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Long-term perturbation of spine plasticity results in distinct impairments of cognitive function

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Abstract

Dendritic spines serve as the postsynaptic structural component of synapses. The structure and function of dendritic spines are dynamically regulated by a number of signaling pathways and allow for normal neural processing, whereas aberrant spine changes are thought to contribute to cognitive impairment in neuropsychiatric and neurodegenerative disorders. However, spine changes within different brain regions and their contribution to specific cognitive functions, especially later in adulthood, is not well understood. Here, we used late-adult *KALRN*-deficient mice as a tool to investigate the vulnerability of different cognitive functions to long-term perturbations in spine plasticity in different forebrain regions. We found that in these mice, loss of one or both copies of *KALRN* lead to genotype and brain region-dependent reductions in spine density. Surprisingly, heterozygote and knockout mice showed differential impairments in cognitive phenotypes, including working memory, social recognition, and social approach. Correlation analysis between the site and magnitude of spine loss and behavioral alterations suggests that the interplay between brain regions is critical for complex cognitive processing and underscores the importance of spine plasticity in normal cognitive function.

Keywords

synapse; kalirin; schizophrenia; sociability; social recognition

Introduction

The majority of excitatory synapses in the mammalian forebrain occur at specialty sites of communication, called dendritic spines. Dendritic spines are highly regulated structures, capable of dynamically responding to sensory input. Alterations of spine structure and function are critical to neural development, maturation, and plasticity across the lifespan (Holtmaat & Svoboda 2009). Even in adulthood, spine remodeling remains active (Alvarez & Sabatini 2007, Roberts *et al.* 2010, Trachtenberg *et al.* 2002). Perturbations in the pathways that regulate synaptic expression have long been suggested to contribute to cognitive deficits, and much evidence now suggests that the synapse serves as a key site of pathology in various psychiatric disorders (Penzes *et al.* 2011).

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While alterations in spine density and morphology have been associated with cognitive dysfunction in humans (Ramakers 2002) and rodents (Barros *et al.* 2009, Chen *et al.* 2008, Jacobsen *et al.* 2006), the differential contribution of spine numbers to various cognitive functions has not been assessed. To address this question, we used adult mice lacking one or both copies of a gene, *KALRN*, encoding a central regulator of spine plasticity (Cahill *et al.* 2009, Xie *et al.* 2007), as a tool to assess the vulnerability of cognitive functions to spine loss.

Spine remodeling is achieved through the coordination of signaling pathways that modulate spine expression. Kalirin, a GEF for Rho-like small GTPases, is brain specific with enriched expression in the cortex and hippocampus (Penzes & Jones 2008). Highly expressed in the postsynaptic density (PSD) of excitatory synapses, kalirin-7 has been shown to regulate spine maintenance and activity-dependent plasticity (Penzes *et al.* 2001, Xie *et al.* 2008, Xie et al. 2007). Interestingly, altered kalirin expression has been linked to psychiatric and neurological disorders that develop later in adulthood. For example, reduced kalirin mRNA expression has been found in patients with schizophrenia (Hill *et al.* 2006, Narayan *et al.* 2008) and Alzheimer's disease (Youn *et al.* 2007a) and genetic studies have found associations with the *KALRN* gene and, in addition to schizophrenia, adult attention deficit hyperactivity disorder (ADHD), and stroke (Krug *et al.* 2010, Kushima *et al.* 2010, Lesch *et al.* 2008, Narayan et al. 2008).

Because we have previously shown that complete absence of the *KALRN* gene leads to significant cortical deficits and behavioral impairments in young mice (Cahill et al. 2009, Xie et al. 2010), we reasoned that analysis of spine densities in different brain regions in both heterozygote and knockout adult mice and their correlation with cognitive phenotypes, might reveal important relationships between spine plasticity and behavior. We found that kalirin-depletion through late-adulthood leads to synaptic deficits in the forebrain that are accompanied by behavioral impairments, with heterozygote mice displaying intermediate impairments for select behaviors. Our findings also suggest that cognitive tasks demonstrate differential sensitivity to spine changes in cortex and hippocampus, and that interplay between brain regions is critical for complex cognitive processing.

Materials and Methods

Mice

Male mice between 11 and 14 months of age were used as subjects in all experiments. Design and generation of the kalirin-deficient mice has been described in detail previously (Cahill et al. 2009). Briefly, a targeting construct was designed in which exons 27-28 were replaced by the neo cassette under an independent PGK promoter. The PGK-neo cassette was inserted in reverse orientation and contained loxP sites at each end to allow for excision. *KALRN* null mice were generated from ES cells by inGenious Targeting Laboratory (Stony Brook, NY) using standard methods. PCR analysis using WT and KO-specific primers indicated that the *KALRN* gene was disrupted. No kalirin proteins were detected by Western blotting of brain homogenates. All experiments involving animals were carried out according to the Institutional Animal Care and Use Committee of Northwestern University in compliance with National Institutes of Health standards.

