Published in final edited form as:

Neurobiol Learn Mem. 2013 October ; 105: 81–92. doi:10.1016/j.nlm.2013.07.011.

Role of Adult Neurogenesis in Hippocampus-Dependent Memory, Contextual Fear Extinction and Remote Contextual Memory: New Insights from ERK5 MAP Kinase

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Abstract

Adult neurogenesis occurs in two discrete regions of the adult mammalian brain, the subgranular zone (SGZ) of the dentate gyrus (DG) and the subventricular zone (SVZ) along the lateral ventricles. Signaling mechanisms regulating adult neurogenesis in the SGZ are currently an active area of investigation. Adult-born neurons in the DG functionally integrate into the hippocampal circuitry and form functional synapses, suggesting a role for these neurons in hippocampus-dependent memory formation. Although results from earlier behavioral studies addressing this issue were inconsistent, recent advances in conditional gene targeting technology, viral injection and optogenetic approaches have provided convincing evidence supporting a role for adult-born neurons in the more challenging forms of hippocampus-dependent learning and memory. Here, we briefly summarize these recent studies with a focus on extra signal-regulated kinase (ERK) 5, a MAP kinase whose expression in the adult brain is restricted to the neurogenic regions including the SGZ and SVZ. We review evidence identifying ERK5 as a novel endogenous signaling pathway that regulates the pro-neural transcription factor Neurogenin 2, is activated by neurotrophins and is critical for adult neurogenesis. We discuss studies demonstrating that specific deletion of ERK5 in the adult neurogenic regions impairs several forms of hippocampus-dependent memory formation in mice. These include contextual fear memory extinction, the establishment and maintenance of remote contextual fear memory, and several other challenging forms of hippocampus-dependent memory formation including 48 h memory for novel object recognition, contextual fear memory established by a weak foot shock, pattern separation, and reversal of spatial learning and memory. We also briefly discuss current evidence that increasing adult neurogenesis, by small molecules or genetic manipulation, improves memory formation and long-term memory.

Introduction

Neurogenesis is a life-long developmental process starting from embryonic development to adult. Under normal physiological conditions, neurons are born in the adult brain from multipotent neural stem cells (aNSCs) residing in the subventricular zone (SVZ) along the

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lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (Altman and Das, 1965; Bull and Bartlett, 2005; Doetsch et al., 1999; Doetsch et al., 1997; Gage, 2000; Gage et al., 1998; Ming and Song, 2005). Recent studies have led to the interesting hypothesis that adult-born neurons may contribute to some forms of hippocampus dependent learning and memory (Aimone et al., 2011; Clelland et al., 2009; Creer et al., 2010; Deng et al., 2010; Deng et al., 2009; Dupret et al., 2008; Farioli-Vecchioli et al., 2008; Guo et al., 2011; Imayoshi et al., 2008; Kageyama et al., 2012; Kim et al., 2011; Kitamura et al., 2009; Ming and Song, 2011; Pan et al., 2012a; Pan et al., 2012c; Sahay et al., 2011b; Saxe et al., 2006; van Praag et al., 1999; van Praag et al., 2002; Zhang et al., 2008; Zhao et al., 2008). Several conditions, including depression, stress, and aging adversely affect hippocampal adult neurogenesis (Ming and Song, 2005; Suh et al., 2009). In contrast, external stimuli including environmental enrichment, voluntary exercise, and treatments with anti-depressants stimulate adult neurogenesis in the SGZ (Brown et al., 2003; Creer et al., 2010; David et al., 2010; Eisch et al., 2008; Li et al., 2008; Malberg et al., 2000; Sairanen et al., 2005; Shirayama et al., 2002; van Praag et al., 1999). Furthermore, increased neurogenesis has been observed after brain injuries including seizure, stroke and DNA damage, promoting the idea that it may be advantageous to recruit endogenous neural stem cells to treat some types of neurodegenerative diseases. Thus, it is important to elucidate molecular mechanisms regulating adult neurogenesis and to generate definitive evidence supporting a role for adult neurogenesis in hippocampus-dependent memory formation.

Adult neural stem cells undergo proliferation, cell-fate specification, differentiation, maturation, migration and functional integration (Belvindrah et al., 2009; Ge et al., 2007; Hsieh, 2012; Kee et al., 2007; Ma et al., 2009; Pan et al., 2012d; Petreanu and Alvarez-Buylla, 2002; Ramirez-Amaya et al., 2006; Schmidt-Hieber et al., 2004; Song et al., 2002; Toni et al., 2007; van Praag et al., 2002; Wang et al., 2013; Whitman and Greer, 2009; Winner et al., 2002; Yamaguchi and Mori, 2005; Yokoyama et al., 2011). Studies have identified cell-type specific proteins in the SGZ as they progress from stem cells, expressing glial fibrillary acidic protein (GFAP), Sox2, and nestin, to transiently-amplifying progenitor cells expressing polysialic acid-neural cell adhesion molecule (PSA-NCAM), doublecortin (DCX), and NeuroD, to immature neurons expressing NeuroD and Calretinin, and to more mature neurons expressing NeuN and Calbindin (Gage, 2000; Kempermann, 2006; Ming and Song, 2005; Taupin, 2006). Newborn neurons in the adult brain are capable of integrating into the existing neuronal circuitry and have been implicated in hippocampus-dependent learning and memory tasks (Epp et al., 2007; Gould et al., 1999; Kim et al., 2011; Leuner and Gould, 2010; Leuner et al., 2004; Pan et al., 2012a; Pan et al., 2012c).

In this review, we briefly discuss several recently identified regulatory mechanisms of adult SGZ neurogenesis and the functional significance of adult neurogenesis for memory formation. More extensive general reviews concerning this topic have recently been published (Faigle and Song, 2012; Hsieh, 2012; Mu et al., 2010; Schwarz et al., 2012; Suh et al., 2009) (Curlik and Shors, 2013; Deng et al., 2010; Glasper et al., 2012; Gu et al., 2012; Kazanis, 2012; Kim et al., 2011; Lee et al., 2012; Marin-Burgin and Schinder, 2012; Ming and Song, 2011; Song et al., 2012). This review focuses on ERK5, one of the newest signaling pathways implicated in adult neurogenesis, fear extinction and persistence of remote memory (Pan et al., 2012a; Pan et al., 2012c; Pan et al., 2012d). Recent data also suggest that ERK5 activation is sufficient to stimulate adult SGZ neurogenesis and to extend long-term, hippocampus-dependent memory (unpublished data).

Regulatory mechanisms implicated in hippocampal adult neurogenesis

Several recent reviews have extensively discussed transcription factors and signaling molecules regulating adult SGZ neurogenesis (Faigle and Song, 2012; Hsieh, 2012; Mu et

al., 2010; Schwarz et al., 2012; Suh et al., 2009). Gene targeting studies have suggested fragile X mental retardation protein (FMRP) and TLX, a cell-autonomous orphan nuclear receptor as regulators of adult SGZ neurogenesis (Guo et al., 2011; Zhang et al., 2008). Using an inducible FMRP knockout mouse to delete FMRP in nestin⁺ neural stem cells in the adult mouse, Guo et al discovered an overall deficit in the total number of adult-born cells in the SGZ, and increases in the number of DCX⁻/Ki67⁺ progenitor cells and GFAP⁺/S100⁺ astrocytes (Guo et al., 2011). They also found decreases in DCX⁺/Ki67⁺ neuroblasts, DCX⁺/Ki67⁻ immature neurons, and NeuN⁺ mature neurons. Additionally, the loss of FMRP in aNSCs resulted in significantly reduced total dendritic length and the number of dendritic branching points and ends.

Up until recently, the role of mitogen-activated protein kinases (MAPK) in adult neurogenesis has not received much attention. MAP Kinases are serine/threonine protein kinases including ERK1/2, ERK5, p38 and JNK and are activated in response to growth factors or stress. They have regulatory roles in cellular proliferation, differentiation, cell-fate specification, cell-cycle progression, survival, and apoptosis (Kuida and Boucher, 2004; Miloso et al., 2008; Raman et al., 2007; Rubinfeld and Seger, 2005; Seger and Krebs, 1995; Xia et al., 1995). MAP Kinases have recently been found to play a role in adult neurogenesis. In one study, adult neural progenitor cells (aNPCs) from the hippocampus were isolated and exposed to HIV-1-associated dementia HIV-envelope glycoprotein gp120. Exposure to gp120 resulted in a significant decrease in the number of proliferating cells. This effect was attributed to the induction of p38 MAPK-MAPKAPK2-Cdc25c and an arrest of aNPCs in the G1 phase of the cell cycle (Okamoto et al., 2007).

