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Social Aggression and Stress-Related Phenotype Formation in the Stress Alternatives Model

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by

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The members of the Honors Thesis Committee appointed

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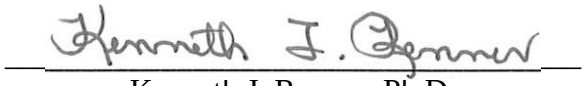

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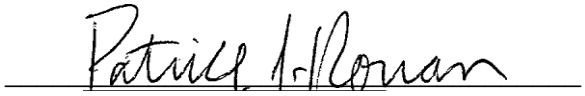

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ABSTRACT

Social Aggression and Stress-Related Phenotype Formation in the Stress Alternatives Model

Tayler L. Modlin

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Stress is a universal reaction. Short-term stress can be viewed as positive, as it can promote survival and encourage positive behaviors; whereas chronic stress that is unpredictable can lead to health defects and emotional pathologies. The Stress Alternatives Model (SAM) was created with the purpose of testing decision-making during socially stressful situations. Over the course of a four-day experiment, test mice are exposed to periods of social stress caused by bites inflicted onto them by a larger aggressive mouse. As a response to these attacks, test mice exhibit an array of behaviors and ultimately develop one of two adaptive phenotypes: Stay or Escape. The adoption of phenotypes results from the test mice having the opportunity to utilize escape holes contained in the SAM apparatus at any point during the experiment. Higher intensity levels of aggression lead to the development of the Stay phenotype. Mice who develop the Escape phenotype demonstrate defensive avoidance behavior, whereas mice who develop the Stay phenotype demonstrate fear-adaptive behavior.

KEYWORDS: Stress, Aggression, Social Interaction, Behavior, Phenotype

TABLE OF CONTENTS

CHAPTER ONE

Introduction

Regulation of homeostasis is critical to survival. Homeostasis maintains balance in physiological and behavioral systems and is constantly being challenged by changes to internal and external environmental conditions. As adaptation to these changes require both additional metabolic, psychological, and behavioral adjustments, they are termed stressors. These stressors can vary in their extent and can be both physical and emotional in nature (Chrousos, 2009). There are many neural, hormonal, physiological, emotional, cognitive, and behavioral responses necessary to adjust to stressors and maintain homeostasis.

Stress has gained a negative reputation, in part, due to its potential effects on health. Stress that is chronic and/or unpredictable can cause adverse health effects, including cardiovascular, metabolic, and immunological diseases (Ebner & Singewald, 2017). On the other hand, short-term stress triggers survival mechanisms in fight or flight situations that can lead to a higher probability of survival (Dhabhar, 2014). Brief episodes of stress can improve memory and serve to motivate positive behavior. For example, an increase in fear, attentiveness, and anxiety when exposed to threatening environments can cause adaptive behavioral responses, such as greater vigilance (McEwen et al., 2012). Due to both the prevalence and variance of stressors and the importance of maintaining homeostasis, there are many diverse reactions to stress, but a common neurocircuitry and a ubiquitous hormonal response. These reactions can be psychological, physiological, and behavioral in nature (Kudielka et al. 2009), and can be positive and help an

individual overcome a situation, or they can be negative and cause further problems for an organism.

Stress and the process of decision-making are highly intertwined. A simple definition of decision-making involves the choice between two or more options. Many decisions must be made under stressful conditions, including how to react appropriately to stressors (Wemm & Wulfert, 2017). Different individuals experience varying reactions to stressful situations. Individuals who are susceptible are not able to adapt appropriately to stressors and are more vulnerable to stress-related pathologies (Ebner & Singewald, 2017). On the other hand, resilient individuals develop responses to stressors that are adaptive in order to maintain normal physiology and emotional state when faced with stressors (Pfau & Russo, 2015). Allostasis is a term used to describe the mechanisms used to conserve homeostasis in the presence of stressors and coincides with resilient phenotypes (Pfau & Russo, 2015). However, even a beneficial response can become exaggerated and/or prolonged; and in this way has the potential to become pathological (Pfau & Russo, 2015).

Chronic stress has been shown to alter regions of the brain used in the decision-making process in mice (Smith et al., 2014), rats (Dias-Ferreira et al., 2009) and humans (McEwen, 2007). Chronic stress causes atrophy of neurons in the prefrontal cortex, hippocampus, and amygdala (McEwen, 2007). These regions of the brain are all involved in stress adaptation (Groeneweg et al., 2011) and also involved in memory, attention, and executive function (hippocampus and prefrontal cortex), as well as fear, anxiety, and aggression (amygdala) (McEwen, 2007). Thus, chronic stress can impair decision making, induce aggressive and anxious behaviors, and cause detrimental health

effects. In contrast, short-term, low-level stress can improve or focus decision-making, and promote anxiolysis, and well-being.

Chronic unpredictable stress as seen in repeated social defeat models is used to examine stress responses in rodents (Pfau & Russo, 2015) and leads to the development of one of two phenotypes: resilient or susceptible, based on whether the rodent develops social avoidance or not (Golden et al., 2011). Social avoidance coexists with adaptive behaviors, whereas the lack of social avoidance is seen as maladaptive. As stress-related behavioral phenotypes are a product of decision-making during a stressful event, our research focuses on distinct behavioral qualities exhibited by resilient and vulnerable subsets of a population.

The Stress Alternatives Model (SAM) was created with the purpose of testing decision making during periods of social stress, as well as alternative response strategies (Carpenter et al., 2009; Robertson et al., 2015; Smith et al., 2014; Staton et al., 2018). Over the course of four days, test mice are repeatedly exposed to social stress from a larger aggressive mouse. Importantly, the aggressive mouse used changes daily, so that test mice are never exposed to an aggressor more than once, to avoid social habituation. Aggression primarily comes in the form of bites, although charging and chasing also occur. Aggressors may direct bites to particular body regions of test mice (back, head, rump, or belly). The apparatus used in the experiment contains two escape holes that are *only* large enough for test mice, preventing the larger aggressor mouse from following. The option to leave presents test mice with a dichotomous choice: utilize the escape holes and evade the stress or remain with the aggressor mouse and submit to the aggression. While the least stressful choice seems obvious, the first entry into the escape tunnel is

stressful, because it is unfamiliar (at least in the first trial). Mice that escape and develop an Escape phenotype, exhibit social preference, as opposed to avoidance, which is evidence of stress resiliency, and therefore considered adaptive behavior (Staton et al., 2018; Yaeger et al., 2018). On the other hand, mice that do not escape display the Stay phenotype. Mice who exhibit the Stay phenotype remain susceptible to aggressive behavior, and subsequently display social avoidance, which is maladaptive (Staton et al., 2018; Yaeger et al., 2018). Importantly, mice choose a phenotype by the end of the second day of SAM exposure and do not deviate from the chosen phenotype for the remaining SAM trials. Using results acquired from experiments using the SAM, I focused on two main objectives: the first aim was to determine how aggressive behaviors in the SAM contribute to stress-related phenotype formation. I hypothesized specifically that the intensity of aggressive behavior would be the catalyst for Phenotype formation. Additionally, I hypothesized that defensive behaviors would be more prominent in Escape interactions. Also, I hypothesized that mice who develop the Stay phenotype would develop fear-adaptive behavioral responses. The second goal was to determine which components of social aggression specifically promote maladaptive behavior.

