EVALUATION OF INNATE ANXIETY IN TA1TUBULIN-CRE/IKBKAP$^{-/-}$ MICE: THE EFFECTS OF THE IKAP PROTEIN DELETION FROM THE CENTRAL NERVOUS SYSTEM

by

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DEDICATION

This paper is dedicated to Dr. Babcock and Dr. Wieseler who have spent countless hours mentoring and training me in many of the techniques used in behavioral neuroscience research while encouraging my independence and curiosity. It is through their encouragement that this current paradigm was proposed and later validated.

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ABSTRACT

Familial Dysautonomia (FD) is a hereditary sensory and autonomic neuropathy (Type III) marked by a mutation within the *Ikbkap* gene encoding the IKAP protein. This mutation is prevalent in 99% of the clinical FD population (Shobhat & Halpern, 2010). Symptoms include emotional labiality, cardiovascular instability, vomiting crises and decreased pain and temperature sensation. One clinical symptom associated with FD is increased anxiety in response to stressful situations (Axelrod, 2006). Dr. Lefcort in the department of Biology and Neuroscience at Montana State University has generated a novel mouse model of FD in which *Ikbkap* is selectively deleted from CNS neurons. The present study characterized the expression of anxiety behaviors in this mouse model using a standard elevated plus maze task. It was observed that FD mice spent significantly more time in the open arms relative to control mice. These mice exhibited significantly greater instances of unprotected head-dipping and fewer protected head-dipping compared to controls. The FD mice also traveled slower than controls but time immobile and distances traveled were found to be similar. These data suggest that the FD mice presented as less anxious, an observation that is inconsistent from observations in the clinical population. Additional research aimed at characterizing the behavioral phenotype of these mice is under investigation.

INTRODUCTION

Familial dysautonomia (FD) is a neurodegenerative, autosomal recessive, hereditary sensory autonomic neuropathy Type III (HSAN-III) disorder that effects development. The specific mutation, implicated in 99% of cases, occurs as a result of incorrect splicing at intron 20 on the *Ikbkap* gene, which is responsible for encoding the IKAP protein (Axelrod, 2010; Shobhat & Halpern, 2010). This error occurs as a result of the insertion of a C instead of a T base pair, causing the improper splicing and removal of exon 20 from the sequence (Slaugenhaupt & Gusella, 2002). This splicing error on the *Ikbkap* gene leads to a dysfunction of the IKAP protein. Because IKAP is present throughout the central and peripheral nervous systems, a mutation can impact many important cellular processes. Mezey et al. (2003) reported that the specific IKAP mutation found in approximately 99% of clinical patients resulted in decreased splicing efficiency, but these cells still retained some capacity for producing normal IKAP protein.

Patients with this mutation can experience what is known as a “dysautonomic crisis”, which is characterized by extreme changes in blood pressure and heart rate, dramatic personality changes, and shutdown of the digestive system (The Dysautonomia Foundation). In addition to autonomic effects, it has been reported that some patients suffer emotional distress characterized by increased general and social anxiety (Axelrod, 2009; Mezey et al., 2003). Although HSAN type III is extremely rare, it is most prevalent within people of Ashkenazi Jewish descent. Approximately 1:32 people of Ashkenazi Jewish descent are carriers for the mutation responsible for familial

dysautonomia and increases to 1:18 in Ashkenazi Jewish individuals with full Polish descent (Shobhat & Halpern, 2010). Due to the progressive neurodegenerative nature of this disorder, treatment is necessary in order to increase life expectancy. A longitudinal study by Axelrod et al. (2002) revealed that the age of entry into a clinical treatment program was a predictive factor in patient outcome; life expectancy decreasing by 3% for each year treatment was delayed. Due to the wide variety of symptomology, there are many reactionary treatments that are used to maintain homeostasis and prevent crisis. Gastrointestinal issues can be treated with various feeding techniques to reduce the possibility of aspirating food while vomiting crisis in response to physical or emotional stress can be treated through the administration of diazepam and chloral hydrate (Axelrod, 1997). In addition, respiratory symptoms can be somewhat alleviated through the use of oxygen tanks and posturing to prevent breath-holding (Axelrod, 1997). Issues of hypotension are most often treated with exercises to aid blood flow and maintaining proper hydration while spinal curvature can be treated using braces (Axelrod, 1997). A major issue with the decreased pain sensitization is the inability to recognize injuries including bone fractures. This is treated primarily by regular check-ups and monitoring (Axelrod, 1997). With so many of the treatments focusing on preventative lifestyle changes, it is necessary to understand exactly how the dysregulation of IKAP influences the symptomology.

To understand the role of the IKAP protein within the central nervous system (CNS), a novel mouse model was generated by the Dr. Frances Lefcort (Montana State University- Bozeman) in which the IKAP protein was selectively deleted from the CNS.

