ESTABLISHING A CONTEXTUAL FEAR CONDITIONING PARADIGM FOR THE TAT TRANSGENIC MOUSE MODEL

Alexandra Maria Proca

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Approved by:

Sylvia Fitting

Kathryn J. Reissner

Ian R. Jacobs

ABSTRACT

Alexandra Maria Proca: Establishing a Contextual Fear Conditioning Paradigm for the Tat Transgenic Mouse Model
(Under the direction of Sylvia Fitting)

HIV-1 Tat is a viral protein produced by HIV that causes structural changes to neurons and inflammation. Tat is implicated in deficits in the functions of several brain areas including hippocampal-dependent memory. Contextual fear condition (CFC) can be used to observe memory by associating a fear-inducing stimulus with a certain place. Studies show that lesioning the hippocampus reduces contextual fear related behaviors, supporting the use of CFC as a measure of hippocampal function. In the current study, three experiments were run to determine the most effective conditioning procedure for the Tat transgenic mouse, a neuroHIV model. A 2x2, 0.4 mA arrangement was found to produce the least variability between subjects. However, the multiple-trial conditioning in this procedure may provide extensive learning, essentially rescuing neurologically compromised subjects. This further raises concerns about the validity of comparisons between the effectiveness of contextual versus cued fear conditioning. Future studies should assess hippocampal neurodegeneration associated with predicted behavioral deficits through quantification of structural proteins, such as MAP2ab, IBA-1, and GFAP.

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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	analysis of variance
cART	combination antiretroviral therapy
CFC	contextual fear conditioning
CNS	central nervous system
CR	conditioned response
CS	conditioned stimulus
CSF	cerebrinal spinal fluid
DC	direct current
DMSO	dimethyl sulfoxide
DOX	doxycycline
DVR	digital video recorder
GFAP	glial fibrillary action protein
HAND	HIV-1 associated neurocognitive disorder
HIV-1	human immunodeficiency virus type 1
IACUC	Institutional Animal Care and Use Committee
IBA-1	ionized calcium-binding adaptor molecule 1
mA	milliamp
MAP2ab	microtubule-associated protein 2
NMDAR	N-methyl-D-aspartate receptor

PFC	prefrontal cortex
rtTA	reverse tetracycline-controlled transactivator
SEM	standard error of the mean
SPSS	Statistical Package for the Social Sciences
Tat	transactivator of transcription
TRE	tetracycline response element
UR	unconditioned response
US	unconditioned stimulus

CHAPTER 1: INTRODUCTION

Human Immunodeficiency Virus (HIV) is a disease that targets the immune system and, without treatment, can result in the development of acquired immune deficiency syndrome (AIDS). Research in the treatment of HIV has rapidly advanced over the past several decades, but there are still approximately 37.9 million people around the world living with HIV (World Health Organization, 2019). Since its discovery in the 1990's, combination antiretroviral therapy (cART) has been used to treat nearly 23.3 million people (World Health Organization, 2019). cART reduces HIV-1 replication, increases chance of survival, and improves the overall life quality of those infected (Cohen et al., 2011). However, cART is limited in its ability to affect the central nervous system (CNS) and therefore is unable to protect against neurological disorders and impairments caused by HIV (Ellis et al., 2017; Heaton et al., 2010). Even after cART treatment, those infected with HIV can develop HIV-associated neurocognitive disorder (HAND), which can lead to neurodegenerative-associated impairments in motor skills, language, abstraction-executive, working memory, attention, and inhibitory control (Clifford & Ances, 2013). In the post-cART era, we see specific effects on memory tasks involving the hippocampus and the prefrontal cortex (PFC) (Heaton et al., 2010, 2011; McArthur et al., 2010).

Transactivator of transcription (Tat) is a viral protein released by HIV-infected cells. Tat has been found in the brains of patients with HIV (Hudson et al., 2000) and is believed to be a major contributor to the neurodegenerative damage observed in HAND (Fitting et al., 2014; Kruman et al., 1994; King et al., 2006). Tat is an important player in HAND, even in the era of