CHAPTER TWO

Materials and Methods

Experimental Design and Protocol – Stress Alternatives Model

To analyze decision-making in response to stress and anxious behaviors, researchers in Cliff Summers' lab developed the Stress Alternatives Model, or SAM (Carpenter et al., 2009; Smith et al., 2014; Robertson et al., 2015; Summers et al., 2020). In this behavioral paradigm, two mice are allowed to interact for 5 min / day over the course of four days. One of the mice is a significantly smaller test mouse (C57BL/6) and the other is a larger, more aggressive mouse (CD1). During the social interaction, the larger and more aggressive mouse attacks the test mouse, causing social stress. In response to this stress, the test mouse can choose either to stay and remain submissively with the aggressor, susceptible to continued social stress or it can escape, demonstrating an adaptive response, which reduces stress hormone response (Smith et al., 2014), suggesting resilience. This leads to the development of two phenotypes, Stay and Escape. The apparatus utilized on this experiment contains an open field area and two holes meant for escaping. The escape holes lead to an enclosed area and are only large enough for the smaller of the two mice to pass through them, not the larger aggressor mouse. An opaque cylindrical divider is added for undetected insertion of the mice and removed from the apparatus to allow social interaction to begin.

Social interaction occurs over four days, each with a novel CD1 social aggressor. Prior to the beginning the experiment, select mice were trained (N = 25; N = 27 control

mice were not trained). The trained mice were introduced to the escape holes in the absence of the aggressor mouse. At the start of the experiment, the aggressor is placed in the SAM arena, but outside of the opaque cylindrical divider. The test mouse is placed in the SAM arena, but within the divider. During this time, the mice are not allowed to interact. A tone is sounded for 15 seconds, followed by 15 seconds of silence. After the silence, the opaque cylindrical divider is removed, enabling the mice to interact.

The mice are allowed to interact for a maximum of five minutes (300 seconds). This maximum interaction time was calculated to minimize injury to the test mouse (Robertson et al., 2015). Interaction time begins when the cylinder is lifted and ends with either the test mouse escaping, or at the end of the five minutes, whichever comes first. Then both mice are removed from the SAM apparatus after five minutes of interaction. If the test mouse escapes before the five minutes is up, it is left in the enclosed area just on the outside of the escape holes for the remainder of the five minutes. The aggressor mouse is also left in the SAM apparatus for the duration of the five minutes, regardless of whether the test mouse has escaped or not. During the interaction time, the test mouse endures attacks in the form of bites from the larger aggressor mouse, inducing social stress. An attack is confirmed when the CD1 aggressor mouse successfully bites the test mouse (Robertson et al., 2015). Bites can be to multiple areas on the test mouse, including the head, belly, back, and rump.

Mice choose a specific type of adaptive response to social stress, Escape or Stay, resulting in establishment of a conserved behavioral phenotype by the end of the second day SAM social interaction and do not deviate from this phenotype on days 3 and 4. Mice are defined as having developed the escape phenotype if they escape on days 1 or 2

of the experiment. Videos (recorded using a GoPro Hero 4) during SAM experiments were scored for specific behaviors using several criteria. Allowing for one mouse that died after day 1 of the experiment (N = 52 mice X 4 days of social interaction), 205 total interactions were scored. Aggressive attacks consisted primarily of bites, classified by their region: head, belly, rump, or back of test mice. We made note of the behaviors of the test mouse in response to these attacks. Interaction time begins when the cylinder that separates the two mice is lifted and ends when either the test mouse escapes, or when the end of the experiment is reached and the mouse is removed from the SAM arena (Summers et al., 2020). During the SAM social interaction, the following parameters were measured: latency to the first attack, latency to escape, number of bites, location of bites, number of fights interrupted, and several behaviors demonstrated by test mice such as turning away from/toward the aggressor, flight, startle, boxing, and jumping.

Statistical Analyses

Escape and Stay mice spend significantly different amounts of time in the SAM arena; with Escape mice averaging 46 s before escaping, whereas mice that stayed spent 300 s in the SAM arena. As Stay mice are in the SAM arena for significantly longer than escape mice, there is more time for behaviors to occur. The data were normalized by dividing the frequency of each behavior by the time that the mouse spent in the SAM arena (in seconds). The data are reported as times each behavior occurred per second.

Average behavior per second for Escape and Stay phenotypes, were analyzed by means of two-tailed Student's t-tests. The null hypothesis was that there is not a significant difference between behaviors scored in Escape interactions and Stay

interactions. The alternative hypothesis was that the results obtained were specifically due to the development of Stay and Escape Phenotypes, and the alteration in social interaction that the establishment of behavioral phenotypes incurred.

CHAPTER THREE

Results

Adoption of Behavioral Phenotype: Escape and Stay

Out of the total number of social interactions, 106 resulted in the test mouse staying throughout the entirety of the interaction; and those test mice are referred to as having developed the Stay Phenotype. In contrast, 99 interactions resulted in the test mouse escaping before five minutes were over; these mice have developed an Escape Phenotype. The interactions were split evenly between the two outcomes, with 51.71% (106 out of 205) of interactions leading to the test mouse staying and 48.29% (99 out of 205) of interactions ending with the test mouse escaping. This is consistent with previous research (Robertson et al., 2015; Summers et al., 2020).

