The Pennsylvania State University

The Graduate School

BEHAVIORAL AND GENETIC ARCHITECTURE OF FEAR CONDITIONING AND RELATED PHENOTYPES

A Dissertation in

Biobehavioral Health

by

Dana Zeid

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2021

The dissertation of Dana Zeid was reviewed and approved by the following:

Thomas Gould
Professor of Biobehavioral Health
Head of Biobehavioral Health Department
Dissertation Advisor
Chair of Committee

Helen Kamens
Associate Professor of Biobehavioral Health

David Vandenbergh
Professor of Biobehavioral Health

Stephen Wilson
Associate Professor of Psychology

ABSTRACT

Classical fear conditioning is a behavioral paradigm with external validity to cognitive and psychiatric variables. Contextual fear conditioning is a hippocampus-dependent form of associative learning in which an assimilated set of cues (context) come into association with an aversive stimulus. This versatile behavior is used in human and rodent research to model anxiety disorders and basic learning processes. In mouse models, conditioned fear is typically assessed through measurement of immobility behavior, which reflects a naturally-selected fear response to predators with limited visual capacity to detect motionless targets. Thus, this dependent variable is the sum of multiple processes including, but not limited to associative learning, configural learning, fear and anxiety, and general activity. These intersecting phenotypes that sum to affect freezing behavior are innate constituents of the fear learning process and should not necessarily be dismissed as potential confounds. That is, contextual fear conditioning in the functioning organism represents the ethologically relevant aggregate of all its underlying effectors. Understanding the behavioral and biological context in which contextual fear conditioning occurs promotes better modeling of parallel human conditions as well as ethologically informed interpretation of this phenotype in animal models.

This dissertation aimed to characterize behavioral and genetic architecture underlying performance in contextual fear conditioning and correlated behaviors using a mouse model of genetic diversity. This research utilized a preliminary fear conditioning dataset in the BxD recombinant inbred strain panel for QTL mapping of contextual fear conditioning. This mapping was used to identify candidate genes for contextual fear learning. High and low fear conditioning strains, as well as intermediate BxD parental strains, were selected for additional testing in a behavioral battery comprising measures of configural learning, anxiety, activity, and fear

conditioning. Expression of candidate genes for contextual fear conditioning was quantified in battery animals. Finally, gene expression and behavioral data were analyzed using an exploratory factor analysis in order to identify underlying phenotypic dimensions impacting fear conditioning and correlated behaviors.

Exploratory factor analysis revealed five distinct phenotypic constructs representing activity/anxiety/exploration, associative fear learning, anxiety, post-shock freezing, and open field activity phenotypes. These findings contextualize fear conditioning within the broader murine behavioral architecture. Associative learning and expression of one candidate gene for contextual fear conditioning emerged as a unique construct within the factor analysis. Post-shock freezing during the fear conditioning training trial and expression of a second candidate gene for contextual fear conditioning emerged as an additional unique construct, highlighting the independence of this measure within the fear conditioning paradigm. These findings additionally support a link between adaptive prey behaviors expressed in anxiety, activity, and exploratory phenotypes. Between-strain comparisons on composite variables informed by the factor analysis further allowed for preliminary behavioral profiling of tested strains. These findings inform understanding of fear conditioning in terms of its secondary measures, underlying biological mechanisms, and interaction with other mouse behaviors.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health (NIH) grant 1U01DA041632, as well as institutional funding from the College of Health and Human Development and the Biobehavioral Health Department. The content of this dissertation is solely the responsibility of the author(s) and does not necessarily reflect the official views of the NIH or Pennsylvania State University.

First and foremost, I would like to acknowledge my Ph.D. advisor, Tom Gould. His excellent mentorship and unwavering support made for a graduate school experience that was both productive and enjoyable. Under your guidance, I have grown as a scientist, a writer, and a member of an outstanding research team. Thank you for supporting my career goals at every stage of this process.

I would like to also acknowledge the members of my dissertation committee, who have supported me through comprehensive examinations, my dissertation proposal, and my dissertation defense. Thank you, Helen Kamens, for providing opportunities to collaborate with your laboratory, and for being an invaluable source of discussion and companionship throughout many conferences, committees, and brown bags. Thanks to David Vandenbergh and Helen Kamens for always being willing to assist when I encountered unexpected wet lab issues. Thank you, David Vandenbergh, for using your exceptional breadth of knowledge in genomics to guide my education in classes, lectures, and as my committee member. Finally, thank you to Stephen Wilson for sharing your expertise and perspectives on human addiction and brain functioning as a member of my committee.

Thank you to previous and current members of Tom's laboratory who contributed to this project. Munir Gunes Kutlu and Lisa Goldberg collected the impressive preliminary dataset

referenced in this dissertation. I would like to express my appreciation especially to Lisa Goldberg, who very patiently trained me in genetic concepts and analysis. I cannot thank Laurel Seemiller enough for suggesting analysis of rearing and grooming behavior and scoring these variables.

Thank you to all members of the Gould lab for being the best teammates and friends I could ask for throughout this process, especially: Sean Mooney-Leber, for your valuable mentorship and enduring positive attitude; Laurel Seemiller, for becoming one of my best friends and my favorite collaborator; Other postdoctoral scholars and graduate students, undergraduate research assistants, and laboratory technicians, who have made the lab a fun and supportive working environment.

I thank the Biobehavioral Health Department and Penn State University for providing me with an excellent education. The wonderful faculty, staff, and students of the Biobehavioral Health Department have made me feel so welcomed. I would also like to acknowledge the Penn State QuantDev Methodology Core, and particularly David Wagstaff, who provided consultation on a portion of the statistical analyses included in this dissertation. Thanks to the National Institutes of Health for funding me through an F31 grant, which allowed me to expand my training in research and professional development during the last few years of my graduate education.

Thank you so much to my family and friends who have always been my number one source of comfort and encouragement. Thanks to my parents for cheering on all my achievements and to my siblings for listening to me ramble about mice and brains. Thank you, Gina Mathew, for being my first friend in graduate school and one of my best friends for life.

Thank you especially to Marc Lindberg, who mentored me as an undergraduate and continues to advise me on matters of science and career development.

Finally, I would like to acknowledge the mice that have served as my experimental subjects. These include the 12 female and 12 male C57BL/6J, 12 female and 12 male DBA/2J, 13 female and 11 male BXD124, 12 female and 13 male BXD56, 11 female and 12 male BXD98, and the 6 female and 14 male BXD64 mice whose lives were sacrificed to produce the current data. These also include the countless mice who have served as subjects on my projects over the past few years. Basic neurobehavioral research would not be possible without these bright and fascinating creatures, and I will always strive to make the science I produce worthy of their sacrifice.

Chapter 1: INTRODUCTION

Classical fear conditioning is a behavioral paradigm with external validity to cognitive and psychiatric variables. Fear conditioning reflects the adaptive tendency to attribute aversion to a previously neutral stimulus following its close temporal presentation with an aversive stimulus. Value of the capacity to learn which stimuli predict danger is evinced in the broad biological conservation of this behavior across species, allowing for translation of fear conditioning research between model organisms. Aberrant fear conditioning is a common manifestation of anxiety disorders, wherein indiscriminate and/or exaggerated fear conditioning becomes pathological instead of adaptive. Classical fear conditioning's constituent cognitive processes additionally make it an attractive model in basic neurocognitive research. Variants of classical fear conditioning test learned fear associations between aversive stimuli and discrete cues (cued fear conditioning), or between aversive stimuli and stimulus assemblies (contextual fear conditioning). Thus, this paradigm can provide insight into multiple underlying cognitive domains, including spatial/configural processing, associative learning, and affective expression. Contextual fear conditioning is a hippocampus-dependent form of associative learning in which an assimilated set of cues (context) come into association with an aversive stimulus (Rudy, Huff, & Matus-Amat, 2004). The translational importance of this form of learning is exemplified in certain anxiety disorders, wherein locations and situations become anxiety-triggering stimuli (Neueder, Andreatta, & Pauli, 2019). Contextual fear conditioning is also valued as a basic model of hippocampus-dependent configural processing, which is critical for spatial learning (McHugh, & Tonegawa, 2007).

As noted above, contextual fear conditioning can be said to represent an intersection of pertinent phenotypes. In rodent models, conditioned fear is typically assessed through

measurement of immobility ("freezing") behavior, which reflects a naturally-selected fear response to predators with limited visual capacity to detect motionless targets (Ilany & Eilam, 2008). Thus, in rodents, the dependent variable "freezing" is the sum of multiple processes including, but not limited to associative learning, configural learning (binding of multiple stimulus elements into a unified representation), fear and anxiety, and general activity.

Contextual fear conditioning has become a nearly ubiquitous model in many neuropsychological fields, often utilized to study anxiety disorders and basic learning processes (Maren, 2008). In the context of rodent studies, the dependent variable "freezing" often acts as the sole surrogate for the phenotype of interest. While immobility behavior is a convenient and ethologically valid proxy for the fear response (Bolles, 1970), it is difficult to dissociate all behavioral factors that may contribute to variation in freezing. In extreme cases, certain organismal abnormalities may entirely confound freezing behavior. For example, freezing indices would be unreliable measures of learning or anxiety in a transgenic mouse line carrying a mutation severely impairing motor behavior.

More typically, however, the intersecting phenotypes that sum to affect freezing behavior are innate constituents of the fear learning process and should not necessarily be dismissed as confounds. Indeed, rodent genetic studies have repeatedly identified overlapping genomic effectors of fear learning, anxiety, and activity (Henderson, Turri, DeFries, & Flint, 2004; Ponder et al., 2007; Sokoloff, Parker, Lim, & Palmer, 2011), a notion that has been supported by additional behavioral work in diverse genetic models (Milner & Crabbe, 2008; Crawley et al., 1997). This does not imply that phenotypes traditionally associated with fear conditioning (e.g., configural and associative learning) do not impact this behavior. Genomic effectors of fear conditioning distinct from known genomic correlates of emotionality/locomotion have been

identified in multiple independent studies (Caldarone et al.,1997; Knoll, Halladay, Holmes, & Levitt, 2016; Owen, Christensen, Paylor, & Wehner, 1997). However, behavioral presentation of fear conditioning in an intact organism represents the ethologically relevant aggregate of all its underlying effectors.

Strategies exist for assessing isolated cognitive processes using fear conditioning as a tool: For example, a researcher interested in configural learning as modeled by contextual fear conditioning may in parallel assess cued fear conditioning, which is a configural processing-independent form of learning. This may control for the portion of variance in anxiety, general activity, and associative learning that overlap between both fear conditioning types, allowing for remaining phenotypic differences between the two paradigms to emerge (Curzon, Rustay, & Browman, 2009).

In the case that fear conditioning is itself the focus of study, however, it should be appreciated that this behavior is an emergent phenotype reflecting multiple intersecting dimensions of anxiety, activity, and learning. Importantly, this may be viewed as a strength of the measure given that the goal of fear conditioning in rodent models of learning and anxiety is to represent the complex human counterparts to these conditions. A number of approaches have been used to examine underlying phenotypic dimensions of rodent fear conditioning and its correlated behaviors. Quantitative genomic analyses (including quantitative trait locus mapping studies, as cited above) involve genome-wide mapping of allelic effectors underlying behavioral traits. These analyses can reveal multiple allelic variants impacting fear conditioning behaviors – which may in turn reflect distinct phenotypic dimensions that sum to control fear conditioning. Genomic mapping strategies are also well-suited for identifying pleotropic loci underlying variance in multiple behaviors (Sokoloff et al., 2011).

Utilization of behavioral batteries comprising measures of anxiety and activity is also recommended for faithful dissection of rodent behavioral architecture (Crawley & Paylor, 1997; van Gaalen & Steckler, 2000). While behavioral batteries are an excellent design for modeling the diversity of rodent phenotypes, the underlying behavioral architecture impacting performance across experimental contexts can only be revealed through combined analysis of battery variables. Data reduction analyses with the capacity to identify overlapping variance across an entire dataset can distinguish latent phenotypic constructs that may not be evident from appraisal of each assay individually. Factor analyses, such as principal components analysis (PCA) and exploratory factor analysis (EFA), are powerful tools for identifying common phenotypic dimensions explaining performance across multiple measures. Genetic diversity is a prerequisite for quantitative genomic analysis, and utilization of a genetically diverse model strengthens analysis of behavioral batteries with factor analysis by introducing phenotypic variability to the dataset (Kline, 2014).

This dissertation aims to characterize behavioral and genetic architecture underlying performance in contextual fear conditioning and correlated behaviors using a murine model of genetic diversity. This research is an extension of our laboratory's initial work using the BxD recombinant inbred strain panel for quantitative trait locus (QTL) mapping of nicotine's effects on contextual fear conditioning (Goldberg, Kutlu, Zeid, Seemiller, & Gould, 2021). The current research utilized QTL mapping of contextual fear conditioning in an expanded BxD RI strain panel to identify candidate genes for contextual fear expression. High and low fear conditioning strains, as well as intermediate BxD parental strains, were selected for additional testing in a behavioral battery comprising measures of configural learning, anxiety, activity, and fear conditioning. Expression of candidate genes for contextual fear conditioning was quantified in

battery animals. Finally, gene expression and behavioral data were analyzed using an exploratory factor analysis in order to identify underlying phenotypic dimensions impacting fear conditioning and correlated behaviors. A secondary goal of the current dissertation was to characterize BxD strain differences in the identified phenotypic dimensions. The findings described in this body of work will facilitate understanding of contextual fear conditioning in the context of the broader murine behavioral architecture, which will inform its use as a translational model for mental disorder and promote better modeling of this behavior in basic animal research. The present work also includes BxD strain behavior and gene expression data that will contribute to public data and analysis resources.

Chapter 2 of this dissertation is a comprehensive literature review of fear conditioning in rodent models, fear conditioning as a model of learning and mental health, and the use of behavioral batteries combined with factor analysis to investigate fear conditioning. Chapter 3 details the preliminary dataset and selection of candidate genes for contextual fear conditioning identified by QTL mapping. Chapter 4 describes the use of a behavioral battery comprising fear conditioning and correlated phenotypes in a sub-panel of extreme phenotype BxD strains and intermediate phenotype parental strains. Chapter 4 also describes an exploratory factor analysis and follow-up mean comparisons to characterize common dimensions affecting gene expression and behavioral performance in this panel. Chapter 5 discusses implications of these findings, dissertation conclusions, and future directions.

Chapter 2: LITERATURE REVIEW

2.1 Classical fear conditioning as a model of learning and mental health

2.1.1. Fear conditioning: Overview of use in human and animal research

Fear conditioning is a subtype of Pavlovian classical fear conditioning, which describes a simple form of associative learning. The classical conditioning process is initiated by the repeated presentation of a biologically neutral stimulus closely prior to a biologically salient event (Pavlov, 1927). Thus, predictive value is attributed to the previously neutral stimulus (conditioned stimulus, or CS) such that it comes to elicit an adaptive response to the associated biologically salient event (unconditional stimulus, or US), even in absence of the US. In Pavlov's classic example, a neutral stimulus like a bell tone may come to elicit salivation following its repeated presentation prior to the availability of food. Mechanisms of associative learning can be scaled down to the neural synapse and further complexed to understand broader learning and memory networks (Maren, 2005; Maren, De Oca, & Fanselow, 1994; Rumpel, LeDoux, Zador, & Malinow, 2005). Thus, variants of this behavioral model revolutionized basic learning and memory research, and its use as an experimental model remains immensely popular today (Maren, 2008).

Classical fear conditioning specifically involves a biologically salient stimulus (US) of an aversive nature, so that the previously neutral stimulus comes to elicit a fear response. As fear behaviors generally represent innate defense responses (Martinez, Carvalho-Netto, Amaral, Nunes-de-Souza, & Canteras, 2008), classical fear conditioning is a highly adaptive behavior that is conserved across animal species, with overlapping neurological underpinnings between mammals (LeDoux, 1996).

In its attribution of emotional valence to previously neutral stimuli, classical fear conditioning guides approach and avoidance behaviors. Emotional dysregulation in the form of exaggerated or otherwise abnormal fear conditioning is a common manifestation of anxiety disorders (Duits et al., 2015; Geller et al., 2017; Grillon, 2002; Grillon & Morgan III, 1999; Lau et al., 2008; McGuire et al., 2016). This family of disorders is defined by pathological attribution of fear and anxiety to innocuous stimuli, or to stimuli that do not pose a proportionate threat to these responses. Fear conditioning can impact both anxiety disorder etiology (e.g., traumatic associations leading to the development of phobias) and symptomology (e.g., exaggerated tendency to attribute anxiety to innocuous stimuli) (Mineka & Zinbarg, 2006). Thus, individual variability in fear conditioning is a focus of great interest in basic cognitive and mental health research.

In addition to this translational relevance, the popularity of classical fear conditioning as an experimental model emerges from the paradigm's practicality in a laboratory setting as well as its ethological relevance. An ongoing challenge in translational research involving rodent models is the identification of rodent behaviors that align with human conditions in addition to being scalable at the level of basic animal research. In the case of classical fear conditioning, the same basic elements of the experimental paradigm can be translated directly from human to rodent research and vice versa. For example, rodent classical fear conditioning models typically utilize a footshock/tone pairing, similar to electric shock/CS pairings commonly used in human research (Glenn, Lieberman, & Hajcak, 2012). Classical fear conditioning is rapidly acquired and well-retained in human and rodent models. Finally, there is some overlap between certain fear responses in humans and rodents: For instance, skin conductance is an oft used proxy for autonomic fear responses in human fear conditioning (Christopoulos, Uy, & Yap, 2019; Grings,

1969) and has been shown to correlate with anxiety responses in rats and mice (Dolu, Acer, & Kara, 2014; Süer, Dolu, Özesmi, Şahin, & Ülgen, 1998).

More often, however, other species-appropriate measures are used to quantify fear response. In rodents, conditioned freezing is favored for its ease of scalability and known ethological validity with regard to the rodent fear/defensive behavioral repertoire (Bolles, 1970; Martinez et al., 2008). In contrast, self-report is usually coupled with quantitative measures of autonomic response, such as skin conductance or fMRI imaging, in human models (Lonsdorf et al., 2017). Importantly, choice of dependent variable is as critical as the assay design, as the measure of fear response dictates interpretation of findings. This is discussed further in the context of the broader rodent behavioral repertoire under section 2.2 ("Investigating rodent behavioral architecture using factor analysis").

2.1.2 Rodent experimental models of fear conditioning: Methodology

A commonly used classical fear conditioning procedure in rodent experimental models involves use of footshocks as the aversive unconditional stimulus and freezing behavior as a measure of learned fear. The footshock/stimulus pairing occurs during a training phase. This training phase is typically designed such that a baseline measurement of activity (freezing) within the chamber is taken prior to any stimulus presentation. This baseline activity measurement is important for determination of baseline freezing behavior within the fear conditioning apparatus. Training trial baseline activity can indicate motor impairments associated with experimental manipulations and is also examined as a proxy of baseline anxiety and activity/exploratory behavior (Ahn et al., 2013; Anagnostaras et al., 2010). Freezing behavior immediately after the initial footshock (called the "post-shock" or "immediate" phase)

is also frequently measured. Post-shock freezing is thought to reflect a combined measure of unconditioned response to the footshock as well as rapid contextual learning (Ballesteros, de Oliveira Galvão, Maisonette, & Landeira-Fernandez, 2014; Fanselow, 1980; Wood & Anagnostaras, 2011). Thus, despite the fact that they are often excluded from data presentation, baseline and post-shock freezing can provide valuable information about the experimental model.

Training trial design specifies which subtypes of fear conditioning can be measured during the testing phase. In training for basic delay fear conditioning, the footshock co-terminates with a distinct stimulus (e.g., an auditory cue). Learned fear of the training context can be tested simply by returning animals to the training chamber and measuring freezing response to this re-exposure. Learned fear of the conditioned cue can be subsequently measured within a distinct testing context (e.g., a separate test chamber with sensory cues unique from the training chamber). Use of a distinct test context is critical for accurate measurement of cued fear learning, as this subtype of conditioning is defined by association of fear with a *discrete* stimulus. The cued testing phase includes an additional baseline measurement of freezing prior to the presentation of the conditioned cue, sometimes called the "pre-CS" or "tone baseline" phase. Importantly, pre-CS freezing is not comparable to the baseline phase of training, which is taken prior to any tone or footshock presentation. Patterns of pre-CS freezing and their associated interpretation vary widely throughout published literature, in part reflecting that pre-CS freezing is a proxy for contextual distinction as a function of training and cued test apparatus design (Baldi, Lorenzini, & Bucherelli, 2004). The general lack of cued testing apparatus standardization across published literature means that degree of generalization between the training and cued contexts will differ from lab to lab. Moreover, tendency to generalize to

training context may vary by experimental model (Jacobs, Cushman, & Fanselow, 2010). As with all measures of freezing in the fear conditioning paradigm, pre-CS freezing is expected to interact with baseline behavioral tendencies such as anxiety and activity. Thus, pre-CS freezing can be viewed as a combined learned fear generalization, anxiety, and activity measure (Jacobs et al., 2010). In sum, context and cued test freezing are often identified as the primary outcomes in fear conditioning models. However, they should be interpreted in the context of baseline, post-shock, and pre-CS freezing, which may reveal interacting behavioral factors that modulate these measures of learning.

2.1.3. Rodent models of fear conditioning: Subtypes and neural underpinnings

As discussed in section 2.1.2, the primary learning outcomes in a basic delay fear conditioning task are the footshock/training context (contextual) and footshock/discrete cue (cued) associations. These categories of learning are mechanistically and translationally distinguishable, although there is a degree of overlap in their expression and underlying effectors. Associative fear learning and fear expression are core components of both contextual and cued fear conditioning. The common neural substrate of stimulus association in fear conditioning is the basolateral amygdala, with the lateral amygdala subnucleus thought to represent the primary area of convergence between sensory representations of the conditioned and unconditioned stimuli (Amorapanth, LeDoux, & Nader, 2000; Maren, 2001; Tsvetkov, Carlezon, Benes, Kandel, & Bolshakov, 2002). Following its reception of stimulus association information from the lateral amygdala, the central amygdala governs fear expression through its projections onto autonomic fear response systems such as the hypothalamus and periaqueductal gray (Iwata, Chida, & LeDoux, 1987; Maren, 2001; Roozendaal, Koolhaas, & Bohus, 1991).

Thus, mechanisms of contextual and cued fear learning largely diverge upstream of amygdala-controlled fear association and expression. In cued fear learning, CS sensory representations delivered to the lateral amygdala are thought to derive directly from sensory processing areas (e.g., auditory cortex and its thalamic relays in the case of an auditory cue), reflecting the discrete nature of the CS (Quirk, Repa, & LeDoux, 1995; Tsvetkov et al., 2002). In contrast, the conditioned stimulus (context) in contextual fear learning represents a highly processed, multisensory stimulus assembly that is primarily constructed via configural processing in the hippocampus (Rudy & O'Reilly, 1999). In rodents and humans, the hippocampus has been shown to consolidate sensory information from diverse brain regions into a unified representation in a process called configural learning. In contextual fear conditioning, this configuration is relayed from the hippocampus to the amygdala, where it comes into association with sensory representations of the unconditioned stimulus (Fanselow, 1990; Young, Bohenek, & Fanselow, 1994).

Existing literature has consistently identified differential involvement of the dorsal and ventral poles of the hippocampus in fear conditioning (Fanselow & Dong, 2010). Specifically, it is thought that the dorsal hippocampus is primarily responsible for configural processing mediating contextual fear conditioning. This is supported neuroanatomically, as pre-hippocampal regions critical for multisensory assimilation project more densely to the dorsal hippocampus versus the ventral hippocampus (Bucci, Phillips, & Burwell, 2000; Burwell, Bucci, Sanborn, & Jutras, 2004). Configural processing in the dorsal hippocampus also mediates allocentric spatial learning, which is dependent upon formation of cognitive spatial maps (Moser, Moser, Forrest, Andersen, & Morris, 1995; Miyoshi et al., 2012). Thus, performance in contextual fear conditioning tasks sometimes correlates with spatial learning phenotypes that can be solved

using an allocentric spatial learning strategy (e.g., Morris Water Maze) (McHugh & Tonegawa, 2007; Podhorna & Brown, 2002). Notably, however, configural learning is not required for many non-associative spatial tasks where egocentric (referential) learning strategies are possible. Accordingly, performance in non-associative spatial learning tasks and contextual fear conditioning do not always correlate within subject or experimental group (Owen, Logue, Rasmussen, & Wehner, 1997, Poplawski et al., 2014), and manipulations affecting learning in a non-associative spatial task do not necessarily impact contextual fear conditioning (Cho, Friedman, & Silva, 1998; Clark et al., 2008). Components of contextual fear conditioning inherent to this task (e.g., associative learning and emotion) may also explain divergent performance between non-associative spatial tasks and contextual fear conditioning (Burwell, Saddoris, Bucci, & Wiig, 2004; Pugh, Tremblay, Fleshner, & Rudy, 1997; Widman et al., 2019).