The model, Ta1tubulin-cre/Ikbkap$^{-/-}$, was developed in the course of creating a related lethal model in which the IKAP protein was deleted from the peripheral nervous system (George et al., 2013). The mice used in the current study were similar to George et al. (2013) where homozygous conditional knockout mice were bred with hemizygous mice that contained one allele for the deletion of Ikbkap within the central nervous system. Littermates were used as controls for the offspring that presented as homozygous for the Ikbkap knockout, following genotyping using PCR analysis. The advantage of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice is that they mature and survive to an age where they can be behaviorally evaluated. These mice are characterized by a smaller stature, high prevalence of scoliosis, and decreased sensitization to pain stimuli (unpublished observation). The behavioral phenotype of these mice has not been extensively studied. Due to the presence of anxiety within the clinical population of FD patients (Axelrod, 2009; Mezey et al., 2003), the present series of experiments focused on evaluating alterations in emotional responses to environmental stressors.

There are several behavioral paradigms that are used to evaluate fear and anxiety in rodent models. Van Gaalen & Steckler (2000) describes a conditioned fear paradigm in which mice are placed into a cage with a floor that could be electrified. On the first day, a flashing light stimulus presented every 100 sec is immediately followed by three successive electrical foot shocks. On the second day, the same procedure is used and the light flashes; however, during this trial no shock follows the flashing light and freezing behaviors in response to the light stimulus are observed. The reduced pain sensitization of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice used in the present experiment are of particular

concern as it eliminates the utility of established fear and anxiety paradigms that utilize electric shock stimuli. The elevated plus maze is another paradigm used to measure behaviors related to anxiety (Walf & Frye, 2007). The elevated plus maze is made up of four arms, two open arms that do not have walls located across from one another and two closed arms with walls. The apparatus is elevated off of the floor such that mice cannot discern where the floor is in order to prevent voluntary jumping from the apparatus. Although the elevated plus maze can be used to measure state dependent anxiety or fear, it is still widely used to measure innate anxiety (Muigg et al., 2009; Rodgers & Cole, 1993). Innate anxiety could be considered a base-line measurement of anxiety. Although the open arms of the elevated plus maze might be considered an anxiety inducing location, the willingness of the animal to explore these areas when there is no immediate threat (such as a noxious smell or sound) is indicative of anxiety level. Because innate anxiety is a unique measurement in that it requires no outside force to alter anxiety levels; this concept is used to describe the anxiety in animals in a non-fear-driven environment.

In the elevated plus maze, measurements indicative of anxiety include the proportion of time spent within the open arms and the frequency of exploratory behaviors such as head dipping, stretch-attends, and rearing (Rodgers & Cole, 1993). These behaviors are important in understanding the anxiety levels and exploration of the apparatus. Head dipping is an exploratory behavior that can be used to evaluate the amount of exploration from the closed and open arms. The location that head dipping behavior occurs in the apparatus will characterize the type of exploration being

performed. Head dips originating within a closed arm or the central platform are behaviorally distinct from head dips originating from an open arm. In the case of head dips from an open arm, the animal is engaging in an exploratory behavior while being unprotected on all sides and is at a higher risk of predation in a natural setting. Stretch-attend behavior, where the mouse extends the body to its full length and retracts back to the original position, is a behavior that allows the animal the safety of being able to retract back to a safe position. Rodgers & Dalvi (1997) suggest that the proportion of time in open arms is associated with reduced anxiety while the total number of stretch-attends related to risk assessment and total head dips related to exploration.

Specific brain regions have been implicated in the expression of anxiety behaviors. Muigg et al. (2009) selectively bred mice to produce high, normal, and low expression of anxiety. Greater c-fos expression was observed within the amygdala and hippocampus regions of high anxiety mice indicative of elevated activation within these regions. More recently the overexpression of corticotropin releasing factor (CRF) has been implicated in anxiety disorders. Flandreau et al. (2012) reported increased behaviors associated with anxiety when CRF was made to overexpress in the hypothalamus and central amygdala. In addition to this, Bruchas et al. (2009) reported increased anxiety with the administration of CRF into the basolateral amygdala of mice. The CRF was used as an anxiogenic—anxiety inducing—drug. Mice in this study exhibited a decreased percent of time within the open arm of the elevated plus maze following CRF administration supporting a role for the amygdala in the expression of anxious behaviors.