Bites

The main source of social aggression in this experiment was bites inflicted on the test mouse by the larger aggressor mouse (Summers et al., 2020). The average number of total bites per second was 0.14 ± 0.002, or 1 bite every 7.14 s directed toward Escaping mice; and in 106 Stay interactions, the mean was 0.075 ± 0.006 total bites/s, or 1 bite approximately every 13.3 seconds directed towards Stay mice (Fig. 1). This difference in the amount of total bites per second in Stay and Escape interactions is significant ($t = 2.76$, $P \leq 0.0063$). Mice that developed the Escape phenotype suffered significantly more bites per second than did mice who developed the Stay phenotype.

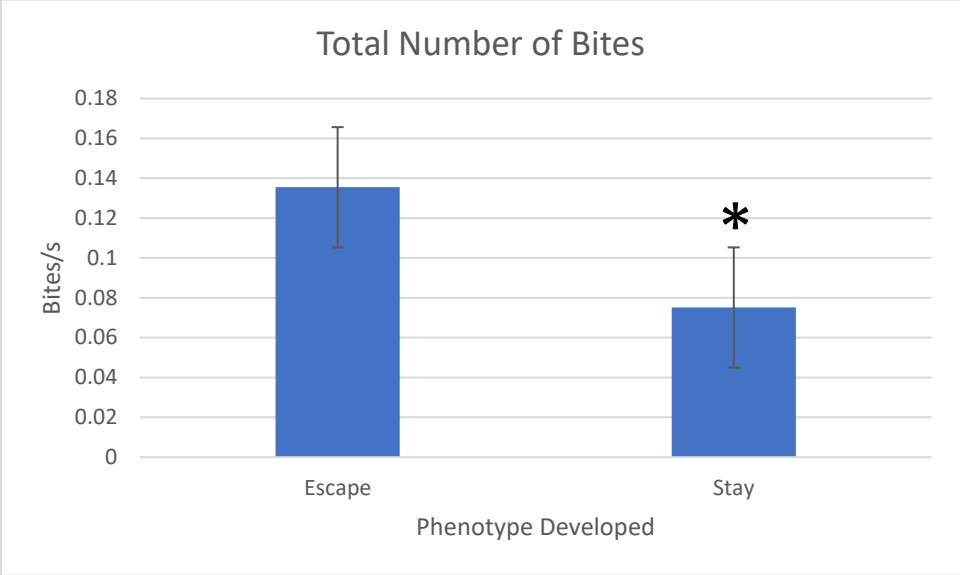

Figure 1. Mean number of total bites inflicted onto the test mouse/s spent in SAM arena in Stay and Escape interactions. Test mice that develop the Escape phenotype experience significantly more bites per second than test mice that develop the Stay phenotype ($t = 2.76$, $P \leq 0.0063$).

The severity and intensity of bites can be determined from their location. From a total of 2,752 bites received by test mice the most common bite location was to the back, which consisted of 1,948 out of 2,752 bites, or 70.78% of bites. The second most common location of bites was to the rump, constituting 661 or 24.02% of bites. A rarer bite location, with 91 instances, or 3.31% of bites, were to the head. This rare bite location obviously has a higher level of intensity. Bites to the belly of the test mouse were the least common, which consisted of 52 out of 2,752 bites, or 1.89% of bites. Bites to the back, the most common biting location, were not significantly different between Stay and Escape mice ($t = 1.43$, $P \geq 0.15$), with a mean 0.054 ± 0.005 bites/s for Stay 0.078 ± 0.02 bites/s for Escape interactions (Fig. 2A). Test mice also received bites to

the head (Fig. 2B), although not very frequently, the rate of biting was significantly greater (t = -1.93, $P \leq 0.05$) for Stay mice, 0.0027 ± 0.0004 bites/s, compared to Escape mice, 0.001 ± 0.0008 bites/s. The mean number of bites to the belly (Fig. 2C) for Stay interactions was 0.0014 ± 0.001 bites/s, and 0.0065 ± 0.006 bites/s for Escape interactions, and not statistically significant (t = 1.43, $P \geq 0.15$). In the second most common attack (Fig. 2D), there was significantly more (t = 3.35, $P \leq 0.00095$) bites to the rump, received by Escape mice, (an average of 0.05 ± 0.01 bites/s) when compared to Stay mice (0.017 ± 0.002 bites/s).

