HHS Public Access

Author manuscript

Brain Res Bull. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

Brain Res Bull. 2018 June ; 140: 72–79. doi:10.1016/j.brainresbull.2018.04.005.

MICE DEFICIENT IN AKAP13 (BRX) DEVELOP COMPULSIVE-LIKE BEHAVIOR AND INCREASED BODY WEIGHT

K. Maravet Baig[a,b,c], **Szu-Chi Su**[a], **Sunni L. Mumford**[c], **Emma Giuliani**[d], **Sinnie Sin Man Ng**[a], **Charles Armstrong**[a], **Margaret F. Keil**[c], **Kamaria Cayton Vaught**[a], **Nils Olsen**[e], **Elyse Pettiford**[c], **Irina Burd**[a], and **James H. Segars**[a,c]

[a]Division of Reproductive Sciences and Women's Health Research, Department of Obstetrics and Gynecology, Johns Hopkins School of Medicine, Baltimore, MD, 21205

[b]Department of Biochemistry and Molecular Biology, Virginia Commonwealth University School of Medicine, Richmond, VA, 23298

[c]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892

[d]Department of Obstetrics and Gynecology, Grand Rapids Medical Education Partners/Michigan State University, Grand Rapids, MI, 49503

[e]Organizational Sciences and Communications Department, The George Washington University, Washington, D.C., 20052

Abstract

Objective—Hormonal contributions to the sex-dependent development of both obsessive-compulsive disorder (OCD) and obesity have been described, but the underlying mechanisms are incompletely understood. A-kinase anchoring protein 13 (AKAP13) significantly augments ligand-dependent activation of estrogen receptors alpha and beta. The hypothalamus and pituitary gland are implicated in the development and exacerbation of OCD and obesity and have strong AKAP13 expression. The AKAP13 localization pattern observed in these key brain regions together with its effects on sex steroid action suggest a potential role for AKAP13 in compulsive-like behaviors. Here we tested the role of AKAP13 in compulsive-like behavior and body weight using an *Akap13* haploinsufficient murine model.

Materials and Methods—Targeted deletion of the *Akap13* gene generated haploinsufficient (*Akap13*+/−) mice in a C57BL6/J genetic background. Established behavioral assays were conducted, video recorded, and scored blindly to assess compulsive-like behavior based on

Corresponding Author: James H. Segars, MD, Division of Reproductive Sciences & Women's Health Research, Department of Gynecology and Obstetrics, Johns Hopkins School of Medicine, 720 Rutland Ave, Ross Building (Room 624), Baltimore, MD 21205, Tel: 410-614-2000, Fax: 410-614-7060, jsegars2@jhmi.edu.

Conflict of interests

The authors declare no competing financial interests.

genotype and gender. Tests included: marble-burying, grooming, open-field and elevated plus-maze. Brain and body weights were also obtained. Mean levels of test outcomes were compared using multi-way ANOVA to test for genotype, sex, genotype*sex, and genotype*sex*age interaction effects with Bonferroni adjustment for multiple comparisons, to further explain any significant interactions.

Results—The marble-burying and grooming assays revealed significant sex-dependent increases in perseverative, compulsive-like behaviors in female *Akap13* haploinsufficient mice compared to female wild type (WT) mice by demonstrating increased marble-burying activity (p=0.0025) and a trend towards increased grooming behavior (p=0.06). Male *Akap13* haploinsufficient mice exhibited no behavioral changes (p>0.05). Elevated plus-maze and open-field test results showed no overt anxiety-like behavior in *Akap13* haploinsufficient mice irrespective of sex (p>0.05, both). No differences in brain weight were found in *Akap13* haploinsufficient mice compared to WT mice (p>0.05). However, female *Akap13* haploinsufficient mice weighed more than female WT mice in the 4 to <7 months age range (p=0.0051). Male *Akap13* haploinsufficient mice showed no differences in weight compared to male WT mice (p=>0.05) at any age range examined.

Conclusion—*Akap13* haploinsufficiency led to sex-dependent, compulsive-like behavioral changes in a murine model. Interestingly, *Akap13* haploinsufficiency also led to a sex-dependent increase in body weight. These results revealed a requirement for AKAP13 in murine behavior, particularly in female mice, and is the first report of AKAP13 involvement in murine behavior. Future studies may show AKAP13 involvement in the pathophysiology of OCD in female humans and may contribute to a better understanding of the role of AKAP13 and sex hormones in the development and exacerbation of OCD.

Keywords

BRX; obsessive-compulsive disorder; estrogen; anxiety; AKAP13; obesity

1. Introduction

Obsessive-Compulsive Disorder (OCD) is a heterogeneous disease and is one of the leading causes of disability worldwide; 1-3% of the US population, or approximately 2-3 million adults and 500,000 teenagers are affected (Kessler et al., 2005). A 1:1 male to female ratio exists in adults with OCD (Kessler et al., 2005). It is a complex disease characterized by the presence of repetitive behaviors and obsessions, such as recurrent, intrusive thoughts, urges or impulses which may cause marked anxiety until a ritualistic activity is performed by the sufferer. These activities can be characterized as behavioral (handwashing, order assembling and incessant checking) or mental (incessant praying/mantras and object counting). Individuals with OCD may feel driven to perform these acts in response to an obsession (American Psychiatric Association, DSM-5, 2013).

The biology of this debilitating disorder primarily involves the limbic-hypothalamic-pituitary-adrenal axis (LHPA), with demonstrable and statistically significant diminution of pituitary volume (Radua et al., 2009; MacMaster et al., 2006). Based on previous findings, regions of the striatum, orbito-frontal cortex and cingulate gyri are abnormal in patients with OCD exhibiting increased grey matter volumes in the bilateral lenticular nuclei and

decreased grey matter volumes in the bilateral dorsal medial frontal and anterior cingulate gyri (Radua et al., 2009). Similarly, pituitary volume is decreased in children (MacMaster et al., 2006) and adults with OCD (Atmaca et al., 2009). Three major neurotransmitter pathways have been implicated in the pathophysiology of OCD: serotonergic, dopaminergic and glutaminergic (Albelda et al., 2012).