cART, and is still present in the cerebrospinal fluid (CSF) of HIV+ individuals after treatment (Henderson et al., 2019). The viral protein activates glutamatergic NMDA receptors (NMDAR), causing excitotoxic influx of calcium and sodium, as well as mitochondrial instability (Fitting et al., 2014; Haughey et al., 2001). The activation of AMPA receptors (AMPARs) by neurotoxic glutamate release upregulates NMDA-mediated toxicity (Longordo et al., 2006), leading to dendritic degeneration and inflammation (Mattson et al., 2005; Green et al., 2018). Extended NMDA exposure results in elevated sodium levels, ionic imbalances and loss of calcium homeostasis in dendrites (Vander Jagt et al., 2008). This synaptodendritic injury manifests as swellings and structural defects caused by excessive levels of sodium and calcium (Greenwood et al., 2007). Under Tat conditions, microtubule associated protein 2 antibody (MAP2ab) is downregulated, signifying loss of dendritic structure, and glial fibrillary action protein (GFAP) and ionized calcium-binding adaptor molecule 1 (IBA-1) are upregulated, signifying increase in neuroinflammatory responses. Tat has been shown to cause neuronal injury and neuroinflammation in various brain regions, dependent on the length of expression induced (King et al., 2006). Specifically, studies have shown neurotoxic damage in the striatum of HIV/Tat transgenic rodent models (Bruce-Keller et al., 2008; Bansal et al., 2000; Hayman et al., 1993), including dendritic varicosities and fragmentation (Fitting et al., 2010, 2014). Exposure to Tat also result in reduced spine density and synapse loss in the hippocampus of Tat transgenic models (Fitting et al., 2013; Kim et al., 2008). These injuries are known to highly correlate with neurocognitive deficits that can be observed in behavioral studies. Hippocampal neurocognitive deficits in animal models can be assessed through tasks, such as the Morris Water Maze and Barnes Maze. In these behavioral tests, spatial learning and memory are quantified by the

distance and latency for animals to reach the platform. Another method, fear conditioning, can be used to study hippocampal deficits related to context or cued stimuli. Spatial learning and memory deficits related to hippocampal damage and impaired consolidation of contextual memory can be quantified using contextual fear conditioning (CFC) experiments (Fitting et al., 2013). Lesioning studies have shown that contextual memory, studied through CFC, is dependent on hippocampal function (Goosens et al., 2001; Phillips et al., 1992).

In the present study we are specifically interested in the effects that Tat has on the hippocampus and its related behavior. HIV-1 Tat's effects on the hippocampus and memory can be studied through CFC, a form of Pavlovian conditioning. CFC can be used to observe contextual memory because it causes healthy subjects to associate a specific context with a fear-inducing stimulus. In Pavlovian conditioning, subjects learn to associate a conditioned stimulus (CS) with an unconditioned stimulus (US). This learning involves presenting a CS with an US that results in an unconditioned response (UR). After sufficient pairings, the CS should elicit a conditioned response (CR) without the presence of the US. In contextual fear conditioning, mice are measured for fear-indicative freezing behavior after being conditioned to an electrical foot shock. This can be done by pairing a CS, the context, with a US of a foot shock. The foot shock (US) elicits freezing behavior (UR), a fear-response in mice. After successful pairing, the context as the CS should produce freezing (CR), regardless of whether or not the foot shock (US) is present. Ultimately, the subject should learn to associate the context it is in to the electric shock, and thus exhibit a fear response of freezing when placed in the context.

As shown by lesioning studies, contextual memory is dependent on hippocampal function (Goosens et al., 2001; Phillips et al., 1992). As Tat has been shown to cause synaptodendritic

injury in the hippocampus, CFC can be used to assess the extent of Tat's effect on contextual memory and potential associated deficits. In this study, we will be testing whether the expression of doxycycline-induced HIV-1 Tat for 3 months decreases fear-conditioned responses using a Tat transgenic mouse model. To measure hippocampal deficits, we will be fear-conditioning Tat(+) and Tat(-) transgenic mice in one context and reintroducing them to the same context 24 hours later, measuring for fear-indicative freezing behavior in order to test for the subject's ability to recall the fearful context. The purpose of these studies is to formalize a fear conditioning protocol in Tat transgenic mice in order to decrease variability, and study whether there are significant differences found between Tat(+) and Tat(-) mice.

CHAPTER 2: MATERIALS AND METHODS

2.1 Subjects

The experiment used 80-day old doxycycline (DOX)-inducible, brain-specific HIV-1IIIB Tat_{1-86} transgenic mice developed on a C57BL/6J hybrid background (Bruce-Keller et al., 2008; Hahn et al., 2015). Subjects were bred and kept in Davie Hall Animal Facility. The mice were single housed when DOX food was administered and provided water and food *ad libidum*. Animals were kept at a 12h light/dark cycle and were tested on during the night cycle. All experimental procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

As summarized in Bruce-Keller et al. (2008) Tat expression, under the control of a tetracycline-responsive promoter controlled by glial fibrillary acidic protein (GFAP) expression, is induced with chow containing 6 mg/g DOX (product TD.09282; Harlan, Indianapolis, IN). Inducible Tat(+) transgenic mice express both GFAP-rtTA and TRE-tat genes, while control Tat(-) transgenic mice express only the GFAP-rtTA genes. All transgenic mice were genotyped to confirm presence of Tat and/or rtTA transgenes. Both control Tat(-) and experimental Tat(+) received DOX for three months to induce Tat expression.