The ERK1/2 signaling pathway is activated in SGZ-derived aNSCs in culture in response to neuropeptide Y (NPY) or vascular endothelial growth factor (VEGF) (Fournier et al., 2012; Howell et al., 2005). A significant decrease in the total number of BrdU⁺ cells 5 h after BrdU administration was observed along the SGZ of NPY^{-/-} mice compared with littermate controls (Howell et al., 2005). The total number of DCX⁺, transiently amplifying progenitors and/or immature neurons, was also greatly reduced in the SGZ of NPY^{-/-} mice. However, since NPY can activate other signaling pathways in addition to ERK1/2, these correlative data alone do not specifically implicate ERK1/2 in adult neurogenesis. VEGF activates ERK1/2 *in vitro* and *in vivo* and increases BrdU⁺/Sox2⁺ neural stem cells (Fournier et al., 2012). The effect of VEGF was blocked by U0126, an ERK1/2 inhibitor, suggesting that the neurogenic effect of VEGF is mediated by the ERK1/2 signaling pathway. These results suggest that extracellular growth factors can stimulate adult SGZ neurogenesis and this effect may be in part mediated by ERK1/2. Nevertheless, there is no direct evidence that ERK1/2 regulates adult neurogenesis under normal physiological conditions.

ERK5 shares high sequence homology to ERK1/2. However, ERK5 contains a unique loop-12 linker between kinase sub-domains VII and VIII and an extended C-terminus making it distinct from other members of the MAPK family (Raman et al., 2007). The unique C-terminus contains a nuclear-localization signal (NLS), transcriptional activation domain, and a proline-rich region. ERK5 is normally localized in the cytoplasm, but upon stimulation by extrinsic factors, it translocates to the nucleus via the NLS sequence (English et al., 1995; Hayashi et al., 2004; Lee et al., 1995; Wang et al., 2006; Zhou et al., 1995). Additionally, ERK5 is capable of auto-phosphorylation through its large C-terminus, the region that is also required for the activation of nuclear substrates (Morimoto et al., 2007). ERK5 is activated specifically by the upstream kinase, MEK5, through dual phosphorylation at ERK5's TEY motif.

Our laboratory and others have demonstrated that neurotrophin-induced activation of ERK5 promotes the survival of newborn neurons during embryonic development (Cavanaugh et al., 2001; Finegan et al., 2009; Liu et al., 2003; Shalizi et al., 2003; Wang et al., 2006; Watson et al., 2001). ERK5 also specifies cortical stem/progenitor cells toward a neuronal lineage during development by phosphorylating and modulating the activity of Neurogenin 1 (*Neurog 1*), a proneural, helix-loop-helix transcription factor (Cundiff et al., 2009; Liu et al., 2006). ERK5 expression in the brain is high during early embryonic development but declines as the brain matures (Liu et al., 2003). It was originally thought that ERK5 is not expressed in the adult brain (Di Benedetto et al., 2007). However, recent data demonstrate specific and discrete expression of ERK5 in the adult neurogenic regions (Pan et al., 2012a; Pan et al., 2012d), implying an important and novel regulatory role of ERK5 in adult neurogenesis. Indeed, using a tamoxifen-inducible knockout mouse strain to conditionally delete *Mapk7*, the gene encoding ERK5, in nestin-expressing aNSCs in the adult mice, it was demonstrated that inhibition of endogenous ERK5 signaling significantly reduces the total number of adult-born neurons (Pan et al., 2012d). This is most likely due to a deficit in neuronal differentiation. Inhibition of endogenous ERK5 signaling in SGZ-derived aNSCs by RNA interference significantly increases the pool of Sox2⁺ and nestin⁺ neural stem cells, as well as PCNA⁺ proliferating cells while concomitantly decreased the pool of β -tubulin⁺ neurons. Furthermore, activating endogenous ERK5 signaling by retroviral infection with a constitutive active MEK5 increases the production of adult-born neurons both *in vitro* and *in vivo*. These results identify ERK5 MAP kinase as a novel signaling pathway that regulates adult neurogenesis in the hippocampus, especially in neuronal differentiation. It has also been shown that Neurogenin 2 (*Neurog 2*) is a downstream target while neurotrophin 3 (NT3) is an upstream activator of ERK5 in this process. Neurogenin 2 belongs to the family of proneural basic helix-loop-helix transcription factors that includes Neurogenin 1. Unlike Neurogenin 1, which is only expressed in the developing brain, Neurogenin 2 is expressed in both embryonic brains as well as in adult hippocampal neural progenitor cells (Hodge et al., 2008; Ozen et al., 2007).

Mechanistic studies highlighted above provide an overview of our current understanding of regulatory mechanisms responsible for adult SGZ neurogenesis. Early studies also identified signaling molecules including Sonic Hedgehog, TrkB/C, Notch, and Wnt/ β -catenin in the regulation of adult SGZ neurogenesis in the rodent brain (Breunig et al., 2007; Breunig et al., 2008; Han et al., 2008; Li et al., 2008; Lie et al., 2005; Pozniak and Pleasure, 2006; Rolls et al., 2007). Transcription factors Pax6, Sox2, Mash1/Ascl1, NeuroD, Tbr2 and the master regulator REST/NRSF are expressed in the SGZ (Faigle and Song, 2012; Gao et al., 2009; Gao et al., 2011; Hodge and Hevner, 2011; Hodge et al., 2008; Hsieh, 2012; Kim et al., 2007; Komitova and Eriksson, 2004; Kuwabara et al., 2009; Osumi et al., 2008; Suh et al., 2007). However, many of these proteins are expressed outside of adult neurogenic regions in the brain. Earlier studies using non-inducible knockout mouse models may be confounded by a lack of specificity in targeting aNSCs in adult animals and/or by compensatory mechanisms during development. Future studies using inducible and conditional knockout mouse models such as those employed in the FMRP and ERK5 studies (Guo et al., 2011; Pan et al., 2012d) should clarify some of those issues and provide a better understanding of signaling mechanisms governing adult neurogenesis.

Adult neurogenesis and hippocampus-dependent learning and memory

Since many recent reviews have addressed issues concerning the role of adult neurogenesis in hippocampus-dependent memory formation (Deng et al., 2010; Kazanis, 2012) (Kim et al., 2011) (Lee et al., 2012) (Marin-Burgin and Schinder, 2012) (Ming and Song, 2011) (Song et al., 2012), we will only attempt to highlight a few of the published studies here. In the past decade, several experimental approaches have been used to ascertain whether

modulating normal levels of adult SGZ neurogenesis affects learning and memory. However, data from different labs have been contradictory and the importance of adult neurogenesis for memory formation remains controversial. For example, although some studies showed that adult neurogenesis plays a role in contextual fear memory formation (Guo et al., 2011; Imayoshi et al., 2008; Saxe et al., 2006), contextual fear memory extinction (Deng et al., 2009), and contextual fear memory transfer out of the hippocampus (Kitamura et al., 2009), several other studies do not support a role for adult neurogenesis in contextual fear memory formation (Deng et al., 2009; Dupret et al., 2008; Jaholkowski et al., 2009; Shors et al., 2002; Zhang et al., 2008). Additionally, one study found that x-irradiated mice did not exhibit a deficit in contextual fear conditioning while rats did (Snyder et al., 2009), thereby suggesting a species-specific difference in the requirement of adult-born neurons for contextual learning. Similarly, a number of studies implicated adult neurogenesis in spatial learning and memory (Clelland et al., 2009; Creer et al., 2010; Dupret et al., 2008; Farioli-Vecchioli et al., 2008; Garthe et al., 2009; Guo et al., 2011; Imayoshi et al., 2008; Jessberger et al., 2009; Zhang et al., 2008), in contrast to others (Denis-Donini et al., 2008; Jaholkowski et al., 2009; Meshi et al., 2006; Saxe et al., 2006; Shors et al., 2002).

It is not totally clear why different studies have led to contradictory conclusions. Some possibilities include differences in the sex and strain of the animals, and details of the experimental design of behavior assays which could influence the level of difficulty of the training tasks. Perhaps one of the important factors responsible for the inconsistent results from earlier studies is the specificity of the methods used to ablate adult neurogenesis. Early studies used x-irradiation or treatment with anti-mitotic drugs. These treatments are effective at reducing adult neurogenesis, but may not necessarily be specific to aNPCs because these methods may also affect neurons and glia. These treatments may also cause side effects including general health deterioration of the animal and neural inflammation. In other studies, specific genes such as cyclin D2, Wnt, PC3/Tis21, TLX and PI3K were deleted or gene products pharmacologically inhibited (Denis-Donini et al., 2008; Farioli-Vecchioli et al., 2009; Jaholkowski et al., 2009; Jessberger et al., 2009; Zhang et al., 2008). However, these genes are also expressed outside of the adult neurogenic regions and the deletions in these genes in transgenic mice or pharmacological inhibition of the specific molecules were not restricted to adult neurogenic regions. The potential off-target effects associated with these various approaches may account for some of the contradictions in the literature.

