**miRNA-132's Role as a Dynamic Regulator of Memory**

Katelin F. Hansen[1]
Department of Neuroscience, Ohio State University, Columbus, OH, USA

## Introduction

MicroRNAs (miRNAs) are small (~22 nt) evolutionarily-conserved single stranded RNA species that function as potent negative regulators of gene expression (Ambros 2004; Bartel 2004; Lim et al. 2005). miRNAs repress gene expression via their ability to hybridize with the 3' UTR of target mRNAs, and, in turn, trigger mRNA degradation and/or translational repression (reviewed in (Bartel 2009; Eulalio et al. 2008; Carthew and Sontheimer 2009)). miRNA expression is often cell-type and tissue-specific (Lagos-Quintana et al. 2002; Kim et al. 2004b; Kosik 2006; Cao et al. 2006), and within the central nervous system (CNS), recent work has indicated that they play key roles in a wide range of physiological processes. Along these lines, during development, the disruption of miRNA expression through genetic deletion of Dicer results in aberrant neuronal development and differentiation, as well as altered morphogenesis and neuronal signaling (Cuellar et al. 2008; Giraldez et al. 2005). Furthermore, recent work reveals specific roles for miRNAs (including miR-124a, miR-134 and miR-375) in dendritogenesis and axonal path finding (Schratt et al. 2006; Abdelmohsen et al. 2010; Fiore et al. 2009; Sanuki et al. 2011).

As more neuronal-enriched miRNA have been identified, the possibility of a prominent role for miRNA in the regulation of neuronal activity and cognition has emerged (Konopka et al. 2011; Im et al. 2010). Among these is the CREB-regulated miRNA132 (miR-132) (Cheng et al. 2007; Remenyi et al. 2010; Wibrand et al. 2010; Nudelman et al. 2010). miR-132 is transcribed from the intron

[1] Additional contributors include: Kate Karelina (OSU), Kensuke Sakamoto (OSU), Gary A Wayman (WSU), Soren Impey (OHSU), Karl Obrietan (OSU)

of a non-coding RNA and has been shown to alter both neuronal morphology and synaptic physiology (Hansen et al. 2010b; Vo et al. 2005). Along these lines, the effects of miR-132 on dendritic morphogenesis appears, in part, to be mediated by its suppression of p250GAP, thus leading to a de-repression of Rac1-PAK-mediatetd spinogenesis (Impey et al. 2010; Wayman et al. 2008). Furthermore, selective deletion of the miR-132/212 locus led to significant alterations in neuronal morphology in new-born adult hippocampal neurons, including decreased dendritic complexity and spine density (Magill et al. 2010).

Given its complex, and context-specific effects on neuronal structure and function, an examination of the potential role of miR-132 as a regulator of cognition is highly merited. In line with this, we recently reported that robust transgenic over-expression of miR-132 in the forebrain increased spine density and caused deficits in novel object recognition memory (Hansen et al. 2010b). Interestingly, in this study, transgenic miR-132 expression levels were quite high (> 5-fold over basal expression), approximating the expression level of endogenous miR-132 following seizure activity (Jimenez-Mateos et al. 2011). This high level of transgenic miR-132 raised the possibility that our reported effects on novel object recognition memory may not serve as a useful guide to understand the role of miR-132 under normal physiological conditions such as following learning/memory tasks. We hypothesized that under normal physiological conditions, miR-132 could couple neuronal activity to enhanced cognition.

To begin to address this question, we employed a combination of *in vivo* detection methods and transgenic mouse models to assess the functional effects of miR-132 on hippocampal dependent learning and memory. Here we show that endogenous miR-132 is induced in response to a spatial memory task, and that this *'induced'* level of miR-132 enhances cognition. Moreover, over-expression (> 3-fold) of miR-132 leads to profound cognitive deficits. Together, these data indicate that miR-132 is part of a normal cellular signaling pathway that

couples associative learning paradigms to memory formation, and that dysregulation of its expression leads to impaired cognition.

## Methods and Materials

### *Ethics Statement*

All animal breeding and experimental procedures were approved by the Ohio State University Animal Care and Use Committee (protocol number: 2008A0227).

