. Additionally, only 8 of the 28 individuals were female. With this in mind, care should be taken when proposing sample-wide, sensory processing effects based on a relatively restricted sub-set of the total sample.

Another significant concern regards the accuracy of coding BR manually. There is very little difficulty in identifying blinks in the TDC sample due to higher levels of behavioral compliance relative to the FXS sample. Muscle- and eye- movement related artifacts are significantly more prevalent in the FXS data. Clear, unambiguous blinks are common in EEG data from TDCs, but, within the FXS group, identification of blinks is complicated by the presence of significantly more artifact along with a much greater co-occurrence of blinks and lateral eye movements that can greatly increase the difficulty of coding blinks in the FXS sample relative to controls. While measures of inter-rater reliability help address this concern, percent agreement, the most common measure of IRR in blink rate literature, is not the most powerful measure of IRR. Given the high degree of agreement between raters in this study, issues associated with blink identification stemming from artifacts in the EEG data seem to be surmountable. However, evaluating IRR using higher resolution approaches would increase confidence in blink rate measures for FXS and help to limit introduction of further sources of variability.

The fact that DA medications are commonly prescribed to FXS patients is also a relevant issue. Of the total FXS sample evaluated in this study, 17 were being treated with DA agonists, antagonists, or both simultaneously. Despite the fact that analyses demonstrated no significant medication effects within that sample, the number of individuals receiving any one medication type was insufficient to examine specific medication effects. Separating medication effects from underlying pathology necessitating the use of those medications is inherently difficult, particularly so in samples of limited size.

The most important concern regarding this research is the validity of blink rate measures as an index of DA signaling. While SBR has extensive support in animal literature as a measure

of DA signaling, and SBR is known to be affected in human diseases involving DA dysfunction, across the healthy adult population a clear correlation between DA signaling and SBR has not been conclusively demonstrated. Indeed, even in animal models with the most robust findings of DA directly modulating blink rate, equivocal and null findings are not uncommon. Furthermore, at least three distinct types of blinking exist: Spontaneous, Reflexive, and voluntary blinks that respond differently in different contexts and the neural processes affecting them individually are poorly understood. There is evidence of a "central blink generator" that is at the core of the different types of blinks, but its existence has not been conclusively established. Without a better mechanistic understanding of the central blink generator and the neural activity that differentiates the different blink classes, any specific hypothesis linking blink rate measures to DA mediation of behavioral effects should be scrutinized. As mentioned previously, a thorough mechanistic understand is not necessary to identify useful biomarkers. What is required, however, is reproducibility and consistency of the correlation between the prospective biomarker and the disease phenotype in question. Within diseases associated with DA dysfunction BR has been demonstrated to be a reliable monitoring biomarker, but, its utility as a diagnostic biomarker to suggest DA dysregulation in atypical populations relative to health controls is questionable without consistent SBR and erBR dynamics for the healthy population.

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Appendix A: Sample Characteristics and Descriptive Statistics

Table 1	**Overall Sample Characteristics**						
FXS n = 68 (31 Female)				Controls n = 54 (26 Female)			
	Mean	Std. Dev.	Range	Mean	Std. Dev.	Range	*t* Statistic (df)
Age	21.7	10.8	6.5 – 54	26.5	14.9	6 – 54	$t(120)=-1.407$, $p<.162$
Full Scale IQ	59.8	19.5	47 – 121	103	10.5	76 – 124	$t(115)=-14.41$, $p<.000$
Verbal Z Score	-2.9	1.9	-6.7 – 1	0.1	0.7	-1.6 – 1.1	$t(113)=-11.15$, $p<.000$
Non-Verbal Z Score	-4.2	2.4	-8.6 – 1.1	0.2	0.7	80 – 121	$t(113)=-12.99$, $p<.000$
Deviation IQ	46	29.6	-10.8 – 116	102	8.6	80 – 121	$t(113)=-13.38$, $p<.000$
SCQ	14.2	8	1 – 30	2.1	2.2	0 – 8	$t(101)=9.95$, $p<.000$

Table 1a.	**EEG Data Sample Characteristics (Habituation task)**						
FXS n=30 (12 females)				Controls n=37 (16 females)			
	Mean	Std. Dev.	Range	Mean	Std. Dev.	Range	*t* Statistic (df)
Age	25.7	10.5	13 - 53	26.8	11.9	12 - 45	$t(65)=0.4$, $p=.69$
Full scale IQ	62.4	21.6	47 - 115	103.1	9.9	85 - 124	$t(62)=10.1$, $p<.001$
Verbal Z	-2.8	1.8	-6.5 – -0.3	0.1	0.6	-1.4 – 1.5	$t(60)=8.9$, $p<.001$
Nonverbal Z	-4.6	2.6	-8.6 – -0.4	0.2	0.8	-1.1 – 1.8	$t(60)=10.3$, $p<.001$
Deviation IQ	43.7	30.2	-10.8 – 94.1	102.1	8.1	88.9 - 120.8	$t(60)=11.1$, $p<.001$
SCQ	13.8	8.1	1 - 29	2.3	2.4	0 - 8	$t(54)=7.7$, $p<.001$

Table 1b.	**EEG Data Sample Characteristics (Chirp task)**						
FXS n=36 (13 females)				Controls n=39 (17 females)			
	Mean	Std. Dev.	Range	Mean	Std. Dev.	Range	*t* Statistic (df)
Age	25.4	10.2	10-53	27.9	12.2	12-57	$t(73)=0.9$, $p=.33$
Full scale IQ	60.7	20.4	47-115	104.2	10.2	85-124	$t(71)=11.8$, $p<.001$
Verbal Z	-3.0	1.9	-6.5 - 0.2	0.2	0.7	-1.4 – 2.0	$t(69)=9.6$, $p<.001$
Nonverbal Z	-4.5	2.4	-8.6 - - 0.4	0.2	0.7	-1.1 – 1.8	$t(69)=11.6$, $p<.001$
Deviation IQ	42.4	29.1	-10.8 – 94.1	102.9	8.3	88.9-120.8	$t(69)=12.3$, $p<.001$
SCQ	14.0	7.9	1-29	2.2	2.4	0-8	$t(62)=8.2$, $p<.001$

Table 2	**Data Availability (Clinical Measures)**			
	FXS n = 68 (31 Female)		Controls n = 54 (26 Female)	
Task	Mean Age	% of sample	Mean Age	% of sample
KiTAP	22.4	86.7%	26.5	100%
ADAMS	20.1	88.2%	22.1	85.6%
ABC FXS	19.7	85.3%	21.2	77.2%
WJ-III	20.8	80.1%	-	-
VINELAND	20.9	82.4%	-	-
CSP	22.9	41.2%	-	-

Note: Only the CSP sample characteristics differed significantly relative to overall FXS sample.

Table 2a.	**Clinical Measure Sample Characteristics (CSP)**						
FXS sample with CSP data n = 28 (8 Female)				Overall FXS Sample n = 68 (31 Female)			
	Mean	Std. Dev.	Range	Mean	Std. Dev.	Range	*t* Stat (df)
Age	22.9	9.9	7.5 – 40.8	21.7	10.8	6.5 – 54	$t(66)=0.757$, p=.452
Full scale IQ	55.2	16.3	47 - 115	59.8	19.5	47 – 121	$t(61)=-1.64$, p=.105
Verbal Z	-3.4	1.8	-6.5 - -0.25	-2.9	1.9	-6.7 – 1	$t(59)=-1.65$, p=.104
Nonverbal Z	-4.9	2.2	-0.7 - -8.6	-4.2	2.4	-8.6 – 1.1	**$t(59)=-2.16$, p=.035***
Deviation IQ	36.5	26.2	-10.8 – 91.4	46	29.6	-10.8 – 116	**$t(59)=-2.27$ p=.027***
SCQ	14.3	6.4	4 - 26	14.2	8	1 – 30	$t(56)=.081$, p=.934

Note: Non-verbal Z score and Deviation IQ differed between CSP and overall FXS samples.

Table 1. Descriptive Statistics

Group	EEG Task	SBR (Stdv)	erBR (Stdv)	Blink Stdv. (Stdv)
FXS	Rest	0.32 (.21)	-	0.83 (.24)
	Chirp	0.33 (.19)	0.29 (.19)	0.86 (.29)
	Hab	0.33 (.20)	0.33 (.19)	0.88 (.28)
TDC	Rest	0.23 (.15)	-	0.76 (.21)
	Chirp	0.25 (.18)	0.22 (.18)	0.71 (.19)
	Hab	0.26 (.19)	0.24 (.17)	0.73 (.19)
FXSmale	Rest	0.32 (.21)	-	0.84 (.26)
	Chirp	0.30 (.17)	0.29 (.18)	0.89 (.31)
	Hab	0.31 (.19)	0.32 (.18)	0.87 (.30)
FXSfemale	Rest	0.33 (.22)	-	0.81 (.21)
	Chirp	0.35 (.20)	0.29 (.20)	0.82 (.26)
	Hab	0.35 (.21)	0.34 (.19)	0.89 (.28)
TDCmale	Rest	0.23 (.16)	-	0.74 (.21)
	Chirp	0.24 (.20)	0.21 (.18)	0.69 (.19)
	Hab	0.26 (.20)	0.26 (.18)	0.73 (.21)
TDCfemale	Rest	0.24 (.14)	-	0.77 (.20)
	Chirp	0.26 (.19)	0.24 (.17)	0.72 (.18)
	Hab	0.26 (.18)	0.22 (.16)	0.73 (.21)

Note: Values represent link rate means (Blinks/sec) and average blink variability (stdv).

