MSK1 regulates environmental enrichment-induced hippocampal plasticity and cognitive enhancement

Kate Karelin1, Katelin F. Hansen,1 Yun-Sik Choi,2 A. Courtney DeVries,1 J. Simon C. Arthur,3 and Karl Obrietan1,4

1Department of Neuroscience, The Ohio State University, Columbus, Ohio 43210, USA; 2Department of Pharmaceutical Science & Technology, Catholic University of Daegu, Gyeongbuk, Republic of Korea, 712-702; 3MRC Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom

Environmental enrichment (EE) has marked beneficial effects on cognitive capacity. Given the possibility that this form of neuronal plasticity could function via the actuation of the same cellular signaling pathways that underlie learning/memory formation, we examined whether the MAPK cascade effector, mitogen/stress-activated kinase 1 (MSK1), could play a role in this process. MSK1 functions as a key signaling intermediate that couples changes in neuronal activity into inducible gene expression, neuronal plasticity, and learning/memory. Here, we show that MSK1 is expressed in excitatory cell layers of the hippocampus, progenitor cells of the subgranular zone (SGZ), and adult-born immature neurons. MSK1−/− mice exhibit reduced spinogenesis and decreased dendritic branching complexity in hippocampal neurons, compared with WT mice. Further, in MSK1−/− mice, progenitor cell proliferation within the SGZ was significantly reduced and, correspondingly, the number of immature neurons within the dentate gyrus was significantly reduced. Consistent with prior work, MSK1−/− mice displayed deficits in both spatial and recognition memory tasks. Strikingly, cognitive enhancement resulting from a 40-d period of EE was markedly reduced in MSK1−/− animals. MSK1−/− mice exhibited reduced levels of EE-induced spinogenesis and SGZ progenitor proliferation. Taken together, these data reveal that MSK1 serves as a critical regulator of hippocampal physiology and function and that MSK1 serves as a key conduit by which enriching stimuli augment cellular plasticity and cognition.

[Supplemental material is available for this article.]
MSK1-deficient mice exhibit significant deficits in learning and memory, which are associated with a reduction in the phosphorylation state of CREB and histone H3 (Chwang et al. 2007). Together, these data raise the prospect that MSK1 could play a key role in EE-evoked cognitive enhancement.

In this study, we employed a MSK1 mouse knockout model to assess the role of MSK1 in memory formation, hippocampal morphological plasticity, and EE-induced cognitive enhancement. We report that MSK1−/− mice perform poorly on memory tasks. Moreover, morphometric analysis of mature hippocampal neurons revealed a marked reduction in dendritic branching complexity of MSK1−/− mice and decreased subgranular zone (SGZ) progenitor cell proliferation. Of note, cognitive enhancement resulting from EE was attenuated in MSK1−/− mice relative to wild-type (WT) littermates. Consistent with this, the effects of EE on progenitor cell proliferation and spinogenesis were blunted in MSK1−/− mice. Together, these data reveal a central role for MSK1 in shaping both basal and inducible changes in cognitive capacity.

Results

Generation of MSK1−/− mice

The targeting construct for MSK1 has been described previously (Arthur and Cohen 2000), and genotyping was confirmed by PCR (Fig. 1A). Western blot analysis of biological replicates and immunohistochemistry further confirmed the absence of MSK1 expression in MSK1−/− mice (Fig. 1A,D). Cresyl violet labeling revealed no gross morphological differences in the hippocampus of WT and MSK1−/− mice, and hippocampus volume was not altered by MSK1 deletion (Fig. 1B,C). Striatal degeneration has been reported in aged (9-mo-old) MSK1−/− mice (Martin et al. 2011); however, at the 6- to 12-wk developmental time point used here, a neurodegenerative profile was not detected. In WT mice, MSK1 expression was detected within both the granule cell layer and the SGZ of the dentate gyrus (Fig. 1E,F). Within the SGZ, marked MSK1 labeling was detected in doublecortin (DCX)-positive immature neurons, whereas in the SOX2-positive stem/progenitor cells (Komitova and Eriksson 2004), relatively weak MSK1 expression was detected.

