Challenging behaviour in Fragile X Syndrome: Investigating its association with environmental and physiological factors.

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Abbreviations

Summary list of abbreviations used throughout thesis (Alphabetised).

Abbreviation	Definition
ABA	Applied Behavioural Analysis
ABC	Aberrant Behaviour Checklist
A-B-C	Antecedent Behaviour Consequence
ACTH	Adrenocorticotropic Hormone
ADHD	Attention Deficit/ Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
Anx-1	Annexin 1
AO	Abolishing Operation
AS	Angelman Syndrome
ASD	Autism Spectrum Disorder
BACB	Behavior Analyst Certification Board
BPI	Behavior Problems Inventory
BPI-S	Behavior Problems Inventory Short Form
CA	Chronological Age (matched)
CAI	Contextual Assessment Inventory
CAR	Cortisol Awakening Response
CARS	Child Autism Rating Scale
CAMHS	Child and Adolescent Mental Health Service
CB	Challenging Behaviour
CBQ	Challenging Behaviour Questionnaire
CBCL	Child Behaviour Checklist
CCG	Cytosine, Cytosine, Guanine (genetic trinucleotide repeat)
CN	Caudate Nuclei
CdLS	Cornelia de Lange Syndrome
CRH	Corticotropin Releasing Hormone
d	Days
DS	Down Syndrome

Abbreviation	Definition
EFA	Experimental Functional Analysis
ELISA	Enzyme-Linked Immunosorbent Assay
EO	Establishing Operation
EPM	Elevated Plus Maze
F	Female
FAI	Functional Assessment Interview
FAO	Functional Assessment Observation
FISS	Family Intensive Support Service
FMR1	Fragile X Mental Retardation 1 (Gene)
FMRP	Fragile X Mental Retardation Protein
FORWARD	Fragile X Online Registry with Accessible Research Database
FXS	Fragile X Syndrome
GR-α	Glutamate Receptor Alpha
h	hour
HADS	Hospital Anxiety and Depression Scale
HPA	Hypothalamic-Pituitary-Adrenal
ID	Intellectual disability
KO	Knockout (mouse)
L-HPA	Limbic Hypothalamic-Pituitary-Adrenal
LTP	Long-Term Potentiation
LNS	Lesch-Nyhan Syndrome
m	Minute
M	Male
MA	Mental age (matched)
MeSH	Medical Sub Heading
mGluR5	Metabotropic Glutamate Receptor 5
MO	Motivating Operation
mRNA	Messenger Ribonucleic Acid
N	Number
N/A	Not available
NFXF	National Fragile X Foundation
NHS	National Health Service

Abbreviation	Definition
ns	Non-significant
PECS	Picture Exchange Communication System
PhD	Doctor of Philosophy
pANS	Parasympathetic Nervous System
PWS	Prader-Willi Syndrome
QABF	Questions About Behavioural Function Scale
R&D	Research & Development
RBS-R	Repetitive Behaviour Scales- Revised
SAA	Salivary α-amylase
sANS	Sympathetic Autonomic Nervous System
SCQ	Social Communication Questionnaire
SD	Standard Deviation
Sib	Sibling
SIB	Self-Injurious Behaviour
SIB-C	Self-Injury Checklist
SMS	Smith-Magenis Syndrome
TA	Teaching Assistant
TARF-R	Treatment Acceptability Rating Form
TD	Typically Developing
VABS	Vineland Adaptive Behaviour Scales
VSC	Vineland Screener
VT	Vagal Tone
w	Weeks
WS	Williams Syndrome
WT	Wild Type (mouse)

Abstract

Challenging behaviours (CBs) are a common issue amongst individuals with Fragile X Syndrome (FXS). The aim of the present thesis was to further understanding of this issue, through exploring physiological factors which may have a motivative influence upon the operant learning of CBs in this group. Analysis of prior literature highlighted that CBs were most commonly negatively reinforced amongst males with FXS. This may reflect an elevated motivation to escape from stressors, associated with atypical stimulus-bound arousal. Accordingly, prior data suggest autonomic hyperarousal and a systematic literature review suggested that the hypothalamic-pituitary-adrenal axis may be implicated in males with FXS. However, the relationship between arousal and escape-maintained CB had not previously been explored, and so was addressed through two empirical studies. In the initial study, CBs were observed in a natural environment, alongside explorations of circadian rhythmicity of salivary arousal measures (cortisol and α-amylase) in boys with FXS and unaffected siblings. Whilst between-group differences were apparent in arousal measures, there were no associations with observed behaviours in the FXS group. In a subsequent study, behaviour and physiological responding were measured in response to a structured demand assessment, amongst individuals with intellectual disability and males with FXS. Despite between-group differences in behaviour, no differences in physiological responding or physiology-behaviour relationships were observed. A final exploratory study of parental reports of the behavioural and emotional timecourse of instances of CBs was conducted, in order to guide future research. Together the results suggest that initial hypotheses were overly simplistic and that a broad range of aspects of the FXS phenotype must be accounted for when explaining CBs in this group. Implications for future research and practice are discussed.

Chapter 1

Incorporating Genetic Influences into Behavioural Explanations of Challenging Behaviour.

Chapter Overview

In the current chapter the significance of challenging behaviour (CB) displayed by some people with intellectual disabilities is established: such behaviours have a negative impact upon the individual displaying the behaviour, as well as those around them. As a result, it is argued that research is warranted to further understand the development and maintenance of these behaviours.

The applied behaviour analytic approach has been used with success to establish maintaining factors, typically in an individual's external environment, associated with the occurrence of CBs. In addition, interventions to provide functionally equivalent alternatives for behaviour have been shown to be successful in many cases, highlighting the value of this approach. However, research has highlighted widely varying rates of CB between different genetic syndromes, with further provisional data suggesting between syndrome differences in the likelihood of exhibiting CBs with different functions. Such differences are not accounted for within the basic operant learning model. As such, alternative theories incorporating genetic influences into the behavioural approach are reviewed. The integrative potential of the motivating operation is discussed.

Finally, the genetic condition Fragile X syndrome (FXS) is introduced. As a condition with a well-established behavioural phenotype, it provides a helpful example through which to further explore syndrome-associated influences upon CBs.

What is Challenging Behaviour?

Challenging behaviours (CBs) are culturally abnormal behaviours that are exhibited to the extent that they place the individual or those around them at risk of harm, or limit access to services or the community (Emerson, 2001). Approximately 10-15% of individuals with intellectual disabilities (IDs) exhibit severe CBs, the most common types being self-injurious behaviour (SIB), aggression and property destruction (Emerson, 2001). Such behaviours create a significant burden on carers: physically, emotionally and financially (Bailey et al., 2012). Furthermore, those who engage in CBs are more likely to be socially excluded and experience a poorer quality of life (Holden & Gitlesen 2006). As a result of these adverse outcomes, it is clear that research to better understand, intervene and prevent such behaviours is of great importance.

Theoretical approaches to understanding challenging behaviour.

A number of theoretical approaches have been taken to understanding the development, maintenance and treatment of CBs, each focussing upon differing causal mechanisms. A broad range of approaches have been considered including the impact of physical health (De Winter, Jansens & Evenhuis, 2011), risk associated with individual characteristics (Holden & Gitlesen, 2007; Hall, McClintock & Oliver, 2003), as well as broader social factors, such as the individual's support setting (McGill, Bradshaw, Smyth, Hurman & Roy, 2016). It has also been suggested that physiological arousal may act as a determinant, accompanying factor and/or consequence of CB (Cohen, Yoo, Goodwin & Moskowitz, 2011; Groden, Baron & Groden, 2006; Groden, Cautela, Prince & Berryman, 1994; Guess & Carr, 1991; Romanczyk, 1986; Romanczyk & Matthews, 1998;

Romanczyk, Lockshin, & O'Connor, 1992). However, the subsequent sections focus upon two prominent explanatory models: operant learning and behavioural phenotypes.

Environmental influences: the operant learning model. Learning theory has been applied to the understanding of CBs through the field of Applied Behaviour Analysis, which aims to understand human behaviour and to improve socially significant behaviour using robust scientific methodology. ABA originates from Skinner's (1953) work on operant conditioning, in which he hypothesised that all behaviour is selected according to a learned history of its associated consequences; a concept mimicking Darwin's (1872/1978) phylogenic theory, in an ontogenic fashion. According to the operant theory, the likelihood of engaging in CBs, like other behaviours, is increased by a history of contingent access to reinforcement. Broadly, there are two types of reinforcement which may be associated with increases in behaviour:

- Positive reinforcement: contingent presentation of a reinforcing stimulus. Or;
- Negative reinforcement: contingent avoidance or escape from a punishing stimulus.

It is believed that behaviours are initially uncommitted, before coming under operant control as a result of their social (mediated by others) or non-social (when the behaviour itself automatically produces the outcome, as opposed to it relying upon the action of another person) consequences. It is believed that CBs may be inadvertently shaped and reinforced by those in close contact with the individual (Guess & Carr, 1991; Oliver, Murphy, Crayton & Corbett, 1993). That is, by their nature, CBs are perceived as concerning or salient and are likely to elicit a response from others; common responses may include provision of attention (for example, through reprimanding or comforting:

positive reinforcement) or removal of the stimulus or situation thought by the onlooker to be associated with the behaviour (such as a demand: negative reinforcement). These responses are typically successful in briefly reducing the behaviour or mediating its consequences but they may, in fact, be reinforcing for the individual engaging in the behaviour and increase the likelihood of the behaviour occurring again in the future. These types of interactions are particularly clear when the individual lacks adequate communicative skills to allow them to communicate their need or desire for the reinforcer in a more functional manner, meaning these more atypical and salient behaviours may develop a communicative function (McClintock, Hall & Oliver, 2003).

The operant model is a prominent and well-supported theory regarding the aetiology of CBs. Much of the empirical evidence for this model involves the use of experimental functional analysis; systematic manipulation of the individual's external environment and measurement of subsequent behaviour, in order to identify variables which are related to the occurrence of an individual's CB. The initial study employing this methodology demonstrated that SIB is functional for many individuals; it enables control of aspects of their environment (Iwata et al., 1982/1994). It has been well established that CB may serve operant functions. In fact, approximately 92% of published experimental functional analyses have been able to identify an operant function for the assessed behaviour. Typically, behaviours are identified as being maintained by one or more of four broad classes of function: access to attention, access to tangibles, escape from aversive stimulation or non-social (automatic; Beavers et al., 2013). In further support of this operant approach, treatments based upon identified functions have been found to be efficacious (Kurz, Boelter, Jarmolowicz, Chin & Hagopian, 2011).

From the discussion above, it is clear that much of the focus of the behavioural approach is upon influences in the external environment. ABA aims to take a scientific approach to the understanding of behaviour and, as such, focuses on identifying objectively measurable variables associated with the occurrence of behaviour. As such, the emphasis in the operant model has typically been placed on temporally proximal external factors which are more readily quantified and assessed than genetic or biological influences, which may influence behaviour both in the short and long term. This has contributed to the common misconception that behaviourism wilfully ignores or discounts the influence of biological and genetic variables (Todd & Morris, 1983). However, even in the early work in this field, Skinner (1971; 1989) recognises the influence of genetics and biological factors upon behaviour. Despite this, there is a paucity of research incorporating biological and genetic influences into the behavioural approach.

Genetic influences: behavioural phenotypes. Alternative theoretical approaches have instead focussed upon internal factors, such as genetics. It is widely recognised that particular syndromes are associated with characteristic patterns of behaviour, which have been called behavioural phenotypes. A behaviour is considered to be part of the phenotype when those with a given syndrome have a heightened likelihood of exhibiting a given behavioural characteristic, relative to those without the syndrome (Dykens, 1995). According to some definitions of these behavioural phenotypes, these behaviours are an integral part of the syndrome and are explicitly described as being "not learned" (Harris, 1998). Therefore, this more biological approach, taken at its most radical, is incongruent with the operant model described above.

Many genetic syndromes are associated with a heightened risk for engagement in CB, relative to idiopathic ID (Arron et al., 2011). Particular CBs are often described as being part of behavioural phenotypes. The archetypal example of this association is Lesch-Nyhan syndrome (LNS: Lesch & Nyhan, 1964); SIB is one of the cardinal characteristics of the disorder and is exhibited almost universally (Nyhan, 1973). In particular, self-biting is exhibited by over 90% of individuals (Anderson & Ernst, 1994). As such, it is argued that the basic operant theory in isolation is unable to explain this uneven profile of CB across groups, due to presumed random distribution of associated environmental variables across these populations (Arron et al., 2011). This highlights a clear need for acknowledgement and incorporation of genetic variables in explanations of CBs. Therefore, an alternative to the behavioural model is that these behaviours are generated by internal, physiological factors. In the example of LNS, it is believed that self-biting is associated with disorder of the brain's dopamine circuits (for instance; Goldstein, Anderson, Reuben & Dancis, 1985; Breese et al., 1984; Khasnavis et al., 2016). Though further research into the exact mechanisms linking genetically-mediated biological variables and CBs in LNS is warranted (Jinnah, 2009).

Integrating theoretical approaches. As described above, genetic and learning models to explain CBs have often been proposed in opposition to one another, reflecting the historical "nature vs nurture" debate. However, realistically this is a false dichotomy, with evidence suggesting that genetic and environmental approaches to understanding CBs cannot, and should not, be viewed in isolation. Even 'phenotypic behaviours', such as self-biting in LNS, may serve operant functions (Bergen, Holborn, & Scott-Huyghebaert, 2002), and may be successfully managed through behavioural strategies (Olson & Houlihan, 2000). Such findings highlighted the need to develop more

sophisticated explanatory models which consider a broader variety of influences upon CBs, including both internal and external influences.

Importantly, there is no theoretical reason why measurable internal variables cannot be included within the behavioural approach. Skinner (1989) acknowledged in the early literature the role less easily measurable genetic influences upon behaviour. With advancements in technology, the ability to quantify such influences objectively is improving. However, a structure in which to incorporate these types of influences into the operant model was lacking. Motivational differences have been proposed as an integrative concept. It is generally recognised that the occurrence of behaviour not only depends upon an individual's ability or knowledge to display the behaviour, but also their motivation for the consequence of the behaviour, at a particular time. This notion is formally incorporated into the ABA framework through the concept of the motivating operation (MO; Michael, 1982). MOs are environmental changes which serve the dual effect of altering the value of reinforcers or punishers and, in turn, altering the frequency of behaviours which have been historically associated with the relevant stimulus. MOs may either be establishing (increasing both the value of the reinforcer and the frequency of behaviour; termed establishing operations: EOs) or abolishing (decreasing the value and behaviour; termed abolishing operations: AOs; Laraway, Snycerski, Michael & Poling, 2003). Seminal work by Vollmer & Iwata (1991) experimentally demonstrated how the recent lack of access to a reinforcer lead to increases in the target behaviour, relative to when the reinforcer had recently been freely available. This concept has helped to further our understanding of the variation in an individual's behaviour over settings and time.

Although the MO was already well-established elsewhere in the behaviour analysis literature, McGill (1999) was one of the first to examine and apply the concept in detail, with respect to CBs specifically, and to incorporate these additional influences into the understanding of such behaviours. For instance, it was hypothesised that environments deprived of interactions are likely to act as EOs for attention, increasing the frequency of behaviours previously associated with the onset of attention from others, which for some individuals will include CBs. These effects have subsequently been experimentally documented for CBs (for instance: Edrishina, O'Reilly, Sigafoos, Lancioni, & Choi, 2011), demonstrating how an individual's external environment can "set the scene" for the occurrence of CBs. However, the focus of research into MOs has particularly focussed upon transient factors in the individual's external environment, as opposed to more enduring changes which may be associated with genetic variations.

During the nineties it was proposed that the presence of a genetic syndrome may influence the perceived value of certain reinforcers for individuals with the syndrome, similar to the value-altering effect of a MO. Specifically, this idea originated from the apparent raised motivation to access food which is displayed by individuals with Prader-Willi Syndrome (Dykens & Kasari, 1997). Sensitivity Theory (Reiss & Havercamp, 1997) also acknowledges the influence which an individual's "traits" have upon their sensitivity to certain types of reinforcement. These trains of thought highlight how internal events can serve to influence behaviour. However, although these ideas are important, the concepts are somewhat vague, which is in contradiction with the scientific approach of ABA.

Instead, these effects of genetic events or "traits" can be more parsimoniously explained as enduring MOs (Langthorne, McGill & O'Reilly, 2007). Although historically,

MOs have been referred to as short-term environmental influences (Laraway et al., 2003), Kennedy and colleagues (Kennedy, Caruso & Thompson, 2001) proposed an expansion of this concept to incorporate genetic variables which enduringly alter the value of external events. By viewing genetic events as MOs, the idea that individuals have differing traits which cause them to be more sensitive to different reinforcement contingencies (Reiss & Havercamp, 1997) can be brought into the ABA framework and assessed accordingly.

As such, Langthorne and colleagues (2007) formulated a new, integrated model of CB, which uses the dual-concepts of aberrant motivations and aberrant contingencies to explain the occurrence of CBs (Figure 1). The latter is based upon evidence from the behavioural literature demonstrating the role of, predominantly external, reinforcement in creating contingencies which support engagement in CB. In addition, the aberrant motivations concept involves a broad consideration of the influence of MOs on behaviour, from the traditional interpretation of MOs being transient factors in the external environment (challenging environments) to a broader consideration of internal factors as enduring motivating operations (challenging needs). It is proposed that genetic events influence the value assigned to certain types of reinforcer and, thus, the individual's interaction with the environment, over time. In turn, this influences the likelihood of the formation of contingencies supporting engagement in CBs. Of note, Figure 1 refers to a range of other biological events or conditions, not further discussed, which are likely to also influence the occurrence of CB.

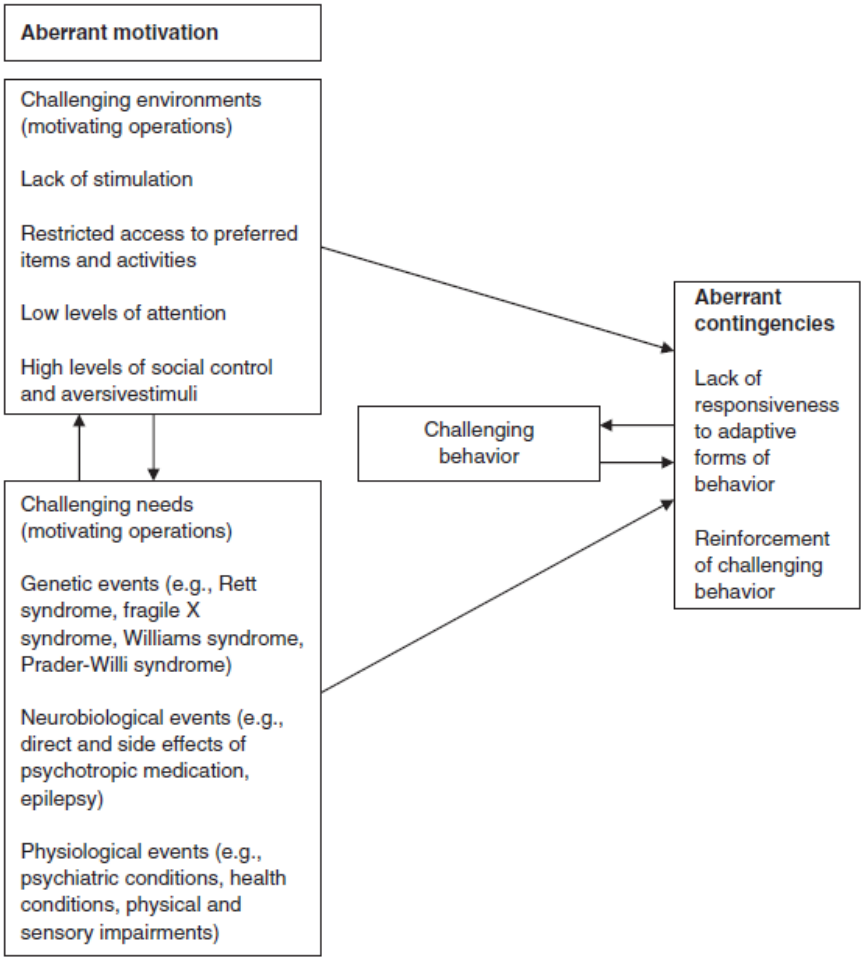

Figure 1. An integrated model of the formation of challenging behaviour (Replicated from: Langthorne et al., 2007, pp. 481)

This broad consideration of causal factors regarding CB is important as a comprehensive understanding of the formation of challenging needs and their contribution to aberrant contingencies and CBs may inform anticipatory environmental manipulations as a preventative strategy, as well as helping to inform and guide behavioural interventions (Langthorne, McGill & O'Reilly, 2007). In addition, as our understanding and tools develop a comprehensive understanding of the influence upon behaviour from all levels (from biological to environmental) may lead to the development of more targeted interventions to directly address the underpinnings of aberrant motivations.

In support of the effect of genetic backgrounds creating challenging needs, using experimental and indirect methods, Langthorne and colleagues observed differences in the prevalence of CBs maintained by different social functions between groups of individuals with different genetic syndromes. For instance, individuals with Fragile X Syndrome (FXS) were found to be less likely to engage in attention-maintained behaviour, when compared to individuals with Smith-Magenis Syndrome (SMS; Langthorne et al., 2011; Langthorne & McGill, 2012; Hardiman, Langthorne & McGill, in press). In addition, individuals with SMS were found to display more behaviour relating to physical discomfort than either comparison group (FXS or idiopathic ID; Langthorne & McGill, 2012). It is proposed that these differences in behavioural function highlight that some types of reinforcement are more valuable for individuals with one syndrome, relative to another. For instance, individuals with SMS are frequently described as being highly motivated by the attention of others, particularly preferred adults. The value of social attention as a reinforcer for individuals with SMS may be enduringly raised, increasing the potential for problem behaviour to occur in conditions of low attention and to be effectively reinforced by attention (Taylor & Oliver, 2008; Langthorne & McGill, 2008).

In an extension of this approach, researchers have begun to draw upon the medical and developmental literature focusing upon physical and cognitive aspects of conditions in order to develop syndrome-specific theories regarding the origins of hypothesized aberrant motivations, or challenging needs. For instance, Oliver and colleagues (2013) suggest a model encompassing phenotype-environment interactions, to explain the high occurrence of CBs, particularly attention-maintained, in SMS (Dykens, Finucane & Gayley, 1997; Smith, Dykens & Greenberg, 1998). It has long been hypothesized that SIB is likely to manifest early in individuals with SMS in response to

discomfort, caused by physical aspects of the syndrome (Finucane et al., 2001). These behaviours, by their nature, are then likely to elicit prompt and reinforcing attention from caregivers and therefore develop a functional element. In addition, Oliver and colleagues highlight that individuals with SMS exhibit other common risk factors for problem behaviour including impulsivity and repetitive behaviours (believed to be related to behavioural disinhibition as a result of yet unspecified brain changes in SMS, suspected to be in the prefrontal cortex: Arron et al., 2011), as well as expressive communication deficits which could lead to decreased availability of, or access to, desired attention or preferred items. In turn, it has been suggested that adults (as opposed to peers) are most likely to consistently understand and respond to approaches, including CBs (i.e. to interpret as an act of communication), making these people particularly reinforcing to the individual. Furthermore, aversion to delay, a characteristic observed in individuals with SMS, may also increase attention-seeking behaviours in situations where attention is not immediately able to be provided by the preferred person. In addition, individuals may learn that approaching adults (particularly those who are familiar) more predictably and quickly brings about a reinforcing response (thus reducing the delay), therefore developing a preference for interacting with these individuals over peers or those who are unfamiliar. It is proposed that these diverse influences may together be associated with the increased levels of attention-maintained behaviour seen in this group. However, little research has been done to link together these influences, beyond this theoretical explanation.

It is clear, despite these important early steps in our understanding of gene-environment interactions that further research is required, and the framework of the integrated model of CB will support this. Therefore, the first steps in investigating this model further will be to conduct further examinations into both within- and between-

syndrome patterns of behavioural function, to identify motivational differences. However, the limitation of the model is that the mechanisms whereby genetic syndromes influence "sensitivity" to reinforcement are not explained, and generally these influences are not well understood. Therefore, once these patterns are identified, research should begin to address the mechanisms whereby particular syndromes influence motivations. Due to the differing aetiologies of the syndromes, such mechanisms are likely to be at least partly specific to individual conditions. As such, further investigations into mechanisms whereby individual genetic syndromes may lead to challenging needs are warranted.

An Introduction to Fragile X Syndrome

In the previous section, it was discussed that particular genetic syndromes are associated with an increased risk for engagement in CB. Fragile X Syndrome (FXS) is one of the genetic syndromes which meet this criterion (for instance, Arron et al., 2011). FXS is a condition with a clearly described behavioural phenotype and with relatively advanced understanding of the genetic and biological underpinnings. As such, this condition provides an ideal example through which to investigate specific mechanisms whereby motivations may be altered in a genetic syndrome, in a way that increases risk for engagement in CB with particular functions. The following section will give a broad overview of the condition before future chapters move on to address CBs in this group (Chapter 2) and possible influences upon this (Chapter 3).

FXS is the most common known inherited cause of ID and the leading known monogenetic cause of autism, affecting approximately 1:4000 males and 1:8000 females (Muhle, Trentacoste & Rapin, 2004; Turner, Wake, Webb & Robinson, 1996; Sherman et al., 2002). The disorder was originally recognised in a small group of males by clinicians

in the early 1940s (Martin & Bell, 1943). Since this discovery, research has provided a substantial amount of knowledge regarding the genetic origin of the disorder and both the associated physical (internal and external) and behavioural phenotypes, which are briefly summarised below.

Genetics and neurobiology. In the late 1960s the understanding of FXS was advanced by the observation of a thin, 'fragile' section of the X-chromosome, in the Xq27.3 region, which gave the syndrome its name (Lubs, 1969). Verkerk and colleagues (1991) later categorized the genetic locus of the disorder further, identifying that those with FXS typically display an expanded CGG repeat on the long arm of the X chromosome, in the 5' untranslated region of the FMR1 gene. The general population have an average of 30 repeats in this region; however, those with FXS have over 200. When the repeat size increases above 55, the gene becomes unstable and prone to expansion during maternal transmission. This may result in children inheriting vastly expanded repeat sizes, relative to the general population. At 200 or more repeats the FMR1 gene becomes abnormally hypermethylated, causing it to be silenced. Depending upon the degree of methylation of the FMR1 gene across the cells in the individual, this leads to either substantial or complete cessation of the production of the gene's protein product: the Fragile X Mental Retardation Protein (FMRP). The lack of FMRP then directly or indirectly leads to the FXS phenotype. Thus, individuals with in excess of 200 CGG repeats are considered to have the full FXS mutation; whereas, those with between 55 and 200 are considered carriers of the premutation (Fu et al., 1991) with its own associated characteristics (Wheeler et al. 2014; Hagerman & Hagerman, 2004; Hall, Leehey, Berry-Kravis & Hagerman, 2016). Genetic testing is available for the condition

(including both the full- and pre-mutation states), typically via a blood test, and is the only sufficient diagnostic tool.

Due to the X-linked nature of FXS, its presentation is quantitatively gender dimorphic: males are typically more severely affected than females (Hagerman & Hagerman, 2002). In females affected by the disorder, one X-chromosome is mutated but the other remains normal. Through the process of X-inactivation in females, one of the two X-chromosomes is inactivated in each cell, which approximately half of the time will be the affected chromosome, thus reducing the impact of the mutation (Lyon, 1961; Berry-Kravis, Potanos, Weinberg, Zhou & Goetz, 2005). However, in males, with their XY genotype, the "fragile" X-chromosome is consistently active, typically leading to greater impairment.

Aside from sex, other genetic factors may create within-group variability in FXS. Individuals may display genetic mosaicism, either in terms of repeat size or in terms of methylation. The former refers to the fact that some individual may display full mutations (>200 CGG repeats) in some cells, but not in others (which may be either clear of FXS of have premutation-size CGG expansions: Nolin et al., 1994). In this case, some cells may be producing FMRP at normal, or near normal levels. Some studies have shown that individuals with repeat mosaicism exhibit milder characteristics (Cohen et al., 1996). Methylation mosaicism refers to cases where some cells in the individual's body, despite having more than 200 CGG repeats (full mutation), are only partially "switched off" (methylated) or not at all. This latter type of mosaicism has also been associated with phenotypic variability (McKonkie-Rosell et al., 1993).

FMRP is a ubiquitously expressed transporter protein which shuttles between cell nuclei and cytoplasm in response to neuronal stimulation (Feng et al., 1997; Irwin,

Galvez & Greenough, 2000), carrying messenger ribonucleic acids (mRNAs) to ribosomes, where the information is decoded to produce specific amino acid chains for protein synthesis (Khandjian, Corbin, Woerly & Rouseau, 1996). As such, the lack of FMRP in FXS leads to the disruption of various pathways of brain development, through disruption of protein production by associated mRNAs. In particular, the mRNAs served by FRMP are largely involved in dendritic structure and function (Feng et al., 1997; Weiler et al., 1997). Evidence from animal research supports that the lack of FMRP in a model of FXS leads to the formation of immature dendritic spines and impaired synaptic plasticity (reviewed by; Schneider, Hagerman & Hessl, 2009). These neuronal differences lead to impairments in learning and memory (Hagerman & Hagerman, 2002). Research demonstrates that FMRP levels are related to developmental outcomes and expression of at least some related symptomatology (Bailey, Hatton, Skinner & Mesibov, 2001; Reiss & Dant, 2003; Dyer-Friedman et al., 2002).

A recent popular theory which has been proposed to help to explain the Fragile X phenotype relates to metabotropic glutamate receptors (mGluRs; Bear, Huber & Warren, 2004). These receptors play an important role in the long-term changes to synapses to allow for learning and memory formation (Bear, 2005). The mGluR theory of FXS suggests that many of the functional consequences of activation of these receptors are hampered by a lack of FMRP, as they rely on translation of FMRP-related mRNAs. In particular, this leads to reduced experiential long-term depression (LTD). This failure to suppress neuronal activation results in widespread neuronal hyper-excitability which, according to the theory, leads to many of the features characteristic of FXS. Though, given the ubiquitous nature of FMRP, it is acknowledged that many other key pathways are also involved.

In addition to the aforementioned changes at the synaptic level, FXS is associated with a broad range of structural (including areas such as the hippocampus, amygdala and cerebellum) and functional brain changes (Hessl, Riviera & Reiss, 2004), which relate to clinically important features (reviewed by: Reiss & Dant, 2003).

Physical phenotype. Males with FXS may exhibit a variety of physical characteristics. With regards to physical appearance, associated features include: large or protruding ears, long and narrow face, prominent forehead, and a high-arched palette may be present (Heulens et al., 2013). Other related features consist of: hyper-extendible joints, poor muscle tone and macroorchidism. These features (less macroorchidism) may also be observed in females but more variably. These features are typically more evident post-puberty (Santos, 1992). However, they are not universal and may be subtle, therefore are not considered to be diagnostically sufficient.

Individuals with FXS are also more prone to certain health conditions such as epilepsy (Berry-Kravis et al., 2010), as well as issues associated with loose connective tissue (mitral valve prolapse, gastrointestinal and digestive issues, strabismus). Recurrent ear infections can also be of high concern, especially in childhood (Kidd et al., 2014). Sleep problems have also been reported in this group (Kronk et al., 2010), which has been corroborated by direct measurement of reduced sleep time, sleep maintenance issues and atypical melatonin profiles (Gould et al., 2000). Finally, motor skills are also often delayed (Kau et al., 2000).

Cognitive and learning features. One of the primary characteristics of FXS is learning difficulty: approximately 90% of males and 50% of females with the full-mutation have IDs (Hessl et al., 2009). Particular challenges may be observed with the processing of sequential information (Burack et al., 1999) and auditory short term

memory (Freund & Reiss, 1991). A syndrome-specific profile of attention and executive function deficits includes: selective attention, divided attention, sustained attention and inhibition (Munir, Cornish & Wilding, 2000). In addition, hyperactivity is seen in approximately 50% of individuals (Alanay et al. 2007). Accordingly, over half of children with FXS (53.7%) have been found to meet the criteria for Attention-Deficit Hyperactivity Disorder (ADHD: Sullivan et al., 2007). Relative strengths are also observed, including: long-term memory retrieval and simultaneous or holistic information processing (Freund & Reiss, 1991). These associated characteristics have important implications for support and education (Braden, Riley, Zoladz, Howell & Berry-Kravis, 2013; Fragile X Society, 2012).

Delays in speech and language are also often observed (Abbeduto, Brady & Kover, 2007), with expressive language typically more affected than receptive (Roberts, Mirrett & Burchinal, 2001). Perseveration (self-repetition of words, phrases and topics; Levy, Gottesman, Borochowitz, Frydman & Sagi, 2006) is a common linguistic feature, as well as high levels of echolalia (Sudhalter & Belser, 2001). As with other features of FXS, these characteristics are typically less clear in females, compared to their male counterparts (Abbeduto et al., 2003).

Behavioural phenotype. Furthermore, individuals carrying the full mutation show marked behavioural features similar to those seen in autism, including: sensory sensitivities; stereotypies such as hand-flapping; shyness and social difficulties, including gaze avoidance (Symons et al., 2010; Lachiewicz, et al., 1994; Bailey, Raspa, Olmsted & Holiday, 2008; Cordiero et al., 2011; Miller et al., 1999). Approximately 25-30% of males and around 6% of females with FXS meet the full diagnostic criteria for autism, with a greater proportion falling on the spectrum (Hatton et al., 2006) As such,

FXS is considered the leading known single-gene cause of autism, underlying about 2-3% of all cases of autism spectrum disorder (ASD: Muhle et al., 2004; Schaefer & Mendelsohn, 2008). Despite these characteristics, in general, those with FXS are described as being sociable and interested in others (Wolff, Gardner, Paccia & Lappen, 1989) with preserved sensitivity to others' facial cues (Simon & Finucane, 1996). As a result, it is hypothesised that, rather than autistic-like behaviours being indicative of social disinterest or a lack of social understanding, phenotypic characteristics such as high levels of anxiety or arousal may lead to these avoidant behaviours (Sudhalter & Belser, 2001). Strikingly, 86.2% of males and 79.6% of females have been found to experience clinically-significant levels of anxiety, which may be generalised, related to social interactions or specific phobias (Cordeiro et al., 2011). Therefore, despite the many similarities between the FXS phenotype and autism symptomatology, these characteristics are often seen as "autistic-like" rather than "truly autistic" (Turk & Graham, 1997) and the applicability of the autism diagnosis to this group has been questioned as a possible category mistake (Hall, Lightbody, Hirt, Rezvani & Reiss, 2010). For instance, it has been found that cognitive ability may confound the assessment and presentation of ASD in FXS, unlike in idiopathic ASD (Abbeduto, McDuffie & Thurman, 2014). However, there remains debate surrounding the nature of the relationship between FXS and autism, with some evidence that those with high levels of autism symptomatology (i.e. meeting the criteria for an autism diagnosis) reflect a distinct sub-group, compared to those with FXS-only (for instance, Roberts et al., 2009). It is also still often asserted that research into FXS may provide valuable insights into non-syndromic ASD (for instance: Belmonte & Bourgeron, 2006).

Additional behavioural features of FXS include CBs such as self-injurious behaviour and aggression, which are reviewed in detail in Chapter 2.

Physiological Arousal. Arousal is a diffuse concept incorporating both overall alertness and that which is secondary to the appearance of emotionally significant stimuli. It has long been hypothesised that atypical regulation of stimulus-bound physiological arousal is central to the behavioural phenotype of FXS (Cohen, 1995). Such hypotheses were based upon the social avoidance, motor and verbal stereotypies and atypical behavioural responses to stressors in this group. Key systems involved in such regulation are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. These distinct but interconnected systems (Young, Abelson & Cameron, 2005; Hinson, 1990; Ottenweller & Meier, 1982) are involved in regulating the body's response to actual or perceived stressors (Ulrich-Lai & Herman, 2009*:* Figure 2). There are a number of ways in which these systems may be implicated in FXS.

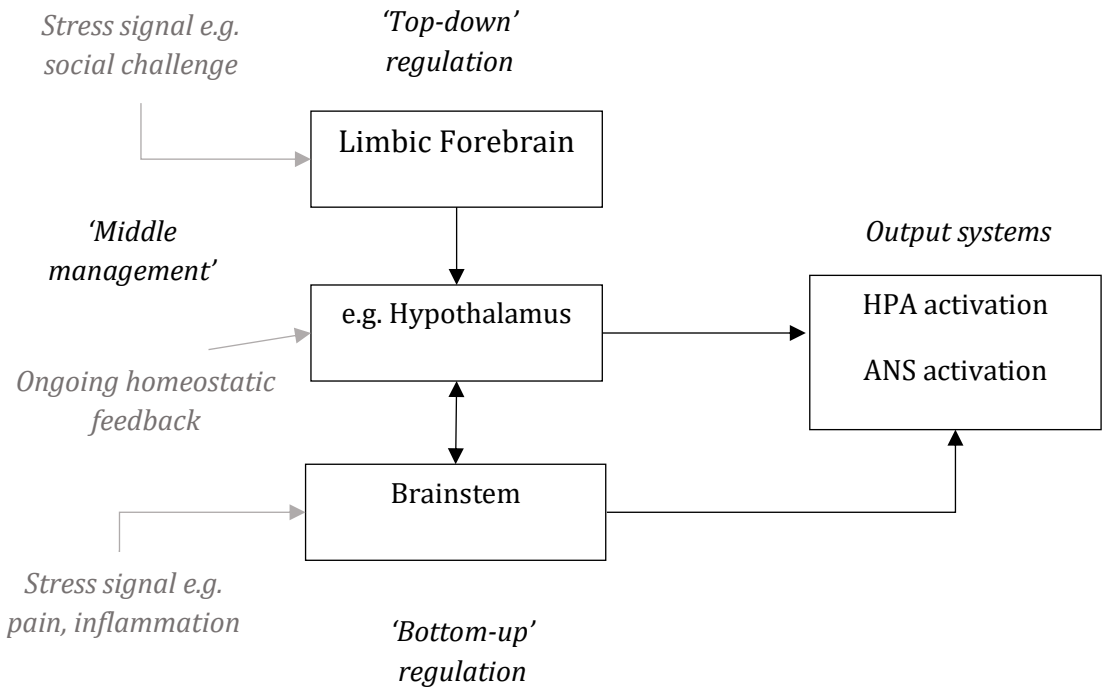

Figure 2. Scheme of brain acute stress-regulatory pathways (based upon: Ulrich-Lai & Herman, 2009)

Firstly, changes to the limbic system (a functionally and structurally connected network of brain areas which coordinate sensory information with higher-order processing centres (LeDoux, 2000)) may affect the emotional evaluation of stimuli. The amygdala is a key component of the limbic system involved in social judgement, anxiety and fear memory (Davis, 1992; Adolphs, Tranel & Damasio, 1998). Research with the FXS mouse model (Paradee et al., 1999; Suvrathan et al., 2010), humans with FXS (Mazzocco et al., 1995) and human Fragile X pre-mutation carriers (Hessl et al., 2006) suggests that amygdala function may be altered in Fragile X. In addition, fear memory formation and long-term potentiation (LTP) in the amygdala are dependent upon mGluR5s (Rodrigues et al., 2002); highlighting a pathway by which this system may be implicated in FXS. Hypothetically, amygdala dysfunction may form part of the

explanation for some of the gaze avoidance (Spezio, Huang, Castelli & Adolphs, 2007), social anxiety or extreme responses to stressors due to atypical fear formation or evaluation of social stimuli. In addition, the hippocampus is a limbic structure involved in memory formation (Lavenex & Amaral, 2000): atypically increased volume of this brain area has been observed in FXS (Kates, Abram, Kaufmann, Breiter & Reiss, 1997). As such, changes to various parts of the limbic system may lead to atypical emotional evaluation of potential stressors that influences physiological responsivity.

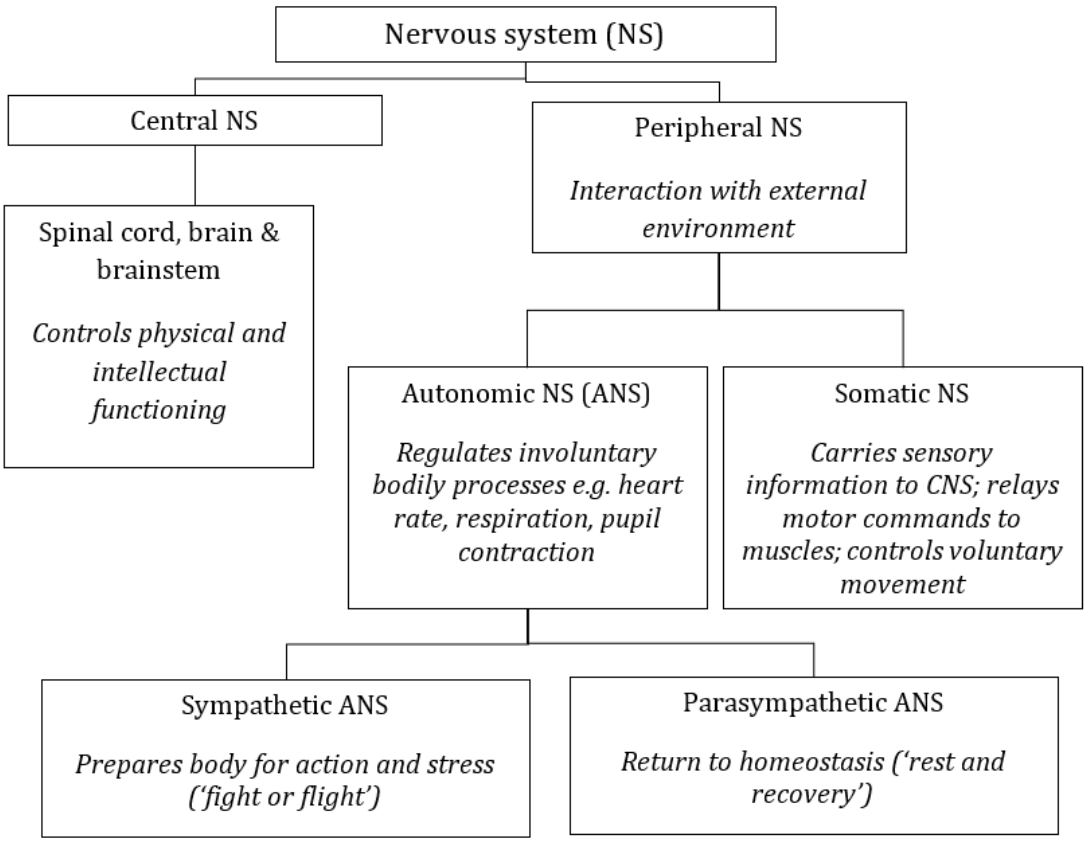

Figure 3. Basic schematic of the nervous system.

The ANS forms part of the peripheral nervous system (Figure 3) and controls the immediate response to challenges, through exerting influence upon a wide range of visceral functions (such as heart and respiration rate, pupillary dilation: Tsigos & Chrousos, 1994). Response of the ANS accompanies increases in arousal of both positive

and negative valence (Kreibig, 2010; Gordis, Granger, Susman & Trickett, 2006). The system has two branches which, for the most part, function antagonistically: the sympathetic branch (sANS) is a predominantly excitatory system (involved in the 'fight or flight' response), whereas the parasympathetic branch (pANS) is responsible for 'calming' or returning the body to homeostasis following increases in sANS arousal. The latter system (in particular, the vagus nerve) appears to be key in behavioural regulation (for instance: Porges & Furman, 2011), including CBs (Manning et al., 2016). In addition, imbalance between these systems is implicated in psychological disorders including anxiety, and ADHD (Klusek, Roberts, & Losh, 2015), features often found in FXS. Findings relating to the ANS in autism are variable (Lydon et al., 2016) which may relate to the existence of both hyper- and hypo-responsive subtypes (Hirstein, Iversen & Ramachandran, 2001). It is possible that changes to these systems are key to the profile of behaviours associated with FXS (Cohen, 1995). For instance, Heilman and colleagues (2011) suggest that atypical autonomic response profiles may result in difficulty with self-calming and contribute to aggression.

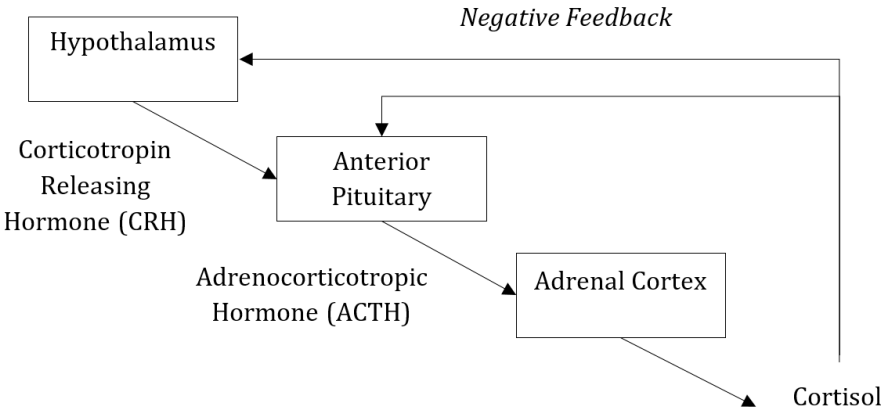

Figure 4. Schematic of the Hypothalamic-Pituitary-Adrenal Axis

The HPA reaction to stressors is slower and is particularly associated with emotional distress or lack of control (Kirschbaum & Hellhammer, 1994). Through a cascade of activity (Jacobson, 2005:

Figure *4*) activation of the axis results in the release of cortisol, which modulates response to stressors over a wide range of time, through effects including the facilitation of energy release (Barett, 2009; Joëls & Baram, 2009). Although temporarily beneficial, long-term effects of cortisol are damaging (McEwen, 1998) and so secretion is controlled by multiple negative feedback loops (Herman & Cullinan, 1997).

There are a number of reasons why the HPA axis may be altered in individuals with FXS. Firstly, investigation of FMRP target mRNAs has highlighted an association with the HPA axis. The absence of FMRP seems to diminish glucocorticoid receptor α (GR-α) numbers (Miyashiro et al., 2003), which may impair negative feedback. Similarly, Annexin 1 (Anx-1), a protein which mediates the inhibition by glucocorticoids on the HPA axis (Jessop, 1999), was synthesised and expressed abnormally in individuals with FXS but not typically developing or intellectually disabled controls (Sun, Cohen and Kaufmann , 2001). The level of dysregulation was closely associated with both FMRP and FMR1 status. Thus, it appears that lack of FMRP may result in excessive activation of the HPA axis, by impairing the negative feedback of glucocorticoids. In addition, wider endocrine investigations in FXS similarly highlight atypical regulation of other hypothalamic-pituitary circuits including atypical hypothalamic-pituitary-thyroid negative feedback (Bregman, Leckman & Ort, 1990) and premature hypothalamic-pituitary-gonadal axis activation (Butler et al., 1988; Kowalczyk et al., 1996; Loesch, Huggins & Hoang, 1995; Moore, Chudley & Winter, 1990). Thus, it appears that the hypothalamus and/or pituitary may be particularly disturbed by the lack of FMRP

(Hessl, Riviera & Reiss, 2004), resulting in subtle endocrine abnormalities as a result of atypical regulation. As a result, HPA axis regulation is thought to be of importance in the FXS phenotype (Hessl et al., 2002).

Of interest in FXS, there exists evidence that cortisol levels are altered in individuals with autism; though variability in the findings indicates that differing sub-groups may exist (Lydon et al., 2016). Abnormality in HPA axis function is one of the most consistent biological findings across a variety of mental disorders (including depression, post-traumatic stress disorder and psychosis) with both hypo- and hyper-activity representing a challenge to wellbeing (Baumeister, Lightman & Pariante, 2014). A paradoxical finding in stress research is that chronic stress may result in suppression of the HPA axis, resulting in hypocortisolism. There is now convincing evidence that the adrenal gland may become hypoactive in some stress-related states (such as Post-Traumatic Stress Disorder or chronic fatigue: Heim, Elhert & Hellhammer, 2000). At this stage, cortisol responses may become blunted and may even decrease in response to stressors (Miller, Chen & Zhou, 2007). Therefore, the relationship between level of cortisol and stress is complex and may be indicated by both hyper- and hypo-secretion.

There are a number of ways in which arousal differences may be associated with behaviour in FXS (based upon: Hessl et al., 2002):

- FXS may lead to behavioural or psychiatric characteristics, via neurodevelopmental changes, which pre-dispose affected individuals to experience greater stress-related affect causing changes in cortisol secretion and ANS activity. For instance, this may occur through changes to the limbic system, or generalised synaptic deregulation.
- Alternatively, FXS might have a direct impact upon the HPA axis and autonomic nervous system, leading to stress-regulation difficulties which, in turn, cause

behaviours characteristic of the syndrome. Evidence of FMRP-associated mRNAs being directly associated with HPA negative feedback supports this approach.

- Finally, there may be a bi-directional relationship between activity of stress-related circuits and behaviour within FXS, whereby both of the above are true.

Summary

The aim of this initial chapter has been to introduce the theoretical background for understanding CBs and to highlight the need to understand and incorporate genetic influences into the understanding of such behaviours, in order to better provide support. Fragile X Syndrome may provide a valuable example through which to explore the pathways by which genetic syndromes influence the operant learning of CBs, via the framework of the Langthorne and colleagues' model (Langthorne, McGill & O'Reilly, 2007). In this initial chapter the syndrome has been broadly introduced, including potential arousal-related changes in this group. In the following chapters the aim will be to address the profiles of CBs observed in this group in more detail, before moving on to considering possible syndrome- specific influences upon these behaviours.

Chapter 2

The Prevalence, Topography and Function of Challenging Behaviour in Fragile X Syndrome: A Systematic Review[1]

Chapter Overview

In the previous chapter, the concept was introduced that genetic variables may influence the operant conditioning of challenging behaviours (CBs) through creating enduring motivational changes. Fragile X Syndrome (FXS) represents an ideal condition through which to explore this theory, given that it is a condition with a well-established behavioural phenotype. In the current chapter, the literature relating to the prevalence, topography and function of CBs exhibited by individuals with FXS is systematically reviewed, with the aim of generating hypotheses to explore aspects of the phenotype which may be associated with their occurrence.

Across studies, a high occurrence of CB was reported, with SIBs being more common than aggression. Males were more likely to engage in CB, compared to females. A within-group pattern of topography was observed whereby self-biting was the most common SIB and hitting was the most common form of physical aggression. Furthermore, CBs were significantly more likely to be negatively reinforced, when compared to other functions. The implications of these findings are discussed in terms

[1] Versions of sections of this chapter are published in:
Hardiman, R. L., & McGill, P. (2017). The topographies and operant functions of challenging behaviours in fragile X syndrome: A systematic review and analysis of existing data. *Journal of Intellectual & Developmental Disability*, 42 (2), 1-14.
Hardiman, R. L., & McGill, P. (2018). How common are challenging behaviours amongst individuals with Fragile X Syndrome? A systematic review. *Research in developmental disabilities, 76,* 99-109.

of furthering the understanding of the interaction between genetic and environmental influences on behaviour in FXS.

Challenging Behaviour in Fragile X Syndrome

FXS is associated with a distinct behavioural phenotype, as outlined in Chapter 1. In addition to the reviewed features such as hyperactivity, repetitive behaviour and social anxiety, behaviours that challenge are also commonly reported in this group (Arron et al., 2011). Notably, carers report that behavioural issues are their most significant concern, when supporting individuals with FXS (Hagerman, 2002). In particular, FXS has been associated with an increased risk for engagement in self-injurious behaviour (SIB) when compared to others with intellectual disabilities (IDs; Arron et al., 2011). Furthermore, physically aggressive outbursts have been reported to be prevalent, particularly amongst males (Wheeler et al., 2016). The aim of this chapter is to provide a comprehensive, systematic review of the literature in order to be able to gain an in-depth understanding of the presentation of behavioural challenges in this group. The findings of this literature review will be used to inform and generate hypotheses in relation to an integrated model (incorporating genetic, physiological and environmental variables), in order to understand the occurrence of CBs in individuals with FXS.

A number of studies have described the prevalence of different classes of CBs within individuals with FXS, such as self-injurious behaviour and aggression. Some studies have described extremely high rates of such behaviours, such as 79% of males with FXS engaging in self-injurious behaviour, and 75% in aggressive behaviour (Hessl et al., 2008). However, the results of individual studies describing the frequency of such behaviours vary widely. In order to better understand the needs of individuals with FXS

and inform future research and discussion, it is important to provide clear epidemiological information regarding these behaviours in FXS. As such, the first aim of this systematic review of the literature was to collate and, therefore, better describe the prevalence of the challenges in males and females with FXS. The findings of this review will then be compared with results from the wider literature relating to CBs in people with intellectual disabilities, in order to identify whether particular challenges may be elevated in individuals with FXS.

Specific topographies of CBs appear to be associated with different neurodevelopmental disorders; such as skin-picking in Prader-Willi syndrome (Dykens & Kasari, 1997) and aggressive grabbing or hair-pulling in Angelman syndrome (Summers, Allison, Lynch & Sandler, 1995). For instance, skin-picking in Prader-Willi syndrome seems to relate to endogenous factors such as itch and pain signalling, and may be the result of a tic-like aberrant 'need to move' in low arousal situations (Klabunde et al., 2015). It has also been proposed that this behaviour relates to a phenotypic obsessive-compulsive insistence on sameness, as blemishes are often targeted (Dykens, Rosner, Martin & King, 1999). In addition, it has been noted that the topographies of physical aggression seen in Angelman Syndrome (AS), which commonly include hair pulling or grabbing, may serve to prolong or initiate social attention, for which individuals with AS are hypothesised to show an enduringly high motivation (Oliver et al., 2013). With regards to FXS, it has been suggested that hand-biting forms part of the behavioural phenotype of the condition (for instance: Hagerman, Amiri & Cronister, 1991). Hand-biting in FXS is often reported to be observed in response to the individuals' "excitement or frustration" (Harris, 2006), though there have not been any more detailed descriptions as to the possible underpinnings of this reported tendency. A review of the existing literature documenting the topographies of CBs may provide data

to clarify the evidence to support a within-syndrome tendency for individuals with FXS to engage in specific topographies of CBs. As such, the second aim of this chapter will be to review studies describing the topographies of different classes of CB displayed by individuals with FXS, and to conduct comparisons to determine whether some topographies of behaviour are more common than others, across the literature. The results of this review will then be considered in light of a model incorporating genetic and environmental influences, in order to highlight possible syndrome- specific factors which may be associated with the distribution of behavioural topographies.

A hypothesis generated from Langthorne and colleagues' (2007) model is that particular functions of behaviour will be more common than others within a certain condition, as a result of genetically-mediated biases in the reinforcing value of particular types of reinforcement, and that the profiles of behavioural function may vary between groups of individuals with different genetic conditions. There are important potential practical applications of this theory. Firstly, whilst not precluding the need for individualised assessments, such biases may direct clinicians as to which environmental influences to investigate as a priority. Secondly, knowledge of altered environmental influences upon behaviour support the development of preventative strategies which are tailored to individuals with particular conditions. For instance, individuals with the condition could be proactively taught an adaptive response to ensure that they are able to access preferred reinforcement (such as attention for people with Angelman Syndrome) appropriately. In addition, carers could be taught to ensure that their responses to CBs minimize inadvertent access to the potent reinforcer. Finally, if a motivational change is found to exist within FXS then this supports the need for research to identify the role played by internal causal mechanisms. The ability to then address aberrant motivations may then reduce the likelihood of individuals engaging in

CBs. As such, the final aim of this review chapter will be to collate information on the function of CB displayed by individuals with FXS in order to be able to identify trends or patterns which may highlight possible motivational differences.

Method

Inclusion & exclusion criteria. The focus of the review was upon three broad classes of CB which are the most commonly reported amongst individuals with intellectual disability: self-injurious behaviour, aggression towards others[2] and destructive behaviour towards property or tangible items.

Manuscripts written in English which included data on humans with a reported diagnosis of Fragile X Syndrome, were included in this systematic review. Where more detailed information was available on genetic status, individuals with mosaicism were included but individuals were excluded if they carried the Fragile X premutation i.e. were reported to have fewer than 200 CGG repeats in the FMR1 region (the diagnostic cut-off for FXS: Verkerk et al., 1991). Data regarding individuals with a diagnosis of a second genetic syndrome, in addition to FXS, were excluded. However, individuals with a diagnosis of autism (in addition to FXS) were included, due to a close association with FXS: approximately 30% of individuals with FXS meet the diagnostic criteria for autism and many more exhibit autistic-like behaviour (for a review, see: Hagerman, 2006). Due to various methods of assessing and reporting, it is not possible to report the prevalence of autism across the samples in this review. Reasons for exclusion during the search

[2] In the prevalence section of the review aggression includes a range of aggressive behaviours, including physical and verbal aggression, due to the broad scope of a number of the common measures. The subsequent sections of the review (topography and function) focus solely upon physical aggression.

process were coded (see Table 1), these codes were tested in a hierarchical order with 1 being tested first, and then the first code on which the manuscript failed was noted.

Table 1

Coding of reasons for exclusion

Rejection Code	Inclusion Criteria	Exclude
1	Manuscript available in English.	Manuscripts not available in English (full text).
2	Human research.	Animal and cell research.
3	Explicitly includes participant(s) with Fragile X Syndrome[3] who do(es) not have additional genetic condition(s).	All human research without participants with FXS, including Fragile X premutation.
4	Original research which includes measure of challenging behaviour relevant to review (see section inclusion descriptions).	Review and conceptual papers. Manuscripts without challenging behaviour measures
5	Data presented or provided by authors with sufficient detail to conduct review analyses, in line with section criteria.	Author does not respond to request for further detail.

[3] It is noted that some studies on challenging behaviour including, for instance, individuals with intellectual disability or autism may have included individuals with FXS without it being noted, however manuscripts were only included for further review, data gathering or inclusion if the inclusion of (a) participant(s) with FXS was specifically recorded.

Rejection Code	Inclusion Criteria	Exclude
6	Other.	Various reasons, including: duplications of the same data published in multiple sources (most recent included, only), insufficient quality of data. Further details provided in results.

Prevalence studies. Published data were included which involved sample sizes of 10 or more individuals reported to have FXS. In addition, in order to be included, the studies were required to have sufficient data to calculate a percentage prevalence of either SIB (including hand-biting), aggression or property destruction. Prevalence statistics of borderline or clinically significant scores on relevant subscales were included. Manuscripts were excluded from this section of the review where participants with FXS had been specifically selected for inclusion because of the presence of CB.

Topography studies. Studies were accepted which included information on the number of participants who engaged in SIB of a particular topography or directed at a particular body site. Studies investigating SIB were not included when they explicitly assessed for only one topography of SIB at a single body site, such as hand-biting, as it was unclear whether either: the same topography of SIB could have also been directed at other body sites (such as biting lip); or other topographies of SIB could have been directed at the same body site (such as skin picking on the hand). Studies were also

included which reported the topography of physically aggressive behaviour or the topography of destructive behaviours (which may cause damage to the individual's physical environment, such as furniture). There was no minimum sample size for inclusion in this section of the review.

Function Studies. In order for data on function to be included, each participant was required to engage in at least one topography of CB being addressed in the review (SIB, physical aggression or property destruction). Evidence regarding behavioural function obtained by direct (experimental or direct observation of individual's behaviour) or indirect (validated questionnaire or interview with parent or caregiver) methods was included. Anecdotal evidence regarding behavioural function was excluded, when it was not assessed via a validated indirect measure. There was no minimum sample size for inclusion in this section of the review.

Requests for further detail. If analysis of a manuscript revealed that data had been collected which could meet review criteria (for instance a standardised measure was used which contained a relevant subscale but only total scores were presented), but were not presented in the manuscript, the review author contacted the corresponding author of the manuscript to request clarification or access to raw data. Initial contacts were followed up once in the event of non-response but were then left and the manuscript was excluded as having insufficient detail (Table 1; criteria 5). If study authors responded with additional data that met review criteria then this was included in the analyses. These cases are noted in the results section.

Literature search. An electronic search was conducted using a search string which included variants on the terms "Fragile X syndrome" and "challenging behaviour". The string required papers to include at least one variant (using the "OR" command) of

the term 'Fragile X Syndrome' (Martin-Bell or Escalante syndrome) and (using the "AND" command) at least one CB-related term, which included: challenging behaviour; problem behaviour; behaviour problems; maladaptive behaviour; aberrant behaviour; self-injurious behaviour; self-injury; self-harm; aggression; aggressive behaviour; disruptive behaviour; destruction of property; or destructive behaviour. Medical Sub Heading (MeSH) terms (in-built additional search vocabulary suggestions) were used, where available in the database. Four databases (PubMed, Web of Science, SCOPUS and PsychINFO) were searched by the first author in November 2012, then updated in April 2017. The search yielded 898 manuscripts, which consisted of 666 unique items (due to database overlap). Unique items were found in all databases, except SCOPUS. Basic details regarding each item identified in the first database search were added to a Microsoft Excel spreadsheet; subsequently, only unique papers (not identified in previous databases) found in further searches were then added to the spreadsheet, in order to identify the total number of unique items.

The titles and abstracts of all 666 unique manuscripts identified in the electronic search then were reviewed to determine potential eligibility and need for further review. After this stage, 202 manuscripts were reviewed to the full text level to determine eligibility. The reference lists of all manuscripts reviewed at the full text stage were examined to identify possible items for review according to titles, which had not been identified in the database search. Four additional manuscripts were examined as a result, two of which were included in the results. The authors of all manuscripts initially rated as having insufficient detail (64: code 5) were contacted to request further detail. Although more replied, only two authors were able to supply additional data which led to the manuscript's inclusion. Examples of "other" reasons for manuscript rejection included: updated data presented in another manuscript, inability

to access full text, and anecdotal report of behavioural function. Finally, 39 manuscripts were included in the systematic review. The full search process is depicted in Figure 5. Of note, there were a number of studies included which contained data relevant to multiple sections of the review (prevalence, topography and/or function). The reliability of the inclusion of papers was checked for 20% of papers in the initial search (17.7% after the later update of the review), by a PhD student at the Tizard Centre with expertise in CB. There was 100% agreement on decisions regarding inclusion and exclusion.

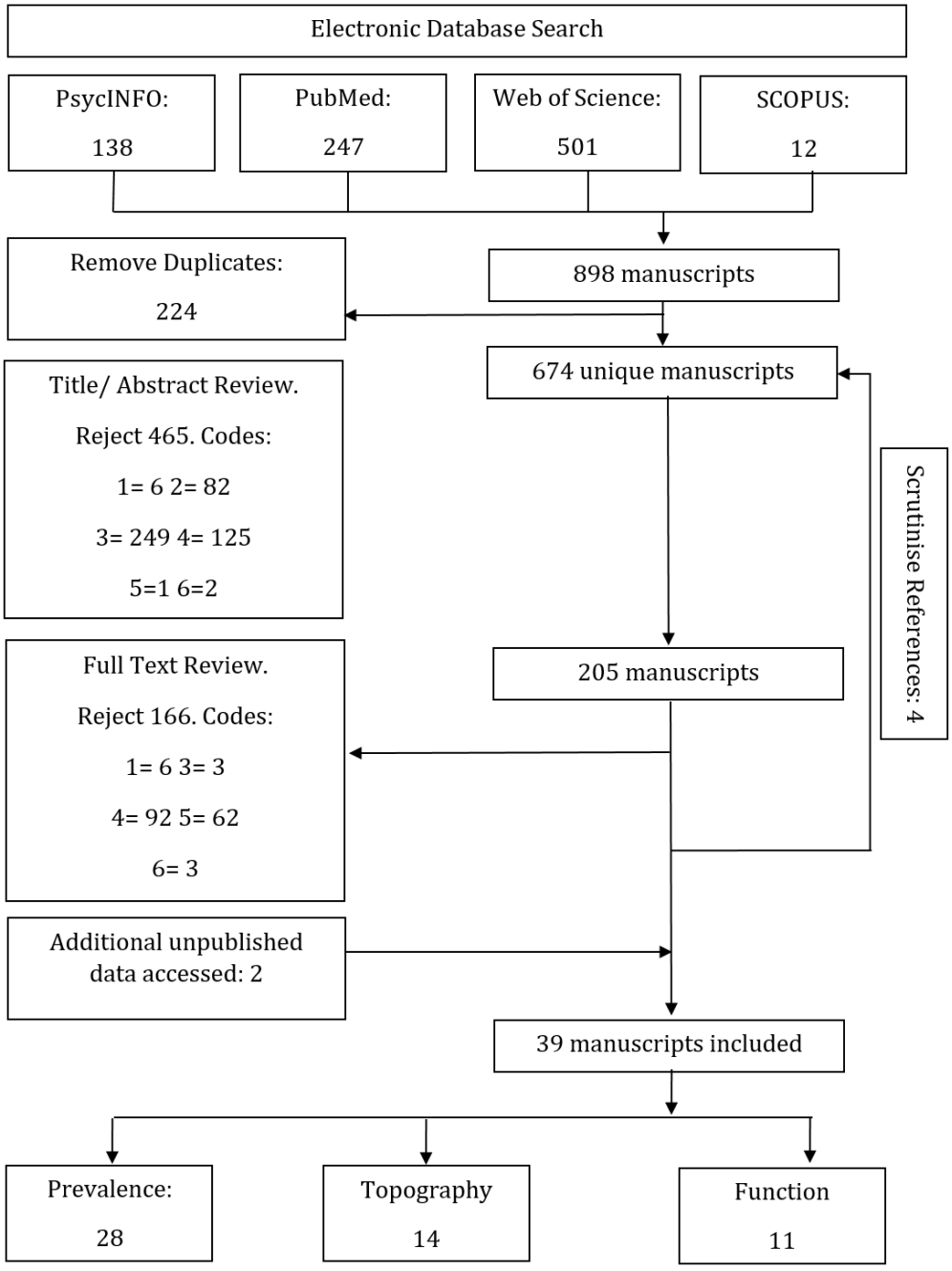

Figure 5. Literature review search process.[4]

Data extraction and analysis. Fifty percent of papers were independently assessed by a second rater to check the reliability of data extraction. Reliability was calculated according to agreement on number of individuals with CB of a particular

[4] See Table 1 for rejection codes.

topography or function in each paper. Initial agreements on individual decisions were 100% for prevalence and function data, and 98.6% for topography data. Final decisions were reached collaboratively for items where raters disagreed.

For papers in all sections of the review basic descriptive information was extracted regarding study participants (number, gender, age) and study method (such as measure used). Across all sections of the review (except behavioural function, for which there was insufficient data relating to females) the findings were split and compared according to participant gender, due to the gender dimorphic severity of presentation of FXS. There were then additional data collection methods for different sections of the review, as described below.

Prevalence. In order to account for the variety of time-frames assessed for occurrence of behaviour, by different measures across studies, prevalence estimates were classified into either point (presence of behaviour evaluated during a set period of up to the past year) or lifetime (evaluation of behaviour over a time longer than the previous year) estimates. In addition, a 'total' summary prevalence statistic was calculated using the results across studies, weighted by study sample size. Where both point and lifetime estimates were available, lifetime estimates were used for total calculations.

Topography. Where information was available, data on the form (for instance: biting, scratching) of SIB and aggression, as well as the body site (for instance: hand, head) of SIB, was recorded. These data were used to calculate a total percentage of participants, out of those who engaged in the relevant class of CB (for instance SIB), who demonstrated a given topography of behaviour. The total percentages were calculated from the number of participants included in studies where the particular topography

was assessed. Where a standardized measure was not used, for instance in a caregiver interview prior to a functional assessment, it was assumed that all topographies of self-injury could have potentially been assessed. Measures used to assess topography are recorded in the tables in the results section. Where a standardized measure was used but the results of all items were not presented, the authors were contacted to request further information. Additional unpublished data about behavioural topography was provided in this respect by Hessl and colleagues (2008).

Exact topographies were then grouped into categories for this review, in order to be able to compare findings across studies. For instance, hitting self with body and hitting self with or against object were collapsed into self-injurious 'hitting'. However, topographies of behaviour were originally grouped differently across individual studies, leading to some uncertainty about the exact number of participants fitting into each review category. If clarification was unavailable after contacting the study authors, the available data were merged to best fit the study categories, acknowledging the potential variation in estimate which this may cause. Specifically, both the maximum and minimum prevalence of a class of behavioural topography (such as self-hitting) was calculated by assuming that cases of the sub-categories of the behaviour (such as hitting self with body and hitting self against object) were either entirely non-overlapping (for instance, none of the participants who hit their bodies were the same as those who hit their heads) or entirely overlapping (all of the participants who hit their bodies also hit their heads), respectively. These potential variations in prevalence estimates are represented as error bars on the graphs.

Function. Conclusions about behavioural functions made in studies were accepted. Where multiple assessments were conducted for an individual participant (for

instance, a questionnaire measure and an experimental measure: Langthorne et al., 2011; Machalicek et al., 2014), the results of the direct measure were used when compiling the findings across studies. The exact functions from studies were noted but, in order to facilitate comparison across studies, functions were also assigned to classes:

- Attention: the individual's behaviour was reported to be associated with the provision of attention
- Social positive (other): the individual's behaviour was reported to be associated with the addition or increase of a reinforcer, other than attention alone, via another person. This included provision of tangible items or adult compliance with mands.
- Social negative: the individual's behaviour was reported to be associated with escape from or avoidance of a situation, such as the presentation of a demand, a social interaction, or a transition.
- Non-social: the individual's behaviour was reported to be associated with internal factors, such as pain or discomfort, or the behaviour itself appeared to be automatically positively reinforcing (indicated, for instance, by it occurring when the individual is alone).

These classes were selected as they correspond closely with the basic functions widely assessed through experimental functional analyses (Iwata et al. 1982/1994).

As with the topography data, this classification resulted in some uncertainty as to the exact prevalence of behaviours serving each class of function, due to variation in categorisation of functions across different papers. Raw data from Langthorne and McGill (2012) was reanalysed to determine whether each participant showed any

topography of behaviour with a particular function; in the original publication the functions for different classes of CB (SIB, aggression and property destruction) were presented at the group level.

The aggregated data give information on the number of individuals with CB at least partly maintained by a particular type of reinforcer. Where individuals had behaviours with multiple functions, they were counted in all relevant categories.

Data analysis. The statistical significance of prevalence differences (for example between males and females) was assessed using Two-sided Tests for the Difference between Proportions (Clarke & Cooke, 2004). This test was selected following consultation with a statistics professional (Dr Diana Cole, Senior Lecturer in Statistics, University of Kent) due to the partially-overlapping sample groups and non-independent behaviour categories. The following formula was inputted into Microsoft Excel 2013 (where: n_1= total number assessed in sample 1, n_2= total number assessed in sample 2; p_1= decimal proportion of individuals assessed who exhibit behaviour of interest in sample 1; p_2= decimal proportion of individuals assessed who exhibit behaviour of interest in sample 2):

$$p = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2} \qquad W = \frac{p_1 - p_2}{\sqrt{\frac{p(1-p)}{n_1} + \frac{p(1-p)}{n_2}}}$$

A p-value was then obtained to evaluate the significance of W using the Excel formula: 1-NORMDIST((cell),0,1,TRUE). A p-value which reached a level of significance indicates that there is a significant difference between the percentage prevalence of the two behaviours.

Where there existed potential variations in the prevalence estimates, comparisons were conducted on the smallest possible difference, in order to minimize type I errors. However, where non-significant findings were obtained, a second test was conducted using the maximum potential difference, in order to evaluate the robustness of the finding. Unless otherwise reported, the comparisons yielded non-significant results for both the maximum and minimum differences. Destructive behaviour was not included in these comparisons, due to the small number of studies identified addressing the subject.

Results

Prevalence of challenging behaviours. The individual results of included studies are summarized in Table 2. In total 28 papers were included in this section of the review. Sixteen studies assessed the prevalence of SIBs, eight of which included male-only samples, the remainder included both male and female participants (four presented compound results, four separated). In addition, five studies were identified which assessed hand-biting as a specific form of SIB (two assessed both males and females, two evaluated only females, and one assessed males). Aggressive behaviour was assessed in 20 studies: eight studies had male-only samples, one had a female-only sample and the remaining eleven included participants of both gender (six presented separated results, five presented compound results). Destructive behaviour was assessed less frequently: one study included male samples and the other two provided compound results from mixed gender samples. The ranges and total estimates from these studies are presented in Table 3.

Table 2

Prevalence of challenging behaviour in FXS

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
Arron et al., (2011)	CBQ (Hyman, Oliver & Hall, (2002))[5]	191	100	Mean 16.57 (SD[6] 8.81)	Point	51.3	52.1	-
Bailey et al., (2012)	Specially developed items to measure proportion of caregivers who have sustained at least one injury inflicted by child.	350	83.4	Mean 19.5 (Range 5-66)	Point	-	Males 31; females 17.	-
Bailey et al., (2008)	"Has ____ ever been treated by a	1235	79.02	6+	Lifetime	Males 41; females 10; total 34.5.	Males 38; females 14; total 32.96.	-

[5] CBQ: Challenging Behaviour Questionnaire
[6] SD: Standard Deviation

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	professional for…?"							
Cronister et al., (1991)	Parent interview	100	0	Mean 32.02	Lifetime	Hand biting: 9	-	-
Dykens, Hodapp & Leckman (1989)	VABS[7] (Sparrow et al., 1984) "too physically aggressive" item.	27	100	Mean 27.4 (Range 3-51)	Unclear	-	33.3	
Eden, de Vries, Moss, Richards & Oliver, (2014)	CBQ	112	100	Mean 10.88 (SD 2.58)	Point	54.5	60.9	-
Fryns, Jacobs, Kleczkowska & Berghe (1984)	'Systematic extensive psychological and	21	100	Mean 9.24 (Range 2-21)	Unclear	Hand-biting 38.1[8]	-	-

[7] VABS: Vineland Adaptive Behavior Scales
[8] Overall SIB data excluded due to discrepancy in data between table and text

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	socio-familial investigation'							
Gillberg et al., (1986)	"meticulously examined clinically by a child physician"	10	100	Range 2-17	Unclear	50	-	-
Gray et al., (2005)[9]	Clinically significant scores and items ABC[10]: Aman, et al., 1985) & CBCL[11] (Achenbach, 1991) combined[12].	57	100	Mean 4.7	Point	31	13	-

[9] Published conference abstract
[10] ABC: Aberrant Behavior Checklist
[11] Child Behavior Checklist
[12] The method of combining the results from the different measures was not expanded upon.

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
Hagerman, (2002)[13]	Parent interview	306	78.1	N/A[14]	Unclear	Hand-Biting: males 50.21; females 20.9; total 43.79.	Males 43.5; females 22.4; total 38.8.	-
Hagerman et al., (1992)	Parent interview	30	0	Mean 8 (range 1-18)	Lifetime	Hand-biting 23.3[15]	-	-
Hall et al., (2006)	Observation of hand-biting during a social demand task	114	64.9	Range 6-17. Male mean: 11.06 (SD 2.68), Female Mean:	Point	Males 25.68; females 15; total 21.93.	-	-

[13] Updated data from Merenstein et al. (1996).
[14] N/A: Not Available

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
				10.42 (SD 3.10)				
Hall et al., (2008)	Self-injury checklist (Bodfish et al., 1995).	60	51.7	Mean 13. 14	Point	Males 58.1; females 17.2; total 38.3.	-	-
Hartley et al., (2011)	Parents asked if child ever diagnosed with or treated for behavioural issue.	328	72.9	Mean 31.14	Lifetime	Males 47.26; females 16.67; total 38.41	Males 43.04; females 12.79; total 34.45.	-
Hartley et al., (2012)	Telephone interview based on Scales of Independent Behaviors (revised). Rate presence/absence behaviour each	76	82.9	Mean 21.4 (12+)	Point	16.9	15.6	14.3

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	day during 8-day diary study							
Hatton et al., (2002)	Clinically significant scores on aggression subscale of CBCL	59	100	Mean 7.22 (SD 2.03)	Point	-	17.6 (+8 borderline)	-
Hessl et al., (2001)	Clinically significant scores on aggression subscale of CBCL	119	66.4	Mean 10.76 (SD 2.83)	Point		Males 12.7; females 12.5; total 12.61.	
Hessl et al. (2008)[16]	BPI[17] (Rojahn et al., 2001)	50	100	Mean 15.6 (SD 4.3)	Point	79	75	36.17[18]
Lachiewicz, (1992)	Clinically significant scores	38	0	Mean 7.43 (Range 4.5-11.9)	Point	-	18	-

[16] Additional unpublished data supplied by author
[17] BPI: Behavior Problem Inventory
[18] Data available for 47/50 participants

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	on aggression subscale of CBCL							
Largo & Schinzel, (1985)	Non-specified parent interview	13	100	Mean 6.5 (Range 2.6-12.5)	Unclear	38.5	53.8	-
Newman, Leader, Chen & Mannion (2014)	BPI-S[19] (Rojahn et al., 2012)	47	75	Mean 7.84 (SD 4.19, Range 2-17)	Point	80.9	85.1	-
Pegoraro, Steiner, Celeri, Banzato & Dalgalarrondo (2014)	Examination of medical charts: data gathered from parent interview	13	92.3	Mean 12 (SD 3)	Unclear	23	53	-

[19] BPI-S: Behavior Problems Inventory-Short Form

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
Reilly, Murtagh & Senior (2015)	Parents rate presence of "challenging aspects" including physical aggression	115	81.7	Mean 11.58	Unclear	-	41	-
Richards et al. (2012)	CBQ: SIB items.	212	100	Mean 15.3 (Range 6-47)	Point	54.5	-	-
Symons et al. (2003)	Self-injury questionnaire (occurrence, age of onset, forms, function (modified from O'Neill et al (1997))	55	100	Mean 6.6 (Range 1.7-12)	Lifetime and point	58 lifetime prevalence (81 of which had continued in past month)	-	-

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
Symons et al. (2010)	SIB: Questionnaire based, in part, on the Self-Injury domain from the RBS-R[20] and a previous SIB and FXS survey (Symons et al., 2003). Aggression: sub-set parents asked one question on historical	1394	Overall 78.2; Lifetime= 78.06; past 30 days 78%[21]	N/A	Lifetime and point	Lifetime[22]: males 41; females 16.7; total 35.7. Past 30 days[23]: males 32; females 11.4; total N/A[24].	Lifetime: males (N=516) 39.75; females (N=96) 18.6; total (N=612)[25] 36.4.	-

[20] RBS-R: Repetitive Behavior Scales–Revised (Bodfish et al., 2000)
[21] There may be small variations in this percentage due to uncertainties about number of missing data items.
[22] SIB lifetime prevalence data available for 1363/1394 participants
[23] SIB data for past 30 days available for 1293/1394 participants
[24] Note: Total cannot be calculated as the proportion of males and females are unclear for the sample for which point prevalence data is available
[25] Data from matched pairs of FXS participants with and without SIB. Matched on gender, age, mutation status and family income.

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	presence or absence of aggression.							
Wheeler et al. (2015)	Parents rated at least one physically aggressive act in past 12 months	774	82.9	Male Mean 19.80 (SD =11.41; range = 3-67); Female mean 16.33 (SD = 9.85; range =3-48)	Point	-	Males 92; females 83; total 90.4.	-
	Parents rated whether diagnosed or	"	"	"	Lifetime	-	Males 38; females 18.	-

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	treated for aggression[26]							
	Proportion of parents sustaining injuries from child. [27]	"	"	"	Point	-	Males 31; females 13.	-
Valdovinos, Parsa & Alexander (2009)	"Has the individual had problems with any of the following behaviours?" Options including: Aggression; Self-injurious behaviour (SIB) (hand biting, skin	392	N/A	N/A	Lifetime	42.9	36.0	21.1

[26] These data were the figures used from Wheeler et al. (2015) in the total calculations across studies.
[27] Further measures of aggression were collected, including peer injuries.

Study	Relevant Measure(s)	FXS Participants			Time-frame of measure	Prevalence SIB (%)	Prevalence Aggression (%)	Prevalence Destructive Behaviour (%)
		Number	% Male	Age (Years)				
	picking, head hitting, etc.)							

Total sample prevalence estimates. Across all studies and participants, the prevalence was 48.8% for SIB (32% for hand-biting, specifically), 35.81% for aggression and 24.5% for destruction (Table 3).

Gender comparisons. Males were significantly more likely than females with FXS to engage in SIB (W=18.43, n=3686, p<.0001; including hand-biting: W=8.75, n=571, p<.0001) and aggression (W=17.15, n=4318, p<.0001)**.**

Behaviour type comparisons. A significantly greater proportion of males in the total samples studied engaged in SIB than aggression (W=4.57 n=6549, p<.0001); however there was no significant difference in the smaller population of females with FXS studied (W=.15, n=1455, p=.88).

Table 3

Summarised prevalence estimates of challenging behaviours in individuals with FXS.

Topography	Study Estimate Range (%)		Entire sample estimate and size					
	Male	Female	Male		Female		Total	
			%	N	%	N	%	N
SIB	31-79	10-17.2	44.6	3010	14.2	676	48.8	4245
Hand Biting	25.7-50.2	9-23.3	44	334	15.2	237	32	571
Aggression	12.7-85.1	12.5-22.4	40.2	3539	13.9	779	35.81	4140
Destruction	36.2	-	36.2	47	-	-	24.5	515

Influence of study methodology.