Golgi Staining

Golgi staining was performed using modified Golgi-Cox impregnation method. Brains from male littermate mice between the ages of eleven and fourteen months were processed in parallel and stained with a FD Rapid GolgiStain kit (FD NeuroTechnologies), following the manufacturer's protocol. Brains were snap frozen and sectioned coronally. For spine density

quantification, the number of spines was counted along apical dendrites of pyramidal neurons. Analysis was focused on secondary dendrites but also included tertiary dendrites and distal segments of the apical trunk. Basal dendrites were not included. For frontal cortex, we examined neurons in layer V from the frontal association, prelimbic and cingulate cortical regions rostral to the genu of the corpus callosum. Hippocampal spine counts were taken from apical dendrites in CA1. Spine density was quantified using ImageJ (rsb.info.nih.gov/ij/) and reported as spine density per 10μm of dendrite. Four to five mice per genotype were used and 6 – 7 cells per mouse were analyzed.

Reagents

The following antibodies were purchased: l/s-Afadin (Sigma), PSD95 (Cell Signaling), Tiam1 (Calbiochem), and actin (Sigma). Hippocampus and frontal cortex were dissected out and cells were lysed in RIPA buffer (in mM: 150 NaCl, 10 Tris-HCl, pH 7.2, 5 EDTA, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, plus protease and phosphatase inhibitors). Lysates were then sonicated and cleared by centrifugation at 14,000 × *g* for 10 min. All samples were boiled for 5 min at 95°C after addition of Laemmli buffer and analyzed by SDS-PAGE and Western blotting. Four samples per genotype were analyzed. Intensities of Western blot bands were quantified by densitometry using Image Lab (Bio-Rad Laboratories).

Behavioral analyses

Y-maze spontaneous alternation—Methods for assessing spontaneous alternation in Y-maze were adopted from previously established procedure (Holcomb *et al.* 1998, Ohno et al. 2004). The Y-maze consists of three arms that radiate from the triangular center area. The arms are spaced 120 degrees apart and are of identical dimensions. Thirteen to fourteen mice of each genotype were tested in this task. Each test mouse (WT, n = 13; HET, n = 14; KO, n = 13) was placed in the first arm of the Y-maze, positioned with its nose towards the center of the maze and allowed free exploration for an 8 min. trial. The sequence and total number of arm entries was recorded and analyzed for spontaneous alternation. This behavior occurs naturally in mice and relies on working memory as animals must maintain and update a mental log of arm entries. No rewards or punishments were used. A mouse was considered to have entered an arm when its hind paws were completely inside the arm where no repeated entries occur. Percentage alternation is the number of triads containing entries into all three arms divided by the maximum possible alternations, with a score of 50% indicating chance level. The Y-maze activity data were automatically collected by LimeLight software (Actimetrics, Evanston, IL).

Social Recognition—The methods for assessing social recognition were adapted from previously established procedures (Kogan et al. 2000). Ten to thirteen mice of each genotype were tested in this task. Each kalirin-deficient or wild-type littermate mouse (WT, n = 13; HET, n = 13; KO, n = 10) was placed in a freshly cleaned arena (56 × 56 cm) and allowed to habituate prior to introduction of the exposure mouse. Male juvenile (3 to 4 weeks old) mice were used as exposure mice and placed in a wire-mesh cylinder in order to minimize aggressive behavior from the adult male mouse being tested. The initial interaction lasted for 5 min. After a 3 hour intertrial delay, the same juvenile exposure mouse was placed back into the test arena for another 5 min trial. Behavior was recorded and later analyzed by a trained experimenter blind to the genotypes of the animals. The amount of time the test mouse spent investigating the juvenile mouse during each exposure was determined. Social investigation behavior included direct contact with the juvenile while inspecting any part of the body surface, sniffing of the mouth, ears, tail and ango-genital area, and orienting of the nose close (<2 cm) to the exposure mouse.

Social approach—To assess social approach behavior, the amount of time a test mouse spent investigating a novel juvenile mouse during trial one of the social recognition protocol was compared across groups (WT, n = 13; HET, n = 13; KO, n = 10). In trial one, the test mouse was exposed for the first time to a juvenile mouse, making the juvenile mouse a novel conspecific. Therefore, assessing the amount of investigation time during trial one served as a measure of social approach. Ten to thirteen mice of each genotype were tested in this task. Social investigation behavior included direct contact with the juvenile while inspecting any part of the body surface, sniffing of the mouth, ears, tail and ango-genital area, and orienting of the nose close (<2 cm) to the exposure mouse.