Dendritic morphology

As part of our analysis of hippocampal physiology and function, we profiled neuronal morphology using Golgi-Cox impregnation. Sholl analysis revealed striking differences between the hippocampal dendritic morphology of MSK1−/− and WT mice. Specifically, relative to WT mice, MSK1−/− mice exhibited reduced branching complexity of dendrites in the dentate gyrus GCL and the CA1/CA3 cell layers (Fig. 2A). The reduction in branching complexity in the CA1 was primarily evident in the proximal apical region, which receives inputs from the Schaffer collaterals of CA3 neurons (which, in turn, receive input from granule cells of the dentate gyrus). Further, MSK1−/− mice exhibited a significantly reduced CA1 and CA3 dendritic spine density relative to WT mice, but no differences in GCL dendritic spine density were observed (Fig. 2B,C). Together, these data reveal that MSK1 plays a role in both arborization and spine density of hippocampal excitatory neurons.

Progenitor cell proliferation and adult-born neurogenesis

The observation that MSK1 is expressed in progenitor cells of the SGZ and in DCX-positive immature neurons (Fig. 1E,F) raises the possibility that MSK1 could regulate aspects of progenitor division and neuronal development. To examine whether MSK1 regulates progenitor cell proliferation, we immunolabeled for Ki-67, a marker of actively proliferating progenitor cells (Scholzen and Gerdes 2000). Representative photomicrographs and quantitative analysis revealed a marked reduction in the number of Ki-67-positive cells in the dentate gyrus SGZ (Fig. 3A). To determine whether these data point to a reduction in the total pool of progenitors, adjacent sections were also labeled for SOX2, a transcription factor expressed by neural progenitor cells. SOX2 labeling within the SGZ was not significantly different between WT and MSK1−/− mice, indicating that MSK1 deletion reduced the proliferative capacity of progenitors, rather than the total number of progenitor cells (Fig. 3B). Finally, in order to examine whether the reduction in proliferating progenitor cells would result in fewer adult-born neurons, adjacent sections were also labeled for DCX. Compared to

Figure 1. Generation of MSK1−/− mice. (A) Genotyping of MSK1 knockout (−/−) and WT (+/+) mice from tail biopsy samples and western blotting of biological replicates (two animals per condition) confirming the absence of MSK1 expression in MSK1−/− mice. (B) Quantification of hippocampal volume from cresyl violet-stained tissue revealed no gross morphological differences in the hippocampus of WT and MSK1−/− mice. (C) Representative cresyl violet-stained hippocampus images, scale bar = 200 μm (low-magnification images), 100 μm (high-magnification images). (D) Immunohistochemical labeling detected MSK1 expression throughout the hippocampus of WT mice; MSK1 labeling was not detected in MSK1−/− mice. (E) In WT mice, within the subgranular zone of the dentate gyrus, relatively weak and limited MSK1 expression was detected in SOX2-positive stem/progenitor cells. (F) Double labeling in WT mice revealed MSK1 expression throughout the GCL and in the doublecortin (DCX)-positive progenitor cells. Scale bar = 20 μm. (GCL) Granule cell layer; (SGZ) subgranular zone.
WT mice, MSK1 deletion resulted in a modest (~14%) reduction in DCX-positive cells (Fig. 3C).

Learning/memory
Changes in hippocampal morphology are closely related to alterations in cognitive function (van Praag et al. 2000). Likewise, the proliferative capacity of the SGZ neurogenic niche is correlated with cognitive capacity (Bruel-Jungerman et al. 2005; Deng et al. 2009). Given that the deletion of MSK1 affected both cell morphology and neurogenesis, we examined the cognitive ramifications of MSK1 deletion. To this end, we initially examined hippocampus-dependent spatial memory of WT and MSK1^-/-^ mice using the Barnes maze. Mice were trained over a period of 5 d to locate and enter an escape hole and were then tested 24 h later via a probe trial, in which the escape box was removed. All data are expressed as mean ± SEM (two-tailed t-tests were performed for each Sholl interval), (*) P < 0.05. (b) Spine density was measured from four to five apical dendrites per animal. Dendritic spine density in the GCL did not differ between WT and MSK1^-/-^ mice but was significantly reduced in MSK1^-/-^ compared to WT mice in the CA1 (t(10) = 2.698, P < 0.05) and CA3 (t(11) = 2.638, P < 0.05) regions. (C) Representative high-magnification images revealing dendritic spines, scale bar = 10 μm.