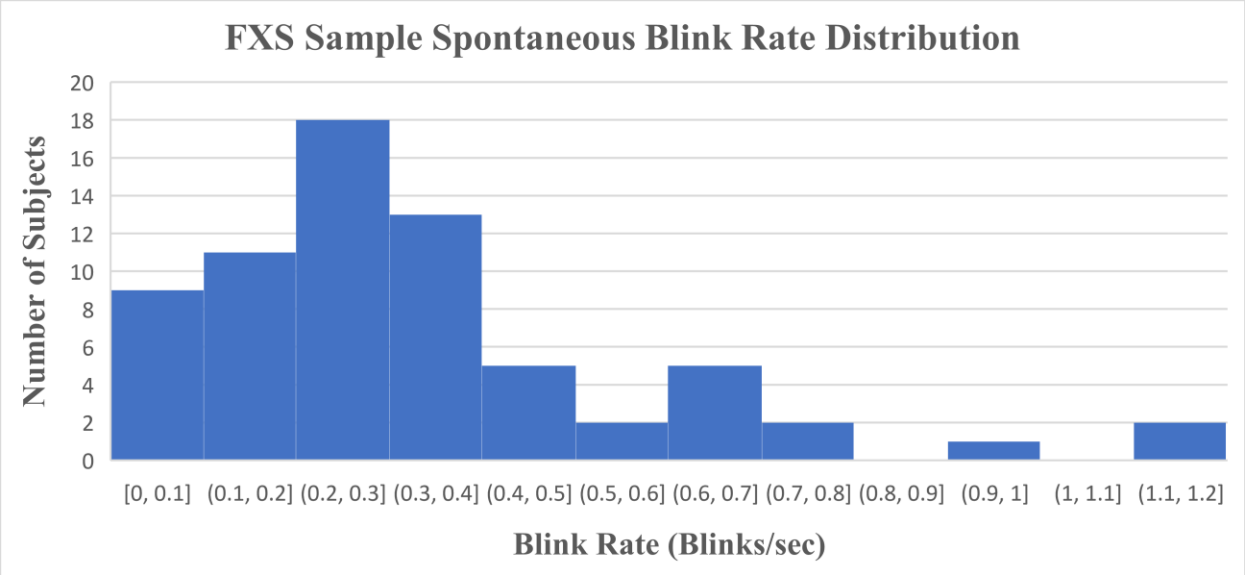


Figure 1: Overall FXS sample SBR distribution.

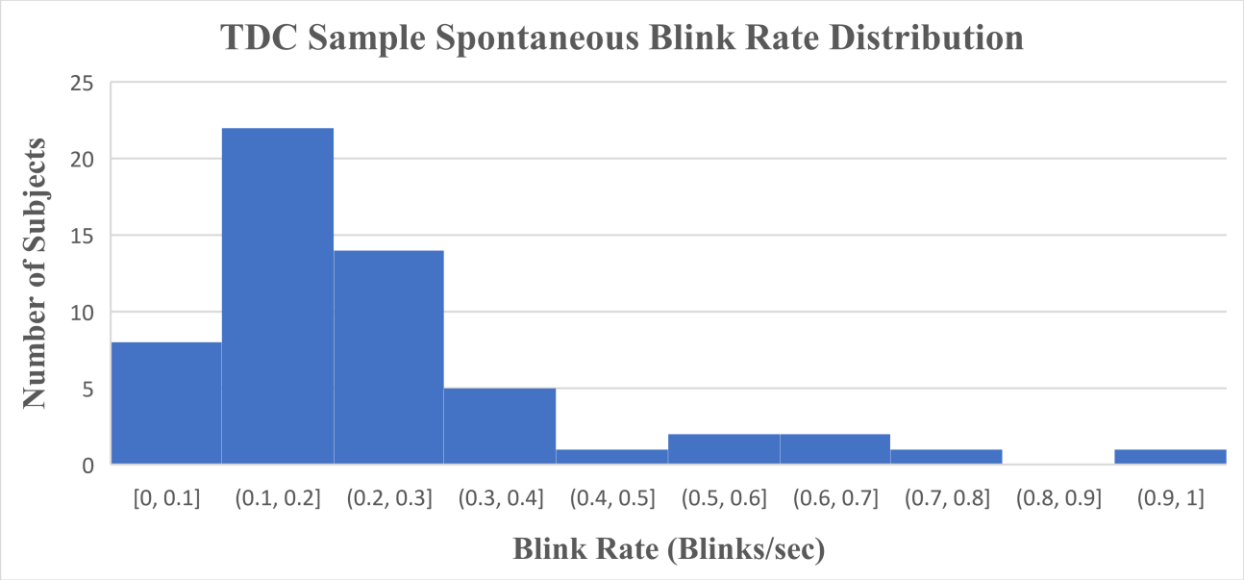


Figure 1a: Overall TDC sample SBR distribution does not appear to be bi-modal.

Appendix B: Exploratory Correlations

Table 4	Clinical Correlations with FXS Blink Rate Measures							
EEG Task	CSP Registration	CSP Avoiding	CSP Sensitivity	ADAMS Manic/Hyperactive Behavior	ADAMS Depressed Mood	Full-scale IQ	Deviation IQ	Verbal Z-score
Rest								
SBR	**-.652****	-.482**	-.423*	-	-.304*	-	-	-
Std. Dev.	-.452*	-	-	-	-.375**	-	-	-
Chirp								
SBR	-	-.538**	-	-	-	-	-	-
erBR	-.475*	-.561**	-	-	-.284*	-	-	-
Std. Dev.	-	-	-	-	-.282*	-	-	-
Hab								
SBR	-.557*	-.526*	-.536*	-.314*	-.282*	-	-	-
erBR	-.584*	-.540*	-.585*	-	-	-	-	-
Std. Dev.	-.480*	-.525*	-.472*	-.401*	-.324*	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 4a	Clinical Correlations with FXS Male Blink Rate Measures							
EEG Task	CSP Registration	CSP Avoiding	CSP Sensitivity	ADAMS Manic / Hyperactive Behavior	ADAMS Depressed Mood	Full-scale IQ	Deviation IQ	Verbal Z-score
Rest								
SBR	**-.740****	.536*	-.560*	-	-	-	-	-
Std. Dev.	-.494*	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	-	-	**-.498****	**-.508****	**-.548****
erBR	-.526*	-.516*	-	-	-	-.455*	-	-.419*
Std. Dev.	-	-	-	-	-	**-.493****	**-.529****	**-.591****
Hab								
SBR	-.518*	-	-.543*	-	-	-	-	-
erBR	-.604*	-	-.604*	-	-	-.409*	-	-
Std. Dev.	-	-	-	-.390*	-		-	-.401*

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 4b	Clinical Correlations with FXS Female Blink Rate Measures							
EEG Task	CSP Registration	CSP Avoiding	CSP Sensitivity	ADAMS Manic / Hyperactive	ADAMS Depressed Mood	Full-scale IQ	Deviation IQ	Verbal Z-score
Rest								
SBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-.542**	-	-	-
Chirp								
SBR	-	-.841*	-	-	-	-	-	-
erBR	-	-.841*	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-.472*	-	-	-
Hab								
SBR	-	-.928**	-	-	-	-	-	-
erBR	-	-.928**	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 5	Vineland Correlations with FXS Blink Rate Measures							
EEG Task	Vineland age equivalence (expressive)	Vineland age equivalence (community)	Vineland age equivalence (receptive)	Vineland age equivalence (coping)	Vineland Composite	Vineland Comm.	Vineland Social	Vineland DLS
Rest								
SBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	-	-	-	-	-
erBR	-.347*	-.291*	-	-	-	-	-	-.330*
Std. Dev.	-.300*	-	-	-	-.304*	-	-	-.355*
Hab								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	.316*	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 5a	Vineland Correlations with FXS Male Blink Rate Measures							
EEG Task	Vineland age equivalence (expressive)	Vineland age equivalence (community)	Vineland age equivalence (receptive)	Vineland age equivalence (coping)	Vineland Composite	Vineland Comm.	Vineland Social	Vineland DLS
Rest								
SBR	-	-.442*	-	-	**-.515****		-.397*	-
Std. Dev.	-	-.383*	-	-	-	-.457*	-.370*	-
Chirp								
SBR	-	-	-	-	-.407*	-.407*	-	-.415*
erBR	-	-	-	-	-	-	-	-.399**
Std. Dev.	-	-	-	-	**-.505****	**-.505****	-	**-.473***
Hab								
SBR	-	-	-	-	-.457*	-.403*	-	-
erBR	-	-	-	**.502****	**-.501****	**-.531****	-	-
Std. Dev.	-	-	-	-	**-.504****	-.441*	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 5b	Vineland Correlations with FXS Female Blink Rate Measures							
EEG Task	Vineland age equivalence (expressive)	Vineland age equivalence (community)	Vineland age equivalence (receptive)	Vineland age equivalence (coping)	Vineland Composite	Vineland Comm.	Vineland Social	Vineland DLS
Rest								
SBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Hab								
SBR	-	-	.527*	-	-	-	-	-
erBR	-	-	.525*	-	-	-	-	-
Std. Dev.	-	-	.510*	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 6	ABC-FXS Correlations with FXS Blink Rate Measures				
EEG Task	ABC FXS Irritability/ Aggression	ABC FXS Lethargy/Social Withdrawal	ABC FXS Hyperactivity/ Non-Compliance	ABC FXS Inappropriate Speech	ABC FXS Social Avoidance
Rest					
SBR	-	-.260*	-	-	-
Std. Dev.	-	-.357**	-	-	-.264*
Chirp					
SBR	-	-	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-	-	-	-	-
Hab					
SBR	-	-.309*	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-.367*	-.402**	-.344*	-.357*	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 6a	ABC-FXS Correlations with FXS Male Blink Rate Measures				
EEG Task	ABC FXS Irritability/ Aggression	ABC FXS Lethargy/Social Withdrawal	ABC FXS Hyperactivity/ Non-Compliance	ABC FXS Inappropriate Speech	ABC FXS Social Avoidance
Rest					
SBR	-	-	-	-	-
Std. Dev.	-	-	-	-	-
Chirp					
SBR	-	-	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-	-	-	-	-
Hab					
SBR	-	-	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-	-	-	-.429*	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 6b	ABC-FXS Correlations with FXS Female Blink Rate Measures				
EEG Task	ABC FXS Irritability/ Aggression	ABC FXS Lethargy/Social Withdrawal	ABC FXS Hyperactivity/ Non-Compliance	ABC FXS Inappropriate Speech	ABC FXS Social Avoidance
Rest					
SBR	-	-	-	-	-
Std. Dev.	-	-.501*	-	-	-
Chirp					
SBR	-	-	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-	-	-	-	-
Hab					
SBR	-	-	-	-	-
erBR	-	-	-	-	-
Std. Dev.	-	-	-	-	-.454*