2.2 Apparatus

Testing was done in a dark room with red fluorescent lighting and an air-conditioner running as background noise. All experimental chambers (MED Associates ENV-307W) were housed in sound and light reducing enclosures (MED Associates ENV-022MD). The chamber

also contained a shock scrambler (ENV-4145: 115 V AC, 60 Hz) on the grid floor. A 28 V DC, 100 mA white house light (MED Associates ENV-215W) was mounted on the wall to illuminate the chamber during testing. Each chamber contained a video camera (Amcrest 1080P Quadbrid) to record each experimental session and a DVR (101AV 16CH 1080p) was used to collect the recorded videos.

2.3 Behavioral Procedure

Mice were habituated to the experimenter by being handled for 2-3 minutes daily, one week before the initial acquisition phase. Weights were recorded weekly for two months prior to acquisition to ensure subjects were eating DOX and maintaining a healthy body weight. Both groups [Tat(+) and Tat(-)] were exposed to the same experimental conditions. During the fear conditioning phase, subjects were placed in the chamber and administered the shock pattern respective to the experiment. Unless otherwise stated, shocks occurred at 5 and 8 minutes.

Testing occurred in the same context 24 hours after the initial fear conditioning. After a 10 second period from initial placement of subjects in the chambers, freezing behavior was measured from the recorded videos. The percentage freezing time was taken from the number of 5 second interval periods within the first five minutes of the trial in which the subject exhibited freezing behavior. Only the first five minutes were used to code freezing because it is known to be the period of time of most animal freezing, as afterwards animals are more subject to within session extinction. Freezing was defined as the subject ceasing all movement for more than one second. Coding utilized the bin method, where a whole 5-second bin was marked as “freezing” if any freezing occurred during that bin, including the overlapping of freezing into two bins. The

videos were coded by one person, and an additional person conducted reliability checks on 31% of the videos to ensure consistent agreement above 80%.

2.4 Statistical Analysis

We measured the percentage of freezing of the testing trial. The independent variables were sex (female, male) and genotype [Tat(+), Tat(-)], and the dependent variable was the percentage freezing time in the testing trial. All descriptive statistics were reported as means (*M*) ± standard error of the mean (*SEM*). The analyses conducted used an alpha value of $p < 0.05$ as a measure of statistical significance. To study test differences between Tat(+)/(-), an independent samples *t*-test was run. A two-way analysis of variances (ANOVA) with Sex (male, female) and Genotype [Tat(-), Tat(+)] as factors was conducted for experiment 3. For all studies, we predicted that the Tat(+) mice will exhibit less freezing behavior compared to the Tat(-) mice in the testing trial, due to a deficit in contextual memory as a result of hippocampal damage. Population effect size was represented using ω^2 as an unbiased estimate (Yiğit and Mendes, 2018). All statistical analyses were conducted in SPSS (SPSS Statistics, Version 26, IBM).

CHAPTER 3: EXPERIMENT 1

Experiment 1 was used as a pilot study to determine whether freezing behavior could be produced from administration of an electrical shock in our model. In their CFC paradigm, Fitting et al. (2013) used a 2 s, 0.7 mA shock after three minutes in an experimental chamber and re-introduced subjects to the same context, measuring freezing 24 h after conditioning. This study found that Tat(+) mice froze significantly less than Tat(-) mice when re-exposed to the context, suggesting that Tat expression impairs contextual memory consolidation and/or retrieval. Hahn et al.'s (2016) cued fear conditioning paradigm consisted of 3 tones of 20 s, each co-terminated with a 2 s, 0.7 mA shock. During the extinction phase, subjects were measured for freezing behavior 24 h after conditioning in a new context, paired with the original tone for 200 s. This study found that there were no significant differences between Tat(+) and Tat(-) mice immediately before and after the shock on the initial conditioning day. Additionally, the study found that Tat(+) mice show delayed extinction of freezing behavior in different contexts, with no significant difference between Tat(+) and Tat(-) after day 3, possibly a result of elevated anxiety behavior in Tat(+) mice, rather than differences in memory consolidation of cued fear. These studies were used to develop the methods utilized in these experiments, as a way to unify both studies' methodology (1 shock vs. 3 shocks) under a single procedure (2 shocks) and investigate the apparent discrepancy in contextual and cued fear conditioning. Based on these previous studies and literature supporting Tat's neurodegenerative effect on the hippocampus, we

hypothesized that Tat(+) mice would freeze significantly less than Tat(-) mice due to failure to consolidate fear into long-term memory.