Open field—Exploratory activity and anxiety-like behavior was assessed in an open field square arena (56 × 56 cm). Ten to thirteen mice of each genotype were tested in this task. Mice (WT, n = 13; HET, n = 13; KO, n = 10) were placed in the center of the arena and were allowed free exploration for fifteen minutes. Ambulation activity was collected by LimeLight software (Actimetrics, Evanston, IL). The open field was divided into three zones (starting from the most peripheral): Outer, mid and inner.

Activity measurement—Animal activity measurements were automatically collected by LimeLight software (Actimetrics, Evanston, IL) for mouse performance in behavioral tests. Measurements could be expressed as total distance traveled, distance traveled in a specified region or percentage of time. For distance traveled in the open field test, measurements were normalized based on the size of the different zones.

Statistical Aanalysis—All data represent means ± SEM. One-way ANOVA followed by *post hoc* Tukey's multiple-comparison test was used to determine the statistical significance of the differences among multiple groups (GraphPad Prism). Two-way ANOVA was used for open field analysis and two-way ANOVA with repeated measures was used for olfactory assessment. Pearson's test was used for correlations of spine density with behavioral performance. Differences were deemed statistically significant when $p < 0.05$.

Results

Late adult kalirin-deficient mice display synaptic deficits in cortex and hippocampus

Kalirin null mice demonstrate specific age-dependent structural and behavioral impairments early in life, but the effect of long-term kalirin depletion is not known. In previous studies, it was shown that young (12-week-old) *KALRN* knockout (KO) mice demonstrate reduced cortical spine density but unaltered hippocampal spine density (Cahill et al. 2009). To determine the effect of long-term kalirin depletion on dendritic spine expression and cognitive function we aged mice for 11 – 14 months. Examination of Golgi impregnated cells revealed a dramatic reduction of spine density in the frontal cortex of *KALRN* KO mice when compared to wild-type (WT) animals (spine density per 10μm: WT, 6.59 ± 0.23; HET, 4.92 ± 0.23; KO, 4.49 ± 0.26; $p < 0.0001$; Fig. 1a, c). Cortical spine density in heterozygote (HET) mice was also reduced (Fig. 1b). For all spine density counts, we examined expression on apical dendrites as they show greater sensitivity to kalirin depletion (Cahill et al. 2009, Xie et al. 2010). Next, we investigated spine expression within hippocampal area CA1 of late-adult kalirin-deficient mice. Surprisingly, we found that *KALRN* KO mice displayed reduced hippocampal spine density, which was 85% of wild-type mice (spine density per 10μm: WT, 6.73 ± 0.23; HET, 6.70 ± 0.26; KO, 5.71 ± 0.31; $p < 0.0117$; Fig. 1b, d). Interestingly, although HET mice experienced reduced density in the cortex, their hippocampal spine density was not different from WT animals (Fig. 1d). We further investigated synaptic alterations by examining the expression of proteins associated with synaptic kalirin signaling. Expression levels of kalirin interacting proteins, such as

PSD-95 and afadin, in lysates from cortex or hippocampus did not significantly differ across genotypes (Figure S1). Furthermore, we assessed the expression of Tiam1. Similar to kalirin, Tiam1 is a RacGEF expressed in the brain. We found protein levels of Tiam1 were unchanged across all genotypes in both cortex and hippocampus, suggesting kalirin depletion may not lead to compensatory increases of other RacGEFs (Figure S1).

Long-term perturbation of spine plasticity leads to distinct cognitive impairments

Signaling pathways regulating spine plasticity are important for normal cognition and spine deficits are associated with various psychiatric disorders. Interestingly, disorders such as schizophrenia and Alzheimer's disease clearly present with synaptic deficits and impaired cognitive function, but also are characterized by delayed onset with symptoms developing later in life. We investigated whether the aberrant structural synaptic alterations observed in aged kalirin-deficient mice were accompanied by cognitive deficits. To assess working memory in 11 – 14 month-old animals, we used a spontaneous alternation task in the Y-maze. Results from the spontaneous alternation task revealed a working memory deficit in both KO and HET mice, with HET mice demonstrating an intermediate impairment relative to WT and KO animals (WT, 68.54 ± 1.47%; HET, 61.00 ± 2.65%; KO, 54.67 ± 1.35%; $p < 0.0001$; Fig. 2a). The total number of arms entered during the test did not differ between groups (WT, 17.29 ± 1.59; HET, 18.93 ± 2.0; KO, 20.07 ± 1.69; $p > 0.05$; Fig. 2b).