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 7	kiTAP Correlations with FXS Blink Rate Measures							
EEG Task	Distractor Correct	Distractor Omissions	Distractor Median	Distractor Errors	Total Distractor Errors	No-Distractor Errors	Flex Correct	gonogo Errors
Rest								
SBR	.270*	-.261*	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Hab								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 7a	kiTAP Correlations with FXS Male Blink Rate Measures							
EEG Task	Distractor Correct	Distractor Omissions	Distractor Median	Distractor Errors	Distractor Total Errors	No-Distractor Errors	Flex Correct	gonogo Errors
Rest								
SBR	-	-	-	**.501****	.450*	.391*	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	.404*	-	-	-	.413*
erBR	-	-	-.405*	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Hab								
SBR	-	-	-	.462*	.460*	.445*	-	**.583****
erBR	-	-	-	**.543****	**.507****	.443*	-	**.596****
Std. Dev.	-	-	-	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 7b	kiTAP Correlations with FXS Female Blink Rate Measures							
EEG Task	Distractor Correct	Distractor Omissions	Distractor Median	Distractor Errors	Total Distractor Errors	No-Distractor Errors	Flex Correct	gonogo Errors
Rest								
SBR	-	-	-	-	-	-	.501*	-
Std. Dev.	-	-	-	-	-	-	-	-
Chirp								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-
Hab								
SBR	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 8	FXS Blink Rate Correlations with EEG Measures								
EEG Task	ERP Onset ITC (Chirp)	ERP Offset ITC (Chirp)	Theta Power (Chirp)	ERP Onset ITC (Hab)	ERP Offset ITC (Hab)	Alpha Power (Hab)	Theta Power (Hab)	P2_3 Latency (Hab)	%habituation N1 amplitude
Rest									
SBR	-	-	-	-	-	-	-	-	.394*
Std. Dev.	-	-	-	-	-	-	-	-	-
Chirp									
SBR	-	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-	-
Hab									
SBR	-	-	-	-	-	-	-	-.463*	-
erBR	-	-	-	-	-	-	-	-.402*	-
Std. Dev.	-	-	-	-	-	-	-	-	.430*

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

Table 8a	FXS Male Blink Rate Correlations with EEG Measures								
EEG Task	ERP Onset ITC (Chirp)	ERP Offset ITC (Chirp)	Theta Power (Chirp)	ERP Onset ITC (Hab)	ERP Offset ITC (Hab)	Alpha Power (Hab)	Theta Power (Hab)	P2_3 Latency (Hab)	%habituation N1 amplitude
Rest									
SBR	-	-	-	-	-	-	.564*	-	-
Std. Dev.	-	-	-	-	-	-	.564*	-	-
Chirp									
SBR	-	-	-	-	-	-	-	-	-
erBR	-	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-	-	-	-	-	-
Hab									
SBR	-	-	-	-	-	-	-	-.514*	-
erBR	-	-	-	-	-	-	-	-	-
Std. Dev.	-	-	-	-.494*	-	**.618****	**.707****	-	.524*

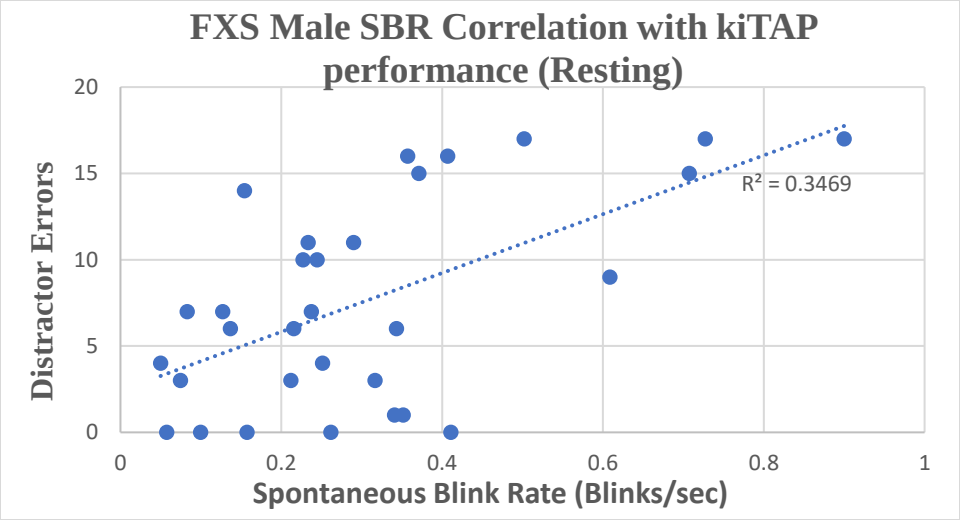
*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

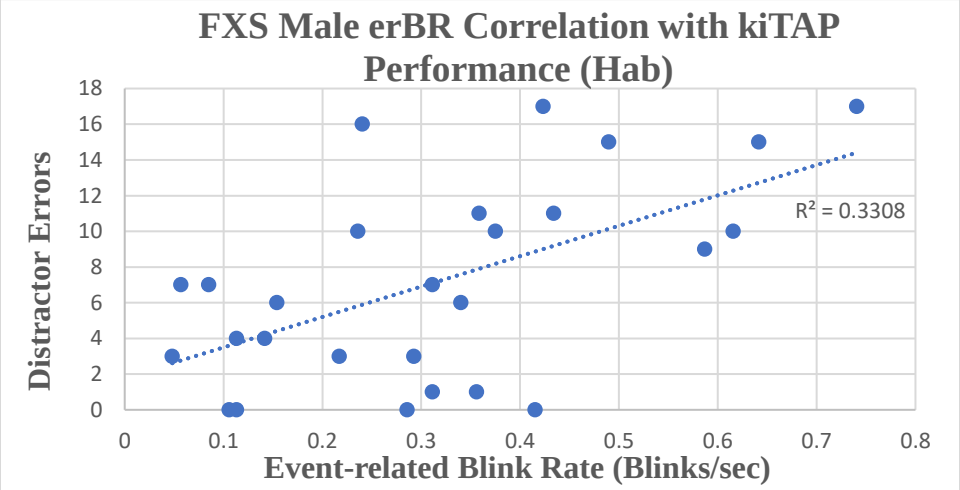
Table 8b	FXS Female Blink Rate Correlations with EEG Measures								
EEG Task	ERP Onset ITC (Chirp)	ERP Offset ITC (Chirp)	Theta Power (Chirp)	ERP Onset ITC (Hab)	ERP Offset ITC (Hab)	Alpha Power (Hab)	Theta Power (Hab)	P2_3 Latency (Hab)	%habituation N1 amplitude
Rest									
SBR	-	.720**	-	**.845****	-	-	-.727*	-	-
Std. Dev.	-	.727**	-	.682*	-	-	-	-	-
Chirp									
SBR	-	.709**	-	-	-	-	-	-	-
erBR	-	.602*	-	-	-	-	-	-	-
Std. Dev.	-	.703**	-	-	-	-	-	-	-
Hab									
SBR	-	-	-	.671*	-	-	-	-	-
erBR	-	-	-	.685*	-	-	-.643*	-	-
Std. Dev.	-	-	-	-	-	-	-	-	-

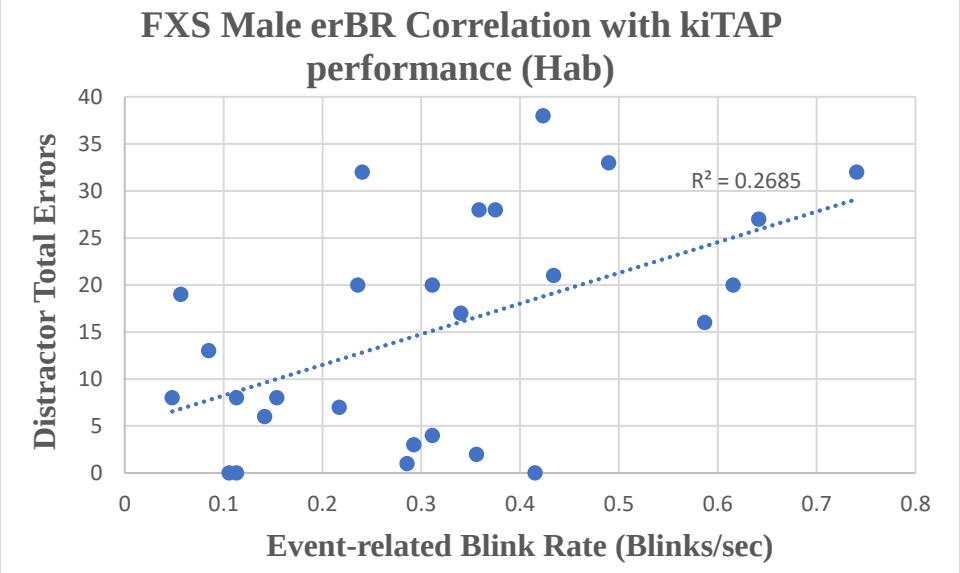
*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the FXS sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*