### *tTA::miR132 mice*

Generation of the tetracycline-regulated bidirectional miR-132/cyan fluorescent protein transgenic mouse line (here referred to as: *miR-132 transgenic mice*) was recently described (Hansen et al. 2010b). To drive transgene expression, the TRE-miR-132 transgenic mice were crossed with the CaMKII tTA driver line (Mayford et al. 1996), which allowed for robust transgene expression within excitatory forebrain neurons (Hansen et al. 2010b). Temporal regulation of tTA (i.e., 'Tet-off') inducible miR-132 transgene expression was accomplished by administration of doxycycline (0.40-200 μg/mL) to the drinking water. Doxycycline was also administered to monotransgenic controls, and was shown to have no cellular level or behavioral effect relative to untreated animals (data not shown).

For the examination of neuronal spine density, tTA::miR132 mice were crossed with a transgenic line expressing green fluorescent protein (GFP) in a subpopulation of hippocampal neurons under the control of the *thy1* promoter (Morris 1985). Thy-1 GFP mice (Feng et al. 2000) were generously provided by Gouping Feng (Duke University). Of note, the Thy-1-driven morphological marker does not affect the electrophysiological or the morphological properties

(i.e., dendrite length and number, spine number and density, soma size) of hippocampal neurons (Vuksic et al. 2008).

*RT-PCR miRNA quantitation*

The NCode™ VILO™miRNA cDNA Synthesis Kit (Invitrogen) was used to create a cDNA library of total hippocampal RNA that was extracted by TRIzol isolation (Invitrogen) from nontransgenic and tTA::miR132 animals. miRNAs were polyadenylated with Poly(A)polymerase and reverse transcribed with universally-tagged 5' oligo-dT primer (Invitrogen). cDNA amplification was performed using SYBR green reporter-based qPCR (Applied Biosystems) and the miScript Primers (Qiagen). Data were normalized to RNU6B_2 cDNA levels.

*Tissue Processing and Fluorescent* in situ *hybridization*

All tissue preparation was carried out under RNAse-free conditions and with use of DEPC-treated solutions. Brain tissue was removed after cervical dislocation and post-fixed in 4% paraformaldehyde for 4 h at 4°C and cryoprotected with 30% sucrose in PBS. Hippocampus-containing sections were then thin-cut (40 µm) on a freezing microtome. Sections were incubated (5 min) with the nuclear stain DRAQ5 (1:10,000; BioStatus Limited, UK) and washed 3x in PBS.

Fluorescent *in situ* hybridization (FISH) against miRNA was carried out according to procedures outlined by Nuovo (2010) (Nuovo 2010). In brief, tissue was mounted onto slides, followed by a short digestion with pepsin. Tissue was then hybridized overnight with fluorescein-conjugated locked nucleic acid (LNA) probes (Obernosterer et al. 2007) to mouse

miR-132, snRNA U6, or an Exiqon-designed ‘scramble’ negative control probe (Exiqon). The signal from the fluorescein-conjugated probes was amplified with an anti-fluorescein Alexa 488 signal detection kit (Invitrogen).

Analysis of FISH intensity was examined using the above equipment and software to acquire images at 40x. Images of the relevant cellular layers of FISH-labeled sections were traced digitally and intensity levels were analyzed with MetaMorph software (Molecular Devices). Intensity levels were normalized by background subtraction and presented relative to staining in naïve animals.

*Immunofluorescent Labeling*

Tissue was fixed and prepared as described above. Sections were then washed and permeabilized in PBS with 1% Triton X-100 PBST (3x, 10 min each). Sections were blocked for 1 h in10% normal goat serum in PBS. Sections were incubated overnight at 4°C in rabbit polyclonal anti-GFP antibody (acquired from Dr. Luc G. Berthiaume, University of Alberta, Canada). The GFP antibody was used at 1:2,500 to detect the TRE-regulated CFP transgene, and 1:20,000 to detect the Thy-1-regulated GFP transgene. Of note, the expression of the tet-responsive CFP transgene is markedly lower than the Thy-1 driven GFP, and thus, by using this relatively low concentration of the primary antibody, we are able to distinguish the Thy-1 GFP transgene from the CFP transgene. A series of control data sets which confirm successful discrimination of the two signals is presented in (Hansen et al. 2010b).

Sections were then incubated in Alexa Fluor-488-conjugated goat anti-mouse IgG antibody (1:1000; Invitrogen, Carlsbad, CA) for three hours at room temperature. Tissue was washed in PBST for a total of 30 min between each labeling step. Sections were mounted on

slides with Fluoromount-G (SouthernBiotech, Birmingham, Alabama, AL). For clarity, CFP transgene immunolabeling (Fig. 3b) is presented as a blue/cyan fluorescent signal.