The ventral hippocampus more generally regulates emotional processing (Bertoglio, Joca, & Guimaraes, 2006; McHugh, Deacon, Rawlins, & Bannerman, 2004), evinced by its reciprocal connectivity with the central amygdala and other brain structures associated with the hypothalamus-pituitary-adrenal axis (Jacobson & Sapolsky, 1991; Kishi, Tsumori, Yokota, & Yasui, 2006). Chemical inactivation and lesion studies have revealed that the ventral hippocampus often modulates performance in both cued and contextual fear conditioning (Bast, Zhang, & Feldon, 2001; Hunsaker & Kesner, 2008; Richmond et al., 1999; Zhang, Bast, & Feldon, 2001). This role for the ventral hippocampus in fear conditioning is feasible considering the inherently emotional nature of the behavior. For example, it has been found that the ventral hippocampus has a critical role in consolidating the unconditioned stimulus representation during contextual fear conditioning (Huff, Emmons, Narayanan, & LaLumiere, 2016). The ventral hippocampus has also been identified as a regulator of emotional behavior in tasks measuring

rodent anxiety (Bertoglio et al., 2006; McHugh et al., 2004). Other work has suggested that situational stress, as processed by the ventral hippocampus, may interact with configural representations in the dorsal hippocampus to modulate contextual fear conditioning (Ritov, Ardi, & Richter-Levin, 2014; Rudy & Matus-Amat, 2005).

In sum, contextual and cued fear conditioning are distinct types of learning with neural substrates unique to each in addition to some overlapping substrates. The dorsal hippocampus is primarily involved in contextual fear conditioning through its control of configural learning. The ventral hippocampus is broadly involved in emotional processing, and thus may modulate both contextual and cued fear conditioning, as well as other emotional behaviors. Both categories of fear learning have translational relevance backing their use in basic learning and mental health research. As noted above, contextual fear conditioning is often utilized as a proxy for configural processing, which overlaps with allocentric spatial learning strategies. As discussed in section 2.1.1, fear conditioning is often identified as a component of emotional dysregulation characteristic of anxiety disorders (Duits et al., 2015; Geller et al., 2017; Grillon, 2002; Grillon & Morgan III, 1999; Lau et al., 2008; McGuire et al., 2016). Contextual fear conditioning in particular is thought to more appropriately represent non-cue-specific anxiety seen in generalized anxiety disorders (Grillon, 2002; Hartley & Phelps, 2010; Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011).

Fear conditioning, as a component of both normal functioning and mental disorder, occurs within the larger behavioral and neural repertoire of a whole organism. Even when fear conditioning is utilized as a tool to approximate specific phenotypes (e.g., configural processing), this behavior is expected to interact within an organism's broader neuro-behavioral context.

Extension of whole organism assessment to animal models may refine interpretation of fear conditioning measures and facilitate understanding of this behavior's role in mental disorder.

2.2. Investigating rodent behavioral architecture using factor analysis

As discussed in previous sections, fear conditioning is the sum of intersecting phenotypes that can be viewed as innate constituents of the fear learning process. Contextual fear conditioning is a behavioral task that well illustrates this convergence in its versatility as an experimental model. In humans and in non-human animal research, contextual fear conditioning has been used to model hippocampus-dependent configural learning, associative learning, and emotional/mental disorder (Maren, Phan, & Liberzon, 2013). Although contextual fear conditioning is often used as a tool to analyze these processes individually, here I argue that it is also beneficial to examine contextual fear conditioning as a whole, naturally-selected behavior. The value of studying contextual fear conditioning as a whole behavior is readily apparent in the context of mental health research, where fear conditioning itself is a component of anxiety disorder etiology and symptomology. However, even in basic research settings for which contextual fear conditioning is used as a tool to model subcomponents of the behavior (e.g., configural learning), it is important to recognize its behavioral and physiological context. Not only can this aid in identifying pertinent phenotypic confounds (e.g., high baseline anxiety affecting freezing in a murine model), but it may also more faithfully model the complexity of animal neural systems, which do not function in isolation.

At the level of basic research, understanding contextual fear conditioning involves scrutinization of both the behavioral model and measurable approximations of performance in this task (dependent variables). As discussed previously, contextual fear conditioning is the

aggregate of configural learning, associative learning, and emotional processes. It may therefore be illuminating to test contextual fear learning alongside assays representing these processes. In rodent models, freezing is the most commonly used proxy for fear response in contextual fear conditioning paradigms, favored for its scalability in a laboratory setting and known ethological validity as a component of the rodent fear/defensive behavioral array (Bolles, 1970). Inherent to fear response as a function of freezing behavior is its expression through the interface of the rodent musculoskeletal system. Thus, assessment of phenotypes that co-express with activity, such as anxiety, locomotor behavior, and exploratory tendencies may reveal broader phenotypic dimensions impacting contextual fear conditioning.

The expression of fear response through the interface of immobility behavior in rodents need not be considered a compromise to measurement of the "pure" fear response. In a functioning organism, no fear response occurs in isolation from interacting neurobehavioral systems. Indeed, freezing behavior's natural occurrence within the rodent's fear/defensive behavioral repertoire suggests this dependent variable is a good candidate for capturing ethologically relevant components of contextual fear learning in rodents. This is supported by extensive literature finding common genomic effectors for contextual fear conditioning, activity, and anxiety in rodent models (Crawley et al., 1997; Henderson et al., 2004). Breeding studies for which rodent lines were selected based on fear conditioning phenotype have found co-selection of anxiety phenotype in high and low fear conditioning lines (Ponder et al., 2007). Principal component analyses have additionally noted co-loading of locomotion, anxiety, and fear conditioning onto the same factor (Milner & Crabbe, 2008; Sokoloff et al., 2011). Neuroanatomical and functional studies additionally suggest that the dorsal hippocampus and its projections regulate diffuse neural systems controlling locomotion and exploratory activity

(Fanselow & Dong, 2010; Swanson, 2000; Zarrindast, Nasehi, Piri, & Heidari, 2011). Thus, performance in contextual fear conditioning may correlate with performance in behavioral assays measuring activity, anxiety, configural learning, and associative learning (Owen et al., 1997b).

This inherent interrelatedness between rodent behaviors can be leveraged using a multi-variable dataset to identify underlying phenotypic constructs that represent shared variance between measures. Data reduction analyses, such as exploratory factor analysis, can empower researchers to move beyond the behavioral proxies represented by individual variables to understanding latent constructs captured by the sum of these variables. Such analyses allow for identification of overarching behavioral variables affecting performance across experimental contexts.

The goal of the current research was to characterize murine behavioral architecture underlying performance in contextual fear conditioning and related behaviors. To enable identification of broader latent constructs involved in contextual fear learning, fear conditioning measures were taken alongside a battery including other behavioral tasks known to correlate with performance in contextual fear conditioning. The resulting dataset was analyzed using an exploratory factor analysis.

Factor analyses are strengthened by the use of phenotypically diverse subjects, which allows for sufficient between-subject variability of the input dataset to capture the range of performance expected from natural populations. Here, I tested a small panel of inbred mouse strains that were chosen based on their contextual fear conditioning phenotype in a previous experiment. Specifically, I selected two strains each to represent the extreme low end of a phenotypic distribution including 31 strains (lowest freezing to conditioned context), two to represent the extreme high end (highest freezing to conditioned context), and two strains to

represent the middle of the distribution (intermediate freezing to conditioned context; Chapter 3). These strains were derived from the BxD recombinant inbred (RI) lines, which were created through a cross of two inbred strains (BxD = derived from parental strains C57**B**L/6J × **D**BA/2J), followed by repeated sib-mating until the point of homozygous fixation at all genomic loci (Taylor, 1989). The resulting RI lines possess a homozygous mosaic genome derived from the two parental strains, producing considerable phenotypic variation across the panel of BxD strains.

Inclusion of biological variables alongside behavioral measures in exploratory factor analyses may reveal mechanistic pathways underlying behavior not otherwise evident from separate analyses. For the current research, expression of two candidate genes for contextual fear conditioning was quantified using qPCR, and expression data was included alongside behavioral measures in the factor analysis. These candidate genes were identified using quantitative trait locus (QTL) mapping of preliminary contextual fear conditioning data available from the 31 initially tested strains (see Chapter 3 for details). QTL mapping as a technique for candidate gene selection was an excellent fit for this dissertation's research goals, as genomic mapping is performed using the behavioral trait – meaning that identified candidates necessarily capture variability in contextual fear conditioning as a whole behavior. Although these genes were already identified as candidates for contextual fear conditioning, the current design permitted greater specification of their role within the murine behavioral architecture.

A secondary goal of the current dissertation was to characterize BxD strain differences in latent phenotypic constructs identified by EFA. The BxD recombinant inbred strain panel enjoys broad popularity in contemporary neurobehavioral research due to the ready availability of the fully genotyped BxD lines. This has enabled construction of publicly available databases

containing rich phenotypic information for many BxD strains, allowing correlation of newly generated data with previous findings. Thus, the data generated for this project will contribute to publicly accessible data and analysis resources. Notably, two of the BxD strains tested for the current research (BXD124 and BXD56) have rarely been included in published datasets. As full BxD strain panels are typically utilized for QTL mapping studies, extended analysis of extreme phenotype strains in terms of the mapped behavior may further inform interpretation of such models. Between-strain statistical comparisons can be challenging in the case of a behavioral/gene expression battery generating a long list of analysis variables, which raises the issue of multiple comparisons. A strength of the current design is that the initial exploratory factor analysis was used to inform between-strain behavioral analyses, a data-driven approach to reducing the number of statistical comparisons.

In summary, this dissertation aimed to characterize phenotypic architecture of contextual fear conditioning, behaviors related to contextual fear learning and representative of the broader murine behavioral repertoire, and expression of candidate genes for contextual fear conditioning using exploratory factor analysis. The present work also produced BxD strain behavior and gene expression data that will contribute to public data and analysis resources. These findings will facilitate understanding of contextual fear conditioning in the context of the broader murine behavioral architecture, promoting improved modeling this behavior in basic animal research.

Chapter 3: PRELIMINARY DATASET AND CANDIDATE GENE IDENTIFICATION

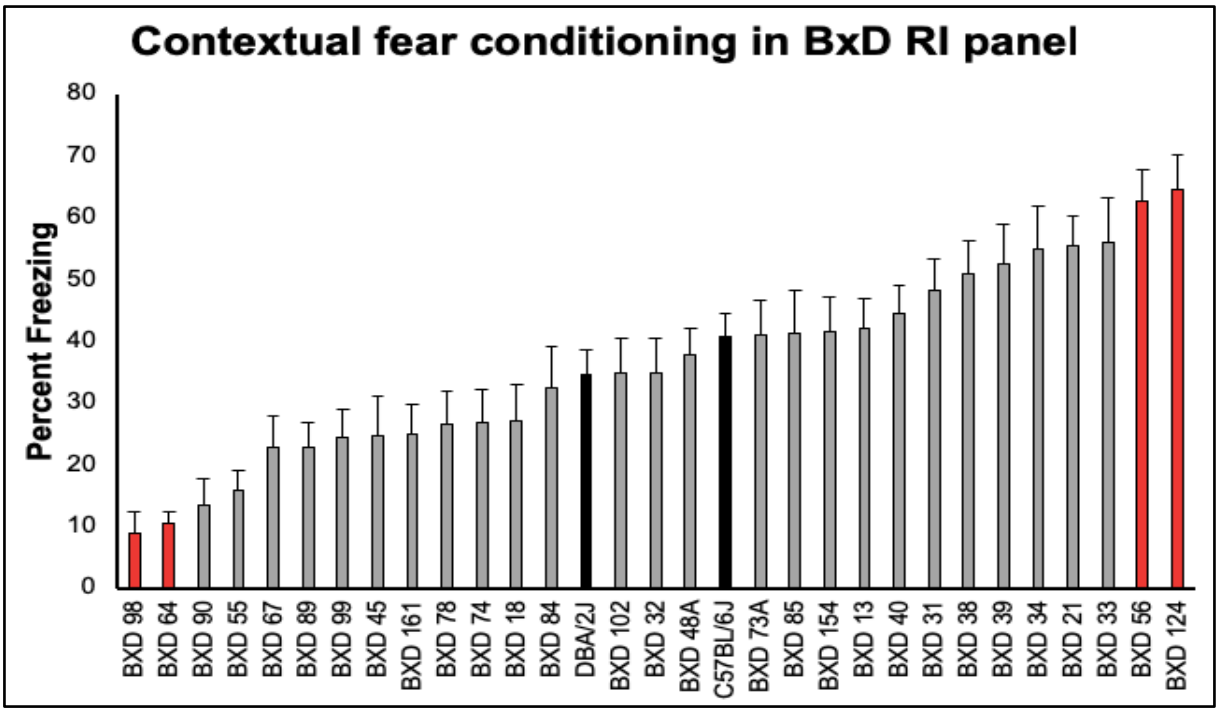
3.1 Introduction

The current research is an extension of a larger project exploring genomic regulators of nicotine withdrawal's impacts on contextual fear conditioning (Goldberg et al., 2021). For this larger project, a panel of 29 recombinant inbred strains from the BxD RI panel, in addition to parental strains C57BL/6J and DBA/2J, were chronically exposed to nicotine or saline via osmotic minipump over a period of 12 days. Contextual fear conditioning was assessed in all animals 24 hours following pump removal, during the nicotine withdrawal period for nicotine-exposed animals. Resulting data from nicotine-withdrawn subjects and saline controls were analyzed using quantitative trait locus (QTL) mapping, which revealed a chromosome 4 peak in both groups. Subsequent analyses suggested that this peak represented a genomic regulator of contextual fear conditioning that may interact with nicotine withdrawal. For the current dissertation, QTL mapping in saline controls was used to identify candidate genomic regulators of contextual fear conditioning in this panel. High and low contextual fear conditioning strains, as well as intermediate phenotype parental strains, were selected from the saline control panel for additional behavioral testing and quantification of candidate gene expression (Chapter 4). Expression of candidate genes was quantified in ventral and dorsal hippocampal tissue from high and low fear conditioning strains, and resulting data were included alongside behavioral measures in an exploratory factor analysis to characterize phenotypic dimensions underlying performance in fear conditioning and related behaviors.

3.2 Preliminary dataset

Contextual fear conditioning was assessed following withdrawal from chronic nicotine or saline in 29 strains from the BxD recombinant inbred mouse panel & the panel's 2 parental strains (C57BL/6J and DBA/2J). Male and female mice from the following BxD strains were tested (n=1009, 6-10 per sex per strain per treatment): BXD13/TyJ, BXD18/TyJ, BXD21/TyJ, BXD31/TyJ, BXD32/TyJ, BXD33/TyJ, BXD34/TyJ, BXD38/TyJ, BXD39/TyJ, BXD40/TyJ, BXD45/RwwJ, BXD48a/RwwJ, BXD55/RwwJ, BXD56/RwwJ, BXD64/RwwJ, BXD67/RwwJ, BXD73A/RwwJ, BXD74/RwwJ, BXD78/RwwJ, BXD84/RwwJ, BXD85/RwwJ, BXD89/RwwJ, BXD90/RwwJ, BXD98/RwwJ, BXD99/RwwJ, BXD102/RwwJ, BXD124/RwwJ, BXD154/RwwJ, and BXD161/RwwJ. Saline control strain means are shown in **Fig 3-1**.

Figure 3-1: Contextual fear conditioning in the BxD RI panel; Saline control means. Parental strains darkened in black and high/low fear conditioning strains highlighted in red.

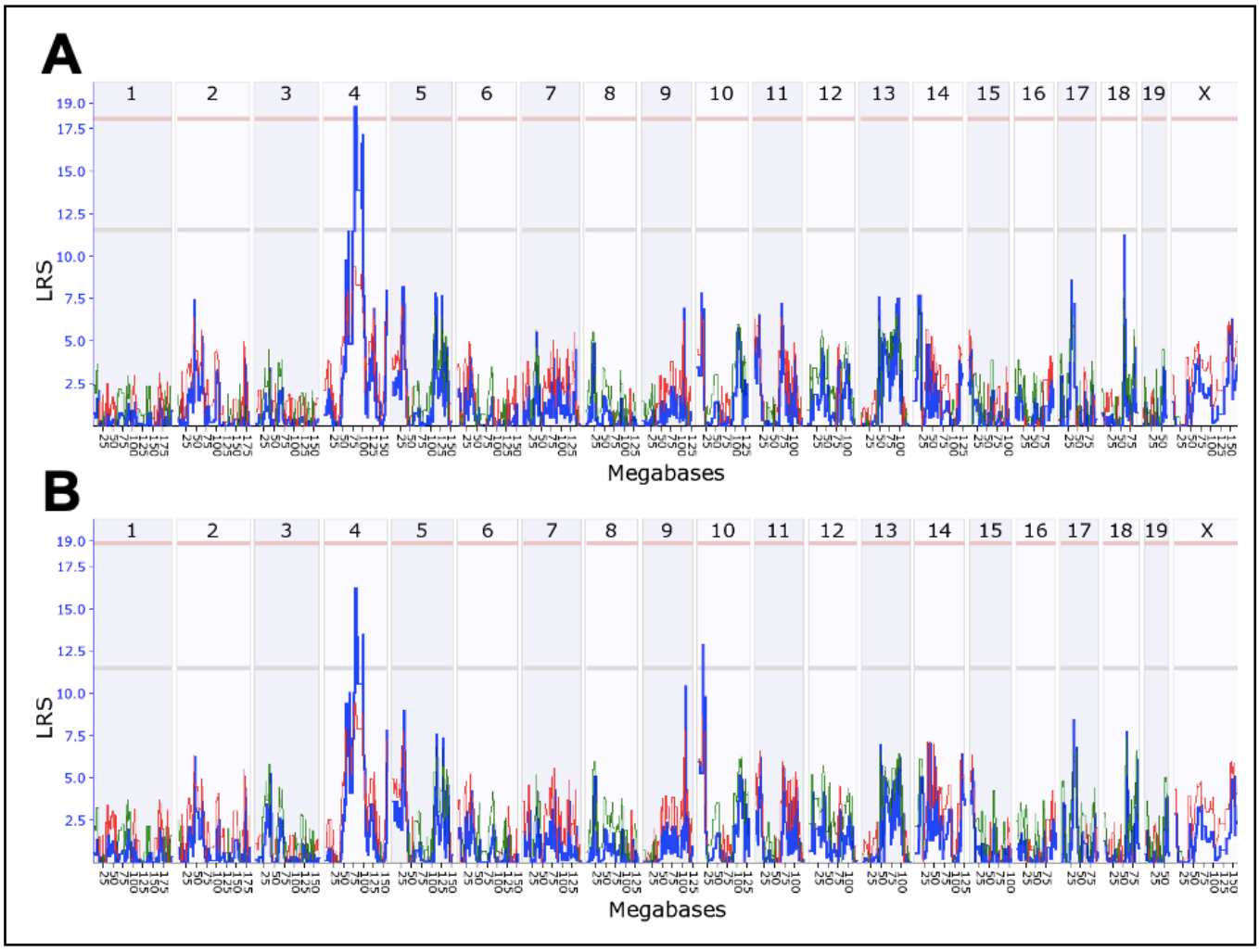


Quantitative trait locus (QTL) mapping was performed using the resulting data, for which the dependent variable was freezing behavior in response to the conditioned context (test day freezing). Briefly, interval mapping was first performed using the GeneNetwork suite of webtools (Mulligan et al., 2017; www.genenetwork.org). Fear conditioning data from nicotine withdrawn animals and saline controls were mapped separately (1000 permutations; GeneNetwork custom BxD mapping algorithm, which uses a mixture of marker regression, linear interpolation, and Haley-Knott interval mapping). With an LRS significance threshold of 18.26 (LOD 3.97), one significant QTL on chromosome 4 was identified using fear conditioning data from nicotine withdrawal animals (**Figure 3-2, A**). In saline control animals, the same chromosome 4 peak was identified, although it only passed the suggestive LRS threshold (significant LRS = 18.88 [LOD 4.1]; suggestive LRS = 11.48 [LOD 2.49]; chromosome 4 peak LRS in saline = 16.26 [LOD 3.53], p = .14; **Figure 3-2, B**). See **Table 3-1** for overlapping peak markers between nicotine and saline. This peak may represent expression of contextual fear conditioning, with a possible interaction of nicotine at this locus to produce its withdrawal effects on fear conditioning (Goldberg et al., 2021). Supporting this interpretation, I mapped baseline BxD contextual fear conditioning data publicly available through GeneNetwork to the same chromosome 4 locus (GN trait ID 20622; unpublished data by S.M. Neuner & C.C. Kaczorowski). As the focus of this dissertation was fear conditioning and related behaviors, candidate gene identification was performed using mapped data from saline control animals.

Table 3-1: Overlapping chromosome 4 peak markers between nicotine-withdrawal and saline control animals. LRS = Likelihood Ratio Statistic.

Marker (SNP ID)	Chr 4 Mb	SAL LRS	NIC LRS	SAL p-value	NIC p-value
rs28019260	75.841	16.257	18.847	0.144	0.039
rs28019230	75.847	16.257	18.847	0.144	0.039
rs28052626	76.013	16.257	18.847	0.144	0.039
rs49734466	76.857	16.257	18.847	0.144	0.039
UNC040517254	78.901	16.257	18.847	0.144	0.039
rs32586854	78.902	16.257	18.847	0.144	0.039
rs13477796	79.052	16.257	18.847	0.144	0.039
rs3708061	81.304	16.257	18.847	0.144	0.039
rs32862298	81.456	16.257	18.847	0.144	0.039

Figure 3-2: (A) QTL mapping of freezing to conditioned context in nicotine-treated BxD RI panel (B) QTL mapping of freezing to conditioned context in saline-treated BxD RI panel. Gray horizontal line = suggestive significance threshold ($p < .63$); Red horizontal line = significance threshold ($p < .05$). Mapping figures created using *GeneNetwork*. LRS = Likelihood Ratio Statistic.



3.3 Candidate gene identification

In order to identify genes whose expression may relate to variation in the fear learning phenotype, the GeneNetwork trait correlation tool (Mulligan et al., 2017) was used to correlate contextual fear learning in our BxD panel with gene expression data available in a public database. For this analysis, contextual fear learning strain means were correlated with publicly available gene expression strain means. Freezing to the conditioned context was correlated with the Hippocampus Consortium PDNN database (Overall et al., 2009), a comprehensive microarray hippocampal gene expression dataset with substantial strain overlap with our panel (24 overlapping strains, including parental). A hippocampus gene expression database was selected due to the established role of the hippocampus in fear learning and anxiety (Hunsaker & Kesner, 2008; Jimenez, 2018). Hippocampal expression of 2117 genes correlated with contextual fear conditioning in our strain panel at a threshold of $p < .05$. The following criteria were used to prioritize positional candidate genes that may contribute to the Chr 4 fear conditioning QTL: 1) localization within the Chr 4 QTL 1 LOD confidence interval of 75.841-96.897 Mb, 2) expression significantly correlating with contextual fear conditioning, and 3) presence of a single nucleotide polymorphism (SNP) between C57BL/6J and DBA/2J. Expression of 24 genes within the Chr 4 QTL correlated significantly with contextual fear learning. Of these, 17 were found to have a SNP between C57BL/6J and DBA/2J (**Table 3-2**). SNPs were identified using the Mouse Phenome Database Mouse SNP Data Retrieval tool (Bogue et al., 2017). Two of these 17 genes (*Ptprd* & *Hacd4*) were identified as final candidates using a Bonferroni adjustment of gene expression correlations with contextual fear conditioning ($p < .0029$, **Table 3-2**, gray-shaded rows). Freezing to the conditioned context was also correlated with a similar amygdala

microarray gene expression dataset available through GeneNetwork (INIA Amygdala Affy MoGene 1.0 ST), which identified no candidates meeting the above criteria.

Table 3-2: Uncorrected chromosome 4 QTL contextual fear conditioning candidate genes. Candidate genes significant at a Bonferroni adjusted p-value of .0029 are shaded in gray.