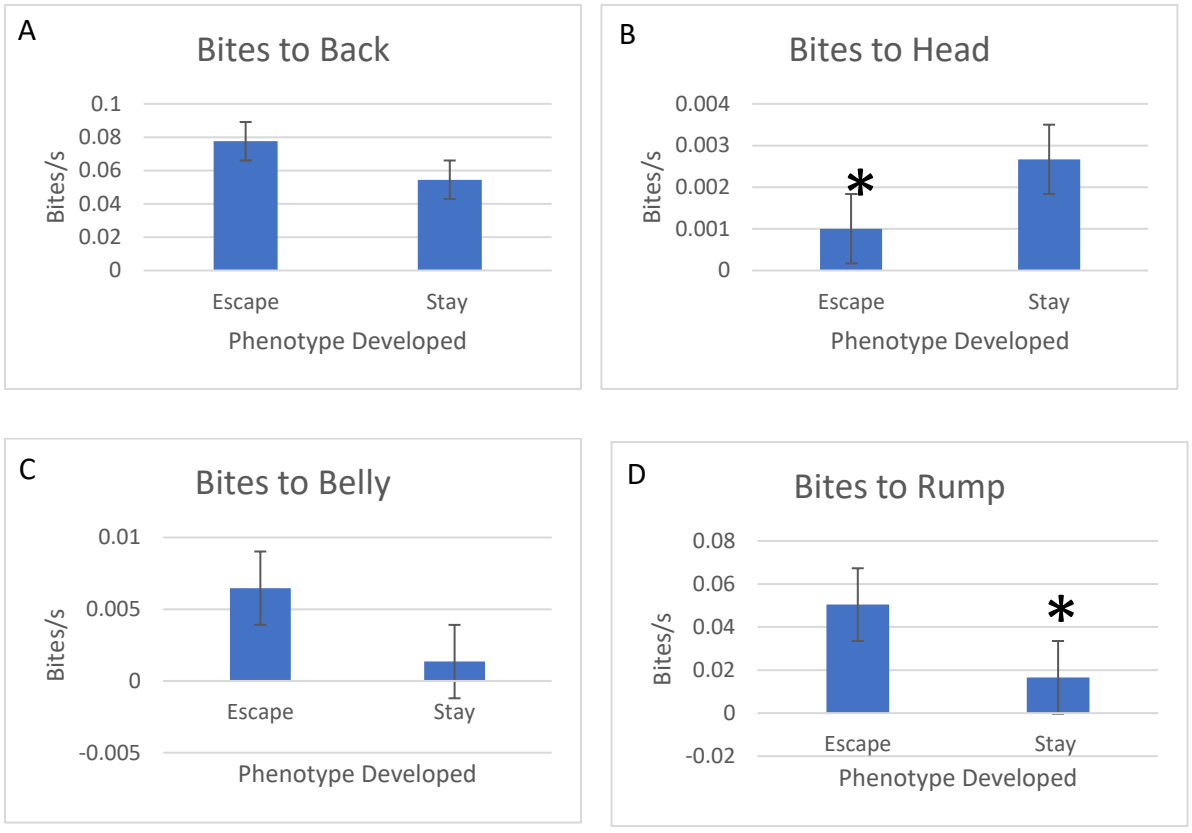

Figure 2. Mean number of bites/s by location: back - A, head - B, belly - C, and rump - D. **A)** There is no significant difference between Escape and Stay Phenotypes in the mean number of bites to the back/s (t = 1.43, $P \geq 0.15$). **B)** The average number of bites to the head of the test mouse per

second is greater in Stay interactions compared to Escape interactions (t = -1.93, $P \leq 0.05$). **C)** There is no significant difference in the average number of bites to the belly per second spent in SAM arena between Stay and Escape Phenotypes (t = 1.43, $P \geq 0.15$). **D)** Bites to the rump of the test mouse were more common in Escape interactions than Stay interactions (t = 3.35, $P \leq 0.001$).

There were a handful of interactions that did not include any attacks. Out of the 205 interactions, 51 of them did not include any attacks (24.88%). Out of the 51 interactions with no attacks, 42 test mice chose the Escape phenotype, whereas only nine chose the Stay phenotype. Out of the 51 no-attack scenarios, 39 of these occurred in Escape mice that had received training prior to the experiment.

Latency to Attack

Latency to attack, is a reliable indicator of motivation for the aggressor (Korzan et al., 2007), which may also be influenced by the behavioral signals of the individual being attacked. Therefore, social stress related phenotypes, like Stay and Escape, may influence latency to attack. For escaping mice being attacked, there was an average of 18 ± 3.6 s between the start of the interaction (removal of the opaque cylinder) and the time of the first attack. This latency was significantly different (t = 2.08, $P \leq 0.04$) for interactions in which the test mouse did not escape (Stay), which averaged 31 ± 4.6 s passed between the cylinder being lifted and the first attack (Fig. 11). This indicates that the aggressive attacks begin sooner in Escape interactions and take longer to occur in Stay interactions.

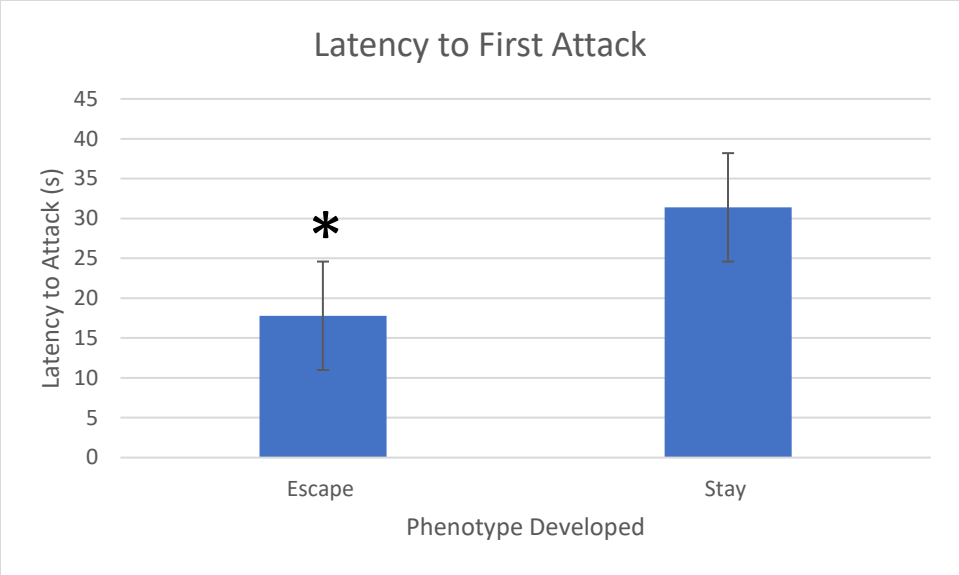

Figure 3. Latency to First Attack was higher (t = 2.45, $P \leq 0.02$) in Stay interactions compared to Escape interactions.

Latency to Escape

For mice that escaped, an average of 45.85 s elapsed following initiation of the interaction to time of escape. This latency was shown to decrease over the course of the four-day experiment. On day 1, test mice took an average of 67.54 ± 16.2 s from the time in which the cylinder was lifted until they escaped. On day 2 of the experiment, test mice took an average of 63.87 ± 17.8 s to escape. On days 3 and 4, the average time to escape decreased significantly (F = 2.99, $P \leq 0.03$), with test mice taking 22.08 ± 6.2 s and 29.92 ± 10.8 s to escape, respectively (Fig. 12). Escape mice often chose to escape quickly after receiving bites, as the average time to escape from the time of the first biting attack was 47 s.

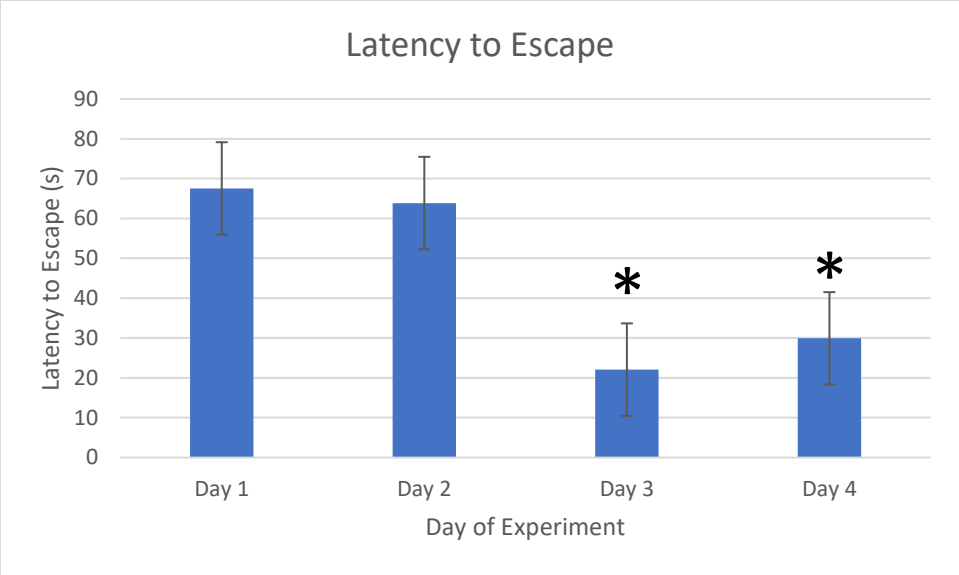

Figure 4. Time to Escape stayed consistent on Days 1 and 2 of the experiment and decreased over Days 3 and 4 ($F = 2.99$, $P \leq 0.03$).