We next evaluated social recognition memory. A social recognition task tests an animal's ability to remember conspecifics of another animal and requires intact olfaction (Matochik 1988), and in mice is hippocampal-dependent (Kogan et al. 2000, Ohno *et al.* 2004). For this task, we exposed mice to the same juvenile in two trials that were separated by a 3 hr intertrial delay. Typically, repeated exposure to the same juvenile leads to reduced investigation during the second trial, thus, the difference in investigation time from trial one to trial two can be used to assess social recognition memory. When we analyzed investigation times, we found the investigation difference was significantly less for KO mice compared to WT and HET mice (WT, 35.21 ± 3.52; HET, 36.32 ± 3.98; KO, 13.61 ± 2.87; $p < 0.0001$; Fig. 3a), meaning KO mice spent more time investigating a familiar mouse. Furthermore, the percent investigation of trial two relative to trial one was significantly elevated in KO mice compared to both WT and HET mice (WT, 33.84 ± 4.66; HET, 32.27 ± 5.92; KO, 67.11 ± 5.30; $p < 0.0001$; Fig. 3b). It should also be noted that investigation during trial two was significantly reduced from trial one for all groups ($p < 0.001$; Fig. 3b), indicating that regardless of genotype the mice demonstrated some degree of social recognition memory but that KO mice were impaired relative to WT and HET mice.

Altered sociability and activity in kalirin-deficient mice

Social interaction is perturbed in various psychiatric disorders and associated animal models (Filali *et al.* 2011, Lieberman *et al.* 2001, O'Tuathaigh *et al.* 2007). Interestingly, our behavioral analysis revealed abnormal social behavior in kalirin-deficient mice. When we compared the amount of time spent investigating a novel juvenile mouse, we found KO mice spent significantly less time investigating a novel mice than did WT or HET mice (WT, 56.69 ± 5.50; HET, 58.73 ± 4.17; KO, 39.60 ± 2.85; $p < 0.05$; Fig. 3c). This signifies that late-adult KO mice exhibit reduced social approach in comparison to WT and HET mice. The decreased sociability displayed by KO mice may influence the altered social recognition memory we observed in these mice and it is possible that these impairments are related. However, that we found a deficit in total investigation difference and an investigation difference relative to each mouse's initial investigation trial could suggest the impairments are related but distinct.

Given that social interaction in mice, including social investigation and social recognition memory, relies on olfactory information, we assessed the animals' ability to smell and distinguish odors using an olfactory habituation/dishabituation test (Yang & Crawley 2009). Both WT and KO mice demonstrated intact olfactory capabilities and there was no significant difference in the habituation rate between these groups [$F(1, 6) = 2.25$; $P > 0.05$] (Figure S2a). Furthermore, decreased social approach and social recognition demonstrated by KO mice cannot be accounted for by reduced exploratory behavior. On the contrary, KO mice displayed locomotor hyperactivity. Measuring free exploration in an open field or during the social recognition task revealed KO mice traveled a significantly greater distance than WT or HET mice in the same period of time (Fig. 4b and Figure S2). The activity levels between HET and WT mice did not differ from one another. Although KO mice demonstrated locomotor hyperactivity, their activity in the Y-maze was not significantly increased when compared to WT or HET animals, when measured by number of arms entered (Fig. 2b). We also analyzed activity in the open field to assess anxiety-like behaviors. We found no differences between genotypes when comparing the normalized total time spent in the different zones of the field [$F(2, 108) = 0.17$; $P > 0.05$] (Fig. 4a, c), indicating kalirin-deficient mice do not have altered anxiety-like behavior.