The field of optogenetics is a fairly new one and involves the use of light stimuli to create activation or inhibition in specific cell types in the brain. Specific cell types can be targeted using a virus and can be made to become light sensitive. This light is transduced via optic fibers and implanted within the brain alongside electrodes which are used to measure the scope or range of activation produced by this light stimulus. Essentially, the use of light transduced through optic fibers can be used to produce a temporary lesion or even excite action potentials. Recent optogenetic research has revealed the connection between activation within the amygdala and hippocampus and the relationship to changes in behavior associated with anxiety in the elevated plus maze. Tye et al. (2011) found a significant increase in the time spent within the open arms of the elevated plus maze when the basolateral amygdala (BLA) was stimulated by administering light to the projections from the central amygdala to the BLA. The anxiogenic response of activation within this pathway could be reversed when light stimuli ceased. Tye et al. (2011) were further able to manipulate this pathway through inhibition. The active inhibition of these central amygdala projections to the BLA led to increased anxiety with greater time being spent in the closed arms of the elevated plus maze. Connections between the BLA and the ventral hippocampus (vHPC) have also been implicated in the expression of anxiety behaviors. Felix-Ortiz et al. (2013) showed that lessened anxiety behaviors could be precipitated by optogenetically inhibiting the basolateral amygdala projections to the ventral hippocampus. Furthermore, by stimulating these inputs to the vHPC they were able to increase anxiety related behaviors. In regards to the origins of anxiety behaviors, it would appear as though the amygdala

and hippocampus play a crucial role in the acquisition of these behaviors. It may be expected then, that any observable changes within one or both of these regions of the brain will be associated with alterations in anxiety.

A recent study by Waller (2013) evaluate the size of various brain structures of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse. Although the total brain volume was smaller for Ta1tubulin-cre/Ikbkap$^{-/-}$ mice, it was found that the proportional volume of the hippocampus in the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice was 27% larger than controls. The amygdala was significantly smaller in Ta1tubulin-cre/Ikbkap$^{-/-}$ mice by 36% compared with control mice. Both the basolateral amygdala and hippocampus have been implicated in anxiety and fear (Bruchas et al., 2009; Muigg et al., 2009; Tye et al., 2011; Felix-Ortiz et al., 2013). Although it is unclear how these regional variations in volume relate to activation within the regions and the expression of anxiety, it is consistent with the human clinical observations and suggests a need for behavioral testing of innate anxiety in the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse.

The observed structural differences in the CNS of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice could support various predictions regarding the animal's behavior. Based on previous optogenetic research, decreased activation within the projections from the central amygdala to the BSA (Tye et al. (2011) and from the BSA to the hippocampus (Felix-Ortiz et al., 2013) could increase time spent within the open arms. Conversely, inhibition in both of these circuits could cause anxiety behaviors. However, it is presently unknown how the volumetric differences influence the projections between the central amygdala and BSA, and the BSA and vHPC. If the mice are more anxious, they

should spend a smaller proportion of time within the open arms of the elevated plus maze and perform fewer exploratory behaviors compared to controls. If, however, these mice are less anxious—a possible interpretation of previous unpublished open field maze data—it is expected that mice will spend a significant amount of time within the open arms of the elevated plus maze and perform more exploratory behaviors than controls.

Aims and Goals

The purpose of the present study was to evaluate the innate anxiety characteristics of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse model. It was predicted that there would be a difference in the level of innate anxiety between the Ta1tubulin-cre/Ikbkap$^{-/-}$ and control mice. Because of the observed increases in levels of anxiety in the clinical population (Axelrod, 2009; and Axelrod, 2010), it was predicted that Ta1tubulin-cre/Ikbkap$^{-/-}$ mice would exhibit significantly greater levels of anxiety. For the purposes of this study, anxiety was measured by evaluating the proportion of time within the open arms in combination with a myriad of other exploratory and locomotor behaviors.

METHODS

Animals

Male and female mice, aged 45 and 365 days (±3 days), young and old respectively were used for this experiment. The Ta1tubulin-cre/Ikbkap$^{-/-}$ mice had a selective deletion of the IKAP protein within the central nervous system. These mice were bred and paired with age- and sex-matched controls. Separate groups of mice were tested at 45 or 365 days of age. Prior handling of mice was minimal for at least 6 months leading up to the elevated plus maze trial. Mice were group housed with 2 to 4 mice per cage on a 12:12 hour (light : dark) cycle with the light period initiating at 0430 hrs and ceasing at 1630 hrs. A couple of the old mice (365 days old) were single housed if their cage-mates had succumbed to the degenerative nature of their disorder. Mice were provided food and water *ad libitum*.

Apparatus

The elevated plus maze was constructed following specifications described elsewhere (Rodgers & Cole, 1993). As seen in Figure 1, the apparatus was elevated 40 cm above the floor level and contained four arms radiating from a central platform (5 cm x 5 cm). Two of these arms, located directly across the central platform from one another, served as open arms (30 cm x 5 cm) with .25 cm high ledges along the three exterior boarders. The remaining two arms (closed arms) were identical to the open arms, except for 15 cm high walls.

The apparatus was placed within a galvanized container with walls placed towards the back half of the testing room. The elevated plus maze was oriented with the open arms running along the seam at the base of the container, which was oriented to run perpendicular with the testing room door. A camera was suspended approximately 4 ft. above the apparatus and connected to a computer positioned adjacent to the door and angled towards the wall of the testing room. Mice were tracked within the apparatus using AnyMaze (Stoelting) tracking software.