Learning/memory following environmental enrichment
Environmental enrichment has been shown to improve cognitive function through enhanced neurogenesis and plasticity (van Praag et al. 2000). Given the reduced degree of hippocampal plasticity in MSK1^-/-^ mice, we assessed cognitive function in WT and MSK1^-/-^ mice following 40 d of EE, in which mice were housed in “enriched” cages consisting of multiple cage-mates, running...
wheels, and plastic toys. Following EE, WT mice exhibited enhanced learning as measured by reduced latency and errors during acquisition compared to MSK1−/− mice (Fig. 5A,B). Specifically, enriched WT mice exhibited faster learning rates on days 2–4 of training. A probe trial conducted 24 h after the final trial revealed increased preference for the target quadrant in WT mice, compared to MSK1−/− mice (Fig. 5C). Finally, using the novel object preference task, we found an enhanced preference quotient in WT but not MSK1−/− mice (Fig. 5D).

To provide a more comprehensive overview of the effects of MSK1 deletion and EE, we also provide a composite graphical comparison of neuronal morphology for WT and MSK1−/− mice under control and EE conditions (Supplemental Fig. S1A). Importantly, compared to control mice, EE enhanced Barnes maze learning in WT mice, particularly on days 2 and 3 of training. In MSK1−/− mice, a similar trending effect of EE was detected, although it did not reach statistical significance. Probe testing revealed that enrichment improved memory for the target quadrant in WT mice but not in MSK1−/− mice (Supplemental Fig. S1C). Similarly, the novel object preference quotient was increased in enriched WT mice (compared to control WT); however, MSK1−/− mice did not exhibit improved object recognition following enrichment (Supplemental Fig. S1D).

Experience-induced changes in dendritic morphology

To establish whether MSK1 regulates experience-dependent neuron plasticity, we assessed the effect of 40 d of EE on hippocampal morphology in WT and MSK1 null mice. Following enrichment, WT mice exhibited greater GCL and CA1/CA3 dendritic branching compared to MSK1−/− mice (Fig. 6A). Dendritic spine density was also significantly greater in enriched WT mice (Fig. 6B,C).

As with the behavioral analysis, we also provide a composite graphical comparison of neuronal morphology for WT and MSK1−/− mice under control and EE conditions (Supplemental Fig. S2). Of note, relative to control, nonenriched mice of the same genotype, EE led to an increase in GCL and CA1/CA3 dendritic branching in both WT and MSK1 null mice (Supplemental Fig. S2A). However, WT mice exhibited a significantly greater increase in dendritic spine density following enrichment than MSK1−/− mice (Supplemental Fig. S2B). Further, EE increased GCL and CA1 spine density to the same degree (~20% increase in GCL and 30% increase in CA1) in both genotypes; however, the total number of spines was significantly greater in WT mice compared to MSK1−/− mice in both hippocampal regions. In the CA3 region, only WT mice increased dendritic spine density following EE, whereas MSK1−/− mice did not exhibit an EE-induced increase in dendritic spines (Supplemental Fig. S2B,C). These data indicate that the deletion of MSK1 does not eliminate experience-dependent hippocampal plasticity but rather reduces efficacy of EE to induce structural plasticity.

Experience-induced proliferation

The finding that EE increases the rate of hippocampal neurogenesis, coupled with our work showing that MSK1 regulates the basal rate of progenitor proliferation in the SGZ, raised the prospect that MSK1 may also function as a regulator of inducible progenitor proliferation. Here, we tested whether MSK1 couples EE to an
increase in the rate of cell proliferation. We utilized the EE method described above as a model for experience-dependent induction of SGZ proliferation. Interestingly, EE significantly enhanced the number of Ki-67-positive progenitor cells in WT mice relative to control mice (Fig. 7A). Of note, a comparison of the Ki-67-positive progenitor cells in MSK1−/− mice under control and EE conditions reveals that inducible progenitor proliferation was completely repressed (Fig. 7A). Together, these data indicate that MSK1 is an important regulator of experience-induced progenitor cell proliferation.