A growing body of evidence supports the involvement of sex-hormones in the modulation of OCD (Flaisher-Grinberg et al., 2009). Previous studies suggested sex differences in the age of OCD onset with increased incidence during puberty, in post-childbearing in women, and during young adulthood in men (Labad et al., 2005; Goodman et al., 2014). Additional evidence points to a differential response to selective serotonin reuptake inhibitors (SSRIs), the gold standard treatment for OCD, between women and men (Kokras et al., 2011) and improvement in OCD symptoms after gonadotropin-releasing hormone (GnRH) agonist therapy (Eriksson 2007; Altemus et al., 1999). Further evidence of the relationship between estrogens and OCD was demonstrated when investigators generated an aromatase knockout (KO) male mouse model; lower levels of estrogen and catechol-O-methyltransferase (COMT) were reported in these mice. Interestingly, the aromatase knockout mice also displayed repetitive, compulsive-like behaviors that were ameliorated with 3 weeks of 17β-estradiol replacement therapy (Hill et al., 2007; Boon et al., 2011). Similarly, BIG mice, a non-induced murine model for OCD, exhibited increased compulsive-like marble-burying and nest-building behavior and increased anxiety-like behaviors post-ovariectomy. Treatment of the post-ovariectomy mice with 17 β-estradiol (E2), but not progesterone (P4), mitigated compulsive-like behaviors (Mitra et al., 2016). In a follow-up study, BIG and SMALL mice exhibited significant strain to strain variation in compulsive-like behavior demonstrating the heterogeneity of compulsive-like behavior in the currently available murine models of OCD (Mitra et al., 2017). Furthermore, another report demonstrated that estradiol alone, or combined with progesterone, yielded complex modulatory actions on serotonergic transmission, which may lead to the observed augmentation of perseverative behavior and suggested that the hormonal effects on OCD-like behaviors is exquisitely complex (Olvera-Hernández et al., 2014).

A-kinase anchoring protein 13 (AKAP13), also known as BRX, was previously identified as a proto-oncogene involved in familial breast cancer (Rubino et al., 1998), ovarian cancer (Miller et al., 2000), osteoporosis (Koide et al., 2015), and Alzheimer's disease (Azorsa et al., 2010). AKAP13 can bind and augment ligand-dependent activity of estrogen receptors (ERα and ERβ) (Rubino et al., 1998; Driggers et al. 2001), progesterone receptors (PR) and glucocorticoid receptors (Kino et al., 2006) through its C-terminal nuclear receptor interacting domain (NRID) and by doing so, increases ligand-dependent activation. In the brain, AKAP13 is expressed in neural areas involved in complex behavior development including: the olfactory bulb, frontal cortex, hypothalamus, thalamus, amygdala, cerebellum, and anterior pituitary gland (Eddington et al., 2006). At the molecular level, the cAMP/protein kinase-A (PKA) signaling pathway is known to play a key role in the neurobiological processes involved in some forms of anxiety (Keil et al., 2012). AKAP13 is the only known AKAP with guanine nucleotide exchange factor (GEF) activity and is a central modulator of the PKA/Rho pathway (Abdul-Azeez et al., 2014).

Similarly, the etiology of obesity has both central nervous system and hormonal components (Berthoud et al., 2008; Shefer et al., 2013). The hypothalamus is one of the main central nervous system centers for weight homeostasis, disbursement of energy, appetite and satiety (Al Massadi et al., 2017). It is well described in the literature that adipose distribution can be strongly influenced by sex hormones; estrogens coordinate hypothalamic activity (Santosa et al., 2015). Furthermore, decreased levels of estrogen have been linked with obesity in menopausal women (Lizcano et al., 2014). In addition to the impact of estrogen on the development of obsessive-compulsive behaviors and obesity, prior studies have revealed an association between AKAP13 and estrogen responsive tissues such as the breast (Rubino et al., 1998), ovary (Miller et al., 2000), heart (Johnson et al., 2015; Lizcano et al., 2014), bone (Koide et al., 2015), and brain (Azorsa et al., 2010). The crossover between spatiotemporal augmentations of estrogen action is implicated in both OCD and obesity, and raised the question: would decreased AKAP13 lead to compulsive-like behavior? The objective of this study was to investigate the association between AKAP13 and its effect on the development of compulsive-like behaviors concomitant with changes in body weight in a murine model system.

2. Materials and Methods

2.1. Murine Model Establishment & Genotyping

All animal procedures were conducted in accordance with standards approved by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and by the Johns Hopkins University School of Medicine IACUC.C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The homozygous *Akap13* null mutation (*Akap13*-/-) was embryonically lethal; mice died from severe cardiac malformations by embryonic day 10.5-11.0 (Mayers et al., 2010). Consequently, *Akap13* haploinsufficient (*Akap13*+/−) mice of both sexes, previously generated by our laboratory (Mayers et al., 2010), were used in this study. Genotypes were confirmed as previously reported (Mayers et al., 2010; Koide et al., 2015). Behavioral studies involved comparisons between *Akap13* haploinsufficient mice to their WT littermates. Mice were housed with same sex littermates, with no more than 5 mice per cage. Mice were tested between 2 and <7 months old. Vivarium conditions included controlled temperature between 22–25 °C and a 12:12 hour light/dark cycle. Food and water were available *ad libitum*.

2.2. Behavioral Assays

The following assays were selected to further study compulsive-like behaviors: marble-burying and grooming; anxiety behaviors were examined by open-field test and elevated plus-maze (Hoffman et al., 2011; Martin et al., 1987). A video-tracking system was installed above the testing environment (Fire-i™ Digital Camera, UniBrain Inc., San Ramon, CA). Video data were subsequently analyzed blindly by investigators. Prior to performing the behavioral experiments, mice were habituated in silent experiment rooms for at least 30 minutes, where appropriate. All experiments were performed during the day time, or light, portion of the light/dark cycle. A new cohort of mice was used for most experiments, where applicable, and did not repeat any single experiment.

2.2.1. Marble-burying—The marble-burying test was conducted essentially as described (Treit et al., 1981; Njung'e and Hadley et al., 1991). Briefly, each testing cage (32.7cm × 19.0cm × 14.3cm) was filled with around 10cm of SANI-CHIP bedding pressed lightly on the cage floor to make a flat, even surface. Eight (8) dark, metallic marbles (1.5cm diameter) were evenly spaced horizontally in the cage. Mice were habituated and tested during the light cycle and as previously described (Njung'e & Handley et al., 1991) in a dimly lit experimental room with red overhead lighting. Mice were placed in the testing cages and could explore for 30 minutes. After 30 minutes, mice were removed from their test cages, and the number of marbles at least two-thirds buried/covered by the SANI-CHIP bedding were counted (Li et al., 2006).

2.2.2. Grooming assays—Grooming in rodents, like behavioral changes observed in humans with OCD, can be assessed for perseverative patterns. Grooming assays were performed during the light cycle and with normal overhead lighting on both male and female mice. Mice were habituated to the experimental room at least 30 minutes before testing. After habitation, mice were singly placed into novel testing cages (32.7cm × 19.0cm × 14.3cm). Each mouse was subjected to two spray mists of sterile, room temperature water. The frequency of induced grooming bouts, coupled with grooming bout types (microstructures), were analyzed essentially as described (Kalueff et al., 2004). Grooming microstructures included: paw licking, nose/face/head wash, body grooming, leg licking and tail/genitals grooming. A completed grooming bout was defined as grooming that occurred in an uninterrupted cephalo-caudal pattern. Immediately following the spray mist, the mouse's grooming activities were video recorded for 5 minutes and subsequently scored. Scorers were blinded to the genotypes of the animals.