Gene	Description	Location (Chr: Mb)	Gene length (bp)	Total SNPs	Missense SNPs	Top correlation with contextual FC (Pearson r)	p-val of top corr
Ptprd	protein tyrosine phosphatase, receptor type, D	chr4:75941237-78211742	2270658	647	1	0.7398	3.46E-05
Hacd4	3-Hydroxyacyl-CoA Dehydratase 4	chr4:88407687-88438936	31249	21	0	0.6177	0.00167
Sh3gl2	SH3 Domain Containing GRB2 Like 2, Endophilin A1	chr4:85205456-85389380	183924	4	0	0.5906	0.00310
Rps6	Ribosomal Protein S6	chr4:86854099-86857367	3268	3	0	0.5585	0.00597
Slc24a2	Solute Carrier Family 24 (Sodium/Potassium/Calcium Exchanger), Member 2	chr4:86983124-87230652	247426	55	0	0.5492	0.00714
Acer2	Alkaline Ceramidase 2	chr4:86874348-86932524	58176	14	0	-0.5380	0.00877
Cntln	Centlein, centrosomal protein	chr4:84881787-85131923	250136	21	3	0.5300	0.01010
Mysm1	Myb Like, SWIRM And MPN Domains 1	chr4:94942037-94979155	37075	90	1	0.5280	0.01046
Frem1	Fras1 related extracellular matrix protein 1	chr4:82897920-83052506	154586	3	0	-0.5197	0.01205
Mpdz	Multiple PDZ domain protein	chr4:81278499-81442897	164398	22	10	0.5188	0.01224
Lurap1l	Leucine Rich Adaptor Protein 1	chr4:80910686-80954301	43615	1	0	0.5156	0.01292
Nfib	Nuclear Factor I B	chr4:82290173-82506751	215606	14	0	0.5111	0.01392
Eqtn	Equatorin	chr4:94875697-94929953	54254	4	1	0.5097	0.01425
Plaa	Phospholipase A2 Activating Protein	chr4:94565139-94603247	38108	36	1	0.5058	0.01517
Mllt3	MLLT3 Super Elongation Complex Subunit	chr4:87769925-88033407	263482	8	1	0.5008	0.01644
Ttc39b	Tetratricopeptide Repeat Domain 39B	chr4:83220300-83324309	103974	19	0	0.4980	0.01718
Psip1	PC4 And SFRS1 Interacting Protein 1	chr4:83455680-83486463	30832	9	0	0.4722	0.02537

Chapter 4: BEHAVIORAL BATTERY AND EXPLORATORY FACTOR ANALYSIS

4.1.Introduction

Contextual fear conditioning is the sum of configural learning, associative learning, and emotional processes that can be viewed as innate constituents of the fear learning process (see section 2.2 for discussion). In rodent models, learning in a contextual fear conditioning task is typically quantified using the proxy of whole-body immobility ("freezing"). Inherent to fear response as a function of freezing behavior is its expression through the interface of the rodent musculoskeletal system. Thus, phenotypes that co-express with activity, such as anxiety, locomotor behavior, and exploratory tendencies also contribute to learned fear expression in rodent models. Although in some cases these co-expressing behaviors can be a considered a confound to measurement of contextual fear learning (e.g., in the case of an experimental manipulation producing severe locomotor deficits), neurobehavioral and genomic studies have also suggested that common mechanisms impact contextual fear conditioning, activity, and anxiety in rodents (Crawley et al., 1997; Fanselow & Dong, 2010; Henderson et al., 2004; Milner & Crabbe, 2008; Ponder et al., 2007; Sokoloff et al., 2011; Swanson, 2000; Zarrindast et al., 2011). Recognizing the neurobehavioral context in which contextual fear conditioning occurs may more faithfully model the complexity of animal neural systems and improve understanding of this phenotype as an intact behavior.

This chapter describes the use of a behavioral battery including assays testing general activity, non-associative hippocampus-dependent configural learning, associative hippocampus-dependent configural learning, associative learning, and anxiety in a panel of BxD inbred mouse strains selected based on their contextual fear conditioning phenotype in a previous experiment

(Chapter 3). Hippocampal tissue was also dissected from a subset of battery subjects for gene expression quantification of candidates for contextual fear conditioning. This work's primary goal was to identify underlying phenotypic constructs representing shared variance between the tested behavioral and biological measures. This approach may promote better understanding of overarching murine behavioral architecture affecting performance across experimental contexts.

The following paradigms were included in the behavioral test battery: open field/object location memory (OF/OLM), elevated plus maze (EPM), contextual fear conditioning, and cued fear conditioning. These assays were chosen based on the phenotypes they traditionally approximate in basic neurobehavioral research: Activity/spatial learning, anxiety, contexual fear learning, and discrete cue fear learning, respectively. Variables representing these primary phenotypic outcomes are often assessed alongside secondary within-assay measures used as proxies for potential behavioral confounds. For instance, as discussed in section 2.1.3, baseline freezing response to the training/testing apparatus is usually measured prior to the CS/US pairing during the fear conditioning training trial. In the context of this work, these variables may also represent valuable proxies for mouse phenotypes of interest, such as anxiety, activity, and exploratory tendencies. Their inclusion in behavioral analyses, especially those utilizing dimension reduction techniques, such as exploratory factor analysis (EFA), may characterize with better accuracy the broader phenotypic structure impacting performance in any one assay.

The behavioral battery was organized such that less invasive assays (OF/OLM and EPM) were run prior to fear conditioning, which is less sensitive to testing history (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001). The behavioral battery took place over a 24-day period, with the first 11 days comprising a combined handling habituation, open field acclimation, and object location memory protocol (based on Vogel-Ciernia & Wood, 2014). This

paradigm was specially designed to reduce initial handling and novelty stress that may inhibit spatial learning in mouse models. Object location memory (OLM) is a test of spatial memory that relies upon a mouse's innate tendency to explore novel stimuli (Crusio, 1995; Vogel-Ciernia & Wood, 2014). The mouse is placed in an open arena containing two identical objects during the assay's training trial. For testing, one of the objects is moved to a novel location within the arena, and time spent exploring the displaced and non-displaced objects is recorded. If the mouse has learned the original (training trial) object configuration, it is expected the subject will spend more time exploring the displaced object during the test trial.

Object location memory was included within this test battery because it is hippocampus-dependent, like contextual fear conditioning, but it is a non-associative and non-fear based form of learning, unlike contextual fear conditioning. Another advantage of this OLM design is its inclusion of an initial open field habituation phase, which here allowed for measurement of baseline activity in battery subjects. Thus, several variables potentially representing phenotypic constructs of interest were derived from the entire OF/OLM protocol: In the initial open field assessment, I measured total horizontal distance moved, proportion of time spent in arena center, supported and unsupported rearing, grooming duration, and number of defecations during the trial. Total horizontal movement, time in arena center, grooming, and rearing have all been associated previously with general activity, exploratory behavior, and anxiety (Careau, Bininda-Emonds, Ordonez, & Garland, 2012; Crawley et al., 1997; Henderson et al., 2004; Milner & Crabbe, 2008; O'Leary, Gunn, & Brown, 2013; Royce, 1977; Sturman, Germain, & Bohacek, 2018), although the contribution of each of these underlying phenotypes to select behaviors can be difficult to judge when they are assessed separately. EFA including all these variables may point to their allocation within broader latent phenotypic dimensions.

Assessment of rearing activity was subdivided into rearing against arena walls (supported) and rearing within arena open space (unsupported), as recent work has noted that supported rearing may better represent general activity and exploration in mice, while unsupported rearing may better approximate anxiety (Sturman et al., 2018). In mice, defecation frequency is associated with autonomic response, and thus is one of the few proxies for murine anxiety not mediated by locomotor behavior (Hall, 1934; Milner & Crabbe, 2008; Mönnikes, Schmidt, & Taché, 1993; O'Leary et al., 2013). For the OLM portion of the assay, general exploratory tendencies can be approximated by measuring total duration of object exploration on training day in addition to the spatial learning index from OLM test day.

Elevated plus maze (EPM) testing was run following the OF/OLM assay, on day 17 of the test battery. The EPM and its variants were designed as ethologically relevant proxies for rodent anxiety. This test leverages the mouse's innate aversion to open and brightly lit spaces, a naturally-selected behavior in many prey species (Hogg, 1996; Lister, 1987; Montegomery, 1955). The primary measure for anxiety-like behavior in the EPM is typically some metric of the proportion of time spent in the EPM apparatus open arms. These data are usually interpreted as a negative relationship between level of anxiety and time spent in open arms (Pellow, Chopin, File, & Briley, 1985). Frequency of entries into the closed arms of the EPM apparatus is sometimes co-assessed as a proxy for spontaneous motor activity during the trial (Walf & Frye, 2007), which can be a useful control for measures of anxiety that are dependent upon movement (like open arm time). However, frequency of closed arm entries in EPM has also been negatively associated with anxiety and positively associated with exploration (Pellow et al., 1985; Schneider, Ho, Spanagel, & Pawlak, 2011), which is perhaps unsurprising given the link between anxiety, activity, and exploratory tendencies in rodents (see section 2.2 for discussion).

Contextual and cued fear conditioning were tested on days 23 and 24 of the behavioral battery. Although the tested strains were chosen based on contextual fear conditioning phenotype in a previous experiment, testing fear conditioning in this independent sample was necessary for inclusion of these data in the exploratory factor analysis. This also allowed for inclusion of other pertinent variables derived from the fear conditioning assay in our analysis (see section 2.1.3 discussion). Baseline freezing (initial freezing response to training apparatus) and post-shock freezing (freezing immediately following the first tone/shock pairing) were included as proxies for activity and anxiety within the fear conditioning paradigm. Freezing during the pre-CS phase (prior to presentation of the conditioned tone during the cued learning test) was included as a measure of activity, anxiety, and generalization of fear learning. Finally, freezing during the cued test trial was included as an additional representative of non hippocampus-dependent associative fear learning.

The purpose of this chapter was to characterize murine behavioral architecture underlying performance in contextual fear conditioning and related behaviors. The current approach to examining contextual fear conditioning – that is, the use of a behavioral battery alongside EFA – was selected because it models contextual fear conditioning as a naturally selected behavior. Activity, anxiety, exploration, and learning are inherently linked in prey animals, as these adaptive tendencies promote behaviors that mediate resource acquisition, socialization, and defensive responses (Crusio, 1995). Thus, their simultaneous investigation, as set up by a behavioral battery, and as analyzed by a factor analysis, may best reveal overarching behavioral and neural systems impacting any one behavior. Inclusion of candidate gene expression alongside behavioral variables in the current factor analysis may further uncover mechanistic pathways underlying contextual fear conditioning and correlated behaviors. Finally, the initial

EFA was used to guide secondary analyses testing behavioral differences between the BxD and parental strains.

4.2. Methods

4.2.1. Subjects

Subjects were male and female mice, aged 8-13 weeks at the start of the behavioral battery. 6-14 subjects per sex per strain were used for behavioral assessment. A subset of these subjects (n = 6-7 per sex per strain) were used for qPCR quantification of candidate genes for contextual fear conditioning. The subset sample was selected pseudorandomly to represent the larger sample; that is, each experimental group represented in the subset contained subjects from all testing cohorts and from multiple cages (housing groups), where sample size permitted. **Table 4-1** displays sample sizes by strain and sex for the full dataset and the qPCR/EFA subset sample.

Table 4-1: Behavioral testing (full) and gene expression/qPCR (subset) sample sizes by experimental group.

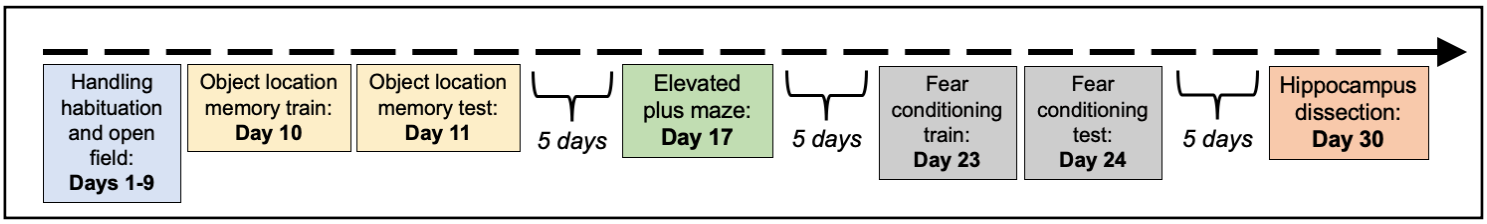
Strain/Sex		**Behavioral testing (full) sample size**	**Gene expression (qPCR)/EFA subset sample size**
C57BL/6J	*F*	12	6
	M	12	6
DBA/2J	*F*	12	6
	M	12	6
BXD124	*F*	13	6
	M	11	6
BXD56	*F*	12	6
	M	13	6
BXD98	*F*	11	6
	M	12	6
BXD64	*F*	6	6
	M	14	5

Tested strains included BxD parental strains C57BL/6J and DBA/2J, as well as previously identified high and low contextual fear conditioning BxD strains (Chapter 3): BXD124 and BXD56 (high contextual fear conditioning); BXD98 and BXD64 (low contextual fear conditioning). All mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Subjects were allowed a colony acclimation period of at least five days post arrival before beginning behavioral testing. All mice were housed in same-sex groups of 2-4 in standard open-top shoebox caging with cob bedding plus a cotton nestlet and red InnoDome igloo (BioServ, Flemington, NJ, USA) for enrichment. The colony was housed in a 12-hour light/dark cycle. All behavioral testing occurred during the hours of 8:00am and 6:00pm, during the colony's light (inactive) period. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Pennsylvania State University IACUC committee.

4.2.2. Behavioral battery overview

Behavioral testing took place over a 24-day period, during which general activity, object location memory (OLM), anxiety, and fear conditioning were assessed (see **Figure 4-1** for experimental timeline).

Figure 4-1: Summarized behavioral battery timeline.



The first 11 days of testing comprised a combined handling acclimation, open field acclimation, and object location memory protocol (based on Vogel-Ciernia & Wood, 2014 mouse OLM protocol). Five days following OLM testing (Day 17), anxiety-like behavior was assessed in the elevated plus maze (EPM). Five days following EPM testing (Days 23-24), animals underwent training and testing in contextual and cued fear conditioning. Animals were run in a total of six cohorts of varying size and strain composition; however, all cohorts included exactly two male and two female mice of both BxD parental strains.

4.2.3 Behavioral assay methodology

Handling acclimation, open field, & object location memory (Days 1-11)

The first 11 days of testing comprised a combined handling acclimation, open field acclimation, and object location memory protocol (based on Vogel-Ciernia & Wood, 2014). The entire 11-day procedure was conducted in the same room, which was dimly lit (light levels ranging from ~20-40 lux). On each day of testing, subjects were transported to the room for a one-hour acclimation period before beginning procedures. Subjects were left in home cages with continuous access to food and water over the acclimation period.

On all arena days, including OLM train and test day, animals were tested in same-sex groups of 2-4. Each individual was tested in the same assigned arena across the entire assay. Arenas were gray acrylic, (45L × 45W × 40H cm; Harvard Apparatus, Holliston, MA, USA) with a strip of black tape placed on one side of the arena to serve as a visual cue. Arenas were cleaned with 10% ethanol between each trial. Fecal boli were manually counted by the experimenter following each trial. Open field activity was recorded using an overhead camera for all OLM arena acclimation days (OLM days 4-9). Distance traveled and % time in center on all

OLM arena acclimation days were tracked using Noldus Ethovision software (Wageningen, Netherlands). Grooming and rearing behaviors on OLM day 4 (first arena trial) were hand-scored from videos using a time-sampling method.

On days 1 through 3, all subjects were briefly handled once daily. Specifically, subjects were gently placed in the experimenter's gloved hand and allowed to explore freely for two minutes. Each subject was handled individually. On days 4 through 5, subjects were first individually handled as described above, then placed in an open field and allowed to explore over a period of five minutes. On days 6-9, subjects were placed directly in the open field (no handling) once daily over a period of five minutes, and open field activity was recorded.

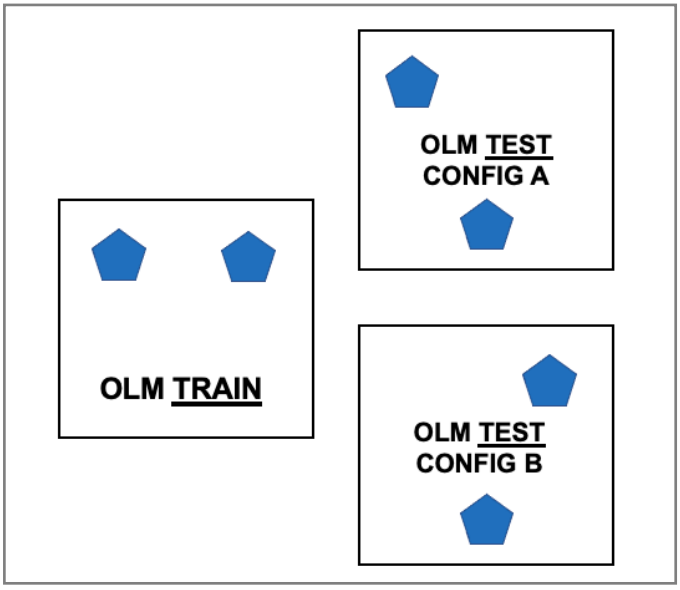
OLM training occurred on day 10, and OLM testing day occurred on day 11. Arena activity on OLM train and test days was recorded using an overhead camera, and total object exploration times were later manually scored from videos. For both OLM training and OLM test day, object exploration was defined as total interaction time with each object; that is, total time the animal's nose was oriented toward the object within ~2 cm of the object. Time spent rearing against objects or sitting on top of objects was not counted toward object exploration time.

For OLM training (day 10), two novel, identical objects (cement-filled 100mL beakers, flipped over) were placed in predetermined arena locations (see **Figure 4-2**, "OLM TRAIN"). Animals were placed directly in the arena and allowed to freely explore the arena and objects over a period of ten minutes. Objects and arena were cleaned with 10% ethanol between trials. Total object exploration time (object 1 exploration time + object 2 exploration time) was calculated for training day.

For OLM testing (day 11), one object was moved to one of two different predetermined arena location (see **Figure 4-2**, "OLM TEST"). Animals were then placed back in the arena and

allowed to freely explore the arena and objects over a period of five minutes. Time spent exploring the displaced object and the non-displaced object were calculated for test day. The displaced object (left vs. right) was counterbalanced across strain and sex. Preference ratio for the displaced object was calculated as: (displaced object exploration time ÷ [displaced object exploration time + non-displaced object exploration time]) × 100.

Figure 4-2: Object location memory training (left) and testing (right) object placement (not to scale).



Elevated plus maze (EPM)

EPM testing occurred five days following OLM test day in the same room used for the OLM assay. Subjects were transported to the room for a one-hour acclimation period before EPM testing procedures. Subjects were left in home cages with continuous access to food and water over the acclimation period. Each animal was tested separately in the same EPM arena. The EPM arena was a 55.5 cm tall gray acrylic structure with two opposing open arms (30L × 7W cm), two opposing closed arms (30L × 7W × 13H cm) and a center area (6L × 6W cm). For

EPM testing, animals were placed in the center area facing a closed arm, and an overhead camera recorded arena activity over a five-minute trial. Fecal boli excreted during the trial were manually counted by the experimenter following each trial. EPM arm entries and time spent in each arm were automatically scored using Noldus Ethovision software. Percent time in EPM open arms was calculated as: (total open arm time ÷ [total open arm time + total closed arm time]) × 100. Total closed arm entries were calculated as the sum of entries into both closed arms.

Fear conditioning

Five days following EPM testing, animals underwent training (day 23) and testing (day 24) in a combined contextual/cued fear conditioning assay. Training in a background context/foreground cued fear conditioning assay took place in chambers (20L × 23W × 15H cm; MED Associates, St. Albans, VT) equipped with ventilation fans for background noise (65 dB). For training, mice received two tone/footshock pairings. Specifically, training sessions consisted of two minutes of free movement within the chamber to measure baseline freezing, followed by 30 seconds of an 85 dB white noise tone. A two-second, 0.57-mA footshock, administered via metal floor grids (0.20 cm width, 1.0 cm spacing), co-terminated with the tone. After a two-minute post-shock period, the tone/footshock pairing was repeated. Animals were removed from the chamber 30 seconds after the second tone/footshock pairing. Freezing behavior across the entire trial was recorded using an overhead camera and automatically scored by trial segment using Noldus Ethovision software.

Approximately 24 hours after training, mice were returned to the training chamber for a five-minute trial to assess freezing to the training context. Freezing across the trial was recorded

using an overhead camera and automatically scored using Noldus Ethovision software. Testing for cued fear conditioning occurred the same day, at least one hour after contextual testing. In order to assess freezing to the conditioned tone, animals were placed in a separate set of chambers designed to be distinct from training chambers in size (15L × 16.5W × 15H cm) and tactile (acrylic floor)/olfactory cues (vanilla scent). The cued test consisted of three minutes free exploration (pre-CS), followed by three minutes of tone presentation. Freezing behavior across the entire trial was recorded using an overhead camera and automatically scored by trial segment using Noldus Ethovision software. Equipment was cleaned with 70% ethanol between all training and testing sessions.

4.2.4 qPCR methodology

Hippocampus dissection and RNA extraction

Dorsal and ventral hippocampal tissue was dissected from a subset of subjects used for behavioral testing (n = 6-7 per sex per strain, see **Table 4-1**). Subjects were euthanized for brain dissections five days following fear conditioning testing (day 30, see **Figure 4-1**). Subjects were euthanized via cervical dislocation, after which hippocampi were rapidly dissected and divided in dorsal and ventral portions (1:1 ratio). Dissected tissue was immediately submerged in RNAlater solution (Sigma-Aldrich, St. Louis, MO, USA) in order to stabilize the sample. Tissue submerged in RNAlater was incubated at 4°C for 48 hours to allow the solution to fully penetrate the sample. Tissue samples were then removed from solution, dried, and stored at -80°C.

Samples were randomized by sex, strain, and cohort for RNA extractions. Total RNA was extracted from hippocampal tissue using a TRIzol (Invitrogen, Carlsbad, CA, USA) and

chloroform extraction in combination with the Qiagen RNeasy Miniprep kit (Hilden, Germany). Specifically, tissue was homogenized in TRIzol reagent using a benchtop rotor-stator at a medium/high setting. Homogenized samples were centrifuged to separate the insoluble portion, and supernatant was transferred to a heavy phase lock tube (Quantobio, Beverly, MA, USA). Cold chloroform was added, and tubes were vigorously agitated, then centrifuged to separate phases. The upper aqueous phase was transferred to a new tube and combined with 70% ethanol. This mix was transferred to an RNeasy spin column, and RNA extraction proceeded following RNeasy Miniprep manufacturer instructions. RNA was eluted in molecular grade water.

RNA quantity and purity were assessed using 260/280 and 260/230 absorbance ratio readings on NanoDrop 2000 (Thermo Scientific, Wilmington, DE). RNA was found to be of high quality, with 260/230 ratios > 2.0 and 260/230 ratios > 1.8. All RNA samples were diluted with molecular grade water to a standard concentration of 100 ng/µL for subsequent cDNA conversion.

RT-PCR and real-time qPCR

Purified RNA was reverse-transcribed to cDNA using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Foster City, CA, USA), which uses random primers for global RT-PCR. See **Table 4-2** for RT-PCR cycling conditions.

Table 4-2: Reverse transcription PCR (RT-PCR) cycling conditions.

Temperature	*Duration*
25°C	10 min
37°C	120 min
85°C	5 min
4°C	Final hold

Ptprd, *Hacd4,* and *Hprt* real-time qPCR primers were purchased as Integrated DNA Technologies (Coralville, IA, USA) custom DNA oligos. Primers were designed such that no SNPs between the tested strains fell in the primer binding region. *Ptprd*, *Hacd4,* and *Hprt* qPCR primers were chosen based on initial testing efficiency and specificity testing (see **Table 4-3** for primer sequences).

Table 4-3: qPCR primer sequences.

Gene	Primer sequence (5'→3')
Hprt	
Forward	GCTGGTGAAAAGGACCTCT
Reverse	CACAGGACTAGAACACCTGC
Ptprd	
Forward	TGAGGGAATTCAAGGTCACG
Reverse	CCTTTGTTTTATGGACTTGGCC
Hacd4	
Forward	AGTCAAACACTCTGGATGCC
Reverse	TCAGCACATAAGGGAAACAGG

Gene expression was quantified via SYBR green fluorescence (Power SYBR Green PCR Master Mix, Applied Biosystems, Foster City, CA, USA) in the Applied Biosystems 7500 Fast Real-Time PCR thermal cycler (see **Table 4-4** for qPCR cycling conditions).

Table 4-4: Quantitative real-time PCR (qPCR) cycling conditions.

Temperature	*Duration*	*Cycle repetitions*
50°C	2 min	
95°C	10 min	
95°C	15 sec	× 40
60°C	1 min	

qPCR plates were designed to approximately balance samples across strain, sex, and testing cohort. Samples were run in triplicate, and average cycle threshold (C_T) values across triplicates was used for subsequent calculations. To calculate ΔC_T values, expression of target

genes *Ptprd* or *Hacd4* was normalized to that of housekeeping gene *Hprt* (target gene C_T – *Hprt* C_T) for each sample. Note that ΔC_T values are inversely proportional to gene expression (higher ΔC_T = lower gene expression). Data analysis was conducted using ΔC_T values.

4.2.5. Statistical analysis

Overview and goals

The two primary goals of this chapter's statical analyses were: 1) Identify latent constructs underlying candidate gene expression and performance across the behavioral battery 2) In the expanded sample size, compare strain means on latent constructs identified by factor analysis.