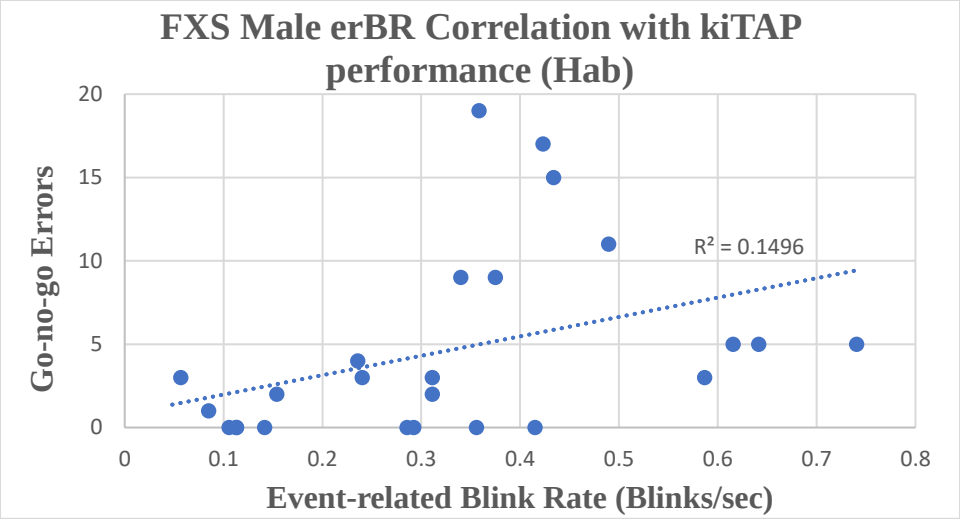
Table 8c	**TDC Blink Rate Correlations for EEG Measures**								
EEG Task	Alpha Power (Hab)	Theta Power (Hab)	N1_2 Latency (Hab)	N1_3 Latency (Hab)	Rep1 ITC (Hab)	Rep2 ITC (Hab)	Rep3 ITC (Hab)	P2_2 Amplitude (Hab)	ERP Onset ITC (Hab)
Rest									
SBR	-	-	-	-.380*	-	-	**-.424***	-	-
Std. Dev.	.338*	-	-	**-.425***	**-.448****	**-.578****	**-.511****	-	-.329*
Chirp									
SBR	-	-	-.424*	-	-	-	-.355*	-	-
erBR	-	-	**-.459****	-	-	-.366*	-.417*	-	-
Std. Dev.	-	-	-.400*	-	-	-.340*	-	-	-
Hab									
SBR	**.519****	-	**-.411***	**-.417***	**-.429***	**-.481****	**-.481****	**-.372***	-
erBR	**.605****	**.374***	**-.491****	**-.443****	**-.378***	**-.404***	**-.464****	-	-
Std. Dev.	**.437****	-	**-.420***	-.362*	**-.471****	**-.497****	**-.475****	-	**-.376***

*Clinical variables with significant correlations to blink measures are included. All correlations are Spearman's Rho and represent the TDC sample only. Correlations that did not survive correction for multiple comparisons are highlighted in red. "-" = N.S., *p < 0.05, **p < 0.01.*


FXS Male SBR Correlation with kiTAP performance (Resting)
Distractor Errors
Spontaneous Blink Rate (Blinks/sec)
R² = 0.3469
0
5
10
15
20
0
0.2
0.4
0.6
0.8
1


FXS Male erBR Correlation with kiTAP Performance (Hab)
Distractor Errors
Event-related Blink Rate (Blinks/sec)
R² = 0.3308
0
2
4
6
8
10
12
14
16
18
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8


FXS Male erBR Correlation with kiTAP performance (Hab)
Distractor Total Errors
Event-related Blink Rate (Blinks/sec)
R² = 0.2685
0
5
10
15
20
25
30
35
40
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8


FXS Male erBR Correlation with kiTAP performance (Hab)
Go-no-go Errors
Event-related Blink Rate (Blinks/sec)
R² = 0.1496
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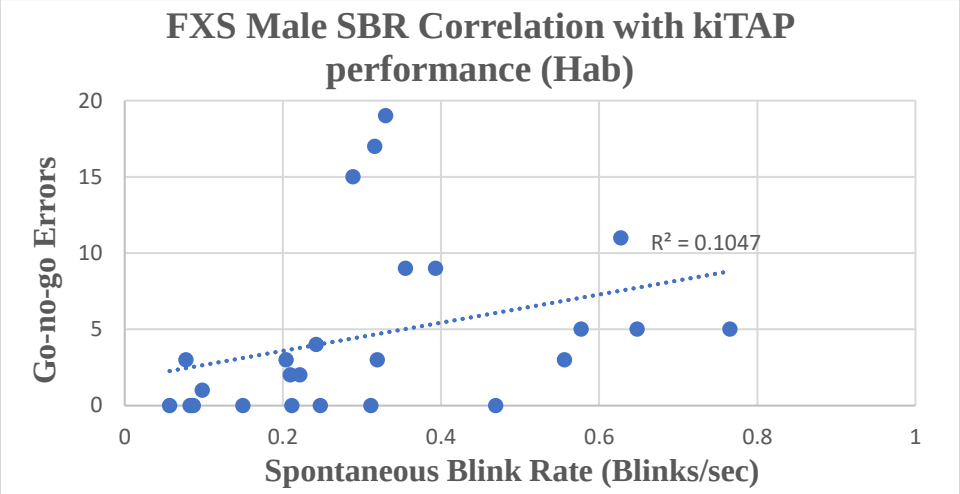
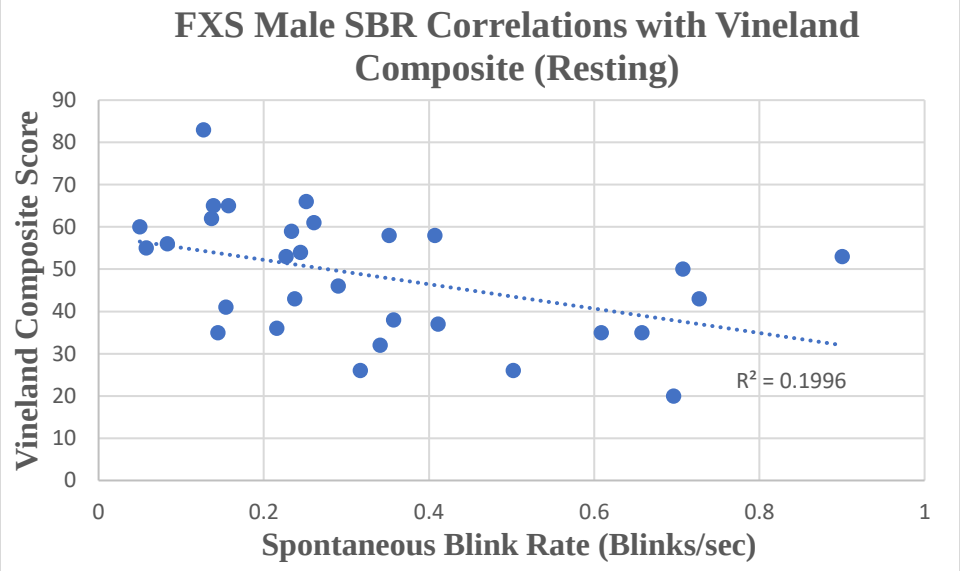
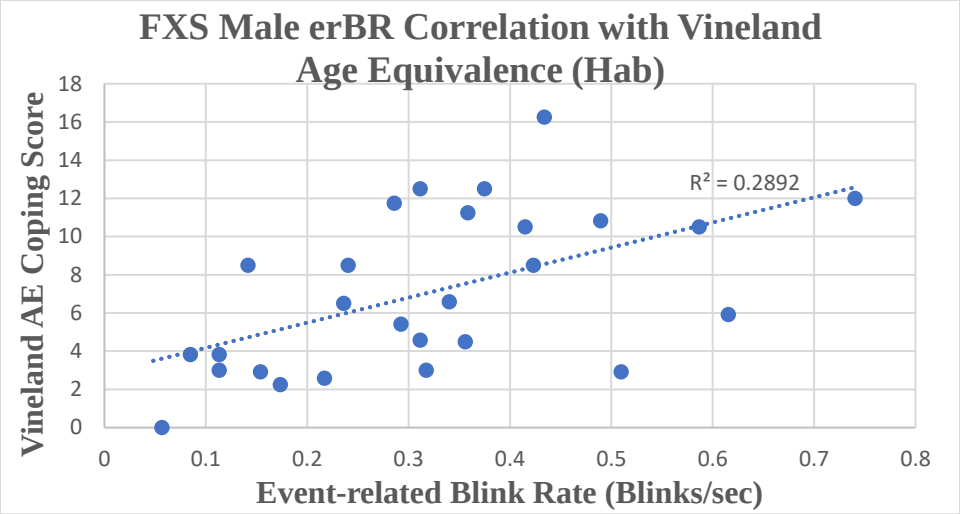
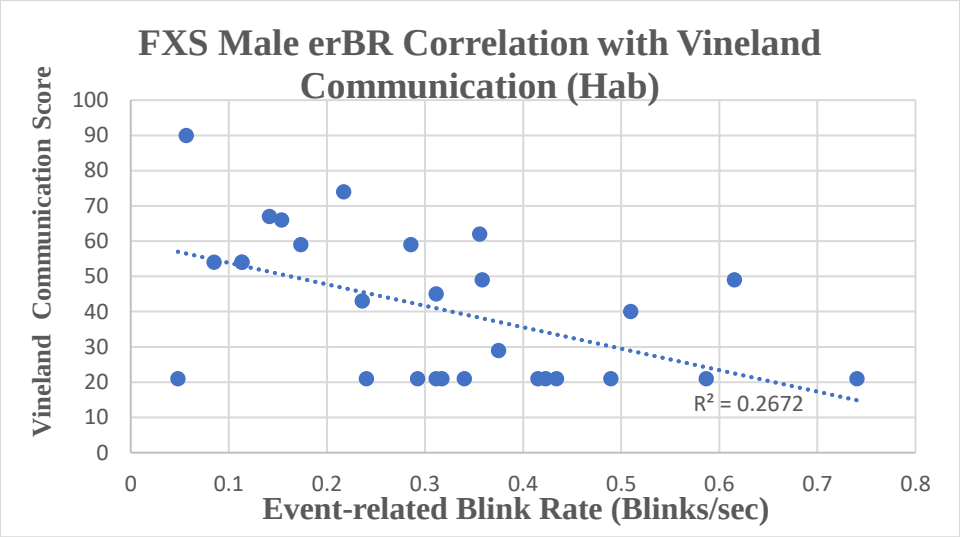
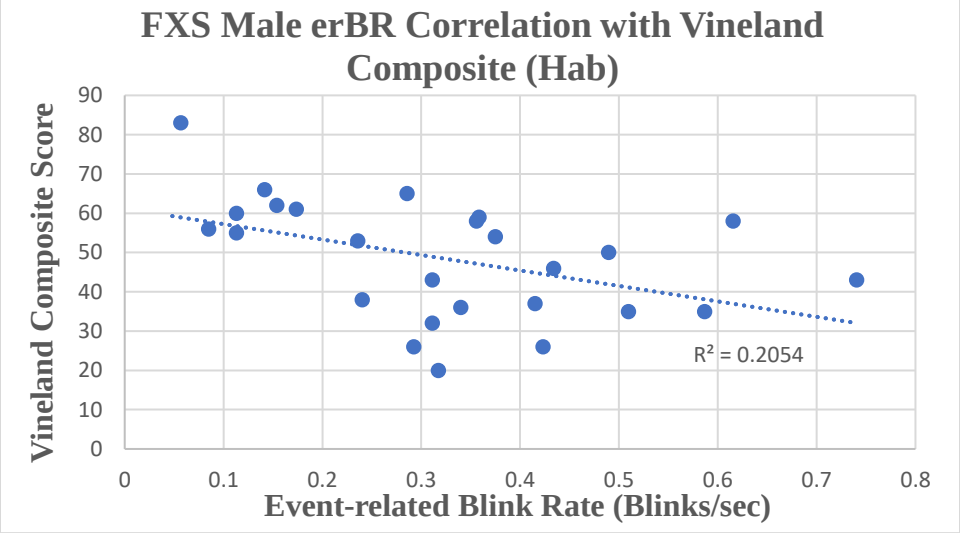
5
10
15
20
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