2.2.3. Open-field test—The open-field test is an established rodent behavioral test for anxiety. Male and female *Akap13* haploinsufficient mice and WT mice were observed both pre- and post-novel object placement. The open-field test was conducted as previously described with nominal modifications (Li et al., 2006). Testing required the following: enclosed test box (50cm × 50cm × 53cm), blue screw cap (33mm) novel object, video recorder and video tracking software (ANY-maze software, San Diego Instruments, San Diego, CA). The box consisted of 4 walls and a floor; the top of the box was left uncovered to video record the mouse's physical movements. The floor area was pre-divided into zones marked by black tape: center zone and peripheral zone. For testing, each mouse was placed alone, under video surveillance, in the open-field at the periphery, and tested for 15 minutes. The recordings were divided into two segments (pre-novel object and post-novel object placement). The pre-object segment lasted for 10 minutes prior to placing the novel object into the 20cm × 20cm center zone, whereas the post-novel object segment was recorded for an additional 5 minutes. The number of times the animal defecated or urinated during testing was recorded. Movement within the open-field was separated into five categories: a) Wall time (time in the peripheral zone), b) Center latency time (time until first entry to the center zone), c) Center time (amount of time in the center zone), d) Center entries (number of times the mouse moved into the center zone) and e) number of defecations and urinations.

2.2.4. Elevated plus-maze—As another test for anxiety, we exposed both male and female mice to the elevated plus-maze as previously described (Pellow et al., 1985; Walf et al., 2007). The maze was elevated 38cm from the floor and was comprised of four arms (two open without walls and two enclosed by 15cm high walls). Each arm measured (30cm × 5cm) (Li et al., 2006). AnyMaze software was used to track the time each mouse spent in the closed arm, open arm, and center of the maze.

2.3. Weight evaluations

Animal and brain weights were obtained using standard procedures and equipment from adult mice between 3-9 months old. We wanted to determine whether any gross anatomical changes were present in the brains of *Akap13* haploinsufficient mice of both sexes. Mouse brains, including the olfactory bulb, were collected post-mortem; standard dissection procedures were followed. The fresh brains were weighed pre-paraffin embedding. Adult *Akap13* haploinsufficient mice appeared visibly larger in size than WT mice. To test our hypothesis, *Akap13* haploinsufficient and WT mice, of both sexes, were observed for up to 6 months of age. Weights were measured and recorded at 4 months or less and 4 to <7 months. For body weight measurements, mice were placed into weigh boats (14.6cm × 7.7cm). Weigh boats were tared before mouse or organ placement.

2.4. Statistical analyses

Data are presented as mean ± standard error of the mean (SEM). SAS version 9.4 (SAS Institute, Cary, NC) was used for all statistical analyses. Differences were considered significant when $p<0.05$. ANOVA was used to test for genotype effects, sex effects, and age effects, and the interaction between genotype, sex, and age. Where significant interaction effects are noted, comparisons between groups were made using t-tests with Bonferroni adjustment for multiple comparisons. A three-way ANOVA was performed on body weight data for mouse genotype, sex, and age interaction effects (genotype*sex*age) as well as genotype by sex, genotype by age, and sex by age interaction effects before doing pairwise comparisons. Fishers exact test was used for analysis of paw licking data.

3. Results

3.1. *Akap13*+/− mice showed sex-dependent compulsive-like behavior

Compulsive-like behaviors were assessed using the marble-burying assay (Thomas et al., 2009). A genotype by sex interaction effect was observed ($F_{1,1}=7.13$, $p=0.01$, ANOVA) so we pursued pairwise comparisons. When stratified by sex, female *Akap13* haploinsufficient mice displayed increased marble-burying activity compared to wild type female mice ($t=-4.13$, $p=0.0025$, Bonferroni corrected). Interestingly, *Akap13* haploinsufficient male mice showed no statistical differences in marble-burying activity when compared to wild type male mice ($t=0.23$, $p=1.00$, Bonferroni corrected) (Figure 1). WT males had higher marble burying activity compared to WT females ($t=-3.09$, $p=0.03$, Bonferroni corrected), as previously reported (Mitra et al., 2017), but no significant differences were observed between *Akap13* haploinsufficient females and males ($t=0.81$, $p=1.00$, Bonferroni corrected). Taken together, these results revealed a sex-dependent increase in marble-burying

activity, such that *Akap13* haploinsufficient female mice buried more marbles than WT female mice.

3.2. Increased grooming patterns observed in female *Akap13*+/− mice

A strong genotype and sex interaction effect was observed ($F_{1,1}$= 8.14, p=0.01, ANOVA). Interestingly, female *Akap13* haploinsufficient mice exhibited a non-significant trend towards an increased number of total grooming bouts compared to female WT mice (t=2.94, p=0.06, Bonferroni corrected) (Figure 2a). When grooming activity was stratified by grooming microstructures, paw licking, comparable to hand washing in humans, was the most repetitive behavior exhibited by 100% of female *Akap13* haploinsufficient mice whereas only 50% of the WT mice exhibited any preference for paw licking. However, this behavior was not statistically significant (p=0.43, Fishers exact test). Results were also stratified based on completion of grooming bouts. Grooming typically proceeds in a cephalo-caudal pattern (paws/nose to genitals/tail). Incomplete grooming bouts did not follow this pattern or were performed only partially. Incomplete grooming bouts were marginally higher in female *Akap13* haploinsufficient mice compared to WT mice (t=2.79, p=0.08, Bonferroni corrected) and showed a strong genotype and gender interaction effect ($F_{1,1}$=7.09, p=0.02, ANOVA) (Figure 2b). Interestingly, male *Akap13* haploinsufficient mice did not demonstrate any changes in grooming behavior in comparison to WT mice (t=−0.80, p=1.00, Bonferroni corrected). These data were consistent with the increased perseverative behavioral pattern seen in female *Akap13* haploinsufficient mice as observed in the MB assay.

3.3. *Akap13*+/− mice do not show increased anxiety in the open-field test

No genotype by sex interaction effects were observed for either pre-novel object placement (center zone: $F_{1,1}$ = 0.00, p=0.95, ANOVA; peripheral zone: $F_{1,1}$=0.39, p=0.54, ANOVA) or post-novel object placement (center zone: $F_{1,1}$= 0.56, p=0.5, ANOVA; peripheral zone: $F_{1,1}$=1.08, p=0.31, ANOVA) and all results include both sexes. No statistical differences were observed for either the center zone pre-novel object placement between *Akap13* haploinsufficient mice and WT mice ($F_{1,1}$=0.12, p=0.73, ANOVA) or post-novel object placement ($F_{1,1}$= 0.00, p=0.87, ANOVA) (Figure 3a). When time spent in the peripheral zone of the open field (wall time) was examined no statistical differences were observed for pre-novel object placement between *Akap13* haploinsufficient mice and WT mice ($F_{1,1}$=0.04, p=0.85, ANOVA). Furthermore, no statistical differences were observed post-novel object placement in the peripheral zone ($F_{1,1}$= 1.16, p=0.3, ANOVA) (Figure 3b). These data show no significant changes in anxiety in *Akap*13 haploinsufficient mice compared to WT mice.