Correlation of behavioral alterations with spine expression across brain regions

Normal cognitive function in rodents, as assessed by behavioral tests, involves multiple brain areas. Evidence suggests the prefrontal cortex is crucial for working memory whereas recognition memory, including social recognition, is thought to depend on the hippocampus (Barker & Warburton 2011, Goldman-Rakic 1995, Kogan et al. 2000). Moreover, changes in spine expression are thought to underlie learning and memory in live animals (Roberts et al. 2010, Yang *et al.* 2009). Given the differential synaptic alterations and behavioral impairments we observed in kalirin-deficient mice and their behavioral impairments, we asked whether changes in distinct brain regions would correlate with performance in specific behavioral tests. To determine this, we first examined correlations between spine density counts in either frontal cortex or hippocampus of an animal and its corresponding behavioral scores in the Y-maze spontaneous alternation task and the social recognition task. Pearson's test was used to determine whether correlations were statistically significant. We found that mean hippocampal spine density did not significantly correlate with Y-maze performance or social recognition performance (Fig. 5c). Cortical spine density also did not correlate with social recognition performance (data not shown). Although mean cortical spine density more closely correlated with Y-maze performance than did hippocampal spine density, this did not reach statistical significance (Fig. 5a). Given the complex nature of cognitive processing, we speculated that spine alterations across brain regions might better correlate with behavioral performance. To test this, we plotted behavioral performance scores against an integrated spine density score, a combined average of hippocampal and cortical spine density means for a given mouse (Fig. 5b and 5d). Interestingly, we found that social recognition significantly correlated with the integrated spine density score ($r = 0.68$; $p < 0.05$; Fig. 5d). This was a specific relationship, as Y-maze performance did not significantly correlate with the integrated spine density score (Fig. 5b). Taken together, these results suggest a relationship exists between spine density and cognitive function and underscores the importance of multiple brain areas in mediating more complex cognitive processes.

Discussion

Here we explored the effect long-term perturbation of spine plasticity in the forebrain has on cognitive function. We used kalirin-deficient mice as a model of impaired spine plasticity since kalirin has an important regulatory role at the postsynapse and kalirin-deficient mice are known to have structural and functional forebrain deficits early in life (Cahill et al. 2009,

Xie et al. 2010, Xie et al. 2011). To examine synaptic and behavioral alterations after prolonged forebrain spine dysregulation, we aged *KALRN* KO and HET mice for 11 – 14 months and compared them to age-matched WT controls. Our data indicate kalirin-deficient mice exhibit profound cortical deficits and delayed hippocampal deficits in late adulthood. Our findings also suggest specific memory tasks rely on multiple brain areas and have varying degrees of sensitivity to regional spine insults.

When we examined synaptic alterations in aged kalirin-deficient mice, we observed that KO and HET mice displayed markedly fewer spines in frontal cortex than WT mice. We chose to focus on apical dendrites because they are particularly sensitive to kalirin perturbations and their spine expression has been correlated with cognitive function (Cahill et al. 2009, Chen et al. 2008, Hains *et al.* 2009, Xie et al. 2010). Although *KALRN* KO mice display changes in spine density, past findings indicate spine length and area do not differ between WT and KO mice (Cahill et al. 2009). In contrast to profound cortical alterations, we observed less severe hippocampal changes. Surprisingly, we found a moderate reduction in spine density within CA1 of the hippocampus in KO mice, but not HET. Hippocampal spine loss thus has a delayed onset as young *KALRN* KO mice show no structural hippocampal deficits (Cahill et al. 2009, Xie et al. 2011). It is worth noting that *KALRN* KO mice have previously been reported to develop age-dependent phenotypes. Specifically, impaired working memory and locomotor hyperactivity are not present at 3 weeks but manifest by 12 weeks. Moreover, cortical spine density is unaltered at 3 weeks of age but significantly reduced by 12 weeks (Cahill et al. 2009, Xie et al. 2010). That aberrant synaptic changes are more severe in the cortex of these mice indicates the frontal cortex is more sensitive to kalirin loss than is the hippocampus, but that prolonged kalirin depletion can engender hippocampal deficits. A potential explanation for differential brain-region sensitivity is that kalirin serves as the predominant RacGEF in the adult cortex whereas in the adult hippocampus other RacGEFs maintain high expression levels (Cahill et al. 2009, Penzes & Jones 2008). In support of this, we have previously shown Rac1 activity is reduced in the cortex but not hippocampus of *KALRN* KO mice (Cahill et al. 2009). To further explore the regional spine differences in *KALRN*-deficient mice, we assessed expression levels of another RacGEF, Tiam1. We found no changes in Tiam1 across genotypes in hippocampus or cortex. Kalirn loss precipitates Rac1 activity deficits in cortex while evidence suggests other RacGEFs may remain stable, and therefore unable to mitigate the profound effects of kalirin loss. Alternatively, greater cortical sensitivity to kalirin loss may portend an intrinsic cortical vulnerability to insults. Interestingly, the prefrontal cortex has been identified as being particularly vulnerable to aging (Burke & Barnes 2006, Wang *et al.* 2011), stress (Murphy *et al.* 1996, Radley *et al.* 2006) and disease (Glantz & Lewis 2000, Tan *et al.* 2007). Studies investigating the effects of stress on cognitive function also report that prefrontal cortex shows greater sensitivity than does the hippocampus (Conrad et al. 1999, Hains et al. 2009). We find the hippocampus is more resilient to the adverse effects of kalirin ablation but still susceptible, especially with age. Notably, 12-week old *KALRN* KO mice display impaired contextual fear conditioning and modest impairments in hippocampal LTP (Xie et al. 2011), perhaps indicative of nascent hippocampal deficits that become exacerbated over time.