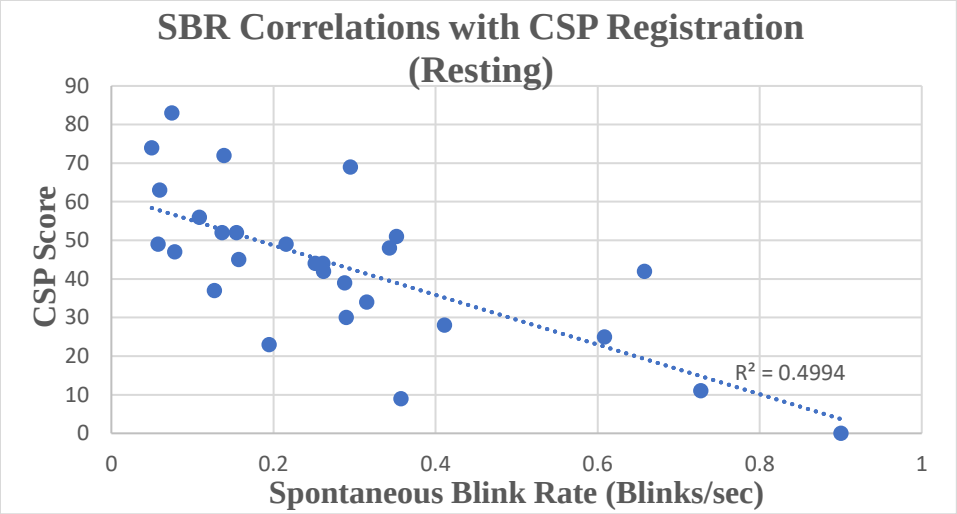

FXS Male SBR Correlation with kiTAP performance (Hab)
Go-no-go Errors
Spontaneous Blink Rate (Blinks/sec)
R² = 0.1047
0
5
10
15
20
0
0.2
0.4
0.6
0.8
1

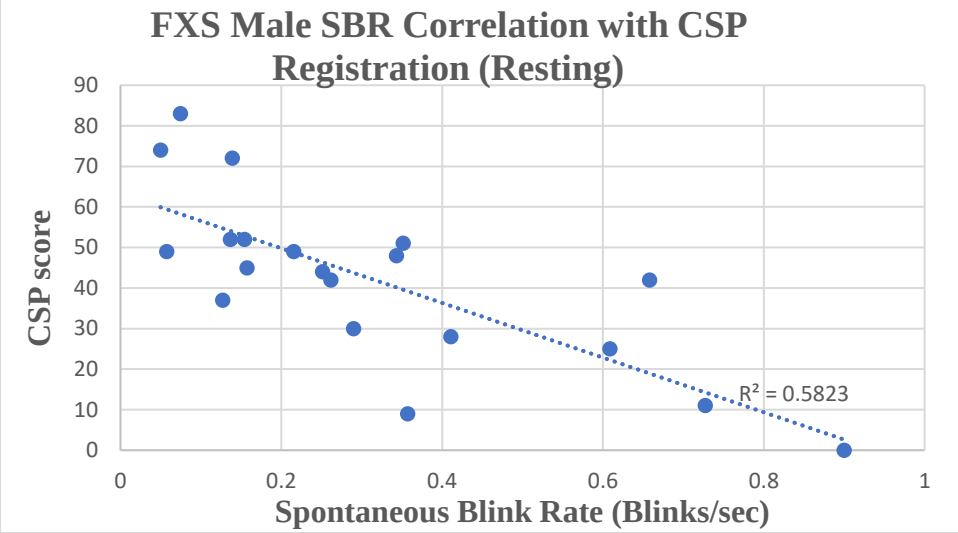

FXS Male SBR Correlations with Vineland Composite (Resting)
Vineland Composite Score
Spontaneous Blink Rate (Blinks/sec)
R² = 0.1996
0
10
20
30
40
50
60
70
80
90
0
0.2
0.4
0.6
0.8
1

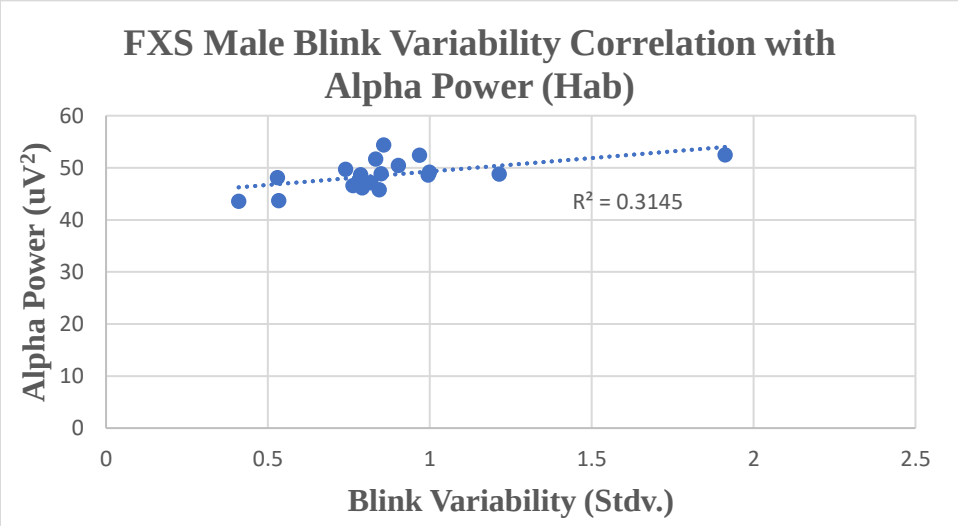

FXS Male erBR Correlation with Vineland Age Equivalence (Hab)
Vineland AE Coping Score
Event-related Blink Rate (Blinks/sec)
R² = 0.2892

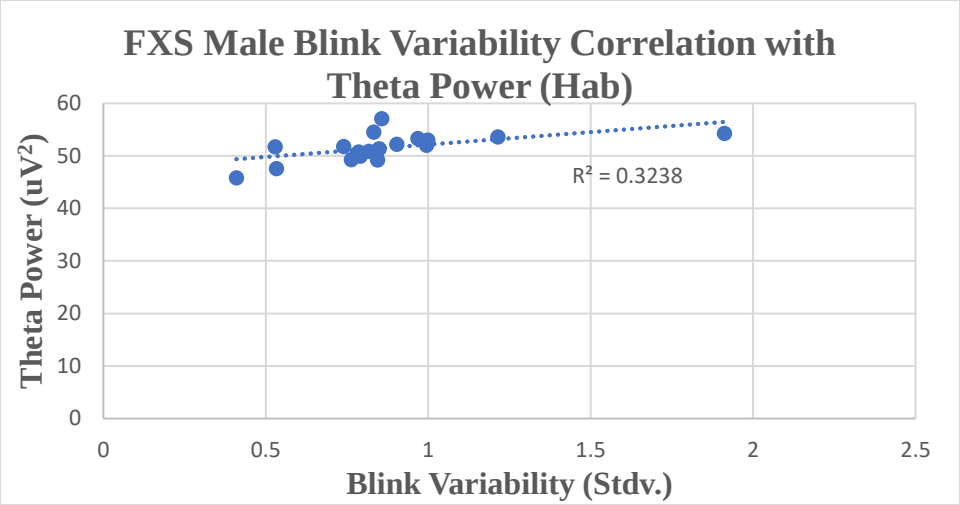

FXS Male erBR Correlation with Vineland Communication (Hab)
Vineland Communication Score
Event-related Blink Rate (Blinks/sec)
R² = 0.2672

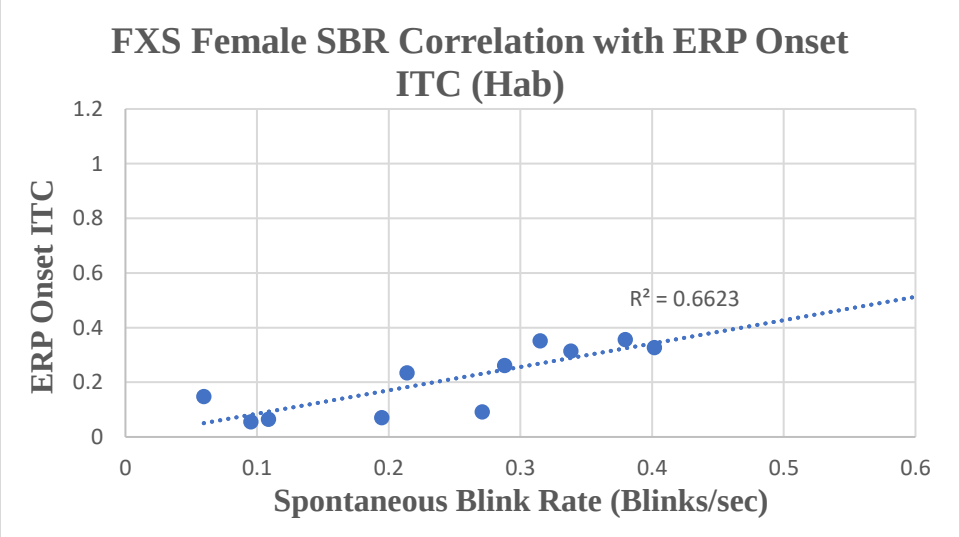

FXS Male erBR Correlation with Vineland Composite (Hab)
Vineland Composite Score
Event-related Blink Rate (Blinks/sec)
R² = 0.2054