3.4. *Akap13*+/− mice do not show increased anxiety in the Elevated plus-maze

As with the open-field, no genotype by sex interaction effects were observed (center time: $F_{1,1}$= 1.62, p=0.3, ANOVA; closed arm: $F_{1,1}$= 0.60, p=0.47, ANOVA; open arm: $F_{1,1}$=0.10, p=0.76, ANOVA), and data are reported as combined sexes. There were no significant differences between *Akap13* haploinsufficient mice compared to WT mice in the elevated plus-maze. Specifically, there were no differences in the mean time spent in the closed arms between *Akap13* haploinsufficient mice compared to WT mice ($F_{1,1}$= 1.21, p=0.3, ANOVA)

(Figure 4a). Similarly, no differences were observed in the open arms of the maze between *Akap13* haploinsufficient mice compared to WT mice ($F_{1,1}$= 0.97, p=0.4, ANOVA) (Figure 4b), or in the center of the maze ($F_{1,1}$= 0.15, p=0.7, ANOVA) (Figure 4c). Furthermore, no significant effects by sex were observed. Collectively, these data indicated that in the elevated plus-maze environment, *Akap13* haploinsufficient mice did not exhibit more anxiety-like behavior than WT mice.

3.5. No change in gross brain weight in *Akap13*+/− mice

No genotype by sex interaction effects were observed for the brain to body weight ratio ($F_{1,1}$= 0.50, p=0.50, ANOVA), nor significant genotype or sex main effects (genotype: $F_{1,1}$= 0.85, p=0.38; sex: $F_{1,1}$= 0.41, p=0.54, ANOVA) (Figure 5). No genotype and sex interaction effects were observed for brain weight ($F_{1,1}$= 0.78, p=0.40, ANOVA). The average brain weights of *Akap13* haploinsufficient mice were similar to WT mice ($F_{1,1}$=1.12, p=0.32, ANOVA) (Appendix).

3.6. *Akap13*+/− mice were heavier than control mice

A statistically significant genotype, gender, and age (genotype*sex*age) three-way interaction effect ($F_{1,1}$= 5.86, p=0.018, ANOVA) was observed. Two-way interactions including genotype by sex $F_{1,1}$= 0.67, p=0.42, ANOVA), genotype by age ($F_{1,1}$= 0.2, p=0.66, ANOVA), and sex by age ($F_{1,1}$= 2.42, p=0.12, ANOVA) were not significant. Statistical differences were observed between the two age groups (<4 months and 4 to <7 months) irrespective of genotype and sex, as expected, with the younger mice in the <4 months age group weighing less than the mice in the 4 to <7 months age group ($F_{1,1}$= 9.24, p=0.0031, ANOVA). Likewise, female mice irrespective of age and genotype weighed significantly less than male mice ($F_{1,1}$= 19.77, < p<0.0001, ANOVA). In the <4 months age range, both female ($F_{1,1}$= −0.20, p=1.00, ANOVA) and male ($F_{1,1}$= 1.36, p=1.00, ANOVA) *Akap13* haploinsufficient mice showed no significant changes in weight compared to WT mice (Figure 6a). Interestingly, female *Akap13* haploinsufficient mice in the 4 to <7 months age range exhibited increased body weight compared to WT mice ($F_{1,1}$=3.91, p=0.0051, ANOVA). However, male *Akap13* haploinsufficient mice in the same age range were not statistically different ($F_{1,1}$=0.25, p=1.00, ANOVA) (Figure 6b). Taken together, these results showed a combined genotype, sex, and age specific phenotype with *Akap13 haploinsufficient* female mice weighing more than WT female mice in the 4 to <7 months age range.

4. Discussion

We are the first to report the importance of AKAP13 in murine behavior. These results are novel because AKAP13 has not previously been associated with murine behavior or any human psychiatric disease. However, given the role of AKAP13 in sex hormone action (Rubino et al., 1998), these findings may provide a possible mechanism for the clinically-observed sexual differences in perseverative behaviors (Mitra et al., 2017) (Kessler et al., 2005) (American Psychiatric Association, DSM-5, 2013). Notably, our laboratory previously demonstrated a 4-fold increase in expression of AKAP13 in the brain and pituitary gland of female mice (Eddington et al., 2006). Based on these data, it may be hypothesized that down

regulation of AKAP13 signaling has a more robust effect in behavioral modulation and neural processes in females (vs. males) yielding more information about the potential differences in the pathophysiology of compulsive-like behaviors. Additionally, reports have shown ovarian sex hormone modulation of compulsive behavior as well as affective and cognitive functions in mice (Mitra et al., 2016). These data may suggest AKAP13 has a regulatory role within the Hypothalamic-Pituitary-Ovarian (HPO) axis.

Grooming and marble-burying are standard behavioral tests for the assessment of perseverative behavior similar to behaviors exhibited in humans with OCD (Kalueff et al., 2005) (Albelda et al., 2012) (Mitra et al., 2016). We observed that *Akap13* haploinsufficient mice exhibited a compulsive-like phenotype as evidenced by increased marble-burying activity and excessive grooming behavior. Specifically, female *Akap13* haploinsufficient mice were more likely to groom compared to WT female mice. That is, Akap13 haploinsufficiency in female mice significantly surpasses female baseline compulsivity. Additionally, female *Akap13* haploinsufficient mice were more likely to initiate grooming behavior, but abort the behavior and begin again, or follow an atypical grooming pattern. Furthermore, more female *Akap13* haploinsufficient mice exhibited perseverative paw-licking than WT mice, though not significantly so possibly due to the small sample size. Nonetheless, the difference in this activity between the two groups was intriguing since excessive grooming/hand washing is a distinct feature of compulsive-like behavior, as previously described in other murine models (Atmaca et al., 2009; Hoffman et al., 2011) and is often exhibited in human patients affected by the disorder (American Psychiatric Association, DSM-5, 2013). Similarly, female *Akap13* haploinsufficient mice demonstrated increased marble-burying activity. These data are particularly interesting since reports have shown male mice to have increased compulsive behavior, such as marble burying, compared to female mice at baseline (Mitra et al., 2016).

When mice were tested in the open-field or in the elevated plus-maze, both of which are known to evoke an anxiety response in rodents due to their fear of open spaces and predation (Li et al., 2006) (Mitra et al., 2017), we observed no significant differences between the groups. These results suggest that reduced AKAP13 did not lead to a purely generalized anxiety or fear phenotype. Future tests should explore time spent in the open-field and elevated plus-maze as an independent variable and with a larger sample size. Furthermore, the comorbidity of anxiety and OCD are incompletely understood; OCD may exist without anxiety thereby highlighting the fact that OCD is a complex, heterogenous disorder both clinically and pathophysiologically (Kessler et al., 2005; Albelda et al., 2012) (Mitra et al., 2016) (Mitra et al., 2017).