Despite the controversy, these earlier studies are interesting and exciting, and provided valuable information regarding the potential function of adult-born neurons on hippocampus-dependent function. However, the contradictory results in the literature underscored the importance of using more specific genetic approaches with less off-target side effects to manipulate adult neurogenesis. More recent studies employed transgenic expression of lethal genes including diphtheria toxin (Imayoshi et al., 2008), thymidine kinase (Deng et al., 2009; Saxe et al., 2006), or the pro-apoptotic protein Bax (Dupret et al., 2008) to selectively kill newborn cells in the adult brain. These transgenic approaches are more focused because the effects are specific to adult neural stem cells. Nevertheless, the controversy persisted. For example, thymidine kinase (tk) was expressed under the control of the nestin promoter (Deng et al., 2009) or GFAP promoter (Saxe et al., 2006) of two lines of transgenic mice. Administration of the antiviral prodrug ganciclovir (GCV) effectively kills dividing, thymidine kinase-expressing neural progenitor/precursor cells. However, these two almost identical approaches for deleting adult born neurons gave opposite conclusions regarding adult neurogenesis and contextual fear conditioning: the GFAP-tk mice were impaired in the contextual fear conditioning when tested 24 h after a 0.7 mA foot shock (1 s shock) while the nestin-tk transgenic mice (2 s shock) were not. Several factors may have contributed to the inconsistent results: the duration of the foot shock (1 s vs. 2 s)

or the different promoters used to direct the tk gene expression. It is also conceivable that the degree of GCV-thymidine kinase triggered cell death exceeded normal physiological level, thus causing side effects.

Recent advances in transgenic mouse technology have made it feasible to conditionally disrupt a gene specifically in adult neurogenic regions. For example, by crossing transgenic mice containing a floxed allele of a gene of interest with the nestin-cre-ER^{T2} or nestin-cre-ERTM driver mice (Kuo et al., 2006, Imayoshi, 2008 #5893; Lagace et al., 2007), the gene of interest can be specifically deleted in nestinexpressing adult neural stem cells by cre-recombinase following tamoxifen drug administration in adult mice. Indeed, a recent study used this technology to delete the *fmrp* gene in aNSCs of nestin-cre-ER^{T2}/FMRP^{lox/lox} mice (Guo et al., 2011). These mice are deficient in pattern separation as well as contextual and tone trace learning. This technology was also used to delete ERK5 in aNSCs, which will be discussed in more detail in the following sections.

Recent studies have begun to dissect the specific role of adult-born neurons in different stages of memory formation. Using transgenic mouse technology to specifically block synaptic output of older granular neurons while leaving the immature granular neurons intact, Nakashiba et al discovered unique functions of old vs. young granular neurons in pattern separation; young neurons mediate pattern separation while old neurons are required for pattern completion (Nakashiba et al., 2012). Using a combination of retroviral and optogenetic approaches to birthdate and reversibly control a group of adult-born neurons, it was found that reversibly silencing 4-week-old neurons after training, but not neurons of other ages, significantly disrupted hippocampal memory retrieval (Gu et al., 2012). These results suggest a specific time window for adult-born neurons in hippocampal memory retrieval.

The role of ERK5 regulated adult neurogenesis in hippocampus-dependent learning and memory and in contextual fear extinction

We generated the nestin-creERTM/ERK5^{loxP/loxP} transgenic mouse model to inducibly and conditionally knockout *Mapk7*, the gene encoding ERK5 (ERK5 icKO mice) specifically in adult neurogenic regions upon tamoxifen administration (Pan et al., 2012a; Pan et al., 2012d). There was a significant reduction in the number of adult-born neurons in the DG *in vivo* for at least four months. This provides an extended time (4 months) for behavior tests. The ERK5 icKO mice provide a unique and powerful tool to investigate the relationship between adult neurogenesis and hippocampus-dependent learning and memory because ERK5 expression in adult brain is generally absent in the adult brain (Di Benedetto et al., 2007) but specific to adult neurogenic regions (Pan et al., 2012a). Furthermore, ERK5 deletion in the adult brain was temporally and spatially regulated and specific to neural stem cells in ERK5 icKO mice (Pan et al., 2012a; Pan et al., 2012d).

To assess the physiological role of ERK5-regulated adult neurogenesis for hippocampal memory formation, adult male nestin-creERTM/ERK5^{loxP/loxP} mice (10–12 weeks old) were treated with tamoxifen to delete ERK5. These mice are designated ERK5 icKO. Several lines of control mice were used for the studies. In one set of experiments, we included only one control group of mice, the adult male nestin-creERTM/ERK5^{loxP/loxP} littermates treated with the vehicle for tamoxifen as a control for the genotype (Pan et al., 2012a). To address the concern that the behavioral defects observed with the ERK5 icKO mice reported in that study might be due to adverse effect of tamoxifen treatment (Fitzsimons, 2012), adult male ERK5^{loxP/loxP} littermates treated with tamoxifen were used as a drug control (Figures 1–7). These new data confirm behavioral defects observed with the ERK5 icKO mice (Pan et al., 2012a) and alleviate the concern regarding tamoxifen toxicity (Fitzsimons, 2012). Our more

recent data includes all possible controls for the ERK5 icKO mice (Pan et al., 2012c). We also confirmed that hippocampus-independent learning and memory, including cued-fear conditioning, was not affected in ERK5 icKO mice (Figures 4 and 5, and (Pan et al., 2012a; Pan et al., 2012c)).

There is no statistically significant difference between ERK5 icKO and either groups of control mice (genotype control or tamoxifen drug control) in their open field activities or their ability to habituate to the open field over three consecutive days (Figure 1 and (Pan et al., 2012a)). ERK5 icKO mice also do not have defects in memory for novel objects measured 1 h or 24 h after training (Figure 2 and (Pan et al., 2012a)). These results suggest that ERK5 icKO mice are not deficient in their general mobility, curiosity to explore a novel environment, or short-term memory. Furthermore, ERK5 icKO mice are normal in the visible platform and hidden platform water maze tests (Figure 3 and (Pan et al., 2012a)), thus they are not ataxic, or deficient in vision, general mobility, ability to swim, procedural learning, or motivation to escape from water. Although ERK5 icKO mice spent significantly less time swimming and searching for the escape platform in quadrant 1 compared with drug control animals during the probe test, their spatial memory for the location of the hidden platform was similar. Finally, ERK5 icKO mice exhibit a normal level of contextual fear memory when trained with a standard 0.7 mA foot shock and tested 24 h post-training (Figure 4 and (Pan et al., 2012a)).

However, unlike control mice, ERK5 icKO animals fail at remembering the novel object 48 h after training (Figure 2 and (Pan et al., 2012a)), implicating adult neurogenesis in long-term memory. Several lines of evidence also suggest that ERK5 and ERK5-regulated adult neurogenesis are important for the more complex and challenging forms of hippocampus-dependent learning and memory tasks, including contextual fear and spatial learning and memory. ERK5 icKO mice show reduced contextual fear memory when trained with 0.3 mA $\times$ 3 foot shocks (Figure 5 and (Pan et al., 2012a)). This training paradigm may be more challenging compared to the single 0.7 mA foot shock because wild type mice have to be shocked three successive times to generate contextual memory as strong as that generated with a single 2 s, 0.7 mA foot shock. The impaired contextual fear memory of ERK5 icKO mice after training using the weak foot shock paradigm (0.3 mA) is due to a deficit in consolidation and persistence of contextual fear memory, but not in memory acquisition or retrieval (Pan et al., 2012c).

Once trained in the hidden platform test of the Morris water maze task, ERK5 icKO mice were subjected to reversal training in which the hidden platform was moved to the opposite location. ERK5 icKO mice swim longer distances than control mice to find the new platform during reversal training and they could not distinguish between the old and new target quadrants during the reversal probe test (Figure 3 and (Pan et al., 2012a)). Reversal training is a complex form of spatial learning and memory because animals need to actively forget the old location of the hidden platform and learn to find it in a new location.

Another well accepted spatial learning and memory assay is pattern separation, which specifically requires the function of DG (Clelland et al., 2009; Farioli-Vecchioli et al., 2008; Guo et al., 2011). Pattern separation is also considered a difficult form of hippocampal memory formation because it tests the process of transforming similar representations of memories into highly dissimilar, non-overlapping representations (Clelland et al., 2009; Guo et al., 2011). ERK5 icKO animals do not perform as well as control animals in the pattern separation assay (Figure 6 and (Pan et al., 2012a)). These data are consistent with other reports implicating adult neurogenesis in pattern separation (Clelland et al., 2009; Creer et al., 2010; Farioli-Vecchioli et al., 2008).

Finally, unlike control mice, ERK5 icKO animals fail to extinguish contextual fear memory even after repeated exposure (twice a day for 8 d) to the training context without foot shock (Figure 4 and (Pan et al., 2012a)). These data suggest that adult neurogenesis may regulate fear extinction, a notion supported by another study (Deng et al., 2009). Contextual fear extinction is a form of hippocampus-dependent learning and memory, distinct from contextual fear conditioning (Fischer et al., 2007). It may be more demanding and complex than contextual fear conditioning because animals learn to actively dissociate the context from the foot shock. For example, while it takes only a single 2 s, 0.7 mA foot shock to establish a strong, thus relatively easy, form of the initial contextual fear memory that persists at least 15 weeks (Pan et al., 2012c), it takes repeated trials of context exposure over the course of 8 d without the foot shock for this memory to undergo extinction in control mice (Pan et al., 2012a)).