SBR Correlations with CSP Registration (Resting)
CSP Score
Spontaneous Blink Rate (Blinks/sec)
R² = 0.4994
0
10
20
30
40
50
60
70
80
90
0
0.2
0.4
0.6
0.8
1


FXS Male SBR Correlation with CSP Registration (Resting)
CSP score
Spontaneous Blink Rate (Blinks/sec)
R² = 0.5823
0
10
20
30
40
50
60
70
80
90
0
0.2
0.4
0.6
0.8
1


FXS Male Blink Variability Correlation with Alpha Power (Hab)
Alpha Power (uV²)
Blink Variability (Stdv.)
R² = 0.3145
0
10
20
30
40
50
60
0
0.5
1
1.5
2
2.5


FXS Male Blink Variability Correlation with Theta Power (Hab)
Theta Power (uV²)
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
Blink Variability (Stdv.)
R² = 0.3238


FXS Female SBR Correlation with ERP Onset ITC (Hab)
ERP Onset ITC
1.2
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Spontaneous Blink Rate (Blinks/sec)
R² = 0.6623

Appendix C: Blink Rate Measures Across Tasks

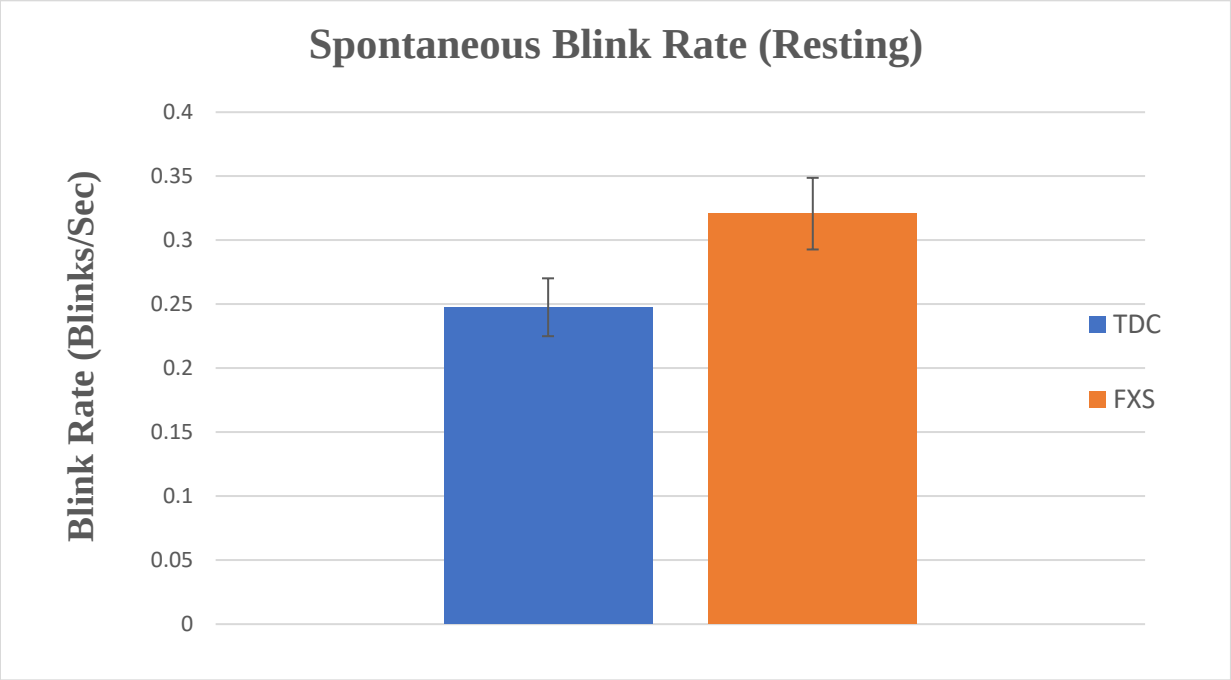


Figure 2. Resting SBR is significantly elevated in the FXS sample relative to TDCs (p = 0.013).

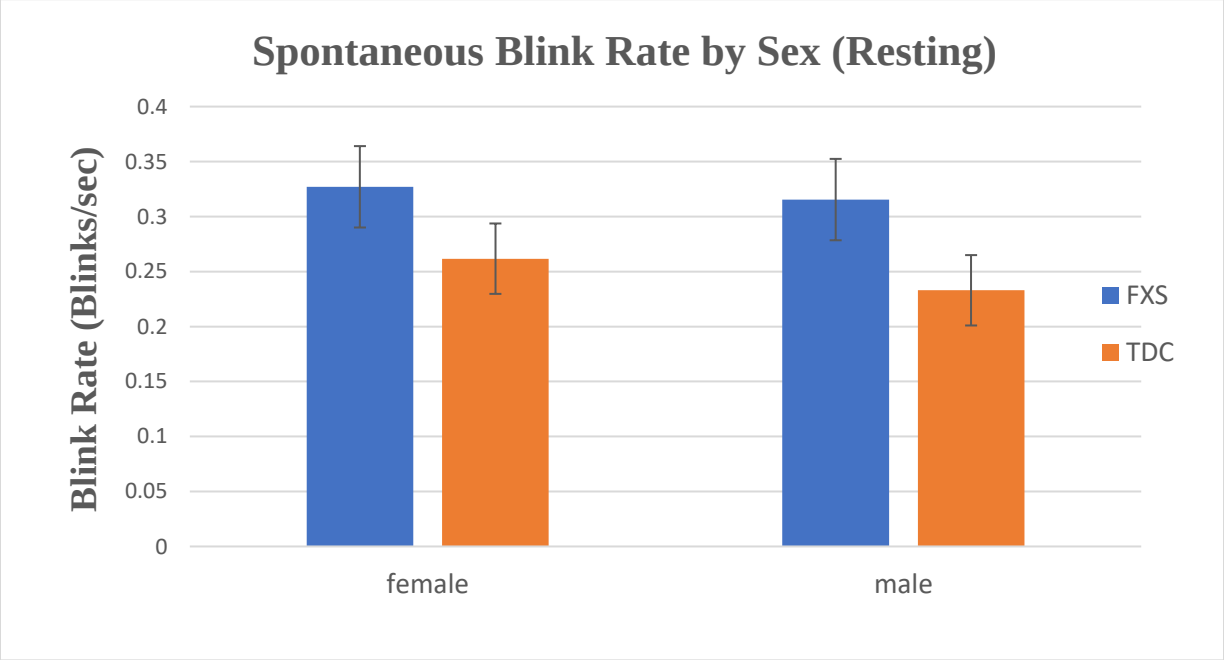


Figure 2a. Resting SBR is not significantly different between genders in either group (p = 0.848).

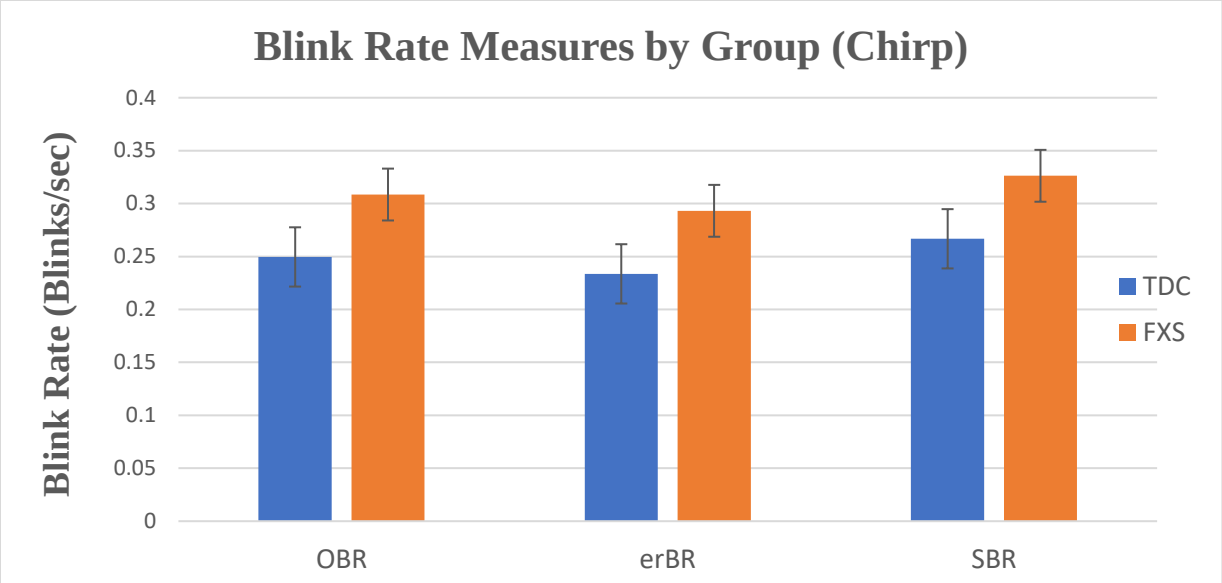


Figure 3. OBR (p = 0.023), and SBR (p = 0.042) are significantly elevated in the FXS relative to TDCs. A marginally significant elevation of erBR in the FXS sample was observed (p = 0.053).