Binge-eating and metabolic syndrome are often comorbid conditions associated with anxiety and depression as well as OCD (Cassin et al., 2005). The exact pathophysiologic mechanisms behind the development of these conditions remain an enigma. Here we showed that female *Akap13* haploinsufficient mice were significantly heavier than WT littermates overall and in the 4 to < 7 months age range, specifically. Interestingly, male *Akap13* haploinsufficient mice were not heavier in either of the age ranges. However, it should be noted that human males exhibit OCD symptoms and onset earlier than females (Kessler et al., 2005; Labad et al., 2005; Goodman et al., 2014). Future studies should include tests for

perseverative behaviors in the <4 months age rage as well as in the 4 to <7 months age range. Future investigations might also include characterization of eating patterns, level of activity, and possible metabolic dysfunctions in *Akap13* haploinsufficient mice. Moreover, we hypothesize that AKAP13 may affect metabolism by regulating nuclear receptors such as estrogen, progesterone, androgen, and peroxisome proliferator-activated receptor alpha (PPAR-α) (Rubino et al., 1998) in peripheral tissues. Lack of estrogen and testosterone signaling decreases fat oxidation and increases fat storage and lipolysis (Santosa et al., 2015). Furthermore, it has been shown that activation of PKA by cAMP enhances the secretion of insulin from pancreatic β -cells, and regulates glucose homeostasis (Kim et al., 2015; Dong et al., 2014). The cAMP/PKA signaling pathway has also been implicated in some anxiety disorders (Keil et al., 2012). Additionally, it is possible that AKAP13 co-localizes and interacts with agouti-related protein (AgRP)/neuropeptide-y (NPY) neurons (Nakazato et al., 2001) in the hypothalamus which regulate feeding behaviors. Further research will investigate the importance of hormone signaling on male/female differences following prenatal hormone exposure, such as progesterone, as well as eating behaviors.

While murine models are not exact replicas of complex human diseases such as OCD, mouse models can allow for a unique understanding of the pathophysiology and behavioral changes that may accompany the disease (Albelda et al., 2012). Additionally, mouse models provide a convenient biological approach to investigate cause and effect relationships, especially when behavioral changes are involved (Hoffman et al., 2011).

5. Conclusion

In conclusion, these findings suggest a novel association between AKAP13 and the development of a sex-dependent, compulsive-like behavioral phenotype with increased body weight in a murine model. This work highlights the importance of the recent NIH emphasis on the assessment of sex as an important biologic variable (U.S. National Institutes of Health, 2015). A Cre-lox deletion strategy targeted to specific regions of the central nervous system and subsequent testing of the model's response to anxiolytic or hormonal therapy may shed light on the role of AKAP13 in neural, behavioral, and metabolic pathways. Future studies using the *Akap13* haploinsufficient model may yield better treatments for OCD and metabolic diseases by providing a novel murine model for the investigation of alternative pathways and targets for drug development, particularly in females.

Acknowledgments

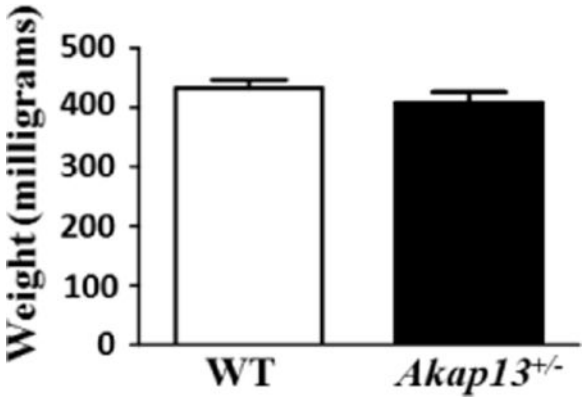
We would like to thank the students, residents, and collaborators who contributed to this endeavor: Tiffany Chu, X. Cat Guo, Tamra Chaillou, Rachel Whynott, Melissa Austin, Saima Rafique, Carmyn Polk, Renee Wah, Anna Venetianer, Jun Lei, Dominca Rubino, and Kathleen McDaniel Sarber. We would also like to give a special thanks to the faculty and staff of the 6B Animal Facility at the National Institutes of Health in Bethesda, MD, especially Dr. Tannia Clark, D.V.M., Phil Trice, and Marcus Anderson.

Funding

This work was supported, in part, by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD [ZIA-HD-008737-12 to JHS] and to SLM, and the Howard and Georgeanna Jones Endowment.

Appendix: Brain weights did not differ between Akap13+/− mice and WT

The average brain weight of *Akap13* haploinsufficient mice (n=6) was similar to WT mice of (n=6) ($F_{1,1}$=0.54, p=0.48, ANOVA). Statistical analyses were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons. Data are presented as mean ± SEM and combined sexes.



Abbreviations

MB	Marble burying
OF	Open-field
EPM	Elevated plus-maze

References

Abdul-Azeez KR, Knapp S, Fernandes JM, Klussmann E, Elkins JM. The crystal structure of the RhoA-AKAP-Lbc DH-PH domain complex. Biochemical Journal. 2014; 464:231–239. [PubMed: 25186459]

Al Massadi O, López M, Tschöp M, Diéguez C, Nogueiras R. Current understanding of the hypothalamic ghrelin pathways inducing appetite and adiposity. Trends in Neurosciences. 2017; 40:167–180. [PubMed: 28108113]

Albelda N, Joel D. Current animal models of obsessive compulsive disorder: an update. Neuroscience. 2012; 211:83–106. [PubMed: 21925243]

Altemus M, Greenberg BD, Keuler D, Jacobson KR, Murphy DL. Open trial of flutamide for treatment of obsessive-compulsive disorder. Journal of Clinical Psychiatry. 1999; 60:442–445. [PubMed: 10453797]

American Psychiatric AssociationDiagnostic and Statistical Manual of Mental Disorders Fifth. Arlington, VA: American Psychiatric Publishing; 2013

Atmaca M, Yildirim H, Ozler S, Koc M, Kara B, Sec S. Smaller pituitary volume in adult patients with obsessive-compulsive disorder. Psychiatry and Clinical Neurosciences. 2009; 63:516–20. [PubMed: 19497000]

Azorsa DO, Robeson RH, Frost D, Meec hoovet B, Brautigam GR, Dickey C, Beaudry C, Basu GD, Holz DR, Hernandez JA, Bisanz KM, Gwinn L, Grover A, Rogers J, Reiman EM, Hutton M, Stephan DA, Mousses S, Dunckley T. High-content siRNA screening of the kinome identifies kinases involved in Alzheimer's disease-related tau hyperphosphorylation. BMC Genomics. 2010; 11:25–35. [PubMed: 20067632]

Berthoud HR. The vagus nerve, food intake and obesity. Regulatory Peptides. 2008; 149:15–25. [PubMed: 18482776]

Boon WC, Horne MK. Aromatase and its inhibition in behaviour, obsessive compulsive disorder and Parkinsonism. Steroids. 2011; 76:816–819. [PubMed: 21477611]

Cassin SE, Von Ranson KM. Personality and eating disorders: a decade in review. Clinical Psychology Review. 2005; 25:895–916. [PubMed: 16099563]

Dong C, Tang L, Liu Z, Bu S, Liu Q, Wang Q, Mai Y, Wang DW, Duan S. Landscape of the relationship between type 2 diabetes and coronary heart disease through an integrated gene network analysis. Gene. 2014; 539:30–36. [PubMed: 24508273]