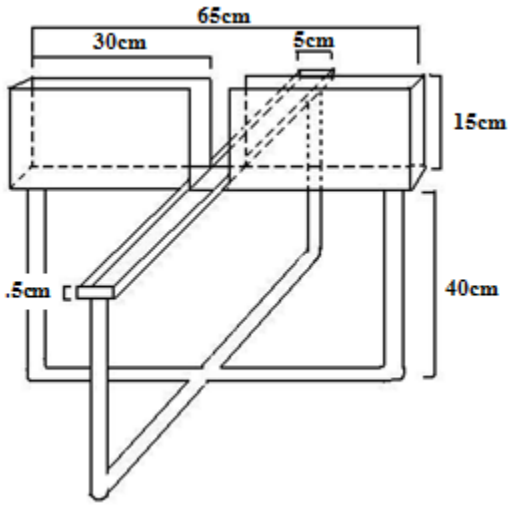

Figure 1: A diagram of the elevated plus maze used within the current experiment. The elevated plus maze is commonly used to measure anxiety in mice and contains two "closed" arms and two "open" arms.

Behavioral Measures

There are a number of behaviors that have been associated with anxiety-like states in mice. The behaviors of interest in this study were similar to those described elsewhere (Walf & Frye, 2007; and Rodgers & Cole, 1993). The primary behavior of interest in this

study was the proportion of time spent in the open arms of the elevated plus maze. Recording of time within a specific arm began once all four of the animal's paws were inside the arm (Walf & Frye, 2007). Some behaviors were visually coded by two individuals that were naïve to the experimental conditions. The following behaviors were adapted from Rodgers & Cole (1993) and Espejo (1997) and evaluated for this study:

Protected Head Dips:

When the head is lowered below the platform of the apparatus with the body of the animal located in the central platform of closed arms.

Unprotected Head Dips:

When the head is lowered below the platform of the apparatus with the body of the animal located in the open arms.

Stretch-Attend:

When the mouse advances, using only the forepaws, to the full length of its body and then retracts its forepaws back to the original position.

Rearing:

When the forepaws are lifted from the platform floor with the exception of when this behavior results in grooming.

Other behaviors that were recorded include time immobile, average speed, and average speed accounting for immobility. In addition, corrected head dips were measured in order to determine whether exploration was a function of time spent within

each arm of the elevated plus maze. Corrected head dips were calculated by taking the total head dips (protected or unprotected) for each animal and dividing this by the total time spent in the open or closed arms of the elevated plus maze respectively.

Procedure

Following the setup of the behavioral room and animal tracking software, animals were transported into the behavioral testing room at the start of their dark cycle. Animals were habituated to the room for 30 mins. The behavioral room was illuminated using an auxiliary light covered in red film in order to maintain a dark phase environment. The computer used for data collection was angled away from the habituation zone and apparatus and covered with a red film in order to prevent white light from entering the environment. Following the 30 min habituation phase, individual mice were placed onto the apparatus with their bodies in an open arm and heads within the central platform. Once mice were placed within the apparatus, the trial began and video recording was initiated for 5 mins. The types and number of head dips were recorded by the experimenter and later confirmed by observers blinded to the condition reviewing video captures. Before each trial, the apparatus was cleaned using Clidox 1:18:1 solution and wiped dry.

RESULTS

Ta1tubulin-cre/Ikbkap$^{-/-}$ mice spent a significantly greater proportion of time within the open arms of the elevated plus maze compared to controls (F(1, 22)=11.73; p=.002; Fig. 2), with no effect of age (p>.05). The number of entries into the open and closed arms were compared and no significant differences existed between age of mice (p>.05) or condition (p>.05). These data suggest that increased proportion of time in the open exhibited by Ta1tubulin-cre/Ikbkap$^{-/-}$ mice was unrelated to visits to arms. The number of unprotected head dips was found to be significantly greater for Ta1tubulin-cre/Ikbkap$^{-/-}$ mice (F(1, 22)= 11.33; p=.003; Fig. 3) compared with controls, with no effect of age (p>.05).

A significant interaction between condition and age was revealed for protected head dips (F(1,22)= 10.61; p=.004; Fig. 4) and subsequent analysis revealed that the 45 day old Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly fewer protected head dips relative to age matched controls (p< 0.05), with no significant difference existed between the conditions within the 365 day old group (p>.05). When both types of head dips were combined, it was found that Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly more head dips (F(1,22)=3.94; p=.035; Table 1). This suggests that the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly more exploration than controls and this was consistent across ages.