Point and lifetime prevalence estimates. Mean prevalence estimates were higher for studies using lifetime estimates for both SIB (point (N=2926): 31.96%; lifetime (N=2153): 35.56%: W=3.27, N=5079, p<.005) or aggression (Figure 6. Point (N=1923): 33.37%, lifetime (N=1351): 36.88%. W=2.55, N=3274, p<.05).

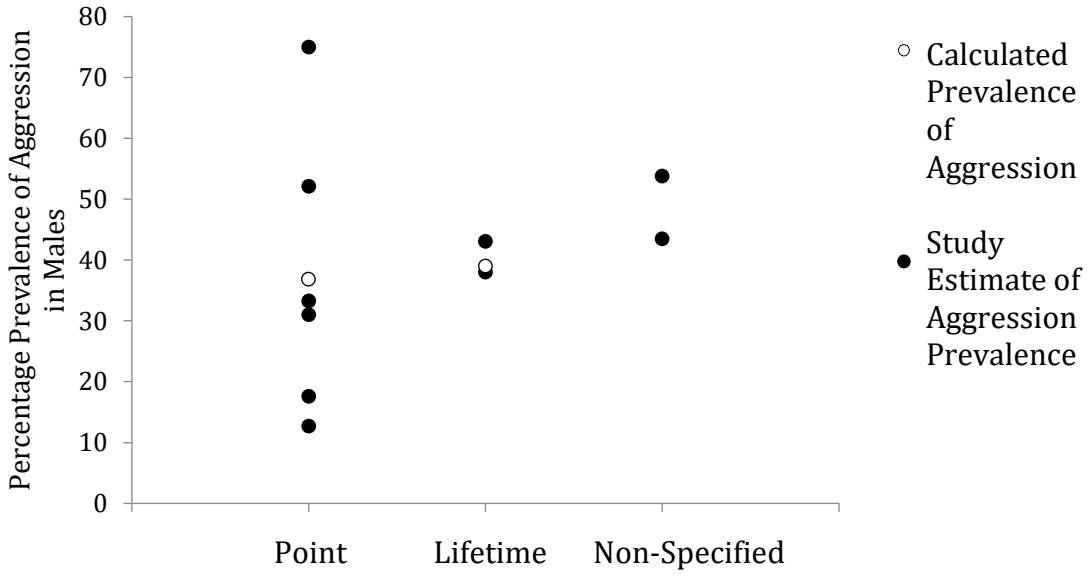

Figure 6. Prevalence estimates obtained by studies assessing over a set period (point) or over the individual's lifetime.

Study sample size. The sample size of studies reporting the prevalence of SIB ranged from ten (Gillberg et al., 1986) to 1394 (Symons et al., 2010). Studies reporting the prevalence of aggressive behaviours included sample sizes of between 13 (Largo & Schinzel, 1985) and 976 (Bailey et al., 2008). There was no correlation between sample size and prevalence estimate for SIB (r_p=-.342, n=12, p=.28) or aggression (r_p=.037, n=12, p=.91). However, visual analysis of the male data for both types of CB shows increased variability of estimates in studies with smaller sample sizes (see Figure 7 for SIB example). Such a trend highlights the issue of drawing conclusions about prevalence

from studies employing smaller sample sizes.

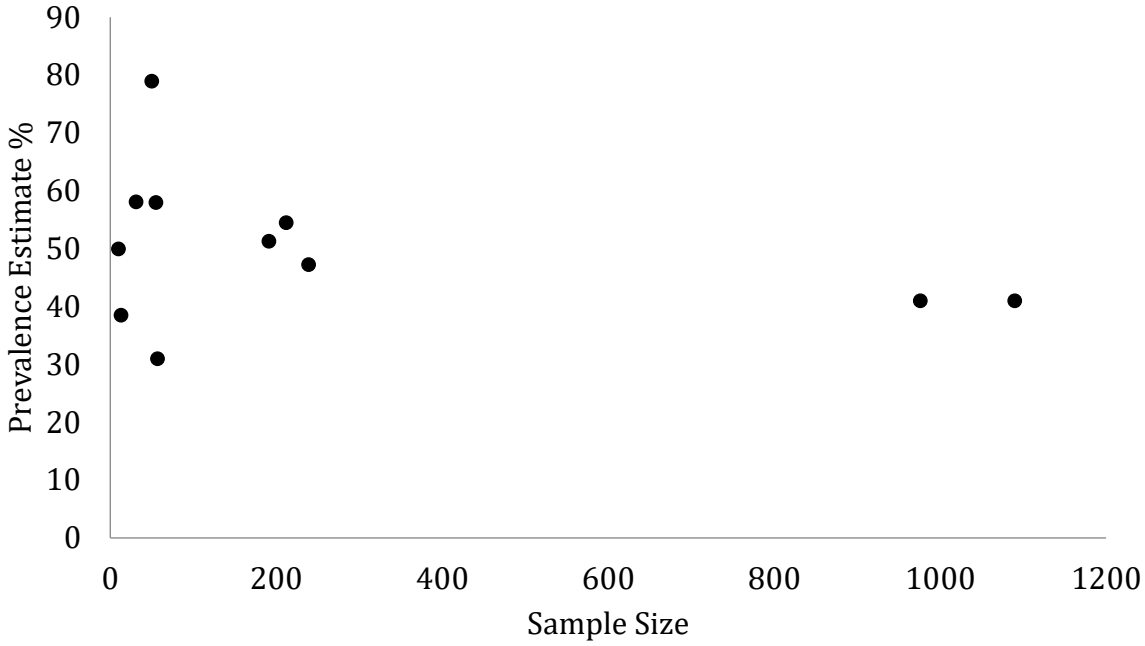

Figure 7. Variation in prevalence estimates according to study sample size.

Topography of challenging behaviour. Fourteen manuscripts were identified in which the topography of self-injurious (including body location), physically aggressive or destructive behaviours were described.

Self-injurious behaviour: topography.

Studies. Fourteen studies reported the topography of SIBs in males with FXS (Table 4); two studies provided data for females (Table 5). A variety of different measures were used to assess the topographies of SIBs (Table 6). Studies explicitly assessing only hand-biting were excluded from this analysis because it was unclear whether other participants may have also bit themselves at another body site, thus under estimating the overall prevalence of self-biting.

Table 4

Topography of SIBs in males with Fragile X Syndrome.

		Number of Participants with Each Topography of SIB											
Study	Study N with SIB	Hitting self (with body or with/ against object)	Biting self	Pulling or picking (Hair/ Skin)	Rubbing/ Scratching	Inserting Objects into Body Openings	Teeth Grinding	Extreme Liquid Drinking	Aerophagia (Excessive Air Swallowing)	Vomiting	Pica	Pulling Nails	Pinching
Hagerman (2002)	1	0	1	0	0	0	0	0	0	0	0	0	0
Hagopian et al. (2004)	1	1	1	0	0	0	0	0	0	0	0	0	0
Hall et al. (2008)	18	5	14	1	7	0	-	-	-	-	-	-	-
Hessl et al. (2008)*	40	25	15	4-7	13-6	3-6	13-5	7-10	1-4	7-10	9-12	5-8	4-7
Kurtz et al. (2015)	8	7	5	0	2	0	0	0	0	0	0	0	0
Langthorne et al. (2011)	8	6	1	0	0	0	0	0	0	0	0	0	0

Study	Study N with SIB	Number of Participants with Each Topography of SIB											
		Hitting self (with body or with/ against object)	Biting self	Pulling or picking (Hair/ Skin)	Rubbing/ Scratching	Inserting Objects into Body Openings	Teeth Grinding	Extreme Liquid Drinking	Aerophagia (Excessive Air Swallowing)	Vomiting	Pica	Pulling Nails	Pinching
Largo & Schinzel (1985)	5	1	4	0	0	0	0	0	0	0	0	0	0
Levitas et al. (1983)	6	-	5	1	-	-	-	-	-	-	-	-	-
Machalicek et al. (2014)	6	5	3	0	0	0	0	0	0	0	0	0	0
Moscowitz et al. (2011)	3	1	2	0	0	0	0	0	0	0	0	0	0
Richards et al. (2012)	67	49	59	18	18	10	-	-	-	-	-	-	-
Sheldon & Turk (2000)	2	2	2	0	0	0	0	0	0	0	0	0	0
Symons et al. (2003)	32	13-19	23-25	11	6	2	-	-	-	-	-	-	-
Symons et al. (2010)	433-6	171-2	301-3	112	130-1	-	-	-	-	-	-	-	-

		Number of Participants with Each Topography of SIB											
Study	Study N with SIB	Hitting self (with body or with/ against object)	Biting self	Pulling or picking (Hair/ Skin)	Rubbing/ Scratching	Inserting Objects into Body Openings	Teeth Grinding	Extreme Liquid Drinking	Aerophagia (Excessive Air Swallowing)	Vomiting	Pica	Pulling Nails	Pinching
Minimum Total	630	268	435	147	176	15	12	7	1	7	9	5	4
Maximum Total	633	293	440	150	180	18	15	10	4	10	12	8	7
Minimum proportion of those assessed for SIB topography (%)		42.7	68.7	23.2	28.0	7.9	16.2	9.5	1.4	9.5	12.5	6.9	5.5
Potential Variance (+%)		4.3	1.1	0.6	0.8	1.5	4.2	4.2	4.2	4.2	4.2	4.2	4.2

* = Additional unpublished data provided; - = Not assessed in study

Male summary. Across the studies, between 630 and 633 males with SIB were included. There were statistically significant differences between the proportions of males who were reported to show each of the four topographies of SIB, which were assessed in all studies. Biting was significantly more likely to be endorsed as being present than all other topographies (compared to hitting: W=9.96, n=1257, p<.00005. Bonferroni adjusted alpha=.008); hitting was more likely to be rated as present than pulling (W=8.65, n=1257, p<.0005) or scratching (W=6.34, n=1251, p<.0005); there was no difference in the number of participants rated as engaging in pulling or scratching.

Table 5

Individual study and review findings regarding the topography of SIBs in females with Fragile X Syndrome.

		Number of Participants with Each SIB Topography			
Study	Study N with SIB	Hitting	Biting	Pulling/ picking (Hair/ Skin)	Rubbing/ scratching
Hall et al. (2008)	5	0	2	1	4
Symons et al. (2010)	48-51	25-27	24-26	20-21	15-16
Total	53-56	25-27	27-29	21-22	19-20
Minimum Proportion of those assessed for SIB topography (%)		44.6	48.2	37.5	33.9
Potential Error (+%)		6.3	6.5	4.0	3.8

Female summary. In total, fewer different topographies of SIB were assessed in females with FXS, therefore the prevalence of other topographies of SIB (such as teeth-

grinding, vomiting and pica) is unclear. Percentages of the four topographies of SIB which were assessed in both studies are displayed alongside the male data in Figure 8. Unlike males with FXS, there were no significant differences between the proportions of those assessed who engaged in the different topographies of SIB.

Table 6

Study measures for assessing topography of SIB

Study	Measure used to Assess Topography of SIB
Hagerman (2002)	Clinical examination
Hagopian et al. (2004)	Parent report (functional analysis)
Hall et al. (2008)	Self-Injury Checklist (Bodfish et al., 1995)
Hessl et al. (2008)	Behavior Problems Inventory (BPI; Rojahn et al., 2001)
Kurtz et al. (2015)	Clinical examination (Caregiver interview and pre-analysis direct observation)
Langthorne et al. (2011)	Clinical examination (Parent report prior to functional analysis)
Largo & Schinzel (1985)	Clinical examination
Levitas et al. (1983)	Clinical examination
Machalicek et al. (2014)	Clinical examination (Parent report prior to functional analysis)
Moskowitz et al. (2011)	Parent Functional Assessment Interview
Richards et al. (2012)	Challenging Behaviour Questionnaire (Hyman et al., 2002)
Sheldon & Turk (2000)	Clinical examination

Symons et al. (2003)	Self-Injury Questionnaire based upon Functional Assessment Interview (O'Neill et al., 1990)
Symons et al. (2010)	Self-Injury Questionnaire based upon Symons et al. (2003)

Gender comparisons. There was no significant difference between the proportion of males or females who self-scratched or self-hit. In contrast, males were significantly more likely to self-bite than females (Bonferroni alpha= 0.0125. Minimum difference: W=2.53, n=686, p=.011. Maximum difference: W=4.01, n=686, p<.001). In addition, a higher percentage of females self-picked, compared to males (minimum difference: W=.2.53, n=686, p=0.011; Figure 8).

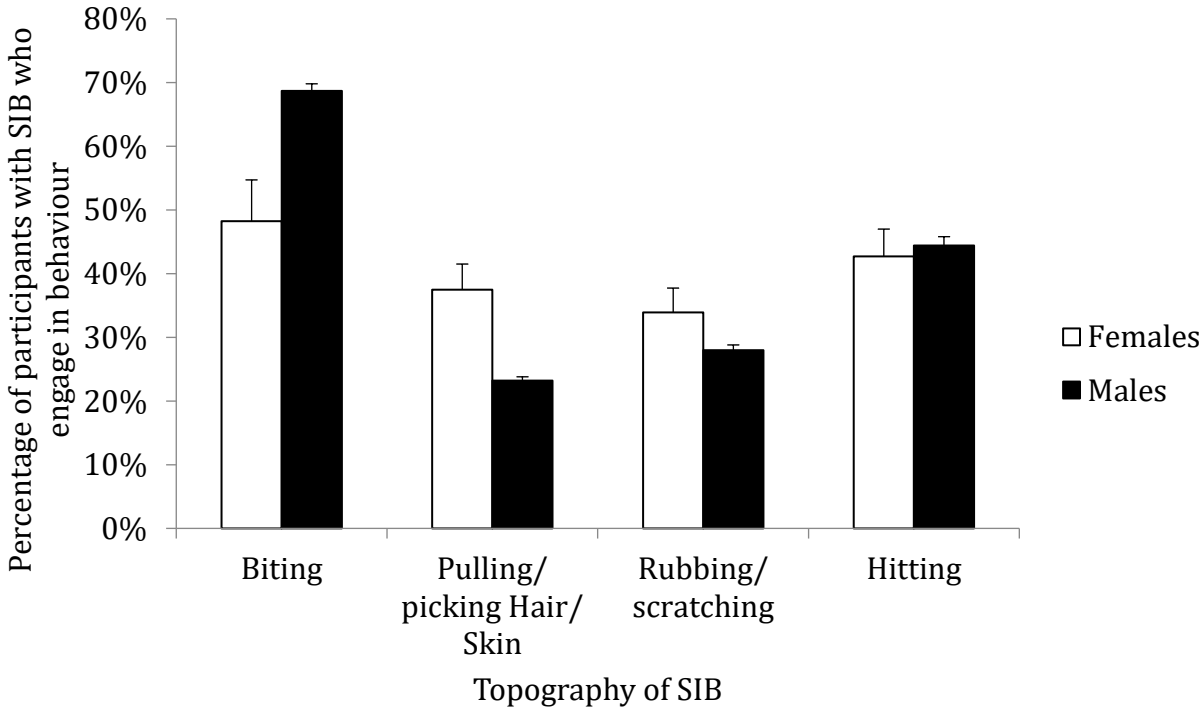

Figure 8. Comparison of SIB topography of males and females with FXS

Self-injurious behaviour: body site.

Studies. Eight studies included data on the body sites of male participants' self-injurious behaviour (Table 7) and one for females (Symons et al., 2010). All studies were deemed to have potentially assessed SIB in all body sites. Studies reporting the prevalence of hand-biting in individuals with FXS were excluded from this analysis due to uncertainty about whether other topographies of SIB were also directed at the hand.

Male summary. In the total sample of 497 males assessed across the seven studies, SIB was significantly most commonly towards the hand or arm followed by the head ($W=13.506$, $n=994$, $p<.0005$; Bonferroni adjusted alpha .008). More male participants injured their head than their legs ($W=13.132$, $n=994$, $p<.0005$) or torsos ($W=11.464$, $n=994$, $p<.0005$); there was not a significant difference in the number of males with FXS who directed their SIB to their legs or torsos.

Female summary. As reported by Symons and colleagues (2010), who investigated 51 females with SIB, the most common body site for SIB in females is towards the arm or hand (75.5%), followed by the head (51%; $W=3.19$, $n=102$, $p<.001$; Bonferroni adjusted alpha=.008). In turn SIB was more common towards the head than legs or feet (30.6%; $W=2.66$, $n=102$, $p<.001$) then torso (18.4%). There was no significant difference between rates of SIB directed towards legs/ feet and torso.

Gender comparison. Gender differences for head, arm and torso were not significant. A higher proportion of females injured their legs than males (Bonferroni adjusted alpha=.0125. $W=2.65$, $n=548$, $p<.01$).

Table 7

Body location of SIBs in males with Fragile X Syndrome.

Study	Assessment Method	N with SIB	Number of Participants with SIB at Body Location			
			Head	Hand/Arm	Leg/Feet	Torso
Hagerman (2002)	Clinical examination	1	0	1	0	0
Hagopian et al. (2004)	Clinical examination	1	1	1	0	0
Langthorne et al. (2011)	Clinical examination	8	2	6	0	0
Kurtz et al. (2015)	Clinical examination	8	8	7	0	3
Machalicek et al. (2014)	Clinical examination	6	5	3	0	0
Moskowitz et al. (2011)	Clinical examination	3	1	2	1	0
Sheldon & Turk (2000)	Clinical examination	2	2	2	0	0
Symons et al. (2003)	Self-Injury Grid (Symons & Thompson, 1997)	32	20	19-32	6	5
Symons et al. (2010)	Based upon Symons et al. (2003)	436	198	348	70	89

Study	Assessment Method	N with SIB	Number of Participants with SIB at Body Location			
			Head	Hand/Arm	Leg/Feet	Torso
Totals		497	237	402-15	77	97
Minimum proportion SIB at body location (%)			47.69	80.88	15.49	19.52
Potential Variance (+%)			0	2.62	0	0

Aggression

Studies. Nine studies gave details of the topographies of physically aggressive behaviours shown by males with FXS (Table 8). One female with FXS was included in Kurtz and colleagues (2015) review of clinical cases. This 7 year old girl was reported to engage in a range of aggressive behaviours: "hitting/ slapping, kicking, hair pulling, biting, scratching, pushing, grabbing, pulling on others/ clothing, spitting, stomping on feet" (Kurtz et al., 2015, p. 153). However, given that only this single case is available, no comparisons between genders can be conducted.

Table 8

Individual study and review findings regarding the topography of physically aggressive behaviours in males with Fragile X Syndrome.

			Number of participants with topography of physical aggression							
Study	Assessment method	Participants with aggression	Hitting	Kicking	Grabbing/Pulling	Spitting	Pinching	Pushing	Biting	Scratching
Hagerman (2002)	Clinical examination	1	1	1	0	0	0	0	0	0
Hagopian et al. (2004)	Clinical examination	1	1	1	1	0	0	0	0	0
Hessl et al. (2008)	Clinical examination	38	24	22	23-6	12-5	13-6	17-20	7-10	10-13
Kurtz et al. (2015)	Clinical examination	7	7	7	7	0	4	5	5	2

Study	Assessment method	Participants with aggression	Number of participants with topography of physical aggression							
			Hitting	Kicking	Grabbing/Pulling	Spitting	Pinching	Pushing	Biting	Scratching
Langthorne et al. (2011)	Clinical examination	8	7	2	2	1	1	1		1
Largo & Schinzel (1985)	Clinical examination	7	5	0	0	0	0	0	2	0
Machalicek et al. (2014)	BPI*	10	7	4	1	0	1	1	3	0
Moscowitz et al. (2011)	Clinical examination	3	1	1	2	0	0	1	0	0
O'Reilly et al. (2000)	Clinical examination	1	0	0	0	0	1	1	0	0

Study	Assessment method	Participants with aggression	Number of participants with topography of physical aggression							
			Hitting	Kicking	Grabbing/Pulling	Spitting	Pinching	Pushing	Biting	Scratching
Total across studies:		76	53	38	36-39	13-6	20-4	26-9	17-20	12-16
Minimum proportion of participants with aggression showing the topography (%)			69.7	50	47.4	17.1	26.3	34.2	22.3	15.8
Potential Estimate Variation (%)			0.0	0.0	3.9	3.9	3.9	3.9	3.9	3.9

*Behaviour Problems Inventory (Rojahn et al., 2001)

Summary. In the total sample of males with FXS and aggressive behaviours (69 individuals), there was a significant difference in the number of participants (based upon minimum estimates) who engaged in different topographies of aggression. In order to minimize the number of comparisons, statistical differences were only investigated between the four most common topographies of aggression. A significantly higher proportion of individuals were reported to hit, compared to other topographies of aggression (Bonferroni adjusted alpha=.008. Compared to kicking: W=3.13, n=152, p<.005). There were no other significant differences other than grabbing being more common than pushing, although this was not robust and only reached significance when the maximum potential difference was considered (minimum difference: W=1.43, n=152, p=.15. Maximum difference: W=2.70, n=152, p<.005).

Destruction of property.

Table 9

Topographies of destructive behaviour

		Number of participants engaging in topography of destructive behaviour					
	Participants with destructive behaviour	Throwing or swiping objects	Hitting/ kicking/ banging objects/ surfaces	Knock over furniture	Ripping/ breaking objects	Spitting/ spraying water	Biting/ mouthing objects
Joy (2009)	1	1	0	0	0	0	0
Kurtz et al. (2015)	5	5	4	1	3	0	1
Langthorne et al. (2011)	8	6	2	0	3	1	0

		Number of participants engaging in topography of destructive behaviour					
	Participants with destructive behaviour	Throwing or swiping objects	Hitting/ kicking/ banging objects/ surfaces	Knock over furniture	Ripping/ breaking objects	Spitting/ spraying water	Biting/ mouthing objects
Machalicek et al. (2014)	7	7	0	0	0	0	2
Moscowitz et al. (2011)	1	0	1	0	0	0	0
Totals across studies	22	18	7	1	6	1	3
Proportion of those engaging in destruction who show topography		81.82%	31.82%	4.55%	27.27%	4.55%	13.64%

Study Details. All studies which provided details on the topography of participants' destructive behaviours gained their information from informants as part of a functional assessment, via either direct (experimental functional analysis: Kurtz et al., 2015; Langthorne et al., 2011; Machalicek et al., 2014; Pairwise analysis and observations: Joy, 2009) or indirect methods (parent interviews: Moskowitz et al., 2011).

Summary. There is very limited data on the topography of destructive behaviours (Table 9) in individuals with FXS. The most common reported topography of destructive behaviour was object-throwing, demonstrated by 82% of those who exhibited destructive behaviour. Statistical comparisons were not conducted due to small sample sizes.

Function of challenging behaviours.

Studies. The function of CBs shown by individuals with FXS (including at least one topography of either: SIB, aggression or property destruction) was assessed in eleven studies (Table 10) using a variety of direct and indirect measures. Many papers assessed the operant function of multiple topographies of behaviour in a single assessment. Therefore, it was not possible to assess the function of each type of CB separately, based on the data available. Data on behavioural function regarding one female with FXS were identified (Kurtz et al., 2015) and, given the small number, were incorporated into the overall analyses with the other males assessed.

Table 10

Function of challenging behaviour in individuals with Fragile X Syndrome

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
Hagopian et al. (2004)	1 male (10y[28])	Direct (Experimental Functional Analysis: Iwata et al., 1982/1994)	Social positive (attention)	1	Access to adult attention	1
			Social positive (other)	1	Access to tangible items	1
			Social negative	1	Termination of "do requests"	1
					Escape from demands	1
Kurtz et al. (2015)	8 males, 1 female (6-15y)	Direct (Experimental Functional Analysis)	Social positive (attention)	3	Access to adult attention	2

[28] y= years

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
					Access to adult physical attention	2
			Social positive (other)	5	Access to tangible items	4
					Adult compliance with mands	1
			Social negative	4	Escape from demands	4
			Non-social	2	Behaviour continued in absence social contingencies	2
Langthorne et al. (2011)	8 males (8-15y)	Direct (Experimental Functional Analysis)	Social positive (other)	4	Access to tangible items	4
			Social negative	5	Escape from social interaction	1

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
					Escape from demands	4
Machalicek et al. (2014)	11 males* (2-4y)	Direct (Experimental Functional Analysis)	Social positive (attention)	3	Access to mother's attention	3
			Social positive (other)	10	Access to tangible items	10
			Social Negative	10	Escape from social interaction	3
					Escape from demand	8
O'Reilly et al. (2000)	1 male (22y)	Direct (Brief Experimental Functional Analysis)	Social positive (attention)	1	Access to attention when parents are interacting with a third person	1
O'Connor et al. (2003)	1 male (14y)	Direct (Experimental Functional Analysis,	Social positive (other)	1	Adult compliance with mands	1

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
		followed by pairwise mand analysis: Bowman et al., 1997)				
Joy (2009)	1 male* (6y)	Direct: (Naturalistic pairwise analysis comparing routine interactions with a familiar person to novel interactions)	Social positive (attention)	1	Gain reactions from mother and sister	1
			Social negative	1	Escape from play with sister	1
					Escape novel social interactions	1
Hills-Epstein et al. (2002)	1 male (8y)	Direct (Non-specified observational assessment)	Social positive (attention)	1	Access to mother's attention when frustrated with an object or bored with a situation	1

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
Langthorne & McGill (2012)	35 males (mean 11y)	Indirect (Questions About Behavioral Function (QABF): Matson & Vollmer, 1995)	Social positive (attention)	4	Access to attention	4
			Social positive (other)	20	Access to tangible items	20
			Social negative	22	Escape from demands	22
			Non-social	16	Pain-related	9
					Automatic reinforcement	12
Moscowitz et al. (2011)	3 males (7-10y)	Indirect (Parent interview: Contextual Assessment Inventory (CAI; McAtee, Carr & Schulte, 2004) & Functional Assessment	Social positive (attention)	1	Access to mother's attention	1
			Social negative	3	Delaying going to bed	1
					Escape from novel or unpredictable places	1

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
		Interview (FAI; O'Neill et al., 1997))			Escape from the toilet	1
Symons et al. (2003)	32 males (1-12y)	Indirect (Questionnaire based on the FAI. Parents asked to rate if challenging behaviour was more likely to occur before, during or after a given series of situations)	Access to attention	12-13	Access to attention	1
					Access to attention when others' attention is divided with a third person	12
			Social positive (other)	19	Access to tangible items	19
			Social negative	28-32	Following changes in routine	28
					Following presentation of a command	21
					Following a difficult task	20

Study	Total Participants (age(s))	Functional Assessment Method	Class Behavioural Function	Number of Participants	Detail	Number of participants
					Following interruption of a preferred routine	18
			Non-Social	5	When left alone	5

* One participant excluded because target behaviours in functional assessment did not include any topographies of self-injury, physical aggression or property destruction.

Summary. The results of individual studies can be seen in Table 10 and are compared overall in Figure 9. Of the 103 individuals studied (102 male; age 22 months to 22 years), 27 or 28[29] engaged in CB at least partly maintained by access to attention (only 13 were reported to engage in these behaviours in a 1-1 scenario, the remainder did so only when the other individual's attention was being divided with a third person. One participant's behaviour was related to the provision of physical attention); 60 engaged in CBs maintained by another source of social-positive reinforcement, beside attention; between 74 and 78 engaged in CBs maintained at least partly by social negative reinforcement; finally, the behaviour of 23 participants was at least partly maintained by non-social sources of reinforcement.

Social-negative reinforcement was significantly the most common category (Bonferroni adjusted alpha = .008) compared to social positive (other), which was the next most common category (W = 2.52, N = 206, p < .005). A significantly higher proportion of participants were reported to have CBs which served a function in the social positive (other) category, compared to attention (W=5.76, n=103, p<.0005) or non-social (W=5.92, n=103, p<.0005). There was no significant difference between the

[29] Possible variation due to uncertainty in data extraction, specifically when calculating numbers from percentages in: Symons et al. (2010).

frequency of non-social and attention functions.

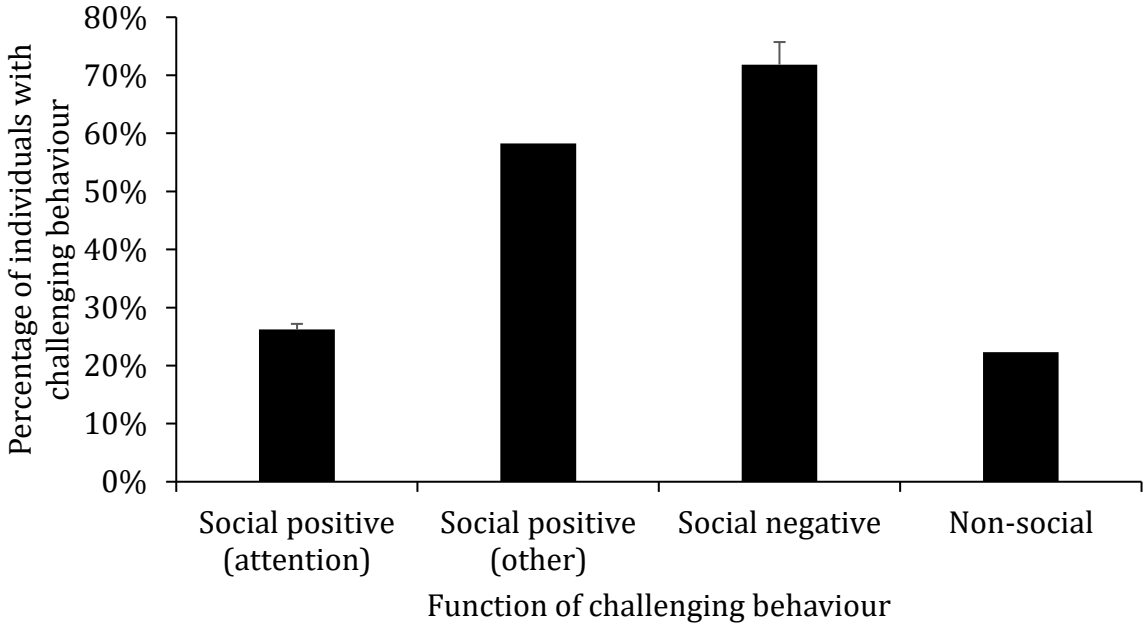

Figure 9. Functions of challenging behaviour of individuals with FXS.

Influence of study methodology: direct vs indirect functional assessment.

Direct functional assessments were conducted with 36 participants (10 studies), and indirect with 67 (2 studies). Visual analysis supported that, across all included cases, different assessment types yielded similar proportions of classes of social function, though the non-social results differed widely (see Figure 10). Of note, however, with the direct assessments there was no difference between the rate of instances of negatively reinforced CB and positive (social, other).

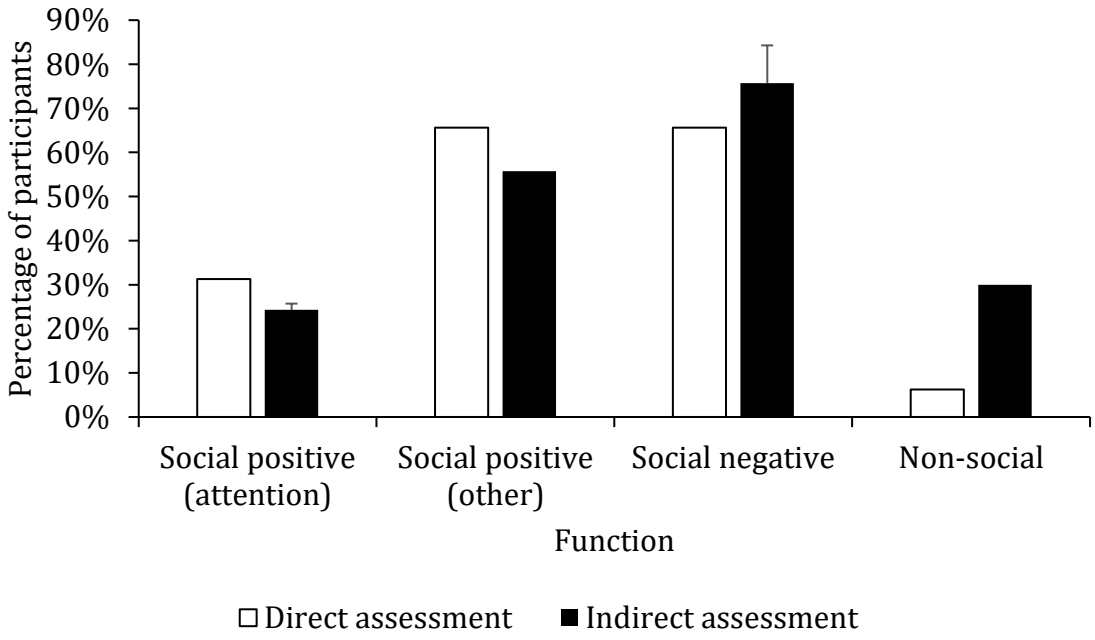

Figure 10. A comparison of the results of direct and indirect assessments of behavioural function, across studies.

Discussion

This review has collated the existing data on the prevalence, topography and function of CBs displayed by people with FXS, in order to provide new insights into influences upon behaviour within the syndrome.

Prevalence. The findings across studies support that CBs are a common issue for individuals with FXS, particularly males. Almost half of the total sample (48.8%; 4245 participants) exhibited SIB and over one third (35.81%; 4140 participants) displayed aggressive behaviour. The estimated prevalence of self-injurious behaviour across the studies (10-81%) in this population appears higher than estimates for individuals with mixed aetiology intellectual disabilities, which typically range from 4% (Emerson et al., 2001) to 24% (Deb, Thomas & Bright, 2001). This is supported by individual study between-group comparisons suggesting that individuals with FXS may be at higher risk

of exhibiting SIB than other groups, such as individuals with Down syndrome and mixed aetiology intellectual disabilities (Richards et al., 2012; Arron et al., 2011). Hand biting was also a prevalent form of SIB reported (32%), consistent with earlier suggestions that this behaviour forms part of the behavioural phenotype (Hagerman et al., 1991). This review also found prevalence estimates for aggression (12.5-60.9%) which are higher than those regarding others with intellectual disabilities (2-20%: Allen, 2000). Although, Arron and colleagues (Arron et al., 2011) found that boys with FXS were not more likely to exhibit aggressive behaviour compared to a group of individuals with intellectual disabilities of mixed aetiology. Finally, the review highlighted that destructive behaviour (such as destruction of items or property), despite being a common topography of behaviour in others with intellectual disability, has received little attention in FXS research.

The findings of this review, which brought together data on large numbers of individuals with FXS, supports the high prevalence of these challenges, which may be higher than in other groups. This has significant implications for provision of supports for this group and highlights the need to better understand risk factors for their occurrence. There are a number of factors which may make individuals with FXS more sensitive or vulnerable to developing behaviour described as challenging. Although a comprehensive review of all of the literature on associated features was not carried out, within the included studies investigating prevalence of CBs in FXS, several factors were identified which were associated with their occurrence. For instance, increased anxiety and autistic behaviour have been identified as correlates with increased SIB in males and females (Arron et al., 2011, Symons et al., 2010). In addition, in males with FXS characteristic features of over activity and impulsivity may be associated with the likelihood of engaging in aggression (Arron et al., 2011). In addition, there may be

factors at the biological level which influence such behaviours. Relating to FXS, lower levels of FMRP (the protein whose production is impaired in FXS), although not found to correlate with the prevalence or number of forms of SIB displayed (Symons et al., 2003; Hall et al., 2008), was found to be associated with earlier onset and increased surface area being targeted (Symons et al., 2003). Furthermore, secondary genetic factors may also play a role in the risk for engaging in CB; Hall and colleagues (2008) identified the status of the 5HTTLPR gene as a mediating factor for aggression in males with FXS. Future research should focus on the factors and characteristics which may lead some to develop such behaviours whilst others do not, in order to better inform strategies for intervention and prevention.

Topography. Across studies, biting was the most common topography of SIB amongst males with FXS. Interestingly, however, females with FXS were not more likely to self-bite, compared to other topographies of SIB. Furthermore, a higher proportion of males self-bit than females. However, across all participants (male and female), SIB was most commonly directed at the hands or arms. This pattern of body sites may be a secondary result of the tendency to self-bite, as there are limited body areas (presumably: arms, hands and lips, cheeks or tongue) that can be easily targeted by self-biting, without requiring high response-effort. However, no studies have conducted a comparison of SIB body sites between individuals with and without FXS and there is a paucity of research with comparable populations investigating body sites of SIB, against which the present findings could be compared. Therefore, the data suggesting within-syndrome patterns of SIB topography are partially consistent with the idea that self-biting is a phenotypic feature (Hagerman et al., 1991), though suggest that its presentation may be mediated by gender. The definition of a behavioural phenotype is that a behaviour is more common in individuals with a condition, relative to those

without (Dykens, 1995). Therefore, inclusion of self-biting in the male FXS phenotype is supported by findings that males with FXS exhibit a significantly higher relative risk of engaging in self-biting, when compared with individuals with either Autism Spectrum Disorders (2.52 times more likely) or Down Syndrome (7.67 times more likely: Richards et al., 2012).

The high prevalence of this specific topography of SIB suggests a motivative influence upon the automatic consequences for this behaviour. A causal model of self-biting in FXS has not yet been established, which should be addressed in future research. Chewing or jaw clenching following a stressful task has been shown to lead to reduced subsequent cortisol levels (when compared to a relaxed jaw: Tahara, Sakurai, & Ando, 2007). Hypothetically, therefore, the clenching action of biting may help to modulate atypical arousal. However, such a speculative association requires further research. In addition, a causal model would need to be able to account for the painful response cost. Within the existing literature, cortisol levels have not been found to be associated with the frequency of self-biting observed during social-demand conditions (Hall et al., 2006). However, given the wide range of situations in which these behaviours occur, relationships between arousal and self-biting should be investigated under a broader range of conditions.

With regards to the topography of aggressive behaviour, the available information suggests that hitting is the significantly most common topography displayed by males with FXS. No studies have directly investigated the prevalence of different topographies of aggression between individuals with FXS and a comparison group. However, comparison of this data with research with other groups reveals similar patterns: hitting was found to be the most common topography of aggression in

samples of individuals with mixed aetiology IDs (Sigafoos, Elkins, Kerr & Attwood, 1994; Emerson & Bromley, 1995) and a sample of individuals with Cri du Chat Syndrome (Collins & Cornish, 2002). Therefore, it does not appear that this expression of physical aggression is unique to males with FXS. Previous studies have noted that aggression may be clinically significant for some females with FXS (for example: Hessl et al., 2001), however there is little information to describe how this manifests.

Function. In this section the findings relating to behavioural function are considered in light of the motivative approach to understanding the presentation of CB in FXS (Langthorne et al., 2007). Within the group of young males with FXS assessed in this research, CBs were significantly more likely to have an escape or avoidance (social negative) function, compared to any other class of function. This suggests that the motivation to escape from or avoid situations may be elevated in males with FXS. When the specific functions assigned to the category of social negative reinforcement are analysed more closely, escape-maintained CBs appeared to be most closely associated with the presence of demands or transitions. Interestingly, despite the high levels of social anxiety and socially avoidant behaviours associated with FXS (Cordeiro et al., 2011), escape from social interactions did not appear to be a particularly common function for CB: only four out of the nineteen participants who participated in an experimental functional analysis, which included a test for a social escape function, showed elevated levels of target behaviours in this condition. However, low levels of attention-maintained behaviours were observed in this review; social positive (attention) was the joint least common class of function for CBs. This reflects earlier suggestions that the motivation to access adult attention may be diminished in FXS, which may reflect that such attention has been previously associated with the onset of stressors (Langthorne et al., 2011). Whilst less common than negative reinforcement,

access to tangible items (which formed the majority of the social positive (other) category) was also a frequent behavioural function.

Comparisons of behavioural function between FXS and individuals without the condition allow assessment of whether this pattern of behavioural function is 'phenotypic'. Langthorne and colleagues (Langthorne et al., 2011; Langthorne & McGill, 2012; Hardiman, Langthorne & McGill, in press) compared males with FXS to other groups (Smith Magenis Syndrome and non-specified ID), finding that the FXS participants were significantly less likely to engage in attention-maintained behaviour. Furthermore, our aggregated findings regarding behavioural function appear to differ in pattern from the pattern of functions seen across a review of all published experimental functional analyses. Beavers and colleagues (2013) found that, of those assessments that were differentiated, 32% of participants' behaviours served a demand escape function, compared to a higher proportion of 65.6% in this review, (of participants with FXS who partook in an experimental functional analysis). This supports that the individuals with FXS in our sample may have been relatively more likely to engage in escape-maintained behaviour than other populations engaging in CBs. Similarly, rates of tangible-maintained behaviour were higher in the present review (63.3%), than in that of Beavers and colleagues (11%). Only 18% of the FXS participants showed elevated levels of problem behaviour in the standard attention condition of a functional analysis, compared to a slightly higher rate of 21.7% in the wider functional analysis literature. Although this difference is small, this finding corresponds with the within group observation that the probability of this function may be lowered. However, it is worth considering that Beavers and colleagues assigned results to function categories according to single functions (any behaviours with multiple functions were classified under a separate "multiple function" category), whereas in the present review functions

were categorised by behaviours that were at least in part maintained by a particular reinforcer. As such, it is highly likely that there is a higher rate of attention-maintained behaviour in Beavers and colleagues' (2013) review. This difference in categorisation may limit the comparability of these findings.

However, the joint consideration of within-syndrome findings and the comparison with results from other populations suggests that there may be motivational changes associated with FXS which influence the operant learning of CBs: the motivation for negative social reinforcement is elevated relative to the motivation for positive reinforcement through the provision of attention. These findings have implications for the intervention and prevention of CBs in FXS. For instance, early training might focus upon teaching communicative behaviours to request time out, in order to provide functional alternatives to escape-maintained CBs, prior to their development. Future research should investigate behavioural function in females with FXS, to determine the applicability of these findings to that group.

It is currently unclear from the available data whether specific behaviours exhibited by individuals with FXS are more likely to be associated with certain functions. Langthorne and McGill (2012) conducted separate indirect functional assessments for self-injury, aggression and property destruction. Visual analysis suggests that aggression was more likely to serve an escape function than self-injury, however the significance of this difference was not evaluated. Future research might investigate, for instance, whether phenotypic behaviours, such as hand-biting, are more likely to be associated with a given function, compared to other behaviours. Understanding topography-function relationships may have implications for future analysis and treatment.

Factors which may be associated with behavioural function in Fragile X syndrome. In the prior section, it was established that individuals with FXS may be particularly likely to exhibit escape-maintained CBs in response to environmental conditions such as demands and transitions. Next, factors which may exert influence upon the value of such negative reinforcement are considered: including aspects of the phenotype of FXS, as well as the broader literature on motivating operations (MOs) and negatively reinforced problem behaviour (Langthorne, McGill & Oliver, 2014; McGill, 1999).

Pain and discomfort. A number of uncomfortable physical states such as allergies (Kennedy & Meyer, 1996), menses (Carr, Smith, Giacin, Whelan, & Pancari, 2003) and physical illnesses, such as otitis media (O'Reilly, 1997), have been associated with increased occurrence of negatively reinforced problem behaviours. Of relevance, therefore, are a number of uncomfortable or painful physical problems which individuals with FXS are at an increased risk of experiencing, such as gastrointestinal problems (such as reflux and constipation, which may be associated with loose connective tissue) and recurrent ear infections, particularly in childhood (Kidd et al., 2014). Although parents do not report high levels of pain-related behaviour directly (Langthorne & McGill, 2012), there may be an interaction between pain and other environmental demands. Namely, in the presence of physical discomfort, which may occur more frequently in FXS as a result of these conditions, an individual's tolerance for aversive situations such as the presentation of demands may be reduced, and as such the conditions may serve as an establishing operation (EO) for escape-maintained behaviour. Of relevance to this point however, are concurrent anecdotal reports of elevated pain thresholds (Lozano, Azarang, Wilaisakditipakorn & Hagerman, 2016) in

individuals with FXS (although this has not been objectively studied), which may counteract this hypothesis.

Sensory issues. In addition, pain resulting from genetically-mediated sensory sensitivity has been found to serve as an EO for escape-maintained behaviour in the context of demands. O'Reilly, Lacey and Lancioni (2000) demonstrated that hyperacusis associated with Williams Syndrome (WS) influenced the likelihood of a young girl engaging in problem behaviour during the presentation of demands. It was found that the individual with WS engaged in elevated levels of CB when demands occurred simultaneously with background noise, when compared to either demands or noise presented alone. Pain-related behaviours similarly varied. This suggests that genetically predisposed sensitivities to environmental stimuli may increase the averseness of tasks, which may otherwise not have been sufficient to establish a motivation to escape. In turn, this additive effect leads to engagement in escape-maintained problems behaviour. With regards to FXS, individuals are at risk of experiencing a variety of sensory integration issues including sensory discrimination and sensory modulation problems (Stackhouse, 1998; Stackhouse, 2014). Elevated physiological arousal in response to sensory stimulation has been objectively demonstrated; individuals with FXS show elevated electrodermal responses to a variety of sensory stimuli (such as: tactile, olfactory and auditory), when compared to controls (Miller et al., 1999). Of interest to the present discussion, Baranek and colleagues (2002) identified that, within a group of 15 boys with FXS, increased sensory processing problems (as assessed by both parent report measures and direct observation) were associated with poorer school-related occupational performance, including increased aversive-avoidance behaviours. As such, enduring sensitivities to various types of environmental stimuli may mean that transient environmental factors (such as noise, smells, textures and heat) act as EOs for

escape-maintained behaviours, which may have an additive effect during the presentation of demands, similar to that demonstrated in O'Reilly and colleagues' (2002) study.

Sensitivity to eye contact. Although in this review low levels of social-avoidant problem behaviours were identified, it is well established that individuals with FXS commonly have an aversion to direct eye contact with others, resulting in high levels of gaze avoidance (for instance, Hall et al., 2015). At the neurobiological level individuals with FXS exhibit aberrant brain activation in response to direct eye gaze, including in areas of the brain associated with fear processing, highlighting a biological underpinning to this behavioural trait (Watson et al., 2008). Although in isolation eye gaze may not be sufficient to commonly elicit problem behaviour within this group (as suggested by the results of this systematic review), it may be that the degree of social interaction during demands may alter the motivation to escape from the demand. Recommended practice during working with individuals with FXS in an educational setting is to reduce eye contact through sitting side-by-side, as opposed to the teacher sitting opposite the pupil (Fragile X Society, 2013). In line with this, preliminary research suggests that for some children with FXS, the aversiveness of eye contact may to be heightened in the context of the presentation of demands, leading to more gaze avoidant behaviour (albeit, no CBs occurred in this situation; Langthorne, unpublished ClinPsyD thesis). This early work suggests that gaze-related factors may interact with the presentation of demands, with a behaviour-altering effect.

Attention problems. One of the key features of the FXS phenotype is problems with inattention and hyperactivity (Thurman, McDuffie, Hagerman & Abbeduto, 2014). Kurtz and colleagues (2015) hypothesise that the presence of hyperactivity and attention problems may establish the motivational value of escape in the context of

demands, as this may make listening to the task instructions and sitting still more challenging, and thus more aversive. The relationship between attention and escape-maintained CBs has not yet been studied, although elevated attention problems have been associated with elevated behaviour problems more generally in FXS (Wheeler et al., 2014).

In addition, individuals with FXS typically exhibit resistance to changes to routine and expectation (particularly when unexpected) (Woodcock, Oliver & Humphreys, 2009). It is suggested that such changes place a high level of demand on a diminished attentional system. Of interest, in the mouse model of FXS resistance to change appears to be related to impulsivity, attention problems and arousability[30] (Moon et al, 2006). However, correlates of such difficulties have not yet been specifically explored in FXS. This characteristic may also relate to elevated difficulty with transitions seen in individuals with FXS, compared to controls (Braden, 1991: unpublished dissertation results, cited in: Braden, 2002). Similarly, transitions were a frequent antecedent to CBs identified in the review.

Arousal regulation. As previously discussed, it has long been hypothesised that atypical regulation of stimulus-bound physical arousal is central to much of the behavioural phenotype of FXS (Cohen, 1995). More recently, the nature of arousal-related difficulties in FXS have begun to be more objectively investigated, with suggestions that the hypothalamic-pituitary-adrenal (HPA) axis (a key circuit in the body's stress-related physiology) may function atypically (Hessl et al., 2002). From

[30] "Regulation of arousal and/or emotion was evaluated in these tasks by examining the reaction of the mice to the unexpected presentation of potent olfactory distractors (in the distraction task), as well as their reaction to committing an error on the previous trial." (Moon et al., p 1368).

clinical experience, Hagerman (1999) noted that avoidant behaviours in FXS typically increase in states of high arousal such as fear, anxiety and agitation. This corresponds with the positive association between cortisol levels (associated with the HPA axis, see Chapter 3 for further details) and levels of behaviour problems (Hessl et al., 2002).

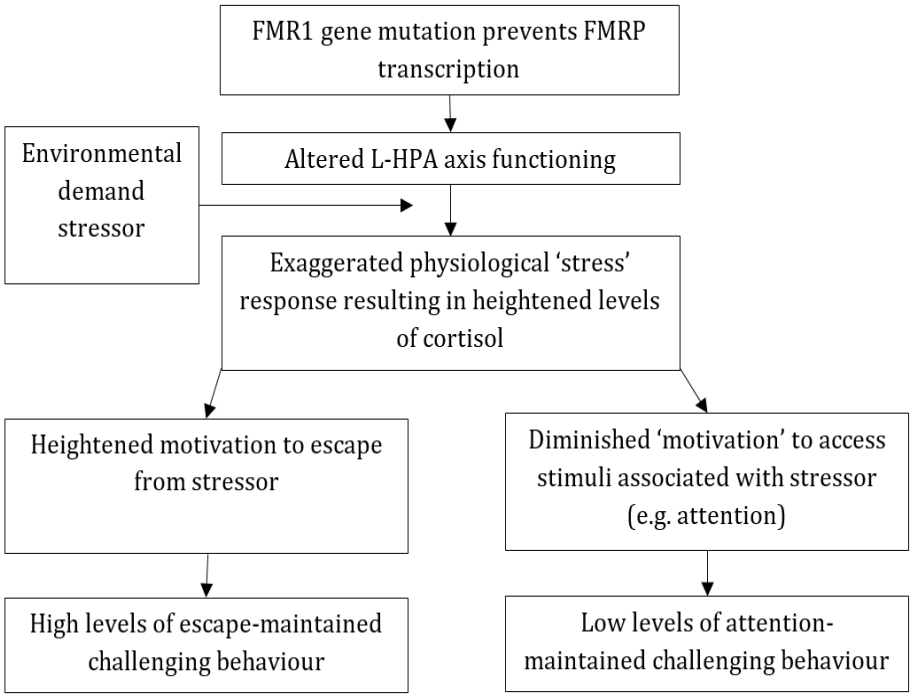

Figure 11. Hypothesised phenotype-environment interaction in FXS (adapted from Langthorne, 2009, p. 288)

The potential for CB to be reinforced through consequential escape or avoidance from external situations or stimuli causing aversive arousal (such as demands or social interactions) has been mentioned in several theoretical accounts of CB (for instance, Groden et al, 2006; Romanczyk, 1986; Romanczyk & Matthews, 1998; Romanczyk, Lockshin, & O'Connor, 1992). Similarly, Langthorne and colleagues (2009; 2011) hypothesised that exaggerated physiological responses to stressors may establish a heightened motivation to escape from aversive stimuli, as well as diminishing motivation for stimuli correlated with the onset of demands, such as attention (Figure

11). Though this model focusses upon the HPA axis, there are wider systems involved in the regulation of responding to environmental stressors (such as the ANS), which should be considered in the same vein. As such, arousal regulation differences may be a key factor influencing the operant learning of problem behaviours in this group. Given that direct, objective measurements of this physiological variable is possible, further research investigating the relationship between physiological arousal and escape-maintained problem behaviour is warranted.

Limitations. There are several limitations with this review that warrant consideration when interpreting the findings. Firstly, by bringing together the results of different studies for analysis, the implicit assumption was made that the heterogeneous measures used corresponded highly to each other. However, definitions of behaviours inevitably differ between studies and different measures, which may limit their comparability. A review of the validated measures of behavioural topography revealed relatively subtle differences in wording of descriptions of behaviour. However, where a validated measure was not used, it was not clear what questions were asked and whether this may have affected the response.

The assumption of the compatibility of findings via different measures may be particularly challenged in the case of the data regarding function of CBs; as previous research has suggested poor correspondence between the outcomes of direct and indirect methods of assessment (Toogood & Timlin, 1996). Though, comparison of the outcomes reported from direct and indirect measures of behavioural function across the FXS sample suggests that there is not a significant difference in the likelihood of each measurement type yielding each type of social function. However, the results of indirect measures were more likely to report non-social functions than direct

assessments. This particular difference may be influenced by the fact that Machalicek and colleagues (2014) did not include a 'no interaction' condition in their experimental analysis, meaning that they may have not been able to adequately detect non-social functions for behaviour. The participants in this study constitute a substantial proportion of individuals with FXS who have participated in a direct functional assessment. Of note, four of these eleven individuals were reported to have automatically reinforced (non-social function) behaviour in an indirect parental assessment. Therefore, this poor correspondence for non-social functions between assessment types may be the result of individual study methodologies. However, it should be noted that the correspondence of direct and indirect measure within studies was mixed, including for social functions (Machalicek et al., 2014; Langthorne et al., 2011). Therefore, it is possible that had all of the participants been assessed using the same measure, a different pattern of results may have been seen.

Furthermore, the results of the functional assessments were only validated by the implementation of function-based interventions for seven participants, all of which were successful at reducing target behaviours (Hagopian et al., 2004; O'Reilly et al., 2000; O'Connor et al., 2003; Joy, 2009; Moskowitz et al., 2011); this equates to 7.4% of the total sample in this review. Information on behavioural function was obtained through direct assessment for four of these participants, and indirect for three. Without validation through implementation, it is unclear whether the conclusions about function were valid; though earlier research has demonstrated the validity of both direct and indirect functional assessments. However, future research might further investigate the utility of functional approaches to behavioural intervention in FXS by assessing the success of function-based treatments for CBs, based upon both direct and indirect assessment findings.

A further limitation with the review is that there may have been small errors in the calculation of numbers of participants who engage in specific behaviours. Firstly, as acknowledged above, by ascribing results into groups to combine the data, some uncertainty was created about the exact number of participants to be assigned to each group, due to unknown overlap of participants. Minor mistakes may have also occurred in calculating the numbers of participants to be placed in each category for the review, due to the calculation of numbers of participants from percentages provided in publications. In addition, where behaviour was assessed by parent report or clinical assessment, it was assumed that all topographies of behaviour could potentially have been assessed. However, it is possible that there may have been reporting biases. For instance, only highly visible behaviours may have been detected by a clinician or behaviours of the highest concern may have been prioritised for assessment, leading to the under-reporting of other topographies of behaviour. In addition, given the earlier suggestion of the specific association between FXS and hand-biting, this topography of behaviour may have been more readily reported.

Finally, the majority of the studies focussed upon children and adolescents with FXS. As such, it is unclear whether these findings are generalizable to older age groups.

Summary. This review chapter has systematically collated the findings of studies of CB displayed by individuals with FXS, to provide new insights into its manifestation. The review highlighted that CBs are a common concern in this group, particularly amongst males. Furthermore, comparisons within the studied groups of individuals with FXS support the existence of a bias towards particular topographies and functions of CBs within the condition, at least for males. As discussed, there are a number of hypothesised reasons relating to the phenotype of FXS which may relate to

the pattern of topographies and functions observed. As such, there are a range of questions which could be addressed in future research. Understanding links between behavioural phenotypes and behavioural function is of primary interest in this thesis, and therefore was the focus of subsequent investigations. However, future research to investigate the genesis and maintenance of self-biting in FXS is also warranted.

Next, specific factors which may underlie this observation of elevated negatively reinforced CBs will be explored. Given the central role atypical arousal is hypothesised to play in the behavioural phenotype of FXS, and earlier hypotheses relating to escape-maintained behaviour (Langthorne et al., 2011), this particular factor will be investigated further in subsequent investigations. Objective assessment of physiological arousal is also possible, which facilitates the identification of possible associations. In Chapter 3, physiological systems of interest are reviewed in terms of their structure and function, and how they may be implicated in FXS.

Chapter 3

Physiological Arousal of the Autonomic System and HPA-axis in Fragile X Syndrome[31]

Chapter Overview

In the previous chapter (Chapter 2), the literature on challenging behaviour (CB) in individuals with Fragile X Syndrome (FXS) was systematically reviewed, in order to investigate whether there exists within-group patterns in its manifestation. Preliminary evidence suggests that there may be a tendency towards individuals exhibiting higher rates of negatively reinforced behaviour when compared to other functions. As such, it has been hypothesised that changes to systems which control the body's physiological response to stressors may be implicated.

In the present chapter, evidence relating to the activity of the Hypothalamic-Pituitary-Adrenal (HPA) Axis and autonomic nervous system (ANS) is considered, in order to inform later investigations. A previous review of the literature on cardiac indicators of autonomic arousal demonstrated a robust pattern whereby males with FXS exhibit reduced vagal tone (parasympathetic regulation) and increased sympathetic activity. Given the lack of previous summaries of data relating to the HPA axis, a systematic review on its function and relationship to behaviour was carried out. The findings across studies are mixed, though trends in the findings can be seen, including elevations in cortisol levels, particularly in males. Preliminary findings also highlight associations between cortisol levels and key behaviours associated with the syndrome,

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such as gaze avoidance. Areas for future research are discussed, including for furthering the understanding of CB in the condition.

Autonomic Nervous System in Fragile X Syndrome.

Do people with Fragile X syndrome exhibit atypical autonomic arousal?

There exists a growing body of literature providing evidence that individuals with FXS exhibit atypical autonomic arousal. ANS activity can be assessed through directly measuring peripheral functions under its control, including: perspiration (skin conductance response), pupillary dilation, and measures relating to heart rate and other cardiac activity. Recently, Klusek, Roberts and Losh (2015) conducted a thorough review of the literature on ANS function in FXS, as indexed by cardiac measures. The researchers identified 11 studies (Baranek et al., 2008; Boccia & Roberts, 2001;Hall et al., 2009; Heilman et al., 2011; Klusek, Martin, et al., 2013; Roberts et al., 2001; Roberts et al., 2006; Roberts, Tonnsen et al., 2012; Roberts, Hatton et al., 2012; Roberts et al., 2013 Tonnsen et al., 2013) comparing cardiac indices of various aspects of ANS activity (heart rate (sANS) and vagal tone (pANS)) between individuals with FXS and controls. Across these studies young males with FXS were compared to typically developing (plus those with idiopathic autism: Klusek et al., 2013) controls matched on gender and age. The authors conclude that there is a strong and well-replicated pattern of physiological dysregulation in males with FXS.

Firstly, cardiac measures indicate that males with FXS exhibit chronic, overall autonomic hyperarousal (as indicated by elevated heart rate) across both baseline (Hall et al., 2009; Heilman et al., 2011; Klusek, Martin, et al., 2013; Roberts et al., 2001; Roberts, Tonnsen, et al., 2012) and stressor tasks (cognitive: Boccia & Roberts, 2000; Heilman et al., 2011; and social: Hall et al., 2009; Klusek, Martin, et al., 2013). Of

note, the review authors highlight that social stressors do not appear to evoke elevated arousal relative to other stressors; rather, the hyperarousal appears to be generalised and non-specific (Klusek et al., 2015).

A number of studies also utilised non-cardiac measures which are associated with sANS function. In a number of these studies, baseline sympathetic activity was found to be heightened relative to typically developing controls, utilising skin conductance (which assesses sANS activity through subtle changes in perspiration and resultant electrical conductivity of the skin. Keysor et al., 2002; Williams et al., 2013: female participants with FXS) and respiration rate (Heilman et al., 2011: young male participants with FXS) measures. In addition, a number of studies observed increased sANS reactivity in individuals with FXS compared to controls. In two studies, Farzin and colleagues (2009; 2011) discovered that males with FXS exhibit greater pupillary dilation in response to faces compared to CA controls, but no group differences were found to scrambled images. This finding was also replicated using skin conductance measures in females with FXS, compared to TD controls matched on chronological age (Williams, Langdon & Porter 2013). This raises the suggestion that individuals with FXS exhibit sANS hyper-reactivity specifically evoked by social stimuli (contrasting with the cardiac findings: Klusek et al., 2015). However, Cohen and colleagues (2015) found generalised hyper-reactivity using skin conductance in males with FXS, relative to TD controls and those with idiopathic ASD, regardless of social valence of the stimuli presented.

In addition to the hallmark, chronic hyperarousal, individuals with FXS appear to exhibit reduced parasympathetic regulation of the ANS, as indicated by reduced vagal tone. Across a number of studies, reduced vagal tone was observed in males with FXS,

relative to typically developing controls. These differences were observed during resting conditions (Boccia & Roberts, 2000; Hall et al., 2009; Heilman et al., 2011; Roberts et al., 2001; Roberts, Boccia, Hatton, Skinner, & Sideris, 2006) and in response to cognitive stressors (Tonnsen et al., 2013; Roberts, Tonnsen, et al., 2012; Heilman et al., 2011; Boccia & Roberts, 2000). According to the polyvagal theory, this reduced regulation may mean that individuals with FXS are less able to regulate their behaviour in response to social expectations (Porges, 1995), for instance leading to autistic-like behaviour (Marshall & Fox, 2006; Porges et al., 2013) or externalising conduct problems (Beauchaine, Gatzke-Kopp & Mead, 2007). Although reduced vagal tone was not universally replicated across all studies reviewed by Klusek and colleagues (2015), overall, dampened pANS activity appears to be a robust finding within males with FXS. There has been no specific research with females with FXS, and as such it is unclear whether these findings generalise to this group.

Interestingly, unlike for heart rate, there was a different pattern of parasympathetic regulation observed between social and non-social stressors. Namely, there were no group differences (Hall et al., 2009; Tonnsen et al., 2013), or increases (Klusek et al., 2013) in VT in response to the social stressor task, compared to the findings of reduced VT in the FXS group during the cognitive stressors. The review authors suggest that this pattern of findings may suggest that social stressors induce a state of hypervigilance and physiological stress in boys with FXS.

Are there gender differences in autonomic arousal in Fragile X Syndrome? As discussed above, the majority of investigations have been conducted with males with FXS, with only few studies including female participants (Farzin et al., 2011; Farzin, Riviera & Hessl, 2009; Keysor et al., 2001; Williams, Langdon & Porter, 2013). Hall and

colleagues (2009) compared heart rate measures between males and females with FXS, and their unaffected siblings. Although both males and females with FXS exhibited lower vagal tone than their siblings, only males with FXS showed lower inter-beat intervals (higher heart rate). As such, preliminary evidence suggests, as with the broader FXS phenotype, that autonomic arousal differences are more pronounced in males.

Associations between autonomic arousal and behaviour in Fragile X Syndrome. The aforementioned studies highlight that individuals exhibit differences in levels of various aspects of autonomic arousal, relative to controls. A number of studies have also investigated whether these differences are associated with some of the behavioural characteristics of this condition. There exists preliminary evidence that physiological activity and autism symptomatology are associated in FXS (dampened vagal tone associated with increased severity: Roberts, Tonnsen et al., 2012; slower heart rate decelerations associated with increased severity: Roberts, Hatton et al., 2012), however the nature of the relationship varies between studies and is not consistently found (Klusek et al., 2013). The review authors suggest that part of the explanation for this variability may a non-linear developmental trajectory underlying the associations between autonomic arousal and behaviour, possibly moving from autistic symptomatology initially being associated with hypoarousal, to hyperarousal. Furthermore, Klusek and colleagues (2013) found increased arousal to be associated with decreased communicative ability in individuals with FXS. Sensory processing problems have also been associated with physiological responding; those with elevated reactions to sensory stimulation also exhibited elevated behavioural reactivity to these sensations (Baranek et al., 2008; Roberts et al., 2013).

The expansion of the FMR1 gene in FXS is associated with the reduction or the cessation of production of FMRP which, through a cascade of effects on other systems, leads to the characteristic features of FXS. Despite the core role of FMRP in FXS, no associations have been found between assessments of ANS function and FMRP levels in FXS (Roberts et al., 2001; Hall et al., 2002), although the assessment of FMRP levels were indirectly assessed in these studies, which may have compromised the validity of the findings. Further research is required to identify factors which may be associated with autonomic arousal differences within this group, as this may help to identify possible risk markers for severity of presentation of at least some aspects of FXS.

Hypothalamic-Pituitary-Adrenal axis in Fragile X syndrome. As discussed in Chapter 1, indirect evidence is indicative of disordered hypothalamic-pituitary regulation of the HPA axis, particularly with regards to impaired negative feedback regulation. As a consequence, research into the secretion of glucocorticoids has begun to emerge within the FXS literature. The function of the HPA axis may be assessed by measuring the levels of cortisol within the blood or, less invasively, can also be assessed through collection and analysis of saliva samples (Jessop & Turner-Cobb, 2008). However, there has not yet been a comprehensive review conducted in order to evaluate the evidence as to whether the HPA axis is altered in FXS.

Aims. The aim of this review is to collate findings relating to HPA functioning in animal models of and humans with FXS. Preclinical literature has been included to allow an in-depth analysis of the potential relationship between FXS and HPA function. The review addresses several questions:

- Do individuals or animals with FXS exhibit atypical levels of glucocorticoids at baseline, or differences in the duration or magnitude of responses to stressors, compared to controls?
- Given the X-linked nature of the condition, are there gender differences in the different aspects of HPA activity in FXS?
- Do measures of HPA activity relate to behaviour in FXS?

Method

Selection criteria for studies. Empirical or observational studies were considered, which assessed measures of HPA output (cortisol in humans or corticosterone in mice, collected via salivary or haematological methods) in humans with full-mutation FXS or an animal model of the human full-mutation, such as the *FMR1* knock-out (KO) mouse. Papers were included if they contained either a group comparison of corticosterone levels or an analysis investigating the relationship between HPA activity and behaviour in individuals or animals with FXS. Case studies were considered when the individual's results were compared to normative data or matched with an individual without FXS. Manuscripts were required to be written in English.

Table 11

Rejection Codes

Rejection Code	Inclusion Criteria	Exclude
1	Manuscript available in English.	Manuscripts not available in English (full text).

2	Includes humans with FXS or relevant animal model (e.g. FMR1 KO mouse)	No FXS-relevant data; FMR1 premutation
3	Includes measure of glucocorticoid	No measure of glucocorticoid
4	Includes comparison group OR within-group exploration of glucocorticoid-behaviour associations	No comparison group or glucocorticoid-behaviour analysis.
5	Original research	Review and conceptual papers.
6	Other	

Search methods for identification of studies.

Electronic search. The following databases were searched: Web of Science, SCOPUS, PubMed, and Academic Search Complete. The search was completed in June 2016.

Search terms. The search terms used for the search were: (("fragile x" OR *FMR1*) AND (glucocorticoid* OR cortisol OR corticosterone)). The fields 'title', 'abstract' and 'keywords' were searched (or closest available option within the database).

Searching other resources. Bibliographies of relevant articles were scrutinised. Furthermore, the titles of studies published in the following journals were searched, using the same terms, to ensure no papers had been missed in the database search: Psychoneuroendocrinology; American Journal of Medical Genetics; Journal of Intellectual Disability Research. These searches yielded no additional papers.

Search Results The search is depicted in Figure 12 (Rejection codes described in Table 11). In total, 79 unique papers were identified in the initial search, of which 17 met the inclusion criteria for this systematic review.

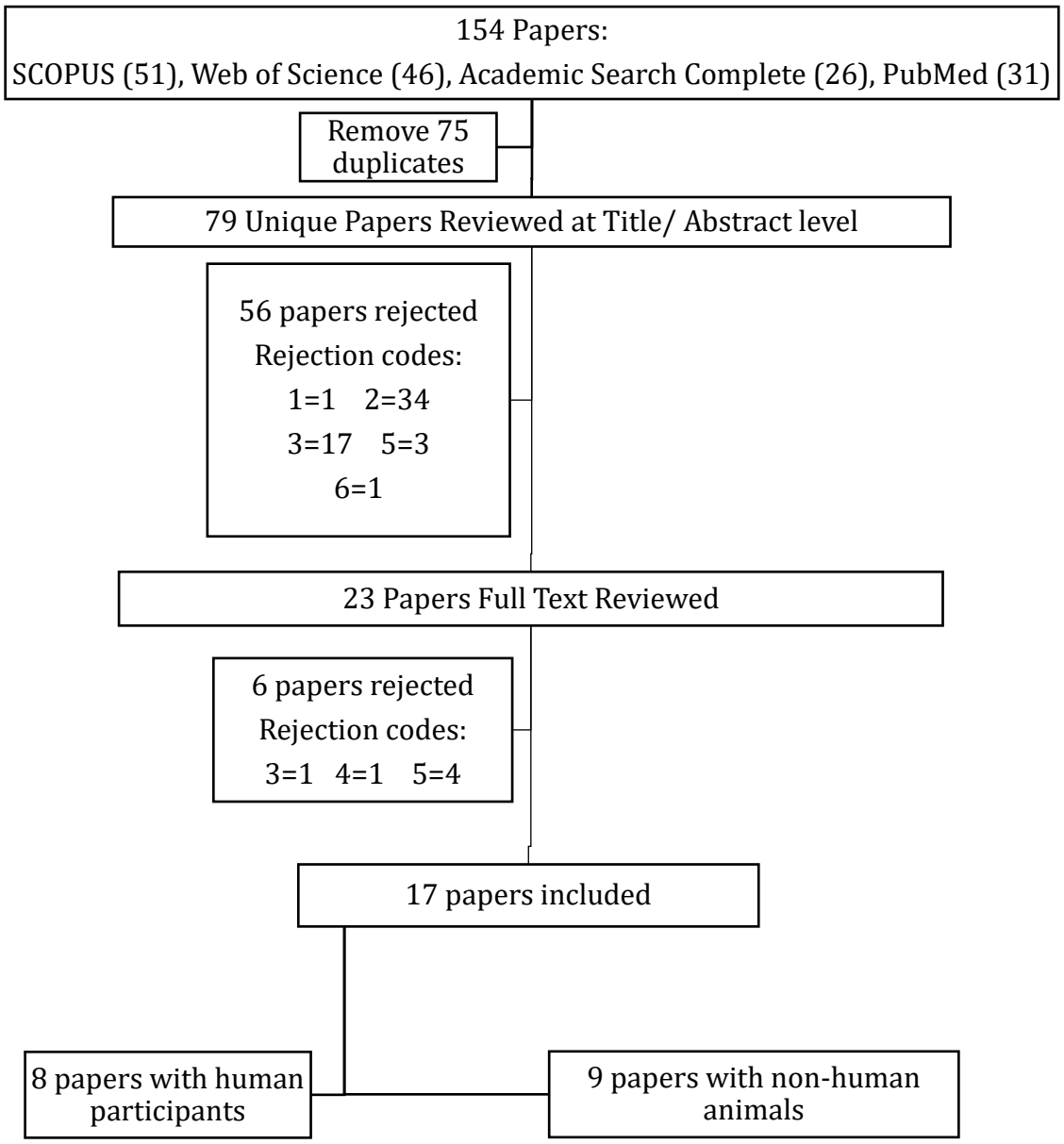

Figure 12. Depiction of the manuscript search process.

Results and Interim Discussion

Do individuals or animals with FXS exhibit atypical levels of glucocorticoids at baseline, or differences in the duration or magnitude of responses to stressors, compared to controls?

Baseline HPA activity and circadian rhythm.

Animal literature. Several studies (Table 12; for further details on study methodology, see Appendix A) have investigated the non-stressed corticosterone secretion of male KO mice compared to wild-type (WT) control animals, in order to identify whether changes exist in the baseline activity of the HPA axis in FXS animal models. The majority of studies found no genotype effect in their comparisons at single time-points, with male animals (Markham et al., 2006; Lauterborn, 2004; Nielsen et al., 2009; Eadie et al., 2009; Ghilan et al., 2015). Furthermore, in a more detailed analysis, Qin and Smith (2008) assessed the baseline circadian rhythm of both genotypes and found no difference at any of the six time-points tested (Qin & Smith, 2008). However, two studies did identify genotype differences, though the nature of the difference contrasted: de Diego-Otero and colleagues (2009) found that KO mice had lower corticosterone levels at baseline than WT controls; in contrast, Qin and colleagues (2011) found a main effect whereby KO mice generally had higher corticosterone than WT controls. As such, there is no evidence to suggest baseline HPA activity is altered in males with FXS, based on the preclinical evidence.

Table 12

Studies investigating corticosterone secretion in FMR1 knockout mice.

Study	Gender (M/F)	Strain	Basal measure	Stress Condition(s)	Recovery Time	Cort. Findings
Ghilan et al. (2015)	M	C57Bl/6	/	Restraint (conditions: 15m/ 30m/ 1h) or control	None: quick sacrifice after restraint	WT mice showed significant elevations only after 30m or 1h of restraint. KO mice showed increases after all restraint periods. After 15m restraint, KO mice significantly higher corticosterone than WT. Suggests even short stress exposures trigger response in KO mice.
de Diego-Otero et al. (2008)	M	FVB-129	/	Social stress (15m) or acute immobilisation stress (15m) or control	None: immediate sacrifice following behavioural. test battery	KO in control and social stress conditions lower corticosterone than WT. Acute stress KO higher corticosterone than WT.

Study	Gender (M/F)	Strain	Basal measure	Stress Condition(s)	Recovery Time	Cort. Findings
Lauterborn (2004)	M	FVB*	/	Restraint (30m/ 2h) control.	-	Following 2h restraint KO higher corticosterone than WT, similar ns trend following 30m restraint
Markham et al. (2006)	M & F	C57/Bl6	Cage mate sham comparisons (no restraint, just moved to test room)	Restraint (30m) or control	Conditions: 0/ 15/ 60m	Male KO protracted return to unstressed baseline (still elevated at 60 m). Female show protracted rise compared to WT. peak secretion does not differ between genotypes.
Nielsen et al. (2009)	M	FVB/NJ x C57/Bl6 (F1 hybrid)	/	Swim Stress (3m) or open field (10m) or restraint (unspecified length). Each condition with control.	Swim: 17m. Open field: 10m. Restraint conditions: 0/ 30/ 60/ 90/ 120m	No genotype difference in magnitude or duration of corticosterone response to any stressor.

Study	Gender (M/F)	Strain	Basal measure	Stress Condition(s)	Recovery Time	Cort. Findings
Qin, Xia, Huang & Smith (2011)	M	FVB/NJ	/	Prior stress: chronic stress (2h/d restraint x10) or control. Acute stressor: spatial novelty (EPM)	-	No interaction between genotype and chronic stress condition. Main effect genotype: corticosterone higher in KO.
Qin & Smith (2008)	M	FVB/NJ	2am, 6am, 10am, 2pm, 6pm, 10pm	Acute restraint stress (30/ 120 m) or spatial novelty (EPM 5m) or control	Conditions: 30/ 120m	WT and KO no circadian rhythm differences (basal measures). Following stressors, no genotype difference in any condition
Eadie et al. (2009)	M	C57BL/6		Acute restraint stress (3 hours) or control	Immediate sacrifice following stressor	No difference in control condition but following stressor NO showed significantly lower corticosterone.

Study	Gender (M/F)	Strain	Basal measure	Stress Condition(s)	Recovery Time	Cort. Findings
Romero-Zerbo et al. (2009)	M	FVB-129		Open field	Immediate sacrifice following stressor	At baseline, KO significantly lower corticosterone than WT but after acute stressor significantly higher.

*= Information obtained from contact with author. -=data not available. /=not tested.

w=weeks. d= days, m= minutes. h= hours. EPM= elevated plus maze. ns=non-significant

Human literature. Research investigating baseline HPA activity in humans has focussed upon profiling the diurnal rhythm of cortisol levels in this group (Table 13). Under typical, non-stressing circumstances, cortisol is released in a pulsatile fashion in a pronounced circadian rhythm: secretion is low during the first half of night time sleep (quiescent period) then rises during the second half; within 30 minutes of awakening there is a sharp increase (Cortisol Awakening Response; CAR) followed by a gradual decrease through the day (van Cauter, 1990). Responses to stressful stimuli are superimposed upon this pattern. Two studies investigated cortisol levels through routine days (without unusual or exciting events). Wisbeck and colleagues (2000) conducted a pilot study involving 7 females and 8 males (between the ages of 6-25 years) with FXS, comparing to a normative sample, Hessl and colleagues (2002) later built upon this with a larger study of 39 females and 70 males with FXS (age 6-17 years) compared to siblings without FXS (58 female, 51 male; age 6-17 years). In both studies, boys with FXS exhibited higher levels of cortisol, resulting from reduced diurnal decline, than their unaffected siblings. These findings may be consistent with the hypothesis, from preclinical literature on mRNA targets, of disordered HPA negative feedback. However, the only way to separate the direct influences of HPA feedback regulation and the influence of broader differences originating from, for instance, atypical emotional evaluation of the environment, would be to directly challenge the HPA axis, for instance with a dexamethasone suppression test (as used by Hoshino and colleagues (1987) with individuals with autism). As mentioned previously, heterogeneity in the preclinical literature in terms of both methodology and results means that it is challenging to draw conclusions about any potential results between the findings in mice and these suggestive findings of blunting of circadian glucocorticoid release in humans with FXS.

Further investigations of circadian rhythmicity in the HPA axis in *FMR1* KO mice may help to provide further evidence to understand these observed differences better.

HPA reactivity to challenges. Early hypotheses suggested that stimulus-bound arousal differences (Cohen, 1995) may play a significant role in the behavioural phenotype of FXS. Evidence to evaluate this claim has been collected across a small number of studies, involving both human and non-human animal participants.

Animal literature. Exposing animals to acute stress paradigms allows for investigation of the magnitude and/or duration of HPA axis reactions, and whether these differ in the *FMR1* KO model of FXS, compared to their WT counterparts. A commonly used trigger for acute stress with mice is to restrain the animal (for instance, in a small tube) for a period of time. A summary of this research is included in Table 12.

Seven studies were identified which had compared the magnitude of responses of male KO and WT mice to this procedure (implemented for between 15 minutes and three hours; Table 12). Three of these studies found that KO mice exhibited higher levels of corticosterone compared to WT controls, following the stressor. De Diego-Otero and colleagues (2008) observed this difference following 15 minutes of restraint stress. In contrast, Lauterborn (2004) found a significant difference in corticosterone responses only after more prolonged restraint (2 hours), however only a trend towards a difference was observed with a shorter stressor (30 minutes). Ghilan and colleagues (2015) observed higher corticosterone levels after a short period of restraint (15 minutes) in the KO mice, compared to the WT mice. However, following more prolonged periods of restraint (30 and 60 minutes) both KO and WT mice showed responses which did not significantly differ in magnitude. Increased stress-related elevations were also seen in response to a different stressor (spatial novelty) by Romero-Zerbo

and colleagues (2009), who found that, despite initially lower baseline corticosterone levels in the KO mice, following stressors the KO mice exhibited higher levels of corticosterone than their WT counterparts.

In contrast to the four studies finding elevations in KO mice responses, Eadie and colleagues (2009) found that KO mice had significantly lower corticosterone than WT, following 3 hours of restraint stress, suggesting a smaller hormonal response to the paradigm. Furthermore, there were no genotype differences observed in seven studies: three studies did not observe any genotype difference in the magnitude of corticosterone responses to restraint stress (Markham et al., 2006; Nielsen et al., 2009; Qin & Smith, 2008) and a further four studies also observed no difference using other acute stress paradigms, including exposure to spatial novelty (Nielsen et al., 2009; Qin & Smith, 2008; Qin et al., 2011) and swim stress (Nielsen et al., 2009).

Of interest, given the atypical social profile associated with Fragile X, de Diego-Otero and colleagues (2008) investigated the mice's reactions to both physical (restraint) and social stressors (housing with between 9 and 11 other animals for 15 minutes), to investigate whether there may be differences in the nature of corticosterone responses. They found that KO mice showed lower levels of corticosterone following the social stressor than WT mice, which differs from the trend for elevations in response to restraint stress in other studies. This preliminarily suggests that the nature of the stressor (social verses physical) may be of importance when investigating stress-related physiology in the FXS mouse model. Finally, another interesting manipulation was included in a study by Qin and colleagues (2011) who exposed both WT and KO animals to chronic restraint stress, before exposure to an acute stressor in the form of a novel environment (Qin et al., 2011). However, no

interaction was found between the genotype and chronic stress, on the corticosterone responses.

In summary, given the high numbers of null findings no firm conclusions can be drawn about the magnitude of responses in FXS mouse models. Where differences were observed, however, the trend was for male animals to exhibit higher levels of corticosterone. A possible reason for this variation in results between studies may be related to the genetic background of the mice used. Mouse strain differences have been previously found to influence both the magnitude and duration of corticosterone responses to stressors (Shanks, Griffiths, Zalcman, Zacharko & Anisman, 1990) and have been hypothesised to be associated with conflicting results more broadly, when using the *FMR1* KO (Pietropaulo, Guilleminot, Martin, Amato & Crusio, 2011). Interestingly, Markham and colleagues (2006) found male KO mice had protracted responses to 30 minutes of restraint when compared to WT mice, using mice of a C57/Bl6 background; however Qin and Smith (2008) did not find any genotype differences after the same stressor when using FVB/NJ male mice. However, clearly, there may have been other methodological differences between the studies which caused the differences in the results (see Appendix A for summary of key study methodology). For instance, the timings of the testing of the animals (when specified) varied between 7am and 2pm. The active phase of mice is typically during the night time, inverse to humans, with a peak at approximately 8pm (Gong et al., 2015), though of course housing and lighting conditions may cause this to vary. The time windows for testing across the reviewed studies overlapped substantially making comparisons challenging. However, this possible influence should be considered in future research and there is a need to establish better evidence on the link between sample timings, circadian rhythmicity and

stress-related corticosterone release in *FMR1* KO mice, in order to facilitate the interpretation of the literature.