Driggers P, Segars JH, Rubino DM. The proto-oncoprotein Brx activates estrogen receptor beta by a p38 mitogen-activated protein kinase pathway. Journal of Biological Chemistry. 2001; 276:46792–46797. [PubMed: 11579095]

Eddington DO, Baldwin EL, Segars JH, Wu TJ. Estrogen effects on the expression of Brx in the brain and pituitary of the mouse. Brain Research Bulletin. 2006; 69:447–451. [PubMed: 16624676]

Eriksson T. Anti-androgenic treatment of obsessive-compulsive disorder: an open-label clinical trial of the long-acting gonadotropin-releasing hormone analogue triptorelin. International Clinical Psychopharmacology. 2007; 22:57–61. [PubMed: 17159461]

Flaisher-Grinberg S, Albelda N, Gitter L, Weltman K, Arad M, Joel D. Ovarian hormones modulate compulsive lever-pressing in female rats. Hormones Behavior. 2009; 55:356–365. [PubMed: 18996389]

Goodman JH, Chenausky KL, Freeman MP. Anxiety disorders during pregnancy: A systematic review. The Journal of Clinical Psychiatry. 2014; 75:1153–1184.

Grohé C, Kahlert S, Löbbert K, Stimpel M, Karas RH, Vetter H, Neyses L. Cardiac myocytes and fibroblasts contain functional estrogen receptors. FEBS Letters. 1997; 416:107–112. [PubMed: 9369244]

Hill RA, McInnes KJ, Gong EC, Jones ME, Simpson ER, Boon WC. Estrogen deficient male mice develop compulsive behavior. Biological Psychiatry. 2007; 61:359–366. [PubMed: 16566897]

Hoffman KL. Animal models of obsessive compulsive disorder: recent findings and future directions. Expert Opinion Drug Discovery. 2011; 6:725–737.

Hudson JI, Lalonde JK, Coit CE, Tsuang MT, McElroy SL, Crow SJ, Bulik CM, Hudson MS, Yanovski JA, Rosenthal NR, Pope HG Jr. Longitudinal study of the diagnosis of components of the metabolic syndrome in individuals with binge-eating disorder. American Journal of Clinical Nutrition. 2010; 91:1568–1573. [PubMed: 20427731]

Johnson KR, Nicodemus-Johnson J, Spindler MJ, Carnegie GK. Genome-wide gene expression analysis shows AKAP13-mediated PKD1 signaling regulates the transcriptional response to cardiac hypertrophy. PLoS One. 2015; 10:e0132474. [PubMed: 26192751]

Kalueff AV, Tuohimaa P. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. Journal of Neuroscience Methods. 2005; 143:169–177. [PubMed: 15814150]

Keil MF, Briassoulis G, Gokarn N, Nesterova M, Wu TJ, Stratakis CA. Anxiety phenotype in mice that overexpress protein kinase A. Psychoneuroendocrinology. 2012; 37:836–843. [PubMed: 22024111]

Kessler RC, Berglund PA, Demler O, Jin R, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). Archives of General Psychiatry. 2005; 62:593–602. [PubMed: 15939837]

Kim MJ, Park SK, Lee JH, Jung CY, Sung DJ, Park JH, Yoon YS, Park J, Park KG, Song DK, Cho H, Kim ST, Koo SH. Salt inducible kinase 1 terminates cAMP signaling by an evolutionarily conserved negative feedback loop in beta cells. Diabetes. 2015; 64:3189–3202. [PubMed: 25918234]

Kino T, Souvatzoglou E, Charmandari E, Ichijo T, Driggers P, Mayers C, Alatsatianos A, Manoli I, Westphal H, Chrousos GP, Segars JH. Rho family guanine nucleotide exchange factor Brx couples extracellular signals to the glucocorticoid signaling system. Journal of Biological Chemistry. 281:9118–9126.

Koide H, Holmbeck K, Lui JC, Guo XC, Driggers P, Chu T, Tatsuno I, Quaglieri C, Kino T, Baron J, Young MF, Robey PG, Segars JH. Mice Deficient in AKAP13 (BRX) are osteoporotic and have impaired osteogenesis. Journal of Bone and Mineral Research. 2015; 30:1887–1895. [PubMed: 25892096]

Kokras N, Sotiropoulos I, Pitychoutis PM, Almeida OF, Papadopoulou-Daifoti Z. Citalopram-mediated anxiolysis and differing neurobiological responses in both sexes of a genetic model of depression. Neuroscience. 2011; 194:62–71. [PubMed: 21839808]

Labad J, Menchon JM, Alonso P, Segalas C, Jimenez S, Vallejo J. Female reproductive cycle and obsessive-compulsive disorder. Journal of Clinical Psychiatry. 2005; 66:428–435. [PubMed: 15816784]

Li X, Morrow D, Witkin JM. Decreases in nestlet shredding of mice by serotonin uptake inhibitors: comparison with marble burying. Life Sciences. 2006; 78:1933–1939. [PubMed: 16182315]

Lizcano F, Guzmán G. Estrogen deficiency and the origin of obesity during menopause. BioMed Research International. 2014; 2014:757461. [PubMed: 24734243]

MacMaster FP, Russell A, Mirza Y, Keshavan MS, Banerjee SP, Bhandari R, Boyd C, Lynch M, Rose M, Ivey J, Moore GJ, Rosenberg DR. Pituitary volume in pediatric obsessive-compulsive disorder. Biological Psychiatry. 2006; 59:252–257. [PubMed: 16140279]

Martin P, Bateson P, Colgan P. Measuring Behaviour, an introductory guide. Behavioural Processes. 1987; 15:347–349.

Mayers CM, Wadell J, McLean K, Venere M, Malik M, Shibata T, Driggers PH, Kino T, Guo XC, Koide H, Gorivodsky M, Grinberg A, Mukhopadhyay M, Abu-Asab M, Westphal H, Segars JH. The Rho guanine nucleotide exchange factor AKAP13 (BRX) is essential for cardiac development in mice. Journal of Biological Chemistry. 2010; 285:12344–12354. [PubMed: 20139090]

Miller BT, Rubino DM, Driggers PH, Haddad B, Cisar M, Gray K, Segars JH. Expression of Brx proto-oncogene in normal ovary and in epithelial ovarian neoplasms. American Journal of Obstetrics & Gynecology. 2000; 182:286–295. [PubMed: 10694326]

Mitra S, Bastos CP, Bates K, Pereira GS, Bult-Ito A. Ovarian sex hormones modulate compulsive, affective and cognitive functions in a non-induced mouse model of obsessive-compulsive disorder. Frontiers in Behavioral Neuroscience. 2016; 10:215. [PubMed: 27881956]

Mitra S, Bastos CP, Chesworth S, Frye C, Bult-Ito A. Strain and sex based characterization of behavioral expressions in non-induced compulsive-like mice. Physiology and Behavior. 2017; 169:103–111.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature. 2001; 409:194–198. [PubMed: 11196643]