EFA methodology

In order to characterize the latent phenotypic structure of this dataset, dependent variables derived from the behavioral battery and gene expression assays described above were analyzed using exploratory factor analysis (EFA). Listed below are all dependent variables that were candidates for inclusion in EFA:

- OF/OLM arena acclimation total distance traveled; arena days 1-6 [6 total variables]
- OF/OLM arena acclimation % time spent in center; arena days 1-6 [6 total variables]
- OF/OLM arena acclimation fecal boli count; arena days 1-6 [6 total variables]
- OF/OLM arena acclimation day 1 supported rears
- OF/OLM arena acclimation day 1 unsupported rears
- OF/OLM arena acclimation day 1 grooming duration

- OLM training day total exploration time
- OLM test day preference ratio
- EPM % time in open arms
- EPM closed arm entries
- EPM fecal boli count
- Fear conditioning training: baseline freezing
- Fear conditioning training: post-shock freezing
- Fear conditioning test: freezing to context
- Fear conditioning test: pre-CS freezing
- Fear conditioning test: freezing to conditioned tone (CS)
- *Ptprd* dorsal hippocampus ΔC_T
- *Ptprd* ventral hippocampus ΔC_T
- *Hacd4* dorsal hippocampus ΔC_T
- *Hacd4* ventral hippocampus ΔC_T

The primary aim for this analysis was to explore latent constructs underlying performance across the entire battery. However, it is generally recommended that the number of input variables is adjusted to sample size for adequate statistical power in an exploratory factor analysis (Velicer & Fava, 1998). Thus, variables were first reduced to a smaller subset to be used for EFA. Variables were prioritized according to:

1) *A priori* selection, with the goal of reflecting the project focus (contextual fear conditioning) and representing each tested behavioral assay. Specifically, all five fear conditioning variables were included. Further, only day 1 OF/OLM arena acclimation variables were included to fully represent baseline open field phenotypes.

2) Based on preliminary analysis, statistical adequacy within the EFA model. Specifically, one variable (OLM training total exploration time) was excluded from the final model due to inadequate statistical validation metrics (see below for details).

For EFA, I aimed to represent phenotypes measured in the open field (OLM arena acclimation), OLM, EPM, and fear conditioning behavioral assays, as well as in the gene expression assays. Thus, EFA was performed with the representative sample that was used for qPCR (see **Table 4-1**). This subset included a selection of subjects from each behavioral cohort, strain, and sex, where sample size permitted.

The following variables were included to represent open field (OLM arena acclimation) phenotypes: [1] Arena day 1 total distance moved, [2] Arena day 1 % time in center, [3] Arena day 1 supported rears count, [4] Arena day 1 fecal boli count [5] Arena day 1 unsupported rears count, and [6] Arena day 1 grooming duration.

Initially, both OLM training and testing variables were included in the analysis; however, it was found that the OLM total exploration on training day exhibited lower than adequate communality (<0.1) and reduced the strength of the model. Thus, only OLM test day preference ratio was included [7]. All EPM variables (% time in open arms, closed arm entries, fecal boli count [8-10]) were included in the analysis. All fear conditioning variables (training baseline, training post-shock, context freezing, pre-CS freezing, CS freezing [11-15]) were included in the analysis. Finally, all gene expression variables (*Ptprd* dorsal and ventral hippocampus ΔCT, *Hacd4* dorsal and ventral hippocampus ΔCT [16-19]) were included in the analysis. Thus, a total of 19 input variables were used for EFA. This variable to sample size ratio is within the acceptable range for exploratory analyses (Sapnas & Zellar, 2002).

EFA extraction and rotation methods

EFA was run using SPSS v27 software (IBM, Armonk, NY, USA). The dataset was initially filtered for outliers, defined as ±2 standard deviations from the mean for a given variable. Principal axis factoring on the correlation matrix was selected as the factor extraction method. Principal axis factoring, which identifies the smallest number of factors accounting for common variance between input variables, is a commonly used EFA extraction method that is more robust to non-normality within the dataset and better recovers weak factors compared to other EFA extraction methods (Coughlin, 2013; Fabrigar, Wegener, MacCallum, & Strahan, 1999; Gaskin, & Happell, 2014). EFA was initially run using an oblique rotation (direct oblimin), which permits correlated factors -- a recommended first step for behavioral factor analyses (Costello & Osborne, 2005). Factor correlation matrices were then examined for between-factor correlations greater than |0.3| to determine if an oblique rotation was a good fit for the analysis. Scree plots displaying factor eigenvalues are used a rough guide for determining the minimum number of factors that can be extracted for any given factor analysis. It is generally recommended that researchers use the eigenvalue cutoff of > 1 only as a preliminary guideline and determine the final number of extracted factors by evaluating analysis output (Fabrigar et al., 1999). Thus, the number of extracted factors was guided by combined assessment of the Scree plot and analysis output.

The suitability of input data for factor analysis was evaluated using the Kaiser-Meyer-Olkin (KMO) test for sampling adequacy and Bartlett's test of sphericity. The KMO test for sampling adequacy compares the sum of partial correlations to the sum of correlations between input variables (Kaiser, 1981), which tests whether variables are adequately interrelated at the level of the model. In a statistically robust factor analysis, the KMO test statistic should be >0.5.

Bartlett's test of sphericity tests the hypothesis that input variables are unrelated and therefore unsuited for EFA (Bartlett, 1950). Thus, Bartlett's test of sphericity should be significant in a statistically robust factor analysis. Depending upon analysis goals, the recommendation for factor loading retention threshold ranges from a minimum cutoff as low as 0.2 and as high as 0.4 (Howard, 2016). The loading value cutoff for interpretation was set to 0.3 for the current analysis as a compromise between statistical lenience and stringency.

Between-strain comparisons

A secondary goal of the current dissertation was to characterize BxD strain differences in latent phenotypic constructs identified by EFA. EFA results were used to inform calculation of linear composite (summed) variables based on each extracted factor, and strain means on each composite variable were compared in ANOVAs using the full behavioral dataset. Unlike selection of single variables to represent each factor, this approach allowed me to further utilize the EFA findings to compare strains on relevant phenotypic constructs.

Linear composite variables informed by each EFA factor were calculated as follows: For each factor, all behavioral variables with loading > 0.3 were summed across subject to create a single composite variable representing that latent construct. Specifically, variables to be summed were first transformed to z-scores in order to standardize their scales. Because the linear composite reflects the absolute loading onto the factor, variables were also reverse coded as needed based on their factor loading. For example, for the composite variable informed by factor 1, the following variables were summed: Percent time in EPM open arms, number of EPM closed arm entries, OF/OLM arena acclimation day 1 supported rears, OF/OLM arena

acclimation day 1 unsupported rears, OLM learning index, and freezing during the baseline phase of the fear conditioning training trial. Using the full behavioral dataset, these variables were all initially transformed to z-scores. Baseline freezing in fear conditioning was then reverse coded so that higher values indicated less freezing to match the loading valence of the other summed variables. Because post-shock freezing in the training phase of fear conditioning was the only behavioral variable that loaded onto factor 4, strain means were simply compared on the standardized version of this variable.

Select individual variables *a priori* determined to be potentially informative at the level of strain mean comparisons were also analyzed. Specifically, strain differences in all gene expression variables were analyzed because these genes were selected as broader candidates for regulation of contextual fear conditioning. Because modeling of object location memory in mice is limited in published literature, strain differences in the OLM learning index were also analyzed. Strain comparisons were performed in contextual fear conditioning because this behavior was the central focus of this project.

All strain comparisons were performed using SPSS v27 software (IBM, Armonk, NY, USA). All analyses were initially run as a mixed-effects ANOVA with sex and strain as between-subjects factors and cohort as a random factor. If no significant effect of cohort was found, the analysis was re-run without the random factor. In the case of a significant sex by strain interaction, follow-up one-way ANOVAs separated by sex were performed. The Shapiro-Wilk test of normality (Ghasemi, & Zahediasl, 2012; Shapiro, & Francia, 1972) and White's test for heteroskedasticity (White, 1980) were used to test for assumptions of dependent variable normality and homoscedasticity, respectively. For analyses in which ANOVA assumptions of homoscedasticity and/or normality were violated, main and interaction effects were verified

using a non-parametric procedure (proportional odds ordinal logistic regression, a ranked data model; Harrell, 2015). Tukey HSD post-hoc tests were assessed as a follow-up to significant main effects in analyses not violating the ANOVA assumptions of homoscedasticity and/or normality. The Games-Howell post-hoc test, which does not assume equal variances between groups (Toothaker, 1993), was assessed as a follow-up to significant main effects in analyses violating the ANOVA assumptions of homoscedasticity and/or normality. The significance threshold for all analyses was *a priori* set to $\alpha = 0.05$.

4.3. Results

4.3.1. EFA

An EFA to examine latent constructs extracted from behavioral and gene expression variables was run with 19 input variables. An initial analysis, specified to extract all factors with an eigenvalue > 1 using an oblique rotation, was run in order to determine appropriate parameters for the final analysis. Although the initial analysis found six factors with eigenvalues > 1 (**Figure 4-3**), the sixth factor was just at the threshold for this criterion, and it was found that cross-loadings were greatly reduced when the number of extracted factors was reduced to five. Thus, a follow-up analysis, specified to extract five factors with an oblique rotation, was run. The resulting factor correlation matrix revealed correlations > |0.3| between multiple factors (**Table 4-5**), confirming that an oblique rotation was the best fit for this dataset. Thus, the final EFA extracted five factors using principal axis factoring as the extraction method and direct oblimin (an oblique rotation) as the rotation method. Missing datapoints (missing value frequency range = 1-6, average number of missing datapoints across all variables = 2.5) were replaced using the variable mean.

Figure 4-3: Scree plot representing Eigenvalues by factor number.

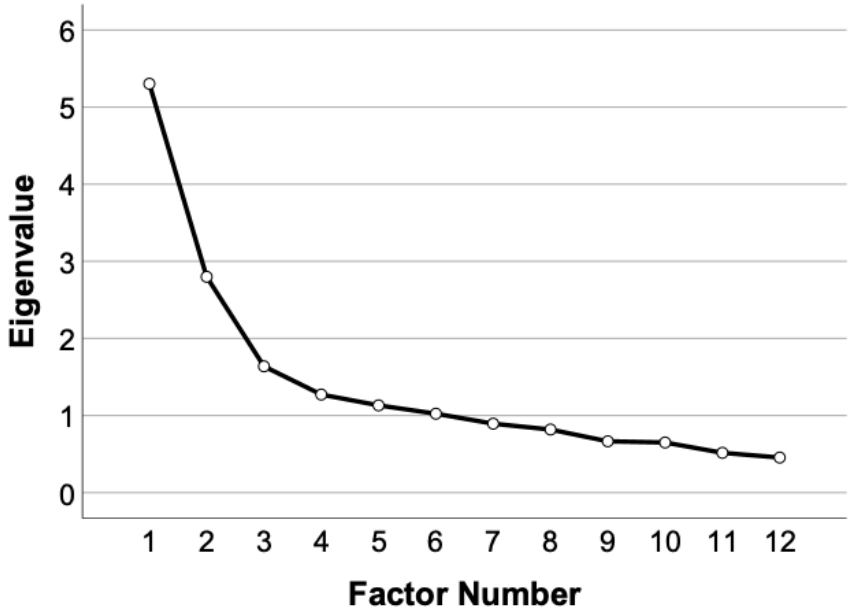


Table 4-5: Factor correlation matrix displaying correlation coefficients between extracted factors.

Factor	*1*	*2*	*3*	*4*	*5*
1	1	-0.09	0.08	0.40	-0.32
2	-0.09	1	0.15	-0.08	0.35
3	0.08	0.15	1	0.22	-0.10
4	0.40	-0.08	0.22	1	-0.35
5	-0.32	0.35	-0.10	-0.35	1

The Kaiser-Meyer-Olkin (KMO) test for sampling adequacy and Bartlett's test of sphericity were examined in order to determine adequacy of the input dataset for EFA. The KMO statistic was found to be adequate at 0.72, well above the minimum recommended value of 0.5. Bartlett's test of sphericity was significant, indicating sufficient interrelatedness among variables. The total variance explained by the 5 extracted factors was 52.25%, with factor 1 explaining 25.83%, factor 2 explaining 12.39%, factor 3 explaining 6.21%, factor 4 explaining 4.18%, and factor 5 explaining 3.64% of the total variance. Factor loadings are shown in **Table 4-6**. Variable loadings with values <0.3 are not shown in the factor loading table and were excluded from interpretation.

Table 4-6: Rotated factor loadings. Variable columns shaded by assay to match *Figure 4-1* (EPM = green; OF/OLM arena day 1 = blue; OLM = yellow; Fear conditioning = gray; Gene expression = orange). Principal Axis Factoring on correlation matrix with oblimin rotation. n = 71. Variable loadings <0.3 not shown.

Variable	F1	F2	F3	F4	F5
EPM % time in open arms	**-0.87**				
EPM closed arm entries	**-0.62**				
OF/OLM arena day 1 unsupported rears	**-0.51**		**0.49**		
OF/OLM arena day 1 supported rears	**-0.51**				**0.39**
OLM learning index	**-0.48**				
FC train: baseline freezing	**0.31**				
FC cue test: freezing to cue		**-0.81**			
FC cue test: pre-cue freezing		**-0.72**			
FC context test: freezing to context		**-0.69**			
DH *Hacd4* expression		**0.59**			
VH *Hacd4* expression		**0.58**			
OF/OLM arena day 1 fecal boli count			**0.60**		
EPM fecal boli count			**0.45**		
VH *Ptprd* expression				**0.73**	
DH *Ptprd* expression				**0.68**	
FC train: post-shock freezing				**0.40**	
OF/OLM arena day 1 distance traveled					**1.00**
OF/OLM arena day 1 % time in center					**0.45**
OF/OLM arena day 1 grooming duration					**-0.39**

Factor 1 (Activity/anxiety/exploration)

Percent time in EPM open arms, number of EPM closed arm entries, arena acclimation day 1 supported rears, arena acclimation day 1 unsupported rears, and the OLM learning index (preference ratio), all loaded negatively onto Factor 1. Freezing during the baseline phase of the fear conditioning training trial loaded positively onto Factor 1.

Factor 2 (Associative learning)

Freezing during context test, during the pre-CS phase of the cued test trial, and freezing during the cued test trial all loaded negatively onto Factor 2. Dorsal and ventral hippocampus *Hacd4* ΔCT loaded positively onto Factor 2. Note that ΔCt values, which are inversely proportional to gene expression, were used for gene expression variables. Thus, *Hacd4* expression loaded negatively onto this factor.

Factor 3 (Anxiety)

Fecal boli count across EPM trial, fecal boli count arena on acclimation day 1, and unsupported rears count on arena acclimation day 1 all loaded positively onto Factor 3.

Factor 4 (Freezing in post-shock phase of fear conditioning training trial)

Dorsal and ventral hippocampus *Ptprd* ΔCT and freezing during the post-shock phase of the fear conditioning training trial all loaded positively onto Factor 4. Note that ΔCt values, which are inversely proportional to gene expression, were used for gene expression variables. Thus, *Ptprd* expression loaded negatively onto this factor.

Factor 5 (Activity in open field)

Supported rears count on arena acclimation day 1, total distance moved on arena acclimation day 1, and percent time in arena center on arena acclimation day 1 loaded positively onto Factor 5. Total time spent grooming on arena acclimation day 1 loaded negatively onto Factor 5.

4.3.2. Strain comparisons

Linear composite scores

Strain means on linear composite (summed) variables calculated based on EFA results were compared in ANOVAs using the full behavioral dataset. The composite variable informed by factor 1 (here called Factor 1 composite) was calculated as the sum of percent time in EPM open arms, number of EPM closed arm entries, OF/OLM arena acclimation day 1 supported rears, OF/OLM arena acclimation day 1 unsupported rears, OLM learning index, and freezing during the baseline phase of the fear conditioning training trial. The Shapiro-Wilk test of normality indicated that the Factor 1 composite variable was normally distributed ($p > .05$). A mixed-effects ANOVA on the Factor 1 composite with strain and sex as between-subjects factors and testing cohort as a random factor revealed a significant random effect of testing cohort ($F[5,123] = 2.35$, $p = .045$) and a significant main effect of strain ($F[5,123] = 23.47$, $p < .001$, **Figure 4-4**). White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$). Tukey-HSD post-hoc tests were used to compare strain means on the Factor 1 composite score as summarized in **Table 4-7**. These comparisons indicated a significant difference in the Factor 1 composite between the BXD panel parental strains C57BL/6J and DBA/2J, with C57BL/6J having a higher Factor 1 composite score versus DBA/2J. These analyses also found no segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on the Factor 1 composite.

Figure 4-4: Factor 1 composite strain means. Homogenous subsets (groups of means not significantly different from each other, Tukey-HSD corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. Error bars express ±1 SEM.

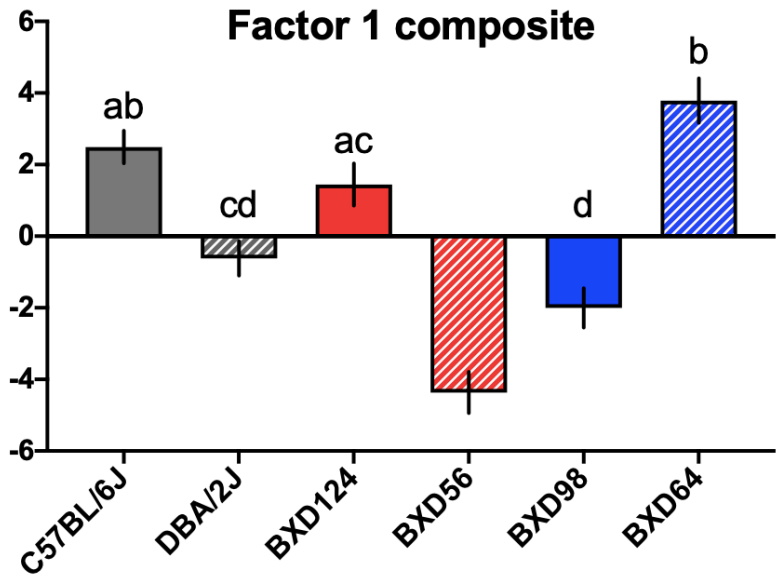


Table 4-7: Factor 1 composite Tukey-HSD post-hoc tests on strain mean. * indicates significance at Tukey-HSD corrected significance threshold of .05; (n.s.) = not significant at Tukey-HSD corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Tukey-HSD corrected significance
C57BL/6J	DBA/2J	3.11	$p = .001$*
	BXD124	1.05	$p = .71$ (n.s.)
	BXD56	6.86	$p < .001$*
	BXD98	4.49	$p < .001$*
	BXD64	-1.30	$p = .54$ (n.s.)
DBA/2J	BXD124	-2.06	$p = .06$ (n.s.)
	BXD56	3.75	$p < .001$*
	BXD98	1.38	$p = .43$ (n.s.)
	BXD64	-4.41	$p < .001$*
BXD124	BXD56	5.8	$p < .001$*
	BXD98	3.45	$p < .001$*
	BXD64	-2.35	$p = .03$*
BXD56	BXD98	-2.36	$p = .02$*
	BXD64	-8.16	$p < .001$*
BXD98	BXD64	-5.79	$p < .001$*

The composite variable informed by factor 2 (here called Factor 2 composite) was calculated as the sum of freezing to fear conditioned cue, freezing to fear conditioned context, and pre-CS freezing in the cued fear conditioning test. An initial mixed-effects ANOVA on the Factor 2 composite with strain and sex as between-subjects factors and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up two-way ANOVA on the Factor 2 composite with strain and sex as between-subjects factors was run. This analysis revealed a significant main effect of strain ($F[5,128] = 43.26$, $p < .001$). However, the Shapiro-Wilk test of normality indicated that the Factor 2 composite variable was not normally distributed ($p = .004$), and White's test for heteroskedasticity indicated that the assumption of homogeneity was violated ($p < .001$). Thus, the main effect of strain was examined using a non-parametric procedure (proportional odds ordinal logistic regression), which confirmed a significant main effect of strain (Wald Chi-Square = 104.72, $p < .001$, **Figure 4-5**). Games-Howell post-hoc tests were used to compare strain means on the Factor 2 composite score, as summarized in **Table 4-8**. These comparisons indicated no significant difference between BXD panel parental strains C57BL/6J and DBA/2J on the Factor 2 composite. However, this analysis supports segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on this associative learning composite variable.

Figure 4-5: Factor 2 composite strain means. Homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. Error bars express ±1 SEM.

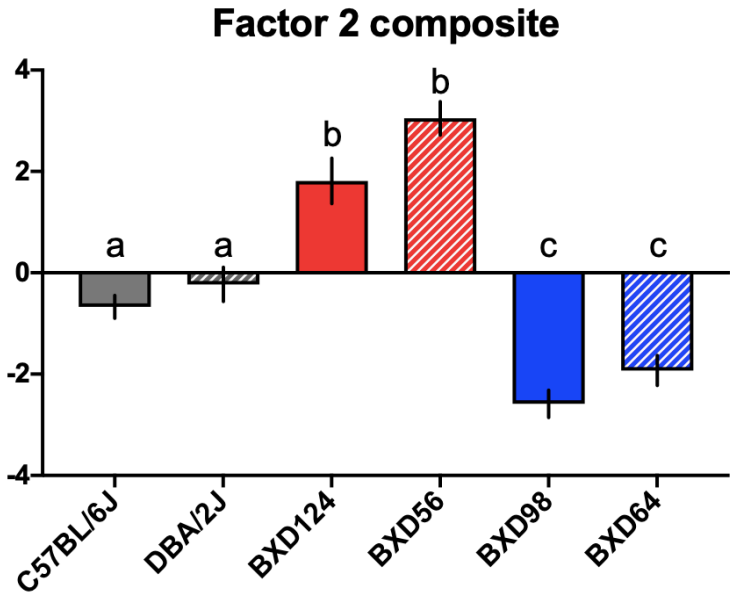


Table 4-8: Factor 2 composite Games-Howell post-hoc tests on strain mean. * indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-0.45	$p = .88$ (n.s.)
	BXD124	-2.49	$p < .001$*
	BXD56	-3.72	$p < .001$*
	BXD98	1.91	$p < .001$*
	BXD64	1.25	$p = .02$*
DBA/2J	BXD124	-2.04	$p = .01$*
	BXD56	-3.28	$p < .001$*
	BXD98	2.36	$p < .001$*
	BXD64	1.70	$p = .01$*
BXD124	BXD56	-1.24	$p = .25$ (n.s.)
	BXD98	4.40	$p < .001$*
	BXD64	3.74	$p < .001$*
BXD56	BXD98	5.63	$p < .001$*
	BXD64	4.98	$p < .001$*
BXD98	BXD64	-0.66	$p = .57$ (n.s.)

The composite variable informed by factor 3 (here called Factor 3 composite) was calculated as the sum of OF/OLM arena acclimation day 1 unsupported rears, OF/OLM arena acclimation day 1 fecal boli count, and EPM fecal boli count. A mixed-effects ANOVA on the Factor 3 composite with strain and sex as between-subjects factors and testing cohort as a random factor revealed a significant random effect of testing cohort ($F[5,123] = 2.59$, $p = .03$), a significant main effect of strain ($F[5,123] = 23.45$, $p < .001$), a significant main effect of sex ($F[1,123] = 28.81$, $p < .001$), and a significant interaction between strain and sex ($F[5,123] = 10.35$, $p < .001$). Although White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$), the Shapiro-Wilk test of normality indicated that the Factor 3 composite variable was not normally distributed ($p < .001$). The main effects of strain and sex, as well as the interaction effect between sex and strain were then examined using a non-parametric procedure (proportional odds ordinal logistic regression), which confirmed significant main effects of strain (Wald Chi-Square = 61.41, $p < .001$) and sex (Wald Chi-Square = 31.43, $p < .001$), as well as a significant interaction between strain and sex (Wald Chi-Square = 45.31, $p < .001$). In order to further explore this strain × sex interaction, follow up one-way ANOVAs on the Factor 3 composite were run separately in females and males with strain as a between-subjects factors and testing cohort as a random factor.

In females, an initial mixed-effects ANOVA on the Factor 3 composite with strain as a between-subjects factors and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up one-way ANOVA on the Factor 3 composite with strain as a between-subjects factors was run in females, which revealed a significant main effect of strain ($F[5,60] = 11.00$, $p < .001$). However, the Shapiro-Wilk test of normality indicated that the Factor 3 composite variable was not normally distributed in females ($p < .001$), and White's test

for heteroskedasticity indicated that the assumption of homogeneity was violated ($p < .001$). The main effect of strain in females was then confirmed using ordinal logistic regression (Wald Chi-Square = 32.97, $p < .001$, **Figure 4-6**). Games-Howell post-hoc tests were used to compare strain means on the Factor 3 composite in females, as summarized in **Table 4-9**. These analyses indicated a significant difference between female BXD panel parental strains C57BL/6J and DBA/2J, with DBA/2J females scoring more highly on the Factor 3 composite. However, these comparisons found no segregation between female high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on the Factor 3 composite.