Next, several studies have investigated the duration of corticosterone responses. This was achieved by conducting timecourse studies involving sacrificing groups of mice at differing lengths of time following a restraint stressor. Interestingly, Markham and colleagues (2006) observed that the male KO animals showed a slower return to unstressed baseline than WT; a pattern which is consistent with the prediction of reduced HPA negative feedback. Though, two other studies did not find any genotype differences between male animals in response duration (Nielsen et al., 2009; Qin & Smith, 2008).

Human literature. Four studies to date have investigated group differences in the release of cortisol in response to cognitive, behavioural or physical testing (see Table 13 for details of study participant characteristics and Table 14 for details of between-group comparisons).

Table 13

Participant characteristics in studies investigating cortisol secretion in humans with Fragile X Syndrome

Study	FXS participants			Control Participants		
	N (M/F)	Age	Number with Autism	N (M/F)	Age	Characteristics
Bricout et al. (2008a)	1 M	24y	N/A	15 (M)	-	"Healthy"
Hessl et al. (2002)	39 (F), 70 (M)[32]*	6-17y (mean: 10.8y)	N/A	58 (F) 51 (M)	6-17y (mean 11.26)	Unaffected siblings. Confirmed absence of FXS or pre-mutation

[32] ns= non-significant. m=minutes. y=years M=male, F=female
*Note: same group of participants in three studies.
**Sub-set of total study participants for whom cortisol data was available.
[+] Fragile X Syndrome and high levels of autism symptomatology (as indicated by a score on the Child Autism Rating Scale (CARS; Schopler, Reicher & Renner, 1988) above the cut-off for an autism spectrum disorder)
[-]Fragile X Syndrome and low levels of autism symptomatology (as indicated by a score below the cut-off for an autism spectrum disorder on the CARS)

Study	FXS participants			Control Participants		
	N (M/F)	Age	Number with Autism	N (M/F)	Age	Characteristics
						using southern blot.
Hessl, Glaser, Dyer-Friedman, & Reiss (2006)	32 (F) 58 (M)*	6-17y (Mean 10.89)	N/A	53 (F) 37(M)*	6-17y (mean 11.13)	Unaffected siblings. (Confirmed absence of FXS or pre-mutation using southern blot.)
Hall, DeBernadis & Reiss (2006)	40 (F) 74 (M)*	6-17y (male mean:11.06y, female mean 10.42y)	N/A	-	-	-
Hall, Lightbody & Reiss (2008)	29 (F) 31 (M)	5-20y (M mean: 13.21, F mean, 13.06)	16 M and 6 F autism (23 M and	-	-	-

Study	FXS participants			Control Participants		
	N (M/F)	Age	Number with Autism	N (M/F)	Age	Characteristics
			13 F autism spectrum)			
Roberts et al. (2009)	51 (M)	FXS-only^{-} mean 3.99y; FXS+ASD^{+} mean 3.55y	18 with autism	21 (M)	Mean: 4.05y	Gender-matched typically developing (TD). No test *FMR1* status.
Scherr, Hahn, Hooper, Hatton & Roberts (2016)	31 (M)**	9.67-14.58y (Mean 12.4, SD 1.29)	N/A	49 (M)**	4.92-9.5y (Mean 7.0y, SD 1.04y)	TD, matched on non-verbal mental age at beginning of longitudinal study.
Wisbeck et al. (2000)	7 (F) 8(M)	6-25y (M mean 13.5y, F mean 13.9y)	N/A	41 (F) 43 (M)	Mean 7.5y	Non-matched normative

Study	FXS participants			Control Participants		
	N (M/F)	Age	Number with Autism	N (M/F)	Age	Characteristics
						sample. Data analysed in same laboratory.

Table 14

Comparisons of cortisol levels between groups of individuals with Fragile X Syndrome or comparison groups

Study	Stressor	Cortisol test		Findings of group comparisons
		Method	Sample Timings	
Bricout et al. (2008a)	Sub-maximal incremental physical exercise treadmill test	Blood (venous Catheter)	At rest (8.30am), start of test, exercise+10m, exercise+20m, exercise+40m, recovery+30m, recovery+60m.	FXS cortisol elevated during the first 20 minutes of the test (start inclusive) compared to controls and showed a decrease at exercise+40m, opposite to controls who showed an increase
Hessl et al. (2002)	-	Saliva (Salivette roll soaked 1-2 m). No citrus <30m, no dairy <60m	*Evaluation day*. 30m after waking, during testing (11am), prior to social challenge (3.30pm), 30m after social challenge, 90m after social challenge, bedtime. Cortisol levels for each sample were standardised by z-score transformation and averaged across the	*Typical Day.* Male FXS cortisol elevated compared to siblings on typical days (as indicated by reduced diurnal decline) but not females. *Experimental day.* Females did not differ from siblings. Males showed higher levels between pre-breakfast and pre-lunch samples.

Study	Stressor	Cortisol test		Findings of group comparisons
		Method	Sample Timings	
			evaluation day to create composite score. *2 consecutive typical non-school days*. Within 30m waking, before breakfast, one hour before lunch, one hour prior to dinner, bedtime. Cortisol levels for each sample were standardised by z-score transformation and averaged across the typical days to create composite score.	
Hessl, Glaser, Dyer-Friedman, & Reiss (2006)	Social Challenge (in home) modified from protocol used by Herbert, Bellack and Hope (1991). Counterbalanced presentation of one 15-20m session of including	Saliva (Salivette cotton roll soaked 1-2 m). No citrus <30m, no dairy <60m	2 samples: prior to social challenge (~3pm) and 30m after beginning social challenge	FXS showed higher pre-challenge levels than siblings. No differences in degree of change or post-challenge levels. FXS participants showed increased cortisol through whole home assessment period (reported in Hessl et al. 2002)

Study	Stressor	Cortisol test		Findings of group comparisons
		Method	Sample Timings	
	the following conditions: child interview, silent reading, oral reading, singing.			
Hall, DeBernadis & Reiss (2006)	Social Challenge. Conducted in-home at approximately 3pm. Fixed order presentation of one 15-20m session of each of the following conditions: child interview, silent reading, oral reading, singing.	Saliva (Salivette cotton roll 1-2 m)	One pre-challenge sample 3pm	-
Hall, Lightbody & Reiss (2008)	In home assessment including intelligence and autism testing.	Saliva (Salivette cotton roll 1-2 m)	Evaluation day pre-breakfast (8am), pre-ADOS-G (3pm), pre-dinner (5pm), and pre-bedtime (9pm).	-

Study	Stressor	Cortisol test		Findings of group comparisons
		Method	Sample Timings	
Roberts et al. (2009)	Naturalistic interactions with experimenter	Saliva (Salivette cotton roll soaked 1-2 m). No citrus or dairy <60m	Pre-assessment and post-social approach assessment.Time of day not specified.	FXS+ASD higher baseline and post-assessment than FXS-only. No group difference in magnitude of response. FXS+ASD higher post assessment and baseline than TD. No differences FXS-only and TD. No differences in magnitude of response.
Scherr et al. (2016)	Neurocognitive assessment battery	Saliva (Salivette, 1-2 m)	Baseline 15m (pre-assessment: 9am) and conclusion of assessment (12pm). Taken in Year 1, 2 and 3 of longitudinal assessment	Visual trend for increase in baseline cortisol over time (each year of longitudinal study). Not seen in TD. Both groups showed lower reactant cortisol than baseline. Year 1: FXS had significantly higher reactant than TD. Not significant at other time points. Non-significant trend for FXS to show greater change in time of cortisol (reactant-baseline) than TD.
Wisbeck et al. (2000)	Social Challenge modified from Herbert and colleagues' protocol (1991). Two 2-minute interpersonal role-play	Saliva (Salivette cotton roll soaked 1-2 m). No citrus <30m or dairy <60m	*Day 1: evaluation day.* Pre-breakfast, 30m post-stress, 90m post-stress, pre-dinner, bedtime.	*Routine Days.* Compared to normative, FXS higher at lunch and bedtime (no pre-dinner sample to compare)

Study	Stressor	Cortisol test		Findings of group comparisons
		Method	Sample Timings	
	tasks: speech/song and reading aloud.		*Days 2&3: routine days*. Pre-breakfast, pre-lunch, pre-dinner (no data for normative sample), bedtime. Average taken at each time-point across 2 days.	

Preliminary evidence for atypical regulation is provided by a case study of an adult male (age 24 years) with FXS who showed an atypical pattern of adaptation in response to physical exercise, compared to healthy controls (15 males; Bricout et al., 2008a). Larger studies have also evaluated differences in the magnitude of cortisol reactions, focussing particularly on the response to social stressors (due to the atypical social behaviour associated with the syndrome). The findings of these studies are mixed. Firstly, Hessl and colleagues (2002) observed that males with FXS (70, age 6-17 years) showed reduced diurnal decline in the period after meeting unfamiliar researchers, compared to the siblings (58 female, 51 male, age 6-17 years), which the authors suggested may have resulted from an increased response to this social challenge. In addition, Scherr and colleagues (2016) found that, in the first year of the longitudinal study, boys with FXS (31, age 9-14 years) showed higher levels of reactant cortisol following an assessment battery, when compared to TD controls (49, matched on non-verbal mental age, 4-9 years). These differences were not observed in the following two assessment years, in which fewer individuals participated. In addition, levels of baseline cortisol were higher in the FXS group than the comparison group, though this difference did not reach a level of statistical significance. The authors also noted differences in the changes of cortisol levels over the longitudinal assessment. Firstly, the degree of change in cortisol levels over the years of the longitudinal assessment (reactant minus baseline levels) increased in the FXS group, as compared to the TD controls. Visual analysis suggested that the baseline levels of cortisol increased over the years of assessment in the FXS group, but not the TD group. As such, the evidence from these two studies, as well as the aforementioned case study, suggest possible differences in the responses of boys with FXS, as well as differences in the development of this regulation over time.

However group differences were not observed in all studies. Further analysis of the data collected in the study by Hessl and colleagues (2002), did not find any differences between the children with FXS and unaffected siblings in cortisol levels in response to, or following, a structured social challenge (Hessl et al., 2006. FXS group: 58 males, 32 females, age 6-17 years. Sibling group: 53 females, 37 males, age 6-17 years). Finally, Roberts and colleagues (2009) conducted an evaluation of 51 males with FXS (mean age 3 years) compared to 21 male TD controls (mean age 4 years). The researchers investigated the magnitude of cortisol responses to a social interaction between children with FXS and their siblings without FXS. In addition, the FXS group was divided according to degree of autism symptomatology for analysis. It was found that, although there were no differences between young boys with FXS and low levels of autism symptomatology (who did not meet the criteria for a dual diagnosis of autism spectrum disorder on the CARS) and their siblings, children with FXS and high levels of autism symptomatology had higher levels of cortisol both prior to and following social interactions with an unfamiliar experimenter (though there were no differences in the magnitude of the response). This suggests that there may be differences in cortisol profiles within the population of people with FXS, relating to the degree of autistic symptomatology. The relationship between cortisol and autism symptomatology is discussed in further detail later in this review.

Thus, as with the findings in the preclinical literature, the findings of the studies in humans are heterogeneous. However, where differences were observed between the 'typical' or baseline cortisol levels of individuals with and without FXS, they were manifested as relative increases, rather than decreases, in cortisol secretion. This corresponds to the preclinical observations of comparatively higher corticosterone responses to stressors in *FMR1* KO mice in four studies; though, as mentioned above,

seven studies found no genotype difference in these animals. However, this potential trend in the findings highlights an avenue for future investigation.

Are there gender differences in the different aspects of HPA activity, in FXS? Given the broad gender differences in the manifestation of FXS, researchers have investigated whether there are differences in cortisol responses between males and females with FXS in four studies (Table 15).

Table 15

Gender comparisons of cortisol levels in individuals with Fragile X Syndrome

Study	Participant Type	N	Aspect of HPA activity measured	Gender comparison findings
Hessl et al. (2002)	Human	39 (F), 70 (M)	Typical day circadian rhythm (average 2 days)	Males and females both exhibited a normal diurnal decline. Males showed slower decline (higher cortisol) post-lunch until bedtime than females.
			Experimental day circadian rhythm (involves novelty and social challenges)	Males had greater response to visit than females: less decline (higher levels) between pre-breakfast and pre-lunch. Possibly related to meeting novel experimenter.

Study	Participant Type	N	Aspect of HPA activity measured	Gender comparison findings
Hessl, Glaser, Dyer-Friedman, & Reiss (2006)	Human	32 (F) 58 (M)*	Reaction to social challenge (pre and post measures)	No gender differences in FXS participants.
Hall, Lightbody & Reiss (2008)	Human	29 (F) 31 (M)	Collection at four time points during evaluation day	No main effect of gender
Wisbeck et al. (2000)	Human	7 (F) 8(M)	Typical day Circadian rhythm (average 2 days)	No male and female difference.
			Experimental day circadian rhythm (involves novelty and social challenges)	Males significantly higher than females 30m post-stressor and before bedtime.

Study	Participant Type	N	Aspect of HPA activity measured	Gender comparison findings
Markham et al. (2006)	Mouse	8-12 per group	Response to acute stressor (restraint)	Different patterns of response and recovery to 30m of restraint stress. Males show protracted return to unstressed baseline; females show protracted rise. Peak secretion does not differ.

In two studies, it was observed that males showed higher levels of cortisol following social challenges (a brief social stressor: Wisbeck et al., 2000; interaction with an unfamiliar experimenter: Hessl et al., 2002) than females. This suggests that atypical responding may be limited to, or at least exaggerated, in males with FXS, compared to females with the condition, mirroring the observations in the preclinical literature on the topic. Of note, statistical comparisons were not conducted in these studies. In contrast, no group differences were observed where statistical comparisons were conducted in other studies (Hessl et al., 2006: reactivity to social challenge. Hall et al., 2008: diurnal decline following social challenge).

Therefore, the results between studies are mixed, which may, in part, reflect the higher variability in the presentation of FXS in females, resulting from processes such as X-inactivation. In the wider literature, there is evidence of gender-related differences in HPA in adulthood, however it is unclear whether robust differences exist in younger individuals (Jessop & Turner-Cobb, 2008), such as those included in the studies reviewed. It is possible that there are also FXS-independent differences which contribute to this gender dimorphism. More detailed exploration of the relationship between other biomarkers (such as FMRP), cortisol and behaviour in males and females with FXS may help to clarify the origins of this variability and verify whether differences do exist.

Is there a relationship between cortisol levels and behaviour within FXS?

Animal literature. To date, there has been no research investigating whether individual differences in corticosterone responses relate to differences in behaviour. However, the utility of such investigations are unclear given that FMR1 KO mice exhibit reduced behavioural indicators of anxiety relative to controls (Elevated Plus Maze

(Pellow, Chopin, File & Briley, 1985): Qin et al., 2011; de Diego-Otero et al., 2008; Qin & Smith, 2008; Eadie et al., 2009), thus differing from the human presentation. Furthermore, correspondence between animal behavioural indicators and clinically significant behaviours in FXS has not been established.

Human literature. Five studies conducted within-group comparisons to investigate the relationship between salivary cortisol and measures of behaviour in individuals with FXS (see Table 13 for participant details and Table 16 for study details).

Table 16

Studies assessing associations between cortisol and behaviour in individuals with Fragile X Syndrome

Study	Behavioural measure		Association of behaviour with cortisol?				
	Topic	Method	Typical Day	Experimental Day			Other
				Pre-challenge	Reactivity	Post-challenge	
Hessl et al. (2002)	Problem Behaviour	Child behaviour checklist (CBCL; Achenbach, 1991). Total and sub-scale scores. Controlled for other factors associated with behaviour problems (See full text)	*Female.* Typical day composite significantly positively correlated with attention problems.	-	-	-	*Males.* Composite cortisol level (unspecified) accounted for 8% of variance in total behaviour problems. Higher levels were associated with increased behaviour

Study	Behavioural measure		Association of behaviour with cortisol?				
	Topic	Method	Typical Day	Experimental Day			Other
				Pre-challenge	Reactivity	Post-challenge	
							problems, especially withdrawn behaviour. *Female.* Cortisol levels account for 14% of variance in behaviour problems. Evaluation composite significantly positively correlated with

Study	Behavioural measure		Association of behaviour with cortisol?				
	Topic	Method	Typical Day	Experimental Day			Other
				Pre-challenge	Reactivity	Post-challenge	
							social and attention problems.
Hessl et al. (2006)	Social Escape	Measurement of gaze, vocal quality, discomfort and non-verbal task avoidance during social challenge.	-	-	Higher cortisol reactivity controlling for pre-challenge levels) associated with more gaze avoidance in siblings but opposite pattern in FXS (blunted response associated with	-	-

Study	Behavioural measure		Association of behaviour with cortisol?				
	Topic	Method	Typical Day	Experimental Day			Other
				Pre-challenge	Reactivity	Post-challenge	
					increased gaze avoidance) for both males and females. No other associations found.		
	Problem Behaviour	Aberrant behaviour checklist (ABC; Aman et al., 1985); CBCL and Autism Behaviour Checklist (Krug,	-	-	Increased cortisol reactivity associated with increased sensory and social relation problems in FXS (no other	-	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
		Arick, & Almond, 1993)			associations). No associations in sibling group.		
Hall et al. (2006)	Social escape	Measurement of gaze, refusals, face-hiding, eye-rubbing, hand-biting, fidgeting, leaving chair during social challenge.	-	In males, increased cortisol associated with decreased eye contact and increased fidgeting. No association with other social escape behaviours or	-	-	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
				number of problem behaviours seen.			
Hall et al. (2008)	Autistic Behaviour	Autism Diagnostic Observation Schedule-General (ADOS-G; Lord et al., 2001).		In males only, more autistic behaviour associated with lower cortisol.	-	-	-
	Compulsions	Compulsive Behaviour Checklist (Bodfish,	-	No association cortisol and prevalence of compulsions.	-	-	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
		Crawford,Powell & Parker, , 1995)					
	Self-Injurious Behaviour (SIB)	Self-injury checklist (SIB-C; Bodfish et al., 1995)	-	No association between cortisol and prevalence or number of forms of SIB.	-	-	-
Roberts et al. (2009)	Autistic Behaviour (AB)	Scores on Childhood Autism Rating Scale (CARS; Schopler,	-	No associations	Decreased cortisol change associated with increased autistic behaviour in FXS+ASD (only)	No associations	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
		Reichler, & Renner, 1986)					
	Social approach	Social Approach Scale- modified (Goldsmith & Lemery, 2000; Roberts et al., 2007): Initial and familiar approach (physical movement, facial expression & eye contact)	-	No associations in FXS group. In TD group increased cortisol associated with increased facial and eye contact during familiar social approach.	No association in FXS group. In TD group increased cortisol change associated with increased facial and eye initial social approach (no other associations)	FXS+ASD higher post-challenge cortisol associated with decreased initial physical approach.	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
Scherr et al. (2016)	Verbal working memory	Score on Memory for Words Sub-test of *Woodcock–Johnson Tests of Cognitive Abilities, Third Edition* (WJ-III, Woodcock, McGrew, & Mather, 2001)	-	Higher baseline cortisol was associated with poorer performance on memory for words working memory test, for both groups.	No significant association.	No significant association.	-
	Verbal working memory	Auditory working memory sub-test of *Woodcock–*	-	Increased baseline cortisol associated with	No significant association.	Overall fixed effects for auditory	-

Study	Behavioural measure		Association of behaviour with cortisol?			
	Topic	Method	Typical Day	Experimental Day		Other
				Pre-challenge	Reactivity	Post-challenge	
		Johnson Tests of Cognitive Abilities, Third Edition (WJ-III, Woodcock, McGrew, & Mather, 2001)		decreased performance in the FXS group, only.		working memory and cortisol change was significant, there were no significant effects of cortisol change or group	

Social and Autistic Behaviours. Many people with FXS display autistic-like characteristics including: gaze-avoidance, repetitive behaviour and shyness. However, not all individuals with FXS display sufficient levels of autistic symptomatology to meet diagnostic criteria for an autism spectrum disorder (Talisa et al., 2014). As such, a number of studies have investigated possible factors associated with the degree of autism symptomatology, including salivary cortisol.

Three studies have observed behaviours exhibited by individuals with FXS during various types of social interaction. Two of these studies, utilising the same group of participants, observed behaviour during a structured social challenge, which involved asking the child to read, answer questions and sing in front of others (Hall et al., 2006; Hessl et al., 2006). Many of the measured behaviours were not found to have relationships with cortisol levels including: vocal quality (including mumbling or intrusive tones: Hessl et al., 2006) discomfort (participant appears in crisis, demonstrating behaviours such as self-injury, crying, aggression: Hessl et al., 2006. Hand-biting was also assessed separately in: Hall et al., 2006), non-verbal task avoidance (physically leaving the situation or covering eyes; Hessl et al., 2006; Hall et al., 2006), verbal refusals (Hall et al., 2006). However, a positive correlation was observed with fidgeting (Hall et al. 2006). Most interestingly, gaze avoidance, one of the characteristic features of the FXS phenotype, was found to relate to levels of cortisol in both studies, though the direction of the associations differed. Hessl and colleagues (2006) found that (across males and females with FXS), after controlling for other potential influences, increased gaze aversion was associated with lower post-challenge cortisol levels. It was noted that the most gaze aversive children exhibited decreases in eye contact in response to the challenge. In contrast, Hall and colleagues (2006) found that *increased* mean levels of cortisol were associated with decreased eye contact.

However, these findings raise two hypotheses as to whether the primary influence on gaze avoidance relates to autistic-like characteristics (i.e. a lack of response to social stimuli) or social anxiety (i.e. an excessive response to social stimuli; Hessl et al.; 2006). Both hypotheses are interesting and warrant further investigation.

Furthermore, the relationship between salivary cortisol and social approach behaviour during naturalistic social interactions has been explored in one study (Roberts et al., 2009). The method involved investigating social approach behaviour (physical approach, facial expressions and eye contact) with an experimenter when they were both unfamiliar (first minute of interaction) and familiar (during last hour of day-long assessment) to the child. Control group children showed the expected pattern of arousal and behaviour. Namely, increased initial approach to the unfamiliar experimenter was associated with increased cortisol reactivity. In addition, those with higher baseline cortisol showed greater social approach to the experimenter later in the assessment, once they were familiar. However, the children with FXS showed a different pattern of association in this study. Firstly, the participants with FXS and low levels of autism symptomatology showed no significant association between cortisol and behaviour at all. Whereas, within the group of children with FXS and high levels of autism symptomatology, boys with higher cortisol levels (following the interaction) showed fewer physical approaches to the unfamiliar experimenter: the opposite pattern to in the control group. As such, this study suggests a possible association between heightened physiological reactions to social situations, and increased social avoidance.

Further evidence on the association between cortisol and autistic behaviour in FXS comes from studies which have utilised broader autism screening or diagnostic measures. Hall and colleagues (2008) utilised a direct observational assessment

measure (ADOS-G; Lord et al., 2001) with their participants. The results of the study indicated that lower baseline levels of cortisol were associated with higher levels of autistic behaviour. Hessl and colleagues (2006) also found a relationship between cortisol and some types of autistic behaviour: increased sensory and social relation problems were positively associated with cortisol reactivity to a social challenge. Roberts and colleagues (2009), in contrast, found that *reduced* cortisol reactivity (which the authors suggest could be related to elevated basal levels) to a social interaction was associated with increased autistic behaviour (as measured on a behaviour rating scale: CARS; Schopler, Reicher & Renner, 1980), only within the group of individuals with FXS and high levels of autism symptomatology; in the group of children with FXS and low levels of autism symptomatology, there was no relationship between cortisol and levels of autistic behaviour.

As such, a number of studies highlight associations between HPA activity and this key part of the FXS behavioural phenotype. However, the nature and direction of this association varies, with some finding increased levels of cortisol to be associated with increased autism symptomatology, others with decreased cortisol levels. The heterogeneity of measures of behaviour (direct observation as compared to informant rating scales) and cortisol may underlie such differences. In addition, the findings of Roberts and colleagues (2009) raise the possibility that levels of cortisol more strongly relate to behaviour in individuals with FXS and high autism symptomatology, as compared to those with lower symptomatology. In fact, the authors suggest that HPA dysregulation may serve as a biomarker of ASD in FXS. This highlights that individuals' levels of autistic behaviour may be important to consider when interpreting the results of studies of the relationship between cortisol and behaviour in FXS. Though, variations in the assessment of autistic behaviour across the other reviewed studies make it

challenging to evaluate this further based on the existing evidence. Future research to study the gradation of ASD in FXS would be valuable to delineate phenotypic boundaries and evaluate the significance of HPA function as a biomarker of ASD in FXS.

There is a growing body of literature relating to idiopathic autism which is also of relevance to this discussion. A review of this literature revealed differences in both HPA rhythm and responsiveness in individuals with autism (Taylor & Corbett, 2014). Typically, in response to social situations, individuals with autism exhibit blunted responsiveness, which corresponds to the patterns seen by both Hall and colleagues (2008) and Hessl and colleagues (2006). Interestingly, however, the differences observed in those with idiopathic autism seem to be moderated by levels of functioning: there is not conclusive evidence that HPA dysregulation observed in lower functioning individuals also applies to individuals with high functioning autism. In Roberts and colleagues' study, the participants with both FXS and high levels of autism symptomatology had lower levels of adaptive behaviour than those with low levels of autism symptomatology (though the significance of the difference was not evaluated), highlighting a potential confound. Future research should examine this potential relationship in the FXS population.

A broader question relates whether autistic-like behaviours in individuals with FXS meaningfully correspond to the characteristics seen with idiopathic autism (Hall et al., 2010). It is possible that autistic-like behaviours in FXS have different causal origins and, as such, the relationships between cortisol and behaviour may differ in those with syndromic and non-syndromic autistic characteristics. Future research might help to address these issues. For instance, comparison of the relationships between cortisol and behaviour in those with autism, including those with non-syndromic autism and those

with FXS who meet the criteria for autism, may help to elucidate whether cortisol and behaviour relations differ in their nature or development, dependent upon genetic status.

Behaviour problems. Behavioural problems and CBs are a key issue of concern for many caregivers of people with FXS. Two studies have utilised the Child Behaviour Checklist (CBCL; Achenbach, 1991) as a broad measure of behaviour problems, and explored relations between scores and cortisol levels. Hessl and colleagues (2002; controlling for other factors which were found to be predictive of the scores) found that a composite score of cortisol significantly predicted 14% of the variance in total behaviour problem scores for females with FXS. Further analyses indicated that increased cortisol levels were specifically associated with increased social and attention problems. Furthermore, a composite score representing cortisol secretion on typical days significantly positively correlated with attention problems and approached significance for somatic complaints and social problems. There were no other relationships between cortisol and other of the measured behaviour problems, including: withdrawn behaviour, anxious or depressed behaviour, thought problems, aggressive behaviour or delinquent behaviour. In the same study cortisol levels accounted for 8% of variance in total behaviour problems in the males with FXS, which approached significance. The strongest association with a subscale score was with withdrawn behaviour. In comparison, in a later study with the same participants (Hessl et al., 2006), no relationship was found between CBCL scores and any cortisol measures (baseline, post-challenge cortisol or magnitude of change) taken in relation to a social challenge.

Other characteristics. Scherr and colleagues (2016) found that increased baseline levels of cortisol were associated with lower verbal working memory performance in boys with FXS, suggesting a possible link between arousal levels and academic-related performance.

Summary Discussion

There are some interesting preliminary findings in the reviewed research. Though findings are heterogeneous, there are some notable observations and trends. In mice, no robust differences in baseline cortisol levels were seen, though there was some evidence of elevated stress-related reactivity. In human studies, baseline differences were observed in several studies (Hessl et al. 2002; 2006; Roberts et al., 2009), as well as some indications of reactivity differences, compared to TD children (Scherr et al., 2016; Hessl et al., 2002; Roberts et al., 2009), though such differences may be mediated by gender and degree of autism symptomatology. Whilst not as robust as the findings relating to the ANS (Klusek et al., 2015), these findings suggest that there may be differences in the secretion of glucocorticoids (indicative of differences in HPA axis function) in FXS.

At present, specific conclusions about the role of cortisol in behaviour associated with FXS are difficult to draw due to the high levels of variability and lack of correspondence between studies. However, there are suggestions of associations between cortisol levels and autistic behaviour, behaviour problems and key cognitive processes (working memory). The variability in findings relating to arousal-behaviour associations is also seen in the ANS literature discussed. Future research will undoubtedly help to clarify some of these uncertainties and strengthen the evidence to clarify the robustness of the observed themes. However, the variability is likely to also

emerge from the complexity of any underlying relationships, potential existence of within-syndrome sub-groups and other factors associated with the activity of these physiological systems. Of particular interest to the present thesis, however, are suggestions of associations between elevated cortisol and behavioural problems (Hessl et al., 2002) especially avoidant behaviour (Roberts et. al., 2009).

Critical evaluation. In addition to the suggestions discussed through the previous sections, there are several limitations with the research exploring the HPA axis to date and, therefore, considerations for future research. Broader investigations of the HPA system highlights its complexity, with individual differences relating to multiple factors, including: medication, pubertal stage, gender, temperament, chronic stress, compliance with the sampling protocol, nature of stressors, familial genetics and BMI (Jessop & Turner-Cobb, 2008; Gunnar et al., 2003; Dickerson & Kemeny, 2004; Kupper et al., 2005). As well as the variation in study methodology, many of these potential influences have not been explored or accounted for in the FXS research and may relate to the observed variability in study findings. Furthermore, the research to date has provided important but limited snapshots of the activity of the HPA axis, with the exception of Scherr and colleagues' (2016) longitudinal study, in small groups of individuals. Prospective or longitudinal studies including further information about an individual's characteristics, behaviour, environment and biology would help to provide a more detailed picture of the role of HPA activity in this population.

In addition, research involving humans has utilised TD comparison groups. However, an important step in future research will be to investigate the specificity of any group differences to individuals with FXS. It is possible that, rather than being directly FXS-related, the differences observed could relate to having an intellectual

disability or autistic behaviour, and may be seen in others without FXS, but with similar characteristics. For instance, earlier research with people with autism has highlighted that level of cognitive functioning relates to the findings: with differences being observed predominantly in those described as "low functioning" (Hoshino et al., 1987; Corbett et al., 2006; Richdale & Prior, 1992; Taylor & Corbett, 2014). In addition, given the aforementioned potential link also between levels of autism symptomatology and cortisol levels in those without FXS, and the high levels of autistic behaviour seen in people with FXS (Clifford et al., 2007), this clearly confound the differences seen. This is particularly pertinent given the findings of Roberts and colleagues (2009), that cortisol differences in FXS, compared to TD controls, were dependent upon levels of autism symptomatology. Thus, future research should employ control groups to help address these potential confounds, including those with non-syndromic autism and those with idiopathic intellectual disabilities.

In addition, in the human literature discussed above, much of the focus has been upon investigating HPA responses relating to social interactions or demands, in individuals with FXS. Given that individuals with FXS are prone to experiencing exaggerated behavioural responses, anxiety or phobias relating to many, varied situations (Cordeiro, Ballinger, Hagerman & Hessl, 2011), it is possible that idiosyncratic circumstances (outside of the examined social challenges or interactions) may also trigger cortisol responses that differ in magnitude or duration, compared to the general population. For instance, individuals with FXS are known to experience atypical sensory processing (Belser & Sudhalter, 1995) and have been shown to show elevated startle responses to sensory stimuli (Miller et al., 1999). Of note, research with individuals with autism has highlighted differential patterns of reactions to social-evaluative and non-social (such as unpleasant sensations) stimuli (Taylor & Corbett,

2014). Therefore, future research should address cortisol responses to a wider variety of situations which may be challenging for individuals with FXS, in order to gain a broader picture of HPA activity in this population, and its potential applicability to day-to-day challenges.

Conclusion. In summary, the evidence suggests that individuals with FXS exhibit alterations in stress-related psychobiology. An earlier review of the literature (Klusek et al., 2015) highlighted robust evidence for cardiac autonomic hyperarousal, and reduced parasympathetic regulation. This has also been supported through other measures of autonomic activity. The present systematic review has been the first to collate the animal and human literature relating to the output of the HPA axis and its significance to behaviour in FXS. Though the findings are variable, there is emerging evidence that cortisol levels differ in males with FXS, compared to typically developing controls. Autism symptomatology appears to be associated with cortisol levels within FXS and must be considered when interpreting results.

For both physiological systems, there is some evidence that levels of activity relate to socially significant behaviours, thus highlighting a number of important avenues for future exploration. There exists some evidence to suggest an association between cortisol and behaviour problems in this group (Hessl et al., 2002) and social escape behaviours such as gaze avoidance (Hessl et al., 2006; Hall et al., 2006). However, to date there have been no investigations which are able to directly inform the previously presented hypothesis relating to cortisol and escape-maintained CB in FXS. This issue will be addressed in subsequent chapters.

Chapter 4

A preliminary study of arousal and behaviour in Fragile X Syndrome: assessing the feasibility and acceptability of saliva sampling.

Chapter Overview

Through the earlier chapters it has been identified that boys and men with Fragile X Syndrome (FXS) display differences in levels of arousal, compared to controls, (reviewed in Chapter 3). It is unclear how this may be associated with an increased propensity to engage in escape-maintained challenging behaviour (CB: reviewed in Chapter 2). One of the primary aims of this first empirical study was to assess the feasibility and acceptability of collecting saliva samples for the purpose of analysing arousal-related measures, in order to investigate this hypothesis further in later studies. Saliva samples were collected across a typical school day from boys with FXS and unaffected siblings, in order to assess circadian rhythmicity of cortisol and α-amylase (an indicator of autonomic activity). During the same day observational data was collected on the occurrence of challenging behaviours, using the Functional Assessment Observation Form (FAO: O'Neill et al., 1990), in order to further understanding of the environmental context of these behaviours in a natural setting.

The collection of saliva samples was deemed to be both acceptable and feasible. In addition, novel preliminary differences were observed between boys with FXS and their siblings with respect to the Cortisol Awakening Response (a previously un-investigated aspect of the cortisol circadian rhythm in FXS). Further differences in arousal-related measures are observed and discussed. A high number of instances of CB were observed across the group of boys with FXS, particularly SIB. In line with earlier literature, demands were the most common antecedents to CB. However, analysis of the

profile of individual primary functions did not reveal clear within-group patterns. The implications of the findings are discussed in relation to earlier research, as well as implications for subsequent arousal-behaviour investigations.

Introduction

Saliva sampling is a commonly used method to comprehensively assess arousal of the stress-regulatory systems. As previously discussed, salivary cortisol is an indicator of hypothalamic-pituitary-adrenal (HPA) axis activation (Jessop & Turner-Cobb, 2008). In addition, salivary α-amylase (SAA) has been identified as a biomarker for autonomic arousal. Whilst its key function is to assist with the digestion of starch in the mouth (Scannapieco, Torres & Levineet al.1993), its release is controlled by ANS activation and has been found to serve as a proxy indicator of autonomic arousal. As such, its measurement via saliva sampling represents an additional low-invasive option for assessment (Nater & Rohleder, 2009). The salivary glands are solely innervated by the sANS and previous literature has demonstrated the ability of a vast range of stressors (physical and psychological) to augment the output of salivary α-amylase (Maruyama et al., 2012; Payne, Hibel, Granger, Tsao & Zeltzer, 2014). As well as secretion related to stress and other bodily processes, release of SAA follows a basal diurnal rhythm, characterised by a decrease in the first 60 minutes after awakening, followed by a steady increase (Nater et al. 2007).

Earlier studies have successfully utilised salivary measures with individuals with FXS (see Chapter 3 for a review). However, in order to investigate the association between physiological arousal and escape-maintained CBs, it may necessary to conduct research with children with FXS who have been specifically selected for engagement in CBs. There may be additional challenges which arise when collecting samples from this

sub-group. For instance, Wheeler and colleagues (2016) found that increased sensory issues were significant predictors for the frequency and severity of aggressive acts, in people with FXS. Similarly, Symons et al. (2010) found that individuals with FXS who engage in SIB have more sensory issues than those who do not. As such, children with FXS and CBs are likely to experience greater sensory issues than the broader FXS groups in earlier research, which may complicate the saliva collection procedure. As such, it is important to conduct an initial feasibility study in order to assess whether methodological refinements will need to be made prior to conducting a larger study. Furthermore, this preliminary study will help to develop the researcher's expertise in, and protocols for, saliva collection and analysis.

In addition to investigating the feasibility of the saliva collection procedures, it is key to assess the acceptability of the method for both parents and the participants themselves. Though generally classed as non-invasive, saliva collection requires participants to either hold a salivette in their mouths or to passively drool, which for some may be challenging. Identifying any issues with acceptability at this stage will allow refinements to be made to the sampling protocol, prior to conducting more extensive research. Few studies have assessed the acceptability of saliva sampling, though Putman and colleagues (2012) found that children with high functioning autism rated both passive drool, salivette and sorbette (a small dart-shaped sponge on a plastic shaft) methods to be acceptable. As discussed, it may be that the experience of children with FXS who exhibit additional behavioural challenges differs from other groups.

One key way of assessing differences in HPA axis function in previous research has been to assess the circadian rhythm of cortisol release in individuals with FXS, compared to unaffected siblings (Hessl et al., 2002; Wisbeck et al., 2000) or wild-type

mice in animal studies (Qin & Smith, 2008). This study aimed to replicate such investigations in order to further clarify group differences. Use of a sibling comparison group allows examination of the effects of FXS upon adrenocortical activity, whilst controlling for the individual's home environment and familial genetics (Wust et al., 2000). In addition, repeated sampling facilitates assessment of the feasibility and acceptability of the procedure.

Researchers investigating circadian rhythmicity in FXS have not yet assessed the Cortisol Awakening Response: a key aspect of the activity of the HPA axis. This brisk, morning rise in cortisol occurs in the majority of people, in approximately the 30 minutes after awakening, and is superimposed upon the basal circadian rhythm (Wust et al., 2000). The exact function and teleological relevance of the CAR is still being determined. However it may function as an anticipatory response, preparing for the up-coming demands of the day (Steptoe & Serwinksi, 2016). This aspect of adrenocortical function is discrete from the rest of the circadian cycle (Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007). Of importance for the present investigation, the CAR is believed to serve as a reliable indicator of the reactivity capacity of the HPA axis (Schmidt-Reinwald et al., 1999). As such, the assessment of the CAR may allow an estimation of stimulus-bound arousal capacity, in the absence of applying a stressful stimulus to trigger a response. Cohen and colleagues (1995) hypothesised that stimulus-bound arousal changes are key in FXS, and may be associated with CBs exhibited in this group.

The CAR has been associated with mental health risk and a range of physical health problems (Steptoe & Serwinski, 2016). For instance, chronic stress and worry appears to be associated with increased CARs (Schlotz et al., 2004) which may be of relevance to the population of people with FXS and CBs, as well as anxiety related to the

behavioural phenotype (for instance; Cordeiro et al., 2011). Interestingly, Brosnan and colleagues (2009) found an absence of the CAR (but typical diurnal decline of cortisol) in adolescent males with Asperger's Syndrome. The authors hypothesised that this absence may be related to an inability of the HPA axis to respond to environmental changes in this group, and that this may be associated with autistic-like characteristics such as resistance to change and social deficits. Though the mechanisms and nature of this potential association are yet to be investigated, this suggests potential relevance of this phenomenon to individuals with FXS, who similarly display these characteristics (Woodcock & Oliver, 2008). However, as with many findings relating to cortisol and behaviour, this difference has not been consistently observed: other studies of children with high functioning autism (Zinke, Fries, Kliegel, Kirschbaum & Dettenborn, 2010) or autism spectrum disorders more broadly (Corbett & Schupp, 2014) did not identify differences in CAR relative to TD controls. As with other aspects of cortisol release, interpretation of findings may be complicated by the non-linear relationship between stress and cortisol: acute stress has been associated with increases in cortisol but the system may become hypoactive with chronic stress, leading to blunting of the response or decreases in cortisol (Fries, Hesse, Hellhammer & Hellhammer et al., 2005).

Therefore, in addition to the broader investigation of adrenocortical activity via diurnal decline, it was proposed to investigate the CAR, in order to further understanding of HPA function in this population. It was hypothesised that, given the broader differences observed in the release of cortisol in this population, there would be differences between the CARs of boys with FXS and their siblings.

Investigation of cortisol levels allows for assessment of the function of the HPA axis, the endocrine stress-effector system. However, the autonomic nervous system

(ANS) also plays a key role in the stress response. As reviewed in Chapter 3, a number of studies have investigated the ANS in FXS and have identified alterations in the activity of both the sympathetic and parasympathetic branches. Furthermore, the balance between these two systems is also important as with high chronic stress or allostatic load, dysregulation can occur between these systems (blunting of cortisol and increases in salivary α-amylase (SAA)), which has been associated with negative psychological states (Ali & Pruessner, 2012). As such, investigating both systems in this single study may provide additional insight into differences in arousal in FXS. Various methods used have been used to assess ANS activity, including through SAA. As such, concurrent assessment is possible in the present study without the need for additional technology and participant burden. Kidd and colleagues (2012) found that young children with autism and low IQs exhibited higher levels of SAA during the day than typically developing comparisons, which the authors hypothesised may relate to some of the symptoms experienced within this group. In the present study SAA levels were measured, along with salivary cortisol, in order to be able to gain a broad picture of the functioning of the stress-effector systems in FXS.

An additional aim of this study was to conduct further investigations into behavioural function in boys with FXS. Spending the day with participants in order to collect samples provides an opportunity to conduct observations of the occurrence of behaviours in their natural setting. Previous investigations of CB in FXS have largely focussed upon indirect reports from caregivers. It is possible that the ability to detect more subtle trends or patterns may have been limited by the use of standardised measures. Information about CBs has also been previously ascertained through experimental functional analyses (Iwata et al., 1994) in order to identify the functions of their behaviours (for instance: Langthorne & McGill, 2011; Machalicek et al., 2014;

Kurtz, Chin, Robinson, O'Connor & Hagopian, 2015). Though this experimental approach is considered the gold standard of functional assessment, the analyses are conducted in artificial, analogue sessions, typically in single settings, which may limit the generalisability of the findings. Descriptions of the occurrence of CBs in a natural setting will help to add further context to previous research aiming to understand the contexts of CBs in individuals with FXS.

Therefore, alongside the saliva sampling, behavioural observations were conducted across a typical school day, including at home and school. The aim was to provide detailed and naturalistic descriptions of the occurrence of CBs in this population, including frequency and topography. Such recordings have been shown to correspond to the outcomes of experimental assessments (Alter, Conroy, Mancil & Haydon, 2008; Sasso et al., 1992), though not in all investigations (Pence, Roscoe, Bourret & Ahearn, 2009). However, observing behaviours in the natural environment provides a valuable addition to prior anecdotal and analogue experimental research and may provide further insight into the nature of behaviours in this group. Based upon prior investigations of behavioural function within this group, it was hypothesised that such behaviours would be most likely to occur in contexts associated with an escape function.

Finally, though the small size of this initial study limited the ability to conduct in-depth investigations, preliminary investigations into associations between arousal and behaviour were possible. Any trends or findings would then guide and inform future research.

Aims and hypotheses. In summary, the aims and associated hypotheses of the present study were as follows:

- Evaluate the feasibility and acceptability of collecting saliva samples from individuals with FXS who exhibit CB.
- Conduct between group investigations to investigate the diurnal rhythm of cortisol (including the CAR) and SAA in boys with FXS compared to unaffected siblings.
    - Based upon prior literature (for instance: Hessl et al., 2002) it was hypothesised that boys with FXS would exhibit reduced diurnal decline relative to unaffected siblings.
    - Due to broader autonomic hyper-reactivity (for instance, as reviewed by: Klusek et al., 2015) it was hypothesised that boys with FXS would exhibit higher levels of SAA compared to unaffected siblings.
- Observe CB in a natural environment in order to collect information on frequency, topography, environmental context and inferred function.
    - Based upon the literature reviewed in Chapter 2, it was hypothesised that escape would be the most common perceived function for challenging behaviours.
- Conduct exploratory, preliminary explorations of possible associations between arousal and CB.

Method

Design. This study used a cross-sectional between-participants design. Exploratory within-group analyses were also conducted.

Ethics and governance. Ethical approval for the study was gained from Kent and Sussex NHS research ethics committee (REC Reference: 13/LO/0244). Local Research and Development approval was obtained from three NHS sites (Guy's and St

Thomas', Birmingham Women's and St George's) to act as Participant Identification Centres. In addition, study approval was obtained from the Fragile X Society's research committee in order to recruit participants through the organisation.

Participants.

Inclusion criteria. Families were eligible to participate who had a son with a genetically-confirmed diagnosis of Fragile X Syndrome, as well as an unaffected sibling (male or female). Both children were required to be school-age (5-15 years) and living in the same home. Children with FXS were not eligible to participate if they had an additional diagnosis of a second genetic condition (such as Down's syndrome) but were eligible if they had a dual-diagnosis of autism or ADHD, due to the frequent associations of these diagnoses with the FXS phenotype (Bailey et al., 2008). Information was gained about diagnostic status from the parents. In addition, parents were asked whether their child with FXS currently (in the past month) engages in CB (self-injurious behaviour, physical aggression or property destruction). Families were eligible to participate who reported the presence of one of more of these types of CB.

Sibling participants were not required to have had a negative genetic test to be classified as 'unaffected' for the purpose of this study. In the absence of symptoms characteristic of FXS, children under the age of 16 are typically not tested via the NHS (Barnicoat, 2016), with the rationale that they should be able to make their own decision as adults as to whether to be tested. Therefore, if a genetic test had not been conducted, siblings were eligible if there was no parental suspicion of them having FXS, namely there was no evidence of learning difficulties and emotional or behavioural problems. Testing was not conducted for the purposes of this study for ethical and resource-related reasons.

Participants were assigned an ID code which consisted of a number (which related to the participant's family) followed by FXS (Fragile X Group e.g. 3FXS) or Sib (sibling group e.g. 4Sib). Two sibling pairs participated from one family and pairs (FXS and non-FXS) are indicated with an 'a' and 'b' depending on pairings for the days of research. This nomenclature is used for reporting of the results.

Recruitment. Families were contacted with study information sheets (Appendix B. Additional information was also provided with more details about cortisol and SAA: Appendices C & D) through several different avenues. Firstly (after approval and review by a committee of parents of people with FXS and specialist advisors) the Fragile X Society, the UK charity supporting people affected by FXS, distributed information packs to members who had previously agreed to be contacted about research and who were known to have a son with FXS between the ages of 5-15 years, according to information provided at membership registration. In addition, short adverts for the study were placed on the charity's website, newsletters and social media. Nine families (ten sibling pairs) were recruited through the Fragile X Society.

Secondly, the researcher contacted Genetics Centres in England whose catchment areas fell within what was deemed to be a reasonable travelling distance from the study base (Canterbury). Local collaborators were identified in three NHS Foundation Trusts: Guy's and St Thomas', Birmingham Women's and St George's. Local collaborators identified families who were known to have a son with FXS and, if known from records, a sibling who was not known to have FXS. The collaborators then posted packs to the families or disseminated via their clinicians. Five families were recruited to the study by this method.

Of note, recruitment was challenging and lasted approximately 18 months. Participation was initially available only to families in the south east of England, however, this was extended to the whole of the UK following insufficient numbers of responses. A number of families expressed interest in participation but were unable to do so due to not meeting the study criteria: one family had a son with FXS who had an additional diagnosis of a second genetic condition; three families had an unaffected sibling who was not within the inclusion age range.

Participant characteristics. In total, 15 pairs of siblings participated in the research. All participants with FXS were male, whereas eight (53.33%) of the sibling participants were female. The two participant groups were closely matched on age; the mean age of the FXS group was 112.60 months (SD= 33.15), compared to 110.36 months (SD=33.56) for the sibling participants. Four boys with FXS attended mainstream schools, one within a Special Education Hub. All remaining participants with FXS attended special education schools. All sibling participants attended mainstream schools. Six of the siblings had received a genetic test and were confirmed to have a number of FMR1 CGG repeats within the normal range. The remaining 9 siblings had not been tested due to not showing any behavioural, emotional or cognitive symptoms warranting evaluation.

Autistic Behaviour. FXS is closely associated with autistic-like features (Harris et al., 2008) which may independently be associated with alterations in differences in arousal levels. As such, descriptive data about the FXS group's autistic behaviour was collected for the purpose of interpreting the findings and comparing with other research. Diagnoses of autism were not considered accurate measures of behaviour due to reported variations in access to diagnostic services across the country and differing

parental attitudes to deciding whether to seek additional diagnoses. Therefore, information was collected directly from parents for the purpose of this study using the Social Communication Questionnaire (SCQ; Rutter, Bailey & Lord, 2003): a 40-item questionnaire which indirectly assesses autistic-like behaviour. Two thirds of participants with FXS (10/15) scored above the autism spectrum disorder cut-off, and 40% (6/15) above the autism cut-off. Descriptive statistics for participants' scores are presented in Table 17.

Table 17

Descriptive statistics for Social Communication Questionnaire

Score	Maximum Possible Score	Mean (SD)	Range
Total	39	19.47 (7.44)	4-31
Reciprocal Social Interaction	15	6.87 (2.39)	4-11
Communication	13	6.2 (2.48)	2-11
Restricted, Repetitive and Stereotyped Patterns of Behaviour	8	5.0 (2.07)	0-8

Medications. Due to the small, exploratory nature of this study, it was not deemed appropriate to ask participants to stop taking medication in order to participate in the study. However, information was gained about medications use. No siblings were taking medication on the day of the study. However, six participants (40%) with FXS were currently using medications. Three participants were taking drugs classified as having a

potential interaction with salivary cortisol: sertraline[33], clonidine[34] or risperidone[34] (Granger, Hibel, Fortunato & Kapelewski, 2009; one participant taking each of aforementioned medications). Five participants were also taking medications not believed to interact with salivary cortisol: 4 were taking melatonin (2 of whom also took a second medication) and one was taking an anti-epileptic medication (sodium valproate).

Measures and procedure.

Initial meeting. Eligibility of respondents was initially screened through a brief telephone interview. Informed consent was gained from parents of eligible families, but assent was later gained, where possible, from the children participating. Even where formal assent could not be gained from a child prior to data collection, no procedures were carried out against the opposition of the child.

Initial visits were then scheduled with families in their own homes. One of the aims of the initial meeting was to allow the families to meet the researcher who would be conducting the observations, to help them feel comfortable with data collection. Furthermore, children were familiarised with the saliva collection procedure and were allowed to trial the available saliva collection methods (described below). A collaborative decision was then made between the parents, researcher and the child as to which was the most suitable method for each individual. When requested by parents,

[33] Classified by Granger et al. (2009) as a medication which has the potential to influence salivary cortisol levels by affecting subjective experience of stress, novelty, threat or pain.

[34] Classified by Granger et al. (2009) as medication with potential to influence salivary composition and availability indirectly by affecting the activity of the Sympathetic Nervous System.

additional materials were given to families to allow continued practice prior to the data collection.

In addition, questionnaires were administered to parents (either an individual parent, or both parents simultaneously) in an interview format. As well as demographic information (including medication status and evidence of diagnosis), parents were asked to report topographies of CB in which their child with FXS engaged, for the awareness of the researcher during the observations.

Social Communication Questionnaire. A validated questionnaire (SCQ) was administered by interview, in order to assess autistic behaviour in the children with FXS. The lifetime form of the measure was used, in order to cover children's developmental histories. Respondents were required to provide yes or no responses to indicate the presence or absence of various characteristics related to autism. Higher scores indicated higher levels of autistic behaviour. The scale yields a total score which may be used as a screening tool, with a score above 15 indicating a high likelihood of the presence of autism spectrum disorder and a score of 22 or over being indicative of possible autism (Rutter, Bailey & Lord, 2003). In addition, items may be divided into three categories to derive sub-scale scores based on the hypothesised triad of impairments associated with autism, namely: restricted, repetitive and stereotyped patterns of behaviour; communication; reciprocal social interaction. The sub-scales have not been formally validated.

Evaluation day. Following the initial meeting, participants' schools were contacted in order to gain permission to assist with the research. Siblings' schools were asked to assist the child with collecting saliva samples at two points during the school day. The schools of the children with FXS were asked for permission for the researcher

to attend for one day to conduct observations and to collect the saliva samples. Subject to school approval, an evaluation day was scheduled with no planned atypical stressful or exciting events (such as a school performance or an exam). Children were required to be physically well on the day of the research and visits were rescheduled in the event of sickness.

During the evaluation day both groups of participants provided saliva samples. However, observational measures of CBs were conducted with the FXS group, only.

Salivary measures. Over the evaluation day all participants were asked to provide saliva samples at six time points. Participants and/or parents were given the following instructions prior to sample collection: avoid consumption of dairy products in the 60 minutes prior to the sample; avoid brushing teeth, eating a meal or consuming anything other than water in the 30 minutes prior to the sample; avoid drinking water 10 minutes prior to the sample. The aim of these restrictions was to minimise sample dilution or contamination.

The first two samples of the day were collected at times in relation to when the child was reported to wake: immediately after (or as close as possible to) awakening and 30 minutes after awakening, in order to assess the Cortisol Awakening Response (Wust et al., 2000). Awakening times were between 5.30am and 7.37am (Mean= 6.50am). Approximately half (53.33%) of the FXS group woke naturally (awakening time was monitored by parents but the exact time was not objectively measured), the remainder were awoken by parents (40%). Awakening type was not recorded for one participant in the FXS group. In the sibling group 53.33% were awoken by parents and 6.67% woke naturally, for the rest of the group the awakening type was not recorded.

The remaining samples were collected at set times throughout the day in order to assess the diurnal pattern of activity: 9am (morning), 12pm (pre-lunch), 5pm (pre-dinner), 7pm (pre-bed). Actual times of collection were monitored through the researcher's recordings or by respondents' recordings (in the absence of the researcher). Issues and experiences were noted for the purposes of assessing feasibility of the method.

Collection methods. Participants were able to choose from two methods of saliva collection: swab or passive drool[35]. Photographic information sheets were available to assist with all procedures (see Appendix E for example). The swab method involved asking the child to hold one end of a Salimetrics Children's Swab under their tongue for 1 to 2 minutes (or for as long as the child would allow). After being allowed to soak, the swab was removed and the wet end placed into a Salimetrics Swab Storage Tube, before any dry section was removed with a clean pair of scissors. The alternative method was passive drool. Participants were asked to pool saliva in their mouth and expel it into a container made of an inert polymer (Salimetrics Swab Storage Tube or 50ml Falcon Sample Collection Tubes, which had wider openings for ease of collection). Salimetrics Saliva Collection Aids were also available to participants, though they were not utilised. All samples were securely sealed within the tubes after collection and labelled with time of collection and a participant ID code. Swabs were selected as the preferred method by 11 participants with FXS, whilst the remaining four opted to use passive drool as their primary method (although, two used swabs in combination with this on at least one occasion due to difficulty with producing enough saliva to drool into the container). In

[35] Although sorbettes have been used in previous research (for example: Zinke, Fries, Kliegel, Kirschbaum & Dettenborn, 2010) they were not deemed suitable for the present study due to their small size and the associated swallowing risk.

contrast, swabs were used by only one of the sibling participants: the remaining 14 used passive drool.

The researcher was always available to assist with the sample collection (with the exception of the two school-time samples for the siblings). However, for some children and time points, the assistance of teachers or a parent was deemed to be most appropriate. At school, sibling participants were supervised by teachers, as the researcher was attending school with the FXS participants. At each time point, the person supervising gained the assent of the child for the sampling.

Storage. Immediately after collection, the sample was sealed in a bag and chilled (in a refrigerator or in a cool-bag with ice packs) or frozen in a domestic freezer (approximately -20°C). Where required, samples were transported between locations (for instance between home and school) in cool bags with ice packs. The samples were then transferred frozen, and on ice, to a -80°C freezer in the Medway School of Pharmacy, for storage until assay.

Evaluation of acceptability of sampling. Saliva Collection Rating Forms were designed to record parents' and children's experiences of the saliva collection procedure, to assess acceptability for future research. The questionnaires were based upon the Treatment Acceptability Rating Form (TARF-R: Reimers, Wacker & Cooper, 1991) and adapted to apply to the procedures in this study. Different versions of the scale were produced in order to suit the needs of the different groups (parents and participants, for example: Appendices F & G). Respondents rated their feelings about different aspects of the sampling procedure on a Likert scale ranging for 1-7 (1 being negative opinion, 7 being positive). A simplified version of the scale was produced for younger participants or those with FXS with simple language and facial expression

symbols as responses. There was also an open space at the bottom of the questionnaire where specific thoughts or recommendations could be recorded. Respondents were given the forms at the end of the evaluation day and were allowed to complete them without the researcher present.

Analysis. Samples were analysed at the Medway School of Pharmacy by the author (Becky Hardiman) supervised by a neuropharmacologist (Dr Alison Bratt). Samples were initially assayed for levels of cortisol using Salimetrics Cortisol ELISA Kits, according to instructions. Samples were defrosted and each analysed in triplicate, or as many times as the sample volume would allow (each repeat requires 25µL of analyte). Cortisol levels were measured in micrograms per decilitre (µg/dL). Samples were then re-frozen until SAA assays could be conducted. These analyses used Salimetrics Salivary SAA Kinetic Enzyme Assay Kits. Samples were analysed in duplicate (with each repeat requiring 10µg/dL). SAA activity is measured in units of activity per millilitre over a two minute incubation period (U/mL). One family (one sibling participant and one participant with FXS) did not respond to requests for additional consent for the SAA analyses, and as such were not included[36].

A high proportion (95%) of planned samples were able to be collected. Low sample volume resulted in an inability to conduct one or both assays for a number of samples. Five samples (2.94% of collected samples) had insufficient volume for any analysis (>10µL) and a further two did not contain enough for a singlet cortisol assay (25µL: 1.20% of collected samples), though these were later tested for SAA activity. After initially running tests for cortisol levels (the maximum number of cortisol assays

[36] The SAA analyses were not included in the original research design, meaning additional consent was required.

were conducted based upon sample volume, up to a triplicate analysis), there was an insufficient volume of saliva remaining for SAA assays, for six of the collected samples.

An additional eight samples did not produce usable data: three samples produced optical density values which were incalculable (the optical density was beyond the values provided in the standard curve); five had optical density values close to that of the zero wells, leading to extremely high estimations of cortisol concentration which were not biologically possible. These latter values were excluded from the analyses as outliers (according to the criterion of >3 standard deviations above the mean).

In total, 67.22% of the planned samples were able to be analysed in duplicate for SAA and in triplicate for cortisol, as aimed (70% of planned samples for participants with FXS, 64.44% for sibling participants). However, values for both analytes could be calculated for 77.22% of the planned samples, with some cortisol values being produced from duplicate or singlet cortisol assays (80% of samples for the FXS group and 74.44% for siblings). Sensitivity analyses were conducted in order to determine whether the findings from reduced assay repeats may have influenced the results: between-group comparisons were run with and without the singlet cortisol results[37]. The findings were robust to reduced repeats as such these samples were retained in the analyses to preserve a larger sample size.

[37] Salimetrics recommend duplicate analysis of each sample when conducting a cortisol assay (Salimetrics, 2016a), though in the present study triplicate analysis was chosen as the preferred method in order to improve the robustness of the analysis. Similarly, in the present study the aim was to conduct duplicate assays for SAA, though Salimetrics recommend singlet assays (Salimetrics, 2016b).

Fidelity of sample timings. Reported awakening time was recorded (parental or researcher report) in order to explore the fidelity of the timing of the first two morning samples (immediately after awakening and 30 minutes after awakening). Of note, the exact time of awakening was not objectively recorded (such as via actigraphy) and is therefore unclear. The time of awakening was recorded for 13 of the FXS participants and for all sibling participants. Across the groups the mean lag to sample collection after awakening was 4.6 minutes (SD=5.23, range= 0-17). The mean timing of the second sample was 34.68 minutes after waking (SD=6.45, range= 19-37). The averages of the absolute number of minutes from which the actual sampling times varied from the planned timings is recorded in Table 18.

Table 18

Timings of saliva samples

	Absolute minutes deviated from planned sample time		
	Mean	SD	Range
9am	8.47	11.12	0-40
12pm	4.73	8.42	0-40
5pm	8.0	9.59	0-35
7pm	14.20	12.94	0-35

Challenging behaviour measures. Information on CB was gained through direct observations of participants in a naturalistic context. Information on three types of behaviour was collected (defined in Table 19): SIB, physical aggression and property destruction. The functions of CBs were hypothesised based on structured observations of the environmental contexts in which they occurred. The researcher shadowed the participants during a typical school day, whilst at home and at school (aside from one participant whose families declined to be observed at home). During this time, the researcher did not initiate interactions with the individual being observed, aside from prompting saliva sample collection and responding to initiated interactions with brief and neutral comments. In addition, interaction with other individuals in the environment was minimised, although for practical reasons conversations were occasionally held. It was ensured that the participant always had a private space to go where they would not be observed and observations were not conducted during private activities.

Observations were conducted continuously, unless a break was required for a particular reason or the child was not able to be observed. Detailed descriptions of individual instances of CBs were made, in an Antecedent-Behaviour-Consequence format (see Functional Assessment Observation form). There was also space to make broader descriptions of the person's activity. The aim of this additional recording was to gain further contextual information to aid the formation of hypotheses about the function of CBs. The total duration of observations varied due to practical limitations and respect for individuals' privacy. The mean total length of observations was 7.7 hours (SD= 1.56; range=5.17-11.17 hours)[38]. The majority of observations took place in school (mean= 5.27 hours; SD= .7 hours; range= 4.25-7.08 hours), as compared to home or locations outside of school or school activities (mean=2.43 hours; SD=1.36 hours; range=0-5.25 hours).

Table 19

Definitions of types of challenging behaviour

Behaviour Type	Definition
Self-injurious behaviour	An action that an individual performs which results in pressure on or impacts to their own body (with their body or an object which they are manipulating, or hitting their body against an object or surface). The action must result in an audible sound or result in damage to the area, including reddening, or would be expected to do so if allowed to continue. These behaviours must

[38] The researcher was typically present from awakening (~6am) until bed time (~7pm), though observations were shorter in duration due to transitions between locations, non-observed activities and breaks.

Behaviour Type	Definition
	not be part of a specified, culturally-normal activity (e.g. clapping or bouncing). Observed SIBs included hand-biting and self-hitting (with own hand or with or against an object). Self-chewing was also recorded as a self-injurious behaviour, as repeated hand-mouthing may have long-term consequences (for instance: Ball, Campbell, & Barkemeyer, 1980). Behaviour was classified as chewing when the hand or finger was inside the mouth and the jaw was moving up and down in a way that suggested repeated, light squeezing between the teeth. In contrast, behaviour was recorded as biting when the pressure on the skin from the teeth appeared to be constant (no repetitive movements of the jaw could be observed).
Physical aggression	The individual performs a behaviour which involves themselves, or an object which they have manipulated, making rough physical contact with another person. Behaviours were also coded when contact was not actually made but would have been expected to if the recipient had not acted to avoid the consequence, or if a thrown object landed within approximately 0.5m of the recipient. The affect of both individuals (the person being observed and the recipient of the behaviour) was noted: behaviours were not coded as aggressive if both individuals were displaying positive affect (for instance, smiling or laughing). However, behaviours were coded as aggressive if at

Behaviour Type	Definition
	least one of the individuals in the interaction (the observed participant or the recipient of the target behaviour) displayed indicators of neutral or negative affect. Observed topographies of aggression included: hitting with body (for instance kicking or slapping), hitting with objects (throwing, ramming, hitting with a held object and slamming door onto recipient), biting and pushing or pulling.
Property destruction	Behaviours were coded as being destructive when they either caused observable damage to an object or involved heavy impacts to objects (as indicated by a loud noise upon contact) which were not designed to be used in that way. Behaviours were not coded as destructive when they were part of a particular activity (for instance, a song which required banging on the table). Chewing of objects was recorded as a destructive behaviour, when the object was not explicitly designed to be mouthed (for instance, use of oral motor toys was not included). Observed destructive behaviours included: throwing (not towards a particular individual) or swiping objects off surfaces, slamming doors, banging or knocking over, chewing, biting, snapping, pulling furnishings or tearing.

Functional Assessment Observation Form (FAO; O'Neill et al., 1997). The FAO form required the topography of the behaviour to be recorded, followed by classifying the

antecedent and the consequence of the behaviour, as well as the perceived function. An entry was made for each individual occurrence of a behaviour. An occurrence of behaviour was counted as being separate from a previous occurrence of the same topography of behaviour when it was separated by at least a manual count of 3 seconds. For instance, an individual record of self-hitting might include several blows to the same area of the body in quick succession.

Table 20

Guidelines for the classification of antecedents for challenging behaviours

Antecedent class	Definition
Lone play	The individual is engaging in a non-work task alone, such as watching a video or playing with toys alone.
No interaction	No direct attention is being received and the individual is not engaging in a playful activity. Situations may include waiting, watching others, listening to an individual speak to a group, compliant engagement in a task alone.
Attention	The observed individual is being spoken to or physically touched by another individual in an interaction which does not involve an explicit request or demand. The observed individual may be actively engaging in the interaction or not.
Social play	Engagement in a non-work, playful activity which includes reciprocal involvement with others.

Antecedent class	Definition
Social proximity	Proximity of, or interaction, with a defined individual (or group of individuals). Information was gained regarding significant relationships or individuals from teachers or parents[39]. Also includes requests which involve approaching the particular individual(s).
Demand	Presentation of a task or a request (such as to stop current activity or to do something), ongoing prompted engagement in a task, or presence of a signal for a demand (such as work being placed on the table or being guided to a work table). May include verbal or physical prompts.
Transition	Specific type of demand which does not involve a work task but involves moving between two physical places.
Lack of access to tangible	A desired tangible item is not available to the individual. Desire is inferred through requests for the item or previous engagement with the item. Non-availability is defined as the item not being present, being removed or it not functioning.
Divided attention	An individual whom the observed individual is paying attention to, or had previously been interacting with, is interacting with a third person.
Unclear	The antecedent was not observed or was uncertain.

[39] Examples include: if challenging behaviours were likely to occur around a particular individual, or a recent disagreement or fight had occurred.

The form was filled in using descriptions then classified according to criteria which were standardised across the group (Table 20; Table 21). However, additional or idiosyncratic environmental influences were recorded for some participants. The definitions were developed prior to data collection based upon common antecedents and responses to CB as assessed in experimental functional analyses, and based upon the previous literature on CB in FXS (see Chapter 2). The data was then reviewed to determine whether any additional categories were required, which resulted in the inclusion of 'social proximity' as an antecedent category. The classifications were designed to be mutually exclusive and the antecedent and consequence for each instance were assigned to one category, only. However, additional or idiosyncratic environmental influences were recorded for some participants. Perceived functions were hypothesised based on antecedents, consequences and wider contextual factors. It is known, however, that CBs frequently result in the provision of attention, but this may not always serve as a reinforcer (St Peter et al., 2005). Accordingly, observational functional analyses methods have been noted to be biased towards yielding an attention function (Thompson & Iwata, 2007). In order to account for this bias, greater weight was given to the antecedent influences when making decisions about the function of individual instances of behaviour, as well as broader contextual information. For instance, if self-injurious behaviour occurred in an antecedent situation which was classed as 'no interaction' (such as, standing alone between activities) and was ignored, then the function would typically be recorded as non-social. However, if the child was clearly looking towards a particular person at the time of the behaviour or was perceived to be making other attempts to access an individual's attention, then the behaviour would be coded as attention-maintained.

Table 21

Guidelines for classification of consequences for challenging behaviours.

Consequence Class	Definition
Ignored	The situation did not change following occurrence of the behaviour: there is no social reaction. In the context of attention, attention may continue but there is no mention of engagement in the behaviour. In the context of a demand, the demand is not removed but additional attention is not given (i.e. no additional prompts)
Prompt (verbal/physical)	The demand is maintained following engagement in the behaviour through presentation of a prompt to continue with the task in question. Prompts are coded separately from ignored due to the potential for the additional attention to be reinforcing. The prompts are either coded as verbal or physical (person stating the demand makes physical contact, such as physically guiding the person or using hand-over hand guidance to complete the task).
Attention	In the context of antecedent attention, attention is given which is directly related to the behaviour. This may include reprimands or comforting statements. In the absence of antecedent attention (none or divided), an interaction begins following engagement in the behaviour.
Escape	A demand or individual (in the context of antecedent social proximity) is escaped or avoided following

Consequence Class	Definition
	engagement in the behaviour. This may involve explicit removal of the demand (e.g. "you don't have to do that now"), cessation of prompting or delay of the onset of the demand. Escape from an individual may involve the person leaving, or being taken away.
Access to tangible	Access to a tangible item is given following engagement in the behaviour. This may include assistance to fix equipment which had previously stopped working thus indirectly gaining access to that item.
Removal of tangible	Removal of tangible item with which the individual had been interacting, following engagement in the behaviour
Removal of attention	Attention which had been present prior to the behaviour is removed following engagement in the behaviour

Reliability. Simultaneous live observations were conducted for two participants, during proportions of the day. In total, two observers were present for 9 hours (7.9% of total time observed across all participants). The length of these observations was limited as, during the presence of two observers, one of the participants withdrew to the pre-defined 'private area' (bedroom), where observations were not conducted. Due to perceived participant burden, believed to be caused by multiple observers, no further live reliability observations were conducted. Reliability statistics were calculated on the rated presence or absence of individual topographies of behaviours during 30 second intervals. Across all dual observations the agreement on occurrence of instances of

behaviour was high (Kappa= .94). For all agreed occurrences of the behaviour (10 instances) the classification of the antecedents, consequences and functions of the behaviours matched. Secondly, reliability data was collected on the categorisations of environmental influences and functions in the FAO. An independent rater was given the text descriptions of the context in which the behaviour occurred (collected in real-time) then asked to assign antecedents, consequences and functions to each instance, using given definitions. This check was conducted for at least 20% of instances of behaviour for each individual (163 instances in total). Across the dataset, agreement on antecedent classifications was 82.82%; consequence classifications 84.05%; and function classifications 85.89%. The agreement on perceived functions (deemed the most important measure) fell below 90% for 4 participants (66.6-75%) and as such a third rater was asked to independently categorise the functions for all instances of behaviour for these individuals (243 instances). Agreement across this wider check was 88.48% (range: 82.35%-93.79%).

Data analysis. The initial data collected provided a detailed, but purely descriptive epidemiological assessment of the occurrence of behaviour across the day. However, subsequent exploratory analyses and methods of collating the data were conducted, to try to identify patterns within the group, and to facilitate comparisons with the wider literature. Initial analyses involved calculating descriptive statistics of frequency of behaviours (presented in terms of mean rate of instances per hour, in order to account for variations in observation length) and number of topographies of types of behaviour observed. In addition, frequency of particular antecedents and consequences were calculated at the group level in order to gain an understanding of the context and responses to the behaviours. In order to analyse behavioural function, for each participant, the proportion of instances of each of their types of CB assigned

with each of the perceived functions was calculated. This data was calculated from data on participants who engaged in at least 3 instances of the class of behaviour across the day, in order to avoid artificially inflating proportions calculated from extremely low values. These proportions for all included individuals were then collated for each of the three types of CB separately, in order to investigate any topography-function links. Finally, exploratory correlational investigations of associations between summary variables indicating key aspects of both behaviour and the salivary measures were conducted.

Results

Feasibility and acceptability of saliva sampling

Feasibility. No participants declined to provide a sample at any of the time points. Therefore, a high proportion of the intended number of samples were collected. However, nine planned samples (10%) were not collected at all: four due to participants consuming dairy before the final pre-bed sample (2 FXS participants and 2 siblings); three were not collected by staff at the sibling school (one participant's school declined to participate and the other made a mistake at one time point); two samples were not collected in the morning (one sibling due to a misunderstanding of times, one FXS participant awoke too early). As discussed in the method section, usable data for both analytes (cortisol and SAA) were able to be obtained from over three quarters (77.22%) of planned samples. As such, the sampling was demonstrated to be feasible.

Acceptability. Table 22 shows the mean and range of responses made by sibling participants and parents of FX participants. Exploratory investigations revealed no clear differences between saliva sampling methodologies and, as such, all the results are presented together. Eleven parents completed the parent version of the acceptability

questionnaire; the remaining three did not complete the measure due to time constraints. All parents found the overall assessment acceptable (i.e. a score of 6 or 7: M=6.8, range 6 to 7). The only items that received scores of lower than 6 were those pertaining to the number of samples, although no parents rated the acceptability as being lower than neutral (i.e. a score of below 4). The majority of parents (90.9%) reported that they would feel confident to be able to collect the samples without the researcher's assistance (a score of 6 or 7), although one parent rated that they would not be confident to do so. On the final item of the questionnaire, all parents rated that they would be happy for their family to take part in a similar procedure in the future (yes/no question format).

Table 22

Participant responses on the acceptability questionnaire.

Question	Siblings		Parents	
	Mean	Range	Mean	Range
Understanding of procedure	5.2	2 to 7	6.7	6 to 7
Information about the procedure	5.2	4 to 7	6.8	6 to 7
Overall acceptability (parents)/ feeling (siblings)	5.7	4 to 7	6.8	6 to 7
Length of time to do the sample	5.7	3 to 7	6.8	6 to 7
Ease of collecting the sample	6.5	4 to 7	6.9	6 to 7
Transporting and storing the samples	6.0	4 to 7	6.9	6 to 7
Number of Samples	5.7	3 to 7	6.3	4 to 7
Morning samples	5.2	3 to 7	6.5	4 to 7

Question	Siblings		Parents	
	Mean	Range	Mean	Range
Confidence to do without researcher	6.8	6 to 7	6.4	2 to 7

Eleven siblings completed the acceptability questionnaire. One additional, young, participant used the simplified form (images of faces) to give feedback. The remaining three either did not do so as a result of time constraints, or unavailability. In general, the siblings' ratings on the measure were more variable than the parents, likely as a result of their greater personal involvement in the procedure. No siblings rated negative feelings (a score of below 4) on the adapted item pertaining to overall acceptability ("Did you feel OK whilst your spit was being taken?"), although three participants gave a neutral score (4). The one participant who gave feedback using the images, selected their happy face when asked to show the researcher how they felt about doing the spit samples.

There were several items where individuals gave low scores, indicating issues to be addressed. Firstly, two participants indicated either that they did not understand what was happening during the procedure (a below-neutral score of 3 or less: 18%). In addition, some participants endorsed lower than neutral scores (3 or less) on the items relating to length of time to do the sample (one participant, who used the swab method), number of samples (one participant) and the morning samples (two participants). At the end of the questionnaire there was the opportunity to write comments and two participants commented upon their experience of doing the samples around peers at school: "people looked", "felt funny at school- made excuses". Reflecting the more varied, reported experience of collecting the samples, not all participants said

that they would do the samples again in the future: one selected no. Therefore, although none rated the procedure as being unacceptable overall, there was some degree of discomfort with the procedure, some of which may have been improved with provision of clearer information.

One of the participants with FXS completed the full acceptability questionnaire. His scores on the items ranged from 4 to 6, therefore he did not endorse any aspects as being worse than neutral. However, when asked if he would do it again, he said no. However, a further six participants rated their experience of the samples by the method of pointing to faces (smile, straight face, sad). All of these participants selected the happy face. The remainder of participants were either unavailable to complete the measure after the final sample, or did not engage when asked.

Salivary measures: cortisol and α-amylase.

Cortisol Assays.

Awakening response. Wust and colleagues (2000) set a responder criterion for the CAR as an increase of at least 2.49nmol/L (0.09μg/dL) in the 30 minutes after awakening. According to this measure, 9 participants in the sibling group (90%) exhibited an awakening response, compared to only 6 in the FXS group (50%). The differenced reached statistical significance, with a medium effect size ($X^2(1)=4.02$, $P<.05$, Cramer's $V=.43$) In addition, five of the participants with FXS showed a decrease in cortisol over the post-awakening period, whereas, all participants in the sibling group showed increases.

The significance of the differences between the CARs (Two levels (Time): Waking, Waking + 30 minutes) of the two groups (FXS and sibling) was examined using a two-way mixed ANOVA. The test showed a significant interaction between time and

group with a large effect size ($F(1,20)=.867$, $p<.01$, partial $\eta^2=.32$), whereby the FXS group showed less change over the 30 minutes after awakening than did their siblings (Figure 13). There was a significant main effect of time ($F(1,20)=8.14$, $p\leq.01$, partial $\eta^2=.30$) but not of group ($F(1,20)=.002$, $p=.96$, partial $\eta^2=.00$).

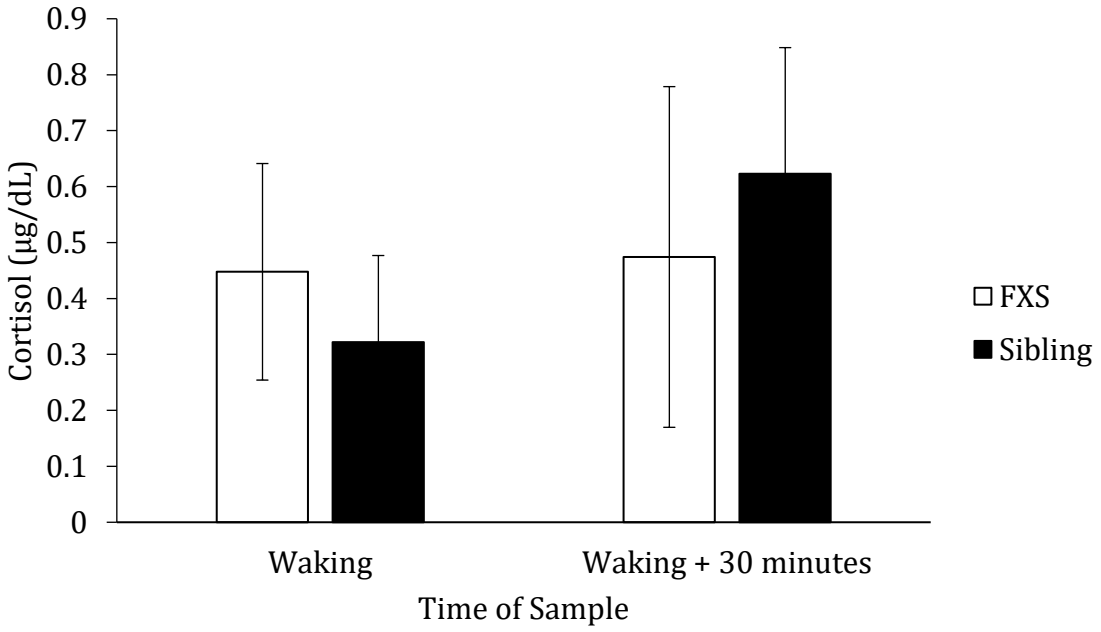

Figure 13. Group-level comparisons of changes in the levels of cortisol in the 30 minutes after awakening.

In order to explore whether the observed pattern may have been confounded by the aforementioned variability in the timings of the samplings (in relation to reported awakening time: depicted in Figure 14 & Figure 15), correlational analyses were performed to assess the relationship between lag to sampling and cortisol percentage change: tests did not reach statistical significance in either the FXS ($r_s(10)=.21$, $p=.58$) or sibling groups ($r_s(10)=.52$, $p=.13$). However, the high positive correlation coefficient in the sibling group suggests a potential association whereby longer sampling lags may

be associated with a greater percentage change in cortisol, only within the sibling group. For comparison purposes, mean CAR values (Wust et al., 2000) are added to the figures.