The regional spine deficits we uncovered, when considered with the distinct behavioral impairments observed, may shed light on brain regions important for specific cognitive functions. Kalirin-deficient mice showed the greatest impairment in working memory, with both HET and KO mice exhibiting a deficit. This might suggest working memory is more sensitive to aberrant alterations in the frontal cortex since both HET and KO mice experienced cortical deficits and both had impaired working memory. In agreement with this, other studies have shown that working memory critically depends on prefrontal cortex and spine expression in this brain region (Goldman-Rakic 1995, Hains *et al.* 2009). That our

results find correlation between frontal cortex spine density and working memory performance does not quite reach statistical significance may be due to differences in experimental approaches, such as species used, brain region or cortical layer investigated, or behavioral test implemented. The prefrontal cortex has also been shown to be particularly vulnerable to aging (Burke & Barnes 2006, Wang *et al.* 2011), stress (Murphy *et al.* 1996, Radley *et al.* 2006) and disease (Glantz & Lewis 2000, Tan *et al.* 2007). In contrast, HET mice showed no hippocampal deficits and performed similar to WT in the social recognition task, whereas KO mice showed impairment in social recognition and displayed decreased hippocampal spine density. However, spine expression on apical dendrites of CA1 pyramidal neurons was not predictive of social recognition memory nor was cortical spine density. Instead, social recognition memory most strongly correlated with combined cortical and hippocampal spine expression and so it seems plausible that interconnected activity from these regions is involved. The brain regions subserving distinct memory types are still being uncovered as is the functional interconnectedness of these regions. Past studies indicate a role for the hippocampus in recognition memory (Barker & Warburton 2011, Kogan et al. 2000, Mansuy *et al.* 1998). Interestingly, recognition memory that also involves place memory or temporal order requires not only the hippocampus but also a functional interaction between hippocampus and perirhinal or medial prefrontal cortex (Barker & Warburton 2011).That some types of memory require functional interaction across multiple brain regions, such as hippocampus and cortex, adds to our knowledge of how memory operates and is supported by our current findings. Thus it seems as memory becomes more complex, the complexity of processing and number of brain regions involved may also increase. In addition to the hippocampus and frontal cortex, the piriform cortex and amygdala have also been implicated in social recognition memory (Ferguson *et al.* 2001, Jacobs & Tsien 2012, Richter *et al.* 2005). We found social recognition deficits in *KALRN* KO mice that may be due in part to disrupted connectivity across brain regions. Since HET mice display cortical but not hippocampal structural deficits, it is tempting to speculate that a functional hippocampus can compensate for some cortical insults in order to preserve social recognition memory. But when hippocampal deficits arise, as in aged *KALRN* KO mice, the relationship becomes dysfunctional and can no longer properly mediate social recognition memory. The correlative nature of the relationships described limits the conclusions that can be drawn, but these findings support a view whereby interplay between brain regions is critical for complex cognitive processing. Furthermore, it remains possible that other brain areas are also involved in the types of memory we tested. In the present study, we focused on hippocampus and the medial and frontal cortex but regions of the lateral cortex, limbic system or other portions of the hippocampal formation may also be involved.