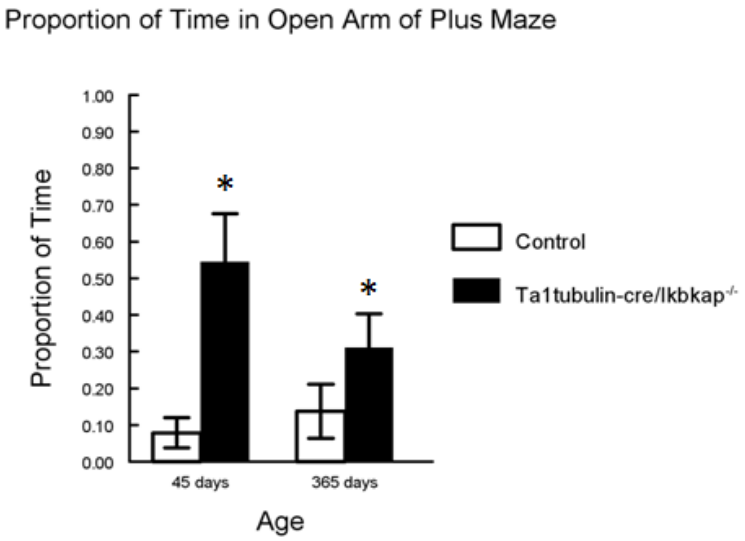

Figure 2: The mean proportion of time within the open arm of the elevated plus maze during the 5 min testing period. A main effect of Condition was found where Ta1tubulin-cre/Ikbkap$^{-/-}$ mice spent a significantly greater proportion of time in the open arm (p<.05). No main effect of Age was found and no main effect of the Condition*Age interaction.
(*= p<.05 vs the control condition)

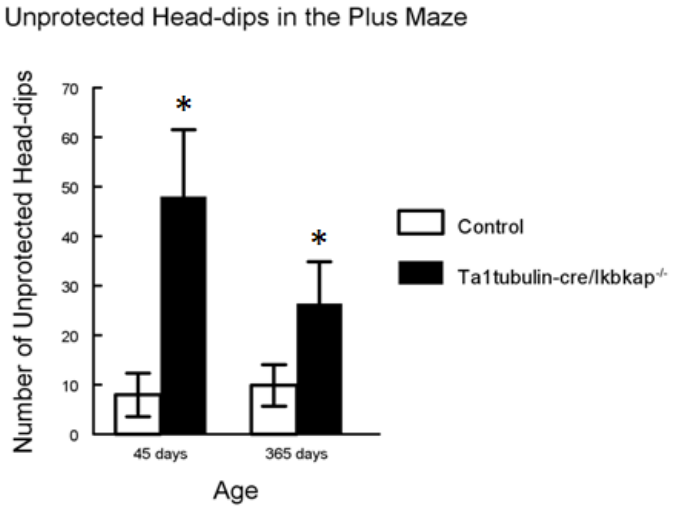

Figure 3: Mean number of unprotected head dips exhibited by Ta1tubulin-cre/Ikbkap$^{-/-}$ and control mice during the 5 min testing period in an elevated plus maze. Unprotected head dips were recorded when the animals lowered their heads below the plane of the apparatus with the body located within the open arms of the plus maze. A main effect of Condition was found where Ta1tubulin-cre/Ikbkap$^{-/-}$ performed significantly greater numbers of unprotected head dips compared to controls (p<.05). No significant main effect of Age or main effect of the Condition*Age interaction was found.
(*= p<.05 vs. controls)