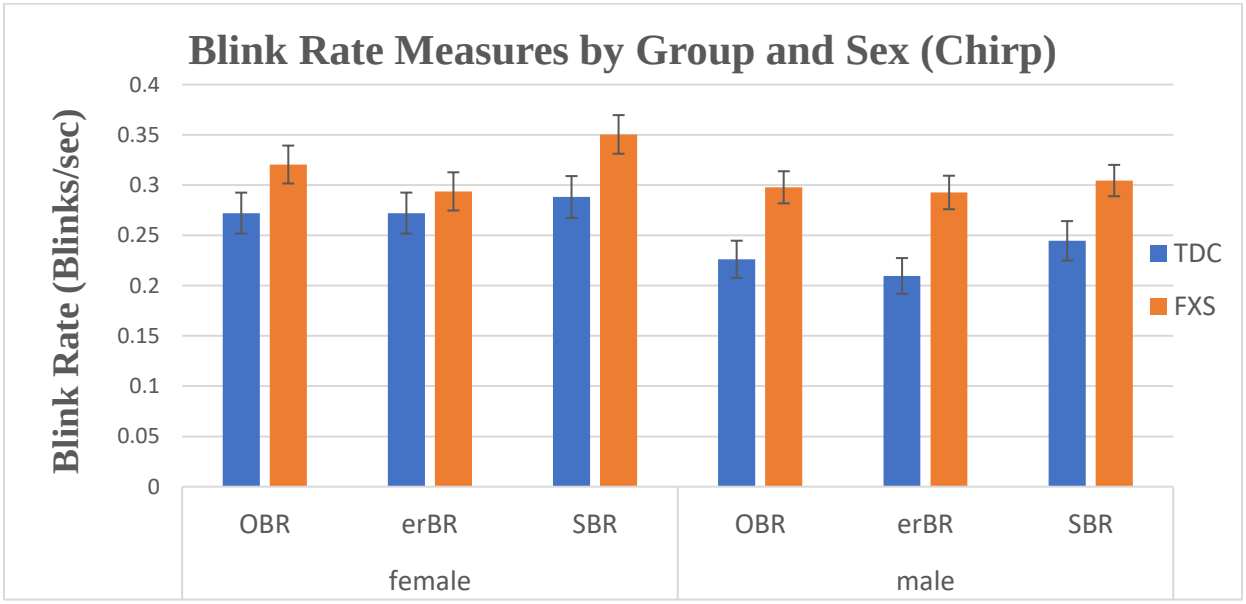


Figure 3a. No significant between-subject effects of sex (p = 0.530) or the group*sex interaction (p = 0.987). A significant within-subject effect of the group*sex*stim interaction was found (p = 0.021) due to a lack of modulation of BR between erBR and SBR in FXS males only.

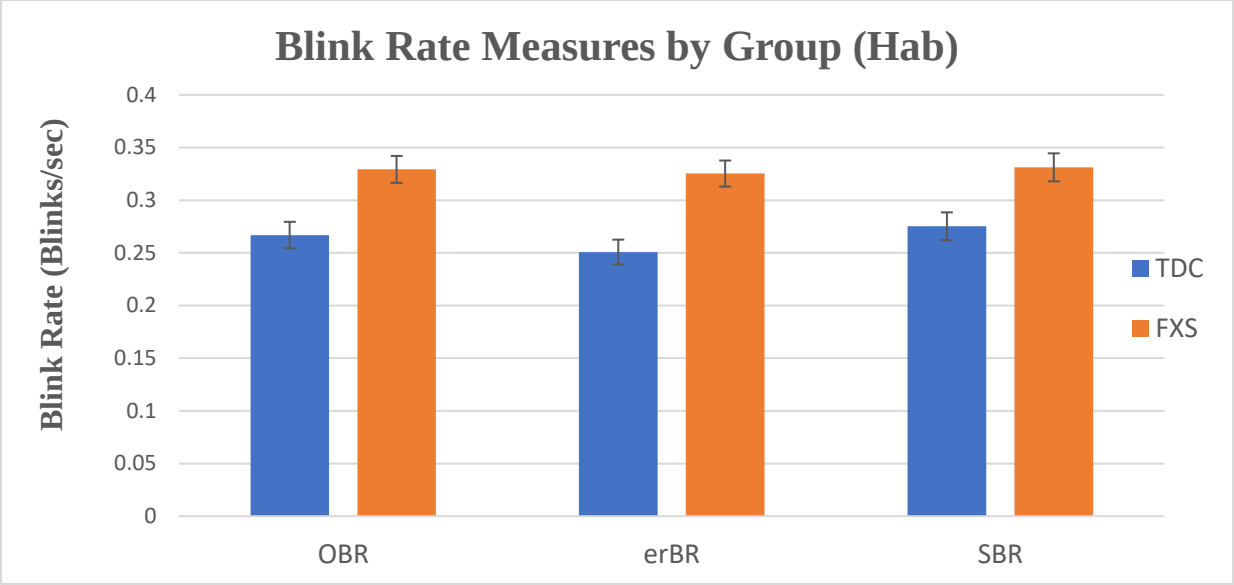


Figure 4.: Significantly elevated OBR (p = 0.023) and erBR (p = 0.012) in the FXS group along with a marginally significant increase in SBR (p = 0.058).

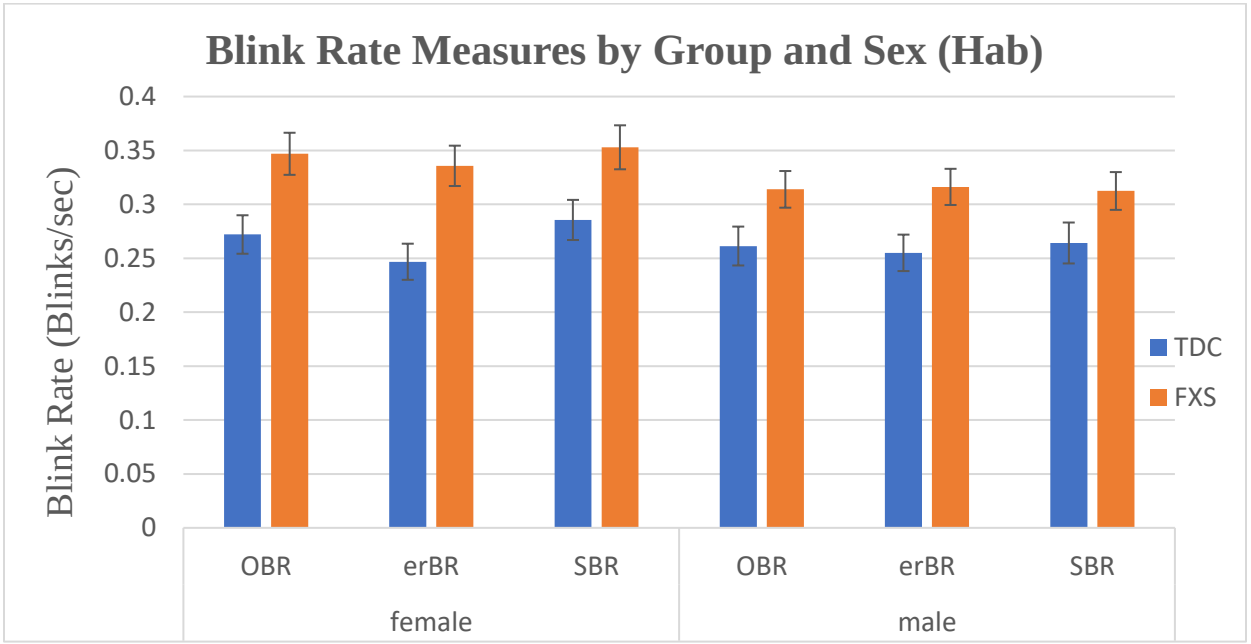


Figure 4a. No significant, between-subject effects of sex (p = 0.823) or the group*sex interaction (p = 0.470). A within-subject effect of the sex*stim interaction approached significant (p = 0.061) with males modulating BR in response to stimuli to a less extent than females. No significant effect of the group*sex*stim interaction was found (p = 0.768).

Appendix D: Blink Identification

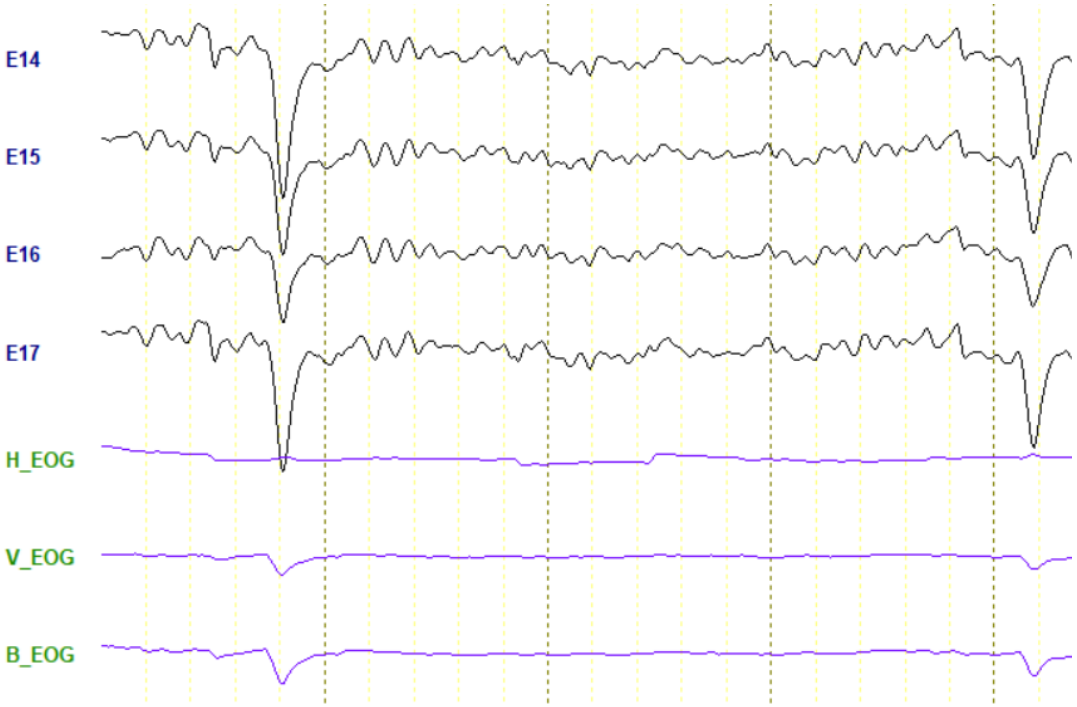


Figure 5. Example of 3d spatial distribution of scalp potentials alongside EEG channel waveforms characteristic of blinks. Virtual Electro-oculogram waveforms are shown below channel data (blue waveforms) and are produced in BESA by applying predefined source model to the data. This creates three topographies accounting for EOG activity (Horizontal, vertical, and blink topographies). vEOG waveforms help disambiguate blinks from other forms of eye-movement related signal.