Figure 4-6: Factor 3 composite strain means by sex. For females, homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase *letters* displayed above mean bars. For males, homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common *symbols* displayed below mean bars. F = Female; M = Male. Error bars express ±1 SEM.

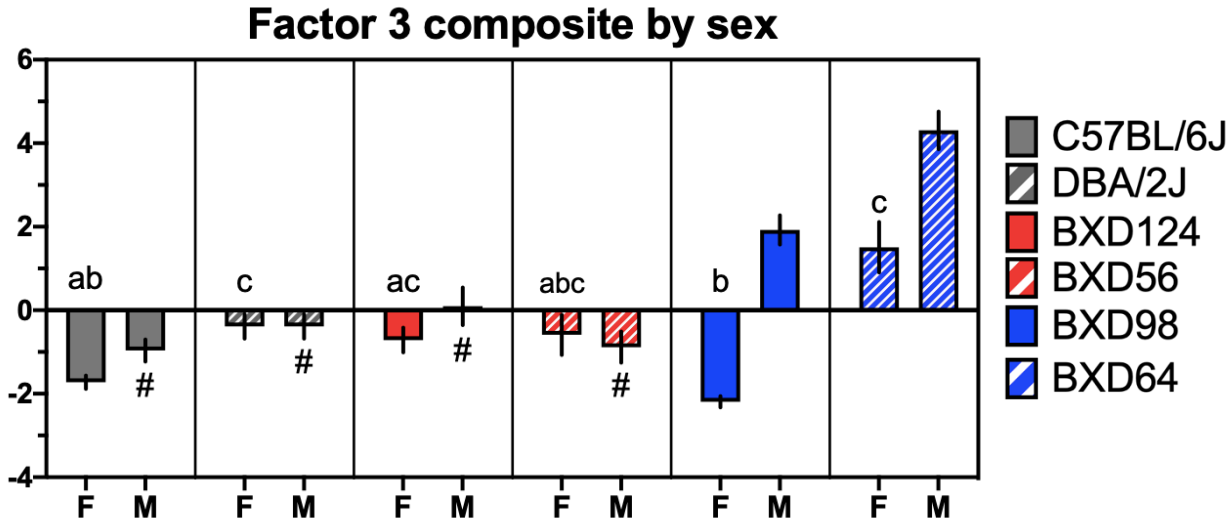


Table 4-9: Factor 3 composite Games-Howell post-hoc tests on female strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-1.34	p = .01*
	BXD124	-1.01	p = .07 (n.s.)
	BXD56	-1.14	p = .28 (n.s.)
	BXD98	0.47	p = .25 (n.s.)
	BXD64	-3.23	p = .02*
DBA/2J	BXD124	0.33	p = .97 (n.s.)
	BXD56	0.20	p = .99 (n.s.)
	BXD98	1.81	p = .001*
	BXD64	-1.89	p = .16 (n.s.)
BXD124	BXD56	-0.13	p = 1.0 (n.s.)
	BXD98	1.48	p = .003*
	BXD64	-2.22	p = .09 (n.s.)
BXD56	BXD98	1.60	p = .06 (n.s.)
	BXD64	-2.10	p = .15 (n.s.)
BXD98	BXD64	-3.70	p = .01*

In males, an initial mixed-effects ANOVA on the Factor 3 composite with strain as a between-subjects factors and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up one-way ANOVA on the Factor 3 composite with strain as a between-subjects factors was run in males, which revealed a significant main effect of strain ($F[5,68] = 32.89$, $p < .001$). Although White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$), the Shapiro-Wilk test of normality indicated that the Factor 3 composite variable was not normally distributed in males ($p = .001$). The main effect of strain in males was then confirmed using ordinal logistic regression (Wald Chi-Square = 50.29, $p < .001$, **Figure 4-6**). Games-Howell post-hoc tests were used to compare strain means on the Factor 3 composite in males, as summarized in **Table 4-10**. These analyses indicate no significant difference between male BXD panel parental strains C57BL/6J and DBA/2J on the Factor 3 composite. However, these comparisons found that male high (BXD124 and BXD56)

and low (BXD98 and BXD64) contextual freezing BXD strains segregate on the Factor 3 composite, with high fear conditioning strains showing lower scoring on this composite variable.

Table 4-10: Factor 3 composite Games-Howell post-hoc tests on male strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-0.58	p = .69 (n.s.)
	BXD124	-1.06	p = .37 (n.s.)
	BXD56	-0.08	p = 1.0 (n.s.)
	BXD98	-2.89	p < .001*
	BXD64	-5.27	p < .001*
DBA/2J	BXD124	-0.48	p = .94 (n.s.)
	BXD56	0.50	p = .90 (n.s.)
	BXD98	-2.31	p = .001*
	BXD64	-4.70	p < .001*
BXD124	BXD56	0.98	p = .56 (n.s.)
	BXD98	-1.83	p = .045*
	BXD64	-4.21	p < .001*
BXD56	BXD98	-2.81	p < .001*
	BXD64	-5.19	p < .001*
BXD98	BXD64	-2.39	p = .004*

Because post-shock freezing in the training phase of fear conditioning was the only behavioral variable loading onto factor 4, strain means were simply compared on the standardized version of this variable. A mixed-effects ANOVA on post-shock freezing (standardized variable representing Factor 4) with strain and sex as between-subjects factors and testing cohort as a random factor revealed a significant random effect of testing cohort ($F[5,121] = 4.36$, $p = .001$) and a significant main effect of strain ($F[5,121] = 20.52$, $p < .001$). Although White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$), the Shapiro-Wilk test of normality indicated that standardized post-shock freezing was not normally distributed ($p < .001$). Thus, the main effect of strain was confirmed using ordinal

logistic regression (Wald Chi-Square = 64.88, $p < .001$, **Figure 4-7**). Games-Howell post-hoc tests were used to compare strain means on standardized post-shock freezing, as summarized in **Table 4-11**. These analyses indicated a significant difference between BXD panel parental strains C57BL/6J and DBA/2J on standardized post-shock freezing, with C57BL/6J mice showing lower post-shock freezing. However, these analyses found no segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on post-shock freezing.

Figure 4-7: Standardized post-shock freezing strain means. Homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. Error bars express ±1 SEM.

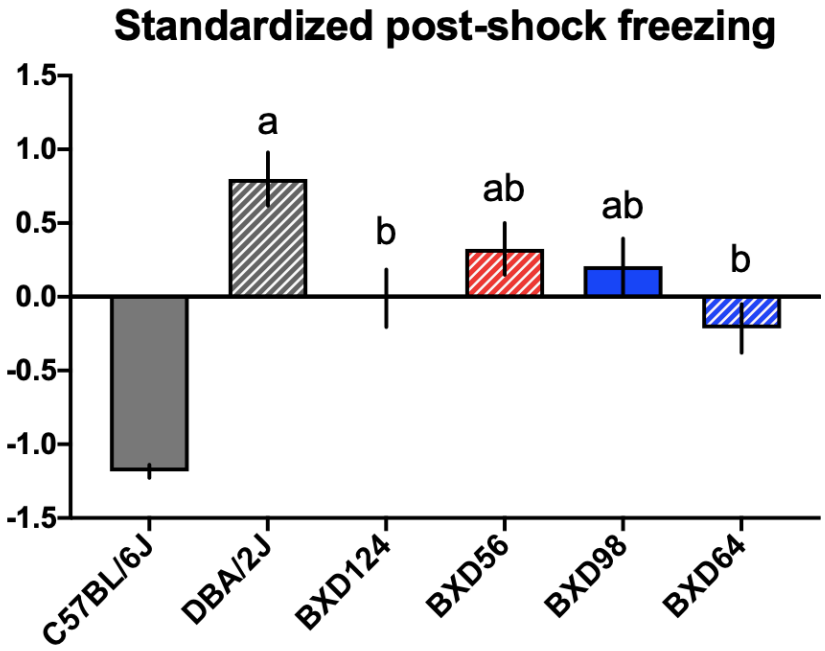


Table 4-11: Standardized post-shock freezing Games-Howell post-hoc tests on strain mean. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-1.98	$p < .001$*
	BXD124	-1.17	$p < .001$*
	BXD56	-1.51	$p < .001$*
	BXD98	-1.39	$p < .001$*
	BXD64	-0.97	$p < .001$*
DBA/2J	BXD124	0.81	$p = .04$*
	BXD56	0.47	$p = .43$ (n.s.)
	BXD98	0.59	$p = .23$ (n.s.)
	BXD64	1.01	$p = .002$*
BXD124	BXD56	-0.33	$p = .80$ (n.s.)
	BXD98	-0.22	$p = .97$ (n.s.)
	BXD64	0.20	$p = .97$ (n.s.)
BXD56	BXD98	0.12	$p = .99$ (n.s.)
	BXD64	0.54	$p = .24$ (n.s.)
BXD98	BXD64	0.42	$p = .55$ (n.s.)

The composite variable informed by factor 5 (here called Factor 5 composite) was calculated as the sum of OF/OLM arena acclimation day 1 supported rears, OF/OLM arena acclimation day 1 distance traveled, OF/OLM arena acclimation day 1 % time in arena center, and OF/OLM arena acclimation day 1 total grooming duration. An initial mixed-effects ANOVA on the Factor 5 composite with strain and sex as between-subjects factors and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up two-way ANOVA on the Factor 5 composite with strain and sex as between-subjects factors was run. This analysis revealed a significant main effect of strain on the Factor 5 composite ($F[5,128] = 53.56$, $p < .001$). Although the Shapiro-Wilk test of normality indicated that the Factor 5 composite variable was normally distributed ($p > .05$), White's test for heteroskedasticity indicated that the assumption of homogeneity was violated ($p < .001$). Thus, the main effect of strain was then confirmed using ordinal logistic regression (Wald Chi-Square = 38.63, $p < .001$, **Figure 4-8**).

Games-Howell post-hoc tests were used to compare strain means on the Factor 5 composite, as summarized in **Table 4-12**. These comparisons indicated a significant difference in the Factor 5 composite between the BXD panel parental strains C57BL/6J and DBA/2J, with C57BL/6J having a higher Factor 5 composite score versus DBA/2J. These analyses found no segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on the Factor 5 composite.

Figure 4-8: Factor 5 composite strain means. Homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. Error bars express ±1 SEM.

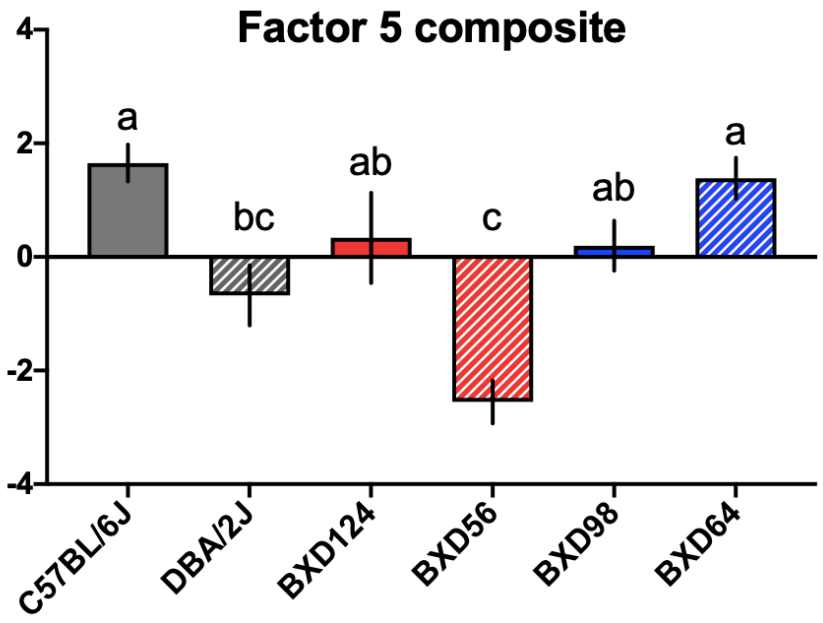


Table 4-12: Factor 5 composite Games-Howell post-hoc tests on strain mean. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	2.33	$p = .01$*
	BXD124	1.32	$p = .65$ (n.s.)
	BXD56	4.20	$p < .001$*
	BXD98	1.45	$p = .11$ (n.s.)
	BXD64	0.27	$p = .99$ (n.s.)
DBA/2J	BXD124	-1.01	$p = .89$ (n.s.)
	BXD56	1.88	$p = .06$ (n.s.)
	BXD98	-0.87	$p = .80$ (n.s.)
	BXD64	-2.06	$p = .03$*
BXD124	BXD56	2.89	$p = .03$*
	BXD98	0.14	$p = 1.0$ (n.s.)
	BXD64	-1.05	$p = .83$ (n.s.)
BXD56	BXD98	-2.75	$p < .001$*
	BXD64	-3.94	$p < .001$*
BXD98	BXD64	-1.19	$p = .31$ (n.s.)

Contextual fear conditioning and object location memory

Contextual fear conditioning (percent freezing to the conditioned context) was initially analyzed using a mixed-effects ANOVA with strain and sex as between-subjects factors and testing cohort as a random factor, which revealed no significant effect of testing cohort. Thus, a follow-up two-way ANOVA on contextual freezing with strain and sex as between-subjects factors was run. This analysis revealed a significant main effect of strain ($F[5,125] = 28.76$, $p < .001$). However, the Shapiro-Wilk test of normality indicated that contextual freezing was not normally distributed ($p = .002$), and White's test for heteroskedasticity indicated that the assumption of homogeneity was violated ($p = .01$). Thus, the main effect of strain was examined using a non-parametric procedure (proportional odds ordinal logistic regression), which confirmed a significant main effect of strain (Wald Chi-Square = 72.53, $p < .001$, **Figure 4-9**).

Games-Howell post-hoc tests were used to compare strain means on contextual freezing, as summarized in **Table 4-13**. These comparisons indicated no significant difference in contextual freezing between the BXD panel parental strains C57BL/6J and DBA/2J. However, this analysis replicates segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains on contextual fear learning.

Figure 4-9: Percent freezing to conditioned context strain means. Homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. Error bars express ±1 SEM.

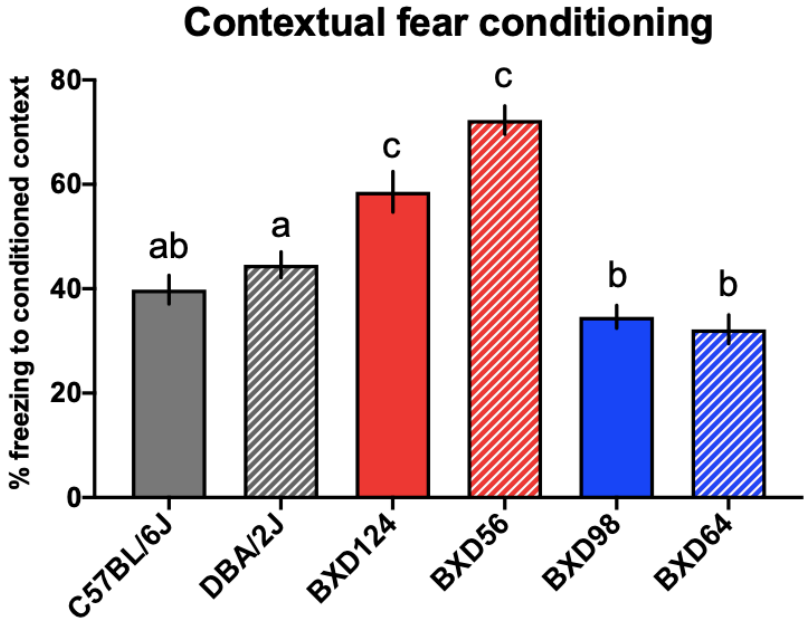


Table 4-13: Games-Howell post-hoc tests on contextual fear conditioning strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-4.78	$p = .79$ (n.s.)
	BXD124	-18.76	$p = .004$*
	BXD56	-32.50	$p < .001$*
	BXD98	5.20	$p = .68$ (n.s.)
	BXD64	7.58	$p = .39$ (n.s.)
DBA/2J	BXD124	-13.98	$p = .045$*
	BXD56	-27.71	$p < .001$*
	BXD98	9.99	$p = .045$*
	BXD64	12.37	$p = .02$*
BXD124	BXD56	-13.74	$p = .06$ (n.s.)
	BXD98	23.96	$p < .001$*
	BXD64	26.34	$p < .001$*
BXD56	BXD98	37.70	$p < .001$*
	BXD64	40.08	$p < .001$*
BXD98	BXD64	2.38	$p = .98$ (n.s.)

Learning in OLM (OLM preference ratio) was initially analyzed using a mixed-effects ANOVA with strain and sex as between-subjects factors and testing cohort as a random factor, which revealed no significant effect of testing cohort. Thus, a follow-up two-way ANOVA on OLM preference ratio with strain and sex as between-subjects factors was run. This analysis revealed a significant main effect of strain ($F[5,124] = 7.29$, $p < .001$) and a significant interaction between strain and sex ($F[5,124] = 3.07$, $p = .01$). However, the Shapiro-Wilk test of normality indicated that OLM preference ratio was not normally distributed ($p = .02$), and White's test for heteroskedasticity indicated that the assumption of homogeneity was violated ($p = .03$). The main effects of strain and the interaction effect between sex and strain were then examined using a non-parametric procedure (proportional odds ordinal logistic regression), which confirmed significant main effects of strain (Wald Chi-Square = 32.79, $p < .001$) and a significant interaction between strain and sex (Wald Chi-Square = 12.32, $p = .03$). In order to

further explore this strain × sex interaction, follow up one-way ANOVAs on the OLM preference ratio were run separately in females and males with strain as a between-subjects factors and testing cohort as a random factor.

In females, an initial mixed-effects ANOVA on OLM preference ratio with strain as a between-subjects factor and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up one-way ANOVA on OLM preference ratio with strain as a between-subjects factors was run in females, which revealed a significant main effect of strain ($F[5,58] = 5.91$, $p < .001$). Although White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$), the Shapiro-Wilk test of normality indicated that the Factor 3 composite variable was not normally distributed in females ($p = .03$). The main effect of strain in females was then confirmed using ordinal logistic regression (Wald Chi-Square = 20.70, $p < .001$, **Figure 4-10**). Games-Howell post-hoc tests were used to compare strain means on the OLM preference ratio in females, as summarized in **Table 4-14**. These analyses indicated no significant difference between female BXD panel parental strains C57BL/6J and DBA/2J, in addition to no segregation between female high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains segregate on the OLM preference ratio.

Figure 4-10: OLM preference ratio strain means. For females, homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase *letters* displayed above mean bars. For males, homogenous subsets (groups of means not significantly different from each other, Tukey-HSD corrected $p > .05$) are denoted by common *symbols* displayed above mean bars. F = Female; M = Male. Error bars express ±1 SEM. F = Female; M = Male. Error bars express ±1 SEM.

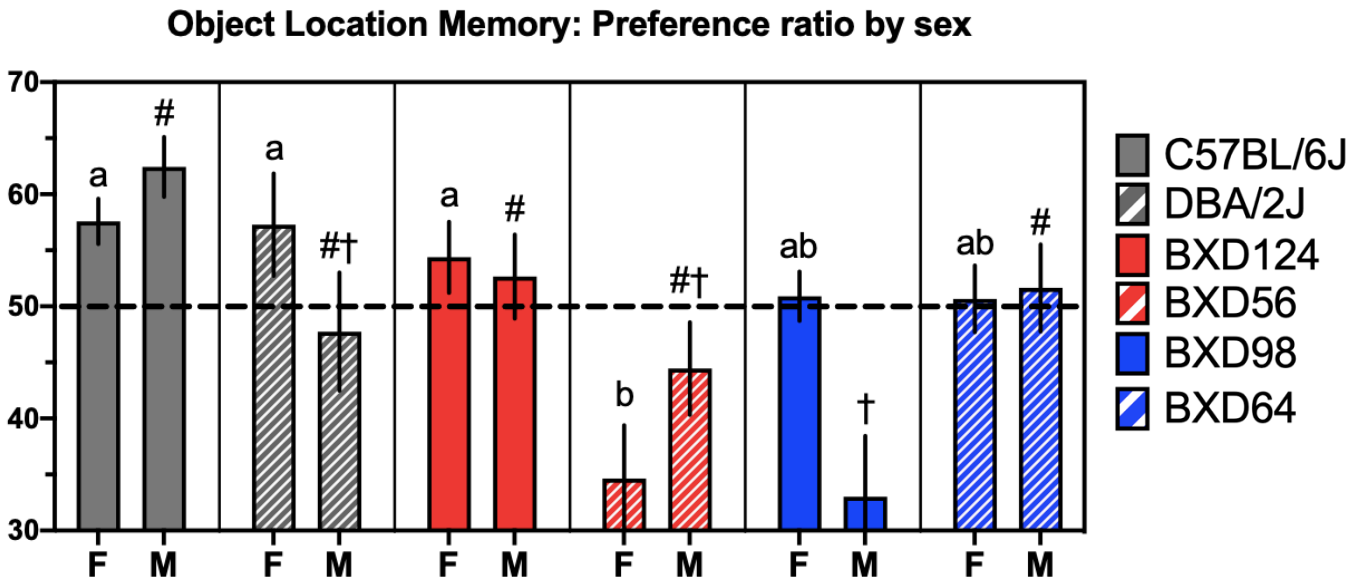


Table 4-14: Games-Howell post-hoc tests on OLM preference ratio female strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	0.30	p = 1.0 (n.s.)
	BXD124	3.20	p = .95 (n.s.)
	BXD56	22.96	p = .01*
	BXD98	6.69	p = .27 (n.s.)
	BXD64	6.92	p = .44 (n.s.)
DBA/2J	BXD124	2.90	p = .99 (n.s.)
	BXD56	22.66	p = .03*
	BXD98	6.39	p = .80 (n.s.)
	BXD64	6.62	p = .82 (n.s.)
BXD124	BXD56	19.76	p = .03*
	BXD98	3.48	p = .94 (n.s.)
	BXD64	3.71	p = .95 (n.s.)
BXD56	BXD98	-16.28	p = .07 (n.s.)
	BXD64	-16.05	p = .10 (n.s.)
BXD98	BXD64	0.23	p = 1.0 (n.s.)

In males, an initial mixed-effects ANOVA on OLM preference ratio with strain as a between-subjects factor and testing cohort as a random factor revealed no significant effect of testing cohort. Thus, a follow-up one-way ANOVA on OLM preference ratio with strain as a between-subjects factors was run in males, which revealed a significant main effect of strain ($F[5,66] = 4.88$, $p < .001$, **Figure 4-10**).The Shapiro-Wilk test of normality indicated that OLM preference ratio in males was normally distributed ($p > .05$), and White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$). Tukey-HSD post-hoc tests were used to compare strain means on OLM preference ratio in males, as summarized in **Table 4-15**. These analyses indicated no significant difference between male BXD panel parental strains C57BL/6J and DBA/2J, in addition to no segregation between male high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains segregate on the OLM preference ratio.

Table 4-15: Games-Howell post-hoc tests on OLM preference ratio male strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05.

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	14.69	$p = .20$ (n.s.)
	BXD124	9.77	$p = .68$ (n.s.)
	BXD56	18.00	$p = .053$ (n.s.)
	BXD98	29.42	$p < .001$*
	BXD64	10.80	$p = .49$ (n.s.)
DBA/2J	BXD124	-4.92	$p = .97$ (n.s.)
	BXD56	3.32	$p = .99$ (n.s.)
	BXD98	14.73	$p = .17$ (n.s.)
	BXD64	-3.89	$p = .99$ (n.s.)
BXD124	BXD56	8.24	$p = .79$ (n.s.)
	BXD98	19.65	$p = .04$*
	BXD64	1.03	$p = 1.0$ (n.s.)
BXD56	BXD98	11.41	$p = .42$ (n.s.)
	BXD64	-7.21	$p = .82$ (n.s.)
BXD98	BXD64	-18.62	$p = .03$*

Gene expression

Relative expression of candidate genes for contextual fear conditioning, *Hacd4* and *Ptprd*, was quantified in dorsal and ventral hippocampus from a subset of behavioral battery subjects. ΔC_T values derived from qPCR are compared here in order to characterize strain differences in expression of these candidates. To calculate ΔC_T values, expression of target genes *Hacd4* or *Ptprd* was normalized to that of housekeeping gene *Hprt* (target gene C_T – *Hprt* C_T) for each sample. Importantly, ΔC_T values are inversely proportional to gene expression (higher ΔC_T = lower gene expression).