3.1 Materials and Methods

3.12 Subjects. The study utilized a sample of 16 male Tat transgenic mice as described above [n = 8 Tat(+)].

3.13 Apparatus. We used the same apparatus as described above.

3.14 Procedure. Due to a programming error, subjects were placed in the chamber for 10 min without a shock, thus introducing a habituation phase into the experiment. After 24 h, the fear conditioning phase occurred in the same context of the chamber. Two unsignaled 0.4 mA shock presentations were administered as described above. Testing and coding were conducted as specified in the general procedure.

3.2 Results and Discussion

An independent samples t-test found that there was no significant difference $t(14) = -.256$, $p = .801$, between the freezing percentages of Tat(+) ($M = 0.14$, $SEM = 0.03$) and Tat(-) ($M = 0.15$, $SEM = 0.32$) mice (**Figure 1**). This indicates that Tat(+) and Tat(-) mice displayed a similar amount of freezing behavior, suggesting that genotype did not affect consolidation and retrieval of the fearful memory.

The study showed no significant differences between the two groups of Tat(+) and Tat(-) transgenic mice. This may have been partially due to the error in programming that resulted in an accidental habituation phase. The former safe encounter with the context may have interfered with the latter fear conditioning, resulting in subjects having less of a fear response to the chamber. Lack of significant differences may also have been a result of the lower intensity shock

of 0.4 mA and later exposure to the shock at 5 min in this study than the higher intensity shock of 0.7 mA and earlier exposure to the shock at 3 min in other studies (Fitting et al., 2013; Hahn et al., 2016). The 0.4 mA shock was used in this experiment because previous studies have shown a 0.4-0.6 mA shock level to produce optimal learning of context in the C57BL/6 mouse (Curzon et al., 2009). An intermediate intensity shock also provides the potential for extinction in future studies. The administration of the shock at 5 and 8 min was done with the goal of eliciting more freezing during the first five minutes of testing, as the timing of the conditioned shock neared; however, it may have contributed to an extended habituation phase and slowed conditioned learning. The use of two shocks, rather than three (Hahn et al., 2016), may have also contributed to the lack of significant results. The two-shock paradigm was used as an intermediate between Fitting (2013) and Hahn (2016) methodology but may have been insufficient when paired with the lower intensity shock. Learning generally increases with the amount of spaced training (Lauterborn et al., 2019) and an additional shock may cause subjects to more closely associate the context with the shocking experience, especially within different strains of mice (Chaudhury et al., 2002). Shock titration experiments are commonly used to ensure an appropriately working apparatus. These experiments entail using different shock intensities and shock recurrences in order to determine the most effective conditioning method for subjects. The lack of replication could be potentially attributed to the fact that the study consisted of different shock exposure/timing and intensity from previous studies.

CHAPTER 4: EXPERIMENT 2

The results of experiment 1 showed that the procedure utilized failed to produce consistent results, as subject percentages had a high variability within groups, and overall freezing was low. In order to determine a more-effective electrical shock method, experiment 2 was used as a shock titration experiment. Four configurations were used: two 0.6 mA shock in one day [2x1 (.6)], two 0.8 mA shocks in one day [2x1 (.8)], four 0.4 mA shocks in one day (4x1), and two 0.4 mA for two days (2x2). Additionally, this study removed the habituation phase at 24 h before fear conditioning, as the initial safe exposure to the context may have decreased the effectiveness of the fear conditioning in experiment 1. We hypothesized that the two 0.4 mA shocks over two days would produce the most reliable freezing percentages, closest to a value of 50% freezing, because of the repeated conditioning to the context. The freezing percentage was selected to be an intermediate value, so that extinction in future studies may be possible. As in the previous study, we also hypothesized that Tat(+) transgenic mice would freeze less than Tat(-) transgenic mice.