These findings highlight the importance of synaptic structural integrity for normal cognitive function. Ablation of kalirin is one approach to compromise spine plasticity and indeed reduced *KALRN* mRNA has been reported in schizophrenia (Hill et al. 2006, Narayan et al. 2008), Alzheimer's disease (Youn et al. 2007a, Youn *et al.* 2007b) and *KALRN* was identified as a genetic risk factor for ischemic stroke (Krug et al. 2010). But kalirin loss, or the loss of other postsynaptic signaling molecules, is likely secondary in importance to disruption of pathways regulating synaptic signaling whose deterioration compromises synaptic stability. Perturbed synaptic signaling has deleterious effects on the structure and function of dendritic spines and likely contributes to impaired cognition observed in disease. This perspective is supported by the fact that various synaptic signaling molecules have been identified to have a role in a host of psychiatric and neurological disorders (Fiala *et al.* 2002, Harrison & Weinberger 2005, Penzes et al. 2011, Penzes & Vanleeuwen 2011, van Spronsen & Hoogenraad 2010). Our present findings support a link between neuronal structural integrity and cognitive function, which may have relevance for cognitive decline in ageing and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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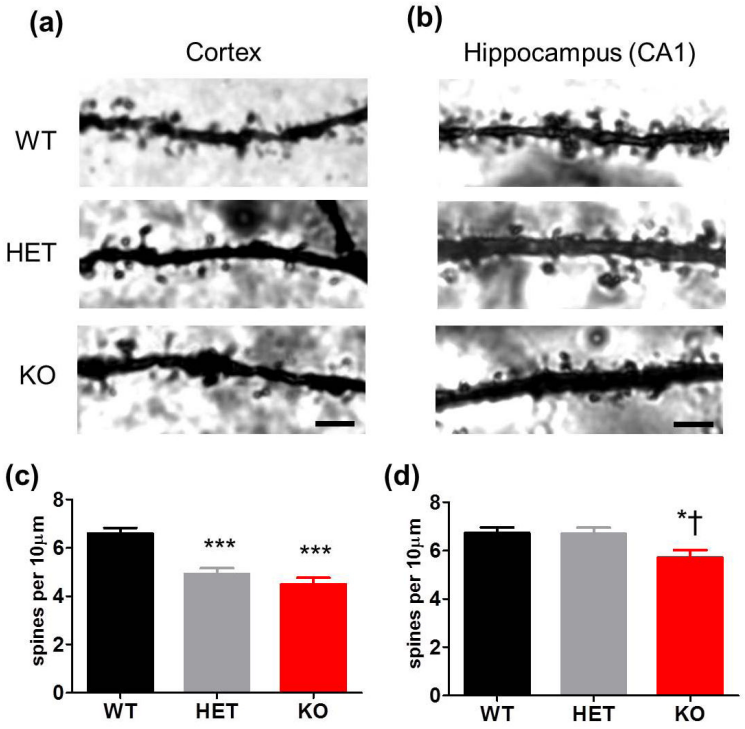

Fig. 1.

Kalirin-deficient mice demonstrate differential synaptic deficits in the forebrain. (a, b) Golgi-impregnated pyramidal neurons from apical dendrites in the frontal cortex and CA1 region of the hippocampus in WT, HET and KO mice. (c) Quantification of spine density reveals substantial reductions in cortex of HET and KO mice (***, $p < 0.0001$). (d) Hippocampal spine density was only decreased in KO mice (*, comparison between WT and KO, $p < 0.05$; †, comparison between HET and KO, $p < 0.05$). Scale bars, 4μm. Data are represented as mean ± S.E.M.

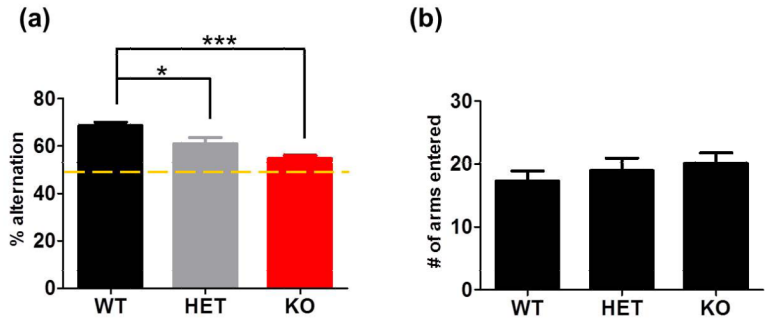

Fig. 2.

Impaired cognitive performance in kalirin-deficient mice. (a) Working memory in 11 – 14 month old kalirin-deficient mice assessed by performance on spontaneous alternation in the Y-maze. The KO mice were significantly impaired compared to WT, and HET mice displayed an intermediate phenotype but were still significantly impaired relative to WT (***, $p < 0.0001$; *, $p < 0.05$). Dashed line indicates chance level performance (50%). (b) Activity in the Y-maze, as measured by total number of arms entered, showed no difference between WT, HET and KO mice. Data presented as mean ± S.E.M.

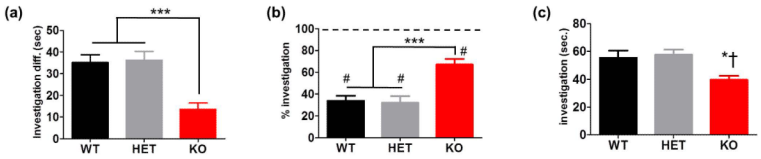

Fig. 3.

Altered social recognition and sociability in kalirin-deficient mice. (a) The difference in investigation between trials of a social recognition task was significantly reduced in KO mice compared to WT and HET mice (***, $p < 0.0001$). (b) The percent time spent investigating the juvenile mouse during trial 2 relative to trial 1 shows KO mice have reduced social recognition when compared to WT and HET mice (***, $p < 0.0001$), but all groups differed from 100 (#, $p < 0.001$). (c) KO mice spent less time investigating a novel juvenile mouse than did WT or HET mice (*, comparison between WT and KO, $p < 0.05$; †, comparison between HET and KO, $p < 0.05$). Data are represented as mean ± S.E.M.