The Shapiro-Wilk test of normality indicated that dorsal hippocampus *Hacd4* expression (ΔCt values) was normally distributed ($p > .05$). A two-way ANOVA on the *Hacd4* expression in the dorsal hippocampus with strain and sex as between-subjects factors revealed a significant main effect of strain ($F[5,58] = 5.41$, $p < .001$, **Figure 4-11**). White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$). Tukey-HSD post-hoc tests were used to compare strain means on dorsal hippocampus *Hacd4* expression, as summarized in **Table 4-16**. Note that comparisons were performed on ΔCt values, which are inversely proportional to gene expression. These comparisons indicated no significant difference between dorsal hippocampus *Hacd4* expression in BXD panel parental strains C57BL/6J and DBA/2J. Some degree of segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains was observed, although this did not reach significance in all strain comparisons.

Figure 4-11: Dorsal hippocampus *Hacd4* ΔCt strain means. Homogenous subsets (groups of means not significantly different from each other, Tukey-HSD corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression). Error bars express ±1 SEM.

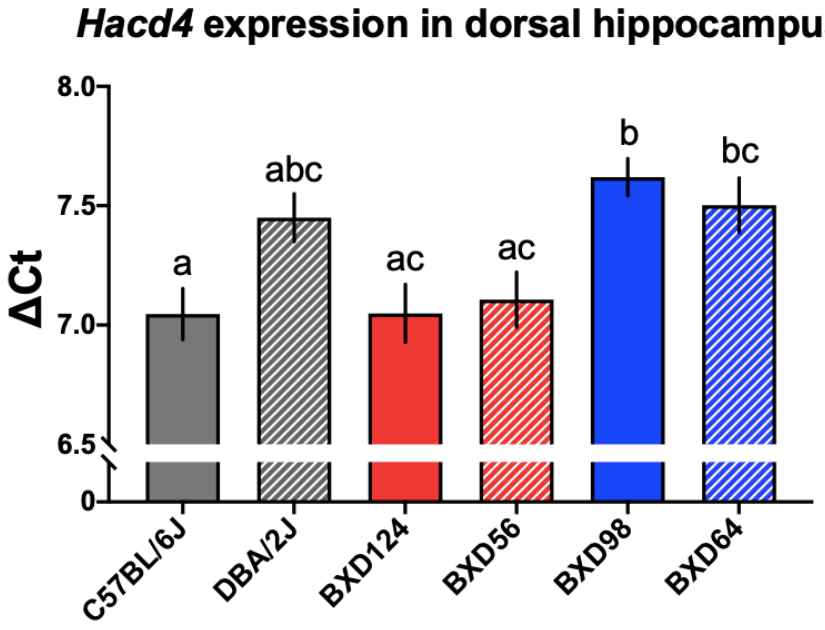


Table 4-16: Tukey-HSD post-hoc tests on dorsal hippocampus *Hacd4* ΔCt strain means. *indicates significance at Tukey-HSD corrected significance threshold of .05; (n.s.) = not significant at Tukey-HSD corrected significance threshold of .05. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression).

Reference strain	Comparison strain	Mean difference	Tukey-HSD corrected significance
C57BL/6J	DBA/2J	-0.40	p = .10 (n.s.)
	BXD124	-0.002	p = 1.0 (n.s.)
	BXD56	-0.06	p = .99 (n.s.)
	BXD98	-0.57	p = .004*
	BXD64	-0.46	p = .049*
DBA/2J	BXD124	0.40	p = .10 (n.s.)
	BXD56	0.34	p = .24 (n.s.)
	BXD98	-0.17	p = .87 (n.s.)
	BXD64	-0.05	p = .99 (n.s.)
BXD124	BXD56	-0.06	p = .99 (n.s.)
	BXD98	-0.57	p = .01*
	BXD64	-0.45	p = .051 (n.s.)
BXD56	BXD98	-0.51	p = .02*
	BXD64	-0.40	p = .14 (n.s.)
BXD98	BXD64	0.12	p = .97 (n.s.)

A two-way ANOVA on ventral hippocampus *Hacd4* expression (ΔCt values) with strain and sex as between-subjects factors revealed significant main effects of strain ($F[5,59] = 13.69$, $p < .001$) and sex ($F[1,59] = 15.97$, $p < .001$). However, the Shapiro-Wilk test of normality indicated that ventral hippocampus *Hacd4* expression was not normally distributed ($p = .002$), and White's test for heteroskedasticity indicated that the assumption of homogeneity was violated ($p = .01$). Thus, the main effects of strain and sex were examined using a non-parametric procedure (proportional odds ordinal logistic regression), which confirmed significant main effects of strain (Wald Chi-Square = 41.33, $p < .001$, **Figure 4-12**) and sex (Wald Chi-Square = 14.53, $p < .001$; Female mean = 7.17, Female SD = 0.50, Male mean = 7.44, Male SD = 0.33) on ventral hippocampus *Hacd4* expression. Games-Howell post-hoc tests were used to compare strain means on ventral hippocampus *Hacd4* expression, as summarized in **Table 4-17**. Note that comparisons were performed on ΔCt values, which are inversely proportional to gene expression. These comparisons indicated a significant difference between ventral hippocampus *Hacd4* expression in BXD panel parental strains C57BL/6J and DBA/2J, with C57BL/6J having a lower average ΔCt (higher expression). These analyses also found that ventral hippocampus *Hacd4* expression segregated between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains, with high freezing conditioning strains having a lower average ΔCt (higher expression).

Figure 4-12: Ventral hippocampus *Hacd4* ΔCt strain means. Homogenous subsets (groups of means not significantly different from each other, Games-Howell corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression). Error bars express ±1 SEM.

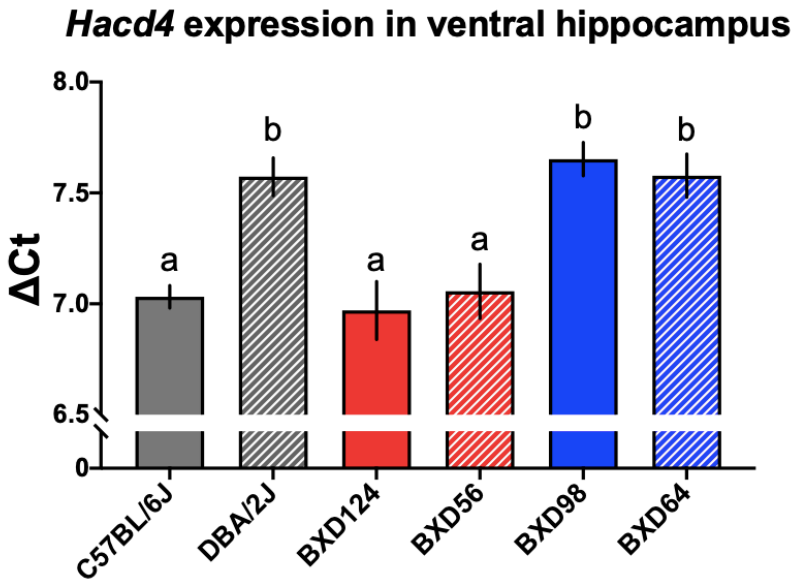


Table 4-17: Games-Howell post-hoc tests on ventral hippocampus *Hacd4* ΔCt strain means. *indicates significance at Games-Howell corrected significance threshold of .05; (n.s.) = not significant at Games-Howell corrected significance threshold of .05. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression).

Reference strain	Comparison strain	Mean difference	Games-Howell corrected significance
C57BL/6J	DBA/2J	-0.54	$p < .001$*
	BXD124	0.06	$p = .99$ (n.s.)
	BXD56	-0.02	$p = 1.0$ (n.s.)
	BXD98	-0.62	$p < .001$*
	BXD64	-0.55	$p = .002$*
DBA/2J	BXD124	0.60	$p = .01$*
	BXD56	0.52	$p = .03$*
	BXD98	-0.08	$p = .98$ (n.s.)
	BXD64	-0.004	$p = 1.0$ (n.s.)
BXD124	BXD56	-0.09	$p = .99$ (n.s.)
	BXD98	-0.68	$p = .003$*
	BXD64	-0.61	$p = .02$*
BXD56	BXD98	-0.60	$p = .01$*
	BXD64	-0.52	$p = .03$*
BXD98	BXD64	0.07	$p = .99$ (n.s.)

The Shapiro-Wilk test of normality indicated that dorsal hippocampus *Ptprd* expression (ΔCt values) was normally distributed ($p > .05$). A two-way ANOVA on *Ptprd* expression in the dorsal hippocampus with strain and sex as between-subjects factors revealed a significant main effect of strain ($F[5,58] = 4.03$, $p = .003$, **Figure 4-13**). White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$). Tukey-HSD post-hoc tests were used to compare strain means on dorsal hippocampus *Ptprd* expression, as summarized in **Table 4-18**. Note that comparisons were performed on ΔCt values, which are inversely proportional to gene expression. These analyses indicated a marginal but non-significant difference between dorsal hippocampus *Ptprd* expression in BXD panel parental strains C57BL/6J and DBA/2J. These comparisons also found no segregation in dorsal hippocampus *Ptprd* expression between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains.

Figure 4-13: Dorsal hippocampus *Ptprd* ΔCt strain means. Homogenous subsets (groups of means not significantly different from each other, Tukey-HSD corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression). Error bars express ±1 SEM.

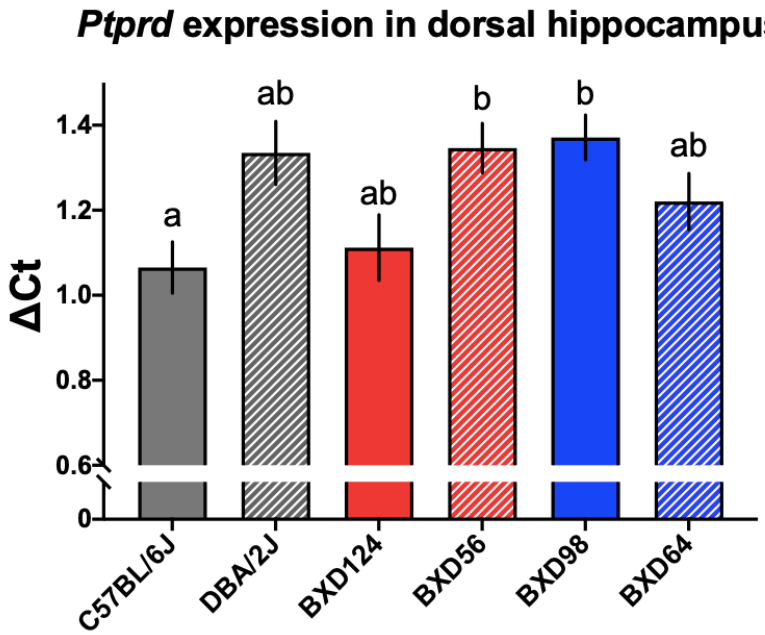


Table 4-18: Tukey-HSD post-hoc tests on dorsal hippocampus *Ptprd* ΔCt strain means. *indicates significance at Tukey-HSD corrected significance threshold of .05; (n.s.) = not significant at Tukey-HSD corrected significance threshold of .05. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression).

Reference strain	Comparison strain	Mean difference	Tukey-HSD corrected significance
C57BL/6J	DBA/2J	-0.27	p = .054 (n.s.)
	BXD124	-0.05	p = .99 (n.s.)
	BXD56	-0.28	p = .047*
	BXD98	-0.31	p = .02*
	BXD64	-0.16	p = .57 (n.s.)
DBA/2J	BXD124	0.22	p = .17 (n.s.)
	BXD56	-0.01	p = 1.0 (n.s.)
	BXD98	-0.04	p = .99 (n.s.)
	BXD64	0.11	p = .83 (n.s.)
BXD124	BXD56	-0.23	p = .15 (n.s.)
	BXD98	-0.26	p = .07 (n.s.)
	BXD64	-0.11	p = .86 (n.s.)
BXD56	BXD98	-0.02	p = 1.0 (n.s.)
	BXD64	0.13	p = .78 (n.s.)
BXD98	BXD64	0.15	p = .61 (n.s.)

The Shapiro-Wilk test of normality indicated that ventral hippocampus *Ptprd* expression (ΔCt values) was normally distributed ($p > .05$). A two-way ANOVA on the *Ptprd* expression in the ventral hippocampus with strain and sex as between-subjects factors revealed a significant main effect of strain ($F[5,59] = 3.84$, $p = .004$, **Figure 4-14**). White's test for heteroskedasticity indicated that the assumption of homogeneity was satisfied ($p > .05$). Tukey-HSD post-hoc tests were used to compare strain means on ventral hippocampus *Ptprd* expression, as summarized in **Table 4-19**. Note that comparisons were performed on ΔCt values, which are inversely proportional to gene expression. These comparisons indicated a significant difference between ventral hippocampus *Ptprd* expression in BXD panel parental strains C57BL/6J and DBA/2J, with C57BL/6J having a lower ΔCt (higher expression) compared to DBA/2J mice. These analyses found no segregation between ventral hippocampus gene expression in high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains.

Figure 4-14: Ventral hippocampus *Ptprd* ΔCt strain means. Homogenous subsets (groups of means not significantly different from each other, Tukey-HSD corrected $p > .05$) are denoted by common lowercase letters displayed above mean bars. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression). Error bars express ±1 SEM.

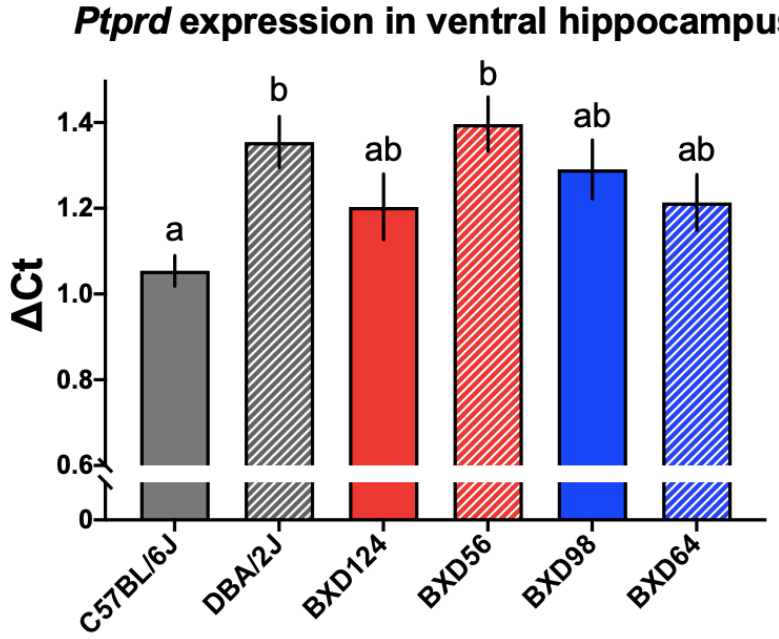


Table 4-19: Tukey-HSD post-hoc tests on ventral hippocampus *Ptprd* ΔCt strain means. *indicates significance at Tukey-HSD corrected significance threshold of .05; (n.s.) = not significant at Tukey-HSD corrected significance threshold of .05. ΔCT values are inversely proportional to gene expression (higher ΔCT = lower gene expression).

Reference strain	Comparison strain	Mean difference	Tukey-HSD corrected significance
C57BL/6J	DBA/2J	-0.30	$p = .02$*
	BXD124	-0.15	$p = .56$ (n.s.)
	BXD56	-0.34	$p = .004$*
	BXD98	-0.24	$p = .10$ (n.s)
	BXD64	-0.16	$p = .51$ (n.s.)
DBA/2J	BXD124	0.15	$p = .54$ (n.s.)
	BXD56	-0.04	$p = .99$ (n.s.)
	BXD98	0.06	$p = .98$ (n.s.)
	BXD64	0.14	$p = .64$ (n.s.)
BXD124	BXD56	-0.19	$p = .27$ (n.s.)
	BXD98	-0.09	$p = .92$ (n.s.)
	BXD64	-0.01	$p = 1.0$ (n.s.)
BXD56	BXD98	0.11	$p = .84$ (n.s.)
	BXD64	0.18	$p = .35$ (n.s.)
BXD98	BXD64	0.08	$p = .96$ (n.s.)

Chapter 5: DISCUSSION

5.1. Overview

This dissertation aimed to characterize phenotypic architecture of contextual fear conditioning and related behaviors using exploratory factor analysis (EFA) in a panel of BXD RI strains selected based on contextual fear learning phenotype. The EFA input dataset included variables from a behavioral battery comprising assays measuring anxiety, activity, configural learning, and associative fear learning. Expression of candidate genes for contextual fear conditioning, selected using QTL mapping in a larger panel of BXD strains, was also included in the factor analysis. EFA results were used to inform calculation of composite variables based on each extracted factor for strain mean comparisons. EFA revealed multiple phenotypic constructs underlying performance across the behavioral battery, and strain comparisons produced behavioral profiles for each of the tested strains. Differential factor loading of candidate genes for contextual fear conditioning pointed to potentially divergent regulation of fear conditioning.

5.2. Exploratory factor analysis (EFA)

EFA was performed in order to examine phenotypic constructs underlying performance in contextual fear conditioning and related behaviors, as well as expression of candidate genes for contextual fear conditioning. Five factors representing distinct phenotypic constructs were extracted from the input dataset, which included variables measured from a combined open field/object location memory assay (OF/OLM), elevated plus maze (EPM), contextual and cued fear conditioning, and qPCR on previously identified candidate genes for contextual fear conditioning, *Hacd4* and *Ptprd*. These input variables segregated into distinct factors

representing a combined activity/exploration phenotype (Factor 1), a phenotype representing associative learning (Factor 2), one representing anxiety (Factor 3), one representing freezing in the post-shock phase of the fear conditioning training trial (Factor 4), and a phenotype representing activity in open field (Factor 5). I begin with an interpretation of EFA results in relation to contextual fear conditioning and candidate genes for contextual fear learning. I then discuss additional EFA findings in the context of the broader murine behavioral repertoire.

5.2.1 EFA findings: Fear conditioning and candidate gene expression

Although the primary aim of this dissertation was to characterize contextual fear conditioning within the context of the broader murine behavioral repertoire, no phenotypic constructs representing processes unique to this form of fear conditioning emerged from the current analysis. Instead, the contextual fear conditioning variable loaded onto a factor that appeared to more broadly represent associative fear learning (Factor 2). These findings do not insinuate that no phenotypic processes unique to contextual fear conditioning (e.g. configural learning) affected contextual freezing in the tested sample, nor do they imply that contextual fear conditioning cannot generally be dissociated from cued fear learning. As detailed in section 2.1.3, contextual and cued fear conditioning have common neural substrates and thus are expected to correlate to the extent that they are both forms of associative fear learning. EFA prioritizes top loading factors and thus may not resolve more nuanced constructs among a larger dataset. That is, if a construct representing configural learning explained only a small amount of unique variance relative to the other extracted factors, it may not meet the threshold for extraction among top factors (Fabrigar et al., 1999). Although the object location memory learning index was included among the current dataset as an additional representative of

configural learning, it did not cluster with contextual fear learning in any of the extracted factors. As discussed previously, however, spatial tasks such as OLM may also be learned using egocentric spatial strategies (Wang, Johnson, Sun, & Zhang, 2005). Further, some variation in OLM performance can be explained by "secondary" phenotypes, such as exploratory tendencies and anxiety, as represented in Factor 1.

Nonetheless, this analysis provides insight into the interpretation of secondary phenotypes in fear conditioning tasks (baseline and post-shock freezing during training, and pre-CS freezing during the cued fear learning test), as well of previously identified candidate genes for contextual fear conditioning. Baseline and post-shock freezing are often found to correlate with contextual and/or cued learning (including in the current dataset) (Owen et al., 1997b), but the nature of this relationship is rarely explored. The current analysis suggests that these variables capture distinct constructs despite their shared variance. Specifically, baseline freezing in the fear conditioning training trial loaded onto a construct capturing general activity, exploratory tendencies, and anxiety (Factor 1). This supports the interpretation of baseline freezing as a metric for baseline anxiety and activity in fear conditioning. Pre-CS freezing in the cued test trial of fear conditioning loaded onto Factor 2 alongside freezing to conditioned context and cue, suggesting that this measure primarily captured generalization of the conditioned context during the cued test trial. Notably, *Hacd4* expression in both dorsal and ventral hippocampus also loaded onto Factor 2, while *Ptprd* expression in either brain region did not.

This may point to *Hacd4* as a better candidate for associative fear learning versus *Ptprd*, a distinction that the original QTL mapping analysis was unable to capture. *Hacd4* (originally named *Ptplad*2) is a protein-coding gene found on mouse chromosome 4 (human analog *HACD4,* aka *PTPLAD2,* on chromosome 9) that encodes the 3-Hydroxyacyl-CoA Dehydratase 4

protein. This endoplasmic-reticulum bound enzyme is member of the HACD family, which catalyzes the dehydration reaction in the very-long-chain fatty acids elongation cycle (Ikeda et al., 2008). These enzymes are important regulators of lipid production critical for many levels of cellular functioning. This gene and its associated protein were named and characterized relatively recently (Ikeda et al., 2008), and literature on its involvement in behavioral processes is limited. Information regarding the regional expression of *Hacd4* is also limited and inconsistent. Initial studies characterizing this enzyme's function noted expression exclusive to immune and peripheral tissue (Ikeda et al., 2008; Sawai et al., 2017). However, protein abundance and RNA sequencing data available in the Human Protein Atlas (http://www.proteinatlas.org; Pontén, Jirström, & Uhlen, 2008) suggest low detectable RNA expression across both the human and mouse brain, and low detectable protein expression in the human brain (mouse protein data unavailable). Low but detectable expression of *Hacd4* was confirmed by the current qPCR data.

Very-long-chain fatty acids constitute a significant portion of brain lipids and are crucial constituents of central myelination and gray matter (Knoll et al., 1999b; Poulos, 1995). Very-long-chain fatty acid chain synthesis, including the 3-Hydroxyacyl-CoA dehydratase-catalyzed dehydration reaction, has been demonstrated in rodent brain microsomes from early development into adulthood (Knoll, Bessoule, Sargueil, & Cassagne, 1999a; Knoll et al., 1999b). It is unclear if or how *Hacd4* gene expression can affect very-long-chain fatty acid chain synthesis or utilization, or indeed how this gene and its encoded protein may impact fear conditioning. In the current sample, higher *Hacd4* expression was observed in the high contextual fear conditioning strains relative to the low contextual fear conditioning strains (**Figure 4-11** and **Figure 4-12**; note that ΔC_T values are inversely proportional to gene expression). A reverse query of BXD

phenotypes correlated with whole brain *Hacd4* expression (GeneNetwork "UTHSC Mouse BXD Whole Brain RNA Sequence [Nov12]" gene expression dataset; searched as "*Ptplad2"*) revealed several neurological and behavioral correlates. The fifth ranked correlation with whole brain *Hacd4* expression indicated a positive relationship between *Hacd4* expression and plasma lipids (GeneNetwork Record ID #19735), which suggests that higher expression of this dehydratase gene can increase fatty acid synthesis. The top two correlations revealed by this query were 1) a positive correlation with freezing to conditioned context in a fear conditioning test (GeneNework Record ID #10901), which replicates the current findings, and 2) a negative correlation with hippocampus volume (GeneNetwork Record ID #10456). The significance of this negative relationship with hippocampus volume is unclear given that hippocampus volume does not associate with contextual learning in similar mouse populations (Yang et al., 2008). One possibility is that this correlation simply reflects of fatty acid regulation affecting overall brain morphology, although this speculation requires experimental validation. Nonetheless, *Hacd4* is a promising novel candidate for regulation of fear conditioning and other neurobehavioral phenotypes.

Post-shock freezing in the fear conditioning training trial loaded onto a factor comprising only one behavior (post-shock freezing) and *Ptprd* expression in both the ventral and dorsal hippocampus (Factor 4). *Ptprd* expression in both hippocampus regions loaded only onto Factor 4, and its expression showed no clear segregation among high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual fear conditioning strains (**Figure 4-13** and **Figure 4-14**). Again, this suggests that *Hacd4* is a more appropriate candidate for associative learning captured by Factor 2. Given that several factors impact post-shock freezing, and that this was the only behavioral variable that loaded onto Factor 4, it is difficult to apply a phenotypic label to this

factor. *Ptprd* was selected as a candidate using QTL mapping of freezing to the conditioned context in a larger BxD strain panel (Chapter 3). Notably, the mapped phenotype was a freezing metric; thus, it is possible that *Ptprd* emerged as a contextual fear conditioning candidate simply due to its broader regulation of freezing behavior. As noted previously, no factor representing processes unique to contextual fear conditioning emerged from the current analysis. Thus, another possibility is that *Ptprd* is indeed a regulator of contextual fear learning, but EFA in this subset of strains was not powered to detect this.