Finally, we assessed CREB phosphorylation at Ser133 (pCREB) following EE. Previous work has detected robust pCREB expression in the SGZ, which has been localized to adult-born immature neuronal cell populations (Merz et al. 2011). Interestingly, WT mice exhibited a much greater level of CREB phosphorylation in the SGZ than MSK1−/− mice (Fig. 7B). Further, in WT mice, EE increased the number of pCREB-positive cells relative to control (nonenriched) conditions. In contrast, the pCREB-positive cell population was not significantly increased following EE in MSK1−/− mice (Fig. 7B).

Discussion

Overview of the findings
CNS neurons exhibit a tremendous range of functional plasticity. Under normal physiological conditions, plasticity can be actuated by brief bouts of synaptic activity, which underlie learning and memory formation, and can also result from ostensibly modest changes in activity that persists over longer periods of time, for example, during EE. Here, the goal was to address the role of MSK1 in both forms of functional plasticity. Our data sets reveal that MSK1 plays a key role in shaping long-term changes in neuronal morphology, processing power, and functional plasticity of the hippocampus.

MSK learning and memory
Recent studies have shown that MSK1 is activated during contextual fear conditioning (Sindreu et al. 2007) and that MSK1

Figure 4. Memory deficits in MSK1−/− mice. Using the Barnes maze and novel object recognition tests, memory was assessed in WT and MSK1−/− mice (n = 6–8/group). During 5 d of training on the Barnes maze, all mice exhibited learning, as evident by a progressive reduction in (A) latency to enter the escape hole (repeated measures ANOVA, F(4,56) = 29.452, P < 0.001) and (B) a reduction in the number of errors (repeated measures ANOVA, F(4,56) = 37.732, P < 0.001), measured as investigation of any other holes. Compared to WT mice, MSK1−/− mice exhibited a relatively slow rate of learning during day 1 (t(14) = 2.249, P < 0.05) and day 2 (t(14) = 2.288, P < 0.05) of training. (C) WT mice displayed a preference for the novel object (shown as percent time spent actively exploring the object); however, MSK1−/− mice do not discriminate between the two objects (t(14) = 1.793, P = 0.05). (D) Analysis of the number of mid-line crossings during novel object testing indicates that MSK1 deletion did not significantly affect locomotor behavior. Data are expressed as the mean ± SEM. (*) P < 0.05.
deletion impairs fear conditioning as well as spatial memory (Chwang et al. 2007). MSK1−/− mice have a deficiency in hippocampal histone H3 phosphorylation and acetylation (Chwang et al. 2007) and CREB-dependent transcription (Wiggin et al. 2000; Zhen et al. 2001; Alonso et al. 2004), whereas the disruption of ERK/MAPK signaling impairs spatial memory retention, disrupts fear conditioning (both in the acquisition and extinction phases), and blocks the consolidation of recognition memory (Kelly et al. 2003). Further, MAPK signaling is essential for long-term potentiation, a change in synaptic strength that is believed to be an underlying cellular mechanism for learning and memory (English and Sweatt 1997). Interestingly, both long-lasting forms of LTP and LTD drive a wave of robust transcriptional activity (Davis et al. 2000; Fonseca et al. 2004; Lindecke et al. 2006; Yilmaz-Rastoder et al. 2010). Given the recognized roles that MAPK signaling plays in transcriptionally dependent forms of learning and memory, it is reasonable to posit that MSK1 is a key conduit in this process. In line with this thought, studies in MSK1−/− mice which focus on the expression of MAPK- and CREB-regulated genes that influence dendrite morphology (e.g., brain-derived neurotrophic factor [BDNF], miR132, MMP9) could provide important clues regarding the mechanism by which MSK1 regulates LTP, neuronal morphology, and, relatedly, cognition.

Here, it is worth briefly noting that the MKS1 phenotypic behavioral effects described here are likely to involve a number of brain regions in addition to the hippocampus. Along these lines, while hippocampal lesions have been shown to significantly impair object recognition (Broadbent et al. 2010), cortical regions such as the perirhinal and medial prefrontal cortex are also implicated in object recognition (Barker and Warburton 2011), as is the basolateral amygdala (Roozendaal et al. 2008). Hence, the involvement of MSK1 in these brain regions cannot be excluded as a contributing factor to the observed object recognition memory deficits.