Thy-1 *GFP Morphometric Analysis*

CA1 basal dendrites (ten per animal) were examined for morphological changes in 40 µm-thick coronal sections. Images of Thy-1 GFP immunofluorescence of 20 µm dendritic segments extending 90-120 µm from the cell soma were captured using confocal microscopy. Processes extending > 0.5 µm from the dendrite were counted as dendritic spines and included both mushroom-shaped and filopodia-like protrusions. Images were acquired at 63x with 4.3x optical zoom, using a Zeiss 510 confocal microscope and LSM Software Zen.

*Behavioral Analysis*

Mature (6-8 weeks), age and gender-matched animals were used for all behavioral paradigms. Novel object recognition was adapted from procedure described by Bevins and Besheer (Bevins and Besheer 2006). Briefly, each animal was allowed a 10 min training session with exposure to two identical, non-toxic objects (glass or hard plastic items). After training, animals were returned to the home cage for a 30-min delay period. After the retention interval, each animal was returned to the testing arena in which one familiar object was replaced with a novel object. Animals were given 5 min to explore while being video recorded for later scoring. Subsequently, exploration time for each object was timed, as identified as the animal's nose being within 2 cm of, and pointed toward, the object. The discrimination ratio was calculated as ['exploration time with novel object' / ('time with novel object' + 'time with familiar object')]. Of note, all animals

demonstrated similar ambulatory capacity, as demonstrated by motility of exploration. All groups exhibited similar total exploration time of their environment.

Barnes maze testing followed procedures outlined by Sunyer et al (Sunyer et al. 2007; Barnes 1979). In summary, a circular and elevated maze platform (92 cm diameter, 76 cm above the floor) with 20 equally-spaced holes was positioned in a brightly lit room. An escape box was placed under one of the holes and visual cues (geometric shapes) were placed on the walls that surround the maze. Bright light (120W in addition to overhead lighting) and an electronic metronome (Boss DB-66, 440 Hz, 65 dB) were used as aversive stimuli. Mice were given five days of training, during which mice experienced three 5-minute trials of free exploration with a 10-min inter-trial interval. A trial ended once the mouse entered the escape box, or after 5 minutes of exploration had elapsed (at which point it was gently guided to the target box). Nose pokes into incorrect holes and latency to reach target hole were recorded as measures of maze performance over all trials. To distinguish the potentially-subtle phenotypic effects of graded transgene expression, Barnes maze testing on animals with titered doxycycline administration (Fig. 3) was conducted under slightly less aversive conditions, relative to those used to test the gross effects of robust transgenic miR-132 (Fig. 4) to avoid any ceiling effect related to minimum training errors and latency to escape.

Successful acquisition of the Barnes maze task was demonstrated by a probe trial that occurred 24 hours after the final acquisition day. Probe trial consisted of a single 90-second exploration of the maze with the escape box removed. All animals demonstrated a capacity to locate the target hole, and no significant differences were found between groups on the probe day (data not shown).

Examination of learning-induced miR-132 expression in wild-type animals was conducted using a modified Barnes maze paradigm in order to capture changes in early miRNA expression. Animals were given two days of Barnes maze training, by which point spatial memory appeared to be largely established, as demonstrated by improved performance across measures of both latency and errors (Fig. 3e). Control animals were also handled and exposed to aversive stimuli, but were not trained to escape to the target box. Tissue was isolated for *in situ* hybridization twelve hours after the last trial on day two, reflecting both the time course of miRNA processing (Schmittgen et al. 2008), as well as similar paradigms used to detect early signaling mechanisms of memory formation (Vázquez et al. 2000; Kim et al. 2004a).

*Statistics*

All values presented here are given as means ± SEM. Unless otherwise noted, a value of $p < 0.05$ was accepted as statistically significant. Comparisons between two groups were made by Student's t-test. Significance for qPCR, spine density, and within-day Barnes maze analysis conducted by SPSS 19.0 and were assessed using one-way ANOVA analysis, followed by Fisher's least significant difference (LSD) test. Repeated Measures Mixed-Model ANOVA was used for multi-day Barnes maze training comparison.