Ptprd is a protein-coding gene found on mouse chromosome 4 (human analog *PTPRD,* on chromosome 9) that encodes PTPδ, the receptor-like type D protein tyrosine phosphatase (see Uhl & Martinez, 2019 for review). Protein phosphorylation is a post-translational modification critical for a diverse range of intracellular signaling. Phosphorylation is regulated by the action of protein kinases and protein phosphatases, which are responsible for phosphorylation and dephosphorylation, respectively. PTPδ is a member of a phosphatase family that specifically targets dephosphorylates tyrosine residues. Receptor-like protein phosphatases, such as PTPδ, are transmembrane proteins with intracellular phosphatase domains.

Regulation of phosphorylation by phosphatases is critical for of several neurological processes, including synaptic plasticity underlying brain development and learning (Riedel, 1999; Sahin & Hockfield, 1993; Xu & Fisher, 2012). It has additionally been found that PTPδ plays a key role in central nervous system synapse organization as a component of the pre/postsynaptic scaffolding complex (Wang & Bixby, 1999; Takahashi & Craig, 2013). Accordingly, *Ptprd/PTPRD* and its encoded protein have been associated with a myriad of neurological phenotypes and disorders in both human and mouse models, including learning/memory (Benoit, Rowe, Menard, Sarret, & Quirion, 2011; Uetani et al., 2000),

addiction (Drgonova et al., 2015; Uhl et al., 2018), obsessive-compulsive disorder (Mattheisen et al., 2015), and restless leg syndrome (Drgonova et al., 2015; Schormair et al., 2008). In the current sample, higher *Ptprd* expression associated with lower post-shock freezing. One mouse model found that PTPδ-deficient animals exhibited enhanced hippocampal long-term potentiation in addition to spatial learning impairments (Uetani et al., 2000). Other research has noted a disruption in anxiety-like phenotypes among PTPδ-deficient animals, although was dependent upon the assay and knockout model in question (Park et al., 2020). Thus, *Ptprd* expression may impact post-shock freezing through pathways related to anxiety or learning. Future investigations may aim to uncover the specific contribution of this pleiotropic gene to post-shock freezing in the fear conditioning training trial.

5.2.2. EFA findings: Factors 1, 3, and 5

The top factor extracted by EFA appeared to represent an aggregate phenotype reflecting general activity, anxiety, and exploratory tendencies. As noted previously, these phenotypes are innately linked in prey animals, as they promote behaviors that mediate resource acquisition, socialization, and defensive responses (Crusio, 1995). This is supported by murine genomic and behavioral studies finding common effectors of activity and anxiety (Crawley et al., 1997; Henderson et al., 2004; Milner & Crabbe, 2008; Sokoloff et al., 2011). One compelling variable loading onto Factor 1 was the object location memory learning index. This index is calculated as a preference ratio for the displaced object, normalized to the non-displaced object. Thus, general activity should not directly impact variability on this index. However, it is possible that anxiety was a prominent mediator of performance in the current design. The OLM procedure used for this research was based on a protocol standardized in C57BL/6J mice (Vogel-Ciernia & Wood,

2014), generally a low-anxiety strain (O'Leary et al., 2013). It has been noted that a square arena shape may encourage more anxious subjects to hide in the arena corners behind the non-displaced object during the test trial (Lueptow, 2017), which may artificially inflate the proportion of time spent exploring the non-displaced object (see **Figure 4-2** for arena configuration used in the current study). Indeed, I observed OLM preference ratios significantly *below* chance for two strains with high anxiety metrics (BXD56 and BXD98). Thus, variability in OLM learning may have been obscured by the more prominent anxiety/exploration phenotype mediating performance for some strains. Future research testing OLM in diverse subjects may consider utilizing a circular arena to avoid this issue.

Factor 1 explained the largest portion of model variance and comprised the most diverse collection of variables among all the extracted factors. With the important caveat that these results should be interpreted in the context of the current sample, this finding further supports that variables used to approximate isolated phenotypes in mice (e.g., EPM open arm time as a proxy for anxiety) may in reality represent one measurement of a broader murine phenotype. This does not necessarily imply that these proxies are confounded or meaningless: Given that this activity/anxiety/exploration system may be the prominent driver of mouse behavior, such assays still reflect an ethologically relevant component of the mouse's anxiety response. However, this may be an issue in the case that researchers are interested in "pure" behavioral proxies for anxiety, activity, or exploration.

It has been suggested that species-typical behaviors, such as grooming, rearing, and defecation, may better reflect murine anxiety (O'Leary et al., 2013). This may be supported by Factor 3, which comprised fecal boli variables from two separate assays and unsupported rearing in the open field. Defecation frequency has long been interpreted as a correlate of autonomic

response in mice that is not directly mediated by locomotion (Hall, 1934; Milner & Crabbe, 2008; Mönnikes et al., 1993; O'Leary et al., 2013). Recent work has found that mouse rearing in a novel environment can be subdivided into supported rearing, which may better represent general activity, and unsupported rearing, which may better approximate anxiety (Sturman et al., 2018). Notably, both types of rearing loaded onto Factor 1, while only unsupported rearing loaded onto Factor 3. Curiously, however, unsupported rearing loaded with the same valence as fecal boli count onto Factor 3 – that is, more unsupported rearing associated with a higher fecal boli count. This is in contrast with Sturman and colleagues' (2018) findings that unsupported rearing correlated negatively with anxiety indices. However, these authors also note that the relationship between anxiety and rearing can follow an inverted-U curve, with rearing suppressed at very low and very high levels of anxiety (Lever, Burton, & O'Keefe, 2006; Sturman et al., 2018).

In the same manner that Factor 3 appears to capture "pure" anxiety, Factor 5 may represent a unique construct of general activity. Four variables, all derived from the OF/OLM day 1 arena acclimation, loaded significantly onto Factor 5. Notably, total distance moved in the arena and supported rearing, but not unsupported rearing, loaded positively onto this factor. This may suggest that this phenotypic construct captured by Factor 5 represents variance in general activity that does not interact with anxiety, as represented by Factor 1. Proportion of time in the open field arena center also loaded positively onto Factor 5. Although time in arena center is traditionally interpreted as a proxy for anxiety, it has also been associated with general activity phenotypes (Milner & Crabbe, 2008). Total grooming duration in the open field was the only variable with a negative loading onto Factor 5. Grooming in mice is a complex behavior that can reflect general activity or anxiety (O'Leary et al., 2013; Spruijt, Van Hooff, & Gispen, 1992). Its

negative loading onto this factor may simply reflect that less active mice had more opportunities to groom because they were not engaging in active behavior (horizontal movement and supported rearing). A notable limitation of these data is that grooming was scored as a broad behavior. It has been suggested that analysis of grooming microstructure may better resolve anxious and active components of the behavior (Kalueff, Aldridge, LaPorte, Murphy, & Tuohimaa, 2007; Kalueff & Tuohimaa, 2005). Finally, another possibility is that Factor 5 captured leftover variance common to the open field task and did not necessarily represent a "pure" activity construct. Future analyses focusing specifically on open field phenotypes may resolve this point.

5.3. Strain comparisons

A secondary goal of the current dissertation was to characterize BxD strain differences in latent phenotypic constructs identified by EFA. The BxD recombinant inbred strain is a popular model in neurobehavioral research due to the ready availability of the fully genotyped BxD lines. The current analyses build upon existing datasets to characterize the behavioral architecture of these inbred lines. As full BxD strain panels are typically utilized for QTL mapping studies, extended analysis of extreme phenotype strains in terms of the mapped behavior may further inform interpretation of such models. A strength of the current design is that the initial exploratory factor analysis was used to inform between-strain behavioral analyses, a data-driven approach to reducing the number of statistical comparisons.

EFA results were used to inform calculation of linear composite (summed) variables based on each extracted factor, and strain means on each composite variable were compared in ANOVAs using the full behavioral dataset. The composite variable informed by factor 1 ("Factor

1 composite") was calculated as the sum of percent time in EPM open arms, number of EPM closed arm entries, OF/OLM arena acclimation day 1 supported rears, OF/OLM arena acclimation day 1 unsupported rears, OLM learning index, and freezing during the baseline phase of the fear conditioning training trial. Between-strain analyses indicated a significant effect of strain (**Figure 4-4**). Post-hoc strain mean comparisons found a significant difference in the Factor 1 composite between the BXD panel parental strains C57BL/6J and DBA/2J, with C57BL/6J having a higher Factor 1 composite score versus DBA/2J. Assuming the Factor 1 composite can be interpreted similarly to EFA Factor 1 (a combined activity/anxiety/exploration construct), this finding is in line with the general consensus that C57BL/6J is a more active and less anxious strain compared to DBA/2J (Crawley et al., 1997; Flanigan & Cook, 2011; Moore, Linsenbardt, Melón, & Boehm, 2011; O'Leary et al., 2013; Sprott, 1975). No segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains was observed on the Factor 1 composite. Indeed, each set included a high (BXD124 and BXD64) and low (BXD56 and BXD98) scorer on the Factor 1 composite. These results suggest no consistent association between activity/anxiety phenotypes and performance in fear conditioning within this sample.

The composite variable informed by factor 2 ("Factor 2 composite") was calculated as the sum of freezing to the fear conditioned cue, freezing to the fear conditioned context, and pre-CS freezing in the cued fear conditioning test. Between-strain analysis indicated a significant main effect of strain on the Factor 2 composite (**Figure 4-5**). Follow-up strain mean comparisons found no difference between BXD panel parental strains C57BL/6J and DBA/2J, which is in line with findings that these strains typically differ in terms of contextual (hippocampus-dependent associative) but not cued (non-hippocampus dependent associative) fear learning if the Factor 2

composite is interpreted as broadly representative of associative learning (Paylor, Tracy, Wehner, & Rudy, 1994). Notably, between-strain analysis only on the contextual learning variable additionally found no difference in freezing to the conditioned context between C57BL/6J and DBA/2J (**Figure 4-9**). Although this replicates data from our original assessment in the full BXD panel (**Figure 3-1**), it is inconsistent with characterization of C57BL/6J mice as superior learners in hippocampus-dependent tasks relative to DBA/2J (Crawley et al., 1997; Paylor, Tipps, Raybuck, Buck, & Lattal, 2014; Tracy, Wehner, & Rudy, 1994). Assessment of OLM preference ratio strain means by sex (**Figure 4-10**) shows greater hippocampus-dependent OLM learning in C57BL/6J males versus DBA/2J males, although this difference was non-significant in corrected post-hoc comparisons (**Table 4-15**). This raises the possibility that studies finding a difference in hippocampus-dependent learning between these two strains have been biased toward male subjects. This would not account for the lack of a sex effect in contextual fear learning, however. Nonetheless, segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains was replicated both in the Factor 2 composite and in analysis of the contextual freezing variable. This segregation was not observed in analysis of the OLM learning index, again supporting that Factor 2 broadly represents associative learning, not hippocampus-dependent configural learning.

The composite variable informed by factor 3 ("Factor 3 composite") was calculated as the sum of OF/OLM arena acclimation day 1 unsupported rears, OF/OLM arena acclimation day 1 fecal boli count, and EPM fecal boli count. Between-strain analysis indicated a significant main effect of strain, as well as a significant interaction between strain and sex on the Factor 3 composite score (**Figure 4-6**). This factor composite was the only one significantly impacted by sex among the five analyzed. Strain mean comparisons by sex revealed a difference between

BXD panel parental strains C57BL/6J and DBA/2J only in females, with C57BL/6J females scoring lower on the Factor 3 composite versus DBA/2J females. This may replicate the general finding that C57BL/6J is a less anxious strain compared DBA/2J, although it is unclear why no effect was observed in males.

The strongest sex effect emerged within the BXD98 strain, whose males and females scored in opposite directions on the Factor 3 composite. Specifically, BXD98 females were low scoring and BXD98 males were high scoring on the Factor 3 composite. A prominent sex difference in the Factor 3 composite also appeared in the BXD64 strain, whose males scored more highly than females. Thus, a sex-dependent segregation between high (BXD124 and BXD56) and low (BXD98 and BXD64) contextual freezing BXD strains was observed. This may be in line with previous findings that fear conditioning and anxious phenotypes are co-selected (Ponder et al., 2007) and is consistent with the central role of emotionality in fear conditioning (see sections 2.1.1 and 2.1.3).

Because post-shock freezing in the training phase of fear conditioning was the only behavioral variable loading onto factor 4, strain means were compared on the standardized version of this variable. Between-strain analysis found a significant main effect of strain (**Figure 4-7**), and post-hoc strain mean comparisons indicated that post-shock freezing in C57BL/6J mice was very low compared to all other tested strains. Because post-shock freezing is understudied, it is unclear what may drive this strain difference. It has been suggested that post-shock freezing is a metric of immediate contextual learning in addition to reflecting anxiety and general freezing behavior (Fanselow, 1980; Wood & Anagnostaras, 2011); however, no segregation between high and low contextual freezing strains on post-shock freezing was observed here. Alternatively, this may simply reflect the fact that the fear conditioning paradigm used for the current study was

primarily optimized using C57BL/6J mice, as conditioning protocols typically aim to minimize non-associative (non-context and cued) freezing (Smith, Gallagher, & Stanton, 2007).

The composite variable informed by factor 5 ("Factor 5 composite") was calculated as the sum of OF/OLM arena acclimation day 1 supported rears, OF/OLM arena acclimation day 1 distance traveled, OF/OLM arena acclimation day 1 % time in arena center, and OF/OLM arena acclimation day 1 total grooming duration. Between-strain analysis indicated a significant main effect of strain on the Factor 5 composite (**Figure 4-8**), and follow-up strain mean comparisons found that BXD parental strain C57BL/6J scored more highly on this factor compared to BXD parental strain DBA/2J. Again, this is consistent with characterization of C57BL/6J as a more active strain versus DBA/2J. No clear segregation between high (BXD124 and BXD64) and low (BXD56 and BXD98) contextual freezing strains emerged on this factor composite. Relative scoring between strains onto the Factor 5 composite appeared similar to the Factor 1 composite, which may be expected given that the Factor 1 composite included measures of general activity.

Preliminary behavioral profiling of tested strains

These strain comparisons informed by EFA allow for preliminary behavioral profiling of the tested strains. BXD parental strains C57BL/6J and DBA/2J exhibited a strong disparity in activity and anxiety, with C57BL/6J being low and DBA/2J being high on activity and anxiety constructs. On the other hand, there was no clear difference between these parental strains in terms of fear conditioning performance. Here, BXD56 and BXD98 were low activity, moderate-to-high anxiety strains (sex-dependent in BXD98). Despite these communalities, they displayed divergent performance in fear conditioning, with BXD56 exhibiting high and BXD98 exhibiting low associative learning. BXD124 was observed as a low-to-intermediate activity and anxiety

strain with good performance in fear conditioning. Finally, BXD64 appeared to exhibit high activity and high anxiety with poor performance in fear conditioning.

In sum, these results suggest no consistent association between activity/anxiety phenotypes and performance in fear conditioning. One interpretation of these findings is that anxiety and activity are not the primary drivers of performance in fear learning tasks despite their association with fear conditioning phenotypes. An alternate explanation is that the degree to which anxiety and activity drive fear conditioning performance is dependent upon strain, or that performance in fear conditioning cannot be accurately compared between some strains. This can be explained using the BXD56 and BXD98 strains as an example. Here, I found that these strains were both high activity and anxiety strains, but BXD98 performed poorly in fear conditioning compared to BXD56. Several underlying processes may drive these behavioral profiles, including:

1. Superior associative learning processes in BXD56 versus BXD98, with no impact of anxiety or activity on fear conditioning.
2. Divergent neurological mechanisms underlying anxiety/activity between BXD56 and BXD98, with mechanisms of anxiety/activity from only one strain having an impact on fear conditioning performance.
3. Identical neurological mechanisms underlying anxiety/activity between BXD56 and BXD98, but divergent impacts on fear conditioning. E.g., high anxiety in BXD56 supports increased attention and better learning, while high anxiety in BXD98 reduces attention and impairs learning.
4. Differential expression of learned fear between BXD56 and BXD98.
5. Some combination of the above factors.

These hypotheticals may apply to any analysis involving the comparison of experimental groups. Thus, in addition to expanding behavioral testing, future investigations should aim to specifically identify mechanisms underlying differences between experimental groups, as each of these possibilities carries a distinct translational relevance. Nonetheless, these findings are a clear demonstration that anxiety/activity phenotypes do not consistently associate with fear learning, even in a panel of strains derived from common parental genomes.

5.4. Limitations and future directions

Limitations of the current experiments are discussed in brief throughout relevant sections and are summarized here:

The current factor analysis identified a number of phenotypic constructs underlying performance across our behavioral battery. Interpretation of factor analysis results is always limited by the fact that the researcher is responsible for applying descriptive labels to extracted factors. A crucial caveat to the current results is that these factor interpretations may be imprecise and are subject to corrections with accruement of additional data. The input sample included commonly tested inbred mouse strains (C57BL/6J and DBA/2J), as well as strains with known phenotypic variation, and thus the current findings have relevance for research using similar models. It is important to interpret these findings within the context of the tested sample, however. That is, different underlying constructs may be extracted from a sample of different strain, sex, and age makeup. The murine behavioral repertoire should always be defined in the context of the tested population. The input dataset used for factor analysis was also limited to variables included in the behavioral battery. Analysis using a different array of phenotypes, or using data collected under different experimental conditions may also return different underlying

constructs. Additional testing in diverse populations representing a variety of behaviors will strengthen understanding of the murine phenotypic architecture.

Expression of candidate genes for contextual fear conditioning was quantified from hippocampus tissue collected at the conclusion of the behavioral battery. Because gene expression can be affected by behavioral testing (Gray, Rubin, Hunter, & McEwen, 2014), it is unclear whether expression in naïve animals would differ. Interpretation of candidate gene results is also limited by the expression quantification at one timepoint. The present candidate genes were identified based on correlation of their baseline expression with contextual fear conditioning in a larger strain panel. Thus, their expression may impact contextual fear learning through developmental pathways (e.g. modulation of hippocampal development) or through regulation of independent genetic programs responsive to learning. Further, expression of these genes themselves may be reactive to learning in a manner that impacts contextual fear conditioning. Nonetheless, the current findings provide valuable insight regarding the potentially differential role of these two candidates in regulation of fear conditioning. Future research may aim to characterize the role of *Hacd4* and *Ptprd* expression in fear conditioning within broader populations.

5.5. Conclusions

This dissertation aimed to characterize the phenotypic architecture of contextual fear conditioning, behaviors related to contextual fear learning, and expression of candidate genes for contextual fear conditioning using exploratory factor analysis. Exploratory factor analysis revealed five distinct phenotypic constructs representing activity/anxiety/exploration (Factor 1), associative fear learning (Factor 2), anxiety (Factor 3), post-shock freezing (Factor 4), and open

field activity (Factor 5) phenotypes. These findings reinforced the link between adaptive prey behaviors represented by Factor 1. Factors 3 and 5 provide candidates for behavioral representatives of pure anxiety and activity. The segregation of *Hacd4* and *Ptprd* expression between Factors 2 and 4, combined with between-strain comparisons of their expression, point to regulation of distinct phases of fear learning between these candidates. Between-strain comparisons on composite variables informed by the factor analysis allowed for construction of behavioral profiles for each of the tested strains. These analyses suggested no consistent association between activity/anxiety phenotypes and performance in fear conditioning. The present work also produced BxD strain behavior and gene expression data that will contribute to public data and analysis resources.

These data contextualize fear conditioning within the broader murine behavioral architecture. I showed that associative learning and expression of one candidate gene for contextual fear conditioning emerge as a unique construct within factor analysis of a behavioral battery comprising multiple behavioral measures. Post-shock freezing and expression of a second candidate gene for contextual fear conditioning emerged as an additional unique construct, highlighting the independence of this measure within the fear conditioning paradigm. Further interpretation of factor analysis findings revealed the interacting structure underlying mouse anxiety and activity phenotypes, and strain comparisons provided preliminary behavioral profiles for rarely tested BXD strains. These findings inform understanding of fear conditioning in terms of its secondary measures, underlying biological mechanisms, and interaction with other mouse behaviors.

References

Ahn, S. H., Jang, E. H., Choi, J. H., Lee, H. R., Bakes, J., Kong, Y. Y., & Kaang, B. K. (2013). Basal anxiety during an open field test is correlated with individual differences in contextually conditioned fear in mice. *Animal Cells and Systems*, *17*(3), 154-159.

Amorapanth, P., LeDoux, J. E., & Nader, K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature neuroscience*, *3*(1), 74-79.

Anagnostaras, S. G., Wood, S. C., Shuman, T., Cai, D. J., LeDuc, A. D., Zurn, K. R., ... & Herrera, G. M. (2010). Automated assessment of pavlovian conditioned freezing and shock reactivity in mice using the video freeze system. *Frontiers in behavioral neuroscience*, *4*, 158.

Baldi, E., Lorenzini, C. A., & Bucherelli, C. (2004). Footshock intensity and generalization in contextual and auditory-cued fear conditioning in the rat. *Neurobiology of learning and memory*, *81*(3), 162-166.

Ballesteros, C. I., de Oliveira Galvão, B., Maisonette, S., & Landeira-Fernandez, J. (2014). Effect of dorsal and ventral hippocampal lesions on contextual fear conditioning and unconditioned defensive behavior induced by electrical stimulation of the dorsal periaqueductal gray. *PloS one*, *9*(1).

Bartlett, M. S. (1950). Tests of significance in factor analysis. *British Journal of statistical psychology*, *3*(2), 77-85.

Bast, T., Zhang, W. N., & Feldon, J. (2001). The ventral hippocampus and fear conditioning in rats. *Experimental Brain Research*, *139*(1), 39-52.

Benoit, C. E., Rowe, W. B., Menard, C., Sarret, P., & Quirion, R. (2011). Genomic and proteomic strategies to identify novel targets potentially involved in learning and memory. *Trends in pharmacological sciences*, *32*(1), 43-52.

Bertoglio, L. J., Joca, S. R. L., & Guimaraes, F. S. (2006). Further evidence that anxiety and memory are regionally dissociated within the hippocampus. *Behavioural brain research*, *175*(1), 183-188.

Bogue, M. A., Grubb, S. C., Walton, D. O., Philip, V. M., Kolishovski, G., Stearns, T., ... & Kunde-Ramamoorthy, G. (2017). Mouse Phenome Database: an integrative database and analysis suite for curated empirical phenotype data from laboratory mice. *Nucleic acids research*, *46*(D1), D843-D850.

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological review*, *77*(1), 32.

Bucci, D. J., Phillips, R. G., & Burwell, R. D. (2000). Contributions of postrhinal and perirhinal cortex to contextual information processing. *Behavioral neuroscience*, *114*(5), 882.

Burwell, R. D., Bucci, D. J., Sanborn, M. R., & Jutras, M. J. (2004). Perirhinal and postrhinal contributions to remote memory for context. *Journal of Neuroscience*, *24*(49), 11023-11028.

Burwell, R. D., Saddoris, M. P., Bucci, D. J., & Wiig, K. A. (2004). Corticohippocampal contributions to spatial and contextual learning. *Journal of Neuroscience*, *24*(15), 3826-3836.

Caldarone, B., Saavedra, C., Tartaglia, K., Wehner, J. M., Dudek, B. C., & Flaherty, L. (1997). Quantitative trait loci analysis affecting contextual conditioning in mice. *Nature genetics*, *17*(3), 335-337.

Careau, V., Bininda-Emonds, O. R., Ordonez, G., & Garland, T. (2012). Are voluntary wheel running and open-field behavior correlated in mice? Different answers from comparative and artificial selection approaches. *Behavior genetics*, *42*(5), 830-844.

Cho, Y. H., Friedman, E., & Silva, A. J. (1998). Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. *Behavioural brain research*, *98*(1), 77-87.

Christopoulos, G. I., Uy, M. A., & Yap, W. J. (2019). The body and the brain: measuring skin conductance responses to understand the emotional experience. *Organizational Research Methods*, *22*(1), 394-420.

Clark, P. J., Brzezinska, W. J., Thomas, M. W., Ryzhenko, N. A., Toshkov, S. A., & Rhodes, J. S. (2008). Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. *Neuroscience*, *155*(4), 1048-1058.

Costello, A. B., & Osborne, J. (2005). Best practices in exploratory factor analysis: Four recommendations for getting the most from your analysis. *Practical assessment, research, and evaluation*, *10*(1), 7.

Coughlin, K. B. (2013). An analysis of factor extraction strategies: A comparison of the relative strengths of principal axis, ordinary least squares, and maximum likelihood in research contexts that include both categorical and continuous variables.

Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., ... & Paylor, R. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology*, *132*(2), 107-124.

Crawley, J. N., & Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and behavior*, *31*(3), 197-211.