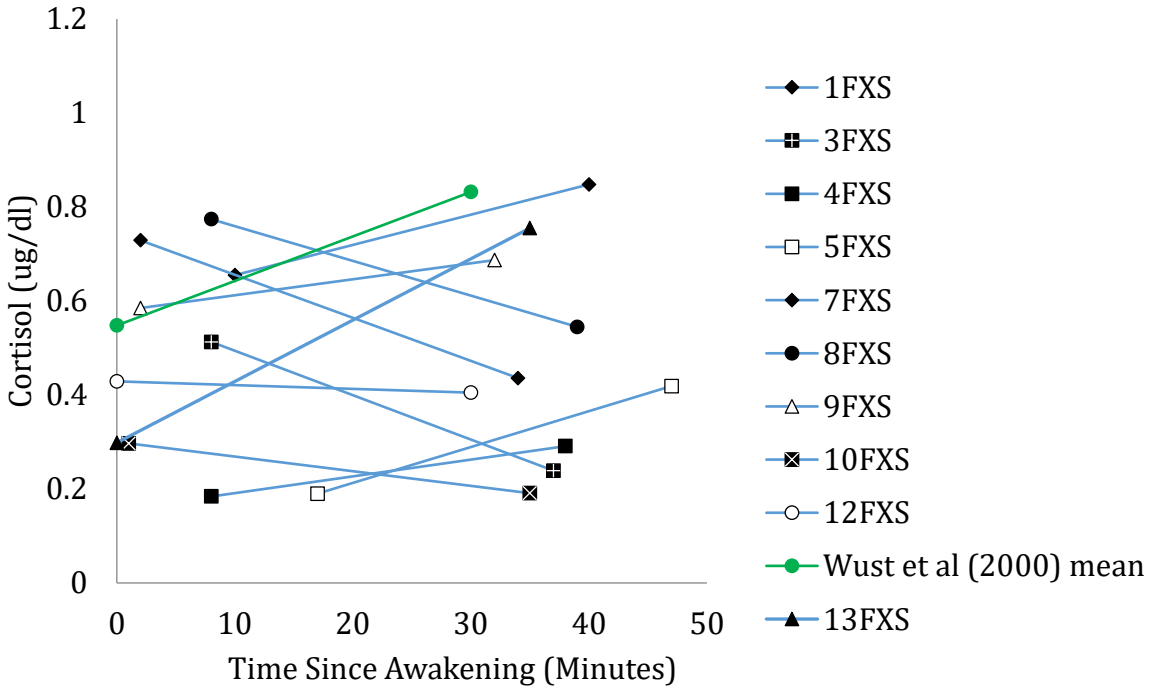

Figure 14. FXS group individual CAR variability

Given that sleep problems may affect the awakening response, visual analysis was conducted to investigate whether there were any clear differences between the participants taking melatonin (a supplement which may be used to decrease latency to sleep, which may imply the presence of sleep issues (Wirojanan et al., 2009), used by participants 5, 7 and 10[40] in the FXS group) and those who were not. There were no clear differences in the results in these sub-groups.

[40] Participant 11FXS was also taking melatonin but had incomplete CAR data.

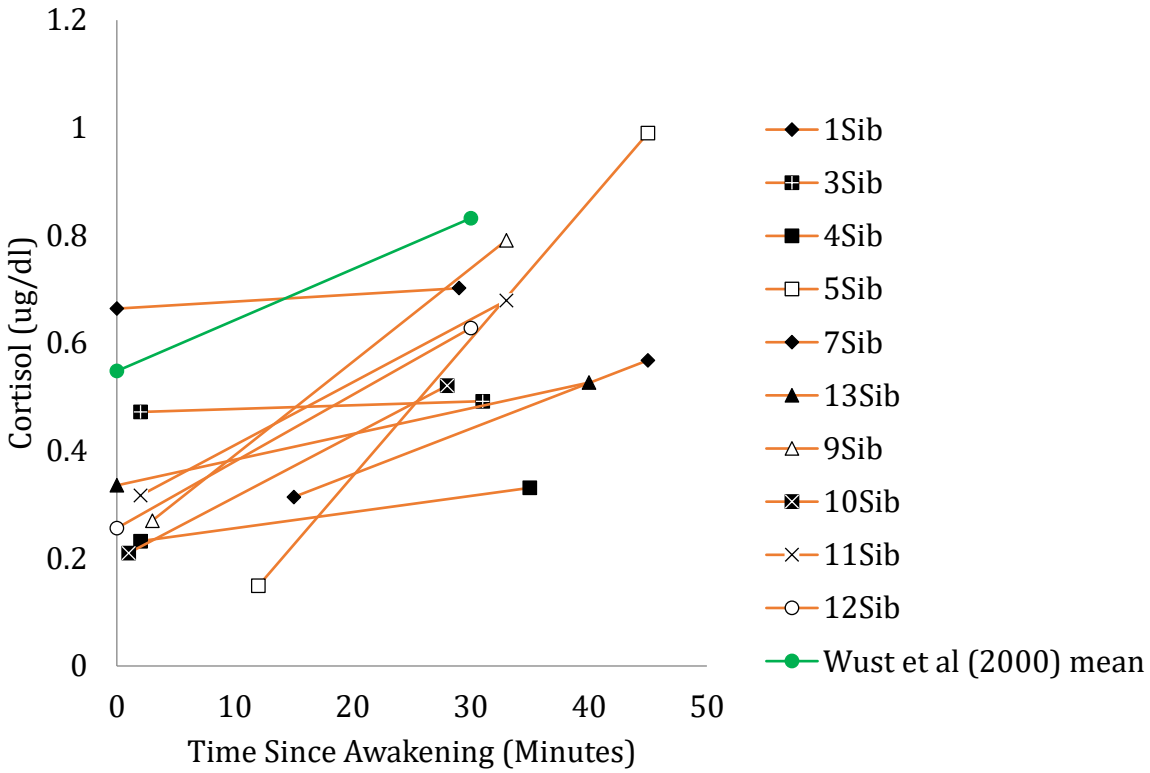

Figure 15. Sibling group individual CAR variability

Daytime Levels. A two-way mixed ANOVA was conducted to examine the effect of participant group (two levels: sibling, FXS) and time (4 levels: 9am, 12pm, 5pm, 7pm) upon levels of cortisol (µg/dL) across the daytime samples (Figure 16). The analyses suggested a significant simple main effect of time, with a medium effect size ($F(3, 51)=7.03$, $p<.001$, partial $\eta^2=.29$). Post-hoc paired t-tests (Bonferroni corrected $\alpha=.008$) supported the expected presence of a diurnal decline in levels of cortisol. Levels of cortisol were significantly higher at 9am (M=.281, SD=.030) than at either 5pm (M=.063, SD= .027: $t(24)=2.88$, $p\leq.008$, d=.57[41]) or 7pm (M=.084, SD=.031; $t(20)=3.76$, $p\leq.001$, d=.82). In addition the 7pm samples were lower than both the 12pm ($t(18)=6.05$, $p<.001$, d=1.39) and 5pm ($t(23)=3.42$, $p<.008$, d=.7). There were no other significant

[41] d= mean difference/ standard deviation

differences in the post-hoc analyses. However, there was no main effect of group ($F(1, 51)=.484$, $p=.50$, partial $\eta^2=.03$) or significant interaction between the two variables ($F(3, 51)=.68$, $p=.42$, partial $\eta^2=.04$).

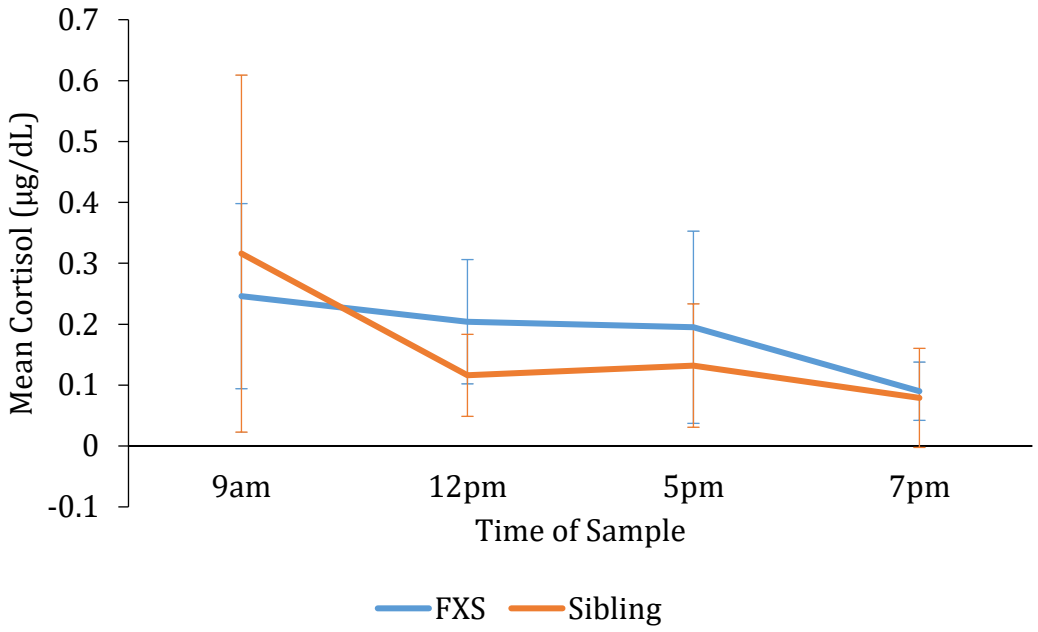

Figure 16. Mean daytime levels of cortisol in the participant groups.

Alpha-Amylase. A two way mixed ANOVA was conducted to evaluate the effect of time (6 levels: waking, waking + 30 minutes, 9am, 12pm, 5pm, 7pm) and group upon levels of SAA. Listwise exclusion of missing data items by SPSS resulted in retention of only 7 participants in the initial analyses[42]. Therefore, missing data were imputed, using participant mean SAA levels across the day. There were no main effects of time ($F(5, 130)=1.30$, $p=.27$, partial $\eta^2=.05$) or group ($F(1,26)=1.58$, $p=.22$, partial $\eta^2=.06$), though the direction of higher mean levels in the FXS group were in line with earlier autonomic findings (Figure 17). In addition, despite visual differences in the mean diurnal profiles

[42] In the analysis with reduced sample size, there were no main effects of group ($F(1)=2.25$, $p=.16$, partial $\eta^2=.16$), though a significant effect of time ($F(5)=2.85$, $p=.02$, partial $\eta^2=.05$) and interaction ($F(5)=2.53$, $p=.04$, partial $\eta^2=.17$), though with small effect sizes.

of the SAA data between groups, the statistical analysis did not reveal a significant interaction between the time and group variables ($F(5,130)=1.40$, $p=.23$, partial $\eta^2=.05$). Of note, however, visual analysis of individual profiles for individuals within each group suggested higher variability within the FXS group (Figure 18 & Figure 19). Additional sensitivity analyses supported the non-significance of the findings[43].

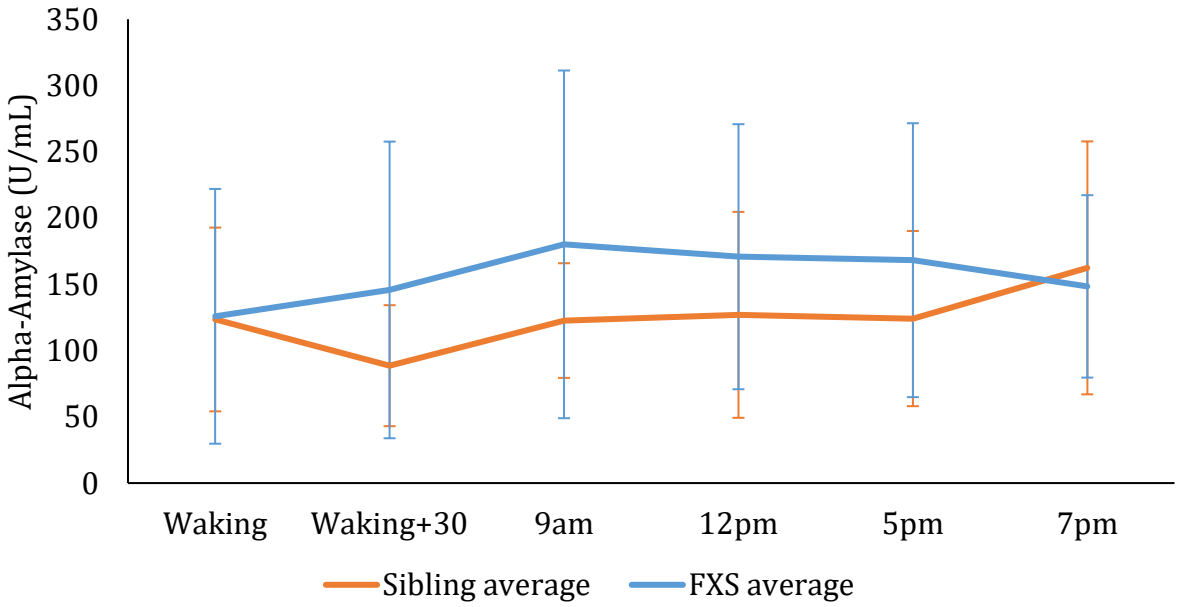

Figure 17. Group mean circadian profiles of SAA activity.

[43] Fixed Effects as derived from a linear mixed model were also explored as an alternative to an ANOVA analysis, given the missing data (Seltman, 2015; unstandardized covariance structure selected for repeated effects). The results were similarly non-significant: Group: $F(1)=.79$, $p=.38$; time $F(5)=1.30$, $p=.30$, interaction $F(5)=1.38$, $p=.28$. Furthermore, there was no significant group difference in participant mean SAA levels across the assessment day ($t(18.124)=1.26$, $p=.23$, $d=.44$).

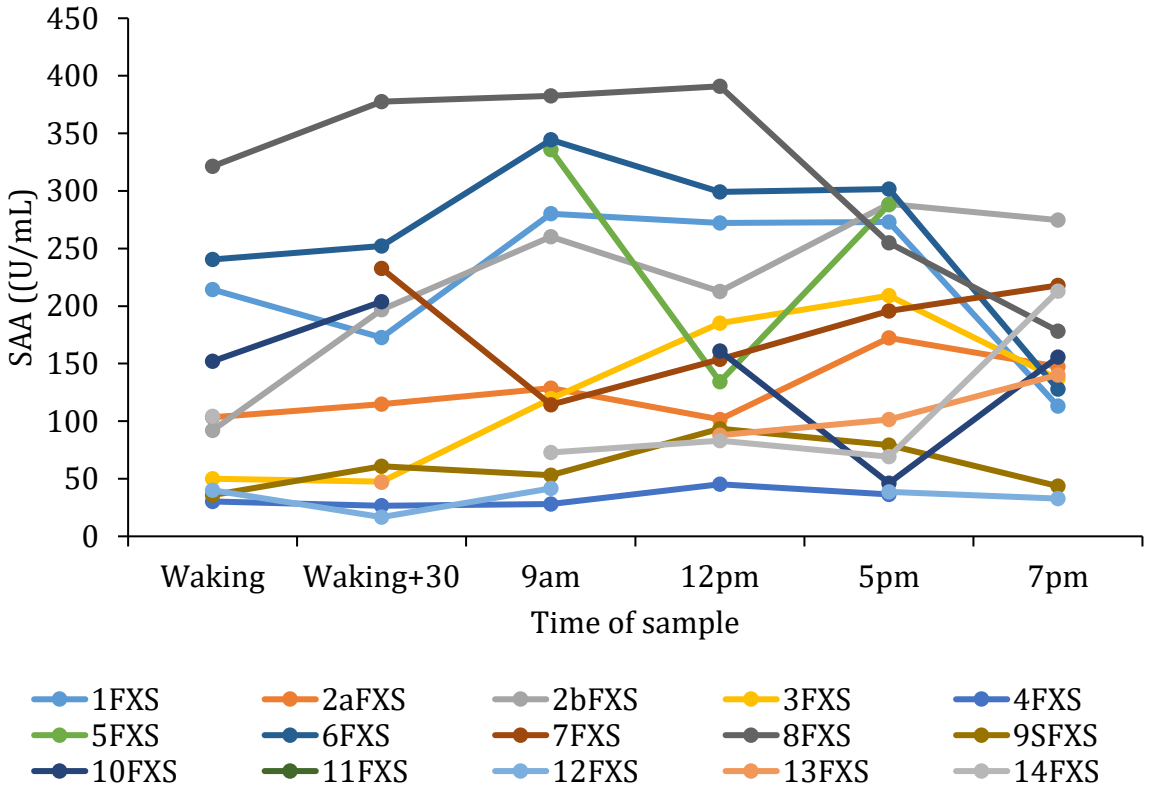

Figure 18. Line graphs to depict within-group variability of SAA circadian profiles for participants with FXS.

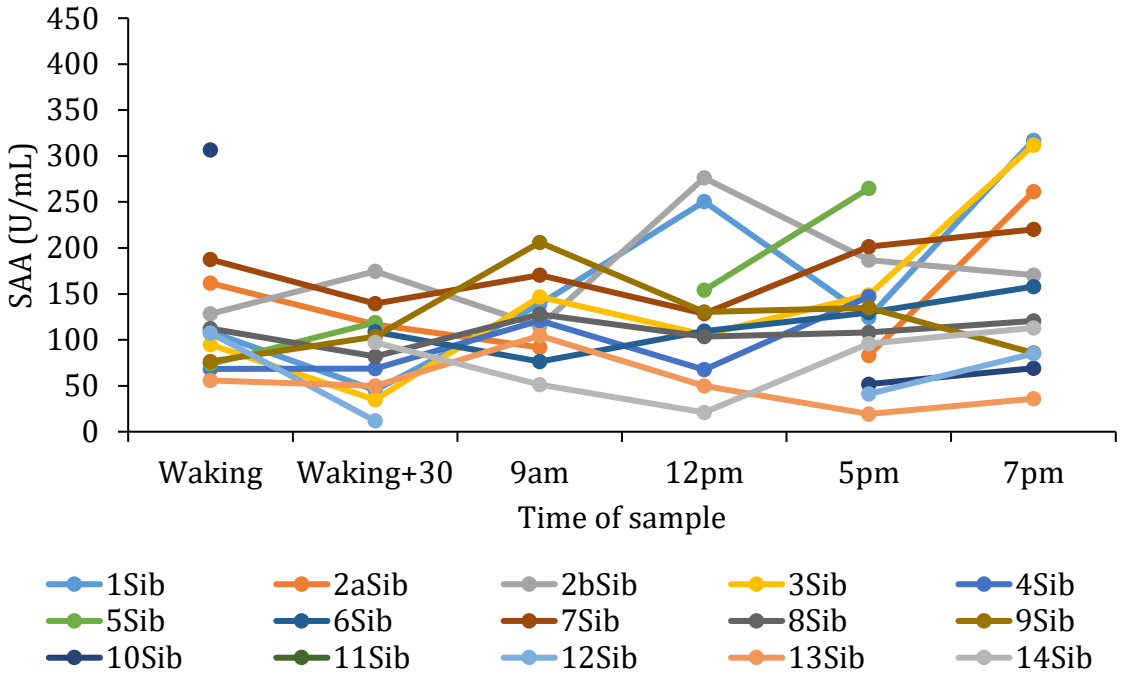

Figure 19. Line graphs to depict within-group variability of SAA circadian profiles for sibling participants.

Associations between cortisol and alpha-amylase. Several variables were calculated which summarised prominent aspects of the arousal-related data in single values, for the purpose of comparison with other data. Given that the CAR is believed to be a discreet aspect of the circadian rhythm of cortisol secretion, this data was summarised separately. Previous research suggested reduced diurnal decline in cortisol in children with FXS (for instance: Wisbeck et al., 2000). As such, the decline between 9am and 7pm was calculated. In contrast, SAA awakening changes appear to correspond to activity across the rest of the day (Nater et al., 2007). Therefore, mean levels of SAA across the evaluation day was used as a summary statistic. Descriptive statistics are depicted in Table 23.

Table 23

Descriptive Statistics for Arousal Summary Variables

Summary Variable	FXS		Sibling	
	N	Mean (SD)	N	Mean (SD)
Mean SAA (U/mL)	14	155.94 (88.84)	14	123.21 (40.26)
CAR (% change)	12	22.58% (64.12%)	9	87.89% (66.57%)
Mean daytime cortisol (µg/dL)	15	.19 (.07)	15	.16 (.11)
Decline in cortisol 9am-7pm (µg/dL)	14	.09 (.17)	12	.18 (.27)

Correlational analyses revealed no significant associations between SAA and cortisol summary variables (Bonferroni adjusted α-level= .008; Table 24). Visual analysis of the data supported these results. There were no significant associations between the CAR and other cortisol summary variables.

Table 24

Tests for association between cortisol and SAA.

	Group	CAR	Cortisol Decline	Daytime Cortisol
SAA	FXS	$r_p(12)=-.06$, p=.86	r_p (14)=.28, p=.34	r_p (14)=.15, p=.61
	Sibling	r_p (9)=-.32, p=.40	r_p (11)=.09, p=.79	r_p (14)=.44, p=.12

Challenging behaviour.

Frequency and topography of observed behaviours. All but one of the participants (14 in total) engaged in at least one instance of CB during the observation period. In total, across all participants, a total of 104 instances of destruction, 78 individual instances of physical aggression and 621 instances of SIB were observed. The mean rate of instances recorded per hour observed was 7.41 (SD=6.33, range=0.12-18.32). Further details about the frequency and topography of observed behaviours are displayed in Table 25.

Table 25

Frequency and topography of challenging behaviours

Type of behaviour	Number showing behaviour	Participant mean rate of instances per hour			Number of topographies per participant			Most common topography
		Mean	SD	Range	Mean	SD	Range	
SIB	11	7.32	5.68	1.02-15.68	1.6	.8	1-3	Hand biting
Aggression	13	.96	.73	.1-2.15	3.1	1.8	1-7	Hitting other with hand
Destruction	11	1.19	1.27	.1-4.29	2.5	1.4	1-5	Hitting, banging or kicking objects or surfaces

Comparison between settings. The rates of CBs observed at school compared to locations outside of school (home or other location. Figure 20; data presented in box and whisker plots). A Wilcoxon Signed-Ranks test indicated non-significant difference in the rate of CB between settings (Z=-.79, p=.43, r=.05). One outlier is marked in the graph: this individual was able to be observed for 25 minutes at home during which time he was watching preferred television and engaged in SIB (hitting and/or biting self) 29 times. After this time, the participant went to his room (designated a non-observation area) and no further data was recorded at home.

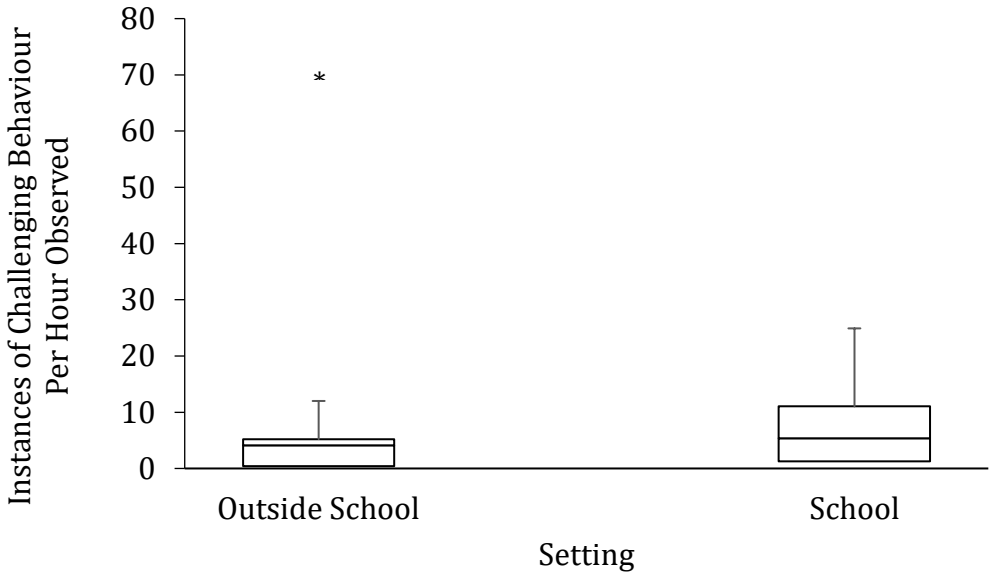

Figure 20. A comparison of participants' average rate of instances (per observed hour) of CBs observed, between settings.

Function of Challenging Behaviours

Antecedents and consequences. The frequencies of different classes of antecedents and consequences for each different type of CB were explored. Figure 21 depicts the percentage of recorded instances of each type of CB, across all participants, which were classified as having different antecedents. Several other idiosyncratic or

unknown antecedents were observed at low frequency (less than 4% of observed instances for any class of behaviour) and are not recorded in the graph. The presence of a demand was the most common antecedent for destructive and aggressive topographies of CB. In contrast, SIBs were most likely to occur during conditions with low environmental stimulation (no interaction) or during play (social or alone).

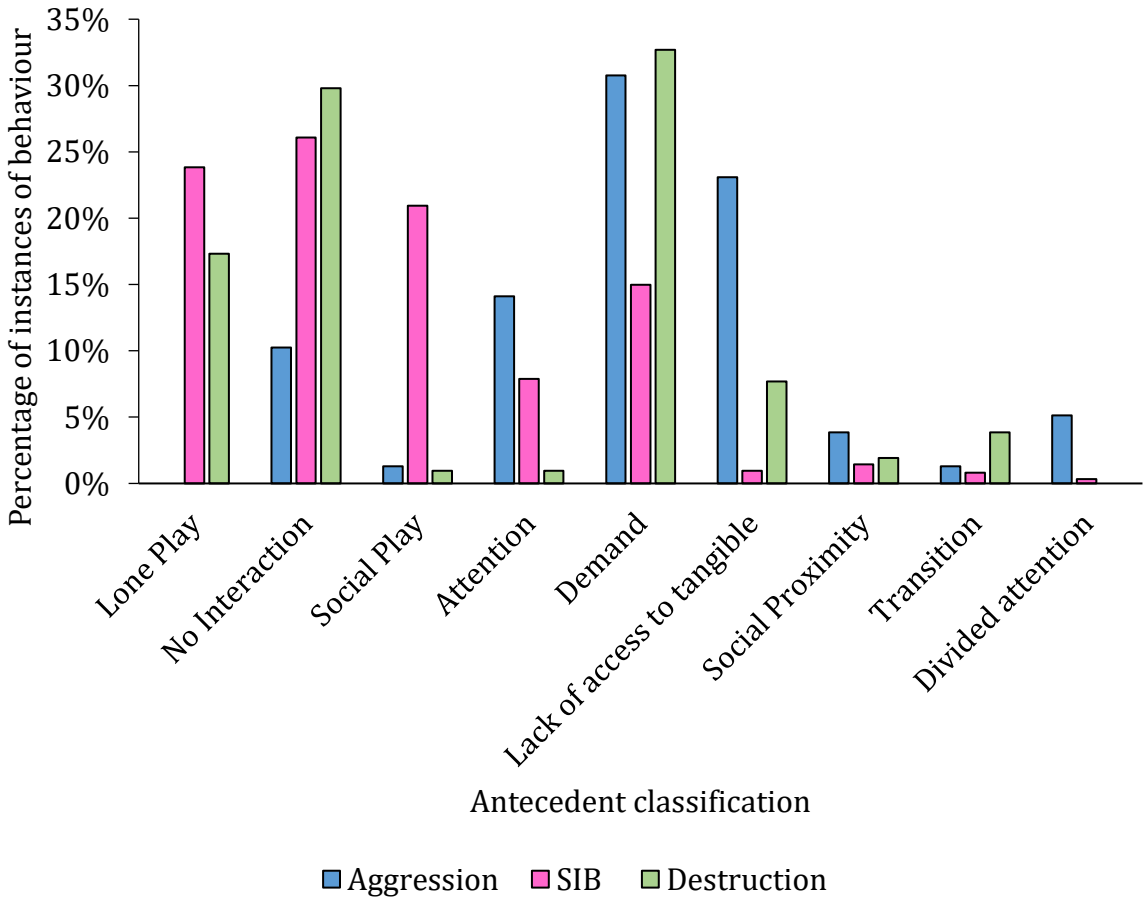

Figure 21. The percentage of occurrences of challenging behaviour with different antecedents.

The FAO data was also evaluated to determine the nature of responses to CBs (Figure 22; several low-frequency consequences are not included in the graph.). Most commonly, behaviours, SIB in particular, did not receive a social consequence (such as

the demand being terminated or provision of attention). Aggressive behaviours were the most likely to result in a social consequence.

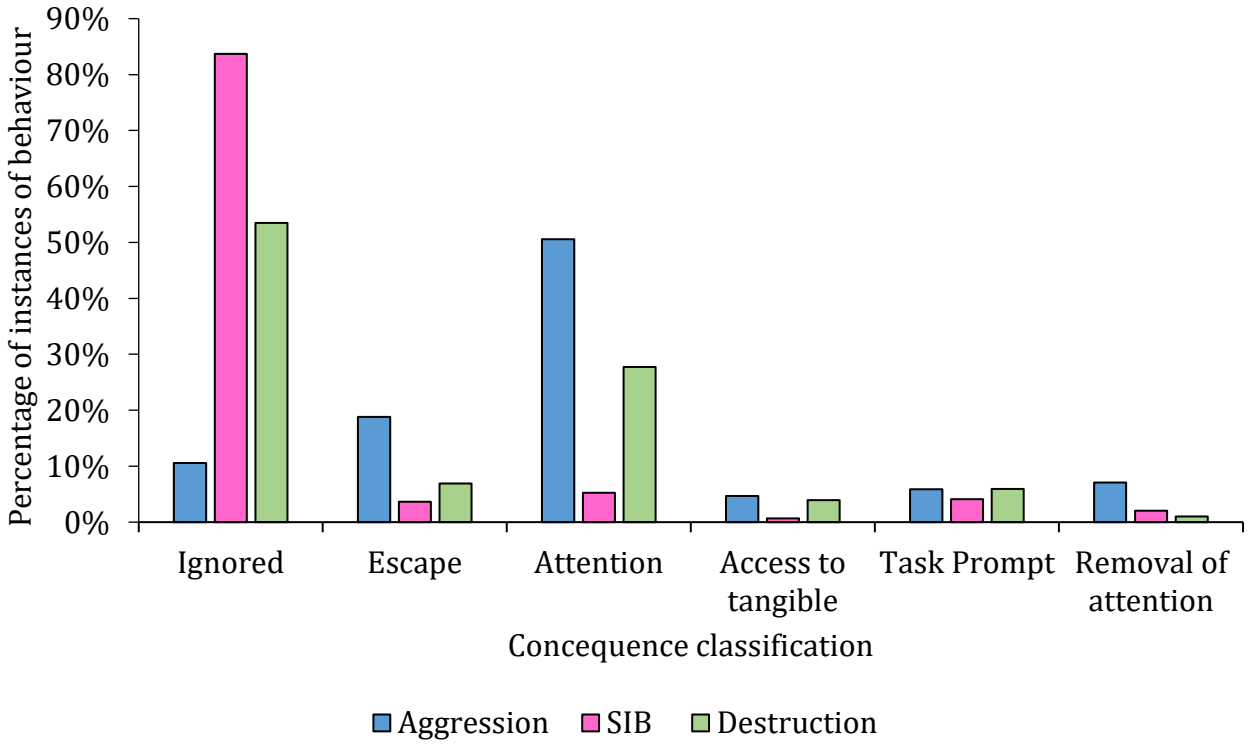

Figure 22. Consequences for challenging behaviour (as recorded on FAO form).

Function. The proportion of instances with different perceived functions is presented in Figure 23. The distribution of proportions are represented as box and whisker plots to account for the positive skew observed. The perceived function was also classified as 'unclear' for 4.77% of instances of SIB, 4.81% of instances of aggression, and 20.69% of instances of destruction. For all types of CB, the social function which the greatest proportion of instances was perceived to serve was escape. However, the highest proportion of instances of SIB across the group, was perceived to have a non-social function.

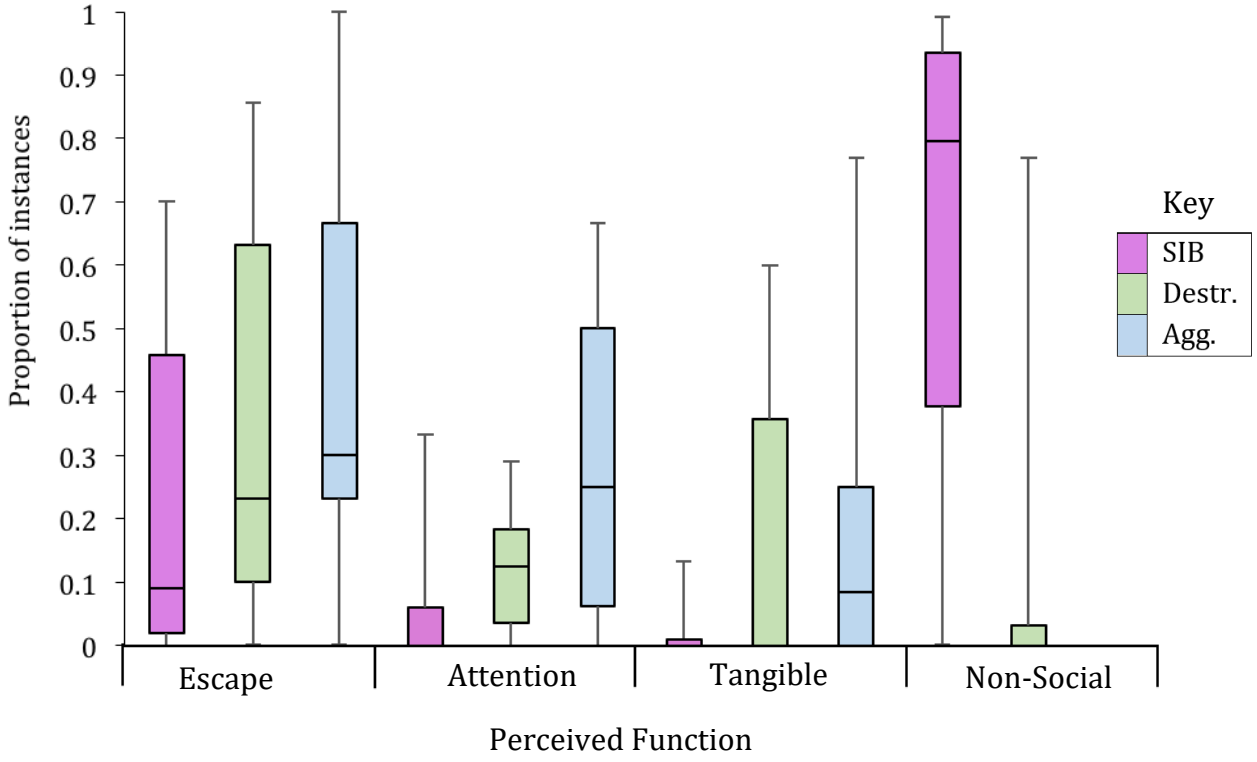

Figure 23. Proportions of behaviour instances with each perceived function (as recorded on the FAO form).

In order to infer the primary function of individuals' behaviours, the data were analysed to identify the function which was assigned to the highest proportion of instances of each topography of behaviour, for each participant (Figure 24). This calculation was conducted for individuals who displayed at least three instances of a given topography of behaviour, in order to avoid inflating percentages through low numbers of observations. A more mixed distribution of functions was revealed through this analysis. Escape was the only primary social function hypothesised for SIBs, though was less common than non-social. No participants' destructive behaviours were assigned attention as a primary function; all other functions were approximately evenly likely. In contrast, attention was the most likely primary function for aggression, though seen only at slightly higher levels than escape.

The proportion of participants who exhibited any class of CB with each of the primary functions was calculated (Figure 24). Eighty percent (8/10) of the participants exhibited differing primary functions for different types of CB.

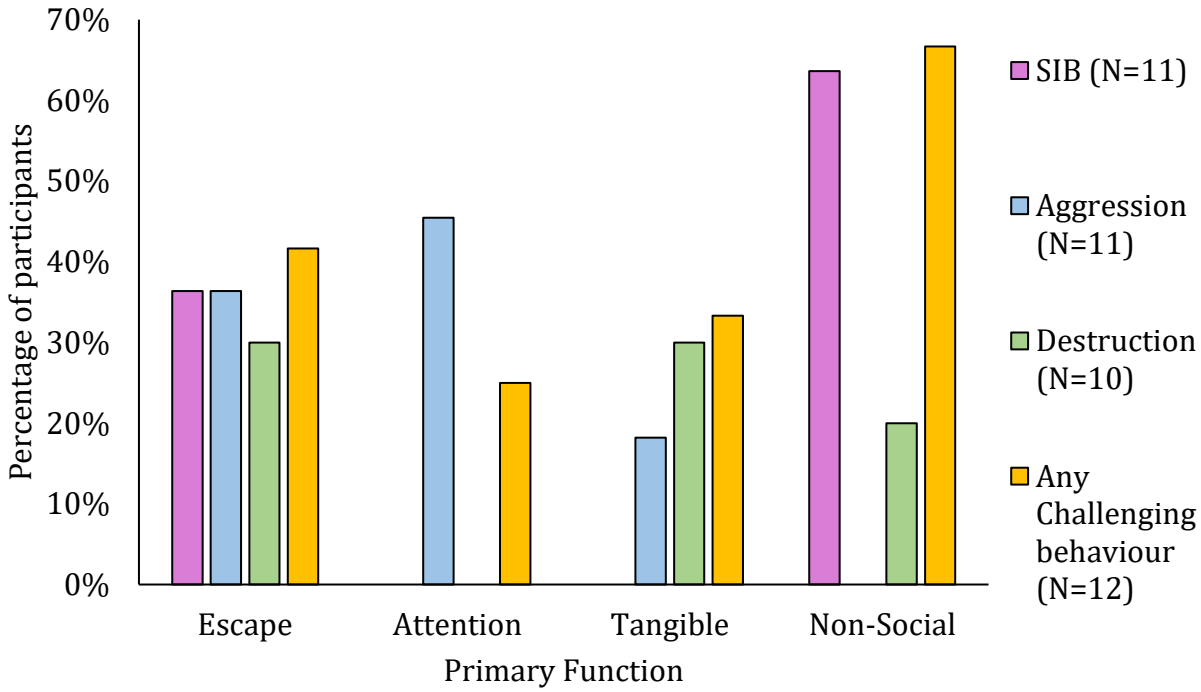

Figure 24. Primary functions of challenging behaviours

Exploratory analyses of associations between physiological arousal and behaviour.

Arousal variables and autistic behaviour. Correlational analyses were conducted between the total SCQ scores and each of the four arousal summary variables, within the FXS group. There were non-significant (Bonferroni adjusted α-level= .0125) associations between total SCQ scores and cortisol decline ($r(14)=-.05$, $p=.85$), average daytime cortisol ($r(15)=-.26$, $p=.36$) or SAA ($r(14)=.18$, $p=.54$). Though the correlation coefficient between autistic behaviour and the CAR was also non-significant ($r(12)=.51$, $p=.09$), the correlation coefficient was quite high, suggesting a potential association whereby individuals with FXS who have higher SCQ scores show

higher CAR percentage change (Figure 25).

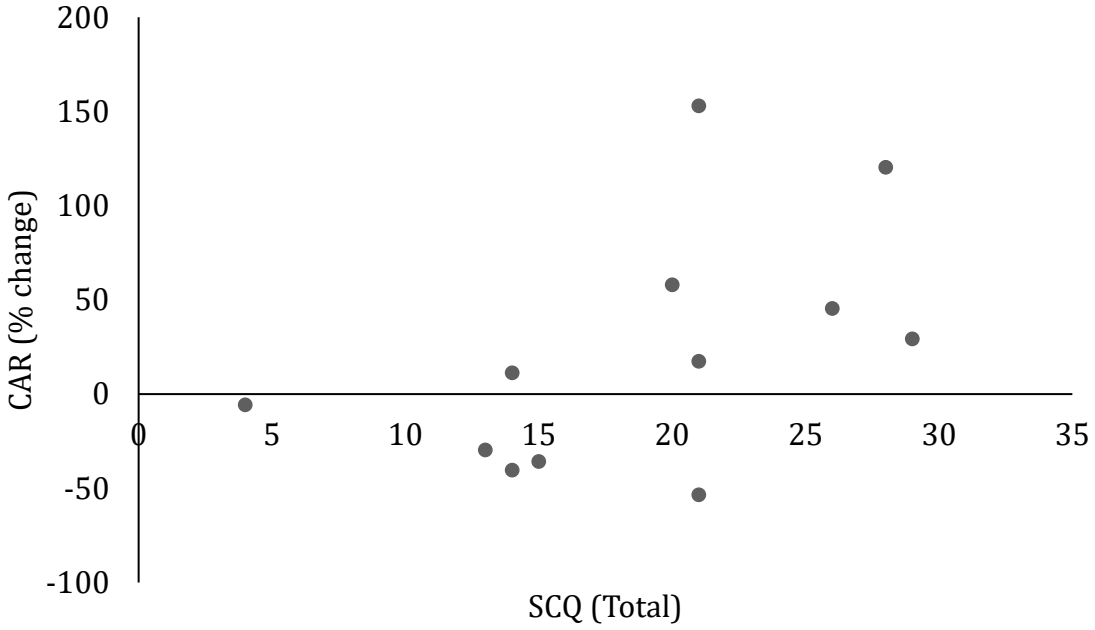

Figure 25. Cortisol awakening response plotted against SCQ total scores.

Arousal and frequency of challenging behaviour. Correlations between each of the arousal summary variables and the rate of each of the types of CB per hour were explored (Table 26). There were non-significant associations between all variables (Bonferroni adjusted α-level= .004). However, the high correlation coefficient associated with the CAR and aggression comparison suggests a potential association (Figure 26). Of note, autistic behaviour is a risk marker for aggression (Hall, McClintock & Oliver, 2003), as such, covariation may influence these possible associations.

Table 26

Arousal and frequency of challenging behaviour.

	SIB	Aggression	Destruction
SAA	r(10)=.27,p=.45	r(11)=-.18, p=59	r(11)=-.12, p=.72
CAR	r(9)=.02, p=.96	r(9)=.47, p=.21	r(9)=.30,p=.43
Cortisol decline	r(10)=-.22, p=.53	r(11)=.36, p=.92	r(11)=-.05, p=.89
Average cortisol	r(11)=-.14, p=.69	r(12)=-.10, p=.76	r(12)=-.20, p=.54

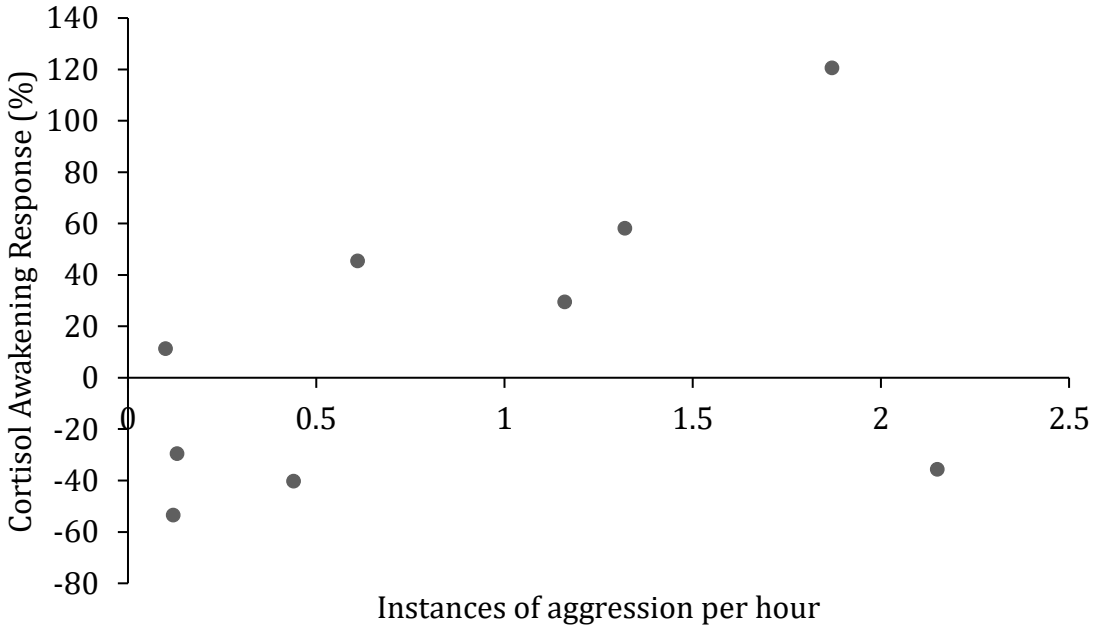

Figure 26. Investigation of association between frequency of aggression and the CAR

Associations between arousal variables and behavioural function. Spearman's rank correlational analyses were conducted due to the positive skew of the results (Table 27). There were non-significant associations between the rate per hour of individuals' CBs with particular functions, and the arousal summary variables. In addition, there was

no clear preliminary indicator of differences in patterns of association between any of the different functions, and the arousal variables.

Visual analyses were conducted in order to investigate whether the variability in the samples across the day may correspond to the occurrence of CBs (for instance, if levels appeared atypically high or low compared to the rest of the individual's samples when CB occurred in close temporal proximity to the sampling). Given the low number of occurrences which occurred in close temporal proximity (±20 minutes) to the samples, the varying environments, the durations between samples (typically several hours) and the unknown baseline diurnal changes in the levels of cortisol and SAA, it is unsurprising that no patterns or suggestions of associations could be identified (Table 27).

Table 27

Arousal and behavioural function

Arousal Variable (N)	Rate per hour of behaviour with function			
	Attention	Escape	Tangible	Non-social
SAA (13)	r=-.11, p=.72	r=.12, p=.69	r=.10, p=.76	r=.12, p=.69
CAR (11)	r=.09, p=.79	r=.04, p=.91	r=.22, p=.52	r=.32, p=.34
Cortisol decline (13)	r=-.27, p=.38	r=-.04, p=.90	r=-.11, p=.71	r=-.19, p=.52
Average cortisol (14)	r=-.18, p=.53	r=-.04, p=.88	r=-.24, p=.40	r=-.02, p=.94

Bonferroni adjusted α-level= .003

Discussion

Feasibility and acceptability of saliva sampling. Though earlier research has utilised salivary analyses with individuals with FXS (for instance: Hessl et al., 2002), none have assessed the acceptability of this method. The results from both parent and participant (FXS and sibling group) report demonstrated the overall acceptability of the sampling. This corresponds with positive ratings of the procedure by adult researchers or graduate students (who rated both passive drool and salivette methods as comfortable, easy to use and unlikely to affect willingness to volunteer: Strazdins et al., 2005) and direct reports from children with high functioning autism (Putnam et al., 2012). However, some sibling participants reported a degree of social discomfort with the procedure, particularly in the school environment, which should be considered and compensated for, where possible, in future research. Overall, however, these findings support the further use of this method within the populations of interest for this research.

This initial study also demonstrated the feasibility of collecting saliva samples from a group of boys with FXS who exhibit CBs. In total, values for both analytes (cortisol and SAA) could be calculated for 80% of the planned samples in the FXS group, which was in fact higher than for the sibling group (74.44%). Anecdotally, the greater challenges in the sibling group were at least in part due to less direct assistance with the sampling, due to the researcher not being present at the school. However, the yield was lower than planned: one of the main barriers to successful analysis was low sample volume. In a natural environment, Hessl and colleagues (2002) were able to collect at least partial data from 102 out of 109 (93.57%) participants on typical (non-school) days in their reported results. Though the proportion of full datasets which were able to be obtained by Hessl and colleagues (2002) is unclear, this seems to be higher than the

present success rate. The reasons for the difference in sampling success are unclear, though may relate to the increased busyness on school days (present study), when compared to non-school days (Hessl et al., 2002). In future research, the issue of sample volume may be addressed by longer absorption periods, or encouraging longer periods of passive drool.

The availability of a variety of methods for sampling was key for ensuring that individuals could comfortably participate, particularly for the participants with FXS. Through parent report and practical experience it was apparent that some individuals were not able to (e.g. could not drool into container) or would not tolerate (e.g. gagged upon trialling the swab in their mouth due to oral sensitivity) use of one of the available methods. The use of this dual methodology is valid: Salimetrics Children's Swabs are validated against results from passive drool (Salimetrics, 2017). Furthermore, investigations within the present dataset similarly did not reveal any clear differences by samples obtained through different methods. Though other studies have used oral swabs (such as cotton swabs or salivettes) across large groups of children with FXS, it may be that the individuals participating in this study, who were specifically selected for engaging in CB, have additional needs which require greater flexibility in the study procedure. Furthermore, the availability of additional sampling aids, such as the visual instructions, were used with success with a number of participants. In summary, the sampling protocols and methods utilised in this preliminary study, including the adaptations and additional resources, have been shown to be feasible for use in future projects.

Salivary cortisol and alpha-amylase. As reviewed in earlier chapters, data from human and non-human animal literature suggests alterations in activity and

responding of the stress-effector systems, including the HPA axis and the autonomic nervous system. One of the aims of the present project was to replicate earlier investigations of the diurnal rhythm of cortisol, including an extension to assess the CAR, as well as novel investigation of daily changes in SAA.

Cortisol awakening response. The CAR is a discrete aspect of the cortisol circadian cycle which had not been previously investigated in individuals with FXS. Therefore, this study is the first to conduct a preliminary investigation of differences between the CARs of individuals with FXS and their unaffected siblings. A smaller than expected proportion of the FXS sample in this study exhibited the expected post-awakening rise in cortisol. In a large investigation of the CAR in healthy adults, Wust and colleagues (2000) found that 77% of their sample showed an awakening response, according to their responder criterion. The frequency of responders observed in the FXS group fell below this, at only half. Accordingly analyses revealed that the children with FXS showed significantly less change in cortisol levels over the awakening period, compared to their siblings. These preliminary findings suggest that there may be an alteration in this distinct aspect of the cortisol diurnal cycle within the FXS population, though further investigation with larger numbers of individuals and repeated sampling days would be needed to confirm this.

It has been suggested that the CAR may act as an indicator of the reactivity capacity of the HPA axis. As such, one interpretation of the present findings is that males with FXS exhibit reduced reactivity capacity, compared to siblings without FXS. This would be in contrast with earlier suggestions of increased reactivity in FXS, from the earlier human and preclinical literature (reviewed in Chapter 3). Alternatively, the present observations may be an indicator of hypocortisolism. It is possible that over

time, in response to chronic activation of the HPA axis due to atypical negative feedback regulation (as is hypothesised to be exhibited in FXS: Hessl et al., 2002), compensatory suppression occurs. Alternatively, those with FXS and CB may be experiencing chronic stress which leads to system burnout (Fries et al., 2005). Flattened CARs have been suggested to be a biological signature of fatigue and exhaustion (for instance: Cleare, 2003) Similar hypocortisolism at awakening has been observed in mothers with the FMR1 premutation who have greater biochemical vulnerability (higher proportion of 'fragile' X chromosomes active), following days where their children engaged in problem behaviour. Interestingly, those with high activation ratios (higher proportion of non-mutated X chromosomes active) exhibited a more typical pattern of increases in awakening cortisol following stress (Hartley et al., 2012). This suggests a link between FMR1 gene mutations and biological vulnerability to stress exposure. However, similar awakening hypocortisolism has been observed in parents of children with autism spectrum disorders (Padden & James, 2017), and so is not unique to this genetic group. Interestingly, the findings of reduced or absent CAR is similar to that seen in people with Asperger's Syndrome (Brosnan et al., 2009), with whom people with FXS may share some characteristics, such as social interaction challenges. Further investigation is warranted to determine whether this observation is replicable and whether it does correspond with system reactivity within this population. This preliminary investigation does suggest however that this aspect of the adrenocortical output may be altered in FXS.

Of note, there are a number of individual factors which may also influence the CAR, which were not monitored or accounted for within our study. One important factor which may influence the CAR is sleep (Fries, Dettenborn & Kirschbaumet al., 2009). Wust and colleagues (2000) suggested that early awakening and dozing before

final awakening may cause the CAR to be missed, similarly Backhaus, Junghanns and Hohagen (2004) found that insomniacs showed reduced CARs. It has been noted that individuals with FXS are at risk of sleep problems; Gould et al. (2000) identified that boys with FXS have an increased tendency to show issues with sleep maintenance, as well as high variability in total sleep time. Though sleep issues were not assessed in this study, the fact that over a quarter (4 out of 15) of boys with FXS were taking melatonin suggests that sleep-related issues were present in this group. Without accounting for sleep-related issues it is unclear whether the differences observed may have been confounded by sleep-related issues, as these may have been more prevalent in the FXS group.

Cortisol diurnal decline. The results of this study show non-significant difference between the daytime cortisol levels, or the diurnal decline of cortisol, between children with FXS and their siblings. This differs from the reduced diurnal decline observed in males with FXS by Wisbeck and colleagues (2000: visual analysis), as well as Hessl and colleagues (2002: statistical analysis). Both studies had larger participant numbers than the present study, therefore it may have been that this study was under-powered to detect any differences, as the mean values were higher during the middle of the day in the FXS group, compared to the sibling group. Interestingly, unlike the earlier studies, average levels returned to being comparable with siblings by bedtime, which contrasts with the impaired negative feedback hypothesis. As with the CAR assessment, repeated measurements of the diurnal rhythm across multiple days could help to determine the stability of these observations (as was done in Hessl and colleagues' study).

Alpha-amylase. This was the first study to assess autonomic arousal in individuals with FXS through measuring SAA. Based upon visual analysis, the mean curves of the sibling group showed the expected fall post-awakening followed by a rise through the day. However, within the FXS group a less typical pattern was seen, on average, with very high variability within the group. Though, across the groups, there was no significant effect of time upon SAA levels, and no interaction between group and sampling time. However, across the day individuals with FXS exhibited higher levels of SAA when compared to the sibling group, though this did not differ significantly statistically. This partially supports observations in previous research (as reviewed by: Klusek, Roberts and Losch, 2015) robust pattern of autonomic irregularities in individuals with FXS, compared to controls: namely, heightened sympathetic arousal paired with reduced parasympathetic vagal tone.

Association between cortisol and α-amylase. Balance in activity between the stress-effector systems is believed to be of clinical importance. For instance, individuals with high chronic stress exhibit a dissociated pattern of blunted cortisol but elevated SAA (Ali & Pruessner, 2012). Exploratory correlational and visual analyses were conducted to investigate possible associations between arousal-related measures in the present study, though there were no significant associations or indicators of trends. The non-significant associations between cortisol and SAA are unsurprising, given earlier findings that SAA does not correspond with cortisol levels (Nater et al., 2006). Though such analyses were not possible in this small sample, in future research with larger groups it may be interesting to investigate the ratios of cortisol levels to SAA (Ali & Pruessner, 2012), in order to investigate possible system dysregulation and its association with key behaviours in FXS. In addition, the lack of association between CAR

measures and daytime cortisol levels observed helps to support that this response is distinct from the rest of the diurnal rhythm, and warrants further investigation.

Challenging behaviour observations. Observational data were collected about the occurrence of CBs of 15 boys with FXS, in natural settings. Across one observed school day, the high frequency of occurrence of these behaviours was notable, with an average number of over seven instances of SIB, aggression or destruction seen per hour. The highest average rate of behaviours for any participant was 18 instances per hour. Self-injurious behaviours were the most prevalent types of CB across the group, and were also engaged in most frequently across the observations. Furthermore, in line with previous descriptions (as reviewed in Hardiman & McGill, 2018; see Chapter 2), the most common topography of SIB exhibited was hand-biting: hand-biting accounted for 86% of observed instances of SIB, and was exhibited by 60% of participants during the observed period. Anecdotally, the severity of observed behaviours was variable but one observed instance of aggression resulted in the recipient having to seek medical attention, and many participants showed hand callouses from repeated self-biting. These effects emphasise the need for greater understanding to aid intervention.

One aim of this study was to collect a natural, descriptive assessment of the behaviour of a group of males with FXS, in order to investigate the distribution of different functions of CB. Based on prior research (as reviewed in Hardiman & McGill 2017; Chapter 2) it was hypothesised that the majority of instances of behaviour would occur in contexts which would suggest an escape function, either from demands or social interactions. This hypothesis was partially supported by our findings as, across the group, the function assigned (based upon the observed antecedents and consequences) to the highest proportion of instances of aggressive and destructive

behaviour was escape. Supporting this observation, demands were the most common antecedents for engagement in these types of CB. Escape (from a demand or interaction) was also amongst the more common consequences for engagement in these behaviours, though most commonly behaviours received no response from others. Interestingly, there was not a clear difference in rate of observed behaviours between school settings, where demands were presumably more common, when compared to settings outside of school. Future research including more detailed information on the individual's environment, including times when CBs did not occur, would allow a more detailed investigation of the relationship between demands and behaviours, through a conditional probability analysis (for instance, see: Taylor & Oliver, 2008).

Additional analyses were conducted in order to attempt to identify the primary functions of individuals' behaviour. Through this analysis, a less clear pattern of results emerged, with a more even distribution of hypothesised functions of individuals' behaviours. Therefore, whilst across the group escape-maintained behaviours were exhibited most frequently (when compared to other social functions such as accessing attention or tangible items), at the individual level participants were not more likely to engage in escape-maintained behaviours when compared to behaviours of other functions. This analysis questions the primacy of escape as a social function in this group. However, of note, a number of the participants whose function was identified as attention displayed low numbers of occurrences of behaviour, which may have affected these results. Therefore, though there were potential trends in patterns of functions and contexts seen, it is important to highlight that there remained substantial within-group variability as to the context and perceived functions of the observed behaviours.

There were differences in the profile of functions relating to the type of CB. Although escape was the most frequent social-function for SIB, the proportion of instances with a non-social function was higher across the group. Namely, for the majority of instances of self-injurious behaviour the function appeared to be unrelated to their external environment. Previous anecdotal evidence has suggested that hand-biting in individuals with FXS is typically exhibited in response to "frustration or excitement" (Harris, 2006). This corresponds to our observation of the high occurrence of SIB during play (alone or with others), and was also seen in Langthorne and colleagues' (2011) study. The internal function of such behaviours is unknown, but it is possible that the biting action may serve an inherent physiological function, such as regulating arousal levels. For instance, chewing (namely, on gum) appears to be linked to reduced chronic stress, with some evidence of a mediating influence on acute stress also (Allen & Smith, 2011). Though it is unclear whether this potential effect could generalise to self-biting or -chewing, it highlights a possible association between chewing actions and altered physiological reactions.

Unfortunately, there are no directly comparable data (collected and analysed in the same manner) on a similar group of children without FXS, to determine the specificity of the findings to this group. It is unclear whether the behavioural contexts and hypothesised functions would differ between the present sample of individuals with FXS, and others who exhibit CBs. However, findings from related investigations may help to shed light upon the findings from the present study. Herzinger and Campbell (2007) conducted a qualitative synthesis of outcomes of different FA methodologies, including those utilising non-experimental methods of behavioural assessment (8/26 of which utilised an A-B-C recording approach similar to that of the present study). Escape was the most commonly identified function (34.62% of

assessments), followed by attention and non-social (15.38%). This differs from the present study which identified non-social as the most common function of behaviours (primarily for SIBs). Though, escape was similarly the most common social function observed. Descriptive observational analyses have also been conducted with individuals with Smith-Magenis Syndrome (SMS), though using a conditional probability approach. This research found that CBs in this group are frequently evoked by low levels of attention and result in the provision of attention, suggesting an attention function (Taylor & Oliver, 2008). As discussed in previous chapters, this corresponds to provisional investigations suggesting differing profiles of behavioural function of individuals with SMS and FXS, based upon indirect assessment (Langthorne & McGill, 2012) and preliminary data from functional analyses (Langthorne et al., 2011; Hardiman, Langthorne & McGill, in press). Across the group, behaviours with a hypothesised attention function occurred at lower frequency than other social functions, which differs from the hypothesised pattern of behaviours which would be expected based upon previous research with individuals with SMS. However, direct between-group comparisons of individuals with and without FXS are required in order to determine whether the condition may affect the likelihood of behaviour occurring in different contexts and with different functions.

Arousal-behaviour associations. The small preliminary investigations which could be conducted in the present study provided no clear indicators for potential associations to be addressed in future research. However, this is unsurprising given the small sample size and the broad nature of the measures. One of the challenges with assessing relationships between arousal and behaviour was the assessment of the diurnal variation in cortisol levels. Given the varying occurrence of CB across the day and timing in relation to the sampling, it was not possible to determine whether arousal

changes may be occurring in temporal proximity to the behaviours, or whether stimulus-bound differences may be apparent compared to controls. As such, now that the viability of saliva samplign has been established, it would be of benefit to utilise this approach in order to investigate system reactivity in response to a more standardised environmental challenge. Future research with larger sample sizes and more controlled measures will be required to investigate the hypothesis that escape maintained behaviour in individuals with FXS is associated with atypical arousal levels.

Limitations. There are a number of considerations which must be taken into account when interpreting the results of this study. The primary limitation of the study is the small and self-selecting sample. Across all measures it is unclear the extent to which a small sample of boys with FXS selected for engagement in CB were representative of behaviour associated with the condition more generally. The self-selecting issue is of particular importance when considering the ratings of acceptability: it is unlikely that individuals who felt that the sampling procedure would be aversive would have volunteered to take part. Furthermore, those who did participate were well-informed and prepared prior to the sampling. Across the broader community it is likely that a more varying range of responses would have been obtained. It is also possible that concerns about the sampling required in the study contributed to the recruitment challenges for this project. The sample size for the acceptability ratings was further limited by the fact that not all participants completed the measures. This reflects the busy data collection of the day and often the child's fatigue from the school day (particularly young children went to bed very soon after the final sample) or the parent's need to conduct caregiving tasks, meaning that they did not have time to complete the measure. It is possible that those who found the procedure less acceptable

were less likely to make availability to complete the measure, though anecdotally this did not seem to be the case.

There is also potential for bias in responding, for various reasons. Firstly, given that people with intellectual disabilities may have a bias towards selecting the most positive option in Likert-type scales (Hartley & MacLean, 2006), the results obtained from the children with FXS should be interpreted with caution. In addition, several of the parents were also known to the researcher outside of the research, through the Fragile X Society (Becky Hardiman was a trustee at the time of this project and a number of the other parents were also on the board or members of the charity). Although the participants were allowed to complete the measure away from the researcher, it is possible that this may have also led to an increased social desirability bias in the responses given that they knew that their responses would be later seen.

The small sample size also extends to the single day on which the saliva samples were conducted. The recommended assessment for saliva sampling for the purposes of assessing the CAR would be to sample on at least two consecutive, similar days (Clow, Thorn, Evans & Hucklebridge, 2004). In addition, CAR may be associated with anticipatory perceived demands about the up-coming day (with increased anticipation typically being associated with higher CARs: Powell & Schlotz, 2012). As such, awareness about the occurrence of the research may have led to alterations of the CAR, relative to typical days. In order to further assess the CAR in FXS in future research, a greater numbers of samples across the awakening period (as validated by objective measures of awakening, such as the use of actigraphy) on at least two days would be required.

In addition, the sample size for the purpose of the arousal analyses was further limited by the fact that a number of samples contained insufficient volumes for any analysis. Values for levels of both SAA and cortisol could be obtained for over three quarters of samples. Focus on compliance with the recommended sampling protocols may help to address this issue in future projects.

There are further limitations relating to the saliva sampling which require consideration. Firstly, there was variation in the timings of the awakening saliva collection in relation to reported awakening. In addition, the correspondence between reported and actual awakening times was unclear. Although there did not seem to be a correlation between the CAR findings and the individual lags between awakening and sampling, these delays may have influenced the results. Stalter and colleagues (2016) found that delays of 5 to 15 minutes could alter CAR measurements, leading to either over- or underestimation of the response. As such, the result may have been affected by this variation in the implementation of the measurement protocol. In addition, although participants were instructed to hold salivettes beneath their tongues (where this collection method was used), anecdotally some of the children chewed the swabs, particularly participants in the FXS group. Although mastication does not affect cortisol levels, it may have confounded the SAA findings. Due to its role in digestion, levels of salivary SAA increase dramatically in response to chewing (or similar jaw movements: Mackie & Pangborn, 1990). This was not systematically evaluated therefore it is not possible to evaluate which samples may have been influenced in this way. However, it is possible that this sampling issue contributed to the variability in levels observed, rather than solely sympathetic autonomic differences between the groups. In future research it will be important to support participants with information and encourage them to hold the swab according to instructions.

Unaffected siblings were selected as a comparison group in the present study, in order to facilitate comparison with earlier research. However, previous research with individuals with autism suggests that intellectual ability may be a key determinant of findings relating to physiological arousal. Namely, those with greater intellectual impairment may show more atypical profiles (Taylor & Corbett, 2014). Therefore, this should be accounted for in future research.

Information was collected on the occurrence of CBs through observation in a natural environment, during a school day. Though the observations were conducted for extended periods, they were conducted on a single day and the behaviour observed may not have been representative of the individual's overall behaviour. Though the participants were naturally exposed to a broad range of situations and possible antecedents to CB through the day, it is likely that there may have been situations which may have evoked CB which were not present.

Furthermore, though the school days were selected as being 'typical', of course the presence of the researcher and the collection of the samples was atypical, and may have influenced behaviour. No structured reports were collected from informants on the perceived representativeness of the observed behaviour. Anecdotally, several of the parents or teachers confirmed correspondence between observed and general behaviour. However, a couple of participants were reported to have been "on best behaviour" during observations, appearing to teachers or parents to have exhibited less behaviour than usual.

The periods during which the data was being collected were extended (lasting on average 7.7 hours during the day) and therefore observer factors, such as fatigue or distractibility, may have influenced the data collection (Barrios, 1993). It is possible that

this could have led to inaccuracies in the recording. Though some real-time reliability data was collected and showed high correspondence between observers, these checks were limited due to practicality issues. It is also possible that other researcher factors may have influenced the collection of the data and its validity. Observer expectations and biases have been shown previously to influence the validity of data collection (Hartmann & Wood, 1990; Kent, O'Leary, Diament & Dietz, 1974).

Finally, given that there was no experimental manipulation of the participants' environments, firm conclusions cannot be drawn regarding the functions of individuals' behaviour. The inclusion of an intervention element could have been beneficial, as successful reduction of behaviour following a function-matched intervention could have validated hypotheses. However, unfortunately this was not possible within the parameters of this particular investigation.

Summary and Future Research. In summary, this initial study has demonstrated the acceptability and feasibility of the use of salivary measures in individuals with FXS and CBs, supporting their use in future work in this area. Despite a small sample size, novel potential differences in the CAR in boys with FXS were identified. Reduced CARs may be an indicator of chronic stress, which may be linked to engagement in CB in this group. In addition, as in previous research individuals with FXS exhibited elevated indicators of sympathetic autonomic activity (SAA). This further validates the existence of differences in physiological arousal which may have clinical significance in this group.

In addition, naturalistic observational data were collected on the behaviour of boys with FXS in both home and school settings. The results of this assessment in part support the hypothesis that escape would be the most frequent function for such

behaviours in this group, but also highlighted important differences relative to the topography of behaviour, as well as substantial within-group variability. This suggests that further investigations into the possible influences upon CBs within this group are warranted.

As mentioned above, sample size limits reaching definitive conclusions. In fact, recruitment was a key challenge for this project. One of the issues relating to this may have been the challenges of identifying families with both a son with FXS and an 'unaffected' child, both within the study age-range (5-15 years). Feedback from non-eligible families and with the local collaborators at the genetic centres, suggested that the issue of sibling inclusion criteria acted as a substantial barrier to participation. The inclusion of siblings as a comparison group helps to control for familial environmental and genetic factors which may influence the stress effector systems (as used in: Hessl et al., 2002). However, research with larger sample sizes will be key for developing our investigations into the influences on behavioural function, particularly upon escape-maintained behaviours, in individuals with FXS. Therefore, this challenge means that the choice of comparison group may need to be re-considered in future research. This may also serve as a key opportunity to investigate the association between behaviour and arousal in other groups comparable on factors such as degree of intellectual disability.

The key aims of subsequent research in this project (see Chapter 5) will be to further investigate associations between arousal and behaviour in individuals with FXS, and comparison groups. As discussed above, the broad measures of behaviour and arousal collected in this study, across a typical day, provide a valuable insight into various aspects of individuals' experiences. However, more consistent and controlled

explorations are warranted in order to further explore hypotheses relating to arousal and escape-maintained behaviour in FXS.

Chapter 5

Arousal and escape behaviour in response to academic and social demands.

Chapter overview

The aim of the present study was to conduct an investigation to further explore the association between escape-maintained behaviour and physiological arousal, in response to academic demands. Previous hypotheses of motivational changes in FXS relate to observations of escape-maintained challenging behaviours (CBs) in this group. However, should such motivational changes exist, it may be expected that any response which is reinforced with escape from demands may be likely to be maintained. As such, in the present study escape motivation was assessed through evaluating the use of an arbitrary taught response, which was reinforced through provision of breaks from academic demands in a structured assessment. Additional measures were also collected relating to the occurrence of CBs and other off-task behaviours during the presentation of demands. Furthermore, previous investigations of both arousal and of CB have been limited by their lack of a control group with comparable intellectual ability to those with FXS. Therefore, in the present investigation a comparison group of children with intellectual disability was recruited.

Between-group differences were observed in relation to the likelihood of engaging in the escape response and in CB, whereby both were seen significantly more commonly in the FXS group. There were no differences between groups in cortisol levels, response or recovery in relation to a challenging academic demand though both groups tended to exhibit low levels of cortisol across samples.

Introduction

Anecdotal, observational and experimental research provide preliminary evidence that individuals with FXS exhibit an increased likelihood of engaging in escape-maintained CBs, compared to other behavioural functions (see review: Chapter 2), and that the profile of behavioural function may differ from other groups (Langthorne & McGill, 2012). It has been hypothesised that an enduring motivational difference underlies these observations, which may arise from changes to systems relating to physiological arousal: the HPA axis and autonomic nervous system (Chapter 3; Langthorne et al., 2011). Specifically, atypical stimulus-bound arousal may be associated with an altered experience of environmental challenges, such as the provision of social and academic demands, leading to a greater reinforcing value of escape or avoidance from these situations. As a result, individuals with FXS may be more likely to engage in behaviours which have previously been negatively reinforced in this manner. In line with this hypothesis, demands were also found to be the most common antecedent for behavioural challenges, via direct observation (see Chapter 4).

There may be a number of different dimensions of a demand context which act as establishing operations for escape-maintained behaviours (Smith, Iwata, Goh & Shore, 1995). Features associated with FXS may make certain aspects of demands particularly challenging. Firstly, FXS is associated with social anxiety (Cordeiro et al., 2011) and individuals with FXS typically exhibit eye-gaze avoidance (Wolff, Gardner, Paccia & Lappen, 1989). Therefore, social factors associated with the presentation of demands may play a role in eliciting escape behaviour, though Langthorne and colleagues (2011) did not find that individuals frequently engaged in higher levels of CB to escape from adult attention in their preliminary study. However, it is possible that the social element of demand presentation may contribute to the motivation to escape, particularly when

presented with difficult tasks requiring a high degree of information processing. Murphy and colleagues (Murphy, Abbeduto, Schroeder & Serlin, 2007) tested the hypothesis that increased task difficulty and increased social demand would have a cumulative effect upon increasing gaze avoidance in individuals with FXS, though their findings did not support this hypothesis. Based upon anecdotal evidence, Fragile X support charities recommend minimising eye contact and sitting beside rather than in front of pupils with FXS, during educational tasks, in order to avoid increasing anxiety and social demand for the child (Fragile X Society, 2013). The utility of this educational strategy, either in increasing task engagement or reducing maladaptive behaviours, has not been assessed. Secondly, given the cognitive and linguistic impairments (Abbeduto & Hagerman, 1997; Crawford, Acuña & Sherman, 2001) and ADHD-like features (Munir, Cornish, Wilding, 2000) associated with the condition, the information-processing demands required by difficult tasks may act as an establishing operation for escape. Understanding the factors which may contribute to the occurrence of escape-maintained behaviours during the presentation of demands in individuals with FXS, and whether these differ from other children, is of value as it may highlight alterations to the nature of the demands which can support the reduction of maladaptive behaviours.

CBs are, by their nature, socially salient and may therefore result in social responses, such as the removal of demands. The hypothesis regarding reinforcement sensitivity in FXS is based upon retrospective analyses of the pre-learned functions of CBs for small groups of individuals with FXS. However, if individuals with FXS do experience a raised sensitivity to negative reinforcement, then the behaviour-altering effect would not be limited to these topographies of behaviour. Rather, any behaviour which results in escape or avoidance from a non-preferred situation would be likely to be reinforced. The range of responses in an individual's behavioural repertoire to access

negative reinforcement depends on a wide range of factors, including communicative ability as well as their individual environment and reinforcement history. Thus, whilst some individuals with FXS may engage in CBs, others may demonstrate more adaptive behaviours which access the same reinforcement. For instance, an individual may avoid or reduce the social aspects of demand through gaze avoidance (Langthorne, 2012), engage in social behaviours (such as changing the topic or "class clown" behaviour, such as that described by: Salend & Taylor, 2002), or verbally request breaks from the task. As such, in order to further assess sensitivity to negative reinforcement in FXS, a wider range of behavioural topographies, beyond just those considered 'challenging', must be considered.

A variety of approaches have been taken to assess motivation and reinforcer strength. Vollmer and Iwata (1991) investigated establishing operations and their short-term influence upon the effectiveness of positive reinforcers. Rather than assessing the occurrence of behaviours already within the individual's behavioural repertoire (which would have been subject to varying reinforcement history), the researchers provided stimuli (food, praise or music) contingent upon the occurrence of an arbitrary behaviour (placing a block in a box). The effects of recent satiation and deprivation of the stimuli were then evaluated by comparing response frequency in these conditions to that at baseline. The researchers were able to identify individual variation in the effectiveness of reinforcers (and thus motivation to access those stimuli), through the reinforcement of an arbitrary response. Similarly, Kodak and colleagues (Kodak, Lerman, Volkert & Trosclair, 2007) investigated the preference for positive reinforcement (provision of a tangible item) or negative reinforcement (break from a task) through teaching individuals to select coupons or items representing the desired reinforcer. Five individuals with developmental disabilities who exhibited

escape-maintained CB were included in the study. High- and low-preference tasks were presented to participants with reinforcement provided contingent upon compliance with a target number of demands. This research demonstrated the utility of teaching a new response to identify individual reinforcement preferences, as individuals were successfully able to discriminate to access desired reinforcers. For the present study, assessment of strength of sensitivity to negative reinforcement (taking a break from a task) through the reinforcement of an arbitrary behaviour would have the benefit of controlling for individual variation in past reinforcement of behaviours already in the individual's repertoire. Though Vollmer and Iwata (1991) employed this approach to assess the influence of transient establishing operations, group level comparisons of individuals with and without FXS may allow for assessment of enduring establishing operations associated with the genetic condition. Furthermore, this assessment avoids the ethical concerns associated with experimental functional analyses (EFAs), which relate to the eliciting and reinforcement of CBs (Poling, Austin, Peterson, Mahoney & Weeden, 2012). If FXS is associated with an increased motivation to escape situations, such as the presentation of academic demands, then one might expect this group to use requests for breaks from such tasks more frequently, when compared to individuals without the condition.

It seemed desirable that the assessment of the strength of demand escape as a reinforcer should take place during a single day, in order to facilitate visits to larger numbers of individuals. However, previous investigations have required lengthy assessments (Kodak et al., 2007: 170+ sessions; Vollmer & Iwata 1991: ≥ 90 sessions). Brief assessment may be facilitated through exposure to single sessions of conditions systematically varied according to key aspects which may influence escape-maintained behaviour, whilst maintaining a consistent response to a target behaviour (based upon:

Smith et al., 1994). The resultant effect of condition upon responding may then be evaluated.

Both information-processing and social demands may be key factors establishing escape motivation during the presentation of tasks. Information processing demand may be altered through the manipulation of task difficulty (Murphy, Abbeduto, Schroeder and Serlin, 2007). In addition, Langthorne (2012) systematically manipulated social demands during the presentation of tasks by varying researcher eye contact (sat opposite or beside the participant) and prompts for eye contact (prompts for eye contact between trails, compared to a control motor response in order to control for instructional demand), in 'high' and 'low' social conditions. Based upon these prior methodologies, a three-condition assessment was adopted for the present investigation: hard task, high social; hard task, low social; easy task, high social. This design facilitates a brief assessment as single repeats of each condition can be completed, with another condition acting as a control: comparison of the two difficult demand conditions allowed for investigation of the influence of social factors upon behaviour, whereas comparison of the two high social task presentations allowed for assessment of the role of information processing demands. The aim of these manipulations was to allow preliminary investigation as to which aspect of demands establishes the motivation to escape from work situations, and whether this differs between those with and without FXS.

As discussed in earlier chapters, males with FXS exhibit atypical endocrine and autonomic arousal in relation to stressors (Chapter 3). In a prior study (Chapter 4), the feasibility and acceptability of assessment of these physiological systems, through the analysis of cortisol and α-amylase levels in saliva, were demonstrated. It has been

hypothesised that enduring changes to these systems act as an enduring establishing operation to escape from or avoid situations which cause unpleasantly elevated arousal or stress. In order to assess the relationship between arousal and behaviour in a demand context, in the present study stimulus-bound arousal was assessed, as opposed to circadian rhythmicity in the prior study (Chapter 4). Furthermore, behavioural indicators can help to compliment salivary measures, when assessing arousal. Yawning may serve the function of increasing arousal and wakefulness: Matikainen and Elo (2008) propose that lowered rates of yawning may indicate a higher level of baseline arousal, conversely higher rates of yawning may reflect a necessity to increase arousal due to the low levels of arousal. Furthermore, fidgeting has been used as an indicator of social anxiety in previous research on FXS, with increased fidgeting being indicative of increased arousal (Lesniak-Karpiak, Mazzocco, & Ross, 2003).

As discussed in Chapter 3, previous studies have used only typically developing controls (Unaffected sibling: Hessl et al., 2002; 2006. Unrelated children: Roberts et al., 2009) when investigating group-level differences in the cortisol responses of children with FXS. Differences in the stress response observed in these studies could have been confounded by the FXS group's elevated intellectual disability (ID) or autism, due to differing experiences of the environment (Roberts et al., 2009). Therefore, it will be valuable to include a control group of people with intellectual disabilities, who are not known to have FXS. Both individuals with ID and with FXS commonly experience challenges with academic tasks as a result of information-processing requirements. As such, by controlling for this, the effects of the FXS phenotype more specifically may be ascertained.

Aims and hypotheses. The aim of this research was to further investigate arousal and escape behaviour through addressing the following questions:

- Do individuals with FXS show a taught response which allows them to escape from, or avoid, the presentation of tasks more frequently than people with IDs that do not have FXS? This question aims to address the proposed motivational change in FXS.
    - It was hypothesised that the participants with FXS would exhibit the taught response more frequently than the comparison group.
- Are there any group differences in other observed behaviours between groups during the presentation of demands? This question aims to account for a wide range of other strategies for avoiding or escaping from task demands.
    - It was hypothesised that individuals with FXS would display higher levels of other topographies of avoidant behaviour, such as gaze avoidance and challenging behaviour.
- Are there differences in responding under conditions varied according to social and information processing demand? In addition, are there any differences between groups? This question aims to investigate potential environmental manipulations which may mediate the motivation to escape from demands.
    - It was hypothesised that increasing the social demand and/ or the difficulty of the task demand may lead to increases in avoidant behaviour.
- Do boys with FXS have different physiological responses to classroom work challenges, compared to other children with IDs? This question aims to expand the research on arousal in FXS by conducting the first comparison which accounts for intellectual ability as a potential confound.

    - Based upon the literature reviewed in Chapter 3, it was hypothesised that boys with FXS would exhibit an elevated cortisol response to the challenge, either in magnitude or duration.
- Is there an association between arousal and behaviour during the assessment? This question aims to investigate whether atypical arousal may be associated with escape behaviour in FXS, as well as more generally across the groups.

Method

Design. This study was a cross-sectional between groups design, with exploratory within-group analyses.

Ethics and governance. The research procedure and materials were designed based upon experiences from a pilot study (ethical approval: Tizard Research Ethics Committee. Local R&D approval from Medway Council) as well as with input from parents of children with FXS, via the Fragile X Society Research Committee (a voluntary panel of parents and specialist advisors of a clinical or research background). The project was then reviewed and approved by an NHS Research Ethics Committee (East Midlands, Derby. Reference: 15/EM/0002). Local Research & Development approval was sought from three NHS Foundation Trusts: Guy's & St Thomas', Birmingham Women's Hospital and Aneurin Bevan University Health Board. Local approval was sought from other organisations supporting recruitment (typically Head teacher at schools and senior representatives of charitable organisations). Additional local council approval was gained from Perth and Kinross council to conduct research in one local area (two participants). Furthermore, the project was independently reviewed and approved by the Fragile X Society's Research Committee.

Participants.

Inclusion criteria. Children were recruited to participate in the study who were between the ages of 4 to 15 years old. Participants were able to be recruited from anywhere in the UK, subject to the researcher's ability to travel (based on time and funding). Individuals were not eligible to participate who were taking medications which directly interacted with the physiological systems of interest (L-HPA axis and autonomic nervous system); this was deduced through literature searches (including Granger et al., 2009) and consultation with one of the study supervisors (neuropharmacologist).

FXS Group. Individuals in the FXS group were required to be male, as in Study 1, due to gender differences in the condition and greater involvement in terms of impact upon the physiological systems of interest (Chapter 3). Parents were asked to provide evidence that their child had received a genetic test to confirm their diagnosis of FXS. Individuals were eligible with a diagnosis of the FMR1 full-mutation, including those with mosaic CGG repeat expansions or methylation status. Those with the pre-mutation were not eligible to participate. Participants were excluded if they had an additional genetic diagnosis associated with ID (such as, Tuberous Sclerosis Complex); one potential participant was not eligible to participate, for this reason.