**MSK1 and progenitor proliferation and neurogenesis**

The expression of MSK1 in progenitor cells as well as DCX-positive cells in the SGZ led us to examine whether proliferation was altered in MSK1−/− mice. Here, we observed that MSK1−/− mice exhibit a reduction in progenitor cell proliferation and hippocampal neurogenesis. As noted, the total number of SOX2-positive cells within the SGZ was not altered by MSK1 deletion, thus suggesting that the pool of mitotically active progenitors was not altered but...
rather that the proliferative capacity of the resident progenitor pool was affected. As expected, the reduction in mitotically active progenitors corresponded with a reduction in the number of immature, adult-born neurons. The reduction in proliferation could have a profound effect on hippocampus-dependent cognitive capacity. Of note, adult-born neurons play an important role in replenishing the pool of granule cells within the dentate gyrus, and a reduction in the generation of adult-born neurons has been shown to affect an array of cognitive processes, including long-term recognition memory, long-term spatial memory, and eye blink trace conditioning (Shors et al. 2001; Bruel-Jungerman et al. 2005; Deng et al. 2009). Interestingly, a number of studies have shown that the MAPK pathway plays an important role in mitotic activity of progenitor cells in the developing CNS as well as in the neurogenic niche of the adult SGZ (Alonso et al. 2004; Choi et al. 2008). Some of the effects of MAPK signaling on proliferation have been ascribed to the effector kinase RSK (Zhang and Liu 2002; Hauge and Frodin 2006), whereas the role of other kinase pathways has not been well established. Clearly, the work presented here provides an interesting context within which to examine the precise mechanism by which MSK1 affects progenitor proliferation as well as neurogenesis.

Environmental enrichment

A number of studies have shown that the cognitive gains resulting from EE correspond to an increase in dendritic complexity and an increased rate of neurogenesis in the hippocampus (Kempermann et al. 1998; Nilsson et al. 1999; Faherty et al. 2003). Given our data showing that MSK1 regulates dendrite morphology and progenitor proliferation, we posited that MSK1 may play a role in coupling EE to enhanced cognitive capacity. Indeed, our data revealed that MSK1−/− mice exhibited a compromised capacity to couple EE to cognitive enhancement. Hence, in Barnes maze testing of hippocampus-dependent spatial memory, the cognitive enhancement resulting from 40 d of EE in WT mice was markedly attenuated in MSK1−/− mice. Particularly noteworthy was the distinct difference in the rate of memory consolidation after 2 d of Barnes maze training, where enriched WT mice rapidly learned to orient to and access the escape box. In contrast, enriched MSK1−/− mice took over twice as long to perform the same task at this time point. Of note, however, Barnes data revealed that enriched MSK1−/− mice exhibited improved performance relative to nonenriched MSK1−/− animals. Together, these data reveal that MSK1 significantly contributes to but is not the exclusive route by which EE drives cognitive enhancement. Other activity-inducible kinase pathways, such as PKA, PKC, and other MAPK-regulated signaling pathways, are also likely to contribute to EE-evoked cognitive enhancement.

Following the acquisition trials, which reflect the speed with which animals learn the task, a probe trial was used as an indicator that spatial memory has been formed (Jeltsch et al. 2001; Kempermann and Gage 2002). As expected, enriched WT mice spent significantly more time in the target quadrant than
nonenriched WT mice; in contrast, enriched and control MSK1−/− mice spent approximately the same amount of time in the target quadrant. Thus, in MSK1−/− mice, although enrichment led to a slight cognitive enhancement during the acquisition phase of the Barnes maze trial, spatial memory retention was not enhanced during the probe trial. A similar distinction was detected using the novel object recognition test, in which EE did not result in cognitive enhancement in MSK1−/− mice. Together, these findings indicate a role for MSK1 as a mediator of spatial and recall memory.

In MSK1−/− mice, the attenuated capacity for EE-evoked cognitive enhancement was paralleled by a blunted morphogenic and cell proliferation phenotype. Exposure to EE is believed to promote dendritic arborization and spine growth through increasing concentrations of neurotrophins, such as brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 (Ickes et al. 2000). Interestingly, ERK/MAPK signaling plays a key role in the inducible expression of neurotrophins and also serves as a downstream kinase effector of neurotrophin receptor activation (Finkbeiner et al. 1997; Kaplan and Miller 2000). For example, the expression of BDNF is mediated by an ERK/MAPK/CREB signaling cassette (Shieh and Ghosh 1999; Su et al. 2011), and signaling via BDNF/TrkB affects cellular morphology via actuation of ERK/MAPK/CREB (Alonso et al. 2004).