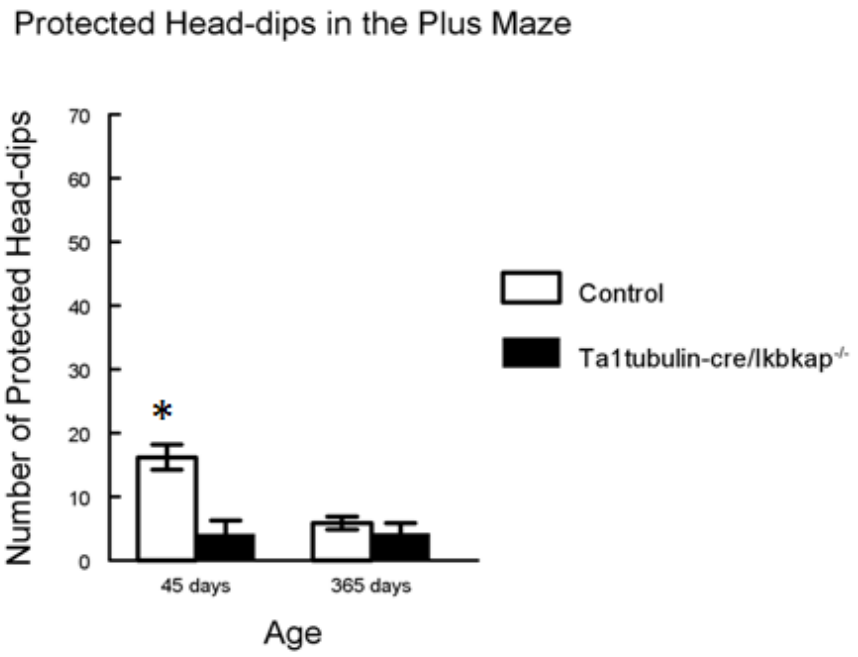

Figure 4: Mean number of protected head dips for Ta1tubulin-cre/Ikbkap$^{-/-}$ and control mice during the 5 min testing period in an elevated plus maze. Protected head dips are those occurring from the central platform or closed arms of the plus maze. Average number of protected head dips was significantly greater for the control group and a significant interaction between age and condition was revealed (p<.05). Pairwise comparisons revealed that 45 day old control mice performed significantly more protected head dips than 365 day old control mice
(*= p<.05 vs Ta1tubulin-cre/Ikbkap$^{-/-}$ mice)

Because the amount of time spent in the open arm was different between groups, additional analysis was conducted to determine if the higher frequency of unprotected head dips was an artifact of time spent in a region of the apparatus that this behavior can occur. A corrected head dip frequency was evaluated by calculating the number of head dips as a proportion of time within the associated arm. The corrected unprotected head dips revealed that Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly more unprotected head dips (F(1,22)=3.65; p=.044; Table 1) than control mice. The corrected protected head dips revealed that control mice still performed significantly more

Exploratory Behaviors and Locomotor Activity

Measurement	Young (45 days)		Old (365 days)	
	Mean (± SEM)		Mean (± SEM)	
	Control	Ta1tubulin-cre/Ikbkap$^{-/-}$	Control	Ta1tubulin-cre/Ikbkap$^{-/-}$
Stretch-Attend	17.2 (±1.02)*	16.33 (±1.50)*	11.25 (±2.35)	12.71 (±1.76)
Rearing	15 (±4.74) ‡	7.5 (±3.2)*	8.25 (±1.26)	2.14 (±0.70)
Average Speed (cm/s)	0.43 (±0.04)	0.35 (±0.05)	0.27 (±0.04)	0.30 (±0.02)
Corrected Speed (cm/s)	0.59 (±0.02) †	0.52 (±0.03)	0.68 (±0.05) †	0.55 (±0.05)
Immobility (s)	80.54 (±20.30)	103.63 (±17.00)	174.96 (±19.67) ‡	129.76 (±15.94)
Distance Travelled (m)	12.82 (±1.15)*	10.36 (±1.39)*	8.09 (±0.99)	9.07 (±0.50)

Table 1: Mean frequency of exploratory behaviors and locomotor activity during the 5 min testing period in an elevated plus maze. A main effect of Age was found for the number of stretch-attends that were found to be significantly greater for 45 day old mice. No significant main effect of Condition or Condition*Age interaction for stretch-attends was found. A significant main effect of Condition was found for rearing which occurred significantly more in control mice. A significant main effect of Age was also found for rearing with greater rearing behavior occuring in mice aged 45 days. No significnat Condition*Age interaction for rearing was found. The average speed did not differ significantly between any of the groups. Immobility was found to be significantly greater in control mice and pairwise comparisons revealed that immobility was significantly greater only in the control condition at 365 days of age. Corrected speed (distance travelled during testing periods, excluding time immoble) was significantly greater for control mice but there was no significant Condition*Age interaction.
(*= $p<.05$ vs 365 day old mice)
(†= $p<.05$ vs Ta1tubulin-cre/Ikbkap$^{-/-}$ mice)
(‡= $p<.05$ vs all other conditions)

protected head dips ($F(1,22)=7.85$; $p=.003$; Table 1) than Ta1tubulin-cre/Ikbkap$^{-/-}$ mice. In addition, the interaction remained significant for the corrected protected head dips ($F(1,22)=5.5$; $p=.029$; Table 1) where the young control mice performed significantly more protected head dips than young and old Ta1tubulin-cre/Ikbkap$^{-/-}$ mice and older control mice. These data indicate that the differences between head dips were not due to the proportion of time spent in the arms that the behaviors occurred.

Head dips

Measurement	Young (45 days)		Old (365 days)	
	Mean(±SEM)		Mean(±SEM)	
	Control	Ta1tubulin-cre/Ikbkap$^{-/-}$	Control	Ta1tubulin-cre/Ikbkap$^{-/-}$
Protected	16.2 (±1.98) ‡	4.17 (±2.17)	5.88 (±0.99)	4.29 (±1.55)
Unprotected	8 (±4.39)	48 (±13.50)*	9.88 (±4.16)	27.43 (±8.40)*
Total Combined	24.2 (±3.93)	52.17 (±11.79)*	15.75 (±3.82)	30.71 (±7.85)*
Corrected Protected	10.06 (±1.98) ‡	1.88 (±1.28)	4.53 (±0.92)	2.58 (±1.01)
Corrected Unprotected	1.35 (±1.15)	33.77 (±13.56)*	3.09 (±1.95)	11.59 (±6.26)*