Previous research has indicated that mice pick their phenotype on the first day of the experiment (Staton et al., 2018). This was consistent with these findings, as only five mice changed whether they stayed or escaped in the duration of the experiment. Only three mice changed their behavior after day 1 of the experiment; one mouse stayed on day 1 but escaped on days 2-4 and two mice escaped on day 1 but stayed on days 2-4.

Interruption of Social Interaction

Aggressive social interaction of exceptional intensity between two mice were interrupted, especially if there was potential physical damage. Situations in which the mice were interrupted included when the test mouse received repeated bites to the head and neck region, or a substantial amount of bites in a certain time period (Staton et al., 2018). Therefore, the number of times in which the interaction was interrupted is

indicative of intense aggression. For Escape mice, there were an average of 0.0011 ± 0.0005 fights interrupted/s of interaction. For Stay mice, there were significantly more (t = -6.03 $P < 0.0001$) fights interrupted; an average of 0.0088 ± 0.001 fights broken up per second, eight times as many (Fig. 4).

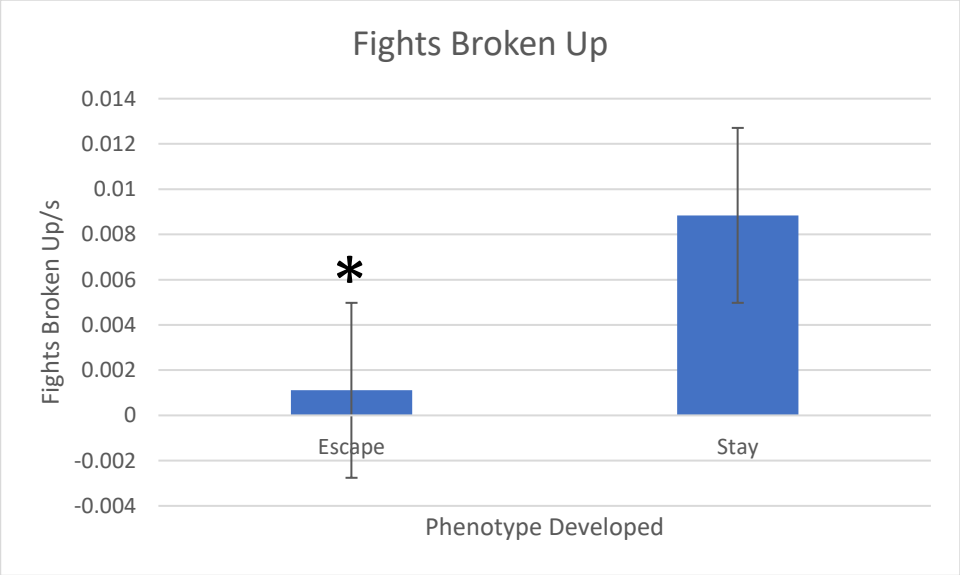

Figure 5. Mean number of aggressive interactions (biting attacks) between the test mouse and aggressor mouse interrupted. Significantly more (t = -6.03, $P < 0.0001$) social interactions between CD1 and Stay mice needed interruption.

Avoiding the Aggressor

As a response to aggression, some test mice turned away from the aggressor mouse. In Escape interactions, the average number of test mice turning away from the aggressor per second of interaction was 0.028 ± 0.007, significantly more (t = 2.11, $P \leq 0.036$) than the average for Stay interactions, which had a mean of 0.014 ± 0.002 episodes/s spent in the SAM arena (Fig. 5). Mice turn away from their aggressor more in situations in which

they develop the Escape phenotype (Fig. 4). Turning away from the aggressor mouse often occurs immediately preceding flight behavior (running away from the aggressor), so it is appropriate that it would occur more frequently in Escape interactions, where flight behavior occurs more ($t = 4.19$ $P < 0.0001$).

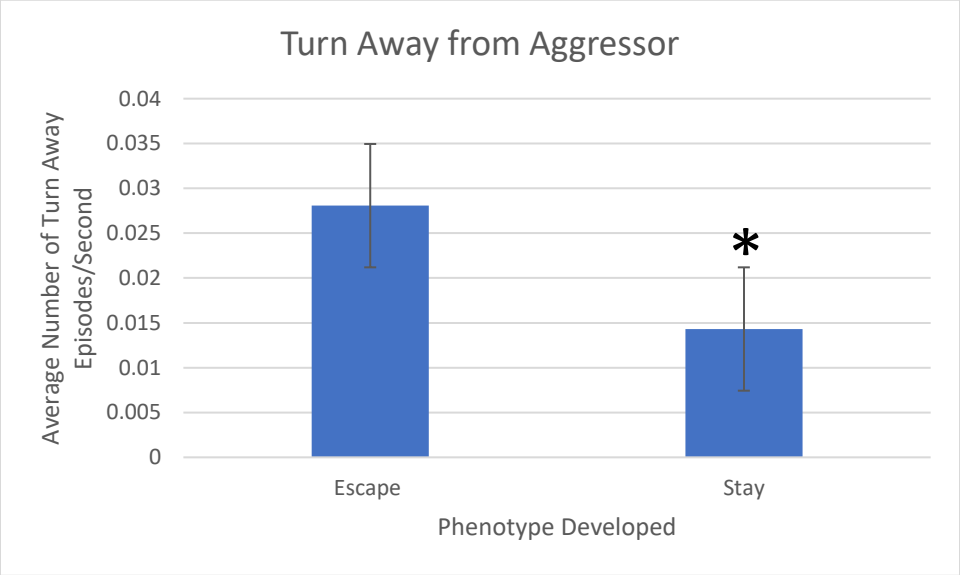

Figure 6. Mean number of times that the test mouse turned away from the aggressor in response to a bite was higher in Escape interactions compared to Stay interactions ($t = 2.11$ $P < 0.0001$).