Intellectual disability (ID) Group. Both males and females were eligible to participate. Children in this group were required to be receiving special education support and have a diagnosis of an ID, made by a professional. Parents were asked to provide details about their child's diagnosis, including genetic diagnoses. Individuals with a genetic syndrome were eligible to participate subject to a literature search confirming that there was no evidence of an association between the condition and

alterations to physiological responses (adrenal or endocrine) to stress. For this reason, individuals with Down's syndrome were not eligible for this control group: this condition is associated with reductions in cortisol levels (Murdoch et al., 1989; Bricout et al., 2008b). In addition, parents were asked whether their child has received a genetic test for FXS. Individuals with the Fragile X pre-mutation were not eligible to participate. For those who had not been tested, or for those where there was uncertainty, testing was not provided to conform the absence of the condition. This was due to ethical and resource-related reasons. However, it may be estimated that if half of all individuals with FXS in the UK have been diagnosed (Pembrey, Barnicoat, Carmichael, Bobrow &Turner, 2001) then of the children attending special education schools in the UK, only approximately 0.7% would be expected to have undiagnosed FXS[44]. As such, the expected prevalence of undiagnosed FXS in a group of 25 children from a special education school is considerably less than one person (0.0175), meaning the risk of selecting an undiagnosed individual in the control group was low.

Recruitment. The aim was to recruit 25 children in the FXS group, and 25 children in the ID group. Given the unknown effect size of the primary outcome variable (escape responding), a detailed power analysis for the present study was precluded. However, using Cohen's (1992) guidelines, in order to achieve an acceptable level of power (0.8) each group would require a minimum of 21 participants for a large effect size ($d = .4$) at $\alpha = .05$. A target group sample of 25 was selected in order to account for feasibility in practice.

[44] This estimate is based upon the following data: 2011 England census data on number of children between the ages of 5-14 years in total, as well as in special education schools; FXS prevalence of 1/4000 males and 1/6000 females; estimates that 95% and 65% of males and females with FXS, respectively, have developmental delay or ID and may therefore attend a special education school (Bailey et al, 2008).

Recruitment for the study commenced following ethical approval of the project in January 2015. The researchers ended recruitment attempts to the study in July 2016 (after 18 months) after extensive efforts, despite not meeting the planned numbers, due to time constraints and lack of further recruitment options. Details of the recruitment process are provided below.

For all recruitment methods, the researchers offered to provide, or cover the costs of, research materials and mailings. However, there were no additional incentives or reimbursements for time for either participants or those assisting with recruitment. Information sheets were developed specifically for centres which were being asked to support study recruitment.

Fragile X-Specific. The Fragile X Society disseminated information about the project to charity members via their newsletter (disseminated in 3 quarterly issues to the charity's membership of approximately 1800 families) website, and social media. In addition, direct mailings containing information sheets (Appendix H[45]) and consent forms were mailed to individuals identified as potentially meeting the study criteria (child with Fragile X age 4-15 years old), who were within travelling distance of the researcher's base (Northamptonshire). In total, 50 packs were directly mailed. The database search and mailings were conducted by another member of staff at the charity, so that the researcher was not aware who had been sent the mailings. All information clearly highlighted that the research (despite the researcher working as CEO of the charity) was independent from the charity and decisions as to whether to participate would not affect their involvement in the organisation in any way. Eighteen participants with FXS were recruited in this way. Three additional families expressed

[45] ID group information sheets can be seen in Appendix I.

interest in participation via this route but were ineligible or unable to participate due to: the individual being diagnosed with the pre-mutation; medication (Guanfacine: a drug which reduces sympathetic central nervous system activation, through action on noradrenergic auto-receptors); the school declining to support the visit.

In addition, all families (14) who had participated in the first study (see Chapter 4) were contacted with information about the study, highlighting that further participation was optional. Five participants (four families) were recruited in this way.

NHS Trusts. Twenty FXS information packs were sent to each Local Collaborator at two sites (Guys & St Thomas' NHS Foundation Trust and Birmingham Women's Hospital) to disseminate. However, there were no responses via this method. Local collaborators at both sites were contacted by the researcher to request further support with recruitment to the ID group, however both declined due to workload reasons.

In addition, following contact with a clinical psychologist who responded to a request for support with recruitment of participants with ID, the Serennu Children's Centre (a centre providing care, research and treatment to children with disabilities in Newport: Aneurin Bevan University Health Board) acted as a Patient Identification Centre. Information about the study was sent to all individuals on the Centre's mailing list in two weekly mailing bulletins, as well as 20 hard copy information packs being disseminated via the Local Collaborator to patients, information flyers available in the waiting room and posters being displayed. However, there were no responses via this recruitment attempt.

All clinicians in the Sussex Partnership NHS Trust Learning Disability Child and Adolescent Mental Health Service (CAMHS) and Family Intensive Support Service (FISS) were contacted with information about the study (following a member of the team

seeing information about the study and offering support). However, due to lack of response, further ethical approval was not sought to recruit.

Schools. Special Education Schools were identified through County Council websites in the following areas (selected according to ease of travel for the researcher) and all were contacted initially by telephone to request an appropriate email address to which to send introductory information: Northamptonshire, Buckinghamshire, Oxfordshire, Bath and North East Somerset, Cardiff, Vale of Glamorgan, Kent. These contacts were then followed up with one phone call and one email. In addition, further specific schools were contacted through personal contacts. Furthermore, schools which had been supportive with facilitating research visits for participants in the FXS group were re-contacted to request support with recruiting further participants for the ID group. In general, responses from schools to these approaches were limited. In total, seven schools agreed to support with recruitment (four identified through earlier involvement in the research; two through local council searches; one through personal contact of Head Teacher of a recruiting school; one through personal contacts). The first school to support with the research (a Special Education School in Oxfordshire) sent out 60 full information packs (information sheets, consent forms) to families they believed may be interested in participating, with no response. As a result, the recruitment information was amended to be more brief and accessible: the remaining schools disseminated brief information flyers to parents (Appendix J). Ten participants in the ID group were recruited with support from a collaborating school: 9 from a generic Special School Academy (age 4-19 years) in Somerset; 1 from a primary school for moderate learning difficulty in Kent. In addition, one participant in the FXS group was recruited through a school attended by another participant in the study.

Support groups. Local and national support groups for people with learning disabilities or autism were contacted to request dissemination of the information flyer to their networks. Representatives of 24 organisations relating to ID or autism were contacted, which were identified through internet searches and personal contacts. In addition, Facebook support groups were approached through messages to group admins to request that either the researcher may post an advert (flyer image plus text), or that the admin did so on their behalf. In total, 15 organisations or online groups agreed to support the study and disseminate information. Four participants were recruited in this way through the Challenging Behaviour Foundation (two participants), Angelman Syndrome Support, Education and Research Trust (ASSERT: one participant) and an online parent support group based in Norfolk (One participant). Two further families expressed interest in the study via this recruitment method: one was ineligible due to lack of ID; the other withdrew due to change in personal circumstances.

Networks. In addition to the methods described above, the researchers and supervisors made requests through their own networks to organisations and contacts who may be able to support with identifying potential participants or advertising opportunities. In addition, all current students and staff at the Tizard Centre were contacted to request support with recruitment. All successful leads identified in this way are listed in the sections above.

Participant characteristics. 24 participants with FXS participated in the study, and 14 in the ID group.

FXS group. All participants were male and had a diagnosis confirmed via genetic test (95.8% full-mutation, 4.2% mosaic expansion). The majority (70.8%) were not taking any medications at the time of the research. Medications taken by the remaining

participants included: melatonin (4), carbamazepine (1), lisdexamfetamine (1), sodium valproate (1), diazepam (1), clozapine (1), fluoxetine (1), folic acid (1), immodium (1). Granger and colleagues (2009) identify fluoxetine as a medication which may affect cortisol through affecting the subjective experience of stress, novelty or threat. Though not identified by Granger and colleagues (2009) there is mixed evidence that benzodiazepines such as diazepam may lead to reduced cortisol levels. A sensitivity analysis was conducted to test the robustness of the whole group findings when excluding the two participants taking these medications: the inclusion of these participants did not alter the findings, as such, these data were kept in order to maintain sample size.

ID group. The majority of participants were male (78.57%). Participants had a range of diagnoses: Global developmental Delay (4); ADHD (3); Autism (6); Anxiety Disorder (1); Angelman Syndrome (1); Hypermobility Syndrome (1); ID with unknown cause (2). The majority of parents were unsure whether their child had been tested for FXS (50%), three participants (12.5%) had not been tested and four (16.7%) had been tested and confirmed clear of FXS (FMR1 CGG repeat expansions within normal range). The majority of the group were not taking any medication at the time of the research (71.4%). Medications being taken included: methylphenidate (2), sodium valproate (1), iron supplements (1). None of these medications were listed by Granger and colleagues (2009) as having a possible effect on cortisol levels.

Participant age. The mean age of the FXS group was 10 years 8 months (Range= 5 years 5 months- 15 years 5 months; SD 39.84 months), compared to 11 years 2 months (Range= 5 years 4 months- 14 years 5 months; SD 34 months) for the ID group.

There was no statistically significant difference in age between groups (t(36)=-.52, p=.61, d=-.15) [46].

Social Communication. As in Study 1, autistic behaviour was assessed by parent report using the Social Communication Questionnaire (Rutter et al., 2003: Table 28). There was no significant difference in levels of autistic behaviour (total SCQ scores) between the two groups (t(32)=.49, p=.63, d=.16). In addition, there was no statistically significant difference between the proportion of the groups which scored above the cut-off for ASD (score of 15 of more: X(1)=1.56, p=.21, Cramer's V=.21. Small to medium effect size) or for autism (score of 22 or more: X(1)=.39, p=.53, Cramer's V=.10), though there tended towards higher proportions above the cut-offs in the FXS group.

Table 28

Autistic Behaviour: Social Communication Questionnaire Scores

Group (N)	SCQ Scores			Above ASD Cut-Off	Above Autism Cut-Off
	Mean	SD	Range		
FXS (23)	20.85	7.43	4-30	81%	57.1%
ID (13)	19.38	10.06	7-35	61%	46.2%

Adaptive Behaviour. Adaptive Behaviour was assessed using the Vineland Adaptive Behaviour Screener (VSC; Sparrow, Carter & Cicchetti, 1993a, 1993b),

[46] Cohen's corrected d used due to small sample size (Durlak, 2009).
$d = \frac{Mean1-Mean2}{Sample\ SD\ Pooled} \times \left(\frac{N-3}{N-2.25}\right) \times \sqrt{\frac{N-2}{N}}$ where $sample\ SD\ pooled = \sqrt{\frac{[(SD1)^2+(SD2)^2]}{2}}$

administered to parents or caregivers in a semi-structured interview. This screening tool, designed for research, gives an estimation of adaptive behaviour, including communication, socialization and daily living skills. Generally, higher scores are indicative of higher adaptive functioning. There are four age versions covering children up to the age of 18. Due to the expected developmental delay of all participants, the age bracket below the child's chronological age was selected as the starting point for the interview. The appropriateness of the age selection was judged based upon scores on the first 5 questions of each section of the interview (maximum score, 10): if the participant scored 2 or less, the lower age bracket was used; if the participant scored 8 or more, the older age bracket questionnaire was selected. The original interview age bracket would be the starting point for each new section of the interview. The questionnaires for ages 6-12 and 13-18 did not include a "Motor Skills" sub-section, as such, the questions from the age 3-5 interview were utilised for all participants. The raw scores were converted to Standard Scores using the Screener Manual and interpreted using the Vineland Adaptive Behavior Scales Full Version manual (Sparrow, Balla and Cicchetti, 1984).

Descriptive statistics are presented in Table 29. There were no differences between groups in levels of adaptive behaviour (Vineland Screener Adaptive Behaviour Composite: $t(31)=-.24$, $p=.81$, $d=-.08$) or age equivalent for adaptive behaviour levels ($t(33)=-.55$, $p=.59$, $d=-.30$).

Table 29

Adaptive Behaviour: Vineland Screener Scores

Group (N)	Communication (mean (SD))		Daily Living (mean (SD))		Socialisation (mean (SD))		Motor Skills (mean (SD))		Adaptive Behaviour Composite (mean (SD))	
	Standard Score	Age Equivalent (months)	Standard Score*	Age Equivalent (months)	Standard Score*	Age Equivalent (months)	Standard Score+	Age Equivalent (months) +	Standard Score	Age Equivalent (months)
FXS (23)	38.59 (12.67)	42 (20.46)	35.63 (16.90)	50.04 (28.71)	52 (13.93)	45.86 (21.83)	65 (21.67)	46.14 (17.95)	63.91 (25.94)	45.97 (22.35)
LD (13)	47.15 (24.97)	53.38 (33.74)	38.07 (23.41)	52.69 (32.38)	47.54 (21.65)	46.30 (25.33)	74 (27.99)	51.15 (18.79)	66.36 (31.81)	53.97 (28.59)

* Note: <20 scores entered as 19 +Note: >5 years 11 months scores entered as 72

Reported challenging behaviour. Parents were asked to report whether their child engaged in any CBs. Responses are detailed in Table 30. There were similar levels of reported behaviour in each group, aside from that SIBs were reported to be markedly more common in the FXS group.

Table 30

Reported Challenging Behaviours

Behaviour	Proportion reported to engage in behaviour (% (N))	
	FXS (22)*	LD (13)
SIB	66.7% (14)	23.1% (3)
Physical aggression	66.7% (14)	53.8% (7)
Destructive behaviour	61.9% (13)	76.9% (10)
Other	Dropping to ground (2) "Shuts down" (1) Verbal aggression or swearing (2)	Tantrum (1) Verbal aggression (1)
Any Challenging Behaviour	100% (21)	92.3% (12)

* Questions were not answered in this section for two participants: One due to researcher error and the other due to time constraints in the interview.

Participant ID. Individual participants were assigned ID codes beginning either with FX or LD, followed by a number. These codes are referred to through individual-level analyses in the results.

Measures and Procedure

In the process of designing the protocol for the present study, a pilot study was conducted.

Pilot Study. The aim of the pilot was to assess the feasibility and utility of assessing sensitivity to reinforcement through assessing engagement in an arbitrary behaviour, in order to access programmed reinforcement. It was hypothesised that children would respond differently in conditions where there were different contingencies for responding, thus demonstrating understanding of the study procedure. In addition, it was hoped that practical insights into the study design could be gained.

Method. The protocol and materials were reviewed and approved by the Tizard Centre Ethics Committee at the University of Kent (Appendix K). Information packs (30) were disseminated via a special education school in Kent, after Research Governance Framework approval from the Medway Council. Five participants with learning disabilities were recruited (Table 31). None of the participants from the pilot participated in the main study.

Table 31

Pilot study participant characteristics

Participant	Gender	Age (years)
1	Male	5
2	Female	7
3	Male	6

Participant	Gender	Age (years)
4	Female	8
5	Male	7

Three condition types were employed in a fixed-order multi-element fashion, using the procedures outlined by Iwata and colleagues (1982/1994): play, demand and attention. In contrast to an EFA, which assesses the strength of pre-learned contingencies, this procedure assessed the acquisition of a new response (placing a block in a box). Planned session length was 10 minutes, with up to four repeats of sessions for each condition, which were conducted during visits on two days. In addition, one participant completed only two sessions of each condition due to requesting not to complete the research on the second visit.

The play condition served as a comparison condition; there were no programmed responses to any behaviour. In the demand condition, a challenging demand was presented (task the child would be unable to complete independently was selected with the class teacher) and use of the target behaviour resulted in provision of a 20 second break from the task (with no interaction from the experimenter). In the attention condition, participants had access to toys, however, the researcher stated that they needed to do work and did not interact with the participant. Use of the target behaviour resulted in the researcher providing attention to the participant for 20 seconds. In order to facilitate differentiation between conditions, the in-session contingencies were explained verbally at the beginning of each session (similar to: Northup; Kodak, Lee & Coyne, 2004), for instance: “It is time to do some work. If you

want a break, put the block in the box". The researcher modelled the response during the explanation. Aside from these additions, the procedure was conducted in the same manner as an experimental functional analysis. There were programmed responses for CBs during any condition, however, criteria for session termination based upon the child's behaviour were decided with key stakeholders prior to conducting the assessment. No sessions were terminated for this reason. The number of occurrences of the target behaviour was manually recorded from videotaped footage of the sessions.

Results. Occurrence of the target behaviour across sessions and conditions for all participants is graphed in Figure 27. The responding of participant 1 is presented as percentage of 10 second intervals in which the target behaviour occurred, due to there being multiple blocks available on the table and the child placing multiple blocks in the box concurrently, therefore exact numbers of responses being unclear. For all participants, there was differentially higher responding in one of the conditions: demand (participants 2 and 4) and attention (participants 1, 3 and 5).

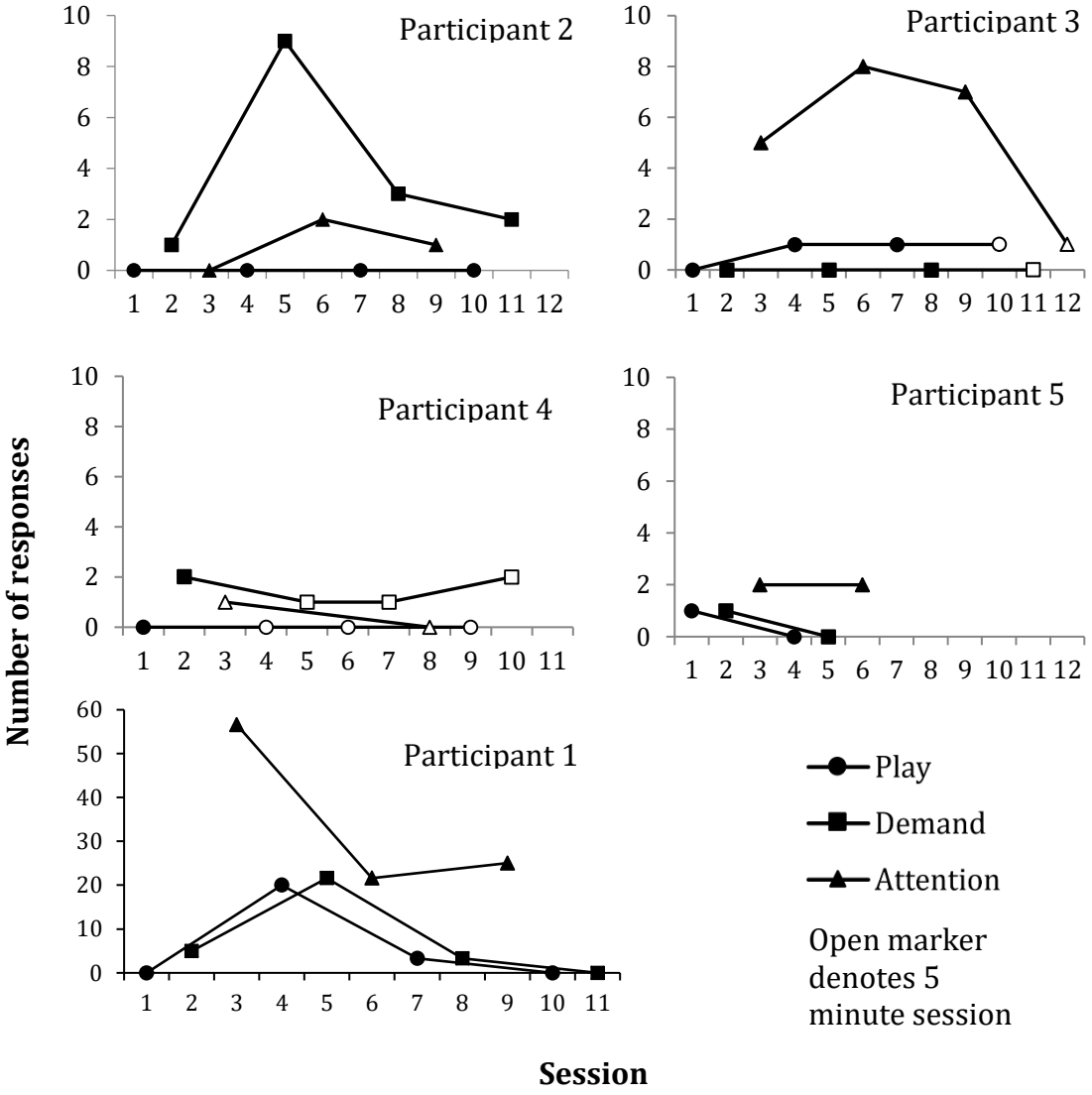

Figure 27. Frequency of engagement in target response[47]

Lessons learned. The pilot highlighted that the approach of reinforcing an arbitrary response was feasible, as participants demonstrated differential responding across different conditions, which differed between participants, thus demonstrating successful learning of the contingencies. In order to facilitate visits to a larger number of participants for the main study, the assessment needed to be conducted across a single

[47] A number of sessions were conducted for 5 minutes, as a result of time constraints for session completion at the school

day. As such, a briefer assessment was required. It was decided that demonstration of the response prior to the session would help to establish the contingency across sessions, and maintaining the same response (removal of the demand) to the target behaviour across sessions would be beneficial. Participant 1 exhibited high rates of responding across all conditions. Anecdotally, this appeared to be related to enjoyment of playing the blocks in the bowl, as opposed to a functional response as per the contingencies of the assessment. This suggested that this topography of target response may not be appropriate for all participants.

Present Study Procedure. Recruitment was conducted as described in earlier sections. Individuals who responded to adverts or the flyer, other than the full information sheets, were sent the full information sheet before they could make a decision as to whether they would like to participate, by returning a signed consent form. An initial, brief interview was arranged to gain basic information (date of birth, diagnosis details, medication use, school information) to confirm eligibility to participate. Participants' schools were then contacted (where not recruited through a participating school) to confirm willingness to facilitate a research visit.

Once eligibility had been confirmed and schools had confirmed support for the study, a second interview was conducted to gain more in-depth information about the participants: autistic behaviour (SCQ), adaptive behaviour (VSC) and questions about CB. The order of these question sets in the interview were randomised. Interviews were conducted with parents or caregivers: mothers (89.2%), fathers (8.1%) or other caregivers (2.7%). These interviews were not conducted for one participant in each group, due to being unable to schedule time with a parent or caregiver. This interview was typically conducted on the phone (78.4%), though face-to-face interviews were

conducted when preferred by the interviewee and logistically possible with travel (21.6%). Parents were offered the option of practice saliva collection materials (including clear photographic information sheets) to familiarise participants with the procedure. This opportunity was taken up by the parents of three participants (one in the FXS group, two in the ID group).

A visit was then arranged to take place in the participant's school (one assessment was conducted at the child's home, in a dedicated play room, due to being home schooled). During the visit, two assessments were conducted which required interaction with the researcher for approximately one hour in total, plus the collection of saliva samples (detailed below). The assessments were conducted in a space in the school away from other pupils (typically a separate and otherwise vacant room, though assessments were conducted in a corridor outside the classroom for three participants, and in a partitioned area of the main classroom for two participants) containing a table and two chairs. As far as possible, access to tangible items not related to the research was restricted. In order to be able to collect data about the child's and researcher's behaviour during the sessions (including measurement reliability and fidelity of implementation), the work sessions were videotaped, using a camcorder on a tripod.

A second adult from the school was asked to be present in the room with the researcher and participant in order to oversee the research and facilitate videoing. The second person was instructed not to interact with the participants during sessions[48].

[48] The Teaching Assistant (TA) of one participant (FX016) interacted with the participant on multiple occasions during the assessment, despite requests to the contrary. Prompts from the TA were coded as prompts using the definition for the prompts by the researcher. Sensitivity analyses were conducted for the behavioural comparisons excluding this participant. Given that the inclusion of this participant did not change the conclusions drawn from analyses, the data for this participant were retained.

However, for the majority of participants it was not possible for a member of staff to be present, primarily due to staffing constraints; members of school staff were present at sessions for eight participants (21.1%). In these cases, it was ensured that the research was conducted in view of members of school staff. It was not possible to arrange for assistance from a volunteer from the University of Kent as a second adult, due to the wide ranging geographical spread of participants.

Prior to the research taking place, the class teacher was consulted (either over the phone or by email ahead of the visit, or in person on the day of the research) in order to select tasks of appropriate difficulty for the sessions (described below). In addition, criteria for session termination (based upon the child's behaviour) were established.

A schematic of the structure of the assessment is presented below (Figure 28), each aspect is described in detail, below.

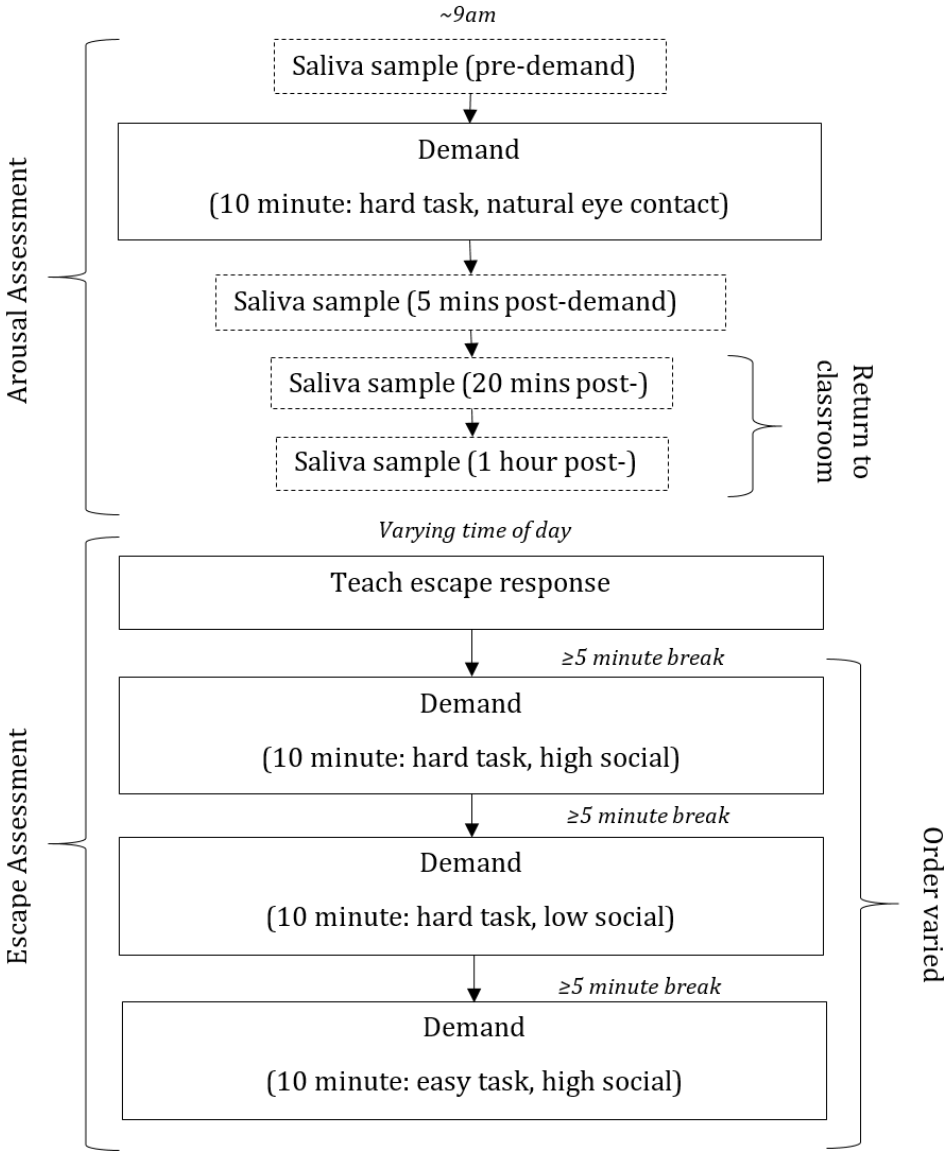

Figure 28. Schematic of experimental day data collection procedure

Arousal assessment. Each participant took part in a standardised procedure to estimate their physiological response to 10 minutes of presentation of a challenging, academic demand.

Demand. This procedure was designed to be a standardised analogue of what the researchers expect would be a fairly typical work procedure for children, within an educational environment. During the presentation of these demands the researcher was

sat opposite the child and looked at the child's face (Mean percentage researcher on screen and looking at child's face= 41.44%, SD=20.66%), although no specific prompts for the participant to make eye contact with the researcher were made. The demands were presented in a discrete trial format, using a three-step prompting procedure (Horner & Keilitz, 1975). Firstly an initial verbal prompt was provided; if accurate compliance did not occur within 5 seconds then the researcher gesturally modelled the correct response or, for more abstract tasks (such as number multiplication) provided verbal guidance as to how to reach the correct answer; finally, if the participant had still not successfully completed the task, then the researcher provided guidance to allow for the task to be successfully completed, such as a hand-over hand prompt or providing the correct response (physical prompt). Verbal praise for completion of the task was given. Across the assessments, prompts were delivered 3.87 times per minute (SD 2.32), with 45% of the prompts being higher-level prompts (gestural or physical prompt: SD= 19.96%).

The demands presented were selected with support from the child's teacher, out of a selection of worksheets and tasks. Teachers were asked to identify tasks which would be sufficiently challenging for the child, so that they would be unable to complete the task without assistance. The tasks which were available to be selected from included a variety of worksheet-based numeracy (varying from counting small arrays, to multiplication of double digit numbers) and literacy (varying from tracing of letters to spelling of complex words) tasks. These worksheets and tasks were selected from online teaching resources across a variety of levels of difficulty (target age groups), during the design of the research. In order to maintain task difficulty and account for learning during the task, a more challenging task was selected if the participant

correctly completed the task without the need for assistance on two consecutive occasions or on over 50% of occasions over at least 4 presentations (e.g. 3 times in 5).

CB was ignored during these sessions, with the exception of the occurrence of behaviours meeting the pre-agreed criteria for session termination. If the child left their seat, they were prompted to return to their seat to continue with the task.

Salivary measures. In order to assess physiological responses to stress, participants were asked to provide saliva samples. The aim was to collect a baseline pre-demand sample, followed by three post-demand samples at 5 minutes, 20 minutes and 1 hour. Due to the circadian rhythms of both cortisol and amylase, the aim was to conduct the assessments at a similar time during the morning; initial pre-demand samples were collected on average at 9.39am (SD 34 minutes, range: 9:05am-11.40am). As shown in Table 32, post-demand samples were collected as planned according to timings. This pattern of collection was designed to facilitate the assessment of response and recovery of both α-amylase (which peaks quickly (5 minutes) and recovers quickly (20 minutes): Almela et al., 2011) and cortisol (which peaks more slowly (20 minutes) and recovers more slowly (60 minutes: Kirschbaum & Hellhammer, 1989). Following the completion of the demand task, participants were allowed to return to the classroom, though teachers were requested not to present the child with challenging tasks.

Table 32

Details regarding saliva sample collection timing and duration.

Sample	Time of sampling			Seconds soaked*	
	Mean (minutes post-demand)	SD (minutes)	Range	Mean	SD
Pre-demand	N/A	N/A	N/A	37.46	21.50
5 minutes post-demand	5.56	1.33	4-9	38.63	24.86
20 minutes post-demand	20.96	3.33	14-34	33.75	20.28
60 minutes post-demand	61.3	7.43	47-84	36.87	23.28

* For those using Salimetrics Children's Swabs.

The protocol for the collection and analysis of the saliva samples was the same as in the prior study (as described in Chapter 4). The primary method for the saliva collection was the use of Salimetrics Children's Swabs (used by 92.1% participants). The approximate length of time swabs were kept in the participant's mouth were timed by the experimenter using a stop watch. Across all samples collected in this manner, the mean length of time swabs were soaked was 36.68 seconds (SD= 22.82). However,

where the participant was not able to tolerate the use of the swab, passive drool was used as the sampling method (this was used by the remaining 7.9% participants).

Following collection, where possible samples were immediately stored in a freezer at the school. Alternatively the samples were stored in a cool bag with ice packs. Samples were then transported to the researcher's base where the samples were stored in a domestic freezer (-18℃) in a locked box, before being transferred (as soon as possible, based upon the researcher's availability to travel) to a -80℃ freezer at the Medway School of Pharmacy, until analysis. Freeze-thaw cycles were minimised as far as logistically possible. Though, Garde & Hansen (2005) found levels of cortisol in saliva to be stable for up to three months at refrigerator temperature (5 degrees C), for at least one year frozen (both at -20℃ and -80℃) and to not be affected by four freeze-thaw cycles (the maximum tested). With regards to α-amylase, O'Donnell and colleagues (2009) also found this analyte to be robust to at least 5 freeze-thaw cycles and to tolerate exposure to room temperature storage for 5 days. As such, both biomarkers are robust and should not have been affected by this handling procedure (Salimetrics, 2016a; 2016b).

Escape assessment. The aim of this assessment was to evaluate individuals' motivation to escape or avoid the presentation of an academic demand, through the evaluation of the use of an arbitrary response (Vollmer & Iwata, 1991), which when emitted resulted in the provision of a 20 second break from the task. The assessment consisted of several phases, based upon the negative-reinforcement assessment used by Zarcone and colleagues (1999), which provided a brief method of determining non-preferred demands.

Teaching the response. A simple motor action was selected which required low response effort. The action that was assigned to be the escape response was based upon discussion with the child's teacher and to align with the child's communication style (e.g. a card exchange if the child used PECS). Card exchange was used as the target response for 73% (27) participants, 9 (24.3%) participants were taught to put a wooden block into a bowl, 1 participant used the sign for "finish" (a sign which the child had not previously mastered). Teachers were made aware of the topography of the response, in order to be aware of the communicative function of the behaviour in case that the participant engaged in the behaviour after the end of the assessment.

The teaching session began by the researcher demonstrating the 'escape response' to the participant, whilst saying "I'm going to teach you how to ask to take a break". A challenging demand task[49] was then initiated and if after 5 seconds following initiation they did not show the escape response, then a verbal prompt was given ("If you want to stop, then (behaviour description)."). If the child did not demonstrate the action, then the researcher gave a gestural prompt ("If you want to stop, then do this." The researcher then demonstrated the action). Lastly, a physical prompt was given to complete the action. Upon completion of the action (independent or prompted), the individual was then given a 20 second break from task. A new trial was then initiated, following the same procedure. This was repeated up to 10 times; fewer repeats were completed if the child demonstrated the action on 3 consecutive occasions, or on at least 80% of trials, over the previous 5 trials.

Evaluating use of the response. The child's behaviour, including the use of the response, was then evaluated during three 10-minute sessions of presentations of

[49] One of the challenging demands selected for later assessment, selected randomly.

academic demands. The style and nature of the three 10 minute demand sessions were systematically varied according to social and information processing requirements (based upon: Murphy et al., 2007; Carr & Durand, 1985; Langthorne, 2012). This included: easy demand-high social, difficult demand high-social, difficult demand-low social. A break of at least 5 minutes was provided between sessions, participants were allowed to leave the study room or area during this time.

The social manipulation was based upon that used by Langthorne (2012). During the 'high social' conditions, prior to the presentation of each new trial, a prompt for eye contact was made (verbal prompt "[name] can you look at me?", followed by a gestural prompt which involved repeating the request and the researcher pointing to their eyes). The researcher sat opposite the child and kept their gaze fixed on the child's eyes as much as possible through the tasks. In comparison, during the 'low social' condition, the researcher sat beside the child. Prior to each trial (to control for the instructional demand of the eye gaze prompt) the child was asked to perform a simple motor response (such as touching their nose), first with a verbal prompt and then with a gestural prompt (the researcher performed the action). The researcher aimed to minimise eye contact with the participant during these sessions.

The task difficulty manipulation was applied by varying the tasks presented for the 'hard task' and 'easy task' conditions. The tasks were selected based on teacher report, but altered based on the child's performance, if necessary. During the 'easy' condition, the researcher presented tasks which the teacher had reported that the child had mastered (they would be expected to be able to independently complete correctly, on at least 90% of trials). During the difficult sessions, the same procedure was applied as in the Arousal Assessment: the child should never or rarely be able to complete the

task spontaneously, without assistance. A mixture of numeracy and literacy worksheets or tasks were selected for each participant and were presented alternately.

Across all conditions, performance of the taught escape response was responded to with a 20 second break from the task. This involved stating “Ok, you don’t have to do that now”, ceasing prompting and turning away from the participant. The researcher did not interact with the participant during the task break. This contingency was verbally explained prior to each session: “It is time to do some work now, but if you want to stop, [describe action]”. Use of the response during a programmed break restarted the 20 seconds of the break, allowing for further avoidance of work.

The order of conditions was randomised by the researcher. However, the distribution of session orders was not even across conditions (Figure 29). This was an inadvertent bias introduced by the researcher due to it being logistically simpler to run the high social tasks consecutively to avoid room rearrangement.

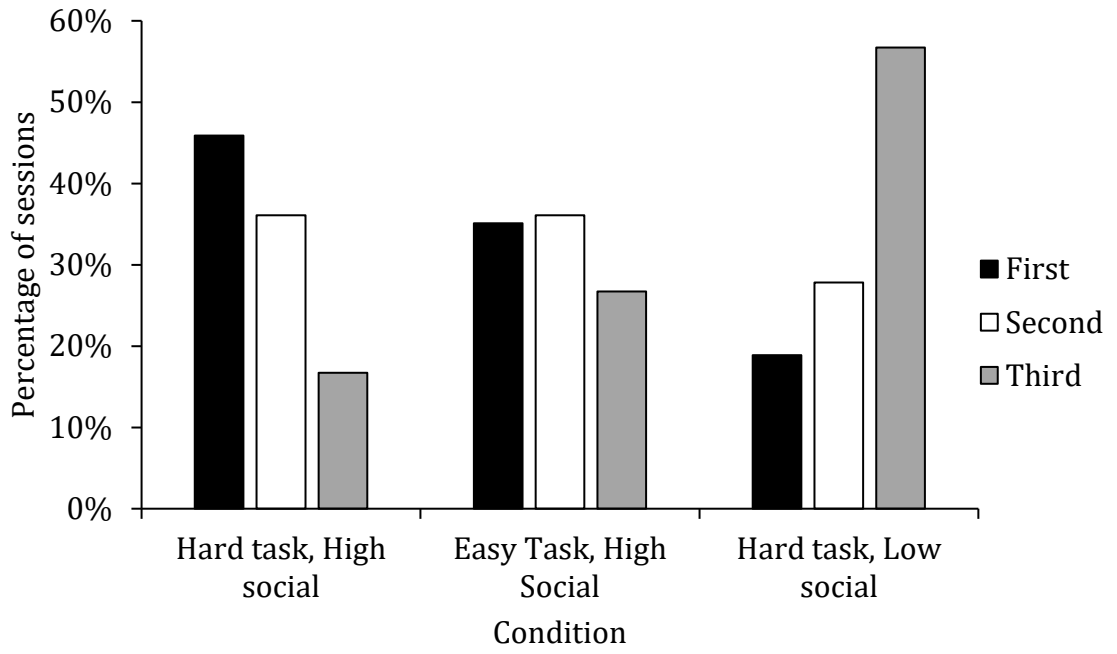

Figure 29. Order conditions were run during the Arousal Assessment.

Due to the uneven distribution of session order, analyses were conducted to assess whether session order had an effect upon observed behaviour. There was no significant association between session order and levels of CB ($F(2,26)=.04$, $p=.96$, $\eta^2=.00$)or gaze avoidance ($F(2,26)=2.43$, $p=.11$, $\eta^2=.16$). The effect of session order upon escape responding is explored in the results.

Data analysis.

Behavioural Measures. The occurrence of the escape response was recorded as an event variable during sessions. In addition, information was collected about the occurrence of CBs (due to the link between FXS and biting or chewing, self-biting was coded separately to SIB and object biting was recorded separately to other destructive behaviours) which may be pre-learned behaviours associated with escape from or avoidance of demands. In addition, information about other off-task behaviours was collected. In addition, information on off-task behaviours was recorded in order to gain

a behavioural comparison for the salivary measures. These included gaze-related variables (looking at the experimenter, head or body turned away from experimenter, eye covering, closing or rubbing), verbal requests for the task to finish and non-verbal signs of arousal (fidgeting; yawning which has an inverse relationship with arousal: Matikainen & Elo, 2008). These measures were selected based upon research by Hall and colleagues (2006) as well as by Langthorne, (2012). Furthermore, information was collected about the researcher's behaviour, including gaze and implementation of the tasks (prompts and task breaks), in order to assess procedural fidelity. Finally, information was recorded about whether the participant (including their face) and the researcher could be seen in the video recording.

Data was recorded from the videotaped footage of the sessions of the Arousal and Escape Assessments using ObsWin (Martin, Hall & Oliver, 2003): a computer program for the collection of observational data. Keyboard keys are assigned to codes and the pressing of the keys allows for the recording of the onset or offset (for duration variables) or occurrence (for event variables) of behaviours of interest. Operationalised definitions were developed for the behavioural codes (Appendix M). All videos were coded by the primary researcher (Becky Hardiman).

Reliability. Reliability was checked for 20% of the videos by a second coder, with a maximum of one session from any one participant. The behaviours were split into three groups (participant gaze-related behaviours; participant behaviour; researcher behaviour) which were each coded by a different person, in order to manage workload for the volunteers (Master's students at the Tizard Centre, University of Kent). The individuals conducting the reliability coding were offered supervision which could be counted towards Behavior Analyst Certification Board (BACB) supervised experience

hours. As the measures of interest were total durations of behaviours, or rates of event variables over the assessment, correlational analyses were conducted on total durations of codes across sessions, in order to assess the correspondence of the researcher's coding and the reliability coding. The desired statistic was a Pearson's correlation coefficient of greater than or equal to .8. The outcomes of the initial reliability are presented in Table 33 and Table 34.

A few variables did not reach acceptable levels of reliability in the initial round of coding. Two variables (destruction and off-task speech) were close to meeting the agreed criteria. Visual analysis of the data identified one participant for whom there was non-agreement on the occurrence of these behaviours. As such, an additional coder re-coded these variables for that participant. Ratings of the occurrence of task refusal showed extremely low correspondence, as such this variable was re-coded for all reliability videos, by an additional volunteer (Master's students at the Tizard Centre, University of Kent). These reliability checks were conducted using the application Behavior Observation Made Easy (Shekhtmeyster, 2017) due to lack of volunteer access to a Microsoft Windows enabled machine on which to use ObsWin. The output of the coding yielded a total duration (second accuracy) across the observation period.

Table 33

Reliability coding outcomes for participant behaviours

Behaviour Code	Pearson Correlation Coefficient (initial)	Pearson Correlation Coefficient (second)
Bite object	.991	
SIB	1.00	
Self-bite/ chew	.939	
Aggression	.992	
Destruction	.777*	.941
Participant on screen	.978	
Participant face on screen	.975	
Gaze to experimenter	.991	
Turned away	.855	
Eyes covered	.846	
Cry	Non-occurrence in reliability sessions	
Stereotypy	.998	
Off-task speech	.625*	.956
Verbal Task Refusal	-.067	.871

Behaviour Code	Pearson Correlation Coefficient (initial)	Pearson Correlation Coefficient (second)
Out chair	.803	
Escape response	.899	
Yawn	.995	
Fidget	.932	

In addition, the coding of occurrence of task prompts did not reach acceptable levels of reliability when divided into levels (verbal, gestural, physical). As such, it was explored whether these variables would reach acceptable levels of reliability when combined: for the task prompts gestural and physical prompts were combined into "higher level" prompts; both levels of gaze prompts were combined; both levels of movement prompts were combined. These combined variables all exceeded the acceptable criteria. It was decided that verbal task prompts (r_p=.68) represented an acceptable level of reliability, as this variable was being used only for the purposes of assessing procedural fidelity.

Further reliability analyses were conducted for the Escape Response variable, in order that the reliability could be ascertained for the purpose of more detailed analyses of the timings of occurrence across sessions. Due to reliability analyses being conducted on Arousal Assessment sessions for some participants (where this variable was not applicable), reliability was conducted on 14% of escape assessment sessions. Due to the

low level of occurrence, r-occ was calculated: the mean agreement for occurrence in 10 second intervals across participants was 93.75% (range 0[50]-100%).

Table 34

Reliability coding for researcher behaviours (procedural fidelity).

Code	Pearson's Correlation Coefficient (initial)	Combined Code	Pearson's Correlation Coefficient (Combined)
Researcher gaze	.835	-	
Verbal prompts (task)	.676	-	
Gestural Prompt (task)	.742	Higher prompts	.819
Physical Prompt (task)	.728		
Gaze prompts (verbal)	.977	Gaze Prompts (all)	.980
Gaze prompts (gestural)	.690		
Movement prompts (verbal)	.782	Movement prompts (all)	.856
Movement prompts (gestural)	.681		

[50] For one participant the reliability coder rated the occurrence of 1 use of the escape response, whereas the researcher did not rate any occurrence. For all other participants agreement was 100%.

Fidelity. In order to assess the implementation of the experimental sessions, information was collected on researcher's behaviour during the sessions, including eye contact and prompts. The task difficulty manipulation was assessed by reviewing the proportion of "higher level" prompts required. A significantly higher proportion of the prompts in the "difficult" task conditions were higher level prompts (gestural or physical: Mean: 43.7%, SD=9.7%) when compared to the easy tasks (Mean= 17.38%, SD=3.9%. $t(16.89)=6.57$, $p<.001$, $d=3.38$), with a large effect size, supporting that the manipulation was valid: tasks used in the difficult task conditions were more difficult than in the easy task condition.

The implementation of the social manipulation was assessed by reviewing the researcher's gaze and the prompts given to make eye contact. Across all conditions, the researcher was looking at the participant's face for an average of 41.0% of the session (during which their face was visible in the video recording). In the low social condition, the researcher looked at the participant's face for mean 13.74% of the session (SD 15.18%), which was significantly lower than in the high social condition (51.95% of session duration, SD=19.72%. $t(39)=6.00$, $p<.001$, $d=2.07$), with a large effect size. Gaze prompts were utilized only in the high social conditions (both hard and easy tasks). These prompts were given an average of 2.33 times per minute (SD= 1.72). Significantly more gaze prompts were given in the easy condition (mean= 3.86 prompts per minute, SD 1.53) compared to the hard condition (mean= 1.22 prompts per minute, SD=.67. $t(15.46)=-5.83$, $p<.001$, $d=2.13$), with a large effect size. This is due to the easier task meaning faster completion of the trials, and therefore prompts for gaze before starting a new trial. As a control for the gaze prompts in the 'high social' conditions, simple movement prompts were given only in the 'hard task, low social' condition. Participants were prompted make the movement (e.g. touch your head) an average of 1.39 times per

minute of the session (SD=.66). There was no statistically significant difference between the frequency of movement prompts in the "low social, hard task" condition, compared to the gaze prompts in the "high social, hard task" condition (t(30)=-.71, p=.483, d=.24).

Salivary Measures. Analyses of the saliva samples were conducted by either the researcher, or by Dr Alison Bratt at the Medway school of Pharmacy, using the methods described in Chapter 4. Cortisol assays were prioritised over α-amylase, as Langthorne and colleagues' (2011) initial hypothesis related to this physiological system, and were therefore conducted initially. As detailed in Table 35, there was a high rate (27.0%) of samples which contained insufficient volume to allow for analysis (recommended triplicate analysis requires 75µl saliva. Single analysis requires 25µl). This occurred with greater frequency in the FXS group. A number of samples were not collected for a variety of reasons: participant being unavailable due to school activity (3 samples), participants declining to provide sample (7 samples).

Table 35

Details regarding saliva sample collection and cortisol assays.

	Pre-demand (%)		5 minutes post-demand (%)		20 minutes post-demand (%)		Hour post-demand (%)		Total (%)	
	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (96)	LD (56)
Triplicate	45.8	64.3	45.8	50	54.2	50	41.7	28.6	46.9	48.2
Duplicate	8.3	7.1	0	14.3	4.2	7.1	0	14.3	3.1	10.7
Single	0	7.1	12.5	7.1	8.3	14.3	12.5	7.1	8.3	8.9
Insufficient volume	37.5	21.4	33.3	14.3	29.2	14.3	29.2	21.4	32.3	17.9
Not collected	4.2	0	8.3	0	4.2	0	12.5	21.4	7.3	5.4
Not detectable	4.2	0	0	14.3	0	14.3	4.2	7.1	2.1	8.9

Sensitivity analyses were conducted to ensure that the singlet cortisol assay results did not influence the results, as duplicate assays are recommended as a minimum. The inclusion of these samples did not influence the findings which could be drawn from the analyses. As such, these samples were retained for the reported analyses.

Where there was sufficient volume, further analysis of the saliva samples was conducted to evaluate levels of α-amylase (Table 36). After the samples had been assayed for cortisol, there was sufficient volume for analysis of 46.5% of the samples which had been collected. However, a human error occurred during the adding of a reagent during the analysis of one of the α-amylase assay plates which meant that the assay was unsuccessful. Dilutions were created where possible. However, there was insufficient volume to re-run the analyses for 19 samples. This issue disproportionately affected samples for the ID group. Due to low levels of successful assays (32.26%), resulting from a variety of issues, these findings were not included in further analyses.

Table 36

Details regarding saliva sample collection and α-amylase assays.

	Pre-demand (%)		5 minutes post-demand (%)		20 minutes post-demand (%)		Hour post-demand (%)		Total (%)	
	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (24)	LD(14)	FXS (96)	LD (56)
Duplicate or triplicate	30.4	35.7	33.3	42.8	33.3	35.7	20.8	28.5	31.3	33.9
Insufficient volume	60.9	42.9	54.2	35.7	62.5	28.6	54.2	50	56.3	39.3
Not collected	4.3	0	8.3	0	4.2	0	12.5	21.4	7.3	5.4
Not detectable/ assay error	4.3	21.4	0	21.4	0	35.7	12.5	7.1	4.2	21.4

Data Analysis. Analyses were conducted to investigate whether there was any change in cortisol across the samples collected in the assessment and whether this differed between groups. For all observational variables, in order to account for varying session length, percentages of the sessions (second accuracy) in which behaviours occurred were calculated (duration variables), adjusting for the proportion of time the participant's face (gaze-related variables. Mean number of seconds per session participant's face off screen= 21.59, SD= 70.33), or entire body (all other participant-related measures. Mean number of seconds participant off screen per session=10.07, SD= 38.67) were not visible in the recording. For event variables, the rate of occurrence per visit was calculated. Group-level differences were investigated in the frequency of use of the escape response, both across the Escape Assessment in totality, as well as according to condition. Furthermore, exploratory analyses were conducted to investigate the effect of group and condition upon other participant behaviours. Finally, within- and between-group exploratory analyses (correlational, comparison of means and visual inspection) to determine whether there is a relationship between the salivary measures and behavioural measures.

Results

The results are presented in order to correspond with the research aims, as detailed in the introduction.

Do individuals with FXS show a taught response which allows them to escape from, or avoid, the presentation of tasks more frequently than people with IDs that do not have FXS?

The taught escape response was utilised in at least one session of the escape assessment by 52.2% (13) of FXS participants, compared to 7.7% (1) of the ID comparison group: the FXS group were significantly more likely to use the response, with a medium to large effect size ($X^2(1)=7.74$, $p<.005$, Cramer's V=.46). There was a low rate of use of the response. Of those who did use the escape response, this was used an average of .18 times per minute by the FXS group (0-1.24, SD= .26), the one participant in the ID group who used the taught escape response did so an average of .13 times per minute across the three sessions of the escape assessment (0-.3, SD=.15). The topography of the escape responses used were card exchange (7 participants) and placing a block in a bowl (6 participants).

Participant characteristics. Exploratory analyses were conducted to investigate whether there were any differences between those participants who utilised the escape response, compared to those without. Due to low levels of responding in the ID group, this investigation was only conducted for those with FXS (Table 37). There was no significant difference in levels of autistic behaviour (those above the SCQ autism cut-off, compared to those below: t(19)=.27, p=.79, d=.11). There was a small to medium effect size for adaptive behaviour, with those who used the response having lower levels of adaptive behaviour, however this difference did not reach statistical significance (t(20)=-1.22, p=.24, d=.50). There was no significant difference in the duration of CB exhibited by participants with FXS who exhibited the escape response (mean= 5.10%, SD=7.18%) and those who did not (Mean= 6.00%, SD=7.26%: t(22)=-.30, p=.77, d=.11)

Table 37

Participant characteristics and escape responding

Measure	Used Escape Response (13)			Did not use response (11)		
	Mean	SD	Range	Mean	SD	Range
SCQ score	21.25	7.83	4-30	20.33	7.30	9-29
Adaptive Behaviour Composite	58.38	27.33	19-109	71.89	22.32	40-113

Are there any group differences in other observed behaviours between groups during the presentation of demands?[51]

Gaze-related variables. The durations (mean percentage of session across Escape Assessment) of observed gaze-related variables are reported for each group in Table 38. There was no statistically significant difference in the proportion of the session participants made eye contact with the researcher ($t(35)=-.66$, $p=.52$, $d=-.21$) or the duration which they closed or covered their eyes ($t(34)=.59$, $p=.56$, $d=.20$). However, the participants with FXS turned away from the experimenter significantly more than the participants in the ID group, with a medium effect size (Levene's test indicated unequal variances ($F=6.95$, $p=.01$): $t(31.81)=2.31$, $p<.05$, $d=.67$).

[51] Figures presented relate to conditions of escape assessment. Descriptive statistics about behaviour during Arousal Assessment available in Appendix M.

Table 38

Mean percentage duration of gaze-related behaviours across Escape Assessment[52].

Behaviour	FXS (24 participants)			LD (13 participants)		
	Mean	SD	Range	Mean	SD	Range
Eye contact	15.89	14.60	1.17-55.51	19.40	17.18	.05-59.76
Turn away	5.93	7.57	0-28.69	1.96	2.72	0-7.42
Eyes covered or closed	6.12	7.94	0-24.86	4.45	8.27	0-28.84

Across both groups, levels of gaze avoidant behaviours (turning away and covering eyes) across the Escape Assessment were significantly positively associated ($r_p(36)=.49$, $p<.005$). As such these variables were combined into an overall gaze avoidance variable (FXS: mean=10.05, SD= 11.78; LD: mean= 5.81, SD= 8.51). On average across the sessions, there was no significant difference between the levels of gaze avoidance between groups ($t(35)=1.14$, $p=.26$, $d=.39$).

Challenging behaviour. During the Escape Assessment, 80% (20) of the FXS group displayed CB in at least one of the sessions, compared to 38.5% (5) of the ID group: the difference reached statistical significance, with a medium to large effect size ($\chi^2(1)=7.75$, $p<.01$, Cramer's V=.46)

[52] Durations are calculated as percentage of session during which the participant's face is visible on screen.

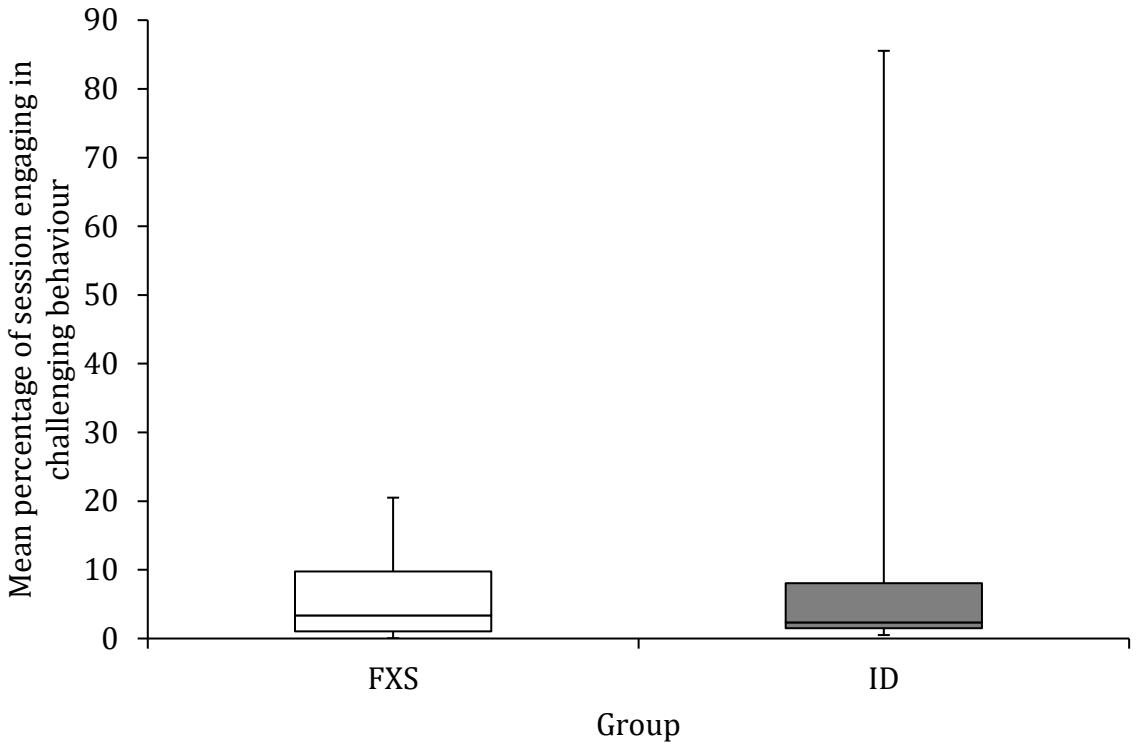

Figure 30. Occurrence of challenging behaviour across Escape Assessment.

For participants who engaged in CBs, there was no significant difference in mean duration of occurrence of CBs across sessions of the Escape Assessment, between groups, though there was a large effect size[53] (Figure 30: Mann Whitney U=35.0, p=.87, η^2=.40), though comparisons were limited by small sample size in the ID group with a high outlier. Details regarding the occurrence of different topographies of behaviour are provided in Table 39.

[53] Effect sizes are interpreted using Cohen's (1998) guidelines as summarised by Watson (2017).

Table 39

Occurrence of challenging behaviour in any session of escape assessment.

Behaviour	FXS (24)			LD (13)		
	Percentage participants showing behaviour (N)	Topography (N)	Mean percentage occurrence* (SD)	Percentage showing behaviour (N)	Topography (N)	Mean percentage occurrence (SD)
SIB (not bite)	0	N/A	N/A	7.7% (1)	Head hit (1)	2.86
SIB (self-bite, chew)	58.3% (14)	N/A	2.40 (5.08)	15.4% (2)	N/A	1.52 (1.16)
Physical aggression	8.3% (2)	Throw objects (1) grab (1)	0.27 (0.01)	15.4% (2)	Grab (2), push (1)	0.6 (0.30)
Verbal aggression	16.7% (4)	N/A	2.44 (4.32)	23.1% (3)	N/A	3.29 (4.55)

Behaviour	FXS (24)			LD (13)		
	Percentage participants showing behaviour (N)	Topography (N)	Mean percentage occurrence* (SD)	Percentage showing behaviour (N)	Topography (N)	Mean percentage occurrence (SD)
Destruction (not bite, chew)	29.1% (7)	Bang object (3) Graffiti desk (1) Break computer mouse (1) Throw or swipe object (4) Slam door (1) Kick object/ surface (1) Screw up work (2)	2.99 (2.21)	30.8% (4)	Throw object (2) Bang object (2) Rip (toys and work materials: 2)	4.84 (8.15)

Behaviour	FXS (24)			LD (13)		
	Percentage participants showing behaviour (N)	Topography (N)	Mean percentage occurrence* (SD)	Percentage showing behaviour (N)	Topography (N)	Mean percentage occurrence (SD)
Bite or chew object	58.3% (14)	N/A	5.30 (7.10)	38.5% (5)	N/A	20.7 (36.5)

* for participants who showed behaviours. Mean occurrence across Escape Assessment sessions.

There were no significant differences between the proportion of the groups which engaged in SIB (X(1)=.017, p=.90, Cramer's V=.02), self-biting (though there was a medium effect size: X(1)=2.55, p=.11, Cramer's V=.26), physical aggression (X(1)=1.33, p=.25, Cramer's V=.19), verbal aggression (X(1)=.13, p=.72, Cramer's V=.06), destruction (X(1)=.09, p=.76, Cramer's V=.05) or either chewing or biting objects (X(1)=.85, p=.36, Cramer's V=.15).

Comparison with reported challenging behaviour. A higher proportion of the participants who were reported to show SIB in the FXS group went on to display SIB, compared to those with ID who were reported to engage in this behaviour (Table 40). For both groups a low proportion of participants who were reported to engage in physical aggression showed these behaviours (FXS: 14.3%, ID: 33.3%). In addition, only 65% of participants reported to engage in destructive behaviour did so during the research. In addition, there were a number of participants who engaged in either SIB or destructive behaviour, that were not reported to do so by parents.

Table 40

Comparison of reported and observed challenging behaviour.

Behaviour observed during assessment	Behaviour Reported		Behaviour Not Reported	
	FXS	ID	FXS	ID
SIB (biting and non-biting)	71.4% (10/14)	33.3% (1/3)	42.9% (3/7)	22.2% (2/9)
Physical Aggression	14.3% (2/14)	33.3% (2/6)	0% (0/7)	0% (0/6)

Destruction (biting and non-biting)	69.2% (9/13)	60% (6/10)	62.5% (5/8)	33.3% (1/3)

Arousal-related indicators. The FXS group yawned significantly more frequently than the ID group, with a medium effect size (U=80.00, p<.01, r=-.43), which may be an indicator of lower levels of arousal. Increased fidgeting may be an indicator of elevated arousal; there was no difference in the duration of fidgeting observed, between groups (U=147, p=.77, r=-.05). Descriptive statistics are presented in Table 41.

Table 41

Occurrence of arousal-related indicators, across all sessions

	Yawn (per minute: median, IQR)	Fidget (percentage of session: median, IQR)
FXS (24 participants)	.07 (0-.28)	1.49 (.15-5.66)
LD (13 participants)	0 (0-.02)	1.39 (.45-6.60)

Task engagement and other target behaviours. There were no significant differences in proportion of sessions not engaged in task, or engaging in other target behaviours (Table 42).

Table 42

Occurrence of off-task behaviours during Escape Assessment

Behaviour	Mean percentage of sessions						Mann-Whitney U		
	FXS (24 participants)			LD (13 participants)					
	Median	IQR	Range	Median	IQR	Range	U	p	η^2
Not Engaged	7.07	1.85-39.42	0-91.22	.94	.31-41.24	0-67.77	108.5	.13	.06
Cry*	0	0-0	0-15.11	0	0-0	0-.3	152.5	.85	0
Laugh	.08	0-.99	0-5.89	.22	.06-.98	0-2.62	134	.47	.01
Off-task speech	.70	.97-4.41	0-11.03	.67	0-1.39	0-2.33	123.5	.30	.03
Refuse	.70	.36-4.11	0-13.13	.28	0-1.82	0-37.25	120	.25	.04
Out chair	.11	0-10.53	0-54.98	0	0-.59	0-29.44	126	.29	.03
Interacting with tangible	.08	0-2.5	0-33.16	.27	0-1.71	0-94.66	152	.89	0

Session Termination and Sessions Not Run.

Session termination. Sessions were terminated early for a variety of reasons. Non-participant-related reasons (such as camera errors or events such as other pupils entering the room) occurred at similar frequency across the two groups (for full details, see Appendix N). However, a number of sessions were terminated either directly due to participants' behaviour (e.g. the participant leaving the room), or due to the researcher ending the session as a result of participant distress or behaviour, in line with agreed session termination criteria. Participant-related reasons for terminating early were more frequent for the participants with FXS (11.5% total sessions, compared to 3.6% ID group). Nine participants in the FXS group terminated sessions early (Eight participants ended one session of the assessment early, one participant ended all three sessions early), avoiding between 21 and 330 seconds of the session (Mean= 189.1, SD= 118.15). In comparison, two participants in the ID group terminated one session early (150 and 279 seconds early).

Sessions not run. In addition, some sessions were not run, both for participant-related and non-participant-related reasons. In the FXS group, 3.1% of planned sessions (3 sessions) were not run, two for participant-related reasons and one for non-participant-related reasons. In contrast, a higher proportion (14.3%) of planned sessions were not run for the ID group, primarily due to school-related reasons. However, there was no statistically significant difference between the proportion of participants in each group for whom a session was not completed or was terminated early as a result of the child's behaviour ($X(1)=.42$, $p=.52$, Cramer's $V=.11$). Reasons for session termination are detailed in Appendix N.

Total off-task behaviour

The mean percentage of sessions of the escape assessment in which participants engaged in any off-task behaviours (including: CB, gaze avoidant behaviour, time avoided due to requested breaks and other off-task behaviours listed above) was calculated. There was no significant difference in the proportion of sessions in which participants in the FXS group (Mean=32.37%, SD= 24%) or ID group (mean= 22.25%, SD=31.49%) engaged in off-task behaviours ($t(27)=1.01$, $p=.32$, $d=.33$).

Summary. In sum, between-group differences in a number of different variables were explored, across the escape assessment. In response to the presentation of demands, compared to the ID group, the children with FXS exhibited higher rates of certain topographies of gaze avoidance (turning away) and a greater proportion of the group engaged in CB. In addition, there was a tendency towards a greater number of sessions being terminated or not run for participant-related reasons (such as behaviour or distress) in the FXS group. No other group differences in off-task behaviours were observed. Interestingly, the participants with FXS yawned significantly more frequently than their ID counterparts, which may be an indicator of reduced arousal, contrary to previous hypotheses.

Are there differences in responding under conditions varied according to social and information processing demand?

Escape Response. For all participants who utilised the Escape Response (N=14: FXS=13, LD=1), there was no significant difference between levels of use of the escape response across different conditions ($F(2, 20)=1.03$, $p=.38$, partial $\eta^2=.09$). As such, further post-hoc group-level comparisons were not conducted to investigate the effect of the task difficulty or social manipulations. Individual variation in responding for

individuals in the FXS group is graphed in Figure 31. It may be expected that the 'Hard Task, High Social' represented the greatest demand (due to combining both high social demand and requiring higher information-processing) and therefore establish the greatest motivation to escape. However, only one participant (FX019) exhibited the highest rate of responding in this condition (of note, the 'hard task, low social' condition was not completed for this participant, due to distress, which limits comparisons).

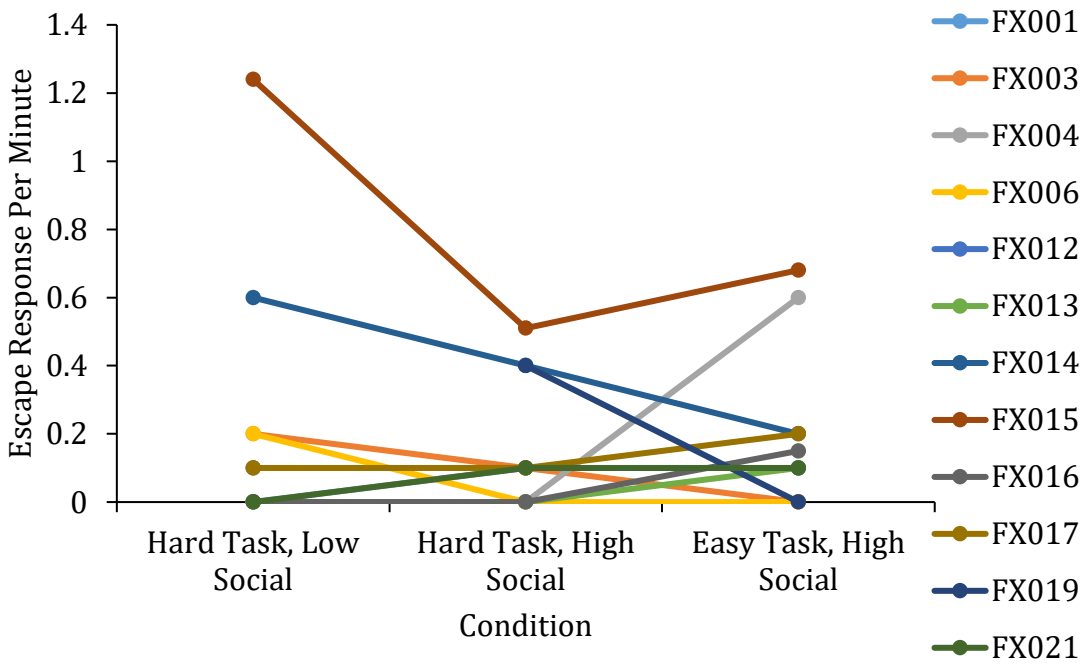

Figure 31. Individual occurrence of escape response across conditions

In addition, the effect of session order upon frequency of responding (rate per minute) was explored (Figure 32), as with reinforcement through provision of a break, one may expect the frequency of responding to increase over time. However, there was no significant difference in the level of responding through the assessment ($F(2,20)=.22$ $p=.80$, partial $\eta^2=.02$).

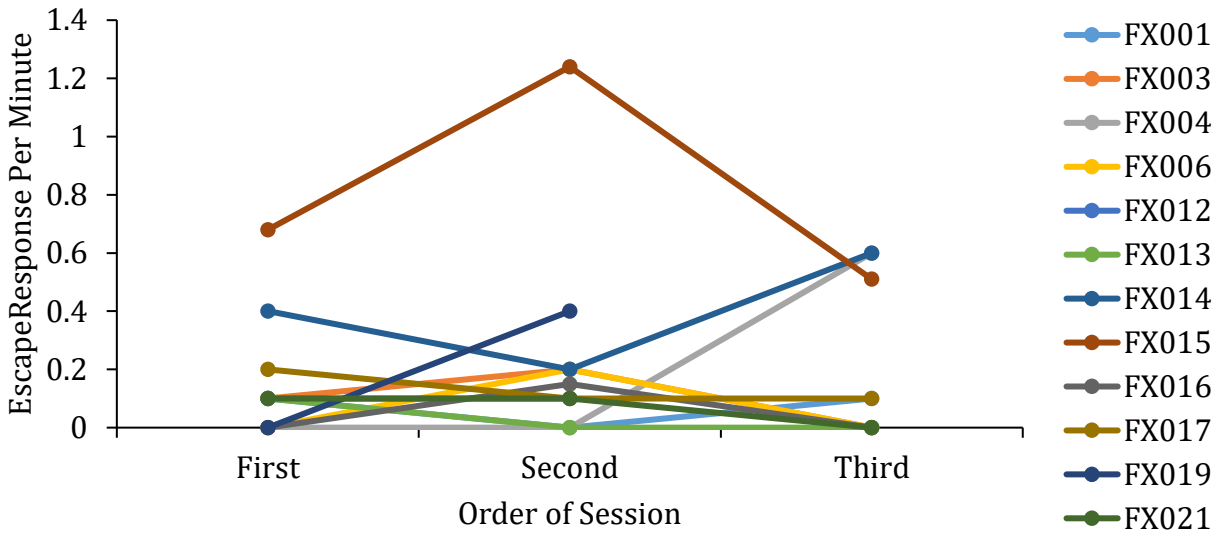

Figure 32. Individual occurrence of escape response across sessions.

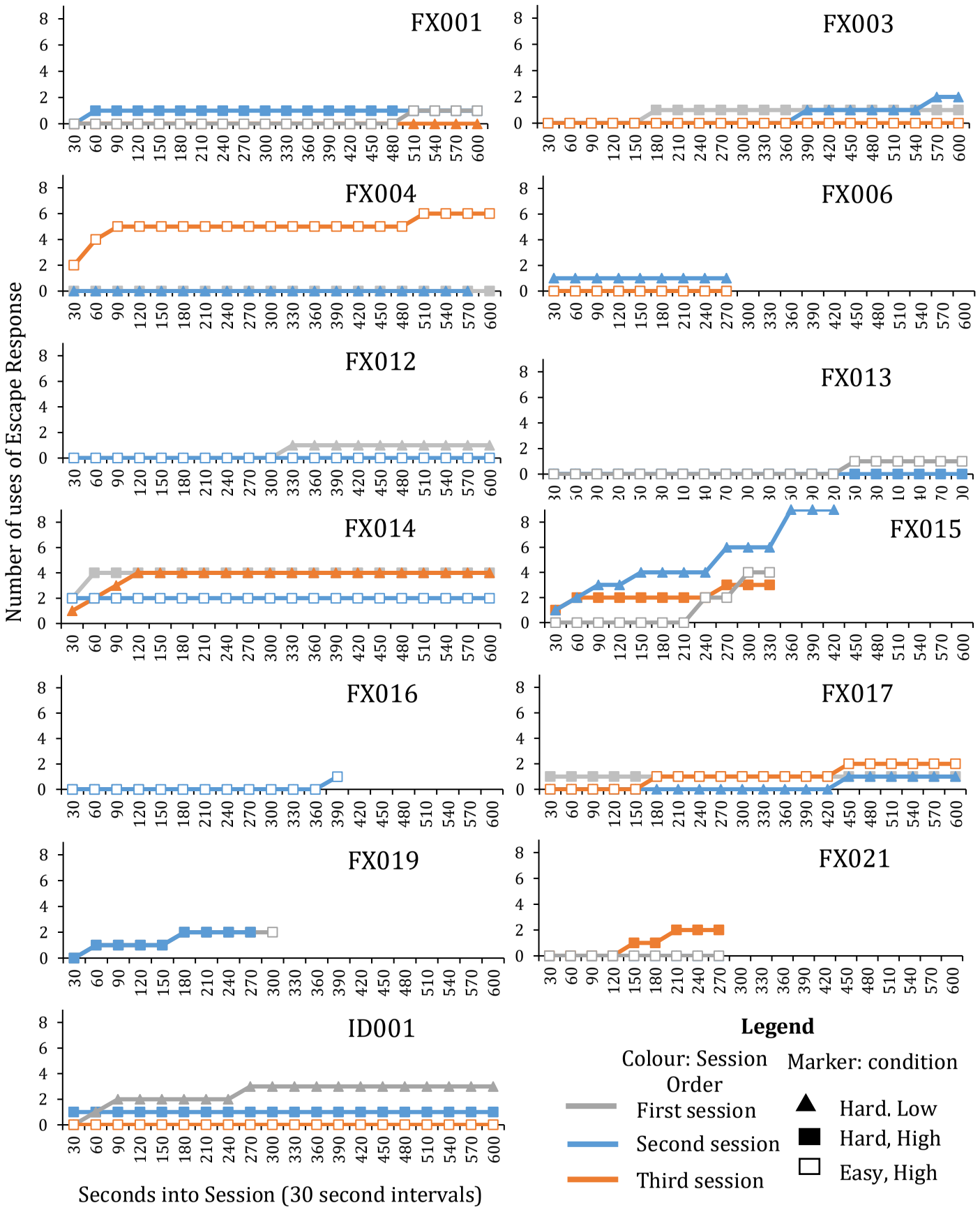

Figure 33. Cumulative frequency graphs of escape responding across sessions

Furthermore, exploratory analyses were conducted to investigate the timings during the sessions when the participants engaged in the target behaviour (Figure 33). Notably, much of the responding occurred at the beginning of sessions: 41% of responses across the group occurred within the first minute. Participant FX015, exhibited the response most frequently of all the participants and did so on multiple occasions throughout all sessions. In addition, for a number of participants responding only occurred under certain conditions. For instance, participant FX003 exhibited the behaviour only under hard task conditions. These variations in responding suggest that different aspects of demands may act as an EO for escape, for different individuals.

Gaze-related behaviour. There was no significant difference in duration of gaze avoidance across conditions (Figure 34) for the ID group ($F(2,16)=.14$, $p=.87$, partial $\eta^2=.,02$), FXS group ($F(2,36)=.26$, $p=.78$, partial $\eta^2=.01$).

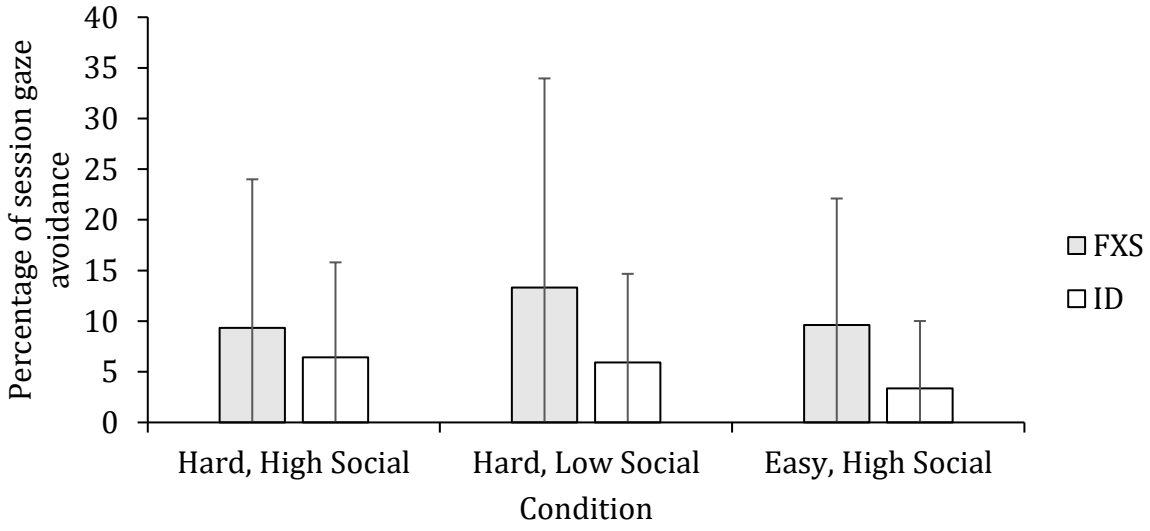

Figure 34. Proportion of session gaze avoidance behaviours demonstrated.

The difference in levels of eye contact across conditions significantly differed for both the FXS group ($F(2,40)=6.6$, $p<.05$, partial $\eta^2=.25$) and the ID group ($F(2,16)=12.71$, $p<.01$, partial $\eta^2=.61$) For both groups, levels of eye contact were greater in the high social conditions (Figure 35).

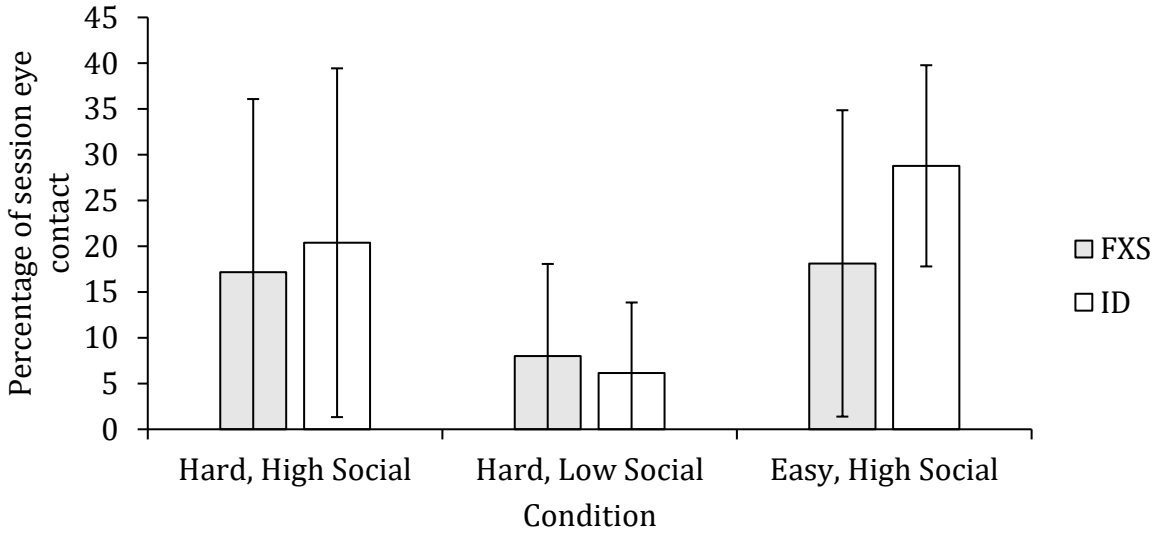

Figure 35. Proportion of session eye contact made with experimenter.

Challenging behaviour. Due to positive skew of the data, Friedman tests were conducted to examine the effect of condition on levels of CB, across the Escape Assessment. There were no significant group-level differences in levels of CB between the different conditions, for either the FXS group ($\chi^2(2)=1.05$, $p=.59$[54]. Condition Medians: Easy, High= 2.0%, Hard High= 1.67%, Hard Low= 1.0%) or the ID group ($\chi^2(2)=.4$, $p=.82$. Condition Medians: Easy, High= 0%, Hard High= 1.17%, Hard Low= 1.67%). Individual response patterns are graphed in Figure 36 and Figure 37.

[54] Effect sizes are calculated for Friedman tests through post-hoc analyses. Due to the non-significant results, post-hoc tests were not conducted, as such effect sizes are not reported. However, condition medians are presented in order to facilitate interpretation.