It is interesting that ERK5 deletion interferes with contextual fear memory extinction and reversal of hidden platform training. It is possible that adult neurogenesis may be particularly important for learning that requires active forgetting of a prior memory.

The role of ERK5 regulated adult neurogenesis in the establishment and maintenance of remote contextual fear memory

How long-term, or remote memory is formed and maintained is an important question. Recent studies have shed light on mechanisms responsible for the formation of recent hippocampus-dependent memories, including a role for adult neurogenesis in this process. However, far less is known how recent memories are transformed into remote memories and how remote memories, once established, are maintained (Frankland and Bontempi, 2005). Contextual fear memory is initially formed in the hippocampus but is thought to be transferred out to extra-hippocampal regions including the cortex for long-term storage. Whether the maintenance of established remote contextual memory requires the function of the hippocampus is controversial with some evidence supporting (Debiec et al., 2002; Goshen et al., 2011; Sutherland and Lehmann, 2011) as well as against its involvement (Anagnostaras et al., 1999; Frankland and Bontempi, 2005; Kim and Fanselow, 1992; Squire and Alvarez, 1995; Squire and Zola-Morgan, 1991). A recent study implicated adult neurogenesis in modulating the duration of the hippocampus-dependent period of contextual fear memory; disruption of adult neurogenesis accelerated the transfer of memory out of the hippocampus but did not affect the strength of the remote memory *per se* (Kitamura et al., 2009).

We discovered that ERK5 icKO mice have no remote memory 21 days after training with a 2 s, 0.7 mA foot shock in the passive avoidance assay although their 24 h memory is completely normal (Figure 7, and (Pan et al., 2012a)). Similarly, their remote contextual fear memory at 5 weeks post-training with the 0.7 mA foot shock is greatly attenuated (Pan et al., 2012c). The deficits in remote memory are specific to hippocampus-dependent tasks and do not occur with hippocampus-independent, remote (up to 16 weeks) cued-fear memory. These data are the first evidence that adult neurogenesis is required for the establishment of full strength remote contextual fear memory.

Interestingly, deletion of ERK5 to attenuate adult neurogenesis at 6 d post-training, a time point when mice had sufficient time to consolidate contextual fear memory but it is still within the window of the hippocampus-dependent period, causes decay of remote contextual fear memory but not remote cued-fear memory (Pan et al., 2012c). Perhaps more profoundly, when tamoxifen is administered 5 weeks post-training, a time point when remote contextual memory has already been established, ERK5 deletion causes retrograde amnesia of remote contextual fear memory in ERK5 icKO mice, again without affecting

remote cued-fear memory (Pan et al., 2012c). These data suggest that ongoing ERK5-regulated adult neurogenesis is required for the maintenance of remote contextual fear memory, even after this memory has been established.

Collectively, studies using ERK5 icKO indicate a critical role for adult-born neurons in both the expression and maintenance of remote contextual fear memory using three different paradigms, exemplified with observed deficits when ERK5 is conditionally deleted prior to training, 6 d after training, or 5 weeks after training. These data supports the hypothesis that interactions between the hippocampus and neocortex, after the formation of recent memory, is critical for the storage of remote memory regardless of the exact storage location for remote memory (Frankland and Bontempi, 2005; Nakashiba et al., 2009; Nakashiba et al., 2008). Together with a recent report using diphtheria toxin-based strategy to ablate mature, adult-generated neurons within the week after training (Arruda-Carvalho et al., 2011), these data suggest that interference with adult neurogenesis can cause retrograde amnesia.

Enhancing memory formation by increasing adult neurogenesis

Enriched environment (Birch et al., 2013; Kempermann et al., 1997), voluntary running (Creer et al., 2010; van Praag et al., 1999), and chronic antidepressant treatment (David et al., 2009) increases adult neurogenesis and improves memory formation. Petrik and colleagues recently discovered that Isx-9, a synthetic small molecule, increases hippocampal neurogenesis and the time spent in the target quadrant in the Morris water maze probe test (Petrik et al., 2012). Metformin, a clinical drug for type II diabetes, also enhances adult hippocampal neurogenesis and spatial learning in the reversal Morris water maze test (Wang et al., 2012). These pioneering studies established a strong correlative relationship and generated much excitement about the possibility that enhancing adult neurogenesis may improve cognition (Curlik and Shors, 2013; Glasper et al., 2012).

Utilizing the Nestin-Cre-ER^{T2}:Bax mice to delete the pro-apoptotic gene *Bax* specifically in adult neural stem cells, Sahay and colleagues reported that more adult-born neurons survived in these mice and that the mice show enhanced pattern separation (Sahay et al., 2011a). We recently generated a conditional knock-in line of mice in which expression of a constitutive active MEK5 can be induced, allowing selective and conditional activation of endogenous ERK5 in adult neurogenic regions (Wenbin Wang, Zhengui Xia et al., unpublished data). These mice show increases in adult hippocampal neurogenesis and most interestingly, extension of long-term spatial memory. This conditional and inducible gene manipulation allows induction of adult neurogenesis without augmenting other biochemical or biological pathways. These studies provide strong evidence that enhancement of adult neurogenesis alone is sufficient to improve cognition.

Potential mechanisms by which adult neurogenesis may regulate long-term memory

ERK5 conditional knockout studies demonstrated that ongoing adult neurogenesis is necessary and required for memory persistence, even after the memory has transferred to extra-hippocampal regions (Pan et al., 2012c). Conversely, conditional knock-in of activated endogenous ERK5 is sufficient to promote adult neurogenesis and long-term hippocampus-dependent memory (Wenbin Wang, Zhengui Xia et al., unpublished data). How do adult-born neurons in the hippocampus contribute to memory persistence? Memory persistence may require recurrent rounds of consolidation because it requires repeated rounds of transcription and protein synthesis, for example, for the production of NMDA receptors (Bekinschtein et al., 2007; Cui et al., 2004). The cAMP/ERK1/2 MAP kinase/CREB pathway is important for memory consolidation and is activated during REM sleep in the

circadian cycle (Eckel-Mahan et al., 2008 ; Luo et al., 2013). The recurrent activation of this pathway during REM sleep may generate repeated rounds of transcription and translation necessary to sustain memory. Interestingly, the same sequence of synaptic firing that occurs during spatial learning is repeated during sleep (Lee and Wilson, 2002). Perhaps new synapses, including those from adult-born neurons, are recruited to the circuitry during repeated synaptic firing in sleep for continued maintenance of established memories. Immature adult born neurons and mature neurons in the dentate gyrus have a different activation threshold. Weak afferent activity activates a significant proportion of the immature adult born neurons but not mature neurons in the dentate gyrus (Marin-Burgin et al., 2012). Since most memory traces, even long-term memories, eventually decay, it is tempting to speculate that the strength of recurrent synaptic firing may gradually reduce over time, leading to memory decay. Because of their higher sensitivity to lower threshold stimulation, immature adult-born neurons may be uniquely suited for new synapse recruitment during recurrent synaptic firing in sleep. When adult neurogenesis is attenuated, fewer synapses from adult-born neurons will respond to the gradually weakening synaptic firing and be recruited to the circuitry, thus accelerating memory decay. However, increasing adult neurogenesis may increase the pool of responding synapses, thus strengthening the circuitry and extending long-term memory.

ERK5 regulation of adult neurogenesis in the SVZ and in olfactory memory

ERK5 is also expressed in SVZ (Pan et al., 2012b). Deletion of ERK5 reduces adult neurogenesis in the SVZ and olfactory bulb (OB) of the ERK5 icKO mice. ERK5 has also been implicated in prolactin stimulation of adult neurogenesis in the SVZ/OB (Wang et al., 2013). Conditional deletion of ERK5 in ERK5 icKO mice has no effect on olfactory discrimination among discrete odorants. However, it reduces short-term olfactory memory as well as detection sensitivity to odorants and pheromones. Furthermore, ERK5 icKO mice show impaired acquisition of odor-cued associative olfactory learning. These data suggest that ERK5 MAP kinase is critical for the regulation of adult neurogenesis in the SVZ/OB, and provide strong evidence supporting a role for adult neurogenesis in several distinct complex forms of olfactory behavior.

Summary and Conclusions

Adult neurogenesis has received much attention in large part because of the therapeutic potential of adult-born neurons. Delineating molecular mechanisms involved in the genesis of functional adult-born neurons will aid in the full understanding of how adult neurogenesis is regulated from stem cell self-renewal, proliferation, cell-fate specification, differentiation, maturation and functional integration into the existing neuronal circuitry. To realize the therapeutic potential of aNSCs, it is important to clarify the roles of various intrinsic and extrinsic mechanisms regulating this process.