In conclusion, the data sets presented here indicate that MSK1 forms a central building block upon which CNS functional plasticity is constructed. Further work examining the precise mechanism by which MSK1 affects dendrite architecture and progenitor proliferation should provide important insights into the cellular processes by which cognition is shaped by experience. In turn, these insights could ultimately help guide the development of therapeutic strategies designed to alleviate an array of acquired and congenital cognitive disorders.

Materials and Methods

Animals

MSK1−/− mice were kindly provided by Dr. J. Simon Arthur (University of Dundee, Dundee, Scotland) and bred at Ohio State University. MSK1−/− mice were generated by crossing heterozygous mutant mice. The MSK targeted strains were backcrossed into the C57/bl6 line over eight generations. Mice were genotyped using the primer sets and cycling conditions described by Wiggin et al. (2002).

Adult (8- to 10-wk-old) MSK1−/− and wild type mice were maintained on a 12:12 light/dark cycle in a temperature- and humidity-controlled vivarium. All mice were allowed ad libitum access to food and water. The study was conducted in accordance with Ohio State University guidelines for the care and use of animals and under protocols approved by the Institutional Animal Care and Use Committee.

Enrichment paradigm

Enriched housing conditions were adapted from van Praag et al. (2000) and consisted of a large rat cage (44.7 × 23.5 × 15.2 cm), plastic tubes, toys, a running wheel, and nesting material. Standard housing conditions consisted of a standard mouse cage (31.8 × 17.1 × 14 cm) and nesting material. Mice were divided into four groups: (1) control wild type (standard cage), (2) enriched wild type (enriched cage), (3) control MSK1−/−, and (4) enriched MSK1−/−. All mice were maintained in assigned housing conditions for 40 d before testing and tissue collection.

Behavioral analysis

Barnes maze

The Barnes maze test of learning and memory was adapted from Sunyer et al. (2007). The maze consists of a circular platform (92-cm diameter) elevated 76 cm above the floor with 20 equally spaced holes. A black acrylic escape box was placed under one of the holes. Visual cues (geometric shapes) were placed on walls surrounding the maze. A bright light (120W, in addition to overhead
lighting) and an electronic metronome (Boss DB-66, 440 Hz, 85 dB) provided the aversive stimuli. A video camera mounted above the maze was used to monitor performance. Testing consisted of five acquisition days followed by a single probe trial 24 h after the last training trial. Each acquisition day consisted of three 5-min trials with a 10-min inter-trial interval. A trial ended when the mouse entered the escape box. If a mouse did not enter the escape box by the end of the trial, it was gently guided to it and assigned a latency of 300 sec. The probe consisted of a single 90-sec trial with the escape box removed. The surface of the maze and escape box were thoroughly cleaned with 70% ethanol after every trial to eliminate olfactory cues.

**Novel object recognition**

The novel object recognition test was performed as previously described (Hansen et al. 2010). Briefly, mice were exposed to two identical objects for 10 min and returned to home cages. One hour after the initial exposure, mice were exposed to the same testing arena in which one of the familiar objects was replaced with a novel object. During a 5-min trial, the time (in sec) spent actively investigating each object was recorded, and a novel object preference quotient was determined [(novel/(novel + familiar)] × 100). The testing arena and objects were thoroughly cleaned with 70% ethanol after every trial.