4.1 Materials and Methods

4.12 Subjects. The study utilized a sample of 32 Tat transgenic mice as described above [n = 16 Tat(+), n = 16 female].

4.13 Apparatus. We used the same apparatus as described above.

4.14 Procedure. Subjects were not DOX-ed prior to the study and therefore were effectively of the same genotype [Tat(-)]. Subjects were placed in the apparatus during the fear conditioning phase. Subjects were divided into four groups. The 4x1 arrangement consisted of an unsignaled 0.4 mA shock presentation administered for 2 s at 5, 8, 11, and 14 min. The 2x2 arrangement consisted of an unsignaled 0.4 mA shock presentation administered for two seconds first after five minutes, and again three minutes following the first shock in the chamber for two consecutive days. The 2x1 (.6) and 2x1 (.8) arrangements consisted of an unsignaled 0.6 and 0.8 mA shock presentation, respectively, administered for two seconds first after five minutes, and again three minutes following the first shock in the chamber. Testing and coding were conducted as specified in the general procedure.

4.2 Results and Discussion

The 4x1 arrangement (M = 0.48, SEM = 0.12) had the least average freezing. The 2x2 arrangement (M = 0.54, SEM = 0.06) was found to have the least variability and average closest to 0.5. The 2x1 (.6) arrangement (M = 0.62, SEM = 0.07) and 2x1 (.8) arrangement (M = 0.70, SEM = 0.06) had the highest average freezing (**Figure 2**).

One study, Curzon et al. (2009), utilized a shock titration experiment, administering either no shock, a 0.17 mA shock, or 0.35 mA shock to mice. The study found that both groups subject to a foot shock froze significantly more than the group without a shock, with the 0.35 mA group freezing the most on average. Similarly, our study found the highest intensity shocks (2x1 0.6 mA, 2x1 0.8 mA arrangement) to produce the highest average of freezing. However, having a high ceiling of freezing may impede results, as differences between groups may be harder to produce. Additionally, experimentally produced extinction is less feasible with higher intensity

shocks, as fear is strongly associated with the context. Another study (Chwang et al., 2006) used a 3x1 0.5 mA arrangement, similar to our 4x1 0.4 mA arrangement, and found that the group treated with DMSO froze significantly more than both the group treated SL327 and the control group. The study indicates that the 3x1 (.5) arrangement was successful in producing differences between different groups. Interestingly, in our study the 4x1 (.4) arrangement had the least average freezing and the most variability, perhaps due to the lower intensity of the shock and lack of multiple trials over multiple days to more strongly consolidate the memory (Kant et al., 2019). The 2x2 arrangement was determined to be the most reliable based on its average being closest to 0.5 (a measure of freezing that could potentially be extinguished) and its variability being the lowest. Experiment 3 utilized the 2x2 arrangement in order to model a more effective version of experiment 1.

CHAPTER 5: EXPERIMENT 3

Experiment 3 was a repeat of experiment 1 using the most effective method, the 2x2 arrangement. Additionally, the study introduced female subjects, as to extend the applicability of the study and analyze whether freezing effects would differ across sexes. Experiment 3 tested the hypothesis that female and male Tat(+) mice would freeze significantly less than female and male Tat(-) mice.

5.1 Method

5.2 Subjects. The study utilized 32 Tat transgenic mice as described above [n = 16 Tat(+), n = 16 female].

5.3 Apparatus. We used the same apparatus as described above.

5.4 Procedure. The procedure was as described above in the 2x2 arrangement in experiment 2. Testing and coding were conducted as specified in the general procedure.

5.2 Results and Discussion

A two-way ANOVA, with sex (2 levels: female, male) and genotype [2 levels: Tat(-), Tat(+)] as factors, found that there was no significant interaction between sex and genotype on freezing, $F(1, 30) = 1.14$, $p = .295$, $\omega^2 = .04$. Female Tat(+) mice ($M = 0.43$, $SEM = 0.10$) froze the least on average, but the difference was not significant. Male Tat(-) mice ($M = 0.51$, $SEM = 0.12$) followed in percentage freezing. Female Tat(-) ($M = 0.55$, $SEM = 0.09$) and male Tat(+) ($M = 0.55$, $SEM = 0.07$) froze the most on average. Overall, female Tat(+) mice froze the least and

female Tat(-) and male Tat(+) mice froze the most, but there was no significant difference or interaction between Tat(+) and Tat(-) subjects and male and female subjects.