The functional impact of adult-born neurons in the SGZ for various forms of hippocampus-dependent learning and memory supports the idea that the brain is not static in its adult form and is capable of continuously generating functional neurons. Studies reviewed in this manuscript identify the ERK5 MAP kinase as a novel kinase signaling pathway regulating adult neurogenesis in the SGZ and in several challenging forms of hippocampus-dependent learning and memory tasks including contextual fear extinction. Furthermore, studies with ERK5 conditional knockout mice provide the first evidence that continual formation of new neurons in the DG is integral for the maintenance of remote contextual fear memory, even after the memory has transferred out of the hippocampus. This conclusion supports the general hypothesis that the hippocampus has a long-term role in the continued expression of contextual fear memory (Sparks et al., 2011; Sutherland et al., 2008). The discovery that

memory formation and persistence can be improved by directly stimulating adult neurogenesis has important therapeutic implications for the treatment of memory disorders. Because of its selective pattern of expression in neurogenic regions of adult brain, modulation of the ERK5 signaling pathway may provide a unique pharmacological window of opportunity for stimulation of adult neurogenesis.

References

- Aimone JB, Deng W, Gage FH. Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. *Neuron*. 2011; 70:589–596. [PubMed: 21609818]
- Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol*. 1965; 124:319–335. [PubMed: 5861717]
- Anagnostaras SG, Maren S, Fanselow MS. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci*. 1999; 19:1106–1114. [PubMed: 9920672]
- Arruda-Carvalho M, Sakaguchi M, Akers KG, Josselyn SA, Frankland PW. Posttraining ablation of adult-generated neurons degrades previously acquired memories. *J Neurosci*. 2011; 31:15113–15127. [PubMed: 22016545]
- Bekinschtein P, Cammarota M, Igaz LM, Bevilaqua LR, Izquierdo I, Medina JH. Persistence of long-term memory storage requires a late protein synthesis- and BDNF- dependent phase in the hippocampus. *Neuron*. 2007; 53:261–277. [PubMed: 17224407]
- Belvindrah R, Lazarini F, Lledo PM. Postnatal neurogenesis: from neuroblast migration to neuronal integration. *Rev Neurosci*. 2009; 20:331–346. [PubMed: 20397619]
- Birch AM, McGarry NB, Kelly AM. Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. *Hippocampus*. 2013
- Breunig JJ, Arellano JI, Macklis JD, Rakic P. Everything that glitters isn't gold: a critical review of postnatal neural precursor analyses. *Cell Stem Cell*. 2007; 1:612–627. [PubMed: 18371403]
- Breunig JJ, Sarkisian MR, Arellano JI, Morozov YM, Ayoub AE, Sojitra S, Wang B, Flavell RA, Rakic P, Town T. Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signaling. *Proc Natl Acad Sci U S A*. 2008; 105:13127–13132. [PubMed: 18728187]
- Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, Kuhn HG. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur J Neurosci*. 2003; 17:2042–2046. [PubMed: 12786970]
- Bull ND, Bartlett PF. The adult mouse hippocampal progenitor is neurogenic but not a stem cell. *J Neurosci*. 2005; 25:10815–10821. [PubMed: 16306394]
- Cavanaugh JE, Ham J, Hetman M, Poser S, Yan C, Xia Z. Differential regulation of mitogen-activated protein kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons. *J Neurosci*. 2001; 21:434–443. [PubMed: 11160424]
- Clelland CD, Choi M, Romberg C, Clemenson GD, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*. 2009; 325:210–213. [PubMed: 19590004]
- Creer DJ, Romberg C, Saksida LM, vanPraag H, Bussey TJ. Running enhances spatial pattern separation in mice. *Proc Natl Acad Sci U S A*. 2010; 107:2367–2372. [PubMed: 20133882]
- Cui Z, Wang H, Tan Y, Zaia KA, Zhang S, Tsien JZ. Inducible and reversible NR1 knockout reveals crucial role of the NMDA receptor in preserving remote memories in the brain. *Neuron*. 2004; 41:781–793. [PubMed: 15003177]
- Cundiff P, Liu L, Wang Y, Zou J, Pan YW, Abel G, Duan X, Ming GL, Englund C, Hevner R, Xia Z. ERK5 MAP kinase regulates Neurogenin1 during cortical neurogenesis. *PLoS ONE*. 2009; 4:5204.
- Curlik DM 2nd, Shors TJ. Training your brain: Do mental and physical (MAP) training enhance cognition through the process of neurogenesis in the hippocampus? *Neuropharmacology*. 2013; 64:506–514. [PubMed: 22898496]

- David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevec IA, Leonardo ED, Hen R. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*. 2009; 62:479–493. [PubMed: 19477151]
- David DJ, Wang J, Samuels BA, Rainer Q, David I, Gardier AM, Hen R. Implications of the functional integration of adult-born hippocampal neurons in anxiety-depression disorders. *Neuroscientist*. 2010; 16:578–591. [PubMed: 20889967]
- Debiec J, LeDoux JE, Nader K. Cellular and systems reconsolidation in the hippocampus. *Neuron*. 2002; 36:527–538. [PubMed: 12408854]
- Deng W, Aimone JB, Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci*. 2010; 11:339–350. [PubMed: 20354534]
- Deng W, Saxe MD, Gallina IS, Gage FH. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *J Neurosci*. 2009; 29:13532–13542. [PubMed: 19864566]
- Denis-Donini S, Dellarole A, Crociara P, Francese MT, Bortolotto V, Quadrato G, Canonico PL, Orsetti M, Ghi P, Memo M, Bonini SA, Ferrari-Toninelli G, Grilli M. Impaired adult neurogenesis associated with short-term memory defects in NF-kappaB p50-deficient mice. *J Neurosci*. 2008; 28:3911–3919. [PubMed: 18400889]
- Di Benedetto B, Hitz C, Holter SM, Kuhn R, Vogt Weisenhorn DM, Wurst W. Differential mRNA distribution of components of the ERK/MAPK signalling cascade in the adult mouse brain. *J Comp Neurol*. 2007; 500:542–556. [PubMed: 17120291]
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999; 97:703–716. [PubMed: 10380923]
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci*. 1997; 17:5046–5061. [PubMed: 9185542]
- Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN, Piazza PV. Spatial relational memory requires hippocampal adult neurogenesis. *PLoS One*. 2008; 3:1959.
- Eckel-Mahan K, Phan T, Han S, Wang H, Chan G, Scheiner Z, Storm D. Circadian Oscillation of MAPK Activity and cAMP in the Hippocampus: Implications for Memory Persistence. *Nature Neuroscience*. 2008 in press.
- Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming GL, Overstreet-Wadiche LS. Adult neurogenesis, mental health, and mental illness: hope or hype? *J Neurosci*. 2008; 28:11785–11791. [PubMed: 19005040]
- English JM, Vanderbilt CA, Xu S, Marcus S, Cobb MH. Isolation of MEK5 and differential expression of alternatively spliced forms. *J. Biol. Chem*. 1995; 270:28897–28902. [PubMed: 7499418]
- Epp JR, Spritzer MD, Galea LA. Hippocampus-dependent learning promotes survival of new neurons in the dentate gyrus at a specific time during cell maturation. *Neuroscience*. 2007; 149:273–285. [PubMed: 17900815]
- Faigle R, Song H. Signaling mechanisms regulating adult neural stem cells and neurogenesis. *Biochim Biophys Acta*. 2012
- Farioli-Vecchioli S, Saraulli D, Costanzi M, Leonardi L, Cina I, Micheli L, Nutini M, Longone P, Oh SP, Cestari V, Tirone F. Impaired terminal differentiation of hippocampal granule neurons and defective contextual memory in PC3/Tis21 knockout mice. *PLoS One*. 2009; 4:e8339. [PubMed: 20020054]
- Farioli-Vecchioli S, Saraulli D, Costanzi M, Pacioni S, Cina I, Aceti M, Micheli L, Bacci A, Cestari V, Tirone F. The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. *PLoS Biol*. 2008; 6:e246. [PubMed: 18842068]
- Finegan KG, Wang X, Lee EJ, Robinson AC, Tournier C. Regulation of neuronal survival by the extracellular signal-regulated protein kinase 5. *Cell Death Differ*. 2009; 16:674–683. [PubMed: 19148185]