Crusio, W. E. (1995). Natural selection on hippocampal circuitry underlying exploratory behaviour in mice: Quantitative-genetic analysis. In *Behavioural brain research in naturalistic and seminaturalistic settings* (pp. 323-342). Kluwer Academic Press, Dordrecht, The Netherlands

Curzon, P., Rustay, N. R., & Browman, K. E. (2009). Cued and contextual fear conditioning for rodents. *Methods of Behavior Analysis in Neuroscience. 2nd edition*.

Dolu, N., Acer, H., & Kara, A. Y. (2014). Investigation of dose-related effects of carnosine on anxiety with sympathetic skin response and T-maze. *Acta Med*, *57*, 112-118.

Drgonova, J., Walther, D., Wang, K. J., Hartstein, G. L., Lochte, B., Troncoso, J., ... & Uhl, G. R. (2015). Mouse model for protein tyrosine phosphatase D (PTPRD) associations with restless leg syndrome or Willis-Ekbom disease and addiction: reduced expression alters locomotion, sleep behaviors and cocaine-conditioned place preference. *Molecular Medicine*, *21*(1), 717-725.

Duits, P., Cath, D. C., Lissek, S., Hox, J. J., Hamm, A. O., Engelhard, I. M., ... & Baas, J. M. (2015). Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depression and anxiety*, *32*(4), 239-253.

Fabrigar, L. R., Wegener, D. T., MacCallum, R. C., & Strahan, E. J. (1999). Evaluating the use of exploratory factor analysis in psychological research. *Psychological methods*, *4*(3), 272.

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *The Pavlovian journal of biological science: Official Journal of the Pavlovian*, *15*(4), 177-182.

Fanselow, M. S. (1990). Factors governing one-trial contextual conditioning. *Animal Learning & Behavior*, *18*(3), 264-270.

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures?. *Neuron*, *65*(1), 7-19.

Flanigan, T. J., & Cook, M. N. (2011). Effects of an early handling-like procedure and individual housing on anxiety-like behavior in adult C57BL/6J and DBA/2J mice. *PLoS One*, *6*(4), e19058.

Gaskin, C. J., & Happell, B. (2014). On exploratory factor analysis: A review of recent evidence, an assessment of current practice, and recommendations for future use. *International journal of nursing studies*, *51*(3), 511-521.

Geller, D. A., McGuire, J. F., Orr, S. P., Pine, D. S., Britton, J. C., Small, B. J., ... & Storch, E. A. (2017). Fear Conditioning and Extinction in Pediatric Obsessive Compulsive Disorder. *Annals of clinical psychiatry: official journal of the American Academy of Clinical Psychiatrists*, *29*(1), 17.

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: a guide for non-statisticians. *International journal of endocrinology and metabolism*, *10*(2), 486.

Goldberg, L. R., Kutlu, M. G., Zeid, D., Seemiller, L. R., & Gould, T. J. (2021). Systems genetic analysis of nicotine withdrawal deficits in hippocampus-dependent learning. *Genes, Brain and Behavior*, e12734.

Gray, J. D., Rubin, T. G., Hunter, R. G., & McEwen, B. S. (2014). Hippocampal gene expression changes underlying stress sensitization and recovery. *Molecular psychiatry*, *19*(11), 1171-1178.

Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biological psychiatry*, *52*(10), 958-975.

Grillon, C., & Morgan III, C. A. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Journal of abnormal psychology*, *108*(1), 134.

Grings, W. W. (1969). Anticipatory and preparatory electrodermal behavior in paired stimulation situations. *Psychophysiology*, *5*(6), 597-611.

Glenn, C. R., Lieberman, L., & Hajcak, G. (2012). Comparing electric shock and a fearful screaming face as unconditioned stimuli for fear learning. *International Journal of Psychophysiology*, *86*(3), 214-219.

Hall, C. S. (1934). Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative psychology*, *18*(3), 385.

Harrell, F. E. (2015). Ordinal logistic regression. In *Regression modeling strategies* (pp. 311-325). Springer, Cham.

Hartley, C. A., & Phelps, E. A. (2010). Changing fear: the neurocircuitry of emotion regulation. *Neuropsychopharmacology*, *35*(1), 136-146.

Henderson, N. D., Turri, M. G., DeFries, J. C., & Flint, J. (2004). QTL analysis of multiple behavioral measures of anxiety in mice. *Behavior genetics*, *34*(3), 267-293.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54*(1), 21-30.

Howard, M. C. (2016). A review of exploratory factor analysis decisions and overview of current practices: What we are doing and how can we improve?. *International Journal of Human-Computer Interaction*, *32*(1), 51-62.

Huff, M. L., Emmons, E. B., Narayanan, N. S., & LaLumiere, R. T. (2016). Basolateral amygdala projections to ventral hippocampus modulate the consolidation of footshock, but not contextual, learning in rats. *Learning & memory*, *23*(2), 51-60.

Hunsaker, M. R., & Kesner, R. P. (2008). Dissociations across the dorsal–ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. *Neurobiology of learning and memory*, *89*(1), 61-69.

Ilany, A., & Eilam, D. (2008). Wait before running for your life: defensive tactics of spiny mice (Acomys cahirinus) in evading barn owl (Tyto alba) attack. *Behavioral Ecology and Sociobiology*, *62*(6), 923-933.

Indovina, I., Robbins, T. W., Núñez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*, *69*(3), 563-571.

Iwata, J., Chida, K., & LeDoux, J. E. (1987). Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. *Brain research*, *418*(1), 183-188.

Jacobs, N. S., Cushman, J. D., & Fanselow, M. S. (2010). The accurate measurement of fear memory in Pavlovian conditioning: resolving the baseline issue. *Journal of neuroscience methods*, *190*(2), 235-239.

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine reviews*, *12*(2), 118-134.

Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., ... & Paninski, L. (2018). Anxiety cells in a hippocampal-hypothalamic circuit. *Neuron*, *97*(3), 670-683.

Kaiser, H. F. (1981). A revised measure of sampling adequacy for factor-analytic data matrices. *Educational and Psychological Measurement*, *41*(2), 379-381.

Kalueff, A. V., Aldridge, J. W., LaPorte, J. L., Murphy, D. L., & Tuohimaa, P. (2007). Analyzing grooming microstructure in neurobehavioral experiments. *Nature protocols*, *2*(10), 2538.

Kalueff, A. V., & Tuohimaa, P. (2005). Mouse grooming microstructure is a reliable anxiety marker bidirectionally sensitive to GABAergic drugs. *European journal of pharmacology*, *508*(1-3), 147-153.

Kishi, T., Tsumori, T., Yokota, S., & Yasui, Y. (2006). Topographical projection from the hippocampal formation to the amygdala: a combined anterograde and retrograde tracing study in the rat. *Journal of Comparative Neurology*, *496*(3), 349-368.

Kline, P. (2014). *An easy guide to factor analysis*. Routledge.

Knoll, A., Bessoule, J. J., Sargueil, F., & Cassagne, C. (1999a). Dehydration of 3-hydroxyacyl-CoA in brain very-long-chain fatty acid synthesis. *Neurochemistry international*, *34*(4), 255-267.

Knoll, A. T., Halladay, L. R., Holmes, A., & Levitt, P. (2016). Quantitative trait loci and a novel genetic candidate for fear learning. *Journal of Neuroscience*, *36*(23), 6258-6268.

Knoll, A., Sargueil, F., Salles, J., Garbay, B., Lucet-Levannier, K., & Cassagne, C. (1999b). Hydroxyacyl-CoA dehydrase and trans-2, 3-enoyl-CoA reductase activities are consistent with long-chain fatty acid accumulation during rat brain development. *Neuroscience letters*, *263*(1), 5-8.

Lau, J. Y., Lissek, S., Nelson, E. E., Lee, Y., Roberson-Nay, R., Poeth, K., ... & Pine, D. S. (2008). Fear conditioning in adolescents with anxiety disorders: results from a novel experimental paradigm. *Journal of the American Academy of Child & Adolescent Psychiatry*, *47*(1), 94-102.

LeDoux, J. (1996). Emotional networks and motor control: a fearful view. *Progress in brain research*, *107*, 437-446.

Lever, C., Burton, S., & O'Keefe, J. (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. *Reviews in the Neurosciences*, *17*(1/2), 111.

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, *92*(2), 180-185.

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., ... & Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience & Biobehavioral Reviews*, *77*, 247-285.

Lueptow, L. M. (2017). Novel object recognition test for the investigation of learning and memory in mice. *JoVE (Journal of Visualized Experiments)*, (126), e55718.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual review of neuroscience*, *24*(1), 897-931.

Maren, S. (2005). Synaptic mechanisms of associative memory in the amygdala. *Neuron*, *47*(6), 783-786.

Maren, S. (2008). Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. *European Journal of Neuroscience*, *28*(8), 1661-1666.

Maren, S., De Oca, B., & Fanselow, M. S. (1994). Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. *Brain research*, *661*(1-2), 25-34.

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nature reviews neuroscience*, *14*(6), 417-428.

Martinez, R. C., Carvalho-Netto, E. F., Amaral, V. C., Nunes-de-Souza, R. L., & Canteras, N. S. (2008). Investigation of the hypothalamic defensive system in the mouse. *Behavioural brain research*, *192*(2), 185-190.

Mattheisen, M., Samuels, J. F., Wang, Y., Greenberg, B. D., Fyer, A. J., McCracken, J. T., ... & Nestadt, G. (2015). Genome-wide association study in obsessive-compulsive disorder: results from the OCGAS. *Molecular psychiatry*, *20*(3), 337-344.

McGuire, J. F., Orr, S. P., Wu, M. S., Lewin, A. B., Small, B. J., Phares, V., ... & Storch, E. A. (2016). Fear conditioning and extinction in youth with obsessive-compulsive disorder. *Depression and anxiety*, *33*(3), 229-237.

McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P., & Bannerman, D. M. (2004). Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. *Behavioral neuroscience*, *118*(1), 63.

McHugh, T. J., & Tonegawa, S. (2007). Spatial exploration is required for the formation of contextual fear memory. *Behavioral neuroscience*, *121*(2), 335.

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: effects of training history. *Physiology & behavior*, *73*(5), 705-717.

Milner, L. C., & Crabbe, J. C. (2008). Three murine anxiety models: results from multiple inbred strain comparisons. *Genes, Brain and Behavior*, *7*(4), 496-505.

Mönnikes, H., Schmidt, B. G., & Taché, Y. (1993). Psychological stress-induced accelerated colonic transit in rats involves hypothalamic corticotropin-releasing factor. *Gastroenterology*, *104*(3), 716-723.

Montegomery, K. (1955). The relation between fear induced by novel stimulation and exploratory behaviour. *Journal of Comparative and Physiological Psychology*, *48*, 225-228.

Moore, E. M., Linsenbardt, D. N., Melón, L. C., & Boehm, S. L. (2011). Ontogenetic differences in adolescent and adult C57BL/6J and DBA/2J mice: Anxiety-like, locomotor, and consummatory behaviors. *Developmental Psychobiology*, *53*(2), 141-156.

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences*, *92*(21), 9697-9701.

Mulligan, M. K., Mozhui, K., Prins, P., & Williams, R. W. (2017). GeneNetwork: a toolbox for systems genetics. In *Systems Genetics* (pp. 75-120). Humana Press, New York, NY.

Miyoshi, E., Wietzikoski, E. C., Bortolanza, M., Boschen, S. L., Canteras, N. S., Izquierdo, I., & Da Cunha, C. (2012). Both the dorsal hippocampus and the dorsolateral striatum are needed for rat navigation in the Morris water maze. *Behavioural brain research*, *226*(1), 171-178.

Neueder, D., Andreatta, M., & Pauli, P. (2019). Contextual fear conditioning and fear generalization in individuals with panic attacks. *Frontiers in behavioral neuroscience*, *13*.

O'Leary, T. P., Gunn, R. K., & Brown, R. E. (2013). What are we measuring when we test strain differences in anxiety in mice?. *Behavior genetics*, *43*(1), 34-50.

Overall, R. W., Kempermann, G., Peirce, J., Lu, L., Goldowitz, D., Gage, F. H., ... & Schalkwyk, L. C. (2009). Genetics of the hippocampal transcriptome in mouse: a systematic survey and online neurogenomics resource. *Frontiers in neuroscience*, *3*, 3.

Owen, E. H., Christensen, S. C., Paylor, R., & Wehner, J. M. (1997a). Identification of quantitative trait loci involved in contextual and auditory-cued fear conditioning in BXD recombinant inbred strains. *Behavioral neuroscience*, *111*(2), 292.

Owen, E. H., Logue, S. F., Rasmussen, D. L., & Wehner, J. M. (1997b). Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neuroscience*, *80*(4), 1087-1099.

Park, H., Choi, Y., Jung, H., Kim, S., Lee, S., Han, H., ... & Kim, E. (2020). Splice-dependent trans-synaptic PTP δ–IL 1 RAPL 1 interaction regulates synapse formation and non-REM sleep. *The EMBO journal*, *39*(11), e104150.

Pavlov, P. I. (1927). *Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.

Paylor, R., Tracy, R., Wehner, J., & Rudy, J. W. (1994). DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behavioral neuroscience*, *108*(4), 810.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of neuroscience methods*, *14*(3), 149-167.

Podhorna, J., & Brown, R. E. (2002). Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. *Genes, Brain and Behavior*, *1*(2), 96-110.

Ponder, C. A., Kliethermes, C. L., Drew, M. R., Muller, J., Das, K., Risbrough, V. B., ... & Palmer, A. A. (2007). Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. *Genes, Brain and Behavior*, *6*(8), 736-749.

Pontén, F., Jirström, K., & Uhlen, M. (2008). The Human Protein Atlas—a tool for pathology. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *216*(4), 387-393.

Poplawski, S. G., Schoch, H., Wimmer, M. E., Hawk, J. D., Walsh, J. L., Giese, K. P., & Abel, T. (2014). Object-location training elicits an overlapping but temporally distinct transcriptional profile from contextual fear conditioning. *Neurobiology of learning and memory*, *116*, 90-95.

Poulos, A. (1995). Very long chain fatty acids in higher animals—a review. *Lipids*, *30*(1), 1-14.

Pugh, C. R., Tremblay, D., Fleshner, M., & Rudy, J. W. (1997). A selective role for corticosterone in contextual-fear conditioning. *Behavioral neuroscience*, *111*(3), 503.

Quirk, G. J., Repa, J. C., & LeDoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron*, *15*(5), 1029-1039.

Richmond, M. A., Yee, B. K., Pouzet, B., Veenman, L., Rawlins, J. N. P., Feldon, J., & Bannerman, D. M. (1999). Dissociating context and space within the hippocampus: effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. *Behavioral neuroscience*, *113*(6), 1189.

Riedel, G. (1999). If phosphatases go up, memory goes down. *Cellular and Molecular Life Sciences CMLS*, *55*(4), 549-553.

Ritov, G., Ardi, Z., & Richter-Levin, G. (2014). Differential activation of amygdala, dorsal and ventral hippocampus following an exposure to a reminder of underwater trauma. *Frontiers in behavioral neuroscience*, *8*, 18.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1991). Central amygdala lesions affect behavioral and autonomic balance during stress in rats. *Physiology & behavior, 50*(4), 777-781.

Royce, J. R. (1977). On the construct validity of open-field measures. *Psychological bulletin*, *84*(6), 1098.

Rudy, J. W., Huff, N. C., & Matus-Amat, P. (2004). Understanding contextual fear conditioning: insights from a two-process model. *Neuroscience & Biobehavioral Reviews*, *28*(7), 675-685.

Rudy, J. W., & Matus-Amat, P. (2005). The ventral hippocampus supports a memory representation of context and contextual fear conditioning: implications for a unitary function of the hippocampus. *Behavioral neuroscience*, *119*(1), 154.

Rudy, J. W., & O'Reilly, R. C. (1999). Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. *Behavioral neuroscience*, *113*(5), 867.

Rumpel, S., LeDoux, J., Zador, A., & Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. *Science*, *308*(5718), 83-88.

Sahin, M., & Hockfield, S. (1993). Protein tyrosine phosphatases expressed in the developing rat brain. *Journal of Neuroscience*, *13*(11), 4968-4978.

Sapnas, K. G., & Zellar, R. A. (2002). Minimizing sample size when using exploratory factor analysis for measurement. *Journal of nursing measurement*, *10*(2), 135-154.

Sawai, M., Uchida, Y., Ohno, Y., Miyamoto, M., Nishioka, C., Itohara, S., ... & Kihara, A. (2017). The 3-hydroxyacyl-CoA dehydratases HACD1 and HACD2 exhibit functional redundancy and are active in a wide range of fatty acid elongation pathways. *Journal of Biological Chemistry*, *292*(37), 15538-15551.

Schneider, P., Ho, Y. J., Spanagel, R., & Pawlak, C. R. (2011). A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. *Frontiers in behavioral neuroscience*, *5*, 43.

Schormair, B., Kemlink, D., Roeske, D., Eckstein, G., Xiong, L., Lichtner, P., ... & Winkelmann, J. (2008). PTPRD (protein tyrosine phosphatase receptor type delta) is associated with restless legs syndrome. *Nature genetics*, *40*(8), 946-948.

Shapiro, S. S., & Francia, R. S. (1972). An approximate analysis of variance test for normality. *Journal of the American statistical Association*, *67*(337), 215-216.

Smith, D. R., Gallagher, M., & Stanton, M. E. (2007). Genetic background differences and nonassociative effects in mouse trace fear conditioning. *Learning & Memory*, *14*(9), 597-605.

Sokoloff, G., Parker, C. C., Lim, J. E., & Palmer, A. A. (2011). Anxiety and fear in a cross of C57BL/6J and DBA/2J mice: mapping overlapping and independent QTL for related traits. *Genes, Brain and Behavior*, *10*(5), 604-614.

Sprott, R. L. (1975). Behavioral characteristics of C57BL/6J, DBA/2J, and B6D2F1 mice which are potentially useful for gerontological research. *Experimental aging research*, *1*(2), 313-323.

Spruijt, B. M., Van Hooff, J. A., & Gispen, W. H. (1992). Ethology and neurobiology of grooming behavior. *Physiological reviews*, *72*(3), 825-852.

Sturman, O., Germain, P. L., & Bohacek, J. (2018). Exploratory rearing: a context-and stress-sensitive behavior recorded in the open-field test. *Stress*, *21*(5), 443-452.

Süer, C., Dolu, N., Özesmi, Ç., Şahin, Ö., & Ülgen, A. (1998). The relation between skin conductance level and plus-maze behavior in male mice. *Physiology & behavior*, *64*(4), 573-576.

Swanson, L. W. (2000). Cerebral hemisphere regulation of motivated behavior. *Brain research*, *886*(1-2), 113-164.

Takahashi, H., & Craig, A. M. (2013). Protein tyrosine phosphatases PTPδ, PTPσ, and LAR: presynaptic hubs for synapse organization. *Trends in neurosciences*, *36*(9), 522-534.

Taylor, B.A. (1989). Recombinant inbred strains. In Lyon M.L. (Ed.) *Genetic Variants and Strains of the Laboratory Mouse* (2nd ed., pp. 773-796). Searle, AG. Oxford: Oxford University Press.

Tipps, M. E., Raybuck, J. D., Buck, K. J., & Lattal, K. M. (2014). Delay and trace fear conditioning in C57BL/6 and DBA/2 mice: issues of measurement and performance. *Learning & Memory*, *21*(8), 380-393

Toothaker, L. E. (1993). *Multiple comparison procedures* (No. 89). Sage.

Tsvetkov, E., Carlezon Jr, W. A., Benes, F. M., Kandel, E. R., & Bolshakov, V. Y. (2002). Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. *Neuron*, *34*(2), 289-300.

Uetani, N., Kato, K., Ogura, H., Mizuno, K., Kawano, K., Mikoshiba, K., ... & Iwakura, Y. (2000). Impaired learning with enhanced hippocampal long-term potentiation in PTPδ-deficient mice. *The EMBO journal*, *19*(12), 2775-2785.

Uhl, G. R., & Martinez, M. J. (2019). PTPRD: neurobiology, genetics, and initial pharmacology of a pleiotropic contributor to brain phenotypes. *Annals of the New York Academy of Sciences*, *1451*(1), 112-129.

Uhl, G. R., Martinez, M. J., Paik, P., Sulima, A., Bi, G. H., Iyer, M. R., ... & Xi, Z. X. (2018). Cocaine reward is reduced by decreased expression of receptor-type protein tyrosine phosphatase D (PTPRD) and by a novel PTPRD antagonist. *Proceedings of the National Academy of Sciences*, *115*(45), 11597-11602.

van Gaalen, M. M., & Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural brain research*, *115*(1), 95-106.

Velicer, W. F., & Fava, J. L. (1998). Affects of variable and subject sampling on factor pattern recovery. *Psychological methods*, *3*(2), 231.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Current protocols in neuroscience*, *69*(1), 8-31.

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature protocols*, *2*(2), 322-328.

Wang, J., & Bixby, J. L. (1999). Receptor tyrosine phosphatase-δ is a homophilic, neurite-promoting cell adhesion molecule for CNS neurons. *Molecular and Cellular Neuroscience*, *14*(4-5), 370-384.

Wang, H., Johnson, T. R., Sun, Y., & Zhang, J. (2005). Object location memory: The interplay of multiple representations. *Memory & cognition*, *33*(7), 1147-1159.

White, H. (1980). A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica: journal of the Econometric Society*, 817-838.

Widman, A. J., Cohen, J. L., McCoy, C. R., Unroe, K. A., Glover, M. E., Khan, A. U., ... & Clinton, S. M. (2019). Rats bred for high anxiety exhibit distinct fear-related coping behavior, hippocampal physiology, and synaptic plasticity-related gene expression. *Hippocampus*, *29*(10), 939-956.

Wood, S. C., & Anagnostaras, S. G. (2011). Interdependence of measures in Pavlovian conditioned freezing. *Neuroscience letters*, *505*(2), 134-139.

Xu, Y., & Fisher, G. J. (2012). Receptor type protein tyrosine phosphatases (RPTPs)–roles in signal transduction and human disease. *Journal of cell communication and signaling*, *6*(3), 125-138.

Yang, Q., Li, L., Yang, R., Shen, G. Q., Chen, Q., Foldvary-Schaefer, N., ... & Wang, Q. K. (2011). Family-based and population-based association studies validate PTPRD as a risk factor for restless legs syndrome. *Movement Disorders*, *26*(3), 516-519.

Yang, R. J., Mozhui, K., Karlsson, R. M., Cameron, H. A., Williams, R. W., & Holmes, A. (2008). Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacology*, *33*(11), 2595-2604.

Young, S. L., Bohenek, D. L., & Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. *Behavioral neuroscience*, *108*(1), 19.

Zarrindast, M. R., Nasehi, M., Piri, M., & Heidari, N. (2011). Effects of cholinergic system of dorsal hippocampus of rats on MK-801 induced anxiolytic-like behavior. *Neuroscience letters*, *505*(2), 65-70.

Zhang, W. N., Bast, T., & Feldon, J. (2001). The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after infusion of N-methyl-D-aspartate or its

noncompetitive antagonist MK-801 into the ventral hippocampus. *Behavioural brain research*, *126*(1-2), 159-174.

ABBREVIATED CURRICULUM VITAE

Dana Zeid

Education

2016-2021 Doctor of Philosophy
The Pennsylvania State University, University Park, PA
Advisor: Thomas J. Gould

2012-2016 Bachelor of Psychology
Marshall University, Huntington, WV

Selected publications (of 12)

1. **Zeid, D.**, Goldberg, L. R., Seemiller, L. R., Mooney-Leber, S., Smith, P. B., & Gould, T. J. (2021). Multigenerational nicotine exposure affects offspring nicotine metabolism, nicotine-induced hypothermia, and basal corticosterone in a sex-dependent manner. *Neurotoxicology and Teratology*, *85*, 106972.
2. **Zeid, D.**, & Gould, T. J. (2020). Impact of nicotine, alcohol, and cocaine exposure on germline integrity and epigenome. *Neuropharmacology*, 108127.
3. Goldberg, L. R.,* **Zeid, D.**,* Kutlu, M. G., Cole, R., Lallai, V., Sebastian, A., Fowler, C., Albert, I., Parikh, V., & Gould, T. J. (2019). Paternal nicotine enhances fear memory, reduces nicotine administration, and alters hippocampal genetic and neural function in offspring. *Addiction Biology,* e12859. **These authors contributed equally to this work.*

Selected Awards and Honors

2021 Gerald E. McClearn Graduate Student Award, The Pennsylvania State University, Department of Biobehavioral Health

2020 NIH Institutional National Research Award (NRSA)

2020 Elizabeth J. Susman Enhancement Fund Award in Biobehavioral Health, The Pennsylvania State University

2019 Dissertation Award, The Pennsylvania State University, Department of Biobehavioral Health

2018 Graduate Research Award, The Pennsylvania State University, Department of Biobehavioral Health

2018 Edward R. and Helen Skade Hintz Graduate Education Enhancement Fellowship Award, The Pennsylvania State University, Department of Biobehavioral Health