After minimizing variability in the CFC procedure, the study still did not find significant genotype or sex differences. This differs from the findings of Fitting et al. (2013), which showed that Tat(+) transgenic mice froze significantly less than Tat(-) mice 24 h after fear-conditioning. Furthermore, there was no significant difference between Tat(+) and Tat(-) mice freezing before or immediately after the foot shock, indicating the results were caused by differences in memory consolidation and retrieval, not pain sensitivity or motor control. Conversely, Hahn et al. (2016) found that Tat(+) mice showed delayed extinction of cued freezing in a different context compared to Tat(-) mice, which could suggest that Tat(+) mice were able to consolidate and retrieve the cued fear memory. Freezing between groups was equivalent after three days of extinction, resulting in the delayed extinction to be interpreted as elevated anxiety in Tat(+) mice (Schneider et al., 2015; Sotres-Bayon et al., 2004). This suggests that CFC deficits may be subtle and thus disappear after repeated training. Although a 2x2 arrangement was shown to decrease variability in experiment 2, the repeated training may have diminished the differences between groups and caused Tat(+) mice to better consolidate the fear memory over multiple trials.

CHAPTER 6: DISCUSSION

The purpose of these studies was to find the most effective CFC procedure for Tat transgenic mice, with the goal of using the established arrangement for future research in contextual memory of HAND models. Experiment 1 found no significant differences between Tat(+) and Tat(-) transgenic mice, although freezing behavior was produced. In the following experiment 2, a shock titration study determined that a 2x2 arrangement of 2 s, 0.4 mA foot shocks produced the least variability in subject freezing out of the other three arrangements [4x1, 2x1 (.6), 2x1 (.8)]. Experiment 3 used these results to replicate the 2x2 arrangement with a larger sample size and the addition of female subjects. There were no significant differences in freezing percentages or interactions between genotype and sex found.

There were no significant differences in freezing found between groups, even after the 2x2 arrangement was identified as the method with least variability. This differs from Fitting et al. (2013), which used a single 2 s, 0.7 mA foot shock, and found that Tat(+) froze significantly less when reintroduced to the context 24 h later. Hahn et al. (2016), instead, used three tone-paired 2 s, 0.7 mA foot shocks, and found that Tat(+) mice froze significantly more than Tat(-) mice when exposed to the conditioned tone in a new context and took a longer time to extinguish the initial tone-paired freezing behavior. Comparing these two studies suggests that Tat(+) mice better consolidated the fearful experience when exposed to it multiple times (1 time vs. 3 times). Lauterborn et al. (2019) also found that spaced training improved learning on semantic and spatial memory tasks in mouse models of intellectual disability. These studies further support the

lack of differences seen in experiment 3, as the 2x2 arrangement exposed subjects to a total of four context-paired shocks, over the course of two days, ensuring better memory consolidation even for the impaired Tat(+) transgenic mice. Perhaps contextual versus cued fear conditioning deficits may need to be rethought and reanalyzed in studies that use a different number of shock-presentations for each type of conditioning, as differences between the two methods may be a consequence of repeated training in one (cued) and not the other (contextual).

Experiments 2 and 3 may have been limited by the focus on solely reducing variability in arrangements, rather than also accounting for arrangements yielding a higher average of freezing. Additionally, conditioning and testing trials may have been limited by their length. Fitting et al. (2013) and Hahn et al. (2016) both administered a foot shock after three minutes in the experimental chamber, while our studies administered shocks at 5 and 8 minutes. This extended amount of time may have served as additional habituation to the chamber that may have reduced the effectiveness of the fear conditioning. Additionally, because our freezing coding is conducted in the first five minutes within the chamber, the percentage of freezing may not account for heightened freezing around the expected time of shock. Future studies should ensure that conditioning and coding occur within the same time frame of subjects' placement in the chamber. Coding errors resulting in the additional habituation phase in experiment 1 also likely reduced the effectiveness of the conditioning phase.

Future directions should utilize one-trial learning, rather than multiple-trial learning, when studying contextual memory in Tat transgenic mice. Graves et al. (2003) found that a high intensity (1.5 mA) single shock in CFC produced significant differences between sleep-deprived and control subjects, supporting the idea that even a salient memory could fail to be consolidated

into memory with only a single presentation. Thus, a high-intensity shock may still have the potential to yield differences between Tat(+) and Tat(-) mice, rather than act as an upper ceiling, and have the potential to be extinguished in future studies. Another study (Drew et al., 2010) investigated the arrest of hippocampal neurogenesis on single- and multiple-trial CFC, finding that only single-trial CFC was impaired, further supporting that multiple-trial learning in CFC overcomes neural deficits. In order for one-trial conditioning to be effective, shock intensity should be at least within the range of 0.6-0.8 mA, as the results of experiment 2 indicate that two-trial learning of this caliber is effective in producing freezing. Time in the conditioning and testing chamber should also be limited so that there is a shorter habituation phase and the context is strongly associated with the shock experience. Providing differences are found, it will be important to account for neurodegenerative changes in the brain that my contribute to the proposed deficit. For instance, investigating proteins associated with structural brain morphology via Western Blot may be useful in this quantification. Additionally, Tat protein expression can be assessed in different brain regions utilizing mass spectrometry.