U.S. National Institutes of Health. 2015 Consideration of sex as a biological variable in NIH-funded research (NOT-OD-15-102). 2015

Njung'e K, Handley SL. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test for anxiolytic agents. British Journal of Pharmacology. 1991; 104:105–112. [PubMed: 1686200]

Olvera-Hernández S, Chavira R, Fernández-Guasti A. Sex- and endocrine-stage-differences in middle-aged rats in an animal model of OCD. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2014; 44:81–87.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods. 1985; 14:149–167. [PubMed: 2864480]

Radua J, Mataix-Cols D. Voxel-wise meta-analysis of grey matter changes in obsessive-compulsive disorder. British Journal of Psychiatry. 2009; 195:393–402. [PubMed: 19880927]

Rubino D, Driggers P, Arbit D, Kemp L, Miller B, Coso O, Pagliai K, Gray K, Gutkind S, Segars J. Characterization of Brx, a novel Dbl family member that modulates estrogen receptor action. Oncogene. 1998; 16:2513–2526. [PubMed: 9627117]

Salunke BP, Umathe SN, Chavan JG. Behavioral in-effectiveness of high frequency electromagnetic field in mice. Physiology Behavior. 2015; 140:32–37. [PubMed: 25496977]

Santosa S, Jensen MD. The sexual dimorphism of lipid kinetics in humans. Frontier in Endocrinology. 2015; 6:103.

Shefer G, Marcus Y, Stern N. Is obesity a brain disease? Neuroscience & Biobehaviroal Reviews. 2013; 37:2489–2503.

Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novely-induced anxiety. Psychopharmacology. 2009; 204:361–373. [PubMed: 19189082]

Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols. 2007; 2:322–328. [PubMed: 17406592]

Weber M, Talmon S, Schulze I, Boeddinghaus C, Gross G, Schoemaker H, Wicke KM. Running wheel activity is sensitive to acute treatment with selective inhibitors for either serotonin or norepinephrine reuptake. Psychopharmacology. 2009; 203:753–762. [PubMed: 19104776]

Highlights

- A new Obsessive Compulsive Disorder (OCD) murine model is described.
- *Akap13* haploinsufficiency caused OCD-like behavior and higher body weight in female mice.
- Novel data suggest a link between *Akap13* and psychiatric disease.

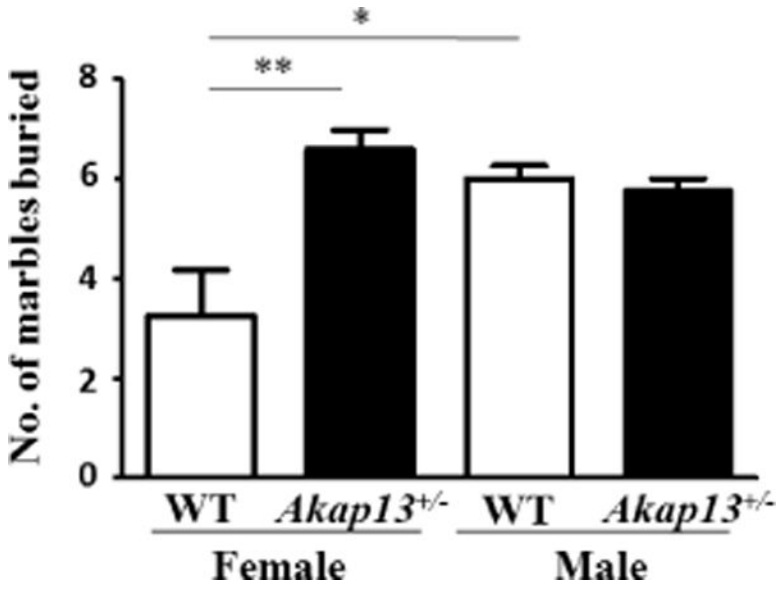


Figure 1. Female *Akap13* +/− mice buried more marbles than WT mice

Akap13+/− and WT mice were compared based on the number of marbles buried. *Akap13*+/− female mice (n=9) buried more marbles than WT female mice (n=8) (t=−4.13, p=0.0025, Bonferroni corrected). No differences were found between male *Akap13*+/− mice (n=4) compared to male WT mice (n=6) (t=0.23, p=1.00, Bonferroni corrected). Statistical analyses were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons and by using a Student's *t*-test. Data are presented as mean ± standard error of the mean (SEM).

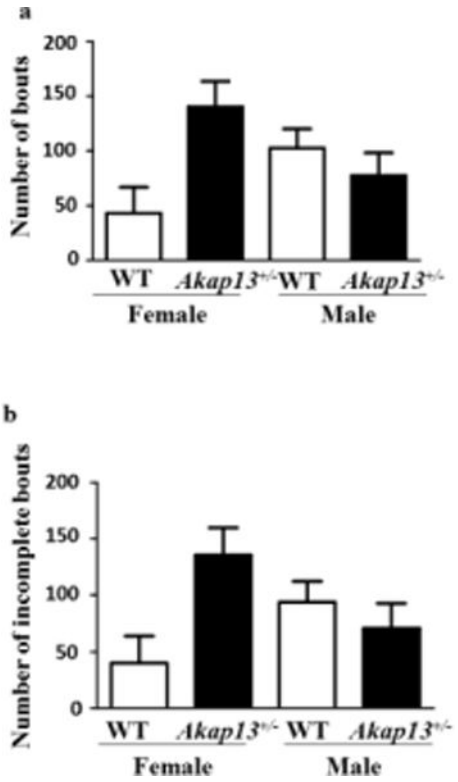


Figure 2. Female *Akap13*+/− mice exhibited increased grooming behavior

(a) Female *Akap13*+/− mice (n=4) performed marginally more total grooming bouts compared to female WT mice (n=4) (t=2.94, p=0.06, Bonferroni corrected). No differences were found between male *Akap13*+/− mice (n=5) compared to male WT mice (n=7) (t=−0.92, p=1.00, Bonferroni corrected). **(b)** Female *Akap13*+/− mice (n=4) performed marginally more incomplete grooming bouts compared to female WT mice (n=4) (t=2.79, p=0.08, Bonferroni corrected). No differences were found between male *Akap13*+/− mice (n=5) compared to male WT mice (n=7) (t=−0.80, p=1.00, Bonferroni corrected). Statistical analyses for (a) and (b) were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons. Data are presented as mean ± SEM.