## Results

### Expression of miR-132 in the hippocampus

To begin our analysis, we profiled miR-132 expression in the mature hippocampus using fluorescent *in situ* hybridization (FISH). To this end, coronal sections from adult wild-type mice animals were incubated with a fluorescein-conjugated locked nucleic acid (LNA) probe against the mature form of miR-132 and labeled with the DNA/nuclear marker DRAQ5 (Fig. 1).

Representative data reveal marked expression of miR-132 in all excitatory sublayers of the hippocampus (i.e., CA1, CA2, CA3, and GCL), as well as in the cortex (Fig. 1a, b). Higher magnification imaging of tissue labeling confirmed that miR-132 expression was largely restricted to neuronal cell populations (Fig. 2). Hence, in contrast to the robust expression detected in the CA1 and GCL sublayers, minimal labeling was detected within cells (largely non-neuronal) located in the molecular layer of the dentate gyrus (Fig. 2A-C). A control set of experiments in which tissue was labeled with a 'scrambled' fluorescein-conjugated LNA probe that does not detect known microRNA species revealed little labeling (Fig. 1 C). As a positive control, tissue was labeled with an LNA-probe against the ubiquitously expressed snRNA U6. As expected, U6 labeling was detected throughout the hippocampus. Together these indicate neuronal enrichment of miR-132 in the adult hippocampus.

**Induction of miR-132 after spatial learning**

A number of studies have shown that miR-132 expression is tightly regulated by CREB (Jimenez-Mateos et al. 2011; Cheng et al. 2007). Given that CREB plays a central role in transcriptionally-dependent forms of learning and memory, it was reasonable to posit that miR-132 may be inducibly expressed during memory formation. To begin to test this idea, we profiled hippocampal miR-132 expression in wild-type animals undergoing Barnes maze spatial memory training (described in detail below). Because the greatest period of memory formation appears to occur during the first two days of training (as demonstrated by both reduced latency and errors, and successful escape to the target box: Fig. 3d), animals were given two days of training (thee trials per day). Tissue was then collected twelve hours later, and miR-132 expression was measured via FISH (Fig. 2). Quantitative analysis of the FISH signal revealed that miR-132

expression was significantly higher (~1.5 fold) in the CA1, CA3, and GCL cell layers of Barnes maze-trained animals, relative to control animals that were naïve to the learning paradigm (Fig. 2a, b and d). In naïve mice, miR-132 expression in the CA1 layer was heterogeneous, with limited expression in some cells, while other cells expressed relatively high levels. Interestingly, training resulted in an elevated and more uniform miR-132 signal across the CA1 sublayer (Fig. 2a). Of note, learning did not increase miR-132 expression in non-neuronal cells, including those occupying the molecular layer of the dentate gyrus (Fig. 2c and d). Together, these data show that miR-132 expression is significantly increased in response to the learning challenge.

**Cognitive enhancement in animals with moderate increases in transgenic miR-132**

Our previous work using a transgenic miR-132 mouse model revealed that robust over-expression (> 3-fold, relative to endogenous levels) of miR-132 impaired learning and memory (Hansen et al. 2010b). Given our work here showing a relatively low-level of miR-132 induction following a learning paradigm, coupled with a number of studies showing that miR-132 affects neuronal plasticity (Impey et al. 2010; Wayman et al. 2008), we hypothesized that robust transgenic over-expression approach may not be a useful model to dissect the contribution of endogenous miRNA-132 to cognition. Hence, in an attempt to provide a more telling model, we returned to our miR-132 transgenic mouse, and tested the effects of low-level transgene expression on Barnes maze learning. As outlined in our previous paper (Hansen et al. 2010b), to drive transgene expression in forebrain excitatory neurons, the tetracycline-regulated miR-132/CFP bidirectional transgenic mouse line was crossed with a αCaMKII tTA driver line (Mayford et al. 1996) (denoted as ‘tTA::miR132’ mice). To negatively regulate transgenic miR-132 expression, varying concentrations of doxycycline were added to the drinking water, and