Test mice often ran away from the aggressor mouse, both in response to a bite and in the absence of one. This demonstrates that bites are not the sole stimulus that provoke test mice. Chasing and approaching often have the same effect as a bite if the test mouse has been bitten before. In some instances, test mice ran away from the aggressor and directly to an escape hole. In Escape interactions, test mice ran away from the aggressor an average of 0.047 ± 0.008 times/s, while surprisingly, running away from the aggressor

occurred significantly (t = 4.19, $P < 0.0001$) less in Stay interactions; an average of 0.013 ± 0.001 times/s (Fig. 6).

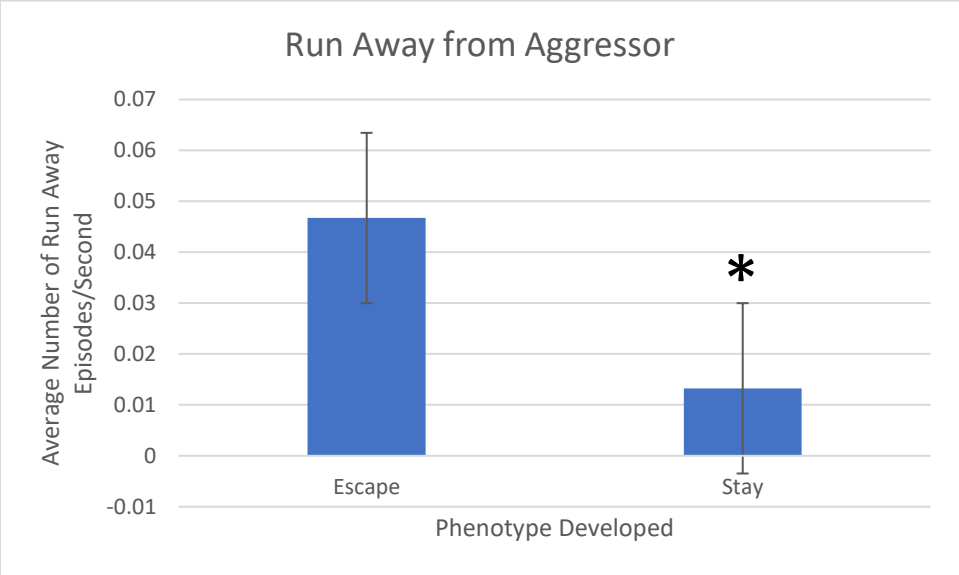

Figure 7. Test mice ran away from the aggressor mouse more often in interactions which they develop the Escape phenotype compared to the Stay phenotype (t = 4.19, $P < 0.0001$).

As an opposite response to aggression (bites), test mice sometimes turned towards the aggressor mouse. This behavior was not significantly different by phenotype (t = 0.37, $P \geq 0.71$) and occurred an average of 0.0047 ± 0.002 times per second in Escape interactions and 0.0039 ± 0.0006 times per second in Stay interactions (Fig. 7).

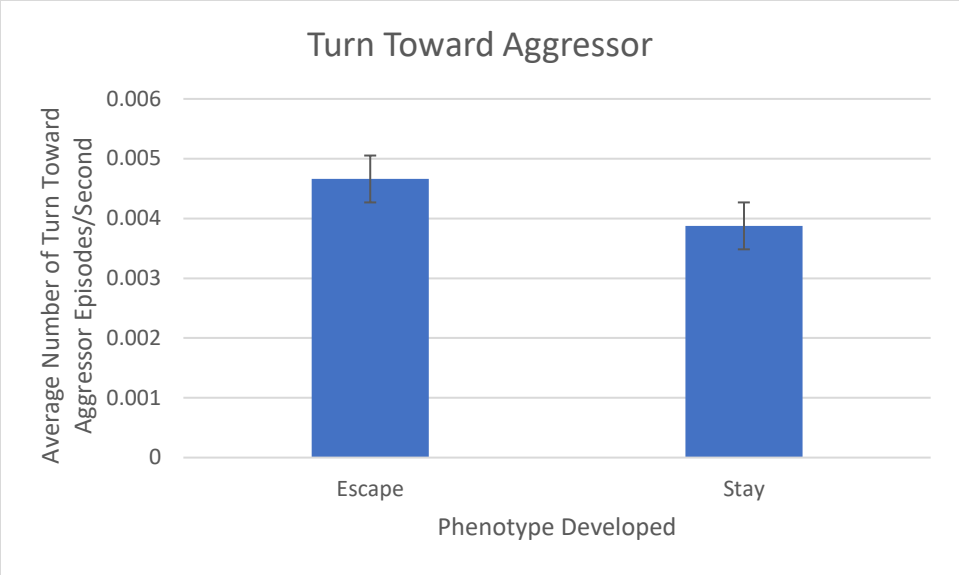

Figure 8. The average amount of turning toward the aggressor episodes per second compared in Escape and Stay interactions; not a significant difference (t = 0.37, $P \geq 0.71$).

Jumping (all four legs are off of the ground at once) occurs as an alternate avoidance behavior (Huang & Wajda, 1975) and (Ryan et al., 2010). In Escape interactions, there were an average of 0.020 ± 0.005 jumps/s; significantly (t = -10.87, $P < 0.0001$) lower than the average for Stay interactions, which was 0.16 ± 0.01 jumps/s (Fig. 8).