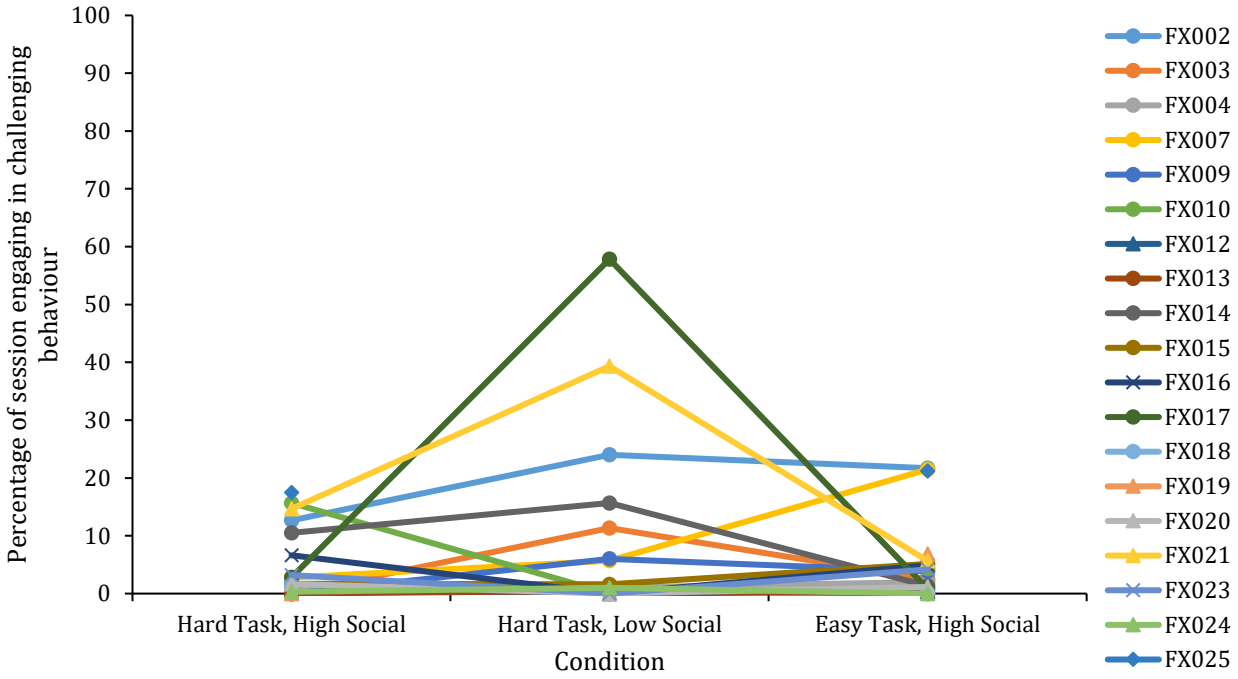

Figure 36. Occurrence of challenging behaviour across conditions (FXS group)

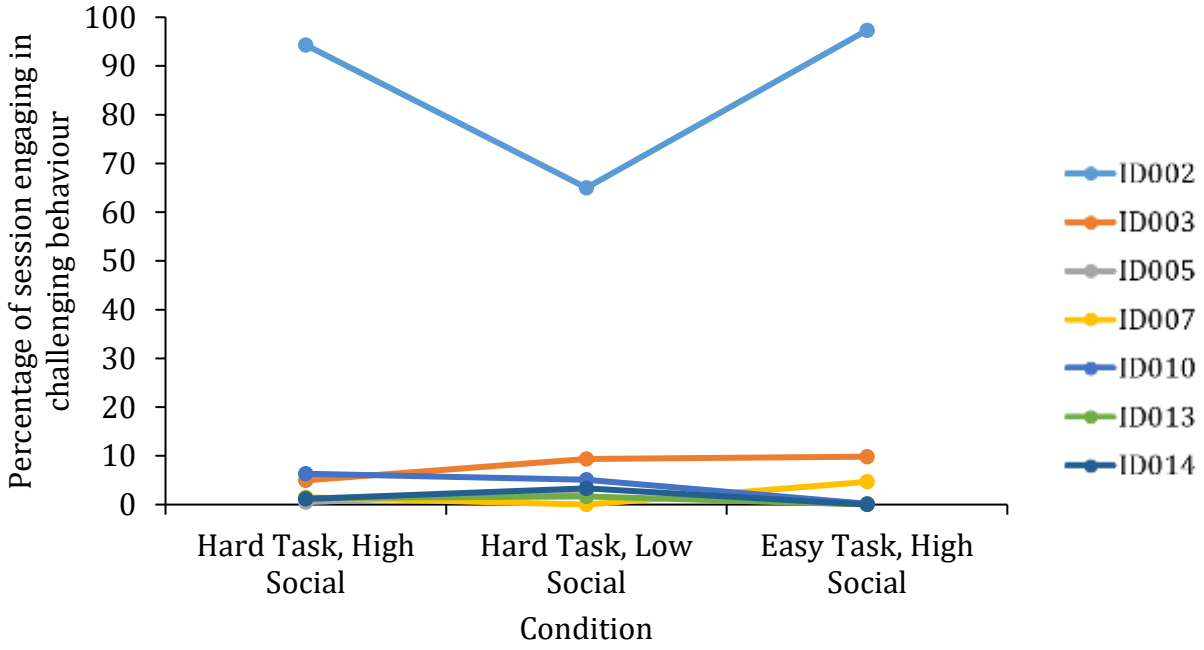

Figure 37. Occurrence of challenging behaviour across conditions (LD group)

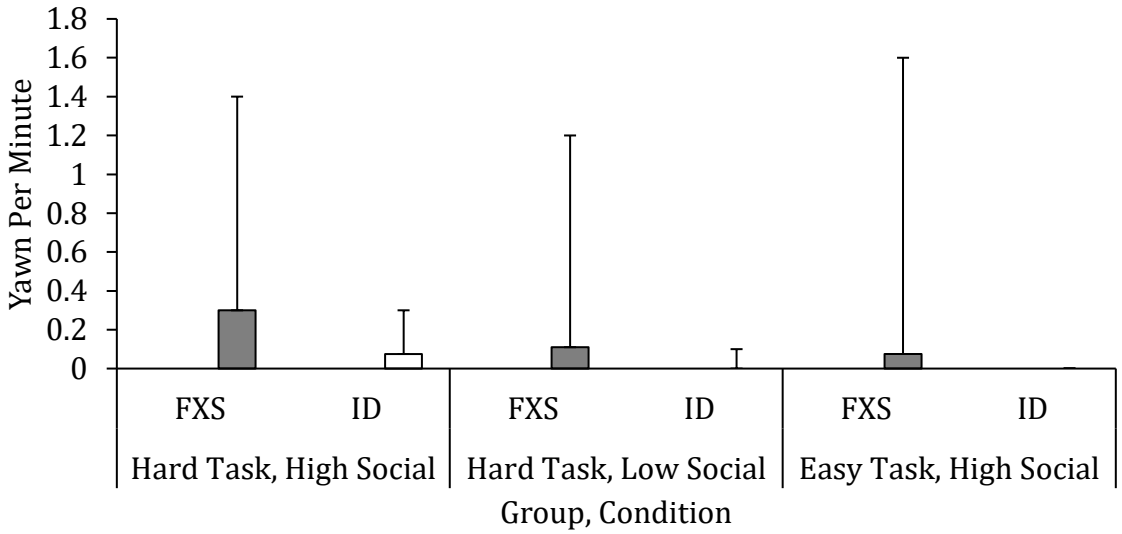

Figure 38. Occurrence of yawning across conditions.

Arousal-related indicators. Due to positive skew of the data, Freidman tests were conducted. There was no significant effect of condition upon frequency of yawning in the FXS group ($\chi^2(2)=3.44$, p=.18). There approached a difference in the ID group, though there was low occurrence of this behaviour and so it is unclear whether this represents a genuine effect ($\chi^2(2)=5.6$, p=.06: Figure 38). There was no difference in the duration of fidgeting across conditions (FXS: $\chi^2(2)=4.1$ p=.13; ID: $\chi^2(2)=.96$, p=.62: Figure 39).

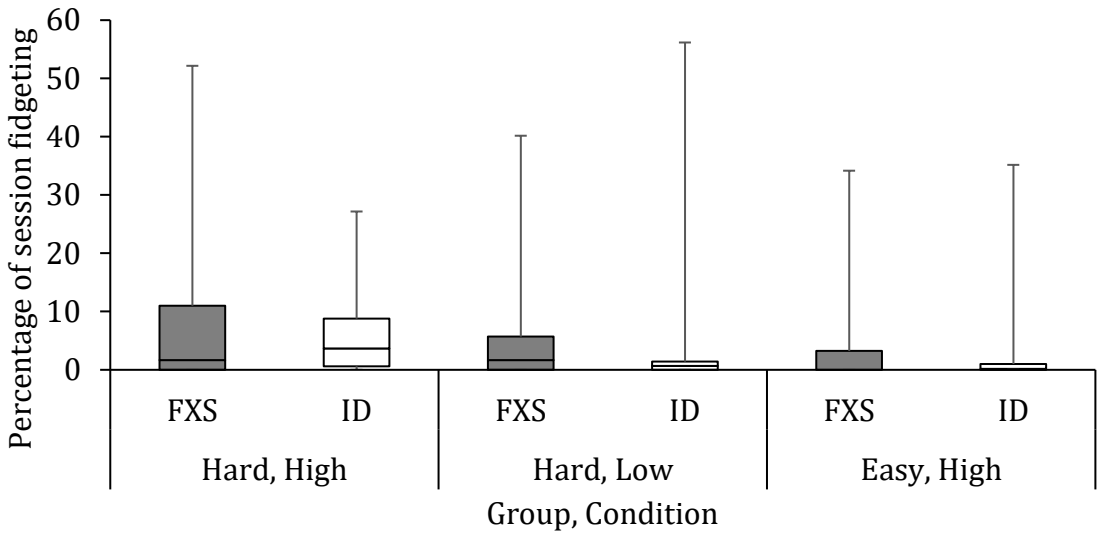

Figure 39. Occurrence of fidgeting across conditions.

Total off-task behaviour. There was no difference in total durations of all off-task behaviours assessed across conditions for the FXS group ($F(2,36)=.38$, $p=.69$, partial $\eta^2=.05$) or the ID group ($F(2,16)=.38$, $p=.69$, partial $\eta^2=..05$).

Summary: effect of condition. There were no significant effects of condition upon off-task behaviours in either group, with the exception of greater eye contact being made in the high social conditions.

Do boys with FXS have different physiological responses to classroom work challenges, compared to other children with IDs?

Cortisol. In order to increase complete sample size, data were truncated to 3 time points (conducted in GraphPad prism[55]). There were no significant main effects of sampling time ($F(1,16)=2.05$, $p=.17$) or group ($F(1,16)=.40$, $p=.54$), or interaction effect

[55] Analyses conducted with support from senior lecturer in Pharmacology, Medway School of Pharmacy. Analyses truncated to two points similarly had non-significant main effects and interactions.

(F(1,16)=.27, p=.61). Sensitivity analyse supported these findings)[56] [57] [58]. The mean levels of cortisol (µg/dL) for each group, across the samples, are displayed in Figure 40 (Standard deviations represented in error bars).

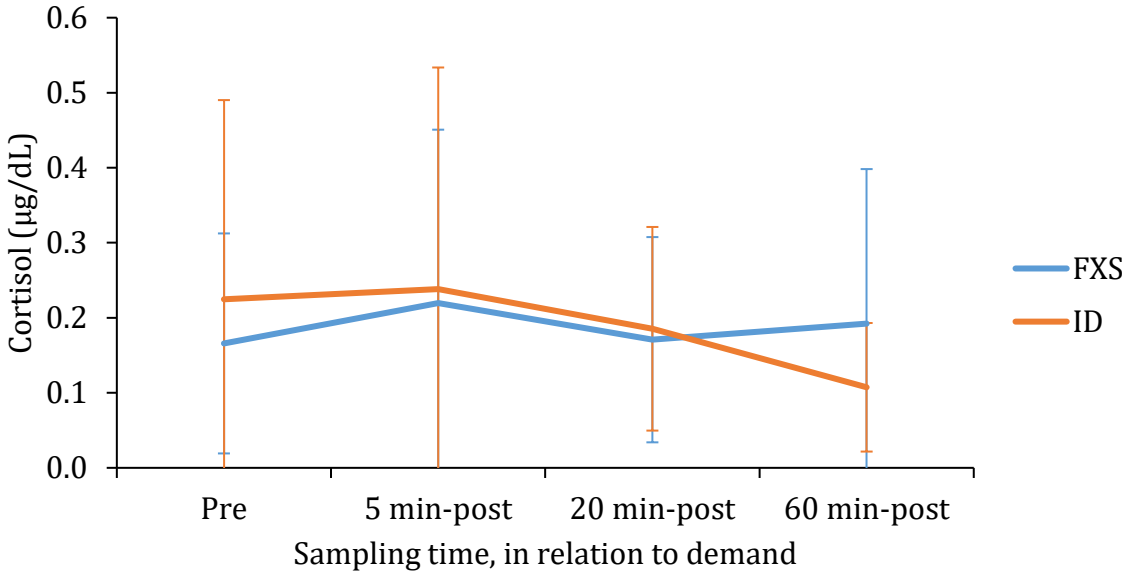

Figure 40. Group-level analysis of cortisol levels across the sampling time period.

Mean levels. Participant's mean levels of cortisol across the samples were .20µg/dL in the FXS group (SD=.14µg/dL, range=.04-.50 µg/dL) and .20 µg/dL in the ID group (SD=.17 µg/dL, range=.02-.64µg/dL). There was no statistically significant difference in mean cortisol levels, between groups (t(33)=.165, p=.87, d=0).

[56] This finding was robust when missing data were replaced with participant mean cortisol levels (excluding participant for whom no data were available: N=3 FXS group). No significant main effect of time (F(3)=1.10, p=.35) or group (F(1)=.03, p=.87) , as well as no significant interaction (F(3)=.28, p=.84).

[57] A sensitivity analysis was conducted in order to investigate the removal of the two participants taking medications which may have affected cortisol levels (Two samples from one participant were excluded). The results remained non-significant.

[58] Fixed effects in a linear mixed model were explored as a sensitivity analysis (Seltman, 2015). There were similarly no significant effects or interaction for fixed effects: time (F(3,80)=.525, p=.666), group (F(1,80)=.002, p=.996), interaction variables (F(3,80)=.411,p=.746).

Reactivity. Cortisol reactivity at 20 minutes post-demand (when a physiological reaction to the demand as a stressor would have been observable), compared to baseline, was calculable (20 minutes post-demand sample level minus pre-demand level) for 10 participants in the FXS group and 5 in the ID group. The mean reactivity in the FXS group was .01µg/dL (SD=.09µg/dL, range= -.20-.14µg/dL) compared to -.07µg/dL in the ID group (SD=.39µg/dL, range= -.69-.38µg/dL).

Individual-level analysis. In light of the lack of statistically significant group findings, individual level results were explored in order to investigate whether there were any trends or within-group differences to inform future investigations. Individual cortisol findings are graphed in Figure 41 and Figure 42. Notably, only one participant (FX005) demonstrated the expected response and recovery pattern: elevations from pre-demand sample at 20 minutes post-demand, only, then return to pre-demand level after 1 hour.

In addition, a number of participants in both groups showed extremely low cortisol levels (<.05µg/dL) and a flattened profile, throughout the assessment (FXS group: FX004 (2 samples); FX009; FX020; FX019; FX011. ID group= LD007 (3 samples) and LD004 (2 samples)). However, visual analysis was conducted in order to investigate any common characteristics amongst these participants (adaptive behaviour, autistic behaviour, reported and observed CB, as well as escape responding) but none could be identified.

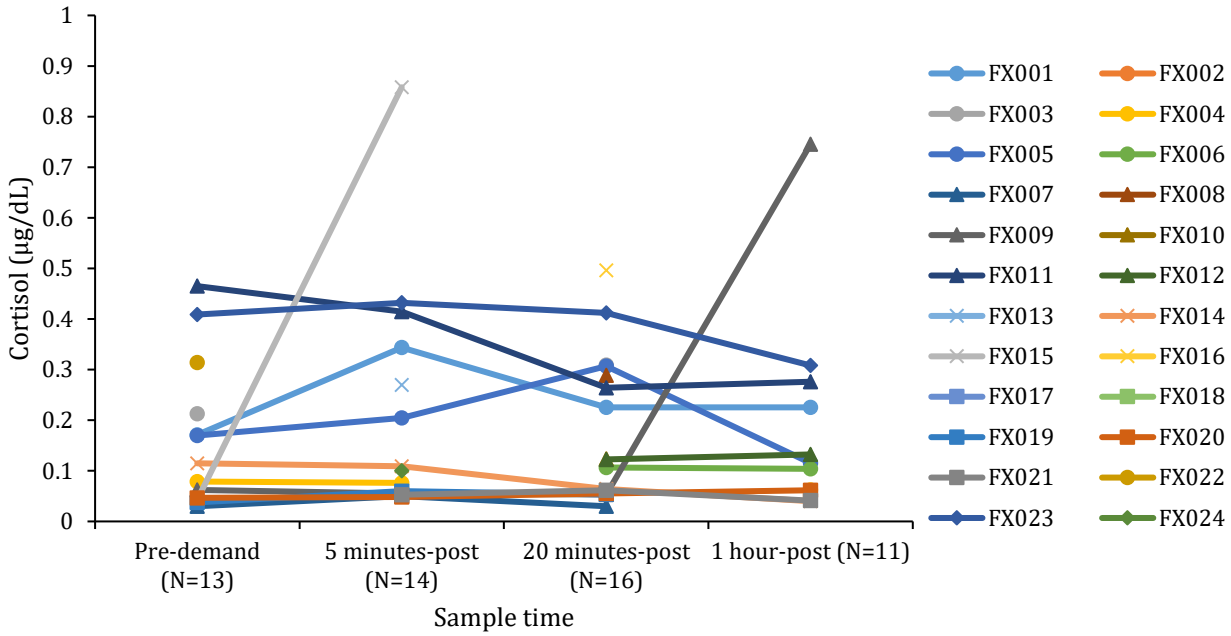

Figure 41. FXS group individual cortisol response patterns.

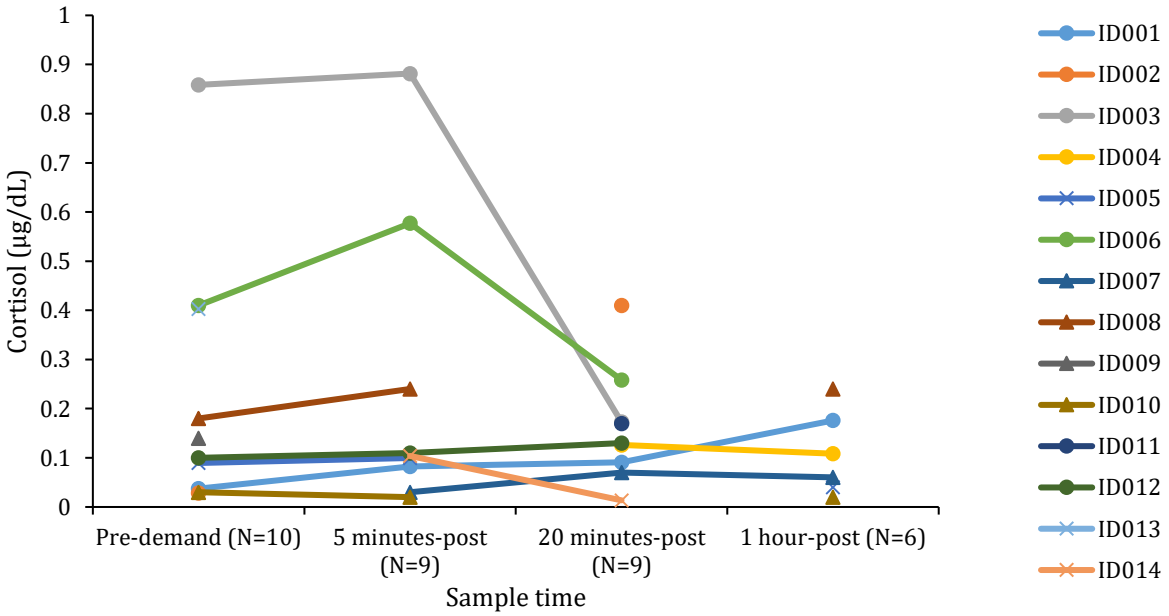

Figure 42. ID group individual cortisol response patterns.

Is there an association between arousal and behaviour during the assessment?

Escape Response. Within the FXS group, there was no significant association between the mean cortisol levels of those participants who later demonstrated the

escape response (N=12. Mean=.22μg/dL, SD=.16μg/dL) and those who did not (N=9. Mean=.19 μg/dL, SD=.12 μg/dL; t(19)=.438, p=.67, d=.20). Statistical comparisons were not conducted for the ID group given that only one participant demonstrated the response[59]. Statistical analyses were not conducted for cortisol reactivity (change pre- to 20 minutes post-demand) due to small sample size. However, visual analyses suggested no association: within the FXS group, those who exhibited the escape response (N=5) had mean cortisol reactivity of .02μg/dL (-.05-.10μg/dL, SD=.06μg/dL), compared to -.01μg/dL (-.20-.14μg/dL, SD=.12 μg/dL) for those who did not (N=5).

Challenging behaviour. Across both groups, there was no association between mean percentage of sessions participants engaged in challenging behaviour, and either mean cortisol levels ($r_s(33)=-.13$, p=.47) or cortisol reactivity ($r_s(15)=-.30$, p=.28).

Autistic behaviour. Given earlier findings relating to autistic behaviour and cortisol levels and reactivity (Matherley et al., 2018; Roberts et al., 2009) the relationship between these variables were explored. Across the groups, there was no association between autistic behaviour (SCQ scores) and mean cortisol levels ($r_p(31)=.20$, p=.28), though, within the small group of individuals for whom data were available, there was a trend towards individuals with decreased reactivity exhibiting increased autistic behaviour ($r_p(12)=.56$, p=.06: Figure 43).

[59] Mean cortisol levels for this participant (LD001) was .09μg/dL, compared to the group mean of .20μg/dL.

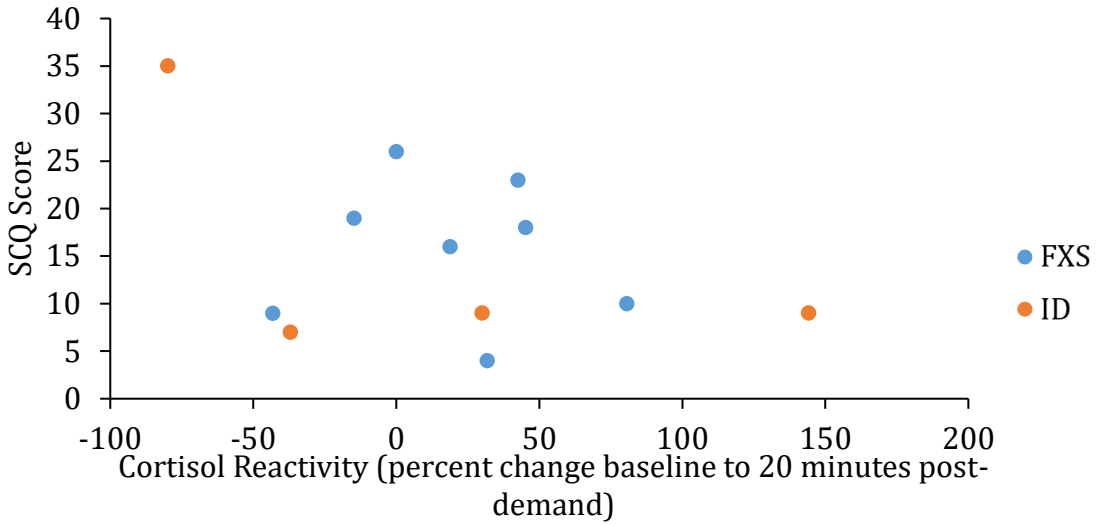

Figure 43. Autistic behaviour and cortisol reactivity.

Discussion

Do individuals with FXS show a taught response which allows them to escape from, or avoid, the presentation of tasks more frequently than people with IDs that do not have FXS? One of the primary aims of this project was to evaluate sensitivity to negative reinforcement, through the provision of a break from a task in response to a target, arbitrary response, and to investigate whether this may differ between children with FXS and children with ID. Based upon earlier investigations of the function of CB, it was hypothesised that those with FXS may exhibit an enduringly raised motivation for escape as a reinforcer and therefore exhibit the taught behaviour more frequently. In the present study, a significantly greater proportion of the FXS group utilised the taught response, with just one participant in the ID group exhibiting the target behaviour. This result could not be accounted for by greater ability in the FXS group, as there was no difference between the two groups in levels of adaptive behaviour. This finding supports the hypothesis that individuals with FXS exhibit a heightened sensitivity to demand escape as a reinforcer, relative to others with ID (Langthorne et al., 2011).

Within the FXS group, there were no participant variables which were associated with engaging in the escape response: adaptive behaviour, autistic behaviour or engagement in CBs. As such, it is unclear which factors may have contributed to some participants utilising this response, whereas others did not. Given the lack of correspondence between engagement in the escape response and CBs, the internal validity of these findings is unclear. Interestingly, however, the participant who exhibited the highest level of responding in the assessment was reported to engage in high levels of severe CB (which in Study 1 had been identified to be likely at least in part maintained by escape from demands). During the present assessment this participant engaged in destructive behaviour but also high levels of off-task behaviour including leaving the room and refusals.

This extends the findings of previous literature which has focussed upon the operant functions of CB, and shown a within-group bias to escape as a behavioural function (Reviewed in Chapter 2). Limited between-group comparisons have been previously conducted. Langthorne and colleagues (Hardiman, Langthorne & McGill, in press; Langthorne et al., 2011; Langthorne & McGill, 2012) showed, through parent report and direct functional analysis, reduced levels of attention-maintained CB in FXS, compared to those with Smith-Magenis Syndrome or non-specific ID (the latter through parent report, only). However in these studies, no between-group differences were observed in relation to escape-maintained behaviours. Therefore, this study is the first to identify that children with FXS may be more likely to engage in escape-maintained behaviour in response to an academic demand (though of a different topography), when compared to others with LD. This builds upon the research suggesting a potential within-group bias towards escape-maintained behaviour. By examining sensitivity to reinforcement through responding to an arbitrary target behaviour, this study controls

for the variability in individual reinforcement histories, which influence whether a participant may engage in CBs. Exhibiting this sensitivity to the acquisition of a new behaviour may show how the inadvertent reinforcement of an instance of CB may lead to future repetition and development of behavioural problems.

However, although a high proportion of the FXS group exhibited the response, engagement in the behaviour occurred at low frequency. Interestingly, across the sessions of the assessment there was no evidence, either at the group level or from examination of individual data paths, of increasing occurrence of the behaviour over time. Such a pattern of behaviour would have suggested strengthening reinforcement of the behaviour across the assessment, through contingent provision of task breaks. Similarly, a disproportionate number (41%) of the responses of the target behaviour occurred within the first minute of the session. In addition, one participant (FX012) exhibited the behaviour on a single occasion in the first session, then in none of the subsequent sessions.

There are a number of possible explanations for the lack of maintenance of the behaviour, within the duration of the session and across the assessment. Perhaps most likely, given that the escape response was recently taught over a short period of time, it is possible that the behaviour was initially exhibited resulting from a recency effect (the contingency was explained at the beginning of sessions). However, given that the response was not well established (given the recency and brevity of the teaching) it may have been that as the demand progressed response competition resulted in reversion to engagement in established responses (such as CBs) which have historically been reinforced with escape more often. An additional, alternative explanation for the higher occurrence of the taught behaviour within the FXS group relates to the reported

tendency towards mimicry and 'desire to please' (Fragile X Society, 2012; Mirrett, Roberts & Priceet al.2003). It may have been that some of the individuals felt that the response was a necessary part of the task, but did not maintain responding due to lack of meaningful reinforcement (i.e. the brief task break was not sufficiently reinforcing that the response was maintained). A further interpretation of the pattern of findings is that the novelty (either of the task or experimenter) is a key element of the aversiveness of the tasks. This corresponds to observations of a social 'warm up effect' seen in FXS: whereby avoidance is initially higher when interacting with an unfamiliar individual, but later reduces (Roberts et al., 2009). Viewing the results in this way, the low use of the behaviour later in the sessions and assessment may reflect a building of rapport or familiarisation with the task which diminished the motivation to request breaks. Further investigation would be required in order to investigate which of these factors may have contributed to the observed patterns of responding.

Are there any group differences in other observed behaviours between groups during the presentation of demands? The environmental conditions in the escape assessment (provision of academic demands) were sufficient to induce CBs (including: SIB, self-biting or chewing, physical or verbal aggression, destruction, biting or chewing of objects) for a substantial proportion (65.79%) of the participants in this study. The occurrence of behaviours in this situation suggests that these behaviours may have been negatively reinforced in the past (though they were not done so during this assessment) and may have an escape or avoidance function. However, the function of the behaviour cannot be concluded with certainty due to a lack of baseline comparison condition to evaluate the occurrence of behaviours in the absence of a demand. The proportion of participants who engaged in any topography of CB, during at least one session of the assessment, was significantly higher in the FXS group compared

to the ID group, though where behaviours did occur, they did so during a comparable duration of the sessions. The greater number of participants who engaged in CB is consistent with the hypothesis of the association between FXS and escape-maintained behaviour, and with the observation of increased levels of use of the escape response in this group.

There was varying correspondence between reported CB and that observed during the assessment. Whilst over half of those with FXS and ID who were reported to engage in destructive behaviour (including biting or chewing objects) did so, fewer (20% of those reported) engaged in aggression. Almost three quarters (71%) of those with FXS with reported SIB (including self-biting), compared to a third of the ID group. For those who did not exhibit reported topographies of CB, it suggests that these may be associated with environmental conditions other than those to which they were exposed in the present study. In addition, a number of participants in both groups engaged in SIB or aggression, which were topographies not previously reported in parent or carer interviews. This may represent altered perceptions between parents' definition of 'challenging' compared to the behaviours coded under each of the topographies, some of which were of lower severity, such as finger chewing. Of note, information collected on prior occurrence of CB was limited, and so the correspondence between observed behaviours and actual situations could not be extensively evaluated. Extensive questions were not added to the lengthy parent interview, in order to reduce participation burden, however in future research it would be valuable to collect more extensive information on this topic.

There were no other group differences observed between other off-task behaviours assessed, or in the mean percentage of the session in which the participants

were engaged with the study task. However, notably, across groups there were a wide range of avoidance behaviours observed including social strategies (such as trying to engage the researcher in an alternative activity through speaking off task or laughing), physical avoidance strategies (such as leaving the chair or the room), as well as idiosyncratic behaviours, such as falling asleep[60]. The lack of correspondence between the duration of occurrence of different topographies of behaviour across the group, suggests that for some participants they represent functional alternatives, whereas others engage in multiple topographies of behaviour. This highlights that, when considering avoidant behaviour in FXS, as well as in others with LD, a broad range of topographies must be considered as any topography of behaviour which results in task avoidance may act as a barrier for the individual to access learning.

Are there differences in responding under conditions varied according to social and information processing demand? The nature of the demand was varied according to task difficulty and social demand (based upon: Murphy et al., 2007; Langthorne, 2012) across a brief assessment consisting of single sessions of three conditions. There was no detectable effect of these manipulations upon measured behaviours during the tasks. The null findings in relation to gaze avoidant behaviours and the altered social conditions differ from the findings of Langthorne (2012), who observed increases in gaze avoidant behaviours under high eye-contact conditions. Though, higher levels of eye contact were observed in the high social conditions for both groups, demonstrating that the manipulation did have an effect on gaze-related behaviour. Murphy and colleagues (2007) did not observe any effect of a social manipulation (interacting face-to-face with an experimenter or with a computer) or

[60] This was reported as a common avoidance behaviour for participant FX006. It was unclear whether the participant was actually asleep or mimicking the behaviour

information-processing factors (task difficulty manipulation) during the presentation of a structured language task (despite overall higher gaze avoidance in the FXS group when compared to children with Down's syndrome or typically developing children). Therefore, the relative contributions of social and information-processing factors upon behaviour could not be established in this study. The findings do not provide empirical support for the current educational recommendation of reducing social demand during academic work through sitting beside, rather than opposite, children with FXS. However, the single repeat of the conditions of the experiment may have been insufficient to detect differences. Future investigations should do more extensive research to determine whether this strategy, or other environmental manipulations, may be effective at reducing maladaptive escape-maintained behaviours for this population.

Do boys with FXS have different physiological responses to classroom work challenges, compared to other children with IDs? One of the aims of the present project was to assess physiological responding to a challenging academic demand in individuals with FXS, compared to those with ID. The findings are discussed in terms of cortisol levels and reactivity, and contrasted with earlier findings.

The levels of cortisol observed in the present study were compared to expected ranges for children in this age group, in order to determine whether there were any relative differences compared to typically developing samples. Salimetrics (2016a) provide example morning ranges for typically developing children (ages 8-11 years; 285 subjects) and adolescents (ages 12-18 years; 403 subjects) as .08-.84μg/dL and .02-.88μg/dL, respectively. In comparison, the mean levels for both groups in the present study were .20μg/dL: within the normal range but at the lower end. Notably, however, a

number of participants in both groups exhibited extremely low values, which fell below the expected age group range (FXS range=.04-.50 μg/dL; ID range=.02-.64μg/dL). In addition, the correspondence between the present findings and those of previous research in males with FXS were explored. In order to facilitate comparison, absolute cortisol levels reported in related studies are reported in Table 43. The values reported are from the sample time which most closely corresponds to that of the present study, and are reported for male-only samples where possible. Reported mean levels varied from approximately .16-.27μg/dL. As such, despite the challenges with missing data in the present study, the validity of the findings are supported by its comparability with similar datasets.

Table 43

Absolute cortisol levels reported in human studies

Study	Sample time (approx.)	FXS			Comparison group		
		Mean Age (years)	Characteristics (N)	Mean Cortisol µg/dL (SD)	Mean Age (years)	Characteristics (N)	Cortisol (µg/dL)
Present (Chapter 5)	9am-11am (mean 4 samples)	10.75	Male (24)	.20 (.14)	11.16	Intellectual disability (14)	.20 (.17)
Present (Chapter 4)	9am	15	Male, selected for engagement in challenging behaviour (15)	.25 (.15)	15	Male and female unaffected sibling (15)	.31 (.06)

Study	Sample time (approx.)	FXS			Comparison group		
		Mean Age (years)	Characteristics (N)	Mean Cortisol µg/dL (SD)	Mean Age (years)	Characteristics (N)	Cortisol (µg/dL)
Matherley et al., 2018	9.30am	18.3	Male (54)	.27 (.22)	18	Autism spectrum disorder (15)	.18 (.15)
Hessl et al., (2006)	3pm	10.89	Male and female (90)	.17 (.12)*	11.13	Male and female unaffected sibling (90)	.14 (.09)
Hall, DeBernadis & Reiss (2006)	3pm	11.06	Male (74)	.22 (.22)	N/A	N/A	N/A
Hall et al., (2012)	2-4pm	21.3	Male (8)	~.25 (N/A)*	N/A	N/A	N/A

Study	Sample time (approx.)	FXS			Comparison group		
		Mean Age (years)	Characteristics (N)	Mean Cortisol µg/dL (SD)	Mean Age (years)	Characteristics (N)	Cortisol (µg/dL)
Wisbeck 2000	6-8am	13.5	Male (8): mean 2 typical days	.69 (.39)	7.5	'young controls' (43 male, 41 female)	~.57 (N/A)+
	11am (30 min post-challenge)	13.5	Male (8): experimental day	~.55 (N/A)+	13.9	Females FXS (7)	~.25 (N/A)+
Hall, Lightbody & Reiss (2008)	10am	13.21	Male (31)	~.16 (N/A)*	N/A	N/A	N/A

Note: data for Hessl et al. (2002), Roberts et al., (2009), Scherr et al., (2016) not presented as transformed values available, only.

* estimated from graphical data and transformed from nmol/L. + = estimated from graphical data.

It has been suggested that individuals with FXS may exhibit elevated or atypical stimulus-bound arousal, which may correspond to high behavioural reactivity to challenges (Scherr et al., 2016). However, in the present study, a lack of reactivity was observed in response to a challenging academic demand for both the males with FXS and those with ID. Furthermore, there were no differences in patterns of responding between groups. Visual analysis of individual response profiles further supported the absence of responding to the challenge. Notably, the 20-minute sample time (the point at which a cortisol response to the demand would have been detectable in saliva) is the point at which there is the lowest variability in the data, reinforcing the null finding. This suggests that the demand task did not elicit a physiological stress response for either group. This lack of physiological response contrasts with the behavioural responses observed, such as the engagement in CBs and requests for breaks, which suggest that the demands may have been sufficiently aversive to induce escape-maintained behaviour.

A number of studies have previously assessed group differences in cortisol responses to demands in humans and animals. In the animal literature, detailed comparisons of response and recovery have been conducted, comparing FMR1 KO mice with wild-type counterparts. As in the present findings, the majority of studies found no effect of genotype was observed (Eadie et al., 2009; Qin & Smith, 2008; Qin et al., 2011; Nielsen et al., 2009). Though, where group differences were found these were characterised by elevated reactivity in the FXS model animals (de Diego et al., 2008; Lauterborn, 2004; Ghilan et al., 2015). Of note, this study was one of the first human studies to assess both the response and recovery of cortisol levels to a challenge, whilst controlling for the presence of ID. Direct comparison with many of the earlier human studies is challenging due to methodological variations including: varying demands or

challenges, varying control groups, as well as more disparate times between the pre- and post-challenge saliva samples. The most comparable previous, in terms of sampling timing and duration of the challenge, is that by Hessl and colleagues (2006). As in the present study, no group differences, were observed in levels or reactivity to the assessment: for both groups, the mean reactivity to the assessment (measured 30 minutes after the challenge) was small: -.003µg/dl in the FXS group, compared to .002µg/dL. However, in contrast to the present study, unaffected siblings formed the comparison group. Scherr and colleagues (2016) observed reduced diurnal decline following a 3 hour assessment battery in their FXS sample, compared to typically developing controls matched on mental age. However, the short-term reactivity to the challenge was unclear due to the length of time between samples. Cortisol reactivity was also assessed by Roberts and colleagues (2009) in response to a social challenge, with elevations in cortisol (both prior to and post-challenge), alongside blunted responsiveness, observed relative to siblings only in those children with FXS who exhibit high autism symptomatology. The absent responsiveness was similarly observed in the present study, though in the context of low levels of cortisol which did not differ from others with ID. The importance of autistic symptomatology in the interpretation of results is discussed in more detail later in the chapter.

There are a number of factors which may be associated with the variable findings. For instance, it is possible that the differences in findings between these studies relate to methodological differences. Increased reactivity (reduced diurnal decline) was observed following a 3 hour assessment (Scherr et al., 2016) but not after challenges of up to 20 minutes (present study; Hessl et al., 2006) suggesting that responsivity may be mediated by the length of the challenge. Accordingly, Lauterborn (2004) found a significant effect of genotype in mice, whereby the FMR1 KO animals

exhibited higher reactivity, only after more prolonged exposure to stressors. As such, it is possible that with longer engagement with demands that group differences would have been observable in the present study. It is also possible that the nature of the demand (the presentation of 10 minutes of challenging academic work in the natural school environment) was insufficient to elicit a physiological response due to familiarity.

Participant characteristics may underlie some of the varying results. For instance, absent responsivity may be an indicator of hypocortisolism. This neuroendocrine profile may relate to the high prevalence of CBs across both groups in this study. It is possible that individuals with ID (with and without FXS) and CB exhibit a distinct profile of neuroendocrine arousal (characterised by low cortisol levels and blunted or absent responsiveness) when compared to others with the same condition. This could reflect the stressful nature of CB itself, or a response due to the individual or environmental characteristics which evoke such behaviour. Although in the present study participants were not specifically recruited for engagement in CB, the aim of better understanding these challenges was referenced in study information sheets, so may have been more likely to attract families with experience and interest in this regard. Of note, no associations between cortisol levels and observed CBs were apparent in the present study. However, the subsequent section discusses a number of limitations which may have precluded detection of such a relationship.

In support of this hypothesis, blunted cortisol awakening responses were observed in boys with FXS who exhibit CB, during the study described earlier in this thesis (see Chapter 4). In addition, Hall and colleagues report similar blunted responsivity in response to functional analysis conditions in their sample of adolescents

with FXS who exhibit severe CB (S. Hall, personal communication, February 23, 2018; NIH project ID: 5R21HD072282-02[61]). It is possible that smaller proportions of participants in earlier studies where elevated reactivity has been observed exhibited these behavioural characteristics. In contrast to this hypothesis, Hessl and colleagues (2002) found behavioural problems to be positively correlated to cortisol levels, though the measure used (CBCL: Achenbach, 1991) includes a broad range of behavioural characteristics.

As previously discussed, interpretation of cortisol findings are also complicated by the changing relationship between cortisol and stress over time. Hypothetically those with elevated cortisol levels or responsiveness may be more likely to engage in CBs, but over time those with more severe behavioural challenges transition to a profile characterised by low levels with blunted responsiveness. Similarly non-linear associations between (autonomic) arousal and behaviour (shift from hypo- to hyper-responsivity) across the lifespan have been observed in FXS in relation to autistic behaviour (Baranek et al., 2008; Roberts et al., 2013; Roberts, Tonnsen, et al., 2012; as reviewed by: Klusek et al, 2015). Such changing associations may also underlie the variability on the literature relating to cortisol and CBs more widely. For instance, both low (Verhoeven et al., 1999), and high cortisol (Symons et al, 2003) has been observed in those with SIB, as well as no associations between cortisol and SIB (Lydon et al, 2015). As such, future research should address this issue longitudinally, or ensure controlling for chronicity of CBs presented. The only longitudinal project to be conducted to date relating to cortisol in FXS suggests an atypical developmental trajectory of cortisol levels, characterised by age-related increases in baseline cortisol

[61] http://grantome.com/grant/NIH/R21-HD072282-02

levels (over 3 years: mean age at year 1 was 10 years), which were not seen in unaffected siblings (Scherr et al., 2016). However, the association with individual characteristics and the possible existence of sub-groups was not explored. It has been suggested that blunted responsiveness may be characteristic of autism in FXS, though in the context of elevated baseline levels. In the present study, the assessment of associations between autistic behaviour and cortisol were limited due to small sample sizes. However, across groups there was a trend towards decreased reactivity being associated with increased autistic behaviour, which is the opposite of the associations seen in earlier studies. However, it is important that autism symptomatology is considered both within samples of individuals with FXS and comparison groups in future research.

Of note, earlier between-group differences have been observed in comparison to typically developing control groups (for instance: Wisbeck et al., 2000; Hessl et al., 2002). The lack of difference observed between groups in the current study may suggest that that the presence of ID may be a key determinant of findings, as has been indicated in research with individuals with autism (Taylor & Corbett, 2014).

Is there an association between arousal and escape behaviour, during the assessment? Across the behaviours and participant characteristics examined, there were no clear associations either at the group-level, or through examination of individual participants, with levels of cortisol, or cortisol reactivity in response to the demand task. Notably, the participant who exhibited the greatest physiological response to the task (FX005) exhibited no CB and did not engage in the escape response. As such, the present study does not provide support for the hypothesis that the escape-maintained behaviours observed are associated with changes in physiological arousal.

A limitation of the measure of arousal utilised in the present study is that there is a lag of approximately 20 minutes between the occurrence of a stressor and the resultant detectable changes in salivary cortisol, which may have hindered the ability to detect more subtle, stimulus-bound changes in cortisol levels. As such, the momentary effects of changes in arousal were not able to be assessed in this study. Lydon and colleagues (2013) conducted an investigation of the relationship between arousal and engagement in CBs for three children with ASD. The use of a heart rate measure allowed for evaluation of arousal before, during and after engagement in CB, on a momentary basis. The researchers discovered differing but specific heart-rate patterns associated with CBs for all participants. In future research, the use of similar methodology comparing groups of individuals with and without FXS would allow for a more detailed assessment of the momentary nature of the relationship between arousal and engagement in escape-maintained behaviours. For instance, a rise in heart rate prior to engagement in behaviour, followed by reduction with escape from the stressor, would support the hypothesis that contingent provision of escape is reinforced through escape from an adverse hyper-arousal state. In addition, use of this measure, which may be simply assessed through wearable technology, may avoid the challenges experienced with saliva sampling in a natural environment in the present study.

In the present study, there was no association between cortisol levels and observed frequency of CBs. Though the small sample size, limited time frame of the observations and the unknown correspondence to the individual's behaviour in the natural environment means that associations may not have been detectable. Investigation of cortisol findings in relation to participants' CB more broadly were precluded by lack of detail in the measures of prior behavioural challenges collected (only details on presence or absence were noted, with no detail on age of onset,

frequency or severity), as well as lack of variability in the findings (almost all participants exhibited some topography of CB). Further investigations into whether sub-groups with differing arousal profiles exist within individuals with FXS relating to engagement in CB, or other characteristics, are warranted.

Limitations. In addition to those raised in the prior discussion, there are a number of further limitations and issues which must be considered when interpreting the results of this study. Most notably, the evaluation of physiological variables was hindered by the high number of samples containing insufficient volume for analysis. This led to not being able to conduct the planned analysis of salivary α-amylase, which would have allowed for a more comprehensive assessment of physiological arousal. Given that concurrent blunted cortisol levels and elevated autonomic indicators may be present in those experiencing chronic stress (Ali & Pruessener, 2012), the availability of this measure might have further informed the aforementioned hypotheses of hypocortisolism. In the previous study (Chapter 4), 80% of the samples collected from the participants with FXS contained sufficient volume to run assays for both analytes; compared to just 32.2% in the present study. As a comparison, a high proportion (92%) successful planned samples were collected and assayed for cortisol, by Matherley and colleagues (2018)[62]. Although the basis of this issue is clear, there are a number of factors which may have influenced sample volumes, discussed below.

A notable difference between the studies described in the present thesis and previous studies investigating cortisol reactivity in humans with FXS, is that the majority of prior studies were laboratory based. As a result, samples were able to be

[62] The study authors were contacted in order to request information on sampling methodology and compliance with protocols to facilitate comparison. However, no response was received.

frozen immediately on-site, then were typically couriered to an external agency for analysis to be completed. In addition, the environment for the collection would have been more controllable and predictable when compared to a busy school. The natural setting of this study was intended to reduce burden upon the participants, both in terms of facilitating participation and to avoid the potential influence of a novel environment as a stressor. However, the disadvantages of this approach are both a less controlled environment in which to collect and store the sample immediately, as well as greater variability in the transport of the samples and resultant temperatures and freeze-thaw cycles. This inevitable variability in sample management, when collecting in natural environments across the country is likely to have contributed to the issues experienced.

However, samples were collected in the natural environment both in the present study, and in the previous study described in Chapter 4. Therefore, the setting alone could not have been the only explanation. Sampling protocols for the use of the Salimetrics Swabs were consistent between the present study and the earlier feasibility study described in Chapter 4. However, in the present study, there was a greater burden in terms of research protocol demands for both the experimenter, and for participants: the present study involved participating in a correctly-implemented demand task in the challenges of a natural setting, when compared to the observational nature of the previous study. As such, this may have affected compliance with the collection protocol. The mean length of time which the swabs were allowed to soak in the present study was 30 seconds, which is shorter than the intended absorption time in the study protocol (1- 2 minutes). The reasons for this anecdotally were challenges with collecting the samples in the school environment, but primarily due to participants not wishing to hold the swabs in their mouths for longer periods of time. Similar measures of sample durations were not collected for the earlier study, so it is not possible to

compare and determine the impact of this aspect of procedural fidelity. In addition, the prior study included a dedicated home visit to allow for familiarisation with the experimenter and trialling of sampling procedures. Due to time and resource constraints, this was not possible in the present study; participants were offered trial materials prior to the experimental day, but there was low take-up. It is likely that this additional preparation was important for facilitating effective sampling.

There are a number of other possible influences upon sample volume, including salivary flow rate. There are a wide range of factors which may influence salivary flow. Triggers for production include mechanical (chewing), gustatory and olfactory (Humphrey and Williamson, 2001). Given that participants did not have access to food in the 30 minutes prior to the sampling, gustatory variables could not have affected the flow rates. Participants were encouraged not to chew the swab, given that to do so would have elevated salivary α-amylase, though doing so would have increased flow and potentially have resulted in higher sample volume, thus facilitating analysis of cortisol. In addition, clearly hydration is a key determinant of unstimulated salivary flow. Many of the visits for the present study were conducted during the summer, and as such potentially in warmer weather where dehydration may have been an issue. Medications may also affect the availability of saliva (Dawes, 1987), though none of the participants in the study were taking medications listed by Granger and colleagues (2009) as affecting saliva composition or flow. Furthermore, salivary flow occurs unevenly throughout the mouth (Humphrey & Williamson, 2001), therefore it is possible that placement of the swabs could have contributed to low absorption. Participants were requested to place the swab under their tongue, though it was not always easy to determine the extent to which participants complied with this instruction. Salivary flow rate was not calculated, as such its potential impact is unclear.

In addition, the aim of the study was to assess both the response and recovery of cortisol levels, following a demanding task, from a pre-demand baseline sample. However, participants may have been experiencing anticipatory worry about taking part in the study, leading to elevations prior to the initial sample. However, given that the pre-demand levels were, as with the rest of the assessment, at the low end of the expected range for the participants' age group (Salimetrics, 2016a) this does not seem a likely interpretation. In addition, following the demand task, participants were allowed to return to the classroom, though teachers were instructed not to place challenging demands upon the pupils during that time. However, compliance with this request was not assessed. In addition, the classroom is a complex social environment and the possible influences of this were not controlled for. Notably, one participant (FX009) exhibited a large increase in cortisol levels between the 20-minute post-demand sample and the final hour post-demand sample, which may have reflected the occurrence of a stressor in the natural environment. As such, it is unclear whether the post-demand profile truly represents a recovery from the challenge presented.

There are also a number of variations in the nature of the environment, demands and conduct of the study which may have confounded the results. The choice to conduct the research in schools meant having a less controlled environment in which to conduct the demand tasks. The physical environment in which the tasks were conducted differed widely (such as: table size, availability of tangible items, and proximity of other pupils). This is reflected in a number of sessions being cut short or being unable to be run due to interruptions or the requirement for the pupil to participate in school activities. Further variation was also introduced by varying the tasks for the demands. The aim of this was to achieve a consistent level of difficulty across all of the participants. However, the variations in the exact nature of the task may have

confounded the results. It is known that subtle changes in the nature of demands can have an impact upon responding, such as: the exact nature of the task demand, individual preferences for demand tasks, prompt frequency (for example: Carr, Yarborough & Langdon, 1997). Further adjustments to the study protocol were also made in order to facilitate participation. In a number of cases, teachers requested that sessions be shortened to 5 minutes, either due to participant behaviour or due to lack of availability due to other tasks. Session duration has been shown to be important when conducting functional analyses (for instance, Wallace & Iwata, 1999) and, similarly, in the present study it is possible that differing patterns of behaviour would have been observed with more extended exposure to the session conditions. In addition, as in the previous study, alternative methods of providing a saliva sample were offered to participants. The use of passive drool was selected less frequently in the present study than in the preliminary feasibility study (Chapter 4). Due to the low level of use of passive drool, statistical comparisons of findings from different collection methods could not be conducted. Although no differences in cortisol levels were observed in the previous study in relation to collection method (Chapter 4), it is possible that this methodological variation could have influenced the results.

The results of this study revealed variability in the frequency of individuals who utilised the taught escape response across sessions. Unfortunately, data were not collected upon participants' acquisition of the response during the teaching sessions (these sessions were not videotaped). The collection of information on this aspect of the procedure would have allowed for further investigation as to whether group differences were reflected in, or related to, the number of trials required to learn the response. Should future research utilise similar methodology in order to further assess motivational differences, such additional measures would be valuable to assess.

A further limitation with the present study is the small sample sizes, particularly for the ID group. The choice of the comparison group was made, in part, due to the challenges with identifying families with sibling pairs eligible to participate in a design with an unaffected sibling control (similar to: Hessl et al.; 2002). Though, the choice of a comparison group with a comparable degree of ID, also allowed for control of this issue as a possible confound. However, there were extensive challenges identifying even the small group who were identified to participate in the ID group. This may be because, for families of a child with a rare condition such as FXS, there is greater motivation to participate in research to better understand the condition, even where there is little immediate, tangible benefit. However, the benefits of participating as part of a comparison group may seem lower, even if some of the findings may be transferrable. Anecdotally, many of the parents of participants in the ID group cited interest in the study due to the investigation of the relationship between arousal and behaviour, which they felt was of concern for their child. As such, it may be that the ID group was not representative of those with ID more widely, in the measures of interest. Furthermore, there were a wide range of diagnoses, as well as unknown causes of ID, in the ID group which introduces unknown variability into the findings. An alternative approach would be to compare between syndromes with more consistent phenotypic profiles (for example: Arron et al., 2011). However, it was decided that this was not feasible for the present study due to challenges with identifying sufficient participant numbers for the FXS group, which has a more common prevalence rate than many other genetic syndromes associated with intellectual disability.

The recruitment challenges experienced in the present study for the ID group in particular may have contributed to the identification of a highly self-selected sample of individuals with atypical physiological arousal. Namely, the study aim of investigating

atypical arousal and CB was referenced in the study recruitment literature (see Appendices H & I). As a result, anecdotally many of the participants in the FXS group expressed interest in participation due to feeling that such physiological differences were of particular relevance for their child. Therefore, the selection of participants in both groups who may have similarly displayed atypical arousal and avoidant behaviour may have contributed to the non-significant group differences observed in this study. Future research should seek to include participants with a broader range of characteristics in order to determine whether the findings of the present study are more widely generalisable.

The small sample size means that the influence of a range of factors which may have influenced cortisol levels could not be explored or controlled for, such as: pubertal stage, age and BMI (Keiss et al., 1995). Furthermore, as discussed in Chapter 4, the literature on the relationship between gender and cortisol levels is unclear. As such, the inclusion of females in the ID group compared to an all-male FXS group may have reduced the comparability of the results. In addition, it is unclear to what extent the findings of the present study may be generalizable to females with FXS. Future research should further address CBs and physiological arousal in this group.

Finally, one of the aims of the present study was to assess the impact of varying the information processing and social demands of the tasks. A brief assessment was conducted in order to facilitate visits to high numbers of participants in a school setting. However, the low number of repeats of the sessions means that the effect of these manipulations cannot be extensively explored. There was no clear effect of the changes to the demands upon behaviour. It remains possible that with repeated exposure to the conditions, differences may have been able to be detected. Future research should

explore this issue, including evaluating the utility of the recommendation to reduce social demand for children with FXS during teaching, by sitting beside rather than opposite, in order to reduce stress and increase task engagement. A further limitation of the social manipulation is that gaze prompts were given more frequently in the easy task condition, meaning that the social demand may not have been even between the variants of the high social condition. The brief assessment also did not include a baseline condition in which to assess the occurrence of behaviour, including the escape response, which limits the conclusions which can be made about the function of the behaviours.

Summary and future research. In conclusion, the findings of this study provide preliminary evidence that, compared to others with LD, individuals with FXS may be more sensitive to demand escape as a reinforcer, and therefore likely to repeat behaviours which have been responded to in this way. This extends upon previous within-group observations of high levels of escape-maintained CB, in relation to other functions. However, the external validity of this finding requires further verification with more extensive investigations of the correspondence between the engagement in the taught response, and behaviour in the child's typical environment. Furthermore, the observation of significantly greater proportion of participants in the FXS group engaging in CB during the demands is consistent with prior observations of high rates of such behaviours occurring in this context.

However, the results of this study did not support the hypotheses relating to physiological arousal. There were no differences in cortisol levels between participants with and without FXS, and there was no evidence that the demand induced a significant stress reaction. In addition, no relationship between physiological arousal (cortisol

levels) and behaviour could be identified. As such, it is unclear whether arousal-related differences do underlie the behavioural differences observed in this study, and the previously discussed profile of CB within individuals with FXS. Future research should investigate in greater detail the situations in which individuals with FXS are engaging in escape-maintained behaviours in order to determine whether the arousal-behaviour hypothesis should be further explored.

Chapter 6

Understanding the context of challenging behaviours: parent interviews.

Chapter Overview

Despite hypotheses relating to atypical arousal and escape maintained behaviour, investigations across a typical day (see Chapter 4) and a structured assessment (Chapter 5) have not been able to demonstrate such a relationship. As such, interviews with parents of boys with FXS were conducted in order to gain further information about challenging behaviours (CBs) and to explore themes which might be addressed in future research. The content, context and time course of episodes of CBs of 12 boys with FXS were described by parents, through a semi-structured interview format. Interestingly, despite earlier hypotheses relating to escape-maintained behaviour, the primary antecedents to behaviours were most commonly being told 'no', or not having access to what they wanted. The findings are discussed in relation to comparable interview data relating to individuals with Prader-Willi Syndrome (Tunnicliffe et al., 2014). The theoretical implications of the findings for understanding and future research, as well as the relation to earlier work, are also discussed.

Introduction

Atypical, stimulus-bound arousal is hypothesised to play a central role in behaviour in Fragile X Syndrome (FXS; Cohen et al, 1995), including establishing the motivation for escape-maintained CBs. Supporting this hypothesis, atypical indicators of arousal have been demonstrated across endocrine and autonomic arousal systems (see Chapter 3). Such arousal differences have been associated with key behaviours associated with the condition; for instance, increased levels of cortisol have been found to be associated with increased behaviour problems (Hessl et al, 2002). However, the

earlier studies described in this thesis (see Chapters 4 and 5) have not been able to empirically demonstrate associations between salivary measures of arousal and escape-maintained behaviours. The ability to detect differences may have been hampered by the challenges with recruiting sufficient participants to allow detection of subtle differences, exacerbated by further difficulties with obtaining data on physiological arousal through the collection of saliva samples. In light of the null findings and these methodological challenges, it would be of value consider whether there may be other key environmental or physiological factors which should be addressed through future research.

Within the population of people with FXS, detailed, structured descriptions regarding the occurrence of behaviours are lacking. Investigations have focussed on reviewing immediate environmental influences, either through questionnaires administered to caregivers, experimental functional analysis or direct observation (reviewed by: Hardiman & McGill, 2017; see Chapter 2). In addition, previous research into CBs in this group may have been limited by the scope and focus of standardised questionnaire measures (such as: Questions about Behavioral Function (Paclawskyj et al, 2000) used by: Langthorne & McGill, 2012) that are not designed for FXS, and therefore may not include aspects of the behavioural phenotype. Furthermore, experimental functional analyses containing few, 'standard' conditions have been conducted (for instance: Langthorne et al, 2011; Machalicek et al, 2014), which assess a relatively limited number of potential environmental influences, in analogue situations which may not represent the individual's natural environment. A narrative approach to describing CBs, including the environmental, emotional and behavioural sequences, and temporal aspects, would be of value to expand upon earlier work, in order to identify avenues for future investigation.

The collection of detailed descriptions (though parent semi-structured interviews and written narratives) of the temper tantrums of typically developing young children have enabled further understanding of these behaviours, in a "bottom up" fashion (Potegal & Davidson, 2003; Potegal, Kosorok & Davidson, 2003). Temper tantrums are a common behavioural feature in typically developing children, with peak occurrence between the ages of 18 to 36 months. Through parent report, it was identified that these tantrums are typically brief (lasting less than one minute) and, on average, occur daily. These outbursts are intense episodes, typically described in terms of physical behaviours (which may include externalising behaviours such as: throwing oneself to the floor, aggressions towards others and crying) and out of control displays of emotion. These tantrums typically occur as a response to frustration; the child appears to lose control and lacks the verbal skill to express or process their frustration (Österman & Björkqvist, 2010). Through examining in detail mothers' descriptions of temper tantrums, Potegal and colleagues were able to establish the Anger-Distress model, which describes the emotional sequence of these episodes, which transition from initial, fast-rising anger, followed by more prolonged distress (including later crying and comfort-seeking). This model has helped researchers to understand that these episodes are developmentally appropriate. Namely, they result from the lack of development of cognitive skills to adequately modulate emotional reactivity: the individual's arousability (in affective, neuroendocrine and autonomic nervous systems) in response to emotionally-relevant challenges (Zentner & Bates, 2008; Trentacosta & Izard, 2007). As these skills develop over time, these behaviours typically begin to wain (particularly the "angry" aggressive components of the episodes), usually by four years of age. However, these behaviours may persist later until older ages in certain groups. For instance, individuals with autism may display tantrum behaviours later into

childhood or adulthood, with greater autism symptomatology being associated with increases in tantrum behaviour (Konst, Matson & Turygin, 2013). In this group, tantrum behaviours are often characterised by attention, escape and tangible functions.

However, the delineation of behavioural sequences and temporal aspects of meltdowns or instances of CB have not been widely studied in individuals with intellectual disability. As an exception, Tunnicliffe and colleagues (Tunnicliffe, Woodcock, Bull, Oliver & Penhallow, 2014) used an approach similar to Potegal and colleagues, in order to gain further insight into the nature of temper outbursts in individuals with Prader-Willi Syndrome (PWS). The semi-structured interview format was designed to assess a comprehensive range of possible antecedents and management strategies, as well as the behavioural and emotional time-course of the outburst, and to take into account aspects of the behavioural phenotype of PWS. Consistencies were identified across the parents' descriptions which supported and extended previous literature on CBs in this condition. For instance, the reports identified that changes to routine or expectations are a common antecedent to temper outbursts, which may result from a deficit in attention-switching associated with the condition (for instance: Woodcock et al, 2009b). In addition, the findings provided preliminary support for the extension of the Anger-Distress model to this group, as CBs (which would be associated with high-intensity anger in the model) typically occurred towards the beginning of the outburst, along with anger as a reported emotion, and distress (including sadness and apologising) at the end of the outburst. The convergent validity of this approach was also verified through confirming correspondence between the interviews with behaviour diaries collected as part of a larger project.

As such, investigations from parent report may help to identify further syndrome-specific influences upon CB in FXS, as well as to help establish whether further investigations into arousal and escape-maintained behaviour in this group are justified. The approach taken to investigate temper tantrums may be broadened to include a broader range of CBs, as is the scope of the present thesis. The interview structure used by Tunnicliffe and colleagues also allows for collection of information about setting events, which may provide further key insights into the motivations underlying the occurrence of behaviours (McGill, 1999). Furthermore, the interview addresses precursors to CB; it will be of interest to investigate whether parents report indicators of arousal levels rising prior to engagement in CB. In addition, the identification of precursor behaviours can be of clinical benefit as this can be a critical point for establishing the use of functionally equivalent behaviours and reducing the likelihood of CBs occurring (Oliver et al, 2009). Finally, there has been little research aiming to understand the use and effectiveness of intervention strategies for behavioural challenges in individuals with FXS. An exception is the work of Moskowitz, Carr & Durand (2015) who demonstrated the effectiveness of behavioural interventions for problem behaviours for 3 individuals with FXS. In addition, a national survey in the United States included questions on the use and perceived effectiveness of a range of interventions for aggressive behaviour (Wheeler et al, 2015), with redirection being the most commonly used and effective strategy. In order to extend our knowledge of this important topic, the sections of the interview addressing this important topic will be valuable.

Aims and hypotheses. The aim of this study was to delineate the nature and course of instances of CBs in people with FXS, in terms of the emotions and behaviours that are reported, precursor behaviours, setting events, antecedents and intervention

strategies. Given the exploratory nature of the study and the mixed findings in relation to behavioural function in earlier studies (Chapters 4 and 5), there were no defined hypotheses being tested. It was hoped that this study would provide a more in-depth understanding of the nature of these behaviours in this population, in order to generate hypotheses to guide future research. Further elucidation of syndrome-specific pathways from phenotypic characteristics to behaviour can help to shift the focus from managing behaviour, to managing motivation and increasing awareness of vulnerability and susceptibility to operant reinforcement associated with genetic syndromes, which facilitates early intervention in these groups (Oliver, 1995; Tunnicliffe & Oliver, 2011).

Method.

Design. This study was a within-group, exploratory, descriptive study, with the aim of supporting the development of hypotheses for future research. The study included both qualitative and quantitative elements: in the context of a semi-structured interview.

Ethics and Governance. During the design of the project, feedback and input were sought from parents of children with Fragile X Syndrome, via the Fragile X Society's Research Committee. The project was then reviewed and approved by the Tizard Centre Ethics Committee (Appendix O), University of Kent. The Fragile X Society's Research committee, including an external Specialist Advisor, reviewed and approved the project, for recruitment through the charity.

Participants. The aim was to recruit parents or guardians of males with FXS, between the ages of 4 and 15 years old, who had exhibited at least one topography of behaviour which was considered to be challenging, or had a "meltdown" on at least one occasion, in the previous month. These behaviours were required to include one or

more of the following topographies of behaviour: self-injurious behaviour, aggression (physical or verbal) or destruction of property or physical environment. Children were included with a dual-diagnosis of autism or ADHD, but were excluded if they had an additional diagnosis of a second genetic syndrome in addition to FXS, such as Tuberous Sclerosis Complex or Down's syndrome. Information on diagnosis was obtained by parent report.

Recruitment. The aim was to recruit the parents or guardians of 10 children for the present study. Participants were recruited through the Fragile X Society by disseminating a summary information flyer (Appendix P). Emails were sent to all members of the charity who had consented to being contacted about research (approximately 1,400). In addition, a notice was added on social media and on the charity's website. In addition, flyers were available on a stand at the charity's annual conference (approximately 200 delegates). Finally, emails were sent to all eligible families who had participated in the previous research projects.

Within one day, 10 eligible families had expressed interest in participation. The total number of participants was extended to 12 due to interest. In total, 20 eligible families (21 eligible children with FXS) expressed interest in participating, though there was not sufficient time and resource to include all interested participants[63]. Three further families made contact wishing to participate, but were ineligible due to their child being older than the inclusion criteria. The methods of recruitment for participants are included in Table 44. Participants were not compensated for participation.

[63] An extension study is being explored to include the interested families.

Table 44

Recruitment Methods

Contact Method	Number Who Expressed Interest	Number Who Participated
Previous Study Participation	10[64]	4[65]
Facebook Advert	6	2
Fragile X Society Email	4	4
Fragile X Society Research Committee	1	1
Fragile X Society Conference	1	1
Fragile X Society Website	1	0
Unclear	1	0

Participant characteristics. All interviews were conducted with the child's biological mother (in addition, Robert's older brother also contributed to the interview). Children were assigned pseudonyms, which are used throughout the discussion and results to aid identification. Details about the characteristics of the individual participants (children) are presented in Table 45. All children had full-mutation Fragile X Syndrome (no methylation or repeat expansion mosaic cases). The mean chronological age of participants was 7 years 2 months (SD 21 months), with an adaptive level of 2 years 9 months (SD= 11 months). The majority of participants scored above the autism cut-off on the SCQ (58%), with a further third (33.3%) scoring above

[64] Five participants from Study 1, five from Study 2.
[65] Two participants from Study 1, two from Study 2.

the ASD cut-off; only one participant (8.3%) scored below any of the clinical cut-offs on this measure.

Measures and Procedure. Upon initial contact expressing interest in participation, full information sheets (Appendix Q) and consent forms were disseminated to the potential participants. Once willingness to participate had been confirmed and any questions had been answered, a brief initial phone call was arranged in which a screening questionnaire was used to gain basic demographic details (age, diagnosis and confirmation of occurrence of relevant behaviours) and to determine eligibility to participate. A full interview was then arranged. Face-to-face interviews were possible where a return journey to the participant's location was possible in one day, from the researcher's base in Cardiff. The majority of interviews were conducted on the phone (8 participants), with 4 face-to-face in the individual's home. No participants expressed preference for face-to-face interviews where this could not be facilitated. The semi-structured interview lasted approximately 1 hour and included the use of three measures, which were presented in a counterbalanced order.

Table 45

Child Characteristics

Pseudonym	Age	Vineland Screener					SCQ	
	(Years, Months)	Communication Age equivalent+ (score*)	Socialization Age Equivalent (score)	Daily Living Age Equivalent (score)	Motor Age Equivalent (Score)	Adaptive Behaviour Composite Age Equivalent (Score)	Score	Cut-off[66]
Laurie	5y 9mo	3y 3mo (66)	2y 8mo (54)	5y 5mo (65)	4y 11mo (59)	4y 1mo (65)	23	Autism
Robert	9y 5mo	3y 3mo (42)	2y 7mo (<20)	3y 1mo (58)	2y 5mo (44)	2y 11mo (44)	27	Autism
Matthew	5y 9mo	1y 3mo (44)	1y 4mo (48)	1y 6mo (54)	2y 0mo (38)	1y 6mo (42)	17	ASD
David	5y 6mo	1y 11mo (44)	1y 9mo (33)	4y 0mo (66)	2y 7mo (46)	2y 6mo (44)	25	Autism
Luke	6y 11mo	2y 0mo (45)	1y 11mo (27)	2y 2mo (55)	1y 10mo (37)	2y 0mo (39)	29	Autism

[66] Autism= above autism cut-off (>20), ASD = above ASD cut-off (>15), Below= below all cut-offs

Pseudonym	Age	Vineland Screener					SCQ	
	(Years, Months)	Communication Age equivalent+ (score*)	Socialization Age Equivalent (score)	Daily Living Age Equivalent (score)	Motor Age Equivalent (Score)	Adaptive Behaviour Composite Age Equivalent (Score)	Score	Cut-off[66]
Paul	9y 2mo	5y 8mo (59)	2y 8mo (<20)	2y 11mo (56)	4y 3mo (73)	3y 9mo (48)	18	ASD
Howard	11y 4mo	2y 2mo (26)	2y 0mo (<20)	2y 2mo (41)	3y 0mo (52)	2y 1mo (26)	31	Autism
Gerald	7y 11mo	4y 6mo (64)	2y 9mo (31)	3y 1mo (58)	2y 10mo (49)	3y 5mo (47)	14	Below
Stephen	8y 2mo	9mo (27)	1y 8mo (<20)	7mo (40)	2y 5mo (44)	1y 0mo (<20)	33	Autism
Tim	7y 1mo	3y 3mo (54)	2y 0mo (39)	5y 11mo (84)	2y 1mo (49)	3y 8mo (54)	25	Autism
Alex	5y 3mo	3y 3mo (72)	2y 7mo (58)	2y 11mo (74)	3y 4mo (61)	3y 0mo (61)	17	ASD
Jonathon	8y 8mo	4y 7mo (53)	3y 1mo (27)	4y 4mo (64)	4y 11mo (81)	4y 0mo (45)	18	ASD

* Equated Standard Score on Vineland Adaptive Behaviour Scale

Behaviour interview. A semi-structured interview format was developed by Tunnicliffe and colleagues (2014) to be used to gather information about temper outbursts relating to change in individuals with Prader-Willi Syndrome. The interview was adapted in order to include questions to capture potential factors relating specifically to the FXS phenotype, including: anxiety, physiological hyper-arousal, social situations and sensory experiences. These adaptations were based upon previous literature on the syndrome as well as input from parents of individuals with Fragile X Syndrome (via the Fragile X Society's research committee) and the Fragile X Society family support workers. Based upon feedback from these individuals, the original terminology of "temper outbursts" was amended to the terms "CB or meltdowns", which reflects terminology more commonly used in this population.

The interview consisted of a series of guide questions and prompts, covering a number of topics in order to gain a comprehensive picture of the emotional and behavioural sequences of meltdowns, including antecedents and interventions (including associated success). The full interview can be seen in Appendix R, but is summarised in Table 46. Questions from the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS: Zigmond & Snaith, 1983), adapted to third person language to suit the parent response format, were included as a broad screener of general anxiety status. However, due to parents' self-reported difficulty with reliably answering questions, these data are not included (See Appendix S).

Table 46

Contents of behaviour interview.

Topic addressed in interview	Example interview question
Antecedent	Thinking about the last meltdown or instance of challenging behaviour that _____ showed, what seemed to trigger it?
Precursors	Are there any physical indicators or signs that you can see that indicate that _____ might be about to have a meltdown?
Emotions prior to behaviour	How would you describe _____'s emotion before a typical meltdown?
Frequency and duration of behaviour	Think about how often meltdowns or instances of challenging behaviours occurred in the last month. If there was no change and you watched this person now, then when would you *definitely* see the next instance?
Topography of behaviours	During a meltdown, what behaviours does X show?
Setting events	What happens on the occasions when it does not trigger a meltdown? What is different about these times?
Emotion During Behaviour	During an instance of challenging behaviour or a meltdown, how would you describe _____'s emotion?

Topic addressed in interview	Example interview question
Consequence and Management Strategies	Do you intervene? And if so at what point would you intervene? i.e. when you saw which behaviour?
Emotion and behaviour following meltdown	What does _____ do at the end of the outburst? Prompt= Do they do anything? Say anything?

Adaptive behaviour. As in the previous study (see Chapter 5), the Vineland Screener was utilised to assess adaptive functioning. The administration and analysis of the interview was conducted as described in the earlier chapter.

Autistic behaviour. As in the previous studies (see Chapters 4 and 5), the Social Communication Questionnaire was utilised as an indicator of autistic behaviour.

Data Analysis. The behaviour section of the interview was recorded using a Dictaphone. A member of staff at the University of Kent was paid to transcribe the interviews. The quantitative elements of the interview were then summed and the qualitative answers were analysed using NVivo 11, by the researcher. Responses were then categorised according to primary nodes, in line with the semi-structured interview format, with lower-order nodes being used to categorise answers and identify themes. Content from responses to any question in the interview were considered when collating responses to individual question items, as additional, relevant details were often given in this way.

Results

Study results are discussed below in sections according to topics addressed in the CB interview.

Antecedents. Respondents were asked to say whether any of a list of situations had ever triggered an episode of CB or a meltdown for their child (Figure 44). The following situations had been reported to trigger behaviour for at least 80% of the boys with FXS: waiting for something (11/12); cannot have something they want (11/12); something not working (12/12); change in own routine (10/12); change in expectation (11/12); cannot have their own way (11/12); asked to do something doesn't want to (11/12). Of the 22 possible antecedents directly assessed, parents identified on average 14.17 (SD=2.51, range= 11-19) situations which had triggered CB for their child. This highlights the wide variety of situations associated with CBs in this group.

Table 47 outlines in greater detail the perceived primary antecedent for the children's behaviours. The most common primary antecedent, identified for half (6) of the participants, was not getting what they wanted or being told "no". Across the participants this could refer to multiple situations including tangible items, food and activities. Where this lack of access to preferred items or activities was the primary antecedent, parents estimated that, on average, it accounted for 60-70% (range= 40%-90%) of all their child's meltdowns. However, the children were not reported to always engage in CB after being told no, with resultant behaviours occurring between 30-80% of instances (two parents could not specify how likely behaviours were). A wide range of mediating factors were identified which appeared to alter the likelihood of CBs occurring once the child had been told "no" , or not been able to access what they wanted (Table 47).

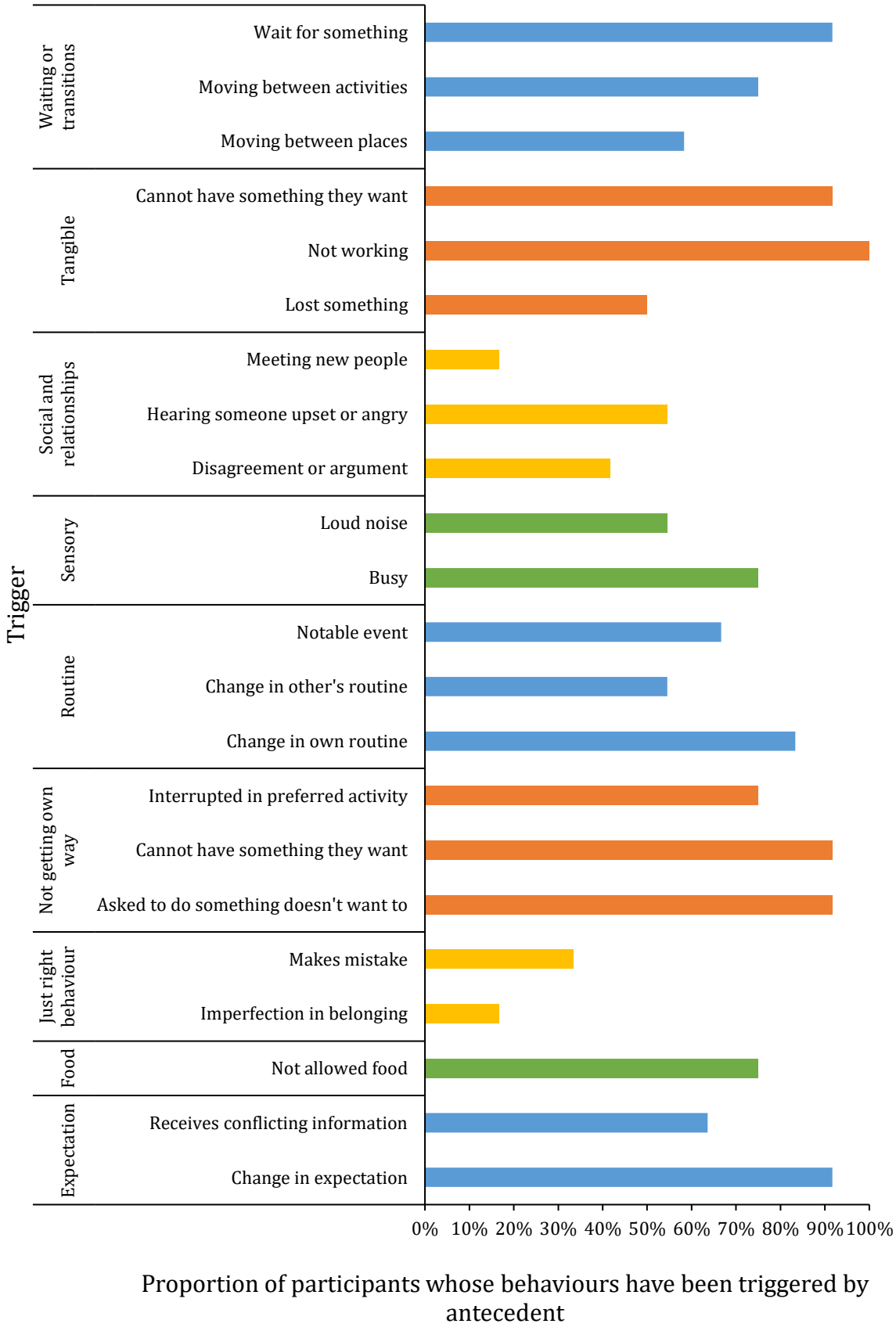

Figure 44. Proportion of children for whom behaviour has been triggered by different situations.

Table 47

Perceived Primary Antecedents for Challenging Behaviours

Participant	Principal antecedents	Proportion of all meltdowns caused by principal antecedent	Does antecedent always lead to a meltdown?	What's different on occasions where antecedent does not cause meltdown?	Other key antecedents
Laurie	Change in Routine	60-70%	No: Could not specify	Who he is with: with certain people e.g. Dad, seems easier to push out of comfort zone and better at managing changes.	None specified
Robert	Being told “no” or not getting what he wants (e.g. toys, food)	40%	No: 30%	How much he wants the item or activity; how well he understands the reason for being told no, or accepts the reason.	None specified

Participant	Principal antecedents	Proportion of all meltdowns caused by principal antecedent	Does antecedent always lead to a meltdown?	What's different on occasions where antecedent does not cause meltdown?	Other key antecedents
Matthew	Frustration of not being able to access something he really wants (e.g. food, iPad) or being told “no”.	50%	No: could not specify	Can’t see anything immediately different.	Gastrointestinal discomfort during the night.
David	Being told “no” (e.g. to “doing something”)	60-70%	No: 50-60%	If there is no flexibility in the situation. Depends on the day	None specified
Luke	Busyness	33%	Yes	Cannot identify anything; found that preparation helps but not enough.	Frustration, transitions
Paul	Cannot have something that he wants (e.g. a toy)	80%	No: could not specify	Where they are; who they are with; what the thing is that he would	Tiredness, noise, busyness.

Participant	Principal antecedents	Proportion of all meltdowns caused by principal antecedent	Does antecedent always lead to a meltdown?	What's different on occasions where antecedent does not cause meltdown?	Other key antecedents
				like (has fixations on things sometimes)	
Howard	Not getting what he wants (e.g. preferred food)	80%	No: 70-80%	If there is something else that he can get instead (can be promise of something in the future).	None specified
Gerald	Could not identify primary antecedent	-	-	-	Somebody not following the rules. "Anything"
Stephen	Could not identify primary antecedent[67]	-	-	-	None specified

[67] Approximates can recognise antecedent for half of instances of behaviour, but could not identify one which was more common than others.

Participant	Principal antecedents	Proportion of all meltdowns caused by principal antecedent	Does antecedent always lead to a meltdown?	What's different on occasions where antecedent does not cause meltdown?	Other key antecedents
Tim	Transition from school taxi into the house	80%	No: 70%	Often unclear; his mood; whether able to distract early enough.	None specified
Alex	Not getting what he wants (e.g. activity)	90%	No: 80%	Tired, hungry.	Falling over in front of someone.
Jonathon	Jealousy (divided attention)	70%	No: 70-80%	Less likely if he is also receiving attention; the physical closeness of the other people (sitting close or if Mum has arm around sister); how much fun the other people seem to be having.	None specified

Precursors. Respondents were asked about whether there were any indicators or changes that they noticed in their child before CB occurred (Table 48). A number of changes in the child's physical demeanour were noted, several of which may be consistent with increased arousal: increased or sped up physical movement (28.33%), reddening of the face (8.33%) jaw clenching or biting (41.66%), deteriorated ability to communicate (16.67%) and appearing "agitated" (8.33%). One respondent explicitly noted arousal (both positive excitement and negative anxiety) as being a key factor in their son's behaviour.

Table 48

Precursor indicators

Sign	N	Participants & Detail
Increased arousal.	2	1 respondent (Laurie): appears "agitated" 1 respondent (David): may appear either anxious or excited (either positive or negative arousal).
Begins biting or chewing self or objects; clenching jaw or grinding teeth.	5	4 respondents (Robert, David, Howard, Stephen): hand biting indicator of likelihood of escalation to other topographies of challenging behaviour 2 respondents (David, Luke): precursor jaw clenching or teeth grinding.
Facial sign.	4	1 respondent (Matthew): patchy reddening of face and ears. 3 respondents: particular facial expression (Paul: stern face; Gerald: grimace; Luke exhibits "fixed smile").