miR-132 expression was profiled two weeks later. As expected, increasing the concentration of doxycycline in the drinking water (0.4 μg/mL-50 μg/mL) resulted in an incremental repression of transgene expression in the hippocampus, as measured by qPCR (Fig. 3a). Consistent with this, administration of 200 μg/mL of doxycycline led to a suppression of CFP expression (Fig. 3b). To approximate the wild-type expression level of miR-132 after 2-days of Barnes maze learning, we chose an intermediate doxycycline concentration (0.4 μg/mL) that yielded an ~ 2-fold increase in transgene expression over control tTA mice (Fig. 3a). For the behavioral assay, tTA::miR132 animals were treated with this dose of doxycycline for three weeks and then tested on the Barnes maze paradigm. Animals were given five consecutive days of training (three trials per day), during which they were allowed to explore the circular Barnes maze platform (maximum of five minutes per trial). Bright lights and metronomic clicks provided aversive stimuli sufficient to motivate escape to a target hole fitted with an escape box. Learning performance was measured by latency to escape, as well as errors made before escaping (nose pokes to incorrect holes). Remarkably, doxycycline-treated tTA::miR132 animals showed excellent Barnes maze performance, exhibiting both reduced latencies and fewer errors relative to monotransgenic (tTA) littermates that also received doxycycline (Fig. 3c and d). Hence, moderate increases in transgenic miR-132 significantly enhanced cognitive capacity, suggesting that the learning-evoked increase in endogenous miR-132 also enhances cognition.

**Cognitive impairment in mice over-expressing miR-132**

As noted, our previous work demonstrated that robust transgenic over-expression of miR-132 (i.e., no doxycycline treatment) impaired novel object recognition memory (Hansen et al. 2010b). Here, we furthered this line of inquiry by testing the effects of transgenic miR-132 over-

expression on spatial memory capacity, and by testing the plastic nature of the learning phenotype.

In the Barnes maze assay, mice that expressed high levels of transgenic miR-132 (no doxycycline) showed significant impairment in spatial memory capacity, as demonstrated by poor performance in maze acquisition. While all groups improved across the five days of acquisition, the transgenic group without doxycycline exhibited both a longer latency to escape to the target hole, as well as an increase in errors made before escape, as compared to monotransgenic littermates (Fig. 4a and b). These data are consistent with our prior work using the novel object recognition test, and thus provide further support for the idea that miR-132 shapes cognitive capacity.

To examine whether the cognitive deficits observed in tTA::miR132 were reversible, a separate group of miR-132:tTA animals received doxycycline through the drinking water at a dose sufficient to fully suppress transgene expression (200 µg/mL). After three weeks of treatment, these animals exhibited Barnes maze performance that was indistinguishable from tTA monotransgenic mice (Fig. 4a and b). Similarly, the poor performance of transgenic miR-132:tTA animals assayed via the novel object recognition test (Hansen et al. 2010b), was reversed by maintaining animals on doxycycline treatment for three weeks (Fig. 4c). For this assay, animals were given 10 minutes to explore two identical objects placed on opposite sides of a testing arena. After removal for a 30-minute delay, animals were returned to the arena, in which one familiar object had been replaced by a novel one. Animals were allowed five minutes of investigation, during which exploration time with each object was recorded. In the absence of doxycycline, tTA::miR132 mice spent equal time with both the novel and familiar objects. However, a separate cohort of animals that had been treated with doxycycline (200 µg/mL) for

three weeks spent significantly more time with the novel object, paralleling the behavior of tTA monotransgenic mice. Thus, these data indicate that cognitive capacity is tightly and dynamically linked to the expression level of miR-132.

**Reversibility of increased spine density in tTA::miR132 animals**

Our previous work demonstrated that, in the absence of doxycycline treatment, tTA::miR132 mice exhibit a marked increase in dendritic spine density relative to monotransgenic mice (Hansen et al. 2010b). Again, although increased spine density is often associated with enhanced cognitive capacity, excessive spine formation has been linked to cognitive deficits (Hutsler and Zhang 2010). Here, we examined spine density in mature tTA::miR132 animals after receiving three weeks of doxycycline treatment (200 μg/mL). For these experiments, the miR-132 transgenic mice were crossed with a *Thy-1* GFP mouse line that has been used extensively to examine neuronal morphology and plasticity (Vuksic et al. 2008; Feng et al. 2000; Hansen et al. 2010b). Morphometric analysis revealed that spine density in CA1 basal dendrites of TA::miR132 mice treated with doxycycline was indistinguishable from tTA monotransgenic mice (Fig. 5). Further, consistent with our prior work, spine density was significantly enhanced in tTA::miR132 mice that were not treated with doxycycline. This ostensible doxycycline-mediated reversibility of the miR-132 spine density phenotype parallels the reversibly of the miR-132 cognitive phenotype, and thus suggests that miR-132 is a dynamic and transient regulator of neuronal plasticity. Together, these data, along with the noted work on miR-132 targets, indicate a key role for this non-coding RNA in activity-inducible CNS physiology and function.