It is well known that HIV-1 crosses the blood-brain barrier (BBB) within macrophages and monocytes and affects the structure and function of multiple neural circuits and systems, especially the PFC and hippocampus (Fitting et al., 2013; Kim et al., 2008). Quantification of proteins in specific brain regions can provide evidence for structural changes caused by HIV. MAP2ab may be used to study dendritic degradation, as downregulation of its binding proteins signifies loss of dendritic structure (Harada et al., 2002). Repeated studies have shown that HIV is an inflammatory disease in various regions of the body, including the brain (Deeks et al., 2013; Tavazzi et al., 2014; Yadav et al., 2009; Li et al., 2007). This neuroinflammation can be

further studied using antibodies for GFAP and IBA-1, well-known markers for astrocytes and microglia, respectively. HIV cannot infect neurons, but can infect macrophages, microglia, and astrocytes (Nath et al., 1999; Yadav et al., 2009; Li et al., 2007). These infected cells release viral proteins including cytokines and chemokines that contribute to functional and structural deficits in the CNS. Of these viral proteins, Tat is believed to be a major contributor to the neurodegenerative effects observed in HAND (Fitting et al., 2014; Kruman et al., 1994; King et al., 2006). Future directions should aim to quantify structural proteins to compare the level of hippocampal damage between subjects expressing Tat [Tat(+)] and control subjects [Tat(-)]. Western Blot can be conducted with MAP2ab, GFAP, and IBA-1 to assess structural changes in the hippocampus.

CHAPTER 7: CONCLUSION

In conclusion, the results of the present study indicate that the 2x2 CFC arrangement produces the least variability and average closest to 0.5 in fear-indicative freezing behavior in the Tat transgenic mouse model. Furthermore, no significant differences or interactions were found between genotype [Tat(+), Tat(-)] and sex (female, male), suggesting that multiple-trial paradigms in CFC may be insufficient to assess differences in Tat(+) and Tat(-) mice, as repeated learning may cause Tat(+) subjects to consolidate and retrieve fear-associated memories. Future studies should determine whether one-trial paradigms are more effective than multiple-trial paradigms in assessing differences in Tat transgenic mice, and whether there is a difference in results between contextual and cued fear conditioning when controlled for the number of learning trials. Based on these results, further experiments are required to assess the neurological damage associated with predicted deficits through the quantification of structural proteins, including MAP2ab, IBA-1, and GFAP.

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FIGURES

Figure 1. Experiment 1 CFC behavioral data

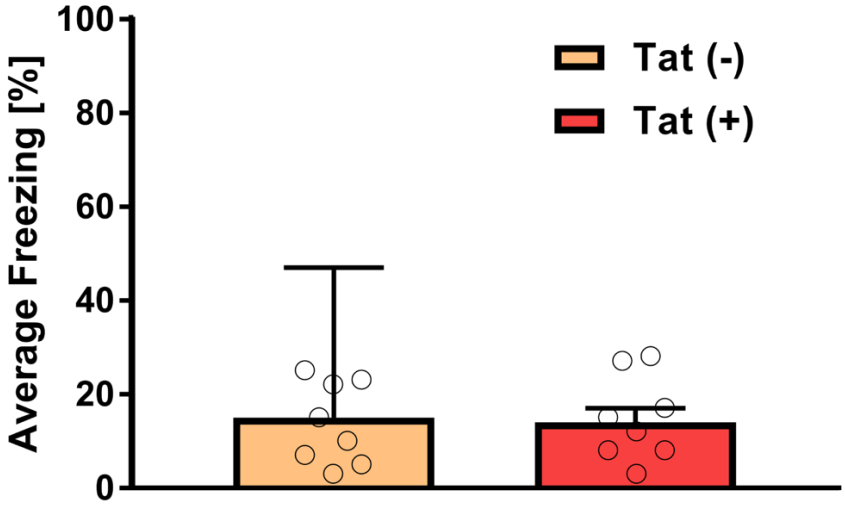

Figure 1. Figure 1 details the results from the CFC pilot study in experiment 1. Data is organized, from left to right, by Tat(-) and Tat(+) male transgenic mice. There was no significant difference between the percentage freezing of Tat(+) and Tat(-) mice using a 2x1 2 s, 0.4 mA foot shock. Tat(-) mice showed high variability in freezing compared to Tat(+) mice.