Sign	N	Participants & Detail
Change in physical movement.	5	3 respondents: increase in physical movement (Laurie: more twitchy, running from one bit of the room to the other, general increased physical movement; Robert: hand-flapping; Stephen: forceful rocking). 1 respondent (Matthew): starts to move more quickly. 1 respondent (Luke): begins stamping/ stomping around.
Postural change: stiffening.	2	1 respondent (David): stiffens body. 1 respondent (Paul): stance changes and he stands up tall.
Sudden onset or no clear signs.	6	Laurie, Robert, Matthew, Tim, Alex, Jonathon
Verbal sign.	7	2 respondents (Laurie, Robert): less verbal language 2 respondents (Laurie, Robert): increased repetitiveness in speech 1 respondent (Stephen): makes more (non-verbal) noises 4 respondents: negative vocalisations (growling (Robert, Gerald), screaming (David) or shouting (Luke)).

Emotions prior to the onset of behaviour. Respondents were asked how they would typically describe their child's emotions prior to the onset of CB. A number of respondents identified multiple possible precursor emotions. Frustration was the most commonly identified emotion prior to the episode of behaviour (Table 49).

Table 49

Reported Emotions Prior to Challenging Behaviour.

Reported precursor emotion	Percent participants[68]	Participants
Frustration	54.54%	Matthew, David, Luke, Paul, Tim, Alex
No clear prior emotional change/ sudden onset of behaviour	27.27%	Laurie, Robert, Jonathon
Excited	18.18%	Matthew, David
Anxious	27.27%	Matthew, David, Stephen
Anger	27.27%	David, Paul, Tim
"Teary"	9.09%	Paul
Disappointment	9.09%	Howard

Setting event. Setting events were split into social, environmental and physiological (Table 50). Predominantly physiological setting events were identified for participants' behaviours (75% of respondents) including pain, tiredness and hunger (tired being the most common of those mentioned). Environmental setting events were noted for 2 individuals, and social for 1. Of note, there was only one question on this topic in the interview and many of these factors were identified through broader questioning on related issues. As such, it is uncertain whether this represents a comprehensive list.

[68] One respondent (mother of Gerald) did not provide an answer: N=11.

Table 50

Reported setting events for behaviour

Setting event		Percent participants	Detail`
Type	Event	(N=12)	
Physiological	Tiredness	75%	Laurie, Matthew, David, Luke, Paul, Howard, Tim, Jonathon
	Hunger	41.67%	Laurie, David, Paul, Luke, Alex
	Gastro-intestinal	16.67%	Matthew (night time pain), Luke (prior to bowel movement)
Social	When with a certain person	33.3%	Laurie: if Dad is around, he becomes very sensitive to his Dad leaving which can be trigger for later behaviour. Robert & Luke: aggression typically targeted at brother Paul: aggression targeted at mother.
	Meeting a new person	8.33%	Luke (may later exhibit behaviour: delated response)
Environmental	Location	16.67%	Luke & Jonathon (more likely to happen at home)

Setting event		Percent participants	Detail`
Type	Event	(N=12)	
	Change at school	8.33%	Stephen (based upon recent change in behaviour).

Frequency and duration of instances of behaviours

Duration of behaviours. The modal length for both the average length and longest lengths of episodes of behaviours were between 15 minutes and 1 hour. Though exploratory analyses had been intended to investigate the association with developmental age, this was precluded by the limited range of responses (Figure 45).

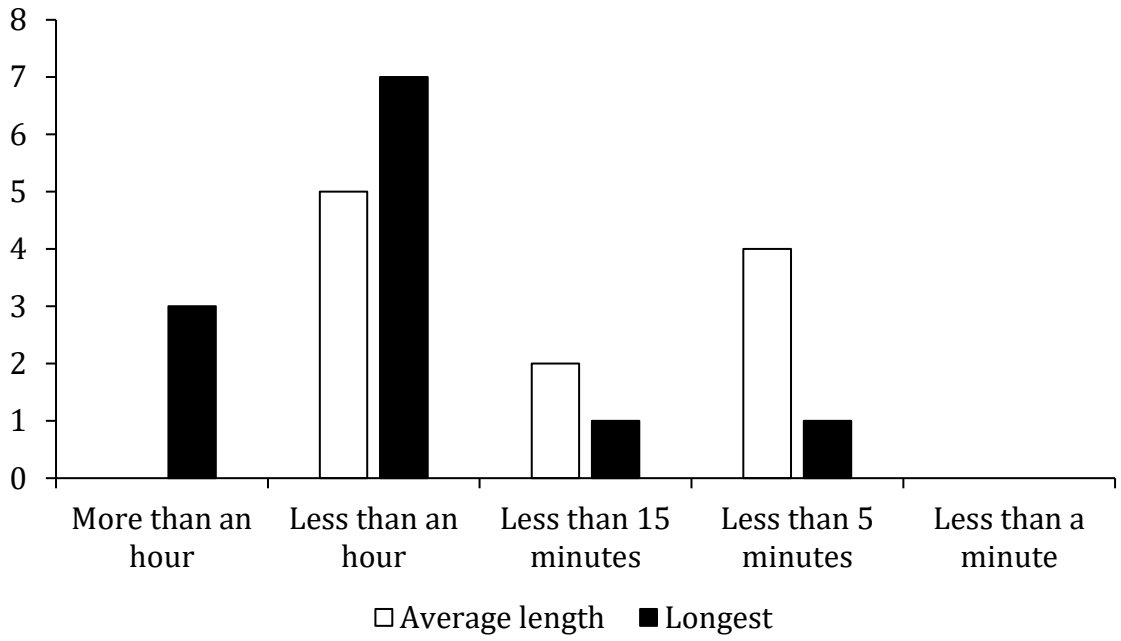

Figure 45. Average length (past month) and longest length of episodes of challenging behaviours.

Frequency of behaviours. When asked when the next instance of behaviour would happen, all but one respondent indicated that this would be seen by this time the following day (91.67%), suggesting at least daily occurrence. The final respondent indicated that, due to preventative strategies being in place, their child's behaviours occurred only around every other day. One respondent indicated that a particular group of behaviours (waking up in night, screaming, lashing out) occurred less frequently, around once a week or fortnight: believed to be in response to physical pain resulting from gastrointestinal problems.

Change over time. Although no questions specifically addressed this issue, seven respondents (58.33%) indicated that there had been a reduction in their child's CB over time. There were a number of reasons cited: 4 referred to improved communication, 1 referred to their child's greater flexibility and tolerance of change, 2 referred to improved management strategies. Conversely, one respondent noted a recent increase in their child's behaviour, which was attributed to a change of class at school.

Topography of challenging behaviours. Parents were asked to report topographies of behaviours exhibited by each individual and the frequency at which respondents reported these behaviours to occur. The group-level prevalence of classes of CB are listed in Figure 46. SIBs were the most common type of CBs exhibited, which most commonly consisted of hand-biting (90% of those who engaged in SIB), followed by self-hitting (60% of those who engaged in SIB). The most common topographies of physical aggression were biting and hitting (both 63% of those who engaged in aggressive behaviour). Four participants were noted to direct their aggression particularly towards specific individuals. In addition, the most common topography of destructive behaviour was throwing objects (78% of participants who exhibited destructive behaviour). Descriptions of CBs at the individual level are available in the appendices (SIB: Appendix T; aggression, Appendix U; destruction: Appendix V; other CBs: Appendix W). The majority of respondents could not identify a predictable chain for occurrence of the behaviours (75%).

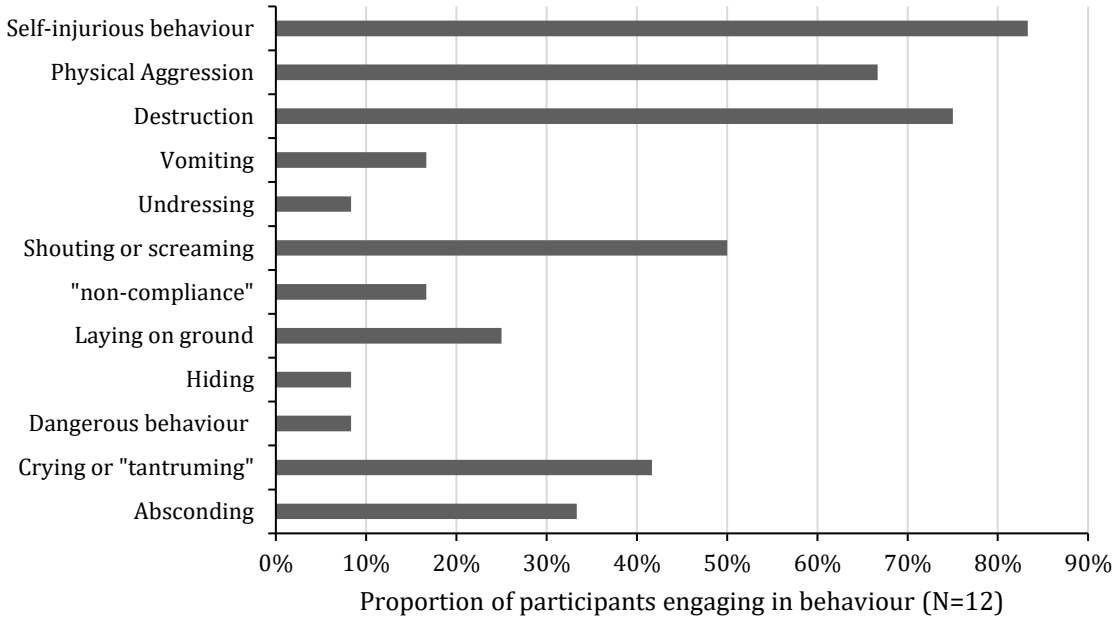

Figure 46. Proportion of participants engaging in topographies of behaviour

Impact of behaviour. Although the impact of the CBs was not directly assessed in depth but the comments of interviewees highlighted the varying physical, interpersonal and environmental effects of the behaviours. For instance:

> "He can really go for [sister] and like he has really hurt her before"… "I don't know how she hasn't ended up in A&E before." –David's mother

> "I mean the thing is you get used to it, don't you, with his behaviour but actually the whole house is [child]'s way because the other children can't watch what they want to watch on TV…everything's got to be his way." –Luke's mother

> "You lose the ability really to be spontaneous and it makes the weekends very challenging"- Laurie's mother

> "[husband] and I are usually most tense when the two of us are around [son] in public…you know, then we'd become tense with each other"- Howard's mother

Emotion during occurrence of behaviour. Eight parents reported the perceived emotion of their child during the occurrence of a meltdown, or episode of CB. The most common perceived emotion of the child during the meltdown/ episode of CB was anger (75%). Other described emotions included: Upset or sad (37.5%), Frustrated (37.5%), anxious and stressed (12.5%) or "in overdrive" (12.5%).

Consequences and management strategies.

What keeps behaviour going? Primarily, children's behaviours were reported to continue for longer times if the "trigger" to the behaviour remained present (50%) This could be due to lack of intervention, lack of compliance with the child's desire (i.e. continuing to not get their own way), for example:

> "It is usually whatever has caused the meltdown is still ongoing, it's still... For him it is usually something that he wants to do. If it's prolonged it's because he wants to do something, he can't do it or for whatever reason we can't do it but the thing he wants to do is still there, he can still see it, he can still... we've not moved away from it." - Gerald's mother

Other factors which may prolong instances of behaviour included: reminders to the child about the original antecedent, telling the child off, physically blocking the behaviour.

Preventative strategy. Respondents were asked what would be the most likely thing to prevent a meltdown from happening, if they had started to see signs that a behaviour might occur or if there was a likely trigger present (Table 51). Distraction was by far the most common strategy used (75%), with high levels of success reported (over half the time for all participants).

Table 51

Preventative strategies and associated success rates at avoiding challenging behaviour.

Participant	Principal intervention	Success	Caveats
Laurie	Distraction (deep pressure, food, tangible, activity)	90%	-
Robert	Distraction	65%	Sometimes too busy to catch early enough
Matthew	Distraction (tangible, activity)	80%	Whether catch at precursor stage (less common because occur at night)
David	(Advance) managing change e.g. visual timetable, verbal explanations	-	-
Luke	Distraction (activity and attention)	-	Depends on whether the trigger is ongoing or has finished
Paul	Remove from situation and give reassurance	25%	-
Howard	Distraction (food)	70%	How much he wants the thing that is the trigger for the behaviour

Participant	Principal intervention	Success	Caveats
Gerald	Removal from situation and deep pressure massage	"most of the time"	-
Stephen	Distraction	50%	How much he wants what he is being distracted with
Tim	Distraction (watching video on phone)	90%	
Alex	Distraction	50%	His emotions, what he wants and how much
Jonathon	Distraction	50%	Whether intervene early enough

Intervention strategies. Parents were asked to describe the most likely action, which could stop an episode of behaviour once it had begun (principal intervention strategies: Table 52). For many this was the same as the preventative strategy (distraction) but there were some differences. Several parents noted that the most likely thing that would stop the behaviour was giving the child what they wanted (the item or activity which had led to the behaviour), though raised concerns about not wanting to 'give in' or reinforce 'bad' behaviour and highlighted that sometimes this was simply not possible.

Table 52

Principal intervention strategies to stop ongoing episode of behaviour

Participant	Principal intervention	Success	Caveats
Laurie	Distraction (preferred person, comfort/ attention)	85%	Earlier intervention more likely
Robert	Distraction (joke or laugh)	55%	
Matthew	*Not stated*	-	-
David	*Not stated*		
Luke	Change of face (person he is with)	90%	
Paul	Give what he wants	100%	If can do but sometimes not possible and don't want to always reinforce
Howard	Explaining what will happen, food	70%	How much he wants the thing
Gerald	Distraction	75%	
Stephen	Distraction	50%	
Tim	Access to phone	99%	
Alex	Give what he wants or completely changing environment	"most"	Sometimes in such a state has forgotten what wanted in first place then doesn't work.
Jonathon	Nothing, leave him alone.	-	

Respondents were also given the opportunity to outline other (reactive) intervention strategies used to try to reduce the maladaptive behaviours (Table 53). Four participants also referenced more proactive (rather than reactive) interventions including: Skills teaching (2 participants e.g. programme to develop ability to wait), preparation for the situation (1), physical activity breaks (1).

Table 53

Frequency of use of intervention strategies.

Intervention	N	Detail (N)
Distraction	10	Attention (Interaction: 5; deep pressure: 5) Food (3) Tangible item or activity (5)
Physically blocking	7	Remove hand from mouth (1) Physical restraint (2) Physically move or pick up child (3) Remove items in environment which may be thrown (2)
Remove original trigger	6	Leave situation/ change of environment (6)
Ignore	4	N/A
Verbal reprimand	4	N/A
Discussion or negotiation	5	Explain situation (3) Explain alternatives to behaviour (1) "Bribe" promise access to desired food in future (1)

Intervention	N	Detail (N)
Redirection	2	Redirect biting to a 'chewy stick', or equivalent (2)
Consequence	1	Threat of not being able to go on holiday (1)

Respondents described a number of factors which influence how they respond to the behaviour. Four parents specified that they would be more likely to respond, or use greater intervention if the child hurt themselves or is at risk of doing so; a further 3 noted that they would do so if another person was at risk of harm. Four parents noted that their intervention strategies were different at home and in public (predominantly requiring a quicker response to contain the behaviour in public, less ability to negotiate or control the situation). Similarly, two parents would respond differently if others were present. The following factors were also noted by one of the respondents: the flexibility of the situation, the stage in the meltdown (intervention early in the meltdown could be counter-productive and escalate the behaviour) and the child's perceived level of distress.

Child emotions and behaviour following the meltdown. Respondents also provided information on the child's state following engagement in CB (Table 54. The most common descriptions of the child's emotion was that they were calm and as if the behaviour never happened (50%), with the remainder being sad or upset (50%). Accordingly, the most common behaviours were either: no notable behaviours (back to normal/ as if never happened: 50%) or apologising (33.3%). There did not appear to be any associations between child characteristics or nature of the outburst which affected the likelihood of different emotional time courses.

Table 54

Participants reported behaviours and emotions following meltdown.

Participant	Emotion	Behaviour
Laurie	Calm and settled	"Back to normal", as if didn't happen
Robert	Initial sadness then, within 30 seconds, over-excited before gradually returning to 'normal keel'.	"Back to normal", as if didn't happen.
Matthew	Typically calm, sometimes exhausted and sad[69]	Crying
David	Sad, remorseful, upset	Curls up in a ball, covers self in a blanket and apologises.
Luke	Sad, remorseful	Crying
Paul	Sad, remorseful, sometimes as if nothing has happened.	Apologising and "grovelling", occasionally "back to normal" and as if didn't happen.
Howard	Calm, as if didn't happen	"Back to normal", as if didn't happen
Gerald	Upset	Initially no clear behaviours, later will talk about what upset him.
Stephen	Calm, as if didn't happen	Carries on with whatever was engaged with before the behaviour.

[69] When referring to hand biting- seems to be less "aroused" afterwards

Participant	Emotion	Behaviour
Tim	Sad, tired	Apologise, seek reassurance, affectionate
Alex	Calm, as if didn't happen	"Back to normal", as if didn't happen
Jonathon	Remorseful	Seek reassurance, affectionate.

Overview. Figure 47 summarises the typical sequences of behaviours and emotions exhibited before, during and after CBs across individuals. Further details on all aspects are presented later in the results.

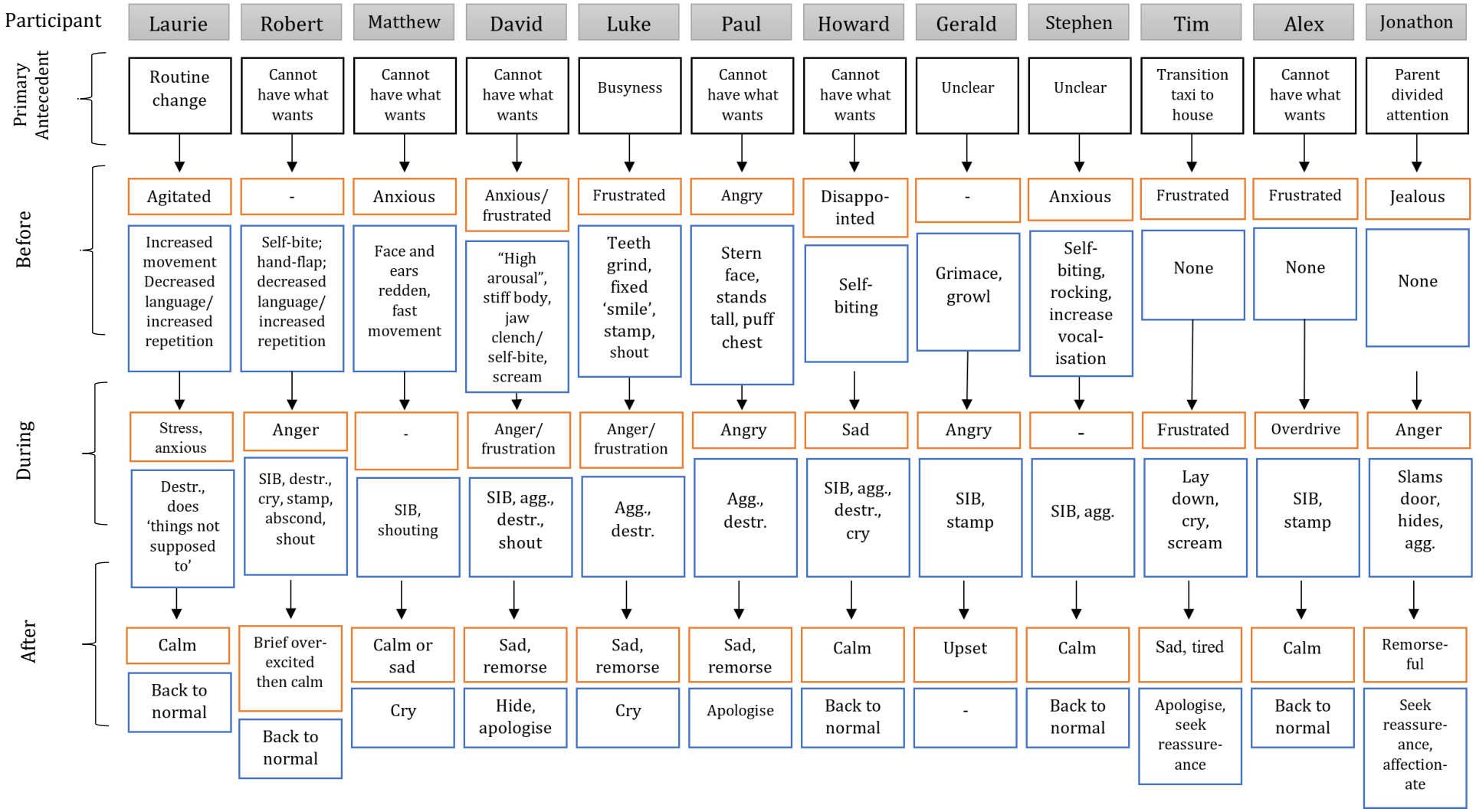

Figure 47. Summary of participant emotions and behaviours before, during and after challenging behaviour

Discussion

The aim of this project was to gain in-depth descriptions of the nature and context of occurrences of CBs from parents of boys with FXS, in order to determine avenues for further research into behavioural issues in this group. The use of a semi-structured interview format enabled a more in-depth analysis of the causes and sequences of CBs, or "meltdowns", when compared to earlier standardised measures or assessments. By utilising a similar interview format to that used to investigate temper meltdowns in individuals with PWS, between-group comparisons were also facilitated.

Notably, a high frequency of behaviours was reported within this group, with all but one participant (11/12) exhibiting behaviours on a daily basis. The behaviours described also appear more common than in children with PWS (Tunnicliffe et al, 2014); only half (7/14) of their participants were reported to exhibit temper tantrums on at least a daily basis. In contrast, the durations of the behaviours (from the episode beginning to the child returning to calm) reported in the FXS sample were shorter: with the modal duration of longest meltdown being between 15 minutes to 1 hour, compared to over 1 hour in the PWS sample. Therefore, in FXS 'meltdowns' appear to be happening more frequently when compared to temper outbursts in PWS, but lasting for shorter durations.

The topographies of behaviours described by parents during interviews reflect earlier findings in FXS (see Chapter 2). As identified in a systematic review of the literature (see Chapter 2; Hardiman & McGill, 2017), self-injurious behaviours were the most common type of behaviour (with hand-biting being the most common topography). The most common forms of aggression and destruction reported were hitting and throwing, respectively. As discussed in the results, a number of parents

referred to the negative impacts of their child's CB, including physical injury, relationship strain and restricted family opportunities or routines. The impact of the aggressive behaviour of boys with FXS has been previously assessed (Wheeler et al., 2016), highlighting that almost one third of parents had been injured by their child in the previous 12 months, with an average of 17.32 injuries being caused per child. Together, these findings further emphasise the need for greater understanding and support for these behavioural challenges.

Due to the retrospective nature of the parent report of the present interviews, information about changes in behaviour over time were not addressed, as the responses may have been biased by the demand on memory (Tunnicliffe et al, 2014). However, a number of parents spontaneously reported improvement in their child's behaviour over time. A number of reasons were cited for this improvement, with improved communication being the most commonly cited. There has been little research understanding age-related changes in the relative risk for engagement in CBs for males with FXS. However, data from the University of Birmingham (Further Inform Neurogenetic Disorders, 2018) supports that the reported, cross-sectional prevalence of physical aggression and destruction of property (during the past month) decreases over time (aggression and destruction, respectively: 71% and 79% 0-5 years; 66 and 62% 6-11 years; 48% and 62% ages 12-15 years; 34% and 39% age 16+ years). The reported rates of SIB also decreased, though less notably (57% age 0-5, 43% age 16+). In a review of the broader literature, Davies and Oliver (2013) found increases in relative risk for engagement in self-injurious behaviour and aggression in individuals with ID, until mid-adulthood, in individuals with intellectual disabilities. Further longitudinal data is required to understand age-related changes in the prevalence of behavioural

problems, both for individuals with FXS and those with ID more widely. In addition, research into individual characteristics which may be associated with improvements in behaviour, or with risk for enduring challenges, may have implications for intervention.

Antecedents Investigation of the antecedents to and triggers for CBs in individuals with FXS allows for greater understanding of possible functions, as well as a potential insight into syndrome-specific factors influencing their occurrence. All parents identified a large number of situations (at least half of the situations listed) which had previously triggered CB for their child, highlighting the broad range of factors likely to be involved in the occurrence of these behaviours. The antecedents which had most commonly led to behaviours occurring across the group included: issues relating to tangible items (cannot have something they want, something not working), routines and expectation (change in own routine, change in expectation), demands (asked to do something doesn't want to) and more broadly 'not getting their own way'. The most common primary antecedent identified for CB was being told "no", or not getting their own way, (identified for half the group) which related primarily to access to tangible items (such as toys, iPad or food). Previous studies gaining information about antecedents and function from parent report did not include the option to rate a primary function (Symons et al, 2003; Langthorne & McGill, 2012). As such, it is possible that the relative prominence of this antecedent has been previously overlooked.

Similar data have been collected relating to a sample of 14 young people with PWS (Tunnicliffe et al., 2014). In contrast to the findings of the present study, principal antecedents to outbursts in PWS were described to be changes in routine or expectation (6/14) or as food-related (4/14). These differences support the hypothesis that environmental stimuli may have differing effects upon behaviour, between diagnostic

groups. Relating to the observations in PWS, it is hypothesised that changes to routine or expectation may be challenging due to the demand which such unexpected changes place on a diminished cognitive ability to switch attention (Woodcock et al. 2009a; 2000b; 2010; 2011). Possible influences upon behaviour in FXS are discussed later in the chapter.

The present findings contrast with the results of a previous review of the literature (see Chapter 2; Hardiman & McGill, 2017), which identified escape (most commonly, from demands or transitions as opposed to social interactions) as the most common function of CB for individuals with FXS. Though all but one parent in this study reported that "being asked to do something that the child doesn't want to do" had previously triggered a meltdown in the past, none reported this to be the primary antecedent for behaviours. Rather, access to tangible items (and "getting one's own way") seemed to play a greater role for this group. This reflects an earlier case study of a young man with FXS whose problem behaviour was positively reinforced by adult compliance with mands (O'Connor et al., 2003). Given that being told 'no' or not getting their own way frequently related to tangible items, it is interesting that functional analysis studies have also identified that CBs may commonly have a tangible function (three quarters of participants: Machalicek et al., 2014; half of participants: Langthorne et al., 2011).

The findings of the present study further support the low prevalence of attention-maintained behaviours in individuals with FXS (see Chapter 2; Hardiman & McGill, 2017, Machalicek et al, 2014; Langthorne et al, 2011), as only one parent identified lack of access to attention as the primary antecedent to behaviour. Similarly, however, no respondents identified this primary antecedent in the PWS sample

(Tunnicliffe et al., 2014). Of note, the scope of the assessment of attention-maintained behaviour was limited in this study, as the interview format (based upon Tunnicliffe et al, 2014), did not address gaining attention as one of the set antecedent questions. Though, parents were given the opportunity to discuss this if they chose, as part of the "other" social and relationship section of the questions regarding triggers, as well as in the broader discussion. As observed in previous studies (where social escape was experimentally assessed as a behavioural function: Langthorne et al, 2011; Machalicek et al, 2014), despite the high levels of social anxiety in FXS, antecedents which may indicate a social avoidance function (meeting new people, hearing someone upset or angry, disagreement or argument) were amongst the least frequently rated as having triggered a past instance of behaviour. Though a number of parents identified atypical responses to social situations, such as becoming overly upset if they hear others angry or crying (6 participants). However, it seems that these antecedents are unlikely to lead to engagement in CBs.

Despite earlier research highlighting the importance of negative reinforcement in this group, antecedent demands were rarely discussed by parents in relation to their child's meltdowns or CBs in the present study. Though, interestingly, when directly questioned these antecedents were endorsed. The descriptive nature of the data means that the focus is upon current behaviour. Therefore, it is possible that the reason that this was rarely discussed was that the presentation of demands was being avoided, due to past history of occurrence of behaviours in these contexts. In comparison, in the previous observational study, described in Chapter 4, demands were the most common antecedents to CBs. This difference may reflect the fact that the majority of these earlier observations were made in school (where demands are presumably a more integral

part of the environment). Though, in the same study, comparable rates of behaviour was observed home and school. In future research, it would be interesting to compare teachers' descriptions of the behaviours of children with FXS with those collected in the present study, in order to assess the effect of setting. In addition, as previously discussed (see chapter 4) conditional probability analyses may further reveal the likelihood of behaviour occurring in response to demands.

Emotional and behavioural sequence. The emotional and behavioural sequence of CBs was also explored, as such information has furthered the understanding of temper outbursts in typically developing children (Potegal & Davidson, 2003; Potegal et al, 2003), The Anger-Distress model developed through these investigations suggests that temper outbursts, which often manifest between ages 2 to 4 years old, relate to an inability to appropriately control emotional responsivity (which includes physiological activation of the autonomic and neuroendocrine systems) as a result of immature regulatory system development. This model is partially supported for older individuals with PWS (Tunnicliffe et al, 2014) which may be the result of developmental delays and/or specific aspects of the phenotype delaying the required regulatory maturation. Half of the children with FXS in the present exhibited the expected sequence of emotions as described in the Anger-Distress model: initial fast-rising anger or frustration followed by later distress, such as apologising or crying. Despite their older chronological ages, participants' adaptive age equivalents were within the expected range of when developmentally appropriate tantrum behaviours may be expected to occur, which may help to explain the nature of these behaviours. The spontaneous reports of improvement with time, including in improvements in the child's adaptive skills, further supports that the developmental delay may be associated with occurrence

of these behaviours. However, a common observation in the present study, which was not seen in those with PWS (Tunnicliffe et al., 2014) was the quick return to 'normal' following engagement in CBs.

In addition, few parents were able to identify a predictable pattern in the time course of their child's meltdown, whereas all in the PWS sample were able to. Though a number of precursor behaviours were described, commonly it was reported that such behaviours seemed to occur without warning. Taken together, these findings suggest that the occurrence of meltdowns are more spontaneous and frequent in FXS when compared to temper outbursts in FXS, but also more transient. The implications of these findings in relation to the FXS phenotype are discussed below.

Consequences and management strategies. There has been little research to identify the management strategies being used by parents of individuals with FXS, and their relative successes. In a large, national Fragile X survey in the United States (Wheeler et al, 2015) parents reported redirection to be the most commonly (tried by 95% of parents of males) and successfully used (rated as very successful by approximately one third, and somewhat successful by approximately a further 55%) intervention for aggressive behaviours, which reflects the high use of 'distraction' as an intervention in the present study. Interestingly, this approach emphasises attempts to either avoid such behaviour in the first place or to stop it as soon as possible. This is consistent with the earlier hypothesis that parents may be avoiding the presentation of demands which, based upon prior research, may be likely to elicit CBs. Given that a sub-group of mothers who carry the FMR1 premutation may exhibit a biological vulnerability to stressors (Hartley et al., 2011), it may be that children's emotional or behavioural reactions are perceived as being particularly aversive. Speculatively, this

may, in turn, influence the strategies used. The high use (41.6% of the sample) of the application of deep pressure (namely, tight hugs or shoulder squeezing) as a preventative strategy was also notable. All parents who reported using this strategy felt that it helped to calm their child and make occurrence of CB less likely. The use of sensory strategies is often advocated amongst the Fragile X community (for instance, Stackhouse, 1998; Stackhouse et al., 2014) though with little empirical evidence to support their use. Future research should address this issue, including validation with physiological measures. A challenge with contingent provision of sensory input may be the inadvertent reinforcement of CB (Lydon, Healy & Grey, 2017), as is the case with other distraction techniques such as provision of attention or tangibles. As such, whilst these techniques may reduce behaviours in the short term, it is important that behavioural function is considered to avoid inadvertent effects on maintenance of the behaviours.

There were a number of other interesting findings in relation to intervention strategies. Only one participant referred to receiving support from a behavioural therapist in the present study. This contrasts with the aforementioned US national survey (Wheeler et al., 2015): support from a behaviour therapist for aggression had been accessed by 30% of the participants, with 71% reporting some level of success with this approach. The effectiveness of this approach has been further supported through direct research (for example: Moskowitz et al., 2011). Given that CBs in FXS are often socially mediated (Hardiman & McGill, 2017), access to this support would be likely to be beneficial. In addition, there was a lower rate of reported use of more restrictive interventions, such as restraint or use of medications, when compared to the PWS sample (Tunnicliffe et al, 2016); though two families reported the use of physical

restraint for their son with FXS (both reported that the use of this intervention depended upon the perceived risk to harm to the child or others).

Linking back to the FXS phenotype. There are a number of aspects of FXS which may relate to the pattern of behaviours observed. In light of previous interest in the relationship between arousal and CBs, this potential influence is initially considered. Based upon previous literature, it was hypothesised that heightened physiological arousal may play a key role in the occurrence of CBs: through establishing a motivation to escape from or avoid situations which create aversive arousal states. This association has been explored in relation to demands. However, a number of situations may result in aversive arousal increases for individuals with FXS. For instance, it has been shown that changes to routine and expectation are aversive for individuals with FXS and may lead to displays of anxiety (Woodcock, Oliver & Humphreys, 2009). It is also possible that, similarly, being denied access to a desired item or activity results in an elevated physiological response. Alternatively, engaging with a tangible item for a long period of time may be associated with the absence of demands and social interaction, which may be anxiety provoking or result in aversive arousal states.

Of note, however, anxiety and arousal were rarely explicitly mentioned by parents. A number of respondents did refer to precursor behaviours which may indicate elevated physiological arousal (reddening of the face and ears: 8.33%; increased movement: 28.33%; appearing agitated: 8.33%). However, similar precursors were also mentioned in the PWS sample (Tunnicliffe et al, 2014), including increased movement (28.57%) and increased arousal (14.29%). As such, evidence from these small samples does not provide support for a heightened link between antecedent arousal and subsequent CBs, relative to other groups. Of note, however, is the implied link to arousal

through the high use of perceived calming strategies like deep pressure squeezing. In addition, anxiety was only mentioned as an emotion associated with meltdowns for a quarter of participants, despite its hypothesised importance in CBs. However, it is possible that such internal changes are not readily identified by others. Alternatively, in the context of enduringly high anxiety (both for the children and for the mothers, who themselves may be at risk for increased anxiety associated with the FMR1 premutation: Wheeler et al, 2014) its presence is seen at the norm, and so its effects are less readily identified.

Relatedly, reduced vagal tone is a robust observation in males with FXS (Klusek et al., 2015). The capacity to manage challenges depends upon the ability to regulate defensive systems such as the autonomic 'fight or flight' response and blunt its maladaptive manifestations. The vagal brake is thought to be a key aspect of this neural regulation (Porges & Furman, 2011). As such, the behaviours described may represent a deficit in these regulatory processes, similar to that which may be apparent in PWS (Manning et al., 2016). This may correspond to the similarity between the nature of at least a sub-set of the present behaviours and those described in young, typically developing children (Potegal & Davidson, 2003; Potegal et al, 2003).

There are a number of alternative or additional explanations which may be related to the pattern of behaviours observed. Individuals with FXS exhibit attention deficits, including reduced inhibitory control (Hooper et al., 2008). More broadly, impulsivity has been identified as a risk-marker for aggressive behaviour in adults with ID (Crocker, Mercier, Allaire & Roy, 2007). In the context of the present study, behaviours which frequently occur without clear precursors in response to challenges may represent a deficit in inhibiting reactions to antecedent challenges or urges (such

as desiring a tangible item). Similarly, impulsiveness and inattention may be associated with the high reported success of distraction in avoiding behaviours. In addition, a number of parents reported that behaviours occurred when the child was told 'no' or didn't get their own way (which often but did not always relate to access to tangible items). An alternative explanation for this pattern of behaviours relates to the high preference for predictability seen in individuals with FXS (Woodcock, Oliver & Humphreys, 2009), which is believed to be associated with anxiety caused by unexpected changes. It may be that the desire to "have their own way", reflects this preference for predictability and that "not getting their own way" results in an aversive state which establishes motivation to engage in behaviours which result in increased control. Of note, the explanations presented are only a few of the possible explanations which may be associated with the occurrence of CBs in this group. Future research would be required to test these hypotheses.

A number of physiological, social and environmental variables may act as establishing or abolishing operations for CBs (McGill, 1999). Of relevance to the FXS phenotype, for whom sleep difficulties may be common (Kronk et al, 2010), parents identified tiredness as being the most common setting event (identified for 75% of participants). In addition, if individuals with FXS are experiencing chronic arousal elevations, it is possible that this may contribute to fatigue and exacerbate CB. Though, tiredness was similarly associated with temper tantrums commonly in the PWS sample (71% participants). Furthermore, in accordance with the wider literature suggesting the importance of pain in the occurrence of CBs (Carr & Owen-DeSchryver, 2007), gastrointestinal issues (which may be a particular concern for individuals with FXS: Kidd et al, 2014) were cited as playing an important role in behaviour for two

individuals. This highlights the need to consider pain as a possible factor in CBs, particularly when there are changes in frequency or severity (Further Inform Neurogenetic Disorders, 2018; Challenging Behaviour Foundation, 2016). Of note, however, setting events described may not accurately reflect the full variety of influences which may alter the motivation for particular reinforcers, and the frequency of associated behaviours. Parents were asked an open question to describe setting events for behaviour and, as such, it may be that more normative attributions were made.

Limitations. This study extends upon previous literature through the collection of detailed descriptions of the behavioural and emotional sequences of CBs in FXS, as well as reported management strategies and their effectiveness, and supports the development of hypotheses to be explored in future research. However, there are a number of limitations to be accounted for when interpreting the results. Primarily, the generalisability of the findings are limited by the small sample size. The interest expressed by potential participants highlights that there is scope and interest in further extending this line of research in the future, in order to help address this issue. In addition, as mentioned previously, the advertisement for participants with children who exhibit behavioural problems may lead to a self-selecting group with more severe behavioural challenges, as such it is unclear whether these findings generalise to all individuals with FXS who exhibit CBs.

In addition, a key limitation of the current study is the reliance on retrospective recollections, and may also have been influenced by the researcher through the semi-structured interview format. Though the convergent validity of the semi-structured interview used by Tunnicliffe and colleagues (2014) was supported through the use of

behavioural observations for a number of their participants, the validity of interview findings were not confirmed in the present study. However, a number of amendments were made to the interview format in the present study in order to account for syndrome-specific characteristics of FXS, as well as to use language more colloquially used in this community (as fed back through consultation with relatives of individuals with FXS during the research design). Though a number of participants had previously taken part in earlier observational research by the authors (see Chapter 4), a substantial amount of time had passed (between approximately 2.5 to 3.5 years) since the observations, which meant that their utility for comparison purposes was invalidated. In addition, the amendments made to the interview format limits the comparability with the sample of individuals with PWS (Tunnicliffe et al, 2014); for instance, it is possible that 'meltdowns and CB' is interpreted differently to 'temper outbursts' and therefore represents a different class of behaviour with differing functions. Similarly, the concurrent assessment of the occurrence of a broad range of behavioural topographies, which may mask differing functions or topography-function relationships.

Conclusion and future research. An aim of the present investigations was to explore parents' descriptions of CBs in a natural context in order to gain further insights to guide future research. The findings are consistent with a number of prior observations: such as low levels of attention-maintained behaviour and topographies of behaviour exhibited. However, the presence of demands were rarely discussed by parents, who instead highlighted that behaviours were most likely to occur when their child does not get their own way, which typically related to lack of access to food or tangible items. In addition to the potential role of atypical physiological arousal in this pattern of behaviours, it is suggested that further aspects associated with the FXS

phenotype should be considered. It is hypothesised that impulsivity and reduced inhibitory control may contribute to the behaviours observed. This broader range of influences should be incorporated into future models of CB in FXS.

Chapter 7

Implications and Future Research

Chapter Overview

In the final chapter of this thesis the findings of the literature reviews and empirical research conducted are summarised. Implications for the understanding of challenging behaviours (CBs) in Fragile X Syndrome (FXS) are initially discussed, including the presentation of a schematic model to incorporate phenotypic characteristics into the understanding of CBs in FXS. In addition, the wider significance of the results for practice and future research is considered. Included in the discussion are reflections upon some of the challenges experienced during the research, such as recruitment difficulties and saliva sampling issues; including their significance for related research and how they may be addressed. The over-arching limitations of the studies conducted as part of this project are reflected upon, as well as broader limitations of research in this field.

Thesis Overview

The operant learning model is widely used to understand CBs exhibited by people with ID (Beavers et al., 2013) and has led to the development of effective, function-based interventions (for example: Kurtz et al., 2011). However, as previously described, the operant learning model cannot account for the variations in presentation and prevalence of CBs across different genetic syndromes (Arron et al., 2011). Langthorne and colleagues (Langthorne, McGill and O'Reilly, 2007) proposed that the concept of motivating operations may act as a unifying concept to incorporate genetic influences into the behavioural model. Specifically, it was hypothesised that genetic

events have a motivative influence on some of the social and non-social consequences that maintain CB. Preliminary evidence to support this association has been collected through indirect (Langthorne & McGill, 2012) and experimental (Langthorne et al., 2011) functional analyses. However, there has been little investigation as to the pathways through which genetic events may have such a motivation-influencing effect.

Given that FXS is a condition with a well-established behavioural phenotype, it was selected as the focus to further explore phenotypic factors which may have motivative influences. Specifically, the role of physiological arousal has been of central interest, given earlier suggestions that exaggerated stress responses may lead to a heightened motivation to escape from stressors, in turn resulting in high levels of negatively reinforced CB (Langthorne et al., 2011). A number of investigations (systematic literature reviews and empirical research) have been conducted in order to explore behavioural function and arousal in FXS, as well as their possible association.

Implications for Understanding Challenging Behaviour in Fragile X Syndrome

Function of challenging behaviours.

Initially, the findings relating to behavioural function are summarised:

- *Systematic review (Chapter 2):* social negative functions were significantly more common than other functions of CB, with attention being the least common social function.
- *Observational study (Chapter 4):* whilst individual instances of CBs occurred most commonly across the group following demands, escape was not a more common primary function for participants' behaviours, when compared to others. In addition, SIBs most commonly appeared to be automatically reinforced.

- *Experimental study (Chapter 5):* During a structured demand, boys with FXS exhibited a taught response to access negative reinforcement (task break) significantly more frequently than children with ID. In addition, a greater proportion of the FXS group exhibited CB during sessions. However, limited other differences were observed in off-task behaviours.
- *Indirect assessment (Chapter 6):* though many parents endorsed that their son with FXS engaged in CB in response to demands, a wide range of antecedents to CB were reported. These included: changes to routine or expectation, waiting and not getting what they want. The most commonly identified primary antecedent was 'being told no', which typically related to preferred activities or tangible items.

The hypotheses on which the present projects have been based were developed based upon the existing literature (as described in Chapter 2). However, this is a developing field and recent research influences the interpretation of the results of the studies conducted. A number of recent or ongoing projects further inform this area of research and so are presented here in order to inform the interpretation of the results.

Provisional data presented by Frank-Crawford and colleagues (poster presentation: Frank-Crawford et al., 2016; M. Frank Crawford, personal communication, Feb. 22, 2018) describe a case series analysis of individuals with varying genetic conditions (Including: FXS, Down Syndrome (DS) and Cornelia De Lange Syndrome (CdLS) [70]) admitted to the Kennedy Krieger inpatient or outpatient program. Within-

[70] CdLS:, N=8, 75% male, mean 14.8 years (range 8.2-21.9 years). DS: N=37, 70.3% male, mean 13.9 years (range 3.1-38.2 years). FXS: N=11, 90.9% male, mean 9.6 years (range 2.9-15.8 years).

group findings are initially discussed. Of note, limited differences were observed. However, by a small margin, escape was the most common function within the FXS group: approximately 35% of participants, compared to approximately 25% for attention, tangible (toy) and automatic.

Further differences were observed between groups in the study, based upon visual analysis. Participants with FXS were less likely than other groups to engage in attention-maintained CB (approximately 55% CdLS participants and 45% DS participants, compared to approximately 25% of the FXS group). This corresponds to previous differences observed between individuals with Smith-Magenis Syndrome and those with FXS (Langthorne & McGill, 2012; Langthorne et al., 2011; Hardiman et al., in press). Individuals with CdLS were more likely to exhibit escape-maintained behaviour (55% of the group) when compared to other groups, but there was no difference between FXS and DS groups in this regard (approximately 35%). Similarly, in earlier research no clear differences between the frequency of escape-maintained behaviour were observed between individuals with FXS and those with ID (Langthorne & McGill, 2012) or those with SMS (Langthorne & McGill, 2012; Langthorne et al., 2011; Hardiman et al., in press).

Furthermore, Hall and colleagues have conducted experimental functional analyses with a group of adolescents, either with FXS (males) or ID, who exhibit severe CB. In addition, measures of physiological arousal (including salivary cortisol) were collected across the experimental sessions (S. Hall, personal communication, February 23, 2018; NIH project ID: 5R21HD072282-02). Though, results of this project are not yet available. In addition, the research team are also conducting a project to evaluate 'Treatment of Disruptive Behaviors in Fragile X Syndrome' (John Merck Fund

Developmental Disabilities Translational Research Program (2016-2020)[71]), which involves in-home, parent-conducted functional analyses. Whilst full data is not yet available, provisional findings (15 males, age 3- 10 years) suggest that functions for CB include: escape from demands and transitions, as well as access to tangible items (Monlux & Hall, 2018, May: up-coming conference presentation). More information from these projects will clearly be of benefit to furthering the understanding of within and between-group patterns of behavioural function, relating to FXS.

Therefore, there appears to be a shift in the pattern of findings as further research emerges in this area. A trend which remains consistent is the low prevalence of attention-maintained CB, both within FXS and compared to a variety of other syndromic groups (SMS, DS and CdLS), as well as those with ID. In contrast, whilst earlier findings appeared to demonstrate the primacy of escape as a social function for behaviours, this pattern appears to have become less prominent with further data, particularly in comparison to other groups. Although there were some suggestive findings in the present thesis to support the hypothesis that motivation for demand escape is elevated, any changes observed were relatively subtle. Of interest, patterns of escape responding in Chapter 5 suggest that motivation to escape from demands may be elevated in FXS relative to ID, though it is unclear how this generalises to naturalistic behaviours. Furthermore, whilst the focus of the present thesis has been upon escape from demands, the findings highlight that escape-maintained behaviours may be elicited in FXS by a wide variety of other situations, including changes to routine or transitions. An emerging theme through studies utilising experimental functional analyses, as well as parent report (Chapter 6) is that CBs associated with tangibles appear to be similarly

[71] https://profiles.stanford.edu/scott-hall

common to escape-maintained behaviours. It remains unclear whether the within-group prominence of escape- and tangible-maintained behaviours is just an outcome of the diminished motivation for attention, as opposed to a direct effect relating to the reinforcing value of escape or tangibles. Further research will be required in order to investigate this issue further.

Another notable area requiring further research is the potential for topography-function relationships. Of particular interest is the function of self-biting: a topography of SIB which was commonly reported in earlier literature (reviewed in Chapter 2), as well as in the present studies through direct observations (Chapters 4 and 5) and parent report (Chapter 6). It is unclear whether the function of this behaviour may probabilistically differ from other SIBs or types of CB. Of interest, observations in the natural environment (Chapter 4) suggested that SIBs (which primarily consisted of hand-biting) were commonly automatically reinforced. Further research is warranted to establish whether the nature or reinforcing value of automatic consequences to biting are quantitatively or qualitatively altered in FXS.

Physiological arousal and its association with challenging behaviour in Fragile X Syndrome. Investigations were conducted in order to explore autonomic and neuroendocrine arousal in FXS, including its association with escape-maintained CB. It has previously been established that males with FXS exhibit a robust profile of cardiac autonomic activity, characterised by elevations in sympathetic activity and reduced parasympathetic regulation (Klusek et al., 2015).

- *Systematic review (Chapter 3):* previous research is characterised by its variability, with a number of studies in both animals and humans demonstrating

no effect of genotype upon cortisol levels. However, there is a trend towards males with FXS exhibiting elevations in cortisol levels (either increases at baseline or reduced diurnal decline, suggesting an increased response, following stressors).

- *Circadian rhythm (Chapter 4):* though there were no differences in daytime cortisol levels between boys with FXS and siblings, those with FXS exhibited blunted or absent cortisol responsiveness in response to awakening.
- *Reactivity (Chapter 5):* there was no evidence of a cortisol response to the presentation of a challenging demand, in either males with FXS or children with ID, or group differences in the findings. This finding was consistent even when results were evaluated at the individual level.
- *Parent report (Chapter 6):* parents did not commonly report an association between their child's CBs or meltdowns and anxiety or arousal.

In sum, in contrast to previous suggestions there was limited evidence for elevated physiological stress responses to stressors through the research. However, as previously discussed, hypocortisolism may complicate the interpretation of these results. In addition, no associations between physiological arousal and CB or escape-maintained behaviour were demonstrated during the studies conducted (as described in Chapters 4 and 5). Though, as discussed through the thesis, there are a number of methodological factors which may have contributed to this inability to detect associations. It remains possible that an association between CBs and/or escape-maintained behaviours and arousal exists in FXS, though further analyses are warranted. Considerations for future research are discussed later in the chapter. Of interest for this research question will be the findings of Hall and colleagues in their

investigation of physiological arousal and behavioural function collected during experimental functional analyses (NIH project ID: 5R21HD072282-02).

Influences upon challenging behaviours in Fragile X Syndrome. The limited findings relating to arousal and escape maintained behaviour across the present thesis, alongside the broad variety of situations in which CBs were reported and observed, highlight that the initial explanatory model relating to arousal and escape-maintained behaviour (Figure 11, p.111: Langthorne, 2009; Langthorne et al., 2011) is insufficient. Rather, a broad range of aspects of the FXS phenotype may relate to the occurrence and function of CB. Initially, it is important to highlight that individuals with FXS exhibit a broad range of risk factors which contribute to the likelihood of CBs being exhibited, a number of which are highlighted below. It is possible that the associations between such characteristics and CBs are similar to other groups, but differ quantitatively due to the FXS phenotype. Alternatively, it is possible that qualitative differences in the associations exist. In addition, aspects of the FXS phenotype may exert a motivative influence and result in the profile of behavioural function observed. The aim of the following section is to discuss these potential influences on understanding CB in FXS. A summary, multi-level, schematic model of potential influences upon CBs in FXS is presented in Figure 48 (Morton, 2004; Oliver et al., 2013). Of note, connections in the model represent a non-exhaustive range of hypothesised indirect or direct relationships, which require further research.

Firstly, as a result of broad neuronal changes associated with a lack of FMRP (such as impaired dendritic spine development and maturation, as well as wide excitatory/ inhibitory imbalances: Irwin, Galvez & Greenough, 2000; Bear, Huber and Warren, 2004) individuals with FXS, particularly males, typically present with an ID

(Hess et al., 2009). In addition, FXS is associated with characteristic deficits in expressive communication (Roberts, Mirett & Burchinal, 2001). In the wider literature, these characteristics have been associated with risk for engagement in CBs (McClintock, Hall & Oliver, 2003). In the absence of effective communication, individuals possess less adaptive means of communicating needs and accessing reinforcement. As such, other maladaptive topographies of behaviour (such as CBs which are socially salient and therefore likely to elicit a reaction) may be exhibited as functional alternatives.

Attention deficits are also associated with FXS, with a distinct profile of impairment characterised by high rates of inattentiveness, restlessness, distractibility, impulsivity (Turk, 1998) and reduced inhibitory control (Hooper et al., 2008). Impulsivity has been identified as a risk-marker for aggressive behaviour in adults with ID (Crocker, Mercier, Allaire & Roy, 2007), and may similarly contribute to behavioural challenges in FXS. Hyperactivity is also frequently observed (Baumgardner, Reiss, Freund & Abrams, 1995) and has been found to predict the frequency of aggressive acts in males with FXS (Wheeler et al., 2015).

Broadly, the presence of increased autism symptomatology has been associated with greater likelihood of a variety of CBs in individuals with ID, including: SIB, aggression and disruption to the environment (McClintock, Hall & Oliver, 2003). FXS is associated with high levels of autistic-like behaviour (particularly stereotyped behaviour, repetitive vocalisations and gaze avoidance: Hall, Lightbody, Hirt, Rezvani & Reiss, 2010). Autistic behaviour has been positively associated with behaviour problems in individuals with FXS (Hatton et al., 2002), though not consistently (SIB: Hall, Lightbody & Reiss, 2008. Aggression: Wheeler et al., 2015). Of note, autistic-like behaviour in FXS appears to be causally linked to anxiety and hyperactivity (particularly

in individuals with decreased intellectual ability who may be less able to employ adaptive, compensatory strategies to manage anxiety during social interactions), more closely than in idiopathic autism (Abbeduto, McDuffie & Thurman, 2014). Similarly, therefore, the association between autistic and CB may quantitatively or qualitatively differ when compared to other groups.

More broadly, anxiety is likely to be a key influencer of CB in this group. Anxiety is a striking feature of the FXS phenotype and, whilst manifesting most commonly as social phobia, may be elicited in response to a broad range of stimuli (Cordeiro et al., 2011). Situations characterised by unpredictability (including changes to routine or expectations) and unfamiliarity, are reported to be triggers for anxiety, potentially as a result of challenges which such situations place on cognitive and attentional systems (Woodcock, Oliver & Humphreys, 2008). This characteristic is thought to relate to changes to the amygdala and other limbic structures (Schneider et al., 2009). In a large survey, increased anxiety was found to predict greater severity of aggressive acts (Wheeler et al, 2015). In addition, SIB has been found to co-occur with displays of anxiety (Woodcock et al, 2008). An individual's specific phobias and situations triggering anxiety should be considered and incorporated into functional assessments for individuals with FXS. It is possible that escape-maintained behaviours are more likely to occur in anxiety-provoking situations, or that it acts as a broader establishing operation for CB.

Furthermore, a particularly high degree of repetitive behaviour is observed in FXS (such as motor stereotypy: Moss, Oliver, Arron, Burbidge & Berg, 2009), which has been associated for risk in engagement in SIB for individuals with ID (Oliver, Petty, Ruddick & Bacarese-Hamilton, 2011). Such behaviours may relate, at least in part, to the

caudate nuclei (CN), which have been implicated in atypical repetitive behaviour (Lewis & Kim, 2009). Those with FXS who exhibit lower levels of FMRP exhibit greater abnormality (increased volume) of the CN (Gothelf et al., 2007) Accordingly, both SIB and repetitive behaviours have been linked to increased CN volume in FXS (Wolff, Hazlett, Lightbody, Reiss & Piven, 2013), demonstrating a gene-brain-behaviour relationship. It is likely that the presence of such traits in FXS may be linked with the elevated risk for SIB in FXS relative to other diagnostic groups (Arron et al., 2011), and other types of CB (Hardiman & McGill, 2018). The reasons for the elevated levels of self-biting as a specific form of SIB have not been delineated, though suggests that the automatic consequences for this behaviour may be qualitatively altered, or their reinforcing value increased. As discussed in Chapter 2, it is possible that biting or chewing has an automatic arousal-management consequence.

A number of further risk factors for CBs should also be considered. It is likely that an individual's wider genetic background also influences the likelihood of occurrence of CB. In the general population, varying polymorphisms of a serotonin transporter gene (5HTTLPR) have been implicated in aggressive behaviour and similarly appear to mediate the occurrence of such behaviours in FXS (Hessl et al., 2008). There are likely to be as yet further un-identified polymorphisms which similarly mediate risk for a variety of CBs. Physical health is also important (De Winter et al., 2011), and specific health conditions associated with FXS should be considered as a priority with changes in behaviour (Kidd et al., 2014). Finally, the individual's environment and the nature of responses to CBs are known to be important in mediating the occurrence of CBs (for instance, Oliver et al., 1993). As noted in Chapter 6, it is possible that the wider familial effects of Fragile X, such as maternal anxiety or

atypical physiological responding to child behaviour problems as a stressor (Hartley et al., 2012), may affect the social responses to CBs and associated operant learning.

In addition to the presence of these broad risk-factors, there are also aspects of the phenotype which may shape the operant conditioning of these behaviours, through altering the experience of particular environmental conditions and the value of associated consequences to behaviours. A number of theoretical explanations have been discussed through this thesis, and are summarised below. Firstly, as previously highlighted, individuals with FXS seem to reliably exhibit relatively lower prevalence of attention-maintained CBs when compared to other groups (Langthorne et al., 2011; Langthorne & McGill, 2012; Frank-Crawford et al., 2016) and to other social functions (Hardiman & McGill, 2016). Of note, individuals with FXS may exhibit atypical fear signalling (due to brain changes e.g. to the amygdala: Schneider, Hagerman & Hessl, 2009) and physiological responses (Farzin et al., 2009, 2011; Hessl et al., 2002)) in response to social interactions. Together, it is hypothesised that these characteristics diminish the value of attention as a reinforcer. However, given that low levels of social-escape behaviour are observed (for instance, Langthorne et al., 2011) it does not appear that interaction represents a highly aversive stimulus sufficient to elicit CBs. However, other topographies of social escape behaviours, such as gaze avoidance, are commonly observed in the group (for instance, Hall et al., 2006).

As outlined in Chapter 2, there are a wide variety of aspects of the FXS phenotype which may make interaction with the environment more challenging, and so be associated with escape-maintained CB. As previously discussed, it is unclear whether escape-maintained behaviour is elevated in this group, though this remains a common behavioural function. Regardless, it is possible that the situations from which

individuals with FXS may be motivated to escape are shaped by aspects of the phenotype. Whilst the focus of the present thesis has been largely upon CB in the contexts of academic demands and its relationship to physiological arousal, a wider range of influences need to be accounted for. For instance, sensory sensitivities may also make environments more challenging (Stackhouse et al., 2014), and contribute to the occurrence of behaviours, including aggression (Wheeler et al., 2015). Environmental conditions such as transitions, waiting, changes to routine and expectations may all be made more aversive as a result of attention deficits (Woodcock et al., 2009; Moon et al., 2006). Similarly, it has been proposed that attention problems may contribute to the motivation to escape from or avoid demands (Kurtz et al., 2015). In addition, ID and working memory deficits (Munir, Cornish & Wilding, 2000) may mean that processing information relating to demand or the environment more broadly is more challenging. These challenges may also be exacerbated by atypical physiological responding to challenges (autonomic and/or endocrine), which further elevates their aversiveness. Jointly or in isolation, these factors may mean that the motivation to escape from or avoid a variety of situations is established.

As previously highlighted, an emerging theme of recent research into behavioural function is the frequency of tangible-maintained behaviour in FXS. A number of hypotheses relating to this pattern are discussed in Chapter 6, and are briefly summarised. Speculatively, attention deficits and resistance to change may be associated with the frequent reports of the primary antecedent to CBs being the child being denied what they want or being told no. Alternatively, interaction with tangible items may result in the absence of challenging demands or social interactions. Further contributors to tangible-maintained behaviour warrant further investigation. However,

as with escape-maintained behaviour, there is not clear evidence to support that this function is elevated in FXS, relative to other groups.

A theme across a number of the influences upon CBs in FXS described above, is the influence of physiological arousal. Atypical physiological arousal (of the autonomic or endocrine stress-effector systems: as described in Chapter 3) has been hypothesised to be directly and/or indirectly associated with a variety of key features in FXS, including: anxiety, autistic-like behaviour and sensory issues. As discussed above, such characteristics are likely to contribute to the occurrence of CBs in this group, suggesting an indirect relationship between arousal and CB. Furthermore, increased arousal, including reduced vagal tone, may contribute to difficulties with behavioural regulation (see discussion in Chapter 6). In the broader literature, arousal differences have been theoretically linked to CBs (as reviewed by: Cohen et al, 2011), with varying results in empirical research (see discussion in Chapter 2). Though associations were not able to be demonstrated through investigations conducted as part of the present project, atypical physiological responding may be directly or indirectly associated with an elevated motivation to escape from or avoid stressors (such as demands, transitions), or be associated with CB more broadly in FXS.

In summary, a broad range of factors associated with the FXS phenotype may contribute to the occurrence of CBs, such as self-injury and aggression (Figure 48[72]). The shaping and reinforcement of these behaviours may also be influenced by motivational changes associated with the phenotype, though this research is in its infancy. Of note, a number of the factors discussed above are likely to be inter-related

[72] The format of the model (based upon Morton, 2004) was amended in order to more clearly highlight emotional influences.

and further research is warranted to explore which aspects are most important in shaping the occurrence of such behaviours. We may have identified many of the factors influencing CBs in this review, however the processes by which these influence the development and maintenance of CB are not well understood.

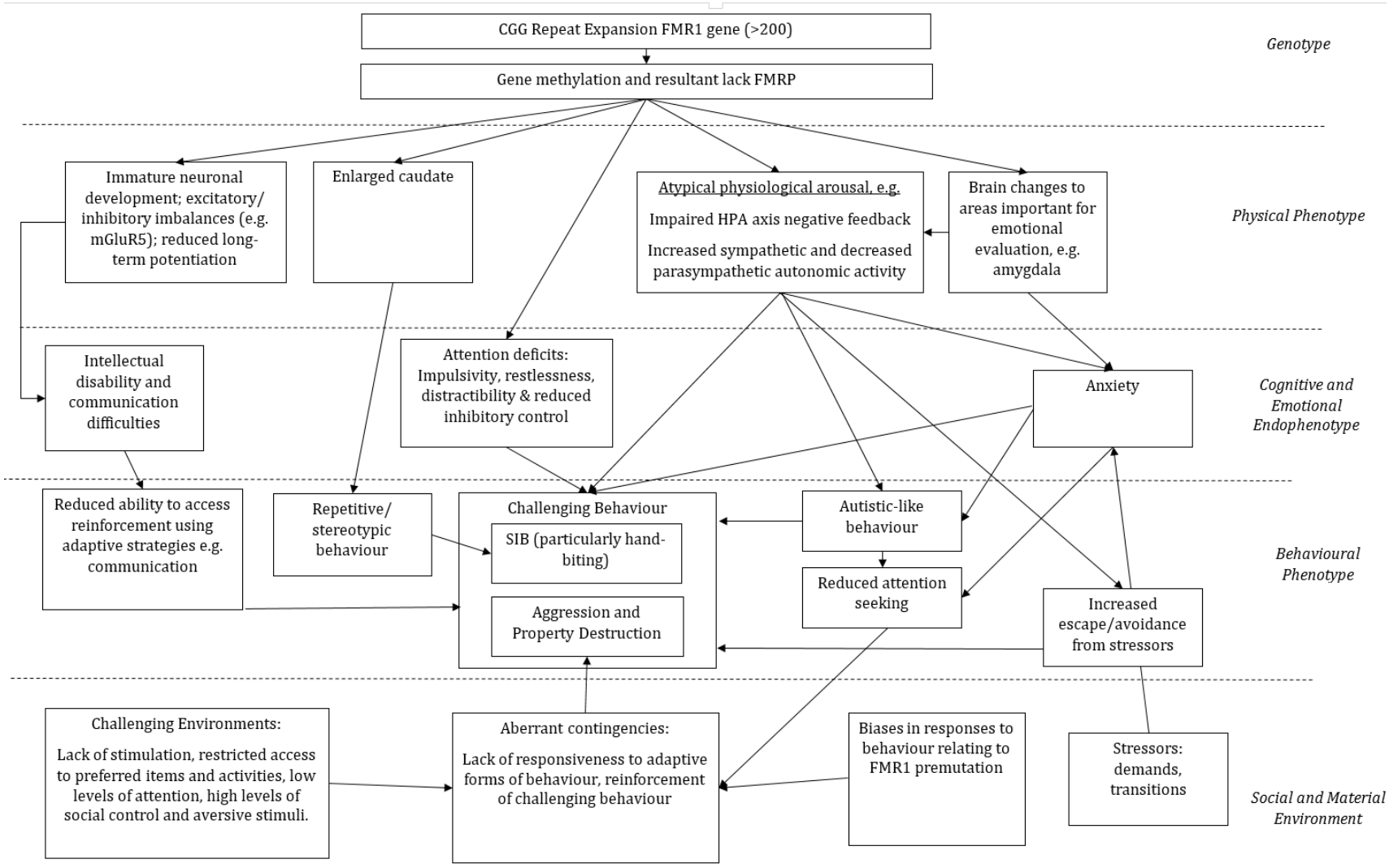

Figure 48. Schematic model outlining possible influences upon challenging behaviours in Fragile X Syndrome.

Implications for Practice

Use of behavioural interventions for individuals with Fragile X Syndrome.

There exists strong evidence that the CBs of individuals with FXS may be socially mediated (Hardiman & McGill, 2017). Accordingly, a number of studies have confirmed that function-based interventions are effective at reducing behavioural problems in this group (Kurtz et al., 2015; Moskowitz et al., 2011). Preliminary data from a number of ongoing studies further support this finding (M. Frank-Crawford, personal communication, Feb. 22, 2018; Monlux, Bujanda, Pollard & Hall, 2016. Up-coming conference presentation: Bujanda & Pollard, 2018, May[73]).

However, the significance of genetic diagnosis for the success or required content of the interventions has not been assessed. Though there may be biases within the group (increases in escape-maintained behaviour) and between FXS and other groups (decreases in attention-maintained behaviour) in the likelihood of different behavioural functions, these changes are relatively subtle and a wide variety of functions of behaviour are also observed. It is clear that the need for individual functional assessment is not precluded by any biases or motivational changes relating to genetic syndromes. However it is possible that the effectiveness of particular approaches to behavioural intervention may vary, when compared to implementation more widely in individuals with ID. Moskowitz and Jones (2015) reviewed 31 studies where behavioural interventions (targeting a variety of skills and problem behaviours) included at least one participant with FXS. In 45% of the studies that also included others without FXS (20 studies), a different response to the intervention was seen in FXS when compared to ID/ idiopathic autism. The authors suggest that this may

[73] https://www.abainternational.org/events/program-details/event-detail.aspx?intConvId=51&by=CE&date=05/28/2018

demonstrate that interventions would benefit from being informed by and tailored to the FXS behavioural phenotype. However, the nature of the differences in response to intervention varied, and so may represent random variation. Interestingly, early data from Frank-Crawford and colleagues (M. Frank-Crawford, personal communication, Feb. 22, 2018) suggests 'very few differences' in treatment components included in intervention plans, between individuals with FXS, DS or CdLS. Though full information, including variations in effectiveness between groups, is not yet available to evaluate this further.

There are a number of aspects of the FXS behavioural phenotype which may influence the conduct or effectiveness of behavioural intervention. For instance, it has been suggested that those with FXS may benefit from more incidental approaches to learning (such as observation) when compared to the more typical structured, direct approach of behavioural interventions (Hagerman & Hagerman, 2002). Though without experimental validation, this may suggest that strategies such as video modelling (requiring less direct social interaction) may be of particular value. Morris, Kondratenko & Griffiths (2014) helpfully summarise the FXS behavioural phenotype and its implications for Applied Behaviour Analysis (ABA) practitioners. For instance, learning tasks are more effective when visual aids are incorporated, as this capitalises on strengths in this group (Schwarte, 2008). Similarly, visual aids may be of particular benefit to be incorporated into CB interventions. Arousal-related differences are discussed in more detail below, but Morris and colleagues (2014) highlight the importance of considering arousal when intervening in escape maintained behaviours in FXS. Namely, they propose that extinction techniques may be less effective or even counterproductive in high-arousal situations, if the underlying cause is not identified

and managed. It may be that alternative approaches such as the differential reinforcement of other behaviours, or managing the environment to reduce the initial anxiety or hyperarousal, may be more effective.

The present findings, alongside the previous literature, suggest that the motivation to access attention may be diminished in FXS. Such information may be useful when considering choice of reinforcers during behavioural interventions. Similarly, preliminary evidence (as indicated by elevated escape responding, described in Chapter 5) that an enduring EO for escape may be apparent has implications for the choice of management strategies. For example, the risk of inadvertently reinforcing behaviours through contingent time-out may be greater in FXS, compared to other groups. In addition, knowledge regarding these potential motivational changes and the wider FXS behavioural phenotype is likely to be of value when investigating behavioural function, and deciding which avenues to explore and prioritise.

Arousal intervention. Of course, should the display of CB in FXS be causally related to arousal, then implications for intervention should be explored. Eliminating or mitigating these physiological conditions may strengthen the impact of behavioural interventions, whose effectiveness may be impeded by their presence (Cohen et al., 2011). Alternatively, there may be direct behaviour-reducing benefit resulting from proactive arousal management; it is possible that strategies to reduce stress and maintain arousal may diminish the occurrence of CBs, particularly in high arousal situations (such as demands, transitions, or being told 'no'). Scherr and colleagues (2016) highlight that individuals with FXS are likely to benefit from targeted arousal-reducing interventions, such as the teaching of coping skills or relaxation techniques.

Mindfulness-based interventions include three major components: self-reflection (such as identification and labelling of one's emotions and sensations), mind-body relaxation exercises (such as breathing exercises and progressive muscle relaxation) and self-regulation (utilising the former aspects to respond to and regulate mood and behaviour: Roberts, 2010). In the general population, mindfulness-based therapy has been shown to reduce anxiety symptomatology (Hofmann, Sawyer, Witt & Oh, 2010) as well as cortisol levels (Lengacher et al., 2012). Mindfulness interventions may be adapted to be more accessible to people with ID (Gore & Hastings, 2016; Roberts, 2010; Singh et al., 2011). Of interest to the present discussion, the effectiveness of a mindfulness intervention has been evaluated for individuals with William's syndrome (WS); a condition which shares features with FXS, such as anxiety and inattention. Adults with WS undertook mindfulness activities for 20 minutes per day, over five days. Acute effects upon reduced self-reported anxiety and concurrent reduced cortisol levels, were observed across individual sessions. In addition, across the week, reductions in SAA were observed. Therefore, these data support that psychological interventions may have a beneficial effect on reducing physiological indicators of stress.

In addition, preliminary evidence suggests that mindfulness interventions may be effective for reducing CBs in individuals with ID. In a randomised waiting list control trial of a mindfulness intervention, Singh and colleagues (2013) observed significant reductions in verbal and physical aggression relative to baseline, in adults with borderline or mild ID. Furthermore, Roberts (2010) reports clinical experience of reductions in SIB and aggression in individuals with more severe IDs, though unfortunately supporting data are not presented. The function of CBs was not assessed in any of the studies. However, it is possible that the effectiveness of such interventions

is mediated by function of the target behaviour. Namely, it may be that such calming techniques may be most effective when CBs relate to escape from high-arousal situations. The effectiveness of the use of relaxation techniques, such as those relating to mindfulness, have not been evaluated in FXS, either in relation to CB or more broadly. However, similar techniques have been recommended, including use of a 'Calm Down Book" which contains brief, visual prompts for simple relaxation exercises (for instance: Epstein, 2016). It is possible that such interventions may form an effective part of interventions for CB in FXS, given that self-management in response to stressors (including demands and not having access to desired items) may be compromised.

Sensory integration approaches are widely utilised for individuals with FXS to manage stress and arousal; 'sensory diets' are often implemented which may include a diverse range of elements such as weighted pressure, deep pressure squeezing or chewable oral support (Stackhouse et al., 2014). Accordingly, deep pressure squeezing was also commonly reported to be used as a management strategy for CB by parents in the study described in Chapter 6. The effectiveness of these interventions has not been assessed in FXS, and there exists limited support in ID more broadly (Lang et al., 2012). However, particularly given the propensity for escape-maintained behaviour within the group, it is important that reactive provision of any calming or sensory activities does not inadvertently reinforce CBs through contingent termination of tasks (Lydon, Healy & Grey, 2017)[74].

[74] Such a pattern of behaviour was observed relating to the hypothesised escape-maintained behaviour of one participant in the earlier observational study conducted as part of this thesis (Chapter 4).

Future research should investigate the effectiveness of using calming strategies (such as mindfulness techniques, given the earlier evidence base) in conjunction with and/ or in comparison to a behavioural approach in FXS. In addition, it would be of interest and value to investigate whether the outcome varies according to behavioural function. Should a relationship between arousal and escape-maintained behaviour be apparent, then one might expect calming strategies to particularly benefit reduction of behaviour with this function. Similarly, interventions for escape-maintained behaviour may be less effective if arousal issues are not concurrently managed. As described by Cohen and colleagues (2011) it may be that adequate assessment to understand and treat CBs will come to include a systematic, comprehensive range of influences, including environmental, genetic and physiological factors.

Implications for future research

Assessing arousal and challenging behaviour relationships. Through the present thesis a number of potential avenues for further research relating to arousal (autonomic and neuroendocrine) and behaviour in FXS have been proposed and discussed. Despite differences in behaviour and arousal being observed between groups in the studies described, no relationship between these two aspects was identified. It is likely that further investigation of CBs and arousal in natural settings, observing temporally and spatially relevant variables that reliably produce the behaviours of interest, will provide valuable insight as to any possible association (Cohen et al., 2011). Such investigations have been conducted with heart rate and SIB (for instance: Hoch, Symons & Sng, 2013; Lydon et al., 2013; Lydon et al., 2015) but warrant further investigation with individuals with and without FXS, including a broader range of behaviours with differing functions (for instance, as identified through experimental

functional analysis). In addition, repeating measurements over a number of observations may help to improve reliability of findings, as well as identify whether fluctuations in underlying physiology may be associated with changes in behaviour. In addition, longitudinal data (or more in depth cross-sectional data investigating the effect of age) may facilitate the understanding of the temporal nature of any associations between physiology and behaviour, and inform cause-effect determination (Cohen et al., 2011). Such investigations may also be of value in light of the possibility that cortisol-behaviour associations change over time, as a result of hypocortisolism (as discussed in Chapter 6).

As discussed in earlier sections, there are a number of challenges in this field of research, including: relatively disparate sampling times; responsivity of the measures of interest (inherent delay in detecting changes via saliva sampling, particularly for cortisol); small sample sizes (participant and sample numbers); and missing data. The implications of these specific challenges are discussed below.

Saliva sampling challenges and arousal assessment methods. Challenges with identifying physiology-behaviour relationships in the present study were compounded by issues relating to the collection of saliva samples. This approach was selected due to its relatively non-invasive nature and ability to assess multiple aspects of physiological arousal. Despite reported acceptability of this project and initial feasibility, in a later study in the school environment a large number of samples contained insufficient volume for analysis. Such challenges are not unique to the present study: a recent project experienced limited success (10% of 51 participants) of collection of saliva samples from children with ID in a home setting (Cooper, 2017). Whilst improved outcomes may be able to be achieved through greater participant

preparation and instruction, this experience demonstrated that collection and management of samples in the natural environment is challenging. Furthermore, as discussed above, in order to further the understanding of the relationship between arousal and behaviour in FXS further investigation of changes in arousal in relation to CBs are likely to be required. The collection of salivary measures at such times of high stress is likely to be more challenging still. As such, alternative measurement methods should be considered.

Developments in wearable technology may facilitate more simple collection of relevant data. Relating to autonomic measures, Yoon, Sim & Cho (2016) have developed a stamp-sized, flexible and wearable "stress monitoring patch" which collects multimodal information about the ANS (temperature, skin conductance, heart rate). Wristbands with similar functions are also available (Kupferschmidt, 2016). Such technology may facilitate the collection of continuous data relating to an individual's arousal state, without the need to disturb participants at times of high arousal in order to collect samples. Furthermore, given broader use of wider biofeedback technology relating to fitness and stress, such as Fitbit PurePulse™, such methods may address the concerns about embarrassment mentioned by some of the participants in the initial study (see Chapter 4).

However, fewer alternative measurement options exist for assessing stimulus-bound changes of cortisol: haematological assessment is notably more invasive when compared to saliva and urinary measures are less suitable to collect repeatedly in relation to a stimulus of interest. Alternative methods are available assessing trait cortisol levels, such as the analysis of hair as an indicator of chronic cortisol levels (Russell, Koren, Reider and Van Uum, 2012). The dual assessment of both the ANS and

HPA axis provides a more holistic picture of an individual's stress-related functioning, with additional insights relative to health and wellbeing (Ali & Pruessner, 2012). Therefore, future research may conduct real-time, stimulus-bound analyses of arousal in relation to CBs in FXS, as well as complementing with additional measures (salivary or hair) to gain a broader picture of the functioning of the HPA axis.

A further consideration relating to this area of research is causality: it remains unclear whether differences observed in measures of arousal relate directly to the FXS phenotype (e.g. through impairment of the negative feedback mechanism: Hessl et al., 2002) or are indirectly associated (e.g. presence of an intellectual disability leading to altered experience of environment and stressors). Further research with comparison groups matched upon key characteristics such as intellectual ability and autistic symptomatology is likely to be of value in this regard. However, to take the specific example of the HPA axis, it may be that such effects can only be teased out through directly challenging the system, such as through use of the dexamethasone suppression test. This test involves ingesting dexamethasone (an exogenous steroid), which binds to glucocorticoid receptors in the pituitary gland. Resultant regulatory modulation and decreases in cortisol levels should be observed (Cole, Kim, Kalman & Spencer, 2000), though hypothetically may be diminished if negative feedback is compromised in FXS. Though there are few side effects of the procedure, the test requires repeated blood draws and would be an unnecessary medical procedure to subject participants to for this purpose. Though, it is possible that such an examination may be able to be conducted with the syndrome mouse model (Koerner, 1997).

As previously discussed, hypocortisolism may occur in response to chronic stress and may complicate interpretation of findings and the detection of associations using

this measure. Methods of addressing this may be to conduct further longitudinal research with individuals with FXS. In addition, concurrent evaluation of autonomic indicators and the balance between the two systems is likely to provide further insight into the functioning of the stress-effector system as a whole (Ali & Pruessener, 2012).

Recruitment challenges. Challenges with recruitment were a substantial barrier when conducting this project. As previously described (see Chapters 4 and 5), the initial two studies both required recruitment efforts across a broad range of organisations and over a wide geographical area, taking longer than a year. In contrast, the latter study was able to recruit sufficient participant numbers in a very short time period. A notable difference between the studies which may relate to the differing response is the use of the saliva sampling, as opposed to an interview-only method. It is highly likely that the inclusion of saliva sampling discouraged many families from participating, despite those who did participate reporting its acceptability. Accordingly, in an international survey of families' perceptions of Fragile X research, the primary concern about research participation was that involvement would be too challenging for the individual with FXS. Specific examples given commonly related to physical procedures such as blood draws (Richstein, Cohen & Hardiman, 2017). If cross-disciplinary research including the collection of physiological measures is valuable, then methods about how to support this should be considered. Of course, the identification of simple and non-invasive methods for assessing measures of interest (such as the wearable technology discussed earlier in the chapter) is likely to be of benefit. In addition, respondents indicated that their primary concern relating to research was that the research had potential practical outcomes and was not being conducted 'for the sake of it' (Richstein et al., 2017). It is therefore essential that, whilst maintaining a realistic

and balanced view of more basic research such as that described, the potential practical applications and rationale for projects are made clear to potential participants.

Improving recruitment material is also likely to help reduce some of the concerns regarding participation. One such approach has been to create recruitment videos in collaboration with parents and participants in order to demonstrate the procedures involved and reduce the associated uncertainty or concern[75]. This corresponds to reports that families would prefer to receive information about Fragile X research opportunities via digital means (Richstein et al., 2017). Anecdotally, the feedback upon the video from Fragile X Society members was positive, though the impact upon participation was not evaluated. The design of initial study information materials was found to be significant in the present project, with the full information sheets (which fully outlines the potential risks involved) being potentially too daunting as a first point of contact.

Collaborations between clinical and research institutions are also key to facilitating participation. In the United States, a Fragile X Clinical and Research Consortium was established in 2006, with the primary aim of developing a network of expertise and local centres for those with Fragile X-associated conditions to access specialised support. However, the partnership also facilitates the harmonisation of measures and recording methods to enable the collation of large-scale datasets, via the Fragile X Online Registry with Accessible Research Database (FORWARD). Example outputs from this collaboration include information relating to health conditions (Kidd

[75] Crawford, H. (2017, Jul 18). *FXS Research Project.* [Video file]. Retrieved from: http://www.findresources.co.uk/research-into-behaviour-emotion-and-movement-in-males-with-fragile-x-syndrome. Created following workshop involving thesis author, parent representative and Dr Crawford.

et al., 2014). In these cases, with appropriate permissions, data may be collected as part of participants' clinical visits and so reduce participant burden. The network also facilitates the identification of potentially eligible participants for other studies. In addition, local collaborators are pre-identified and committed to ensure wide promotion of the project. Understandably, due to clinical workload, identification of individuals to act in this capacity for a small, single study in the present project was challenging.