## Discussion

The central goal of this project was to further our understanding of the role of miR-132 in hippocampal-dependent learning and memory formation. To this end, we demonstrated that endogenous miR-132 is expressed in excitatory cell layers throughout the hippocampus, and that miR-132 is induced in response to a spatial learning task. Using these data as a guide, we employed a tetracycline-regulated operon to drive the expression of transgenic miR-132 and assess its effects on cognition. These data reveal that cognitive capacity is tightly regulated by miR-132. Hence, physiological levels of miR-132 enhance cognition, whereas supra-physiological levels of the miRNA lead to cognitive deficits. Together, these data indicate that miR-132 plays a key role in shaping cognitive capacity.

Next, we turned to the question of miR-132 inducible expression following the presentation of a spatial learning task. For this assay, we used the Barnes maze paradigm, and profiled miR-132 expression after two days of training. One key motivation for choosing a relatively short time point following initiation of the learning paradigm was the desire to capture ostensible early-stage gene expression, which would be expected to play a central role in the induction of learning. At this time point, significant upregulation of miR-132 was detected in the excitatory cell layers of the hippocampus, suggesting that miR-132 induction occurs during a period of memory consolidation. Interestingly, these data are consistent with recent PCR-based profiling data which showed that contextual fear conditioning triggers a rapid increase in the expression of the primary transcript of miR-132 (Nudelman et al. 2010), although a learning-evoked increase in the mature form of miR-132 was not detected in the noted study. Together, these data suggest that miR-132 is part of the CREB-regulated, learning evoked transcriptional

response, and, given the time course of induction, raises the prospect that miR-132 plays a role in CREB-dependent memory consolidation.

In our prior report, we found that transgenic miR-132 expression led to an increase in overall dendritic spine density in the hippocampus, and a deficit in novel object recognition memory (Hansen et al. 2010b). Here, the examination of transgenic miR-132's action on learning and memory was extended to the Barnes maze, where we found that over-expression of miR-132 (no doxycycline treatment) led to a profound deficit in spatial learning capacity. On its face, pairing our current data showing that learning induces an increase in miR-132 expression with the our prior data showing that transgenic over-expression of miR-132 triggers cognitive deficits would suggest that endogenous miR-132 functions as a negative regulator of learning and memory. However, a more circumspect examination of the data raises issues with this interpretation. Central to this concern is the marked difference between the miR-132 induction level evoked by learning and the level driven by the tet-operon. Notably, wild-type animals showed learning-evoked increase in expression of ~1.5-fold, whereas in transgenic mice, miR-132 expression was elevated > 3-fold over control levels (in the absence of doxycycline). This transgenic, supra-physiological, level of miR-132 could profoundly dysregulate neuronal physiology and, in turn, cognition. To address this possibility, we employed the doxycycline regulatory system to modulate transgenic miR-132 levels, and assess the effects of moderate miR-132 induction that approximates the learning-evoked expression level. Under these conditions, tTA::miR132 animals showed significantly enhanced spatial memory capacity relative to nontransgenic littermates. These expression-level-specific effects of miR-132 led us to envision miRNA functionality as an inverted U-shaped curve, where low or supra-physiological levels of the miRNA result in cognitive impairment, and where miR-132 enhances cognitive

capacity only within a fairly limited expression range (Fig. 6). Our data raise the prospect that a limited range of miR-132 expression leads to morphological plasticity conducive to normal cognitive capacity, and that relatively high levels disrupt cognition via aberrant spinogenesis/synaptic connection. Consistent with this idea, our data reveal that doxycycline suppression of transgenic miR-132 reversed *both* the cognitive deficits and the spinogenesis phenotype.

Given the complexity of miRNA expression and the plethora of miR-132 targets that have yet to be validated, newly developed approaches, including array and deep sequencing assays designed to quantify gene expression levels and identify gene networks, may be required to begin to unravel the mechanisms by which miR-132 sculpts cognition.

**Figures**