**Tissue processing and immunohistochemistry**

Brain tissue was removed following behavioral analysis, and the right and left hemispheres were immediately separated in order to process one hemisphere for immunolabeling analysis and the other for Golgi analysis. For immunohistochemistry, one hemisphere was post-fixed in 4% paraformaldehyde for 6 h and cryoprotected with 30% sucrose in PBS. Brain sections were then thin-cut (40 μm) on a freezing microtome. Adjacent sections were washed and incubated in 0.3% hydrogen peroxide in 20% methanol for 20 min. Following PBS washing, sections were blocked with 10% normal goat serum followed by overnight incubation at 4°C with the following antibodies: rabbit anti-MSK1 (1:1000, Cell Signaling), rabbit anti-p-CREB (1:1000, Cell Signaling), or rabbit anti-Ki67 (1:2000, Vector Labs). Sections were processed using the ABC staining method (Vector Labs) and visualized with nickel-intensified DAB (Vector Labs). For immunofluorescence, sections were washed with PBS and blocked with 10% normal horse serum followed by overnight incubation at 4°C with the following antibodies: rabbit anti-MSK1 (1:2000), goat anti-doublecortin (DCX: 1:500, Santa Cruz Biotechnology), or goat anti-SOX2 (1:500, Santa Cruz Biotechnology). Following PBS washes, sections were then incubated with AlexaFluor secondary antibodies conjugated with Alexa 488 or Alexa 594 (1:500, Invitrogen). Fluorescence images were captured using a Zeiss 510 confocal microscope.

**Cresyl violet staining**

A separate cohort of WT and MSK1−/− mice were transcardially perfused and their brain tissue processed for cresyl violet staining. Sections were mounted on gelatin-coated slides, dehydrated in graded alcohol solutions, and incubated in 0.3% cresyl violet solution. Sections were then destained with 0.1% glacial acetic acid in 95% ethanol, cleared in xylene, and mounted with Permount. Every fifth 40-μm section was mounted and stained for hippocampal volume quantitation.

**Cell quantitation**

To quantitate Ki-67, SOX2, DCX, and pCREB expression in the SGZ, cells were counted unilaterally in three dorsal hippocampal sections. Cell counts are reported as the average of three counts per animal. Hippocampal volume was estimated from 10× magnification photomicrographs of cresyl violet-stained sections (Paxinos and Watson [2007] coordinates: Bregma – 1.22 mm to –2.70 mm) using the Cavalieri method (Cruz-Orive 1999). Using Imagej software (Rasband 1997–2012), a grid was superimposed onto each image. Briefly, the hippocampal area was manually outlined in seven slices per animal, the total number of pixels was converted into μm², then multiplied by the distance between sections to obtain hippocampal volume (in mm³). Left and right hippocampal volumes were calculated separately, then summed together. All quantitation analyses were performed by an experimenter blinded to genotype and housing conditions.

**Golgi labeling and analysis**

The remaining brain hemisphere was processed for Golgi analysis using the FD Rapid GolgiStain Kit (FD NeuroTechnologies) according to the manufacturer’s protocol. Apical dendrites of GCL and pyramidal neurons in the CA1/CA3 regions of the hippocampus were selected for Sholl and spine density analysis. Pyramidal neurons were identified by their characteristic triangular shaped soma and apical dendrites extending toward the pial surface. Neurons were selected for analysis when all dendritic arbors were found intact and unobstructed by overlapping dendrites of neighboring cells. Sections were visualized using a Nikon E800 brightfield microscope, and 4–5 neurons per animal that met the selection criteria were traced (20× magnification) using NeuroLucida software (MicroBrightField). The morphology of the reconstructed cells was quantified using NeuroExplorer. Sholl analysis was performed by counting the number of intersections of dendrites on concentric Sholl rings (10-μm intervals). Total dendritic length and cell body area were also calculated. For spine density analysis, high-magnification (1000×) images of secondary apical dendrites were captured. Spines were counted across a 20-μm section beginning ~100 μm from the cell body. All analyses were performed by an experimenter blinded to genotyping and housing conditions.

**Western blot**

Isolated hippocampal tissue was processed using Western blotting as previously described. Briefly, hippocampi were lysed in radioimmunoprecipitation assay buffer and protein extracts (5 μg/μL), electrophoresed into a 10% SDS-PAGE gel, transblotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore) and blocked in 5% milk in Tris-buffered saline with 0.1% triton (TBS-T). The membranes were then incubated overnight at 4°C in TBS-T with goat anti-MSK1 antibody (1:1000, Santa Cruz), followed by anti-goat IgG horseradish peroxidase-conjugated antibody (1:2000, PerkinElmer Life Sciences).

**Statistical analysis**

Immunohistochemistry and morphology data comparisons were made using a Student’s t-test. Behavioral analysis data were compared using a repeated measures ANOVA (for latency and error Barnes Maze data) or one-way ANOVA followed by a post-hoc Tukey analysis. Data were considered significant for P-values <0.05.

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