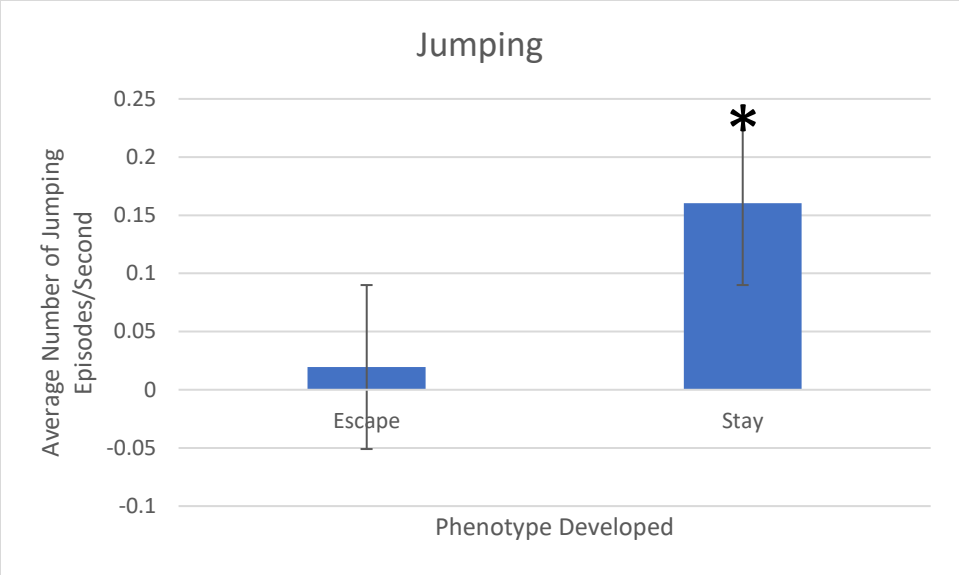

Figure 9. The average number of jumping episodes per second was significantly higher in Stay interactions compared to Escape interactions (t = -10.87, $P < 0.0001$).

Startle

The startle reflex is common to all vertebrates, including humans, and perhaps all animals. The startle behavior seen in test mice is an involuntary, whole-body flinch (Risbrough et al., 2004) and occurs in response to an abrupt, intense stimulus, such as bites (Moberg & Curtin, 2009) but also to more benign but abrupt environmental stimuli, such as sound (Davis et al., 1997) or the appearance of the shadow of a flying predator suddenly appearing. Corticotropin-releasing hormone is involved in the stress response (Orth, 1992) and has been shown to heighten startle response when administered to rats (Moberg & Curtin, 2009; Davis et al., 1997). Thus, the startle reflex is prominent during stressful conditions. It is categorized as an anxious behavior (Davis et al., 1993) and can be modulated by fear and stress (Sallinen et al., 1998). There are different types of stimuli that cause a startle response (Sallinen et al., 1998). The startle reflex is also

categorized as a defensive behavior that can be used to protect the body from impact during an attack (Risbrough et al., 2004). Hits to the head, neck, and upper body produce acoustic, vestibular, and tactile stimuli which elicit the startle response (Yeomans et al., 2002). Besides simply serving as a protecting mechanism, the startle reflex can trigger other behavioral responses necessary to escape further attacks (Yeomans et al., 2002). As previously mentioned, there are considerably more bites in Escape interactions than Stay interactions ($P \leq 0.0063$). As startle behavior occurs in response to a strong stimulus (e.g., bites) and serves as a method of protection, it is plausible that it would occur more frequently in situations with increased biting attacks (Escape interactions) as test mice have a heightened need for protection in situations with more aggression. Escape mice were startled an average of 0.033 ± 0.006 times/s of interaction; not significantly different ($t = 0.37$, $P \geq 0.72$) from Stay mice, which were startled 0.031 ± 0.003 times/s (Fig. 9).

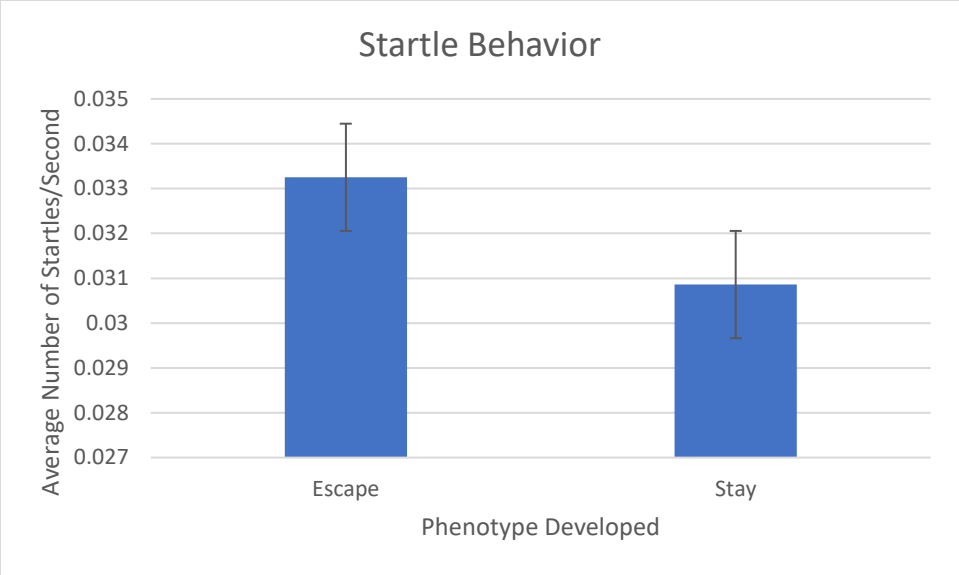

Figure 10. The difference in the average number of Startles in Escape and Stay interactions was not significant (t = 0.37, $P \geq 0.72$).

Boxing

Boxing is a defensive posture, defined as rearing upright and extending forepaws and is assumed by a subordinate mouse (Scott, 1966). Boxing is an aggressive behavior (Ragnauth et al., 2005) and (Couppis & Kennedy, 2008) and can be used for defense avoidance (Wersinger et al., 2007). Test mice demonstrated the boxing behavior an average of 0.010 ± 0.01 times/s in Escape interactions, not significantly different (t = 0.66, $P \geq 0.51$) compared to an average of 0.0073 ± 0.007 times per second in Stay interactions (Fig. 9).

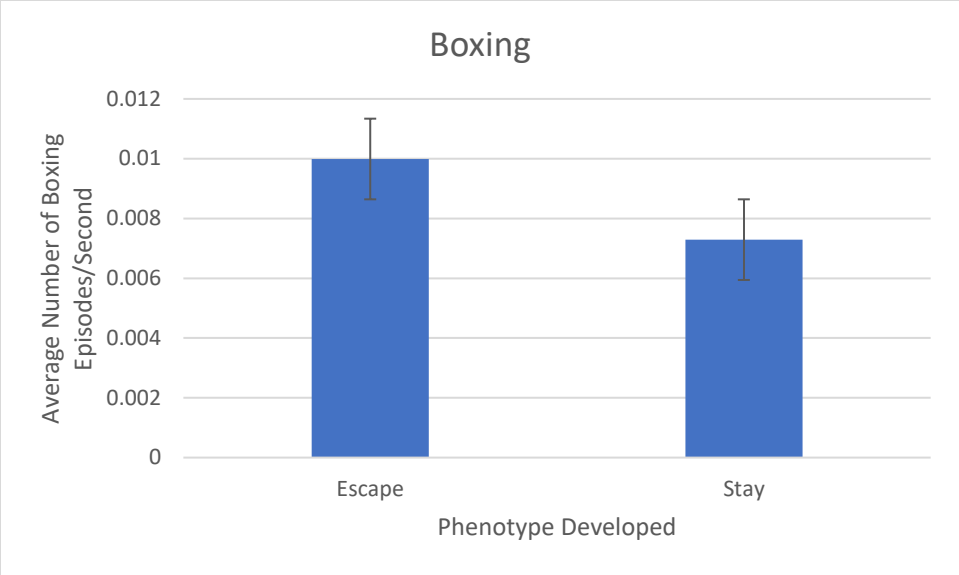

Figure 11. We could not conclude that there was a significant difference in average number of boxing episodes per second in Escape and Stay interactions (t = 0.66, $P \geq 0.51$).