- Fischer A, Radulovic M, Schrick C, Sananbenesi F, Godovac-Zimmermann J, Radulovic J. Hippocampal Mek/Erk signaling mediates extinction of contextual freezing behavior. *Neurobiol Learn Mem.* 2007; 87:149–158. [PubMed: 16979915]
- Fitzsimons CPVE, Lucassen PJ. Inhibition of adult neurogenesis through ERK5 knockdown impairs complex hippocampus-dependent spatial memory tasks. *Future Neurology.* 2012; 7:531–535.
- Fournier NM, Lee B, Banasr M, Elsayed M, Duman RS. Vascular endothelial growth factor regulates adult hippocampal cell proliferation through MEK/ERK- and PI3K/Akt-dependent signaling. *Neuropharmacology.* 2012; 63:642–652. [PubMed: 22580375]
- Frankland PW, Bontempi B. The organization of recent and remote memories. *Nat Rev Neurosci.* 2005; 6:119–130. [PubMed: 15685217]
- Gage FH. Mammalian neural stem cells. *Science.* 2000; 287:1433–1438. [PubMed: 10688783]
- Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. Multipotent progenitor cells in the adult dentate gyrus. *J Neurobiol.* 1998; 36:249–266. [PubMed: 9712308]
- Gao Z, Ure K, Ables JL, Lagace DC, Nave KA, Goebbels S, Eisch AJ, Hsieh J. Neurod1 is essential for the survival and maturation of adult-born neurons. *Nat Neurosci.* 2009; 12:1090–1092. [PubMed: 19701197]
- Gao Z, Ure K, Ding P, Nashaat M, Yuan L, Ma J, Hammer RE, Hsieh J. The master negative regulator REST/NRSF controls adult neurogenesis by restraining the neurogenic program in quiescent stem cells. *J Neurosci.* 2011; 31:9772–9786. [PubMed: 21715642]
- Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLoS One.* 2009; 4:e5464. [PubMed: 19421325]
- Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron.* 2007; 54:559–566. [PubMed: 17521569]
- Glasper ER, Schoenfeld TJ, Gould E. Adult neurogenesis: optimizing hippocampal function to suit the environment. *Behav Brain Res.* 2012; 227:380–383. [PubMed: 21624398]
- Goshen I, Brodsky M, Prakash R, Wallace J, Gradinaru V, Ramakrishnan C, Deisseroth K. Dynamics of retrieval strategies for remote memories. *Cell.* 2011; 147:678–689. [PubMed: 22019004]
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci.* 1999; 2:260–265. [PubMed: 10195219]
- Gu Y, Arruda-Carvalho M, Wang J, Janoschka SR, Josselyn SA, Frankland PW, Ge S. Optical controlling reveals time-dependent roles for adultborn dentate granule cells. *Nat Neurosci.* 2012; 15:1700–1706. [PubMed: 23143513]
- Guo W, Allan AM, Zong R, Zhang L, Johnson EB, Schaller EG, Murthy AC, Goggin SL, Eisch AJ, Oostra BA, Nelson DL, Jin P, Zhao X. Ablation of Fmrp in adult neural stem cells disrupts hippocampus-dependent learning. *Nat Med.* 2011; 17:559–565. [PubMed: 21516088]
- Han YG, Spassky N, Romaguera-Ros M, Garcia-Verdugo JM, Aguilar A, Schneider-Maunoury S, Alvarez-Buylla A. Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat Neurosci.* 2008; 11:277–284. [PubMed: 18297065]
- Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Abel ED, Eliceiri B, Yang Y, Ulevitch RJ, Lee JD. Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. *J Clin Invest.* 2004; 113:1138–1148. [PubMed: 15085193]
- Hodge RD, Hevner RF. Expression and actions of transcription factors in adult hippocampal neurogenesis. *Dev Neurobiol.* 2011; 71:680–689. [PubMed: 21412988]
- Hodge RD, Kowalczyk TD, Wolf SA, Encinas JM, Rippey C, Enikolopov G, Kempermann G, Hevner RF. Intermediate progenitors in adult hippocampal neurogenesis: Tbr2 expression and coordinate regulation of neuronal output. *J Neurosci.* 2008; 28:3707–3717. [PubMed: 18385329]
- Howell OW, Doyle K, Goodman JH, Scharfman HE, Herzog H, Pringle A, Beck-Sickinger AG, Gray WP. Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. *J Neurochem.* 2005; 93:560–570. [PubMed: 15836615]
- Hsieh J. Orchestrating transcriptional control of adult neurogenesis. *Genes Dev.* 2012; 26:1010–1021. [PubMed: 22588716]
- Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itoharu S, Kageyama R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci.* 2008; 11:1153–1161. [PubMed: 18758458]

- Jaholkowski P, Kiryk A, Jedynak P, Ben Abdallah NM, Knapska E, Kowalczyk A, Piechal A, Blecharz-Klin K, Figiel I, Lioudyno V, Widy-Tyszkiewicz E, Wilczynski GM, Lipp HP, Kaczmarek L, Filipkowski RK. New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. *Learn Mem.* 2009; 16:439–451. [PubMed: 19553382]
- Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, Squire LR, Gage FH. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem.* 2009; 16:147–154. [PubMed: 19181621]
- Kageyama R, Imayoshi I, Sakamoto M. The role of neurogenesis in olfaction-dependent behaviors. *Behav Brain Res.* 2012; 227:459–463. [PubMed: 21557970]
- Kazanis I. Neurogenesis in the Adult Mammalian Brain: How Much Do We Need, How Much Do We Have? *Curr Top Behav Neurosci.* 2012
- Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat Neurosci.* 2007; 10:355–362. [PubMed: 17277773]
- Kempermann G. Neurogenesis in the Adult Olfactory System. New York: Oxford University Press; 2006. Adult Neurogenesis; p. 140–167.
- Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. *Nature.* 1997; 386:493–495. [PubMed: 9087407]
- Kim EJ, Leung CT, Reed RR, Johnson JE. In vivo analysis of Ascl1 defined progenitors reveals distinct developmental dynamics during adult neurogenesis and gliogenesis. *J Neurosci.* 2007; 27:12764–12774. [PubMed: 18032648]
- Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. *Science.* 1992; 256:675–677. [PubMed: 1585183]
- Kim WR, Christian K, Ming GL, Song H. Time-dependent involvement of adult-born dentate granule cells in behavior. *Behav Brain Res.* 2011
- Kitamura T, Saitoh Y, Takashima N, Murayama A, Niibori Y, Ageta H, Sekiguchi M, Sugiyama H, Inokuchi K. Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory. *Cell.* 2009; 139:814–827. [PubMed: 19914173]
- Komitova M, Eriksson PS. Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. *Neurosci Lett.* 2004; 369:24–27. [PubMed: 15380301]
- Kuida K, Boucher DM. Functions of MAP kinases: insights from genetargeting studies. *J Biochem (Tokyo).* 2004; 135:653–656. [PubMed: 15213239]
- Kuo CT, Mirzadeh Z, Soriano-Navarro M, Rasin M, Wang D, Shen J, Sestan N, Garcia-Verdugo J, Alvarez-Buylla A, Jan LY, Jan YN. Postnatal deletion of Numb/Numbl reveals repair and remodeling capacity in the subventricular neurogenic niche. *Cell.* 2006; 127:1253–1264. [PubMed: 17174898]
- Kuwabara T, Hsieh J, Muotri A, Yeo G, Warashina M, Lie DC, Moore L, Nakashima K, Asashima M, Gage FH. Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. *Nat Neurosci.* 2009; 12:1097–1105. [PubMed: 19701198]
- Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, Donovan MH, Fischer SJ, Farnbauch LA, Beech RD, DiLeone RJ, Greer CA, Mandyam CD, Eisch AJ. Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci.* 2007; 27:12623–12629. [PubMed: 18003841]
- Lee AK, Wilson MA. Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron.* 2002; 36:1183–1194. [PubMed: 12495631]
- Lee JD, Ulevitch RJ, Han J. Primary structure of BMK1: a new mammalian map kinase. *Biochem. Biophys. Res. Commun.* 1995; 213:715–724. [PubMed: 7646528]
- Lee SW, Clemenson GD, Gage FH. New neurons in an aged brain. *Behav Brain Res.* 2012; 227:497–507. [PubMed: 22024433]
- Leuner B, Gould E. Structural plasticity and hippocampal function. *Annu Rev Psychol.* 2010; 61:111–140. C111-113. [PubMed: 19575621]