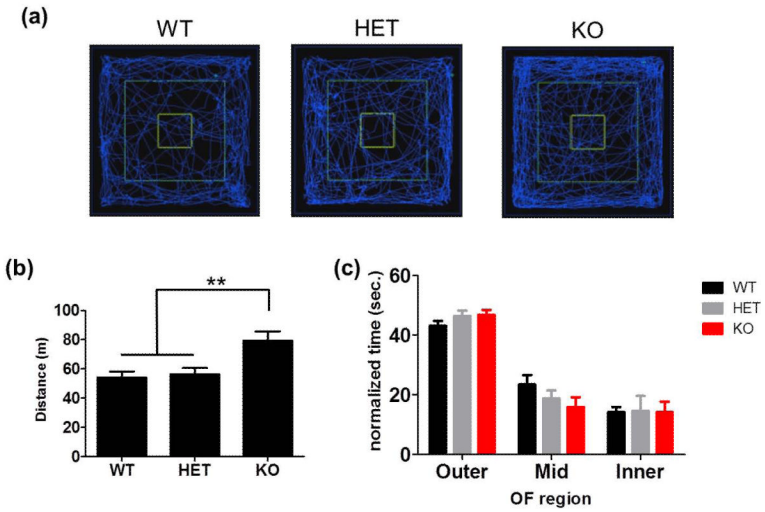

Fig. 4.

Kalirin-deficient mice display locomotor hyperactivity (a) Traces tracking the activity of WT, HET or KO mice during free exploration in an open field. The open field was divided into three zones (starting from the outermost): Outer, mid and inner. (b) Activity measurements in the open field demonstrating increased locomotor hyperactivity in KO mice when compared to WT and HET (**, $p < 0.01$), with WT and HET mice displaying similar activity levels. (c) Quantification of normalized time spent in each open field zone reveals no differences between WT, HET and KO mice. All mice spent the majority of their time in the outer zone, typical of mouse behavior, yielding a main effect for this open field zone [$F(2, 108) = 100.00$; $p < 0.0001$]. Data are represented as mean ± S.E.M.

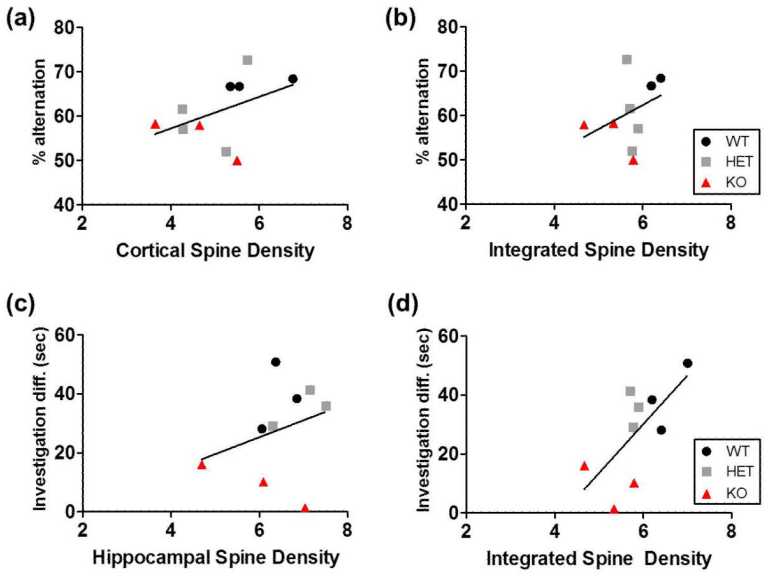

Fig. 5.

Correlations of spine density to behavioral phenotypes. (a) Working memory performance plotted against mean spine density in the frontal cortex of individual WT, HET and KO mice ($r = 0.435$, $p > 0.05$). Working memory was assessed by the spontaneous alternation task in the Y-maze, and reported as percent alternation. (b) Working memory performance plotted against an integrated spine density score ($r = 0.361$, $p > 0.05$). (c) Correlation between social recognition and mean hippocampal spine density from CA1 apical dendrites in WT, HET and KO mice ($r = 0.298$, $p > 0.05$). The difference in investigation time from trial 1 to trial 2 yielded the social recognition score, reported as investigation difference. (d) Correlation between social recognition and an integrated spine density score revealed a significant relationship ($r = 0.682$, $p < 0.05$). Pearson's test was used to determine correlation for all data sets.