Effects of Training

As previously mentioned, 25 test mice were trained before the start of the experiment and 27 test mice were not trained. In training, the test mice were introduced to the escape holes with no aggressor mouse present. Not surprisingly, 21 out of the 25 (84%) trained test mice chose to escape on the first day of the experiment. All but three of those mice also chose to escape on the following days of the experiment as well: one mouse stayed on day two and returned to escaping on days three and four and the other two mice escaped on the first day but stayed on days two, three, and four. Out of the 27 mice that did not receive training, 21 (or 77.78%) stayed on every day of the experiment. Out of these mice, one chose to stay on the first day, but escaped on the remaining days, and one mouse escape on days one, three, and four, but stayed on day two of the experiment.

Mice that did not receive any prior training stayed more often than escaping, whereas mice that did receive the training escaped more than they stayed.

CHAPTER FOUR

Discussion and Conclusion

Discussion

In interactions which lead to the test mice developing the Escape phenotype, we observed more total bites/s. As for specific location of bites, Escape interactions had more bites to rump/s and Stay interactions had more bites to head/s. These bites to the head are exceptionally intense for the test mice. Also indicative of intense aggression was the number of interactions interrupted/s, which was significantly higher in Stay interactions. In Escape interactions, test mice engaged in defensive avoidance behaviors such as turning away and running away from the aggressor more than in Stay interactions. Jumping behavior, which shows abnormal psychology, was observed more in Stay interactions.

The intensity of aggressive behavior influences Phenotype development. Test mice in Escape interactions received more bites/s when compared to test mice in Stay interactions. Latency to first attack was significantly shorter in Escape interactions compared to Stay interactions. Based on these findings, it seems plausible that increased amounts of aggression lead to the development of the Escape phenotype; however, it seems also that the intensity of the aggression is equally influential on the development of phenotypes. Exceptionally intense bouts of aggression lead to the development of the Stay phenotype. Although there is higher quantity of aggression in Escape interactions, the aggression observed in Stay interactions was especially intense. Interactions between

the aggressor mouse and test mouse were interrupted when the life of the test mouse was threatened. It follows that increased amounts of social interactions interruptions demonstrate intense aggression. The number of interaction interruptions/s was significantly higher in Stay interactions compared to Escape. Bites to the head of the test mouse are considered to be exceptionally intense and are significantly more common in Stay interactions compared to Escape interactions. These data show that increased intensity of aggression leads to the development of Stay phenotype.

Fleeing is a defensive behavior (Eilam, 2005) and helps the mouse to remove itself from a predator (Eilam, 2005). Along with boxing, it can also be categorized as a defensive avoidance behavior (Wersinger et al., 2007). This behavior is more prominent in Escape interactions compared to Stay interactions. Along with running away from the aggressor, turning away from the aggressor was also observed more frequently in Escape interactions as well. These behaviors are categorized as defensive and more commonly seen when the test mouse develops the Escape phenotype.

Jumping is classified as a stereotypic behavior, meaning it is an abnormal behavior that does not have an apparent function (Garner et al.). It is a consequence of being placed into an abnormal environment, and tends to occur in repetitive bouts (Garner, et al.). Repetitive behaviors can be critical for survival and normal functioning in certain animals, but some repetitive behaviors are considered to be abnormal (e.g., jumping in mice) (Langen et al., 2011). Abnormal repetitive behaviors can be a consequence of dangerous environmental circumstances, especially confinement (Langen et al., 2011). Stress is also a risk factor for developing abnormal repetitive conditions (Langen et al.,

2011). In some neuropsychiatric disorders, abnormal repetitive behaviors (e.g., jumping) are prominent (Langen et al., 2011).

Mice who develop the Stay phenotype participate in jumping behavior remarkably more than mice who develop the Escape phenotype. Jumping behavior is seen in abnormal and adverse environments and is not considered to be an adaptive coping mechanism (Langen et al., 2011). For this reason, it follows that this fear-adaptive behavior is observed more in situations in which the test mice develop the Stay phenotype.

Conclusions

Phenotype formation in the Stress Alternatives Model is comparable to the stress response in humans. Humans can be thought to develop either resilient or susceptible phenotypes as a reaction to stress. Short-term stress can increase survival through activation of fight or flight mechanisms and can increase vigilance and positive, defensive behaviors. When exposed to brief period of stress, resilient individuals develop adaptive behavioral responses which allow them to maintain homeostasis and a normal emotional state during adverse conditions. This is demonstrated by Escape mice, as these mice experience a greater number of bites/s and receive attacks quicker when compared with Stay mice; however, they choose to adapt to the situation by utilizing the escape routes provided and remove themselves from the aggressive environment. They also demonstrate defensive behaviors, such as fleeing and turning away from the aggressor in response to an attack, more often than Stay mice. On the other hand, chronic and/or unpredictable stress leads to adverse health conditions and leaves individuals more vulnerable to stress-related pathologies, such as depression and anxiety disorders.

Chronic stress damages areas of the brain that are involved memory, attention, and executive function (hippocampus and prefrontal cortex) along with fear, anxiety, and aggression (amygdala). Mice who choose to remain in the SAM arena during the experiment remain exposed to chronic stress and are comparable to susceptible individuals. These mice are also exposed to more intense stress than Escape mice, as the number of bites to the head/s and number of interactions interrupted/s are both higher in Escape interactions compared to Stay interactions. This long-term and intense stress leaves the test mice vulnerable to developing emotional pathologies, similar to anxiety and depression in humans. Stay mice demonstrate jumping, an abnormal behavior, significantly more than Escape mice.

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