In relation to the aforementioned recruitment issues, is the ethical consideration of the researcher's concurrent work as trustee or CEO[76] of the Fragile X Society, through which many of the participants were recruited. A number of participants in the latter studies of this project (once greater rapport had been built with the community) said their taking part had been influenced by a desire to 'give back' based upon the work conducted at the charity. Alongside the methodological differences, this is likely to have contributed to greater ease with recruitment in the later study. In addition, through all the empirical studies the researcher had a personal (friendships developed through joint working) or professional relationship (for example, they were a trustee of the employing charity) with a number of the participating families. The importance of researcher-community rapport and perceptions of shared collaborative goals (benefit for the community in question) have been previously recognised in terms of facilitating recruitment (Levkoff, Levy & Weitzmann, 2000). However, associated concerns with the present relationship, as in clinical research, include the perception that improved

[76] Commenced volunteering for the charity in September 2012 before being co-opted as a Director in December 2012 and being formally elected September 2013 at the charity AGM. The role of CEO commenced from October 2014. Thus, the initial empirical study was conducted whilst the researcher was a charity trustee, and the latter whilst CEO.

treatment (in this case, charity support) may have been accessible through participation (Steinke, 2004). This was managed in the present case through transparency and clarity in communications about the duality of the roles, and any requests for support were directed to appropriate colleagues (Family Support Workers), as would be in other cases. A number of practical steps were taken to ensure that the dual role did not benefit the researcher or compromise charity members or participants, such as: ensuring that projects were independently, externally reviewed by a specialist advisor prior to supporting recruitment; ensuring other members of staff managed database searches and study information dissemination; ensuring that communications relating to the research and charity used separate telephone numbers and email addresses. However, clearly the dual role raised a number of additional ethical considerations when compared to acting as an external researcher.

Limitations

As highlighted throughout the thesis, small sample sizes have been one of the primary limitations of this project, as well as research more broadly in this field. The hypotheses underpinning the present research were themselves based upon a relatively limited number of studies with small sample sizes and relatively subtle within and between-group differences. Though this was addressed through conducting analyses across studies (as in the review of the literature relating to CB, presented in Chapter 2), this in itself is problematic due to the differing methodology across studies. This is an inherent issue with research into rare genetic conditions (despite FXS being one of the more common amongst these). However, in light of this concern and the aforementioned recruitment challenges, further efforts to collaborate as a research community and harmonise measures to facilitate data sharing will all be of importance

to further the understanding and support for behavioural challenges in this population. In addition, the present research, as well as many related projects in this field, has focussed upon the behaviour of young males with FXS. Generalisability to adults and females with FXS is uncertain and requires further exploration.

Relatedly, the direct insight that individuals with FXS may be able to provide on their experiences has not, as yet, been formally explored. However, preliminary work with self-advocates at the National Fragile X Foundation (NFXF) conference (workshop hosted by: Braden, Cohen, Cohen, Epstein & Finucane, 2014[77]. Cited in: Cohen, Cohen & Cohen, 2015) demonstrated that young people and adults with FXS (N=40) were able to select and self-rate factors which were likely to trigger anxiety, as well as how it feels and how they behave when anxious. Of interest, the top 5 things which the workshops participants rated that they did when anxious were (most common first): avoidance, cranky, texting or fiddling, obsessions or repeating, aggression or hurting self[78]. More controlled investigations of this kind may provide insight into the associations between anxiety and behaviour in this group and whether such experiences quantitatively or qualitatively differ when compared to other groups.

In addition, whilst specific challenges relating to the collection of physiological data have been previously discussed (i.e. deficient measurement techniques), it is also possible that the challenge in identifying physiology-behaviour relationships results from the attempted use of simple measures to represent a complex system. It is clear that a wide range of factors are likely to influence the occurrence of CBs and, whilst

[77] A similar workshop was also hosted at the NFXF 2016 conference.
[78] 'Top 10' table available at: http://www.wecommunities.org/tweet-chats/chat-details/1547

arousal related changes may play a role in their expression in FXS, detection is likely to be complicated. This challenge is reflected in broader variability in behaviour-physiology findings, such as those relating to heart rate and SIB (Lydon et al., 2015; Symons et al., 2003; Verhoeven et al., 1999) as well as physiological reactivity in autism (Lydon et al., 2016). Similarly, the term 'arousal' has been used through the present thesis in order to refer to changes to stress-related physiology, including the autonomic nervous system and the HPA axis. However, it is likely that there are differing relationships with different aspects of these systems and behaviour and that discussing this issue as a single concept is overly simplistic.

A further limitation reflects breadth of the concept of CB. Namely, in the present thesis a broad range of topographies of behaviour have been considered concurrently (such as SIB and aggression), whereas such behaviours may have differing causal origins (for instance: Hall, McClintock & Oliver, 2003). By compiling these behaviours for the analyses assessing arousal-behaviour relationships, it is possible that more specific associations may have been missed.

Final comments.

The explorations conducted through this thesis have highlighted the need to gain a broader understanding of influences upon CB in FXS. Though no associations between physiological arousal and CBs have been identified, further investigations into this potential association and its implications for practice are warranted. The complexity of phenotype-environment interactions has also been highlighted in other groups. For instance, despite an enhanced drive for adult attention in Angelman's syndrome, as indicated by high levels of laughing and smiling which appear to serve the function of increasing adult attention (Oliver et al., 2007), this does not translate to elevated

attention-maintained CBs (Strachan et al., 2009; Radstaake et al., 2012). However, the utility of exploring syndromic influences upon behaviours are highlighted in recent investigations relating to skin picking in PWS: which is a prevalent topography of SIB seen in individuals with the condition (for instance, 86%: Didden Korzilius & Curfs, 2007). Functional analyses reveal that such behaviours are likely to have an automatic function (Hustyi, Hammond, Rezvani & Hall, 2013; Hall, Hustyi, Chui & Hammond, 2014). This has led to further investigations which highlight that such behaviours may represent an aberrant 'need to move' in low-arousal situations, suggesting atypical interoceptive sensation and homeostasis regulation (Hall, Hammond & Hustyi, 2013; Klabunde et al., 2015). Greater understanding of the pathological neural and reinforcement processes underlying such behaviours are likely to aid the identification of more effective interventions.

Within- and between-group differences in behaviour observed and discussed during the present thesis support the value of considering genetic diagnosis. In general, the field of ABA has been slow to integrate growing knowledge of genetic conditions into practice. However, this may be changing. A recent handbook for ABA practitioners (Griffiths, Condillac & Legeree, 2014) outlines the behavioural and physical phenotypes of eight genetic syndromes and their relevance to ABA. In addition, guidelines are provided for incorporating syndrome information into practice (Boyd et al., 2014; p. 258-262). Promisingly, a number of the recommendations relate to considering motivational differences which may be associated with conditions, and the implications which these might have when considering environmental manipulations, choices of consequences and required compensatory skills or behaviours. The creation of this resource represents a growing recognition of the importance of recognising behavioural

phenotypes in practice. As our knowledge of phenotype-environmental interactions deepen, the ability to put this knowledge into practice to best support individuals and families living with genetic syndromes will grow.

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Contents: Appendices

Appendix A

Methodological details relating to mouse corticosterone studies.

Study	Cort. measure	Method Sacrificed	Gender (M/F)	Mice per group	Strain	Age of Mice	Basal measure	Stress Condition(s)	Recovery Time	Time Tested
Ghilan et al. (2015)	Blood serum from trunk	Decapitation following anaesthetisation by isoflurane	M	7-18	C57Bl/6	55-65d	/	Restraint (conditions: 15m/ 30m/ 1h) or control	None: quick sacrifice after restraint	9am-11am
de Diego-Otero et al. (2008)	Blood plasma	Retro- orbital puncture	M	8-12	FVB-129	60-180d	/	Social stress (15m) or acute immobilisation stress (15m) or control	None: immediate sacrifice following behavioural. test battery	-
Lauterborn (2004)	Blood plasma from right ventricle	Overdose with ethanol	M	-	FVB*	-	/	Restraint (30m/ 2h) control.	-	10am-2pm

Study	Cort. measure	Method Sacrificed	Gender (M/F)	Mice per group	Strain	Age of Mice	Basal measure	Stress Condition(s)	Recovery Time	Time Tested
Markham et al. (2006)	Blood serum from trunk	Rapid decapitation	M & F	8-12	C57/Bl6	40-45d	Cagemate sham comparisons (no restraint, just moved to test room)	Restraint (30m) or control	Conditions: 0/ 15/ 60m	10am -12.30 pm
Nielsen et al. (2009)	Blood plasma from trunk	Rapid decapitation	M	5-12	FVB/NJ x C57/Bl6 (F1 hybrid)	11-12w	/	Swim Stress (3m) or open field (10m) or restraint (unspecified length). Each condition with control.	Swim: 17m. Open field: 10m. Restraint conditions: 0/ 30/ 60/ 90/ 120m	7am-9am
Qin, Xia, Huang & Smith (2011)	Blood plasma from trunk	Rapid decapitation	M	19-24	FVB/NJ	96±1d	/	Prior stress: chronic stress (2h/d restraint x10) or control.	-	-

Study	Cort. measure	Method Sacrificed	Gender (M/F)	Mice per group	Strain	Age of Mice	Basal measure	Stress Condition(s)	Recovery Time	Time Tested
								Acute stressor: spatial novelty (EPM)		
Qin & Smith (2008)	Blood plasma from trunk	Rapid decapitation	M	10-12	FVB/NJ	100±10d	2am, 6am, 10am, 2pm, 6pm, 10pm	Acute restraint stress (30/ 120 m) or spatial novelty (EPM 5m) or control	Conditions: 30/ 120m	Before 11am
Eadie et al. (2009)	Blood plasma from trunk.	Rapid decapitation	M	4	C57BL/6	-		Acute restraint stress (3 hours) or control	Immediate sacrifice following stressor	9am-1pm
Romero-Zerbo et al. (2009)	Blood serum	Cervical dislocation	M	10-11	FVB-129	90-120d		Open field	Immediate sacrifice following stressor	-

Appendix B

Study 1 Information Sheet

Tizard Centre, University of Kent

Canterbury, Kent, CT2 7LR

Researcher: Becky Hardiman

Email: rh432@kent.ac.uk

Tel. number: 01227 82 4770; 07948 047785

Supervisors: Peter McGill & Dr Alison Bratt

Email: P.Mcgill@kent.ac.uk;

A.M.Bratt-54@kent.ac.uk

Information Sheet

Title: *Investigating the Stress Response and Challenging Behaviour in Boys with Fragile X Syndrome*

Dear Parent,

Your children are being invited to take part in a pilot research study conducted by Becky Hardiman, who is a PhD student at the Tizard Centre. Her supervisors are Professor Peter McGill, a co-director of the Tizard Centre, and Dr Alison Bratt, from the Medway school of Pharmacy. Becky does not have your name and address.

Your family's participation in this study is entirely voluntary. Before you decide whether you wish for your children to take part, it is important for you to understand why the research is being conducted and what it will involve.

What is the purpose of the study?

The researcher is investigating challenging behaviours (such as self-injurious, aggressive and destructive behaviour) in boys with Fragile X Syndrome (FXS). This a study investigating whether there is a link between the stress response and challenging behaviours, in boys with FXS. As well as getting some preliminary information, this study will allow the researcher to develop the procedure for a larger follow-up study. When you find something stressful, your "fight or flight" response is activated, which helps you to deal with the situation in the short-term. The body also releases the hormone cortisol, to help your body deal with the stress in the long-term. In the short term these responses help you to cope with the stressful situation; however, abnormal or extended responses can be unhelpful. Previous research has suggested that boys with FXS have higher activity of these stress-response systems than their siblings. Furthermore, individuals with FXS may show challenging behaviours, such as hand-biting or physical aggression. Research has shown that individuals with FXS often use these behaviours in order to communicate that they wish to escape from things that they find unpleasant or stressful. It has been suggested that this pattern of use of challenging behaviours to escape might be related to the stress response systems, in boys with FXS.

What does the study involve?

You can directly measure the level of cortisol in saliva and you can estimate the "fight or flight" activity by looking at the amount of another chemical in saliva, which is called alpha amylase. Therefore, this study will involve measuring these chemicals in saliva, to investigate the stress response.

Saliva can either be collected using a foam swab (a child-friendly swab which will need to be soaked in the mouth for 1-2 minutes), by using a pipette to suck a sample of saliva

from the mouth, or by asking participants to spit into a provided container. Levels of cortisol and alpha-amylase change through the day, therefore six samples will be collected during a typical school day (e.g. without any trips or performances) in order to get a detailed picture of how they are released in people with and without Fragile X. Samples will be collected from your son with FXS and their sibling without FXS, in order to be able to compare levels of the chemicals between people in the same family. The samples will be taken between waking up and going to bed, including two samples before school: immediately after waking and 30 minutes after waking. These early morning samples will measure the Cortisol Awakening Response.

The researcher, Becky Hardiman, will spend a full day, morning until bedtime, with your son with Fragile X, including attending school with him and will collect the two saliva samples during school-time. At home, Becky will supervise a parent in collecting the samples. For the sibling group, arrangements will be made on an individual basis regarding collection during school-time. If you decide to take part in this study, we will request contact details for your children's schools, in order that we can send them information about the study, including how to collect and store the saliva, and request consent to conduct the research at their school. Questionnaires will be used to collect information from parents and children about their experience of the saliva collection, in order to highlight any changes that could be made to make the experience better for those participating in a future study.

A record will be kept of activities in which your son participates through the day. If any challenging behaviours (self-injurious, aggressive or destructive) occur during the observation, Becky will record what occurred immediately before the behaviour (e.g. the participant was asked to do something) or immediately afterwards (e.g. received adult attention), in order to try to understand the reasons behind the behaviour.

The researcher will also perform a short visit prior to the day of the study, in order to decide the most appropriate saliva collection procedure, to discuss the study procedure with your family, and to allow your son with FXS to meet the researcher. Furthermore, the researcher will ask you to complete a short questionnaire about autistic behaviour shown by your son with FXS; this should take approximately 10-minutes.

Why have I been contacted about this study?

Your family have been invited to take part as you have a son affected by FXS between the ages of 5-15 years old.

Is my family eligible to take part in the study?

In order to be eligible for the present study, your son must have been diagnosed with FXS by a professional and you will be asked to show proof of this diagnosis to the researcher. Furthermore, your son with FXS must display at least one form of challenging behaviour weekly. This might include: hand biting, self-hitting or other self-injurious behaviour; physically aggressive behaviour; or behaviour that is destructive to property. Furthermore, to be eligible your family must also have a child, between the age of 5 and 15 years, who does not have a diagnosis of FXS, any diagnosable intellectual impairment or emotional health needs.

What do I need to do if I wish my family to take part?

Firstly, if you have any questions about the study please contact Becky (rh432@kent.ac.uk; 07948047785; 01227824770), who will happily provide further information. If you wish your family to take part in the study please sign the consent form enclosed in the information pack and return it, including your contact details, in the prepaid envelope provided. Alternatively, it can be posted to: Becky Hardiman, Tizard Centre, Giles Lane University of Kent, Canterbury, CT2 7LR... If you do consent,

you will be contacted by Becky by phone or email to discuss dates that would be convenient for you, on which to conduct the study.

Do I have to take part?

Your family's participation in this study will be entirely voluntary. It is up to you whether you decide to take part. If your family decide to take part you are still free to withdraw at any time, without giving any reason. If so your family's data will be destroyed and not included in any analysis.

Will what I say be kept confidential?

Material gathered during this research will be treated as confidential and securely stored. Only the researcher and her supervisors will have access to the completed forms, logs and personal information.

What are the risks and benefits of taking part in the study?

You might be worried that your son will swallow the swab whilst the saliva sample is being taken. The swab chosen is long enough to allow it to be held at one end by an adult during the collection in order to prevent this. Furthermore, saliva has previously been used as a measure in this age-group of boys with FXS without any issues.

What will happen to the results of the research study?

The information you provide us with will be used to produce part of the researcher's PhD, and may be published in a scientific journal. Furthermore, the experiences and results of this pilot study will help to develop the protocol for a larger study in the near future. Specific individuals will not be identifiable from the results as pseudonyms or ID numbers will be used. At the end of the study the researcher will send you a summary of the results.

Who is organising and funding this research?

The University of Kent is funding this research and will hold the findings as well as the actual report of the current study. This study has been approved by an NHS Ethics Committee.

What if something goes wrong?

All participants have the right to complain if they feel they have been badly or unfairly treated by researchers. It is advised that, in the first instance you should seek to resolve any complaint with the researcher. If this is not appropriate or you are still not satisfied then you should contact the research supervisors: Alison Bratt (amb54@kent.ac.uk) or Peter McGill (p.mcgill@kent.ac.uk). If you remain unsatisfied then complaints can be sent to the University of Kent Director of Research Services, Simon Kerridge (S.R.Kerridge@kent.ac.uk; 01227 823229).

Conflict of Interest

The researcher, Becky Hardiman, is a director of the Fragile X Society. Becky was not involved in the Society's decision regarding their involvement with this study. Your decision regarding participation in this study will in no way affect your membership of the Fragile X Society, if applicable.

Contact

Please do not hesitate to contact the researcher (Becky Hardiman, e-mail: rh432@kent.ac.uk, tel. number: 01227 82 4770) or the supervisors of the research (Peter McGill: P.Mcgill@kent.ac.uk, Alison Bratt: A.M.Bratt-54@kent.ac.uk) if you have any queries. If you wish to take part please complete the consent form enclosed in the information pack and return this in the envelope provided.

Thank you for taking the time to read this

Becky Hardiman

Appendix C

Additional Information about Cortisol (Chapter 4)

University of Kent | Tizard Centre

T: +44 (0)1227 827373
F: +44 (0)1227 763674
E: tizard-info@kent.ac.uk
www.kent.ac.uk/tizard

Additional Information

What is cortisol?

Cortisol is a steroid hormone produced in glands just above the kidney. Cortisol is released in a normal daily rhythm: in the morning we produce high levels of cortisol in order to make us awake and alert, then through the day this secretion declines to a low point before bedtime (Figure 1). On top of this cycle, cortisol is released in response to events or stimuli that are perceived to be stressful. Cortisol has widespread effects on the body, including increasing release of energy from stores in the body and suppressing the immune system. In the short term, these effects serve to help prepare your body to cope with a stressor and to protect your body by shutting down the initial 'fight or flight' response. However, too much cortisol can have negative effects, such as increased susceptibility to illness (due to over suppression of the immune system) and increased anxious or depressive moods. Normally, the release of cortisol is controlled like a thermostat; when it is detected that a sufficient level of cortisol is present, the release of cortisol is terminated (as a thermostat turns off the heating when the right temperature is reached).

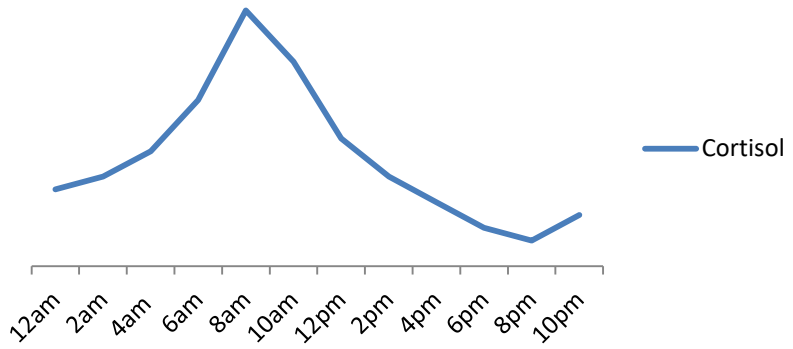

Figure 1: A graph showing the approximate baseline pattern of cortisol release through the day.

Cortisol responses in individuals with Fragile X

It has been noted that individuals with FXS may have difficulty controlling their level of arousal and their response to stressors. Anecdotally, some parents report that their son or daughter experiences 'meltdowns' in response to situations that they find stressful. Some studies have looked at cortisol in people with FXS and it seems that boys with FXS show longer cortisol responses to stressful situations than their siblings. Therefore, it may be that the "thermostat control" of cortisol is functioning abnormally, leading to larger increases in cortisol after a stressful event. This, in the long run, can increase the amount of adrenaline that the body produces, leading to a frequent feeling of nerves, similar to how others may feel only before an exam. Alternatively, it may be that, due to the difficulties experienced by individuals with FXS, events are perceived to be more stressful than they would be by others.

Appendix D

Additional Information about Salivary Alpha-Amylase

Tizard Centre, CT2 7LR
Researcher: Becky Hardiman (rh432@kent.ac.uk)
Tel. number: 01227 82 4770; 07948 047785

Dear [insert name]

Thank you, again, for taking part in our study investigating arousal and challenging behaviour. I am writing to you again to ask whether it is okay to look at an additional hormone, which is related to the "fight or flight" response, in the saliva samples that we collected.

Fight or Flight Response

When we find something stressful, our "fight or flight" response is immediately activated. This causes short-term changes in our bodies, such as the heart beating faster. This system is also activated when we find things exciting. Research with people with Fragile X shows that they generally have faster heart rates, as well as bigger increases in heart rates in response to challenges.

As well as looking at heart rate, you can also measure this type of activity by looking at a chemical in saliva, called alpha-amylase. Nobody has looked at levels of alpha-amylase in people with Fragile X, before. Therefore, we would like to see whether levels of this chemical are related to the measures of behaviour which we collected.

If I agree, what will happen?

If you agree, if there is enough saliva left after we analyse it for cortisol, we will analyse the amount of alpha-amylase in the rest of the sample. We will then feed-back what we

find to you, like we will with the rest of the data. The analysis will not require you or your family to do anything extra.

Do I have to agree?

No, this is a voluntary additional element to the study. Your decision will not affect your involvement in the rest of the study.

If I want to agree, what do I need to do?

Firstly, feel free to ask me any questions about this analysis. You can also contact my supervisor: Alison Bratt (A.M.Bratt-54@kent.ac.uk). Once your questions have been answered, you can sign the attached consent form and send it back to me.

Thanks! Becky

Appendix E

Example Saliva Collection Photo Information Sheet: Assisted Child Swab

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Collecting a Saliva Sample

For the present study samples will be collected at the following times:

- Immediately after waking: Approximately 7am
  Please don't allow the participant to go back to sleep after this sample is collected
- 30 minutes after waking: Approximately 7.30am
- 2 hours after waking: Approximately 9am
- Before lunch: Approximately 12pm
- Before dinner: Approximately 5pm
- Before bed: Approximately 8pm

Please avoid:

- Brushing teeth 30 minutes before the sample
- Eating or drinking anything apart from water 30 minutes before the sample
- Eating or drinking dairy products 60 minutes before the sample
- Drinking or rinsing mouth with water less than 10 minutes before the sample.

It is therefore recommended that breakfast is eaten and teeth are brushed

between 7.30am and 8am

Instructions

We will supply the following:

Scissors

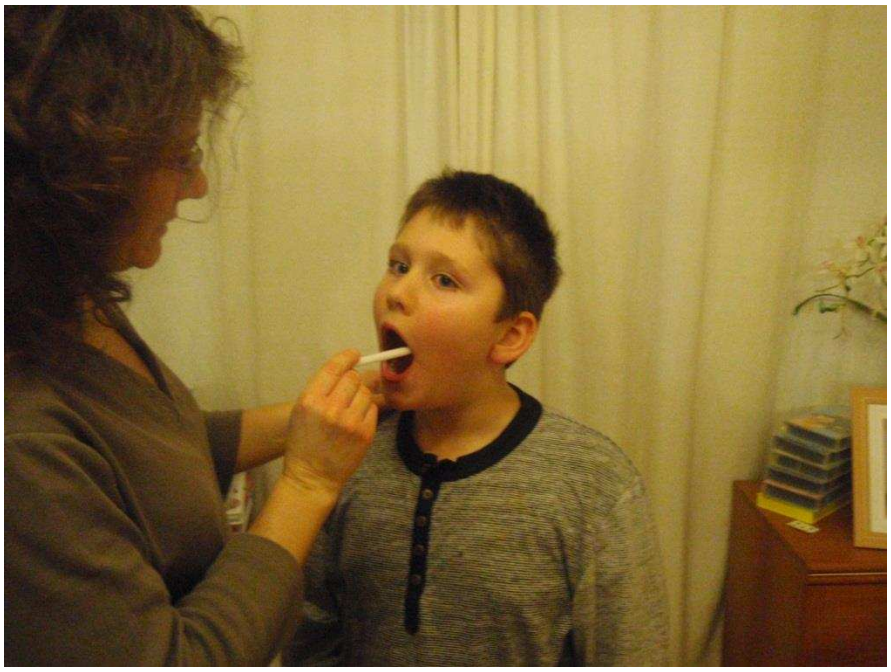
Swab

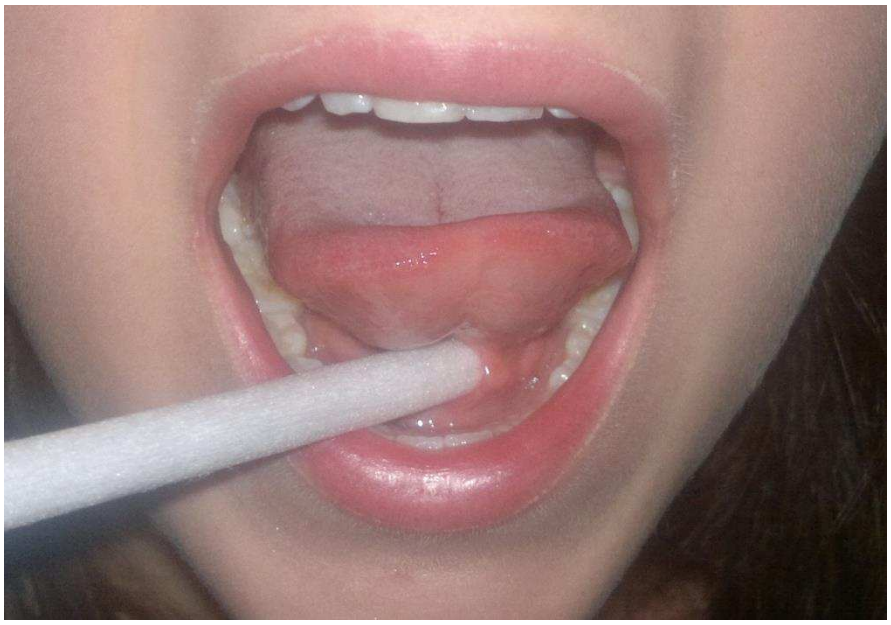
Storage tube

Timer

1. Remove the swab from the packet

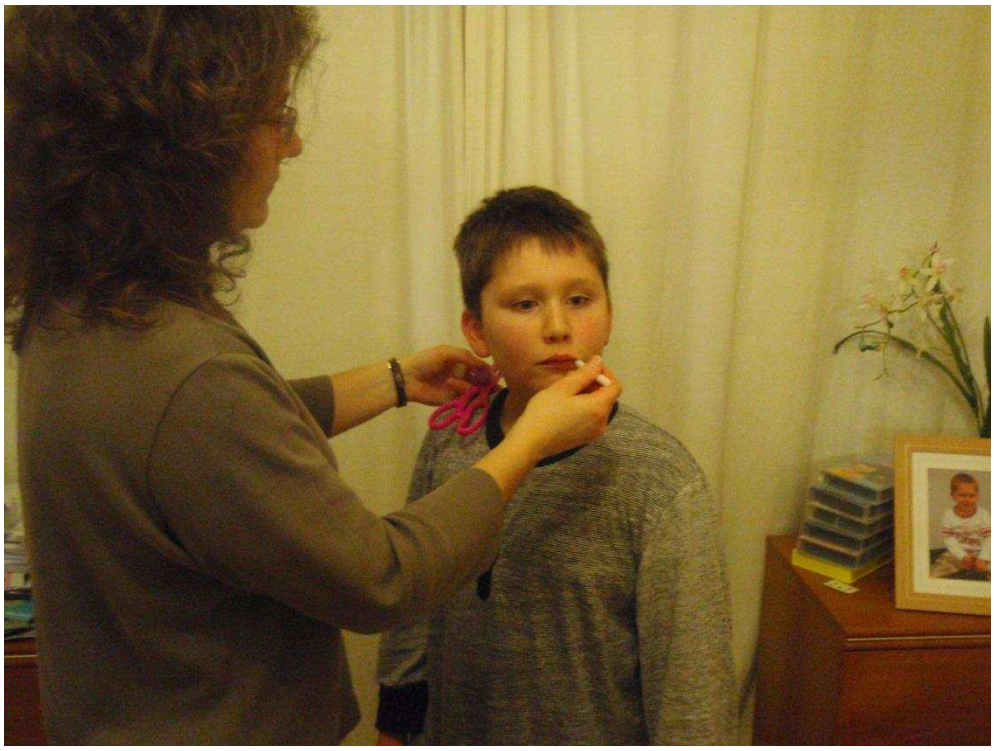
2. Hold on to one end of the swab and position the other end under the front of the participant's tongue



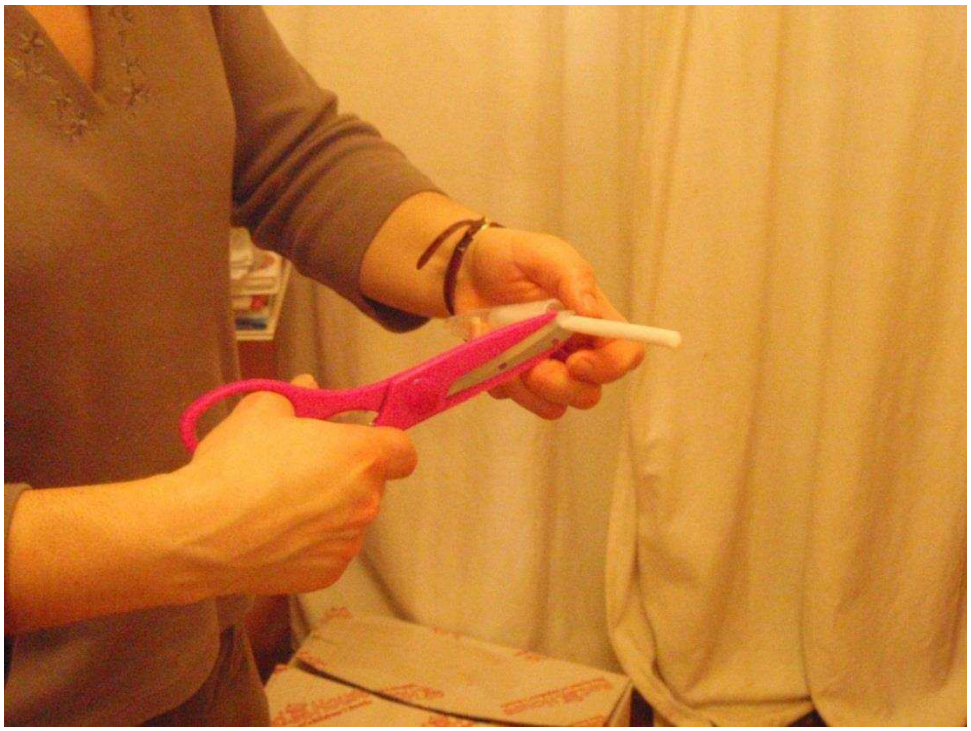


3. Allow the swab to soak under the tongue for 2-3 minutes whilst holding onto the other end.

4. Once the swab has soaked, remove from the participant's mouth.



5. Place the wet end of the swab into the storage tube and, using clean sharp scissors, cut off the remaining dry end of the swab



6. Seal the wet part of the swab in the storage tube and discard the dry end. Please try not to touch the wet end of the swab, but use the top of the storage tube to push it into the tube before sealing.

7. Label the tube with the participant's ID number _____, EXACT TIME of sample collection (even if it is earlier or later than instructed) and length of time swab soaked in the mouth.

8. Immediately freeze the sample or, alternatively chill in the fridge or provided cool-box.

Thank you!

Appendix F

Child Saliva Collection Rating Form

Participant Saliva Collection Rating Form

Please work through this questionnaire with your child who participated in the study. Circle the answers that best represent his/her feelings towards the saliva collection procedure.

1. Did you understand what was happening when your saliva was being taken?

1 2 3 4 5 6 7

Not clear at all Neutral Very clear

2. Was the information about taking saliva clear?

1 2 3 4 5 6 7

Not clear at all Neutral Very clear

3. Did you feel OK whilst your saliva was being taken?

1 2 3 4 5 6 7

Not OK at all Neutral OK

4. Was the length of time it took to give the saliva OK?

1 2 3 4 5 6 7

Not OK at all Neutral OK

5. How easy did you find it for your saliva to be taken?

1 2 3 4 5 6 7

Not easy at all Neutral Very easy

6. Was the number of samples we took OK?

1 2 3 4 5 6 7

Not OK at all Neutral OK

7. Were the samples that we took in the morning OK?

1 2 3 4 5 6 7

Not OK at all Neutral OK

8. Would you let people take your saliva again?

Yes No

Further comments:

_____

_____

_____

_____

_____

_____

_____

_____

Appendix G

Adult Saliva Collection Rating Form

Parent Saliva Collection Rating Form

Please complete the following form, circling the numbers which represent your feelings towards the saliva samples that were collected from your children.

1. How clear was your understanding of the saliva collection procedure?

1 2 3 4 5 6 7

Not clear at all Neutral Very clear

2. How clear was the information provided to you regarding saliva collection?

1 2 3 4 5 6 7

Not clear at all Neutral Very clear

3. How acceptable was the overall saliva collection procedure?

1 2 3 4 5 6 7

Not acceptable at all Neutral Very acceptable

4. How acceptable was the length of time taken to collect each sample?

1 2 3 4 5 6 7

Not acceptable at all Neutral Very acceptable

5. How easy was it to supervise the collection procedure?

1 2 3 4 5 6 7

Not easy at all Neutral Very easy

6. How easy was it to transport and store the samples according to instructions?

1 2 3 4 5 6 7

Not easy at all Neutral Very clear

7. How acceptable was the number of saliva samples that were collected?

1 2 3 4 5 6 7

Not acceptable at all Neutral Very acceptable

8. How acceptable was the disturbance caused to your family's morning routine?

1 2 3 4 5 6 7

Not acceptable at all Neutral Very acceptable

9. How confident would you feel, as a family, to be able to collect samples in this way without the help of the researcher?

1 2 3 4 5 6 7

Not confident at all Neutral Very confident

10. Having experienced the procedure, would you be happy to consent for your family to have saliva samples collected from them in this way again?

Yes No

Further comments:

_____

_____

_____

_____

_____

_____

_____

Appendix H

Participant Information Sheet (FXS: Chapter 5)

Becky Hardiman
Tizard Centre, University of Kent
Canterbury, CT2 7LR
rh432@kent.ac.uk; 07756547751

Information about New Research Project:

Arousal and Escape Behaviour

This letter contains information about new study that is taking place, looking influences on behaviour in boys with Fragile X Syndrome. My name is Becky Hardiman and I am leading this project, along with Professor Peter McGill (P.McGill@kent.ac.uk) and Dr Alison Bratt (A.M.Bratt-54@kent.ac.uk).

What is the research about?

Background: Arousal is how physically and mentally alert someone is. The focus of this study is on the influence that arousal has on behaviour. Our level of arousal is controlled by lots of systems in the body, including those which react when we are stressed. People with fragile X are often described as showing hyper-arousal, which means that they may be over alert and tense. It is thought that this has a strong effect on their behaviour. Part of the reason for this heightened arousal may be that the systems that control the body's response to stress are changed by fragile X.

Earlier research suggests that children with fragile X might be more highly motivated to try to escape from situations that they might find stressful, like people asking them to do school work. These might be situations which other people would not find stressful. This could be because their body reacts to these day-to-day challenges in a different way, making them want to leave that situation more. This is important because it might make children with fragile X more likely to learn "challenging behaviour" (such as aggressive outbursts), because it allows them to escape from these situations quickly.

This Study: This study will test whether boys with fragile X show more escape behaviour (requests to leave the situation or behaviours which suggest that they want to leave) when they are being asked to do work, compared to other children with

learning disabilities. Also, we will look into whether it is the difficulty of the work or the social demands (such as making eye contact with the person teaching) that influence this. In order to see whether escape behaviour might be related to hyper-arousal, measurements will be taken to see if people's bodies show a stress reaction in response to being asked to do the work. We will look to see whether boys with fragile X show a different stress reaction compared to other children.

The aim of this research is to better understand how arousal affects escape behaviour in fragile X, but also more generally for people with learning disabilities. If an association is found, we hope that it will encourage people to scientifically investigate ways of reducing escape behaviours by managing arousal.

Why have I been contacted?

This information is being sent to you either because you have contacted Becky about research before, or because an organisation (such as genetics centre or charity) you are involved with are sending out information about the study on behalf of the research team. Organisations were asked to contact boys with Fragile X Syndrome, between the ages of 5-15 years. Children with learning disabilities but without fragile X are also being contacted.

What does the study involve?

1. **Phone interview.** If you decide to take part, I will organise a time to do a telephone interview with you, which will last approximately one hour. This can be done over multiple calls, if required. During this time, I will ask some basic questions about your child such as name, date of birth and whether they take any regular medication. In addition, I will ask for contact details for the child's school, so that I can check that they are happy for me to come in to do the study. I will also read two questionnaires: one about day-to-day skills that the person has and the other on autistic behaviour. These are being done to see how similar the two groups of participants (with and without fragile X) are, apart from their diagnosis. Finally, I will ask whether your child has any 'challenging behaviours' and, if so, some short questions about the situations when they occur.
2. **School visit.** The main part of the study will involve me visiting your child at school on one day. In total, this will involve about 2 hours activities, spread across the day, including sessions of work and play. A second person will be present when we are doing the activities, which may be a member of staff from the school or another student from the Tizard Centre. The work sessions will be videoed so that we can re-watch and record data for the study.

Recordings will only be seen by people involved in the study, including those named above and a small number students from the Tizard Centre acting as research assistants, all of whom will have signed confidentiality policies and have an up to date DBS check.

Measure levels of arousal. The first part of the visit will involve me asking your child to do some difficult worksheets for 10 minutes. They will then be able to rest and watch a video of their choice. In order to measure levels of arousal, participants will be asked to do some spit samples before (one) and after (three, spread over the next hour). The spit samples can be done in different ways, depending on what your child would prefer. The first way is to ask them to hold a child-friendly foam swab in their mouth (see picture) for 1 to 2 minutes. The other way is to spit into a container, there are special 'funnels' that they can use if they need help to aim! I can send you any of these materials to see, if you would like. Later, the levels of two "stress" chemicals will be measured in the spit: α-amylase (which measures the "fight or flight" response) and cortisol (which measures the other stress reaction in the body). Contact me if you would like more information about these measures.

Measure escape behaviours. Next, I will teach your child an easy action (like putting a block into a box, passing a card or clapping their hands) which lets them take a break from work. If they understand this then I will do three more 10 minute sessions of work tasks with them. The sessions will be spread out with breaks in-between. During these sessions, your child will be able to request to take breaks as much as they like, using the action which I taught them (each time they do it they will get to have a 20 second break). This will be a measure of the child's motivation to 'escape' from the situation. From the video of these sessions, I will compare the amount that children with and without fragile X request for breaks, as well as other behaviours associated with arousal (such as gaze avoidance and fidgeting). We will then see whether there is a relationship between the arousal measures and behaviour.

I will change the level of the work difficulty and the amount of social interaction (such as, eye contact) between each of these three sessions, to see whether this makes children ask for breaks more or less often. This might provide evidence to show the best way of giving work tasks to children with fragile X. For instance, showing whether the amount of eye contact should be minimised when presenting work.

What are the risks and benefits of taking part?

The aim is that this study will provide information that will be helpful for those supporting individuals with fragile X. For instance, the study may provide support for changing the way that tasks are presented, in order to reduce children's desire to escape from them. However, there are likely be few immediate, direct benefits to your family for taking part.

The study will involve asking your child to do some spit samples. If they choose to use the swab, this will involve them putting something into their mouth, meaning there is a very small chance that they might swallow it. However, the swabs are 10cm long and have been designed to be safe for use with young children. We used these swabs in a study of children with fragile X before without problems and parents rated them as being highly acceptable. However, I would supervise the child at all times when they are doing the sample. Also, I will not force participants to do a sample, if they do not want to.

The other potential risk of the study is that your child might not like being asked to do the difficult work. Over the day, the study will involve 40 minutes of being asked to do work, spread out over four 10 minute sessions. The aim of the research is to mimic situations which we expect are happening in schools on a regular basis. However, I will discuss with you, and the class teacher, rules for deciding if the task should be stopped, based on the child's behaviour.

Can my child take part?

For the Fragile X Group, we are looking for boys who have a confirmed diagnosis of Fragile X Syndrome i.e. they have had a genetic test done by a professional which confirms that they have the condition (full-mutation or mosaicism). The age range for the study is 5-15 years old. Boys with a dual-diagnosis of autism or ADHD will be able to take part. I will ask about medication use and will research whether those medications might affect the spit samples, if they do, then they will not be able to take part. Please contact me if you have any questions about this. Also, it does not matter whether you think your child shows hyper-arousal or not; we would like a variety of people to take part.

Do we have to take part?

No! This is entirely voluntary and unrelated to your involvement in the either earlier research, or to the organisation who contacted you. Your decision to take part will not affect any services that you are receiving.

What do I need to do to take part?

Firstly, you should make sure that you understand what the study involves and read the additional information which includes your rights as a participant. You can also contact me (rh432@kent.ac.uk) to ask questions or to discuss the study further. If you are happy to take part, then sign the consent form and send it back to me in the post or scanned in an email.

Appendix I

Participant Information Sheet (ID: Chapter 5)

Becky Hardiman
Tizard Centre, University of Kent
Canterbury, CT2 7LR
rh432@kent.ac.uk; 07756547751

Information about New Research Project:

Arousal and Escape Behaviour

This letter contains information about new study that is taking place. My name is Becky Hardiman and I am leading this project, along with Professor Peter McGill (P.McGill@kent.ac.uk) and Dr Alison Bratt (A.M.Bratt-54@kent.ac.uk)

What is the research about?

Background: Arousal is how physically and mentally alert someone is. The focus of this study is on the influence that arousal has on behaviour. Our level of arousal is controlled by lots of systems in the body, including those which react when we are stressed. Some children show hyper-arousal, which means that they may be over alert and tense. Part of the reason for this heightened arousal may be that the systems that control the body's response to stress are functioning differently.

Some children seem to be more highly motivated to escape from situations which would not normally be seen as being highly stressful, such as being asked to do work. In addition, sometimes children learn to escape from demands by engaging in "challenging behaviour". We believe that people whose bodies react differently to day-to-day challenges (such as being asked to do work), may have an increased motivation to try to leave the situations. This idea has come from research with children with a genetic condition called Fragile X Syndrome and this project will investigate the idea by comparing children with learning disabilities, with and without fragile X.

This Study: This study will test whether children with fragile X show more escape behaviour (requests to leave the situation or behaviours which suggest that they want to leave) when they are being asked to do work, compared to other children with learning disabilities. Also, we will look into whether it is the difficulty of the work or the

social demands (such as making eye contact with the person teaching) that influence this. In order to see whether escape behaviour might be related to hyper-arousal, measurements will be taken to see if people's bodies show a stress reaction in response to being asked to do the work. We will look to see whether children with fragile X show a different stress reaction compared to other children.

The aim of this research is to better understand how arousal affects escape behaviour in fragile X, but also more generally for people with learning disabilities. If an association is found, we hope that it will encourage people to scientifically investigate ways of reducing escape behaviours by managing arousal.

Why have I been contacted?

Organisations, such as special education schools, were asked to send information about the study on behalf of the research team. Organisations were asked to contact families with a child with a learning disability who is between the ages of 4-15 years old.

What does the study involve?

3. **Phone interview.** If you decide to take part, I will organise a time to do a telephone interview with you, which will last approximately one hour. This can be done over multiple calls, if required. During this time, I will ask some basic questions about your child such as name, date of birth and whether they take any regular medication. In addition, I will ask for contact details for the child's school, so that I can check that they are happy for me to come in to do the study. I will also read two questionnaires: one about day-to-day skills that the person has and the other on autistic behaviour. These are being done to see how similar the two groups of participants (with and without fragile X) are, apart from their diagnosis. Finally, I will ask whether your child has any 'challenging behaviours' and, if so, some short questions about the situations when they occur.
4. **School visit.** The main part of the study will involve me visiting your child at school on one day. In total, this will involve about 2 hours activities, spread across the day, including sessions of work and play. A second person will be present when we are doing the activities, which may be a member of staff from the school or another student from the Tizard Centre. The work sessions will be videoed so that we can re-watch and record data for the study. Recordings will only be seen by people involved in the study, including those named above and a small number students from the Tizard Centre acting as research assistants, all of whom will have signed confidentiality policies and have an up to date DBS check.

Measure levels of arousal. The first part of the visit will involve me asking your child to do some difficult worksheets for 10 minutes. They will then be able to rest and watch a video of their choice. In order to measure levels of arousal, participants will be asked to do some spit samples before (one) and after (three, spread over the next hour). The spit samples can be done in different ways, depending on what your child would prefer. The first way is to ask them to hold a child-friendly foam swab in their mouth (see picture) for 1 to 2 minutes. The other way is to spit into a container, there are special 'funnels' that they can use if they need help to aim! I can send you any of these materials to see, if you would like. Later, the levels of two "stress" chemicals will be measured in the spit: α-amylase (which measures the "fight or flight" response) and cortisol (which measures the other stress reaction in the body). Contact me if you would like more information about these measures.

Measure escape behaviours. Next, I will teach your child an easy action (like putting a block into a box or clapping their hands) which lets them take a break from work. If they understand this then I will do three more 10 minute sessions of work tasks with them. The sessions will be spread out with breaks in-between. During these sessions, your child will be able to request to take breaks as much as they like, using the action which I taught them (each time they do it they will get to have a 20 second break). This will be a measure of the child's motivation to 'escape' from the situation. From the video of these sessions, I will compare the amount that children with and without fragile X request for breaks, as well as other behaviours associated with arousal (such as gaze avoidance and fidgeting). We will then see whether there is a relationship between the arousal measures and behaviour.

I will change the level of the work difficulty and the amount of social interaction (such as, eye contact) between each of these three sessions, to see whether this makes children ask for breaks more or less often. This might provide evidence to show the best way of giving work tasks to children with fragile X. For instance, showing whether the amount of eye contact should be minimised when presenting work.

What are the risks and benefits of taking part?

The aim is that this study will provide information that will be helpful for those supporting individuals with fragile X as well as others with learning disabilities.

However, there are likely be few immediate, direct benefits to your family for taking part.

The study will involve asking your child to do some spit samples. If they choose to use the swab, this will involve them putting something into their mouth, meaning there is a very small chance that they might swallow it. However, the swabs are 10cm long and have been designed to be safe for use with young children. We used these swabs in a study of children with fragile X before without problems and parents rated them as being highly acceptable. However, I would supervise the child at all times when they are doing the sample. Also, I will not force participants to do a sample, if they do not want to.

The other potential risk of the study is that your child might not like being asked to do the difficult work. Over the day, the study will involve 40 minutes of being asked to do work, spread out over four 10 minute sessions. The aim of the research is to mimic situations which we expect are happening in schools on a regular basis. However, I will discuss with you, and the class teacher, rules for deciding if the task should be stopped, based on the child's behaviour.

Can my child take part?

We are looking for children (boys and girls) between the ages of 5-15 years old who have a professional diagnosis of a learning disability. Children with a diagnosis of autism or ADHD will be able to take part. If your child has a particular genetic condition, I will investigate whether there is evidence that the condition might affect the stress response. Children who have genetic conditions which are known to affect the stress response (apart from Fragile X Syndrome) will not be able to take part. For instance, children with Down's syndrome will not be able to take part as it is known that they have a reduced stress response. Please contact me if you wish to discuss whether your child is eligible to take part.

I will ask about medication use and will research whether those medications might affect the spit samples, if they do, then they will not be able to take part. Please contact me if you have any questions about this. Also, it does not matter whether you think your child shows hyper-arousal or not; we would like a variety of people to take part.

Do we have to take part?

No! This is entirely voluntary and unrelated to your involvement in the either earlier research, or to the organisation who contacted you. Your decision to take part will not affect any services that you are receiving.

What do I need to do to take part?

Firstly, you should make sure that you understand what the study involves and read the additional information which includes your rights as a participant. You can also contact me (rh432@kent.ac.uk) to ask questions or to discuss the study further. If you are happy to take part, then sign the consent form and send it back to me in the post or scanned in an email.

Appendix J

Recruitment Flyer (Chapter 5)

NHS Ethics Study Reference: 15/EM/0002

Opportunity to Help with Research

Researchers: Becky Hardiman, Prof. Peter McGill & Dr. Alison Bratt

TIZARD University of Kent medway school of pharmacy

Some children with learning disabilities show behaviour described as challenging. Different situations trigger these behaviours for different children. One common situation is when a child is asked to do something different or difficult. We are conducting a new research project to help understand why some children are more likely to develop these behaviours, so that in the future this might be prevented.

We are looking for children with learning disabilities, between the ages of 4 and 15 years old, and their parents/ guardians, to help with this project. The research involves a telephone interview with the parent lasting approximately 1 hour, followed by Becky visiting the child on one day at school. During the visit:

- We work on some hard and easy classroom work tasks (such as worksheets), chosen based upon the child's abilities. During these tasks we vary the amount of social interaction, for example, we make lots of eye contact with the child or keep this to a minimum. We record how the child behaves during these sessions.
- We also measure children's physical reactions during these demands, to see whether this relates to their behaviour. We ask children if they are happy to use a child-friendly swab to provide spit samples (most are very happy to do so!). We analyse the samples to see how children are responding, physically, to different kinds of situations.
- Most children who take part seem to enjoy the activity. We never force a child to do something they don't seem to want to do.

If you would like more information about the study and to find out more about taking part, please contact Becky:

Email: rh432@kent.ac.uk **Phone: 07756547751**

Research Information Flyer Version 2 12th May 2016

Appendix K

Tizard Centre Ethics Approval (Pilot Study: Chapter 5).

TIZARD
University of Kent

Tizard Ethics Feedback Form

Student Name:	Rebecca Hardiman
Supervisor:	Peter McGill
Title:	Assessing sensitivity to positive and negative reinforcement

Following receipt of the amended papers the Tizard Ethics Committee confirm that the above proposal now has ethical approval.

Signed: J.Ruffels **Date: 10.02.14**

On behalf of Tizard Ethics Committee

Alterations approved by Supervisor	**Signature** **Date**
Final approval On behalf of Tizard Ethics Committee	 **Signature** **Date 10.02.14**

Appendix L

Operationalised Definitions of Codes (Study described in Chapter 5).

Variable Type	Behaviour	Definition/ coding instructions.
Event	Yawning	Participant inhales deeply with mouth wide open or whilst actively keeping mouth closed (nostrils likely to be flared). Code as soon as notice behaviours beginning. Code as new instance each time there's a new inhale.
Event	Verbal prompt for physical gesture	*[Hard Task, Low Social condition, only]* Researcher verbally instructs participant to engage in physical gesture (in low social conditions) e.g. touch your nose. Code at onset of prompt (Code as gestural prompt if concurrent gesture)
Event	Gestural prompt for physical gesture	*[Hard Task, Low Social condition, only]* Researcher physically imitates a physical gesture for a participant to copy (e.g. touching nose), accompanied with verbal instruction. Code at onset of prompt
Event	Verbal prompt for eye contact	*[High Social conditions, only]* Researcher verbally requests for a participant to "look at me" (or phrase of same meaning) (in high social conditions). Code at onset of prompt (Code as gestural prompt if concurrent gesture-code 6)

Variable Type	Behaviour	Definition/ coding instructions.
Event	Gestural prompt for eye contact	*[High Social conditions, only]* Researcher verbally requests for a participant to "look at me" (or phrase of same meaning) whilst also pointing towards own eyes (in high social conditions). Code at onset of prompt
Event	Participant demonstrates escape response	Participant completes action which has been taught to request a break. May include card exchange, sign or putting a block in a bowl.
Event	Verbal prompt for task activity	Researcher verbally instructs participant to engage in a task demand (e.g. "what is 2 + 2?" "How do you spell cat?")
Event	Gestural prompt for task activity	Experimenter physically models correct response to task, or how to reach correct response (includes holding up fingers of numbers for addition).
Event	Physical prompt for task activity	Researcher guides participant to successfully complete trial. Includes hand-over-hand prompt to demonstrate correct task response or writing or saying correct answer.
Duration	Physical aggression	Participant makes rough contact with researcher (or other person) with own body or with a thrown object. Contact with other person should make an audible sound or (for behaviours such as pinching and scratching)

Variable Type	Behaviour	Definition/ coding instructions.
		indent the skin of the recipient (or be expected to do so if skin is covered e.g. by clothing).
Duration	Off task speech	Participant speaks about topic unrelated to the current task. May include statements (e.g. "it's sunny outside" or "I like planes") or questions (e.g. What are you doing at the weekend?). Includes off-task signing (e.g. pointing at the window, signing to ask for a toy).
Duration	Participant engaged in task activity	Participant actively engaged in task as prompted by experimenter. May include: directly interacting with work materials in way congruous with task demand; looking at work materials; looking at experimenter (only if presenting a demand at the time); or speaking about work task. Task engagement ends after 3 seconds where none of these behaviours have occurred.
Duration	Stereotypy	Stereotyped, repetitive, *rhythmic*, unusual seemingly purposeless movements of their body or objects.
Duration	Participant out of chair	Whole body out of chair (or work area) and at least one step away from being in front of chair.
Duration	Participant refusal or negative verbalisation	Participant makes comment expressing negative views about the task (e.g. "this is boring", "adding is stupid"), verbally refusing to complete the task (e.g. saying "no" following a request from the researcher). Includes

Variable Type	Behaviour	Definition/ coding instructions.
		comments on task difficulty: e.g. “this is hard”, “this is tricky”, “I don’t know this”. Also code for non-verbal negative vocalisations such as exaggerated sighs, growling, tutting (do not code crying as has separate code). Code irrespective of actual participation in the task (i.e. they may say no but engage in the task regardless).
Duration	Participant face off screen	Participant’s face is not visible to the extent that eye gaze cannot be determined (if eyes are being covered actively, e.g. with hands or object, do not code- instead use: eye cover)
Duration	Chewing or biting object	Object inside participant’s mouth with teeth making contact with it (if teeth cannot be seen may be indicated by chewing motion of jaw or indicators of jaw clenching).
Duration	Chewing or biting body part	Participant has a body part (such as hand or finger) inside their mouth so that pressure is being exerted by teeth (if teeth cannot be seen may be indicated by chewing motion of jaw or indicators of jaw clenching).
Duration	Experimenter looking at participant	Experimenter’s eye gaze is directed towards participant’s face. Turn off coding if experimenters eyes look away for >1 second (approx.)
Duration	Destructive behaviour	Participant rips, snaps, crumples or otherwise breaks or damages an object (including paper work materials) or

Variable Type	Behaviour	Definition/ coding instructions.
		engages in behaviour which might be expected to break the object if not blocked by experimenter or other person (NB if biting object then code object biting instead). Includes knocking over furniture. Also code if participant forcefully bangs object or surface (e.g. table) with sufficient force that it moves or makes loud noise.
Duration	Self-Injurious behaviour (SIB)	Participant engages in behaviour which either causes them physical harm (such as skin reddening) or has the potential to do so (for instance, if the result of behaviour cannot be seen through clothing or if behaviour blocked before escalating to point of actual harm). This includes (but is not limited to) hitting or punching self, scratching self (if breaks skin), picking skin or scabs, banging head or body against objects or surfaces. (If self-biting, code as biting)
Duration	Participant off screen	Participant's whole body not visible in recording.
Duration	Experimenter face off screen	Experimenter face not visible on screen to allow determination of eye gaze direction.
Duration	Participant fidget	The participant displays restless, repetitive, non-rhythmic, non-functional motor movements, such as, moving their hands, hand wringing, touching their face or

Variable Type	Behaviour	Definition/ coding instructions.
		hair or moving an object, or wriggling in their seat. This code *does not* include stereotyped behaviours, which are *rhythmic*, unusual seemingly purposeless movements of their body or objects (code as stereotypy).
Duration	Participant cries	Participant crying which may include vocalisations (sobbing, wailing, whimpering) and/or tears.
Duration	Participant turned away from researcher	Head/body turn away from experimenter/table top >45 degrees. If participant is sat beside experimenter, code when turn >45 degrees away from straight on, opposite direction from experimenter.
Duration	Verbal aggression	Negative speech directed at experimenter personally (appearance or characteristics e.g. "you're stupid"), includes use of expletives. For participants with no verbal language, includes negative vocalisations (such as a growl) made whilst making eye contact with the researcher.
Duration	Participant makes eye contact with experimenter	Participant looking at face/eyes of experimenter
Duration	Participant laugh	Loud burst of sound from expulsion of air from lungs, to a series of quiet chuckles, may be accompanied by

Variable Type	Behaviour	Definition/ coding instructions.
		characteristic facial (smiling or mouth open) and bodily movements (e.g. shoulders moving up and down).
Duration	Experimenter initiates task break	Researcher initiates task break e.g. “ok we don’t have to do that now”, removes task from participant and turns away. Stop coding when task resumed
Duration	Participant covers eyes	Both eyes covered by hands/objects/surface OR prolonged closure of eyes i.e. 3 seconds plus.

Appendix M

Observed behaviours during Arousal Assessment and comparison with frequency of occurrence in Escape Assessment

Descriptive statistics regarding occurrence of behaviour during the initial arousal assessment are presented. There were significantly lower levels of some measures of challenging and off-task behaviours during this initial demand session (see Table below), when compared to the mean of the subsequent sessions of the Escape Assessment.

Behaviour (Percentage of session)	Group				Comparison with Mean Occurrence Escape Assessment (all Ps)		
	FXS (24)		ID (13)				
	Median	IQR	Median	IQR	Z	p	Effect (r)
Gaze to experimenter	5.07	16.66	11.05	28.00	-1.29	.20	.21
Turn Away	.51	2.79	0	3.67	-2.73	.006	.45
Eye Cover	.78	4.86	.70	3.17	-2.23	.025	.37
SIB	0	0	0	0	-1.34	.18	.22
Self-bite	0	.45	0	.33	-1.15	.25	.19
Aggression	0	0	0	0	-1.60	.11	.26
Verbal Aggression	0	0	0	0	-1.52	.13	.25
Destruction	0	0	0	0	-2.20	.03	.36

Behaviour (Percentage of session)	Group				Comparison with Mean Occurrence Escape Assessment (all Ps)		
	FXS (24)		ID (13)				
	Median	IQR	Median	IQR	Z	p	Effect (r)
Bite object	0	5.96	0	5.85	-1.49	.14	.24
Not engaged	.33	24.37	0	8.08	-3.43	.001	.56
Laugh	0	.25	0	.25	-2.39	.017	.39
Off-task speech	0	1.33	.33	1.08	-1.76	.078	.29
Refuse	.25	1.52	0	.83	-2.72	.006	.45
Out Chair	0	0	0	.17	-3.05	.002	.50
Interact Tangible Item	0	3.04	0	1.21	-.60	.55	.10
Yawn (rate per minute)	.05	.3	0	0	-.75	.46	.12
Fidget	.44	9.45	0	7.5	-.53	.60	.09

Appendix N

Details of Shortened Sessions, Sessions Terminated Early and Sessions Not Run (Chapter 5)

Session Length	FXS (24 Ps)			ID (13 Ps)		
	Proportion (N sessions, N participants)	Percentage of sessions of conditions	Reason (N sessions, N participants)	Proportion (N sessions, N participants)	Percentage of sessions of conditions	Reason (N participants, N sessions)
5 minutes	13.5% (13, 6)	AA[1]: 0% HH: 16.7% HL: 20.8% EH: 16.7%	*Participant* Teacher suggest couldn't cope 10 min (7,3) Based on previous session behaviour (3,2) *Other* Time constraint: School activity (3,1)	12.5% (7, 3)	AA: 0% HH: 14.3% HL: 14.3% EH: 21.4%	*Other* Time constraint: School activity (7,3)
Session not run	3.1% (3, 3)	AA: 0% HH: 4.2% HL: 8.3% EH: 0%	*Participant* Distress (1,1) Reported anxiety (1,1) *Other*	14.3% (8, 5)	AA: 0% HH: 14.3% HL: 14.3% EH: 28.6%	*Participant* Wouldn't come to room (4,2) *Other*

[1] AA= arousal assessment; HH= high social, hard task; HL= hard task, low social; EH= easy task, high social.

			School activity (1,1)			School activity (2,2) Previous session terminations (2,1)[2]
Participant terminated early	11.5% (11, 9)	AA: 8.3% HH: 8.3% HL: 8.3% EH: 20.8%	Left room or work area (5,5) Asked to go toilet (2,1) Remove clothes (1,1) Researcher terminate due to distress (1,1) Researcher terminate due to behaviour (1,1) Participant asleep (1,1)	3.6% (2, 2)	AA: 0% HH: 7.1% HL: 0% EH: 0%	Left room or work area (1,1) Participant distress (1,1)
Other reason early terminated	7.3% (7, 6)	AA: 0% HH:12.5% HL: 8.3% EH: 8.3%	Camera error (4,3) Others enter room (2,2) Researcher unwell (1,1)	7.1% (4, 3)	AA: 7.1% HH: 14.3% HL: 14.3% EH: 0%	Mistiming (1,1) TA terminated session (2,1)[1] Others enter room (1,1)

[2] A Teaching Assistant (TA) was present in the room for the research with this participant (ID005). Though there was non-occurrence of any target behaviours for session termination, the Teaching Assistant requested that the session was stopped, due to feeling that the protocol was not suitable for the child. One further session was attempted, again with the TA suggesting that the session should be terminated. As such, no further sessions were conducted.

Appendix O

Tizard Ethics Approval Documentation (Chapter 6)

TIZARD
University of Kent

Tizard Ethics Feedback Form

Student Name:	Rebecca Hardiman
Supervisor:	**Peter McGill**
Title:	*Understanding challenging behaviour or 'meltdowns' in boys with Fragile X syndrome*

The Tizard Ethics confirm receipt of the minor amendments and that the proposal now has ethical approval.

Signed: J.Ruffels **Date: 20.09.16**

On behalf of Tizard Ethics Committee

Alterations approved by Supervisor	**Signature** **Date**
Final approval On behalf of Tizard Ethics Committee	 **Michelle McCarthy** **Signature** **Date 20.09.16**

Appendix P

Study Information Flyer (Chapter 6)

University of Kent | Tizard Centre

We need your help with a new research project:

Understanding Challenging Behaviours or "Meltdowns" in Boys with Fragile X Syndrome

Are you the parent or guardian of a boy with Fragile X Syndrome?

Is your child between the ages of 4 and 15 years old?

In the past month, has your child engaged in challenging behaviour, or had a "meltdown"?

If the answer to these questions is yes, then you may be interested in taking part in our research. We want to understand in more detail the triggers, nature and interventions used for these behaviours. The research involves a semi-structured interview, which can be done in person or over the phone. This interview will take approximately an hour and a half.

If you would like to find out more about the project, or might be interested in taking part, then contact Becky Hardiman for an informal chat and for full information (rh432@kent.ac.uk; 07948047785)

Research Flyer v2 31st August 2016

Appendix Q

Participant Information Sheet (Chapter 6)

Researchers: Becky Hardiman & Prof Peter McGill

Email: rh432@kent.ac.uk

Phone: 07948047785

Understanding Challenging Behaviours or "Meltdowns" in Boys with Fragile X Syndrome

What is the project about?

A substantial minority of boys with Fragile X engage in challenging behaviours (such as hurting themselves, being aggressive to others or breaking things). Sometimes these behaviours occur in episodes people describe as "meltdowns". We want to interview parents and guardians of boys with Fragile X who engage in challenging behaviours, to get an in-depth description of the triggers for these behaviours, the nature of the behaviours themselves and interventions used. The aim is that this will help to further highlight trends and patterns of behaviours in this group and to guide future research and development of interventions.

Who can take part?

We are looking for parents or guardians of boys with a confirmed diagnosis of Fragile X Syndrome, who are between the ages of 4-15 years. As this is a project about challenging behaviours or meltdowns, we are looking for people to take part whose children have shown one or more episodes of this nature, in the past month.

Who is doing the research?

The research is being conducted by Becky Hardiman, supervised by Professor Peter McGill, at the Tizard Centre, University of Kent. This project is part of Becky's PhD. Of note, Becky works part-time as CEO of the Fragile X Society, but this project is unrelated to the Fragile X

Society. Your decision about whether to take part is unrelated to your involvement with the charity.

The study has been independently approved by the Tizard Centre Ethics Committee at the University of Kent. The study is funded by a Tizard Centre bursary.

What does the study involve?

The study will involve an initial phone call with the researcher (about 10 minutes) to answer any questions you have and check that your family fits the study criteria. The researcher will then arrange a time to do the main interview with you. This can be done in person (the researcher will travel to you[3]) or over the phone, depending upon your preference. The interview will last approximately an hour and a half and will include questions about your child's daily living skills (45 minutes), as well as questions about the nature of their challenging behaviours or meltdowns (45 minutes) including: triggers, signs that the behaviour might be about to happen, frequency, duration and how you respond or manage the behaviour. The interviews will be audio recorded to help with analysis of the data, but these recordings will only be available to the researchers.

The advantages and disadvantages of taking part

The aim of this study is to get detailed descriptions about individuals' behaviour, and the situations surrounding it. We want to do this to get a better understanding of the triggers and results of these behavioural challenges for boys with Fragile X syndrome, to help with future research and interventions. However, the direct, immediate benefit to those taking part is minimal.

However, some people may find it upsetting to talk about their child's behaviour. If this is something that you would rather not do, then it is OK not to take part. The study also involves committing approximately an hour and a half to speaking with the researcher, though we will work with you to ensure that this is done at a time convenient for you, if you would like to take part.

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[*]Dependent upon your location. Though, if face-to-face is your preference then the will researcher do their best to do that.

What can I do if I decide I don't want to take part, or want to stop taking part?

That's absolutely fine. It is completely optional whether you take part and you can decide to withdraw from the study any time. If you decide you no longer want to take part, we will delete or destroy any data that you have provided to the project. All you need to do is to let the researcher know, no questions asked.

What can I do if I have any questions about the project?

Please get in touch! We are happy to talk about the project and happy to answer any questions that you may have. You can either telephone (07948047785) or email rh432@kent.ac.uk

If I was unhappy with something that happened during taking part, who could I contact?

Initially, it would be important to speak to the lead researcher, Becky, to see if she could resolve your issue. Alternatively you could speak to the project supervisor, Peter McGill (P.McGill@kent.ac.uk; 01227823838). If your issue is still not resolved, you can send a complaint to the University of Kent Director of Research Services, Simon Kerridge (S.R.Kerridge@kent.ac.uk; 01227 823229).

What do I need to do if I want to take part?

If you would like to take part, you will need to fill in a consent form and send it back to the researchers. If you have a consent form, please complete it and send it back to the researchers via the details on the form. If you do not have one, please contact us and we will send one to you.

Although it is the parents giving consent to take part in the research, if possible, we ask parents to check with their child that they are happy for them to take part, as the study involves discussing aspects of their behaviour.

Thank you for taking the time to read this

Appendix R

Fragile X Syndrome: Challenging Behaviour Interview (Used by researcher)

The aim of this interview is for us to get a better understanding of _____'s behaviour. In particular, we are interested in challenging behaviour or meltdowns and what behaviours _____ shows during a typical episode. We are also interested in how often challenging behaviours or meltdowns occur, how long they last and what seems to trigger them. In addition, I will also ask some questions about the ways in which these behaviours can be managed. The interview should take no longer than 45 minutes. Do you have any questions before we begin?

Name: _____ Gender: Male ☐ Female ☐

Age: ___ Date of interview: _____

Name of respondent: _____

1) **Think about how often meltdowns or instances of challenging behaviours occurred in the last month. If there was no change and you watched this person now, then would you <u>definitely</u> see the next instance:**

In the next 15 minutes	In the next hour	By this time tomorrow	By this time next week	By this time next month

2) **In the last month, for how long did the <u>longest</u> meltdown or instance of challenging behaviour last?**

Less than a minute	Less than 5 minutes	Less than 15 minutes	Less than an hour	More than an hour

3) **In the last month, how long have meltdown or instances of challenging behaviour <u>typically</u> lasted on average?**

Less than a minute	Less than 5 minutes	Less than 15 minutes	Less than an hour	More than an hour

4) **Thinking about the longest meltdown or instance of challenging behaviour in the last month that continued for over an hour- how long did it last?**
.....
.....

5) **What keeps a meltdown or challenging behaviour going for long periods of time.....**
.....
.....

6) **Thinking about what you would consider to be the last difficult episode of challenging behaviour or meltdown that _____ showed, can you describe the sequence of events and behaviours starting with _____ being calm.**
.....
.....
.....
.....
.....
.....
.....
.....

7) **Do any behaviours occur before the outburst?**
.....
.....
.....
- **Prompt (if verbal): does their language or speech change?**

Do these always happen?
.....
.....

8) **Are there any physical indicators or signs that you can see that indicate that _____ might be about to have a meltdown?**
……
……
……

Prompts:
- **Changes in movement?**
- **Changes in appearance?**

Do these always happen?
……
……

9) **How would you describe _____'s emotion before a typical meltdown?**
……
……
……

Is this always the case?

10) **When you see these indicators (emotions, behaviours or physical changes) is there anything that you could do to prevent a meltdown from occurring?**
.....
.....
.....
.....

How likely is it to be successful? (out of 10)
.....
.....

11) How do you know, or at what point do things go critical? i.e. you know a meltdown is happening or you can no longer intervene?

.....
.....
.....
.....

12) During a meltdown, what behaviours does X show?

	Frequency			
Behaviour	**Always** (during an episode)	**Often**	**Sometimes**	**Other comments**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

13) Do any of the above behaviours occur together or in a predictable chain?

.....
.....
.....
.....

14) During a instance of challenging behaviour or a meltdown, how would you describe _____'s emotion?
Prompt= how do you think that they feel during the outburst?

.....
.....

15) How would you describe _____'s emotion at the end of the meltdown?
Prompt= how do you think _____ is feeling?

.....
.....

16) What does _____ do at the end of the outburst?
Prompt= Do they do anything? Say anything?

.....
.....

17) Do you intervene? And if so at what point would you intervene? i.e. when you saw which behaviour?

.....
.....

What would you do?

.....
.....
.....

18) Roughly how many times after an instance of challenging behaviour or a meltdown would you respond in this way?

Always ☐ More often than not ☐ Sometimes ☐

Occasionally ☐ Rarely ☐

19) In what other ways might you respond? When would you respond in these ways?

Prompt: e.g. are there any differences at home compared to in public?

1.....2.....
.....3.....
.....4.....
.....
5.....

20) What are the reasons that you might respond in different ways?

.....
.....
.....

21) Would you respond differently at different stages of the outburst?

.....
.....
.....

22) What is the thing that is most likely to stop an outburst?

.....
.....

23) Out of 10, how successful would this be?

.....
.....

Thinking about the last meltdown or instance of challenging behaviour that _____ showed, what seemed to trigger it?

.....
.....

24) Would you say that this is the most common trigger? If not, what is?

.....
.....

25) Out of 10, what proportion of all meltdowns that _____ shows seem to be caused by the trigger that you have identified?

.....
.....

26) Does the trigger that you mentioned always result in a temp meltdown or instance of challenging behaviour?

Yes ☐ **No** ☐

26) If no, out of 10, how often does the trigger that you mentioned result in a meltdown or instance of challenging behaviour?

.....

27) What happens on the occasions that it does not trigger a meltdown? What is different about these times?

Leave free response initially

.....
.....
.....
.....
.....

Prompts:

- Is there something different about the day in general? Less happening or more routine?
-Is the trigger definitely the same?
-Are there different people present?
-Is X's mood different in some way?
-Does X do something that means that they are not thinking about the trigger or do not notice that it occurs?
-Do you do anything to prevent the outburst from occurring?

28) We have established that …..is a common trigger to _____'s meltdowns. Are there other triggers?

Yes☐ No ☐

If yes, list below in order of most frequent to trigger meltdown/challenging behaviour.

1)……
2)……
3)……
4).....

29) Is _____ more likely to have a meltdown at a certain time of day?

Yes☐ No ☐

If yes, why?……..
.....
.....
.....

Tiredness?
Hunger?
Certain people around?
Certain activities happening?
Any times of day when challenging behaviour or a meltdown would definitely or definitely not occur?

30) I'm going to read through a list of situations that are examples of other

things that can trigger meltdowns or challenging behaviour. As I read them one by one, I'd like you to say whether the situation has ever triggered a meltdown or challenging behaviour for X.
If it has then I'll note it and I'll ask you a little more about it.

<u>Have the following triggered a meltdown or challenging behaviour in the last 12 months?</u>

Routine

1) **When there is a change in X's routine?** ☐
2) **When there is a change to someone else's routine? Not X's** ☐
3) **When there is a notable event (such as: Christmas, Birthday) that they are excited about currently happening, or coming up?** ☐
   Any other examples of related to routine?
   .....
   .....

Waiting/ Transitions

4) **When the person has to wait for something?** ☐
5) **When moving between two places?** ☐
6) **When moving between activities?** ☐
   Any other examples related to waiting or transitions?
   ……
   ……

Expectation

7) **When there is a change in X's expectation? I.e. X is expecting something to happen and either it doesn't happen or something different happens- would this cause a problem?** ☐
8) **When X receives conflicting information?** ☐
   Any other examples of related to expectations?
   .....
   .....

Food

9) **When X is told that he/she is not allowed food?** ☐
   Any other examples of issues relating to food?
   .....
   .....

'Just right behaviour'

10) **When there is an imperfection in something that belongs to X?** ☐
11) **Making mistakes in their work e.g. something that they have written or made.**
    Any oth ☐ **examples of related to 'just right behaviour?**
    .....
    .....

Tangible items

12) **When X has lost something, or thinks that they might have done?** ☐
13) **When they aren't allowed access to an item, such as a toy or a computer** ☐
14) **When something isn't working?** ☐

Any other examples of related to losing things?
.....
.....

Not getting own way

15) **Asked to do something that X does not want to do** ☐

☐

16) X is told that he/she cannot have something that they want (not food)
17) Interruption of preferred activity ☐
Any other examples of related to not getting their own way?
.....
.....

Social and relationships
18) Following a disagreement with family member, other resident, staff member?
19) After being asked to do something by someone else ☐
20) Following being in an argument? ☐
21) Following hearing someone else be upset or angry ☐
22) After being teased? ☐
23) After meeting or interacting with someone new? ☐
Any other examples of related to social interactions?
.....
.....

Sensory
24) When somewhere busy? ☐
25) Following loud noises? ☐
Any other examples related to sensory issues?
……
……

27) Is there anything else about meltdowns that you would like to mention that has not been asked about?
.....
.....
.....
.....

Anxiety

Finally, I am going to ask a couple of general questions about X's anxiety. I am going to ask a few questions about X's emotions over the past week.

Q1 Has X seemed to feel tense or wound up?

- ❍ Most of the time
- ❍ A lot of the time
- ❍ Time to time, occasionally
- ❍ Not at all

Q2 Has X seemed to feel at ease and relaxed?

- ❍ Definitely
- ❍ Usually
- ❍ Not often
- ❍ Not at all

Q3 Has X seemed to be frightened?

- ❍ Not at all
- ❍ Occasionally
- ❍ Quite often
- ❍ Very often

Q4 Has X seemed to feel restless or as if they have to be on the move?

- ❍ Very much indeed
- ❍ Quite a lot
- ❍ Not very much
- ❍ Not at all

Q5 Has X seemed to get sudden feelings of panic?

- ❍ Very often indeed
- ❍ Quite often
- ❍ Not very often
- ❍ Not at all

Q6 Has X seemed to be preoccupied with worrying thoughts?

- ❍ Very often indeed
- ❍ Quite often
- ❍ Not very often
- ❍ Not at all

Appendix S

Anxiety indicators (past week)

Participant	Tense or wound up	At ease and relaxed	Frightened	Restless and on the move	Sudden feelings of panic	Preoccupied with worrying thoughts	Total
Laurie	Occasionally	A lot of the time	Not at all	Most of the time	Not at all	-	5
Robert	Occasionally	A lot of the time	Occasionally	Most of the time	Not at all	Occasionally	7
Matthew	Occasionally	Most of the time	Not at all	Most of the time	Not at all	-	4
David	A lot of the time	A lot of the time	Not at all	A lot of the time	Occasionally	Not at all	6
Luke	A lot of the time	A lot of the time	Not at all	Most of the time	Not at all	Not at all	6
Paul[4]	Occasionally	A lot of the time	Not at all	Most of the time	Not at all	Occasionally	6
Howard[5]	Occasionally	A lot of the time	Not at all	-	Not at all	-	2
Stephen	Occasionally	Most of the time	Not at all	Most of the time	Not at all	Not at all	4

[4] Respondent notes past week was school holidays so may not be representative
[5] Respondent notes this week unusual as is the lead up to a highly anticipated trip.

Participant	Tense or wound up	At ease and relaxed	Frightened	Restless and on the move	Sudden feelings of panic	Preoccupied with worrying thoughts	Total
Tim	Occasionally	Most of the time	Not at all	Most of the time	Not at all	Not at all	4
Alex	Occasionally	Most of the time	Occasionally	Most of the time	Not at all	Not at all	5
Jonathon							

- parent could not answer

Appendix T

Topographies of SIBs (Chapter 6)

Participant	Topography of SIB (Frequency[6])
Laurie	Hand-biting (sometimes)
	Head-hitting (sometimes)
Robert	Hand-biting (Always)
	Self-hitting (Always)
	Hair pulling (Always)
Matthew[7]	Hand-biting (Often)
	Chin pushing or hitting (Often)
David	Hand-biting (Often)
Howard	Self-biting (Often)
Gerald	Head-hitting (Always)
	Self-biting (Always)
Stephen[8]	Hand-biting (Always)
	Head- and body-banging against object or surface (Always)
Tim	Self-biting (Sometimes)
	Vomiting (Sometimes)

[6] Response options: Always, often, sometimes. '–' indicates no frequency information provided.
[7] Matthew was reported to exhibit two distinct "classes" of behaviour with separate functions: SIB with at least part automatic function and "meltdown" (crying etc) related to physical discomfort, often at night.
[8] Faecal smearing was also a behaviour of concern, though did not occur concurrently with other 'meltdown' behaviours and was anecdotally more related to sensory stimulation.

Participant	Topography of SIB (Frequency[6])
Alex	Self-biting (Sometimes)
Jonathon	Head banging (Sometimes)

Appendix U

Topographies of physical aggression (Chapter 6)

Participant	Topography of Physical Aggression (Frequency)
Laurie	Physical aggression to others, particularly sister (topography not specified; sometimes)
Robert	Hitting (Particularly brother: Sometimes)
David	Hitting Mum in face and hitting sister (Always)
	Kicking others (-)
	Biting others (sometimes)
Luke	Hitting others (-)
	Biting others (-)
	Head-butting others (-)
	Pinching (Always)
Paul	Hitting (Always)
	Pushing/ shoving others (-)
Howard	Kicking others (Often)
	Pushing and pulling others (-)
	Punching Others (Often)
Stephen	Biting others (-)
	Hitting others (Often)

Participant	Topography of Physical Aggression (Frequency)
Tim	Hitting and pushing others (Sometimes)
Alex	Scratching others (Always)
	Biting others (Sometimes)
	Pinching others (Sometimes)
Jonathon	Pushing other people (Often)

Appendix V

Topographies of destructive behaviour (Chapter 6)

Participant	Topography of Destruction (Frequency)
Laurie	Breaking sister's possessions (sometimes)
	Grabbing things (often)
Robert	Throwing glasses or other objects (Always)
	Slamming doors (-)
David	Grabbing and throwing objects "rampaging" (-)
	Biting objects (sometimes)
	Throwing things (Always)
Luke	Throwing (Always)
	Pushing things over (Always)
Paul	Bites clothing (Always)
	Slams door (-)
	Hits and kicks walls and doors (Always)
Howard	Pushing objects out others' hands (Often)
	Throwing Objects (-)
Gerald	Hitting walls and surfaces (Sometimes)
	Throwing objects (-)
Stephen	Throwing objects or swiping off table (-)
Jonathon	Door slamming (Often)
	Throwing objects (-)

Appendix W

Other reported topographies of challenging behaviour.

Participant	Topographies of other challenging behaviours reported (Frequency)
Laurie	Absconding (sometimes)
	Doing things "knows not supposed to[9]" (often)
Robert	Crying and rubbing eyes (Always)
	Stomping (Always)
	Absconding (Always)
	Shouting (Always)
Matthew	Crying (Sometimes)
	Tantruming: lying down, throwing limbs, shaking head (Sometimes)
	Shouting (Often)
David	Shouting and screeching (-)
Paul	Absconding (sometimes)
	Stamping feet (-)
Howard	Dangerous behaviour (grabbing car steering wheel; sometimes)
	Laying on ground (-)

[9] *"And then he'll start to do things that he knows he's not allowed to do and he'll say he's not allowed to do them almost... So he'll go and like touch the TV and say, "Don't touch the TV," or he'll try and go out the front door and say, you know, "Leave the door. Leave the door." So it's really... You know, he will do behaviours that he knows he's not supposed to do and he'll say that while he's doing them and look for a response."*

Participant	Topographies of other challenging behaviours reported (Frequency)
	Protesting/ non-compliant behaviour (-)
	Screaming (-)
	Vomiting (-)
	Crying (Always)
	Absconding (Sometimes)
Gerald	Feet stamping (Always)
	Shouting and growling (-)
Stephen	Crying (-)
Tim	Crying (Always)
	Laying down (Always)
	Screaming (Always)
	Removing clothes (Sometimes)
Jonathon	Hiding (e.g. in wardrobe or under bed; Often)