- Leuner B, Mendolia-Loffredo S, Kozorovitskiy Y, Samburg D, Gould E, Shors TJ. Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. *J Neurosci*. 2004; 24:7477–7481. [PubMed: 15329394]
- Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, Bassel-Duby R, Parada LF. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron*. 2008; 59:399–412. [PubMed: 18701066]
- Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH. Wnt signalling regulates adult hippocampal neurogenesis. *Nature*. 2005; 437:1370–1375. [PubMed: 16251967]
- Liu L, Cavanaugh JE, Wang Y, Sakagami H, Mao Z, Xia Z. ERK5 activation of MEF2-mediated gene expression plays a critical role in BDNF-promoted survival of developing but not mature cortical neurons. *Proc. Natl. Acad. Sci. USA*. 2003; 100:8532–8537. [PubMed: 12826611]
- Liu L, Cundiff P, Abel G, Wang Y, Faigle R, Sakagami H, Xu M, Xia Z. Extracellular signal-regulated kinase (ERK) 5 is necessary and sufficient to specify cortical neuronal fate. *Proc Natl Acad Sci U S A*. 2006; 103:9697–9702. [PubMed: 16766652]
- Luo J, Phan TX, Yang Y, Garelick MG, Storm DR. Increases in cAMP, MAPK Activity, and CREB Phosphorylation during REM Sleep: Implications for REM Sleep and Memory Consolidation. *J Neurosci*. 2013; 33:6460–6468. [PubMed: 23575844]
- Ma DK, Kim WR, Ming GL, Song H. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Ann N Y Acad Sci*. 2009; 1170:664–673. [PubMed: 19686209]
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*. 2000; 20:9104–9110. [PubMed: 11124987]
- Marin-Burgin A, Mongiat LA, Pardi MB, Schinder AF. Unique processing during a period of high excitation/inhibition balance in adult-born neurons. *Science*. 2012; 335:1238–1242. [PubMed: 22282476]
- Marin-Burgin A, Schinder AF. Requirement of adult-born neurons for hippocampus-dependent learning. *Behav Brain Res*. 2012; 227:391–399. [PubMed: 21763727]
- Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat Neurosci*. 2006; 9:729–731. [PubMed: 16648847]
- Miloso M, Scuteri A, Foudah D, Tredici G. MAPKs as mediators of cell fate determination: an approach to neurodegenerative diseases. *Curr Med Chem*. 2008; 15:538–548. [PubMed: 18336268]
- Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci*. 2005; 28:223–250. [PubMed: 16022595]
- Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*. 2011; 70:687–702. [PubMed: 21609825]
- Morimoto H, Kondoh K, Nishimoto S, Terasawa K, Nishida E. Activation of a C-terminal transcriptional activation domain of ERK5 by autophosphorylation. *J Biol Chem*. 2007; 282:35449–35456. [PubMed: 17928297]
- Mu Y, Lee SW, Gage FH. Signaling in adult neurogenesis. *Curr Opin Neurobiol*. 2010; 20:416–423. [PubMed: 20471243]
- Nakashiba T, Buhl DL, McHugh TJ, Tonegawa S. Hippocampal CA3 output is crucial for ripple-associated reactivation and consolidation of memory. *Neuron*. 2009; 62:781–787. [PubMed: 19555647]
- Nakashiba T, Cushman JD, Pelkey KA, Renaudineau S, Buhl DL, McHugh TJ, Rodriguez Barrera V, Chittajallu R, Iwamoto KS, McBain CJ, Fanselow MS, Tonegawa S. Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell*. 2012; 149:188–201. [PubMed: 22365813]
- Nakashiba T, Young JZ, McHugh TJ, Buhl DL, Tonegawa S. Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science*. 2008; 319:1260–1264. [PubMed: 18218862]
- Okamoto S, Kang YJ, Brechtel CW, Siviglia E, Russo R, Clemente A, Harrop A, McKercher S, Kaul M, Lipton SA. HIV/gp120 decreases adult neural progenitor cell proliferation via checkpoint

- kinase-mediated cell-cycle withdrawal and G1 arrest. *Cell Stem Cell*. 2007; 1:230–236. [PubMed: 18371353]
- Osumi N, Shinohara H, Numayama-Tsuruta K, Maekawa M. Concise review: Pax6 transcription factor contributes to both embryonic and adult neurogenesis as a multifunctional regulator. *Stem Cells*. 2008; 26:1663–1672. [PubMed: 18467663]
- Ozen I, Galichet C, Watts C, Parras C, Guillemot F, Raineteau O. Proliferating neuronal progenitors in the postnatal hippocampus transiently express the proneural gene Ngn2. *Eur J Neurosci*. 2007; 25:2591–2603. [PubMed: 17466019]
- Pan YW, Chan GCK, Kuo CT, Storm DR, Xia Z. Inhibition of Adult Neurogenesis by Inducible and Targeted Deletion of ERK5 Mitogen-Activated Protein Kinase Specifically in Adult Neurogenic Regions Impairs Contextual Fear Extinction and Remote Fear Memory. *J Neurosci*. 2012a; 32:6444–6455. [PubMed: 22573667]
- Pan YW, Kuo CT, Storm DR, Xia Z. Inducible and Targeted Deletion of the ERK5 MAP Kinase in Adult Neurogenic Regions Impairs Adult Neurogenesis in the Olfactory Bulb and Several Forms of Olfactory Behavior. *PLoS One*. 2012b; 7:e49622. [PubMed: 23185386]
- Pan YW, Storm DR, Xia Z. The Maintenance of Established Remote Contextual Fear Memory Requires ERK5 MAP Kinase and Ongoing Adult Neurogenesis in the Hippocampus. *PLoS One*. 2012c; 7:e50455. [PubMed: 23189204]
- Pan YW, Zou J, Wang W, Sakagami H, Garelick MG, Abel G, Kuo CT, Storm DR, Xia Z. Inducible and conditional deletion of extracellular signal-regulated kinase 5 disrupts adult hippocampal neurogenesis. *J Biol Chem*. 2012d; 287:23306. [PubMed: 22645146]
- Petreanu L, Alvarez-Buylla A. Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J Neurosci*. 2002; 22:6106–6113. [PubMed: 12122071]
- Petrik D, Jiang Y, Birnbaum SG, Powell CM, Kim MS, Hsieh J, Eisch AJ. Functional and mechanistic exploration of an adult neurogenesis- promoting small molecule. *FASEB J*. 2012; 26:3148–3162. [PubMed: 22542682]
- Pozniak CD, Pleasure SJ. A tale of two signals: Wnt and Hedgehog in dentate neurogenesis. *Sci STKE*. 2006; 2006:pe5. [PubMed: 16434726]
- Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. *Oncogene*. 2007; 26:3100–3112. [PubMed: 17496909]
- Ramirez-Amaya V, Marrone DF, Gage FH, Worley PF, Barnes CA. Integration of new neurons into functional neural networks. *J Neurosci*. 2006; 26:12237–12241. [PubMed: 17122048]
- Rolls A, Shechter R, London A, Ziv Y, Ronen A, Levy R, Schwartz M. Toll-like receptors modulate adult hippocampal neurogenesis. *Nat Cell Biol*. 2007; 9:1081–1088. [PubMed: 17704767]
- Rubinfeld H, Seger R. The ERK cascade: a prototype of MAPK signaling. *MolBiotechnol*. 2005; 31:151–174.
- Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton AA, Dranovsky A, Hen R. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature*. 2011a; 472:466–470. [PubMed: 21460835]
- Sahay A, Wilson DA, Hen R. Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. *Neuron*. 2011b; 70:582–588. [PubMed: 21609817]
- Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci*. 2005; 25:1089–1094. [PubMed: 15689544]
- Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD, Sofroniew MV, Kandel ER, Santarelli L, Hen R, Drew MR. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci U S A*. 2006; 103:17501–17506. [PubMed: 17088541]
- Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature*. 2004; 429:184–187. [PubMed: 15107864]
- Schwarz TJ, Ebert B, Lie DC. Stem cell maintenance in the adult mammalian hippocampus: a matter of signal integration? *Dev Neurobiol*. 2012; 72:1006–1015. [PubMed: 22488809]
- Seger R, Krebs EG. The MAPK signaling cascade. *Faseb J*. 1995; 9:726–735. [PubMed: 7601337]

- Shalizi A, Lehtinen M, Gaudilliere B, Donovan N, Han J, Konishi Y, Bonni A. Characterization of a neurotrophin signaling mechanism that mediates neuron survival in a temporally specific pattern. *J. Neurosci.* 2003; 23:7326–7336. [PubMed: 12917366]
- Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci.* 2002; 22:3251–3261. [PubMed: 11943826]
- Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus.* 2002; 12:578–584. [PubMed: 12440573]
- Snyder JS, Choe JS, Clifford MA, Jeurling SI, Hurley P, Brown A, Kamhi JF, Cameron HA. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. *J Neurosci.* 2009; 29:14484–14495. [PubMed: 19923282]
- Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat Neurosci.* 2002; 5:438–445. [PubMed: 11953752]
- Song J, Christian KM, Ming GL, Song H. Modification of hippocampal circuitry by adult neurogenesis. *Dev Neurobiol.* 2012; 72:1032–1043. [PubMed: 22354697]
- Sparks FT, Lehmann H, Hernandez K, Sutherland RJ. Suppression of neurotoxic lesion-induced seizure activity: evidence for a permanent role for the hippocampus in contextual memory. *PLoS One.* 2011; 6:e27426. [PubMed: 22110648]
- Squire LR, Alvarez P. Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol.* 1995; 5:169–177. [PubMed: 7620304]
- Squire LR, Zola-Morgan S. The medial temporal lobe memory system. *Science.* 1991; 253:1380–1386. [PubMed: 1896849]
- Suh H, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH. In Vivo Fate Analysis Reveals the Multipotent and Self-Renewal Capacities of Sox2(+) Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell.* 2007; 1:515–528. [PubMed: 18371391]
- Suh H, Deng W, Gage FH. Signaling in adult neurogenesis. *Annu Rev Cell Dev Biol.* 2009; 25:253–275. [PubMed: 19575663]
- Sutherland RJ, Lehmann H. Alternative conceptions of memory consolidation and the role of the hippocampus at the systems level in rodents. *Curr Opin Neurobiol.* 2011; 21:446–451. [PubMed: 21592780]
- Sutherland RJ, O'Brien J, Lehmann H. Absence of systems consolidation of fear memories after dorsal, ventral, or complete hippocampal damage. *Hippocampus.* 2008; 18:710–718. [PubMed: 18446823]
- Taupin P. Neurogenesis in the adult central nervous system. *C R Biol.* 2006; 329:465–475. [PubMed: 16797452]
- Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH. Synapse formation on neurons born in the adult hippocampus. *Nat Neurosci.* 2007; 10:727–734. [PubMed: 17486101]
- van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci.* 1999; 2:266–270. [PubMed: 10195220]
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature.* 2002; 415:1034.
- Wang J, Gallagher D, Devito LM, Cancino GI, Tsui D, He L, Keller GM, Frankland PW, Kaplan DR, Miller FD. Metformin Activates an Atypical PKC-CBP Pathway to Promote Neurogenesis and Enhance Spatial Memory Formation. *Cell Stem Cell.* 2012; 11:23–35. [PubMed: 22770240]
- Wang W, Pan YW, Wietecha T, Zou J, Abel GM, Kuo CT, Xia Z. Extracellular Signal-regulated Kinase 5 (ERK5) Mediates Prolactin-stimulated Adult Neurogenesis in the Subventricular Zone and Olfactory Bulb. *J Biol Chem.* 2013; 88:2623–2631. [PubMed: 23223235]
- Wang Y, Su B, Xia Z. Brain-derived Neurotrophic Factor Activates ERK5 in Cortical Neurons via a Rap1-MEKK2 Signaling Cascade. *J Biol Chem.* 2006; 281:35965–35974. [PubMed: 17003042]
- Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA. Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nat. Neurosci.* 2001; 4:981–988. [PubMed: 11544482]