Table 2: Mean frequency of head dipping behaviors during the 5 min testing period in an elevated plus maze. Head dips were separated by location of the animal with protected head dips occurring within the central platform or closed arms and unprotected head dips occurring within the open arms. Corrected head dips were a function of the type of head dip over the time spent within the respective arm needed to preform them. A significant main effect of Condition*Age interaction was found for protected head dips were control mice at 45 days of age performed significantly more protected head dips than any other condition. A main effect of Condition was found for unprotected head dips with Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performing significantly more regardless of age. A main effect of Condition was also found for total head dips with the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly greater head dips regardless of age. Correcting for the proportion of time spent in each arm revealed that control mice were still found to have performed significantly more protected head dips and Ta1tubulin-cre/Ikbkap$^{-/-}$ mice performed significantly greater unprotected head dips. These corrected measures revealed that differences in exploratory head dips were not an effect of the time spent in each arm, an aspect inherent to the type of head dip.
(*= p<.05 vs control mice)
(‡= p<.05 vs all other conditions)

A main effect of age (F(1,22)=9.06; p=.001) and condition (F(1,22)=5.06; p=.035) were found regarding the frequency of rearing behaviors. Pairwise comparisons revealed the frequency of rearing behavior was significantly greater for young mice and that control mice exhibited the behavior more frequently than Ta1tubulin-cre/Ikbkap$^{-/-}$ mice. Stretch-attends were significantly more frequent in younger mice irrespective of condition (F(1,22)= 6.02; p=.022; Table 2). In addition, immobility was significantly greater for the old age group (F(1,22)=10.23; p=.004; Table 2) than the young age group,

and differences between the Ta1tubulin-cre/Ikbkap$^{-/-}$ and control mice were not significant (p>.05). Although average speed revealed no significant differences between the groups, a corrected speed (distance travelled/time mobile), revealed that control mice were significantly faster than Ta1tubulin-cre/Ikbkap$^{-/-}$ mice. (F(1,22)= 4.99; p=0.036; Table 2). Distance travelled was found to be significant only between the age groups (F(1,22)=7.42; p=.012; Table 2) with young mice travelling further than the old group (see Table 2).

DISCUSSION

The results of the present study suggest that Ta1tubulin-cre/Ikbkap$^{-/-}$ mice are less anxious and explored the environment of the elevated plus maze significantly more than their control counterparts. For the most part, it would appear that the ageing process of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice is fairly normal. The older Ta1tubulin-cre/Ikbkap$^{-/-}$ mice did not differ, for the most part, in their locomotor activity compared with the old control group. It is interesting that, although older control mice were found to be significantly faster, their Ta1tubulin-cre/Ikbkap$^{-/-}$ counterparts also spent significantly less time immobile. So, although the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice have a slower speed, they are not necessarily less active. In addition, despite the slower speed, the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice were found have significantly greater exploratory behaviors than controls. Overall, the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse appears to have a heightened level of exploration. This is supported by the increased proportion of time spent within the open arms of the elevated plus maze, a behavior considered to be indicative of low anxiety (Rodgers & Cole, 1993; Rodgers & Dalvi, 1997). In addition, the increased number of total head dips is suggestive of reduced anxiety and increased levels of exploration (Rodgers & Dalvi, 1997). Because the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice exhibited greater proportion of time in the open arms it was important to exclude the possibility that high frequency of unprotected head dips was a function of the time spent within the open arms. When transformed into a proportion of head dips as a function of the time spent within the associated arms, these exploratory behaviors remained significantly elevated. This indicates that the elevated exploration was independent of

the time spent in the open arms. This further supports the interpretation that these Ta1tubulin-cre/Ikbkap$^{-/-}$ mice were significantly less anxious than controls.

The original prediction that Ta1tubulin-cre/Ikbkap$^{-/-}$ mice would exhibit increased levels of anxiety was not supported. Indeed, the results indicated lower levels of innate anxiety, an observation that is incongruent with what is observed within the clinical population. Data from the present study suggest that the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice have an atypical response to environmental stimuli that naturally evoke anxiety or are impaired in their ability to interpret their environment could perhaps point towards a mouse that is uninhibited by the exposure to a novel environment or a differential expression of anxiety.