Figure 2. Experiment 2 shock titration behavioral data

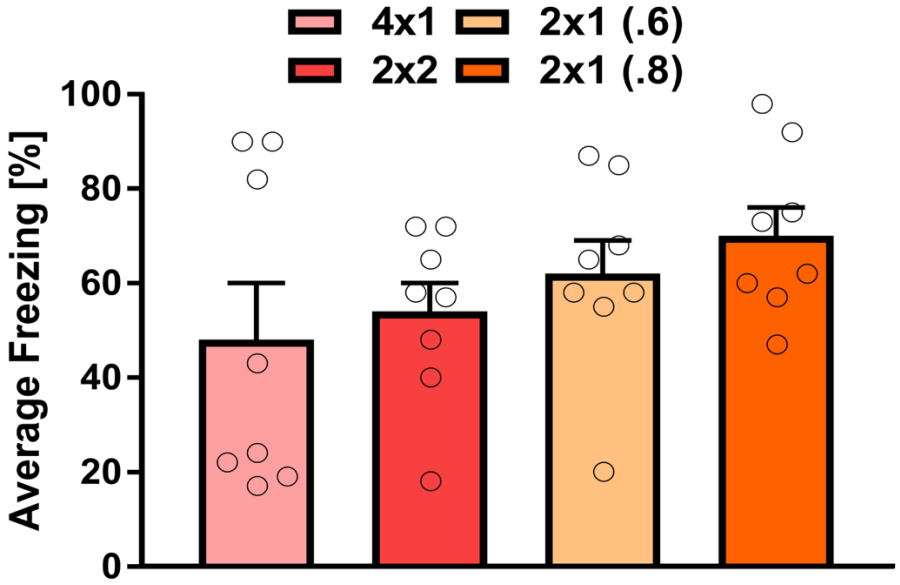

Figure 2. Figure 2 details results from the shock titration in experiment 2. Data is organized, from left to right, as arrangements of 4x1, 2x2, 2x1 (.6), 2x1 (.8) of Tat transgenic mice (not administered DOX). The 4x1 arrangement produced the least average of freezing with the highest variability. The 2x2 arrangement had a freezing average closest to 0.5 and had the least variability out of all arrangements. The 2x1 0.6 mA and 2x1 0.8 mA arrangements produced the highest average freezing, with similar variability.

Figure 3. Experiment 3 2x2 arrangement behavioral data

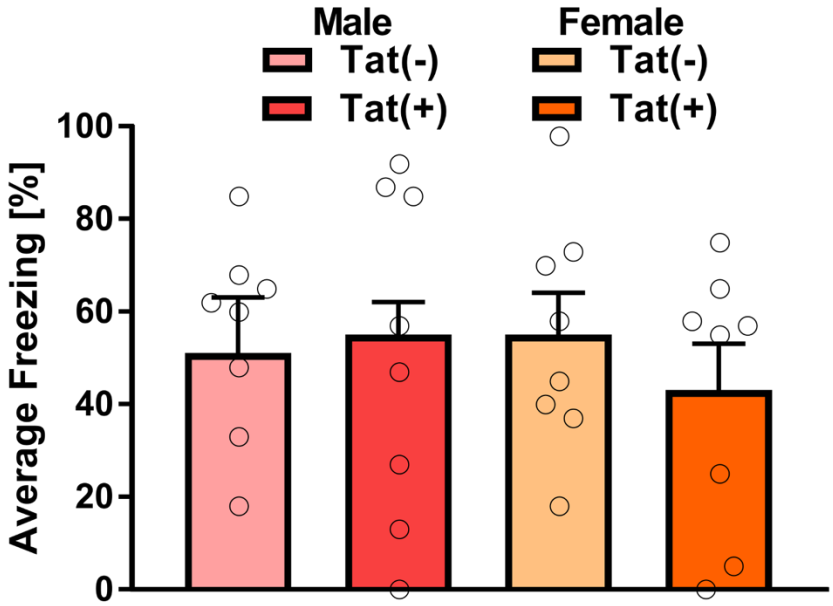

Figure 3. Figure 3 details results from the 2x2 arrangement in experiment 3. Data is organized, from left to right, as male Tat(-), female Tat(-), male Tat(+), and female Tat(+) transgenic mice. Although female Tat(+) mice froze the least, followed by male Tat(-) mice, there were no significant differences or interactions between sex (female, male) or genotype [Tat(-), Tat(+)].