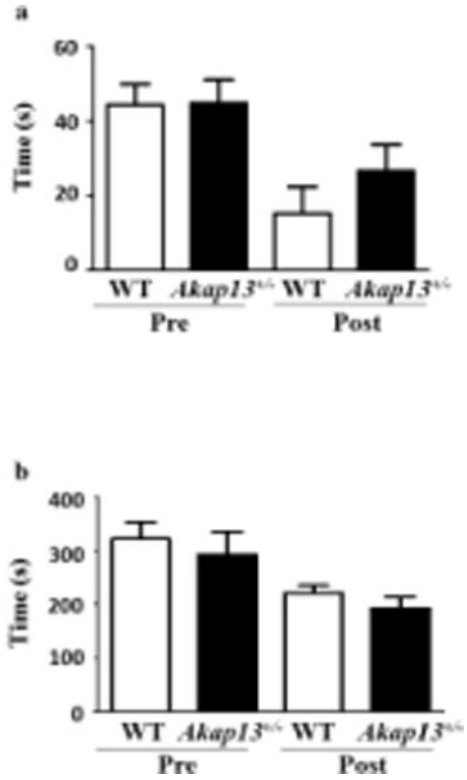


Figure 3. *Akap13*+/− mice spent marginally more time in the Center Zone post-novel object placement

(a) No differences in time spent in the Center Zone were observed pre-novel object placement by *Akap13*+/− mice (n=11) compared to WT mice (n=10) ($F_{1,1}$ =0.00, p=0.95, ANOVA). *Akap13*+/− mice showed no statistical differences in the center zone post-novel object placement compared to WT mice (n=10) ($F_{1,1}$ =1.28, p=0.3, ANOVA) **(b)** Time in the peripheral zone did not differ between groups pre- or post- novel object placement (pre-novel object placement: $F_{1,1}$ =0.04, p=0.85; post-novel object placement: $F_{1,1}$ =1.16, p=0.3, ANOVA). Statistical analyses for (a) and (b) were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons. No genotype and sex interaction effects were observed. Data are presented as mean ± SEM and combined sexes.

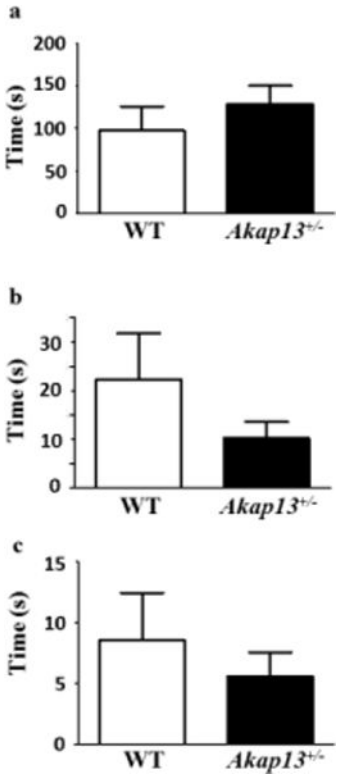


Figure 4. No significant differences were observed in the Elevated plus-maze

(a) No changes were observed between *Akap13*+/− mice (n=5) and WT mice (n=5) in the closed arm ($F_{1,1}$ =1.21, p=0.3, ANOVA), **(b)** open arm ($F_{1,1}$ =0.97, p=0.4, ANOVA), or **(c)** time spent in the center of the maze ($F_{1,1}$ =0.15, p=0.7, ANOVA). Statistical analyses for (a), (b), and (c) were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons. No genotype and sex interaction effects were observed. Data are presented as mean ± SEM and combined sexes.

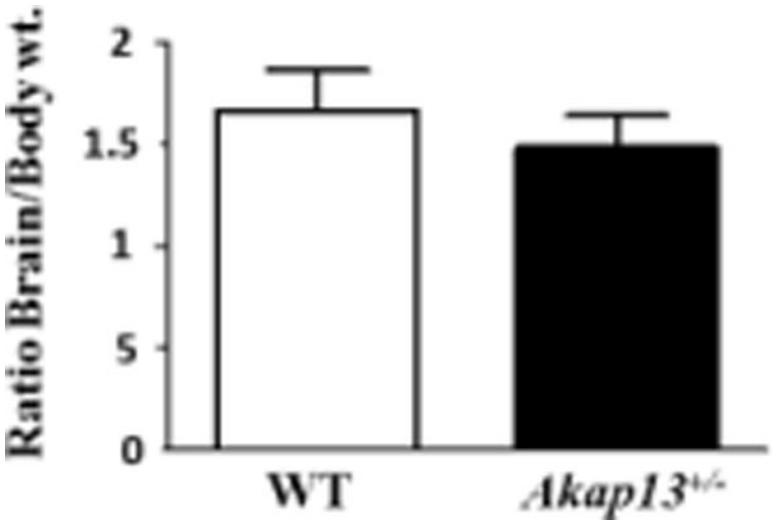


Figure 5. No changes in brain to body weight ratio in *Akap13* +/− mice

There were no differences in the brain to body weight ratio between *Akap13*+/− mice (n=6) and WT mice (n=6) ($F_{1,1}$= 0.85, p=0.38, ANOVA). Statistical analyses were performed using a two-way ANOVA with Bonferroni adjustment for multiple comparisons. Data are presented as mean ± SEM and combined sexes.

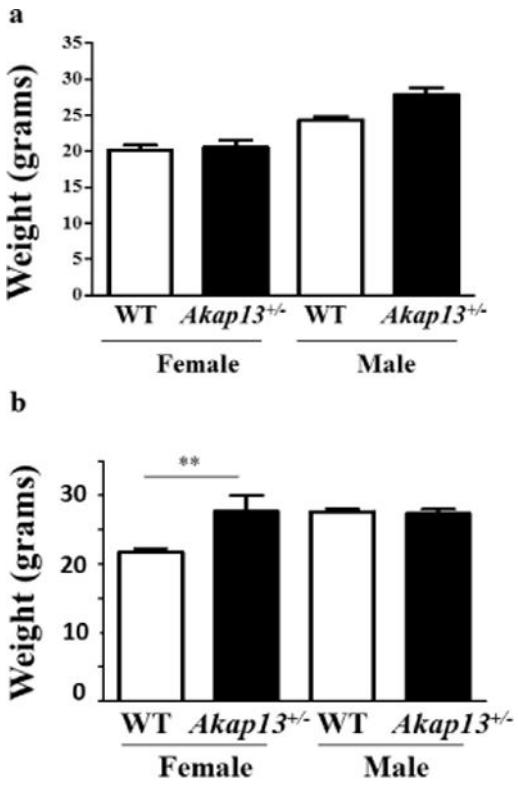


Figure 6. Female *Akap13*+/− mice weighed more than female WT mice at 4-7months of age

(a) Weights for mice <4 months age range. Both female (n=7) ($F_{1,1}$= −0.20, p=1.00, ANOVA) and male (n=3) ($F_{1,1}$= 1.36, p=1.00, ANOVA) *Akap13* haploinsufficient mice showed no significant changes in weight compared to WT mice, respectively (female, n=7; male, n=11). **(b)** Weights for mice aged 4 to <7 months. Female *Akap13* haploinsufficient mice (n=14) in the 4 to <7 months age range exhibited increased body weight compared to female WT mice (n=13) ($F_{1,1}$= 3.91, p=0.0051, ANOVA), though there was no difference in the males (*Akap13* male, n=8; WT male n=3) ($F_{1,1}$=−0.25, p=1.00, ANOVA). Statistical analyses were performed using a three-way ANOVA with Bonferroni adjustment for multiple comparisons. Data are presented as mean ± SEM.