Although our findings are incongruent with report of high anxiety in clinical familial dysautonomia cases, it is possible that our mouse model may differ in several ways. First, Ta1tubulin-cre/Ikbkap$^{-/-}$ mice have a deletion of the *Ikap* protein only within the central nervous system, while the clinical population of FD is characterized by a partial deletion and dysregulation of the *Ikap* protein throughout both the central and peripheral nervous system (Axelrod, 2004; Mezey et al., 2003). Perhaps full deletion of the *Ikap* protein in our mice generates a different behavioral syndrome compared to the partial deletion in humans. There is also a more general issue of comparing mice to humans. The possibility exists that the anxiety observed within FD patients is manifested as a result of some social factor as opposed to occurring innately. FD patients know they have a serious neurological problem while the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice do not. Because of this possibility, it is necessary to further investigate the traits of anxiety and

fear. Although the reality of a behaviorally identical model to the population is quite narrow, by understanding the behavioral phenotype of this Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse, it is possible to isolate the behaviors that are similar and dissimilar to the clinical population. Eventually, by characterizing this model, it could set the ground work for possible treatment applications specifically relating to this disorder and lead to a greater understanding of the behavioral implications associated with the *Ikap* protein.

The decision to evaluate anxiety in these mice was based in part on previous work that has associated activity within the amygdala and hippocampus to performance in the elevated plus maze. Although there are clear volumetric differences in the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice (Waller, 2013), it is difficult to predict how this would be responsible for the changes in behaviors we observed in the present study. The possible explanations for what may be occurring within the brain rely heavily upon what has been found in studies by others.

Based on the findings of this study, it will be important is to investigate how these animals respond in other paradigms that can evoke anxiety. Although Axelrod (2010) reported increased prevalence of anxiety in the FD clinical population, it appears more common in social situations rather than more generalized anxiety. This would indicate the necessity to develop methods to evaluate anxiety in response to a threatening situation. The idea of a fear or threat conditioning paradigm becomes complicated by the fact that the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice have decreased sensitization to pain, eliminates the utility of the social defeat paradigms (Rodgers & Cole, 1993) or classical conditioning techniques involving foot shock (Van Gaalen & Steckler, 2000; Rosen,

2004). Due to these model-specific concerns it was necessary to develop methods involving unconditioned fear stimuli to evaluate anxiety in response to fear stimuli. Dr. Babcock, of the Psychology Department at Montana State University is working towards a new direction of behavioral research with these Ta1tubulin-cre/Ikbkap$^{-/-}$ mice and the focus have shifted to an unconditioned fear paradigm. Rosen (2004) discussed the neurophysiological effects of conditioned versus unconditioned fear paradigms and describes a far lessened response in the lateral amygdala to conditioned fear induced by foot shocks and unconditioned fear induced by trimethylthiazoline (TMT), a derivative of fox feces. The direction of future research will focus on the response of Ta1tubulin-cre/Ikbkap$^{-/-}$ mice to a predator odor. In particular, cat odor will be explored as a possible odorant. Ideally, this unconditioned fear response will be brought on using cat odor as the unconditioned fear stimulus and will be based previous studies using this stimulus (Areda et al., 2006; Haquemand, Choffat, & Brand, 2013). By observing the unconditioned fear response to predator odor, a more complete picture of the behavioral phenotype of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse can be compiled. Based on the results of the current study, it is clear that these Ta1tubulin-cre/Ikbkap$^{-/-}$ mice differ in their innate anxiety and perhaps in their ability to interpret anxiety provoking stimuli. Now the question becomes how this fear response relates to the anxiety characterized within the current study. In addition, perhaps further research into the activation within the basolateral amygdala and hippocampus will yield greater clues as to how the volumetric differences reported by Waller (2013) intertwine with the anxiety and fear characteristics of the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse.

The results of this study provided evidence for a less anxious behavioral phenotype in the Ta1tubulin-cre/Ikbkap$^{-/-}$ mouse that is contrary to the original prediction and opposite of what is observed within the clinical population. However, the finding of lowered innate anxiety, as it related to hippocampus and amygdala volume, would appear to indicate different activation within these regions. Although it is yet unknown how these regional volume differences influence the activation within these regions, increased hippocampal and decreased amygdala volumes are associated with lower levels of innate anxiety. Based on previous research by Felix-Ortiz et al. (2013) it would appear probable that this lowered anxiety behavior in the Ta1tubulin-cre/Ikbkap$^{-/-}$ mice may be due to inhibition from the basolateral amygdala and projected to the hippocampus. Perhaps the decreased volume of the lateral amygdala, observed by Waller (2013), might be associated with lowered activation within the projections to the hippocampus. It is necessary to further investigate these cortical regions as well as the projections and inputs between them in order to assess the nature of activity between these regions.

Future research aimed at evaluating the fear response of these Ta1tubulin-cre/Ikbkap$^{-/-}$ mice will provide additional insight into the impaired response to environments that evoke a typical anxiety response. Although the interconnectedness of brain regions responsible for anxiety and fear may make it difficult to distinguish between the two, it is probable that an internally mediated behavior is distinctively different from an externally mediated behavior. Although lower levels of innate anxiety might be predictive of a decrease in fear-response, the behaviors and brain regions associated with anxiety and fear are distinctive.

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