- Whitman MC, Greer CA. Adult neurogenesis and the olfactory system. *Prog Neurobiol.* 2009; 89:162–175. [PubMed: 19615423]
- Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG. Longterm survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur J Neurosci.* 2002; 16:1681–1689. [PubMed: 12431220]
- Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing Effects of ERK and JNK-p38 MAP Kinases on Apoptosis. *Science.* 1995; 270:1326–1331. [PubMed: 7481820]
- Yamaguchi M, Mori K. Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. *Proc Natl Acad Sci U S A.* 2005; 102:9697–9702. [PubMed: 15976032]
- Yokoyama TK, Mochimaru D, Murata K, Manabe H, Kobayakawa K, Kobayakawa R, Sakano H, Mori K, Yamaguchi M. Elimination of adult-born neurons in the olfactory bulb is promoted during the postprandial period. *Neuron.* 2011; 71:883–897. [PubMed: 21903081]
- Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature.* 2008; 451:1004–1007. [PubMed: 18235445]
- Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell.* 2008; 132:645–660. [PubMed: 18295581]
- Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. *J. Biol. Chem.* 1995; 270:12665–12669. [PubMed: 7759517]

Figure 1. Motor behavior and habituation in the open field assay is not affected in ERK5 icKO animals compared to the drug control mice

Adult male nestin-creERTM/ERK5^{loxP/loxP} and ERK5^{loxP/loxP} littermate mice (10–12 weeks old) were treated with tamoxifen as in (Pan et al., 2012a) and designated ERK5 icKO or drug control respectively. Mice were exposed to the open field assay for three consecutive days to examine habituation to a novel environment. (A) Total floor plane activity on day 1 (d1), d2, d3. (B) Total amount of time spent along the margin of the open field chamber. (C) Total distance traveled during each exposure to the open field chamber. (D) Average traveling speed during each exposure. Data represent mean ± SEM of two independent experiments with n = 10 animals per treatment group per experiment.

Figure 2. Drug control animals retain, while ERK5 icKO mice lack memory in the novel object recognition assay tested 48 h after training

As described in (Pan et al., 2012a), animals were trained for two objects (A and B) during the training session for 5 min (A–B) or 10 min (C–F). Following an interval of 1 h, 24 h, or 48 h, object B was replaced with object C and animals were permitted to explore both object A (familiar) and object C (novel) in the testing session. Total exploration time of each object was recorded identically during the training (A, C, E) and testing sessions (B, D, F). A unique set of objects A–C was used for each time point tested. Memory for the familiar object A is manifested as more time spent in exploring the novel object C than the familiar object A. (A, B) ERK5 icKO mice have normal 1 h memory. (C, D) ERK5 icKO mice have

normal 24 h memory. (E, F) ERK5 icKO mice have no memory 48 h post-training. Data represent mean exploration time $\pm$ SEM from three independent experiments with n = 8 animals per treatment group per experiment. n.s., not significant; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3. ERK5 icKO animals are deficient in acquiring newer spatial information during reversal training in the Morris water maze

(A) As described in (Pan et al., 2012a), mice were subjected to 8 d of training (4 sessions/day, 30 min inter-session interval, from d1 to d8) in the hidden platform Morris water maze. Target (in quadrant #1) acquisition, quantified as swim distance to the hidden platform, was similar in drug control and ERK5 icKO mice, demonstrating similar spatial learning. (B) A probe test was conducted 24 h later, which demonstrated that drug control and ERK5 icKO mice both acquired spatial memory for the location of the hidden platform. (C) Mice were then subjected to 7 d of reversal training (4 sessions/day, 30 min inter-session interval) where the escape hidden platform was relocated to the opposite quadrant (quadrant #3) of

the water maze. ERK5 icKO mice swam longer distances to find the hidden platform in the newer location, suggesting impaired learning of newer spatial information. (D) ERK5 icKO animals are unable to differentiate between the new and old locations of the escape platform in the reversal probe test, conducted 24 h after reversal hidden platform training. Data represent mean $\pm$ SEM from two independent experiments with $n = 10$ animals per treatment group per experiment. n.s., not significant; *, $p < 0.05$; ***, $p < 0.001$.

1X 0.7mA foot shock

Figure 4. ERK5 icKO mice are deficient in contextual fear memory extinction

As described in (Pan et al., 2012a), all animals were trained in the contextual fear conditioning assay using a single 0.7 mA foot shock lasting 2 s. A 30 s tone was presented prior to foot shock delivery and co-terminated with the foot shock. (A) Drug control and ERK5 icKO animals have similar levels of contextual fear memory 24 h after training. (B) Fear memory is context specific since neither drug control nor ERK5 icKO mice froze when placed in a novel context 24 h after training. (C) Cued-fear response is similar between drug control and ERK5 icKO animals when tested 24 h after training. (D) Fear extinction is impaired in ERK5 icKO animals compared to drug controls. Day 0: the day of contextual fear testing. Day 1: the first day animals were subjected to fear extinction training. (E) Following fear extinction training, mice were placed into a novel context on Day 9. Both drug control and ERK5 icKO animals froze minimally when placed in the novel context,

suggesting that the persistent fear memory associated with ERK5 icKO mice is context-specific behavior. Data represent mean freezing behavior $\pm$ SEM from two independent experiments with $n = 8$ animals per treatment group per experiment. n.s., not significant; ***, $p < 0.001$.

3X 0.3mA foot shock

Figure 5. ERK5 icKO animals have reduced contextual fear memory when trained using a more challenging training paradigm

As described in (Pan et al., 2012a), animals were trained using a weaker electric foot shock (0.3 mA) delivered 3 consecutive times. Each foot shock was paired with a tone, which co-terminated with the foot shock. (A) Freezing behavior was monitored in the shocking context 24 h after training. ERK5 icKO mice froze less compared with drug control animals. (B) This deficit was context-specific since both groups of animals did not freeze when exposed to a novel context 24 h after training. (C) Cued-fear conditioning was also not affected in either group of mice when tested 24 h after training. Data represent mean freezing behavior $\pm$ SEM from two independent experiments with $n = 6$ animals per treatment group per experiment. ***, $p < 0.001$.

Figure 6. ERK5 icKO mice show impaired pattern separation

As described in (Pan et al., 2012a), mice were trained in the delayed-non-matching-to-place radial arm maze assay as a test for pattern separation (Pan et al., 2012a). When the food reward was moved 180° from the original sample arm location (Separation 4), ERK5 icKO performed nearly as well as drug control animals in acquiring this spatial memory. However, ERK5 icKO mice were unable to distinguish between the sample and test arms when the food reward was placed in the test arm 90° from the original sample arm location (Separation 2), which is a more difficult test than separation 4. Data is from the last day of a 5 d experimental paradigm and represents mean $\pm$ SEM with $n = 10$ per treatment group. *, $p < 0.05$.

Figure 7. Remote memory is impaired in ERK5 icKO mice when tested in the passive avoidance assay

As described in (Pan et al., 2012a), mice were trained in the passive avoidance assay by placing animals in the light side of a light/dark box. The light/dark chambers are separated by a small trap door. When mice entered the dark side of the box, the trap door immediately closed and a single 0.7 mA foot shock was delivered for 2 s. Following a 1 min memory acquisition period, mice were removed from the dark side of the box. Mice were placed back to the light side of the training box 24 h later to assess short-term, 24 h memory for the aversive stimulus, manifested as increased latency to cross over into the dark side. No deficits exist between drug control and ERK5 icKO animals at 24 h. Mice were then returned to their home cages. At 21 d post-training, animals were again placed back to the light side of the box to assess for remote memory. Unlike drug control mice, ERK5 icKO animals had no remote memory. Data represent mean crossover latency $\pm$ SEM with $n = 10$ per treatment group. ***, $p < 0.001$.