Appendix E: EEG Channel Montage and Previous Results

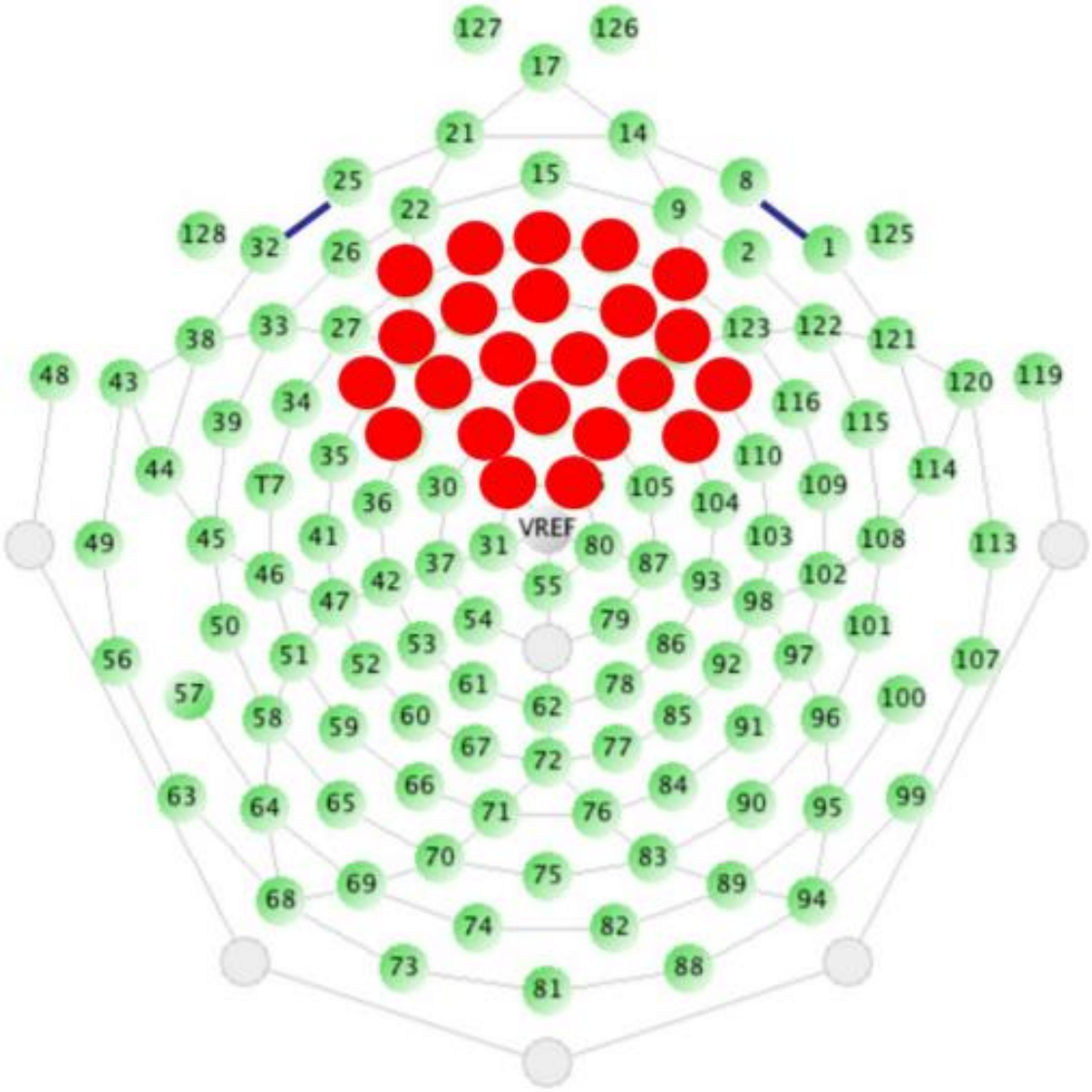


Figure 6. Sensory layout of the EGI 128 channel system used for data collection. Sensors selected for further EEG analyses are highlighted in red. Sensor selection was based on standards used in previous aimed at detecting N1 ERP components originating in the auditory cortex. Reproduced with permission (Ethridge et al., 2019). Copyright (2019), [*Front. Integr. Neurosci.*].

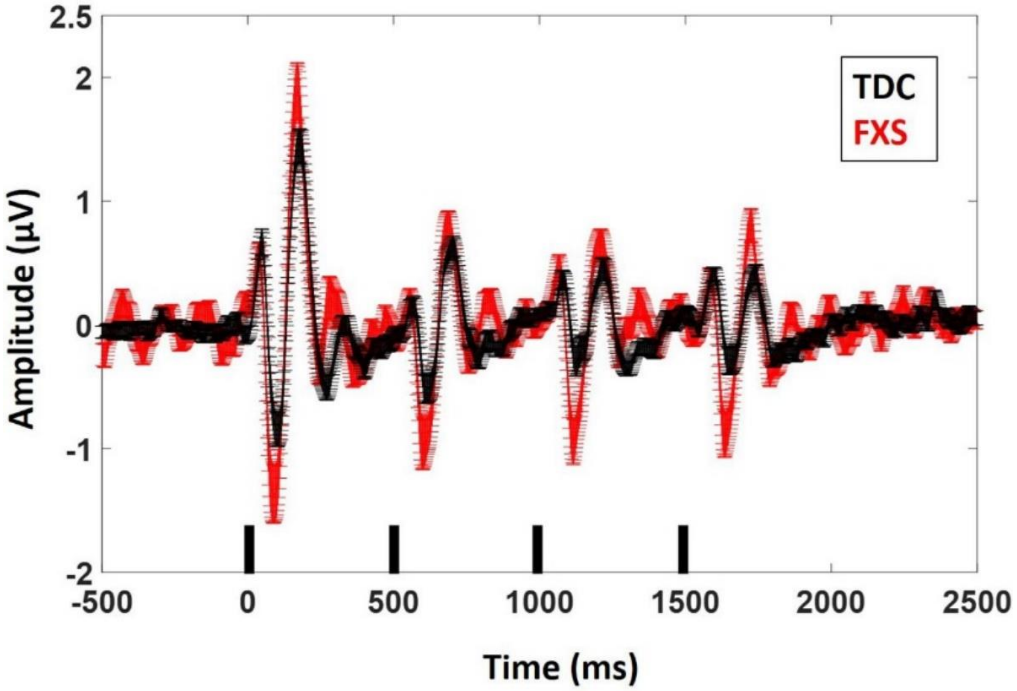


Figure 6a. Average ERP waveforms for FXS (red) and TDC (black) samples for the habituation task. Black bars on the horizontal axis represent stimulus presentation of each burst of the train. Significantly increased N1 and P2 amplitude in FXS as compared to controls. Reproduced with permission (Ethridge et al., 2019). Copyright (2019), [*Front. Integr. Neurosci.*].

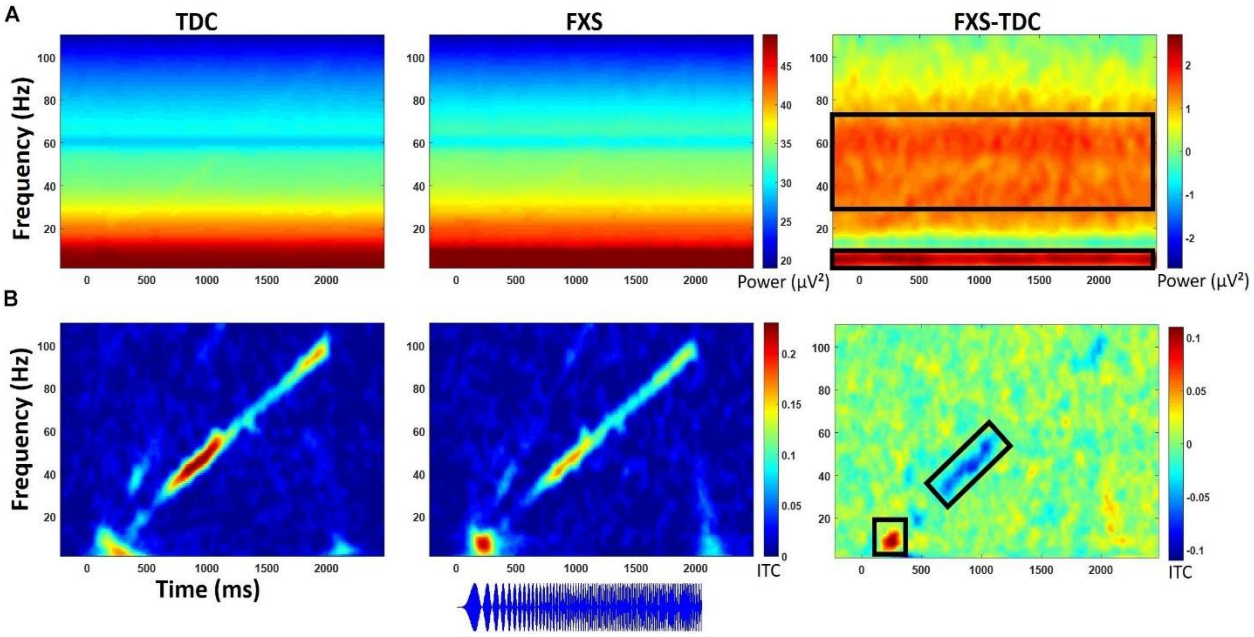


Figure 6b. a) Single trial power (STP) and difference map (FXS – TDC) for the chirp task. b) ITC for TDC and FXS samples along with difference map. Clusters with significant group differences in the difference maps are indicated by black boxes. Reproduced with permission (Ethridge et al., 2019). Copyright (2019), [*Front. Integr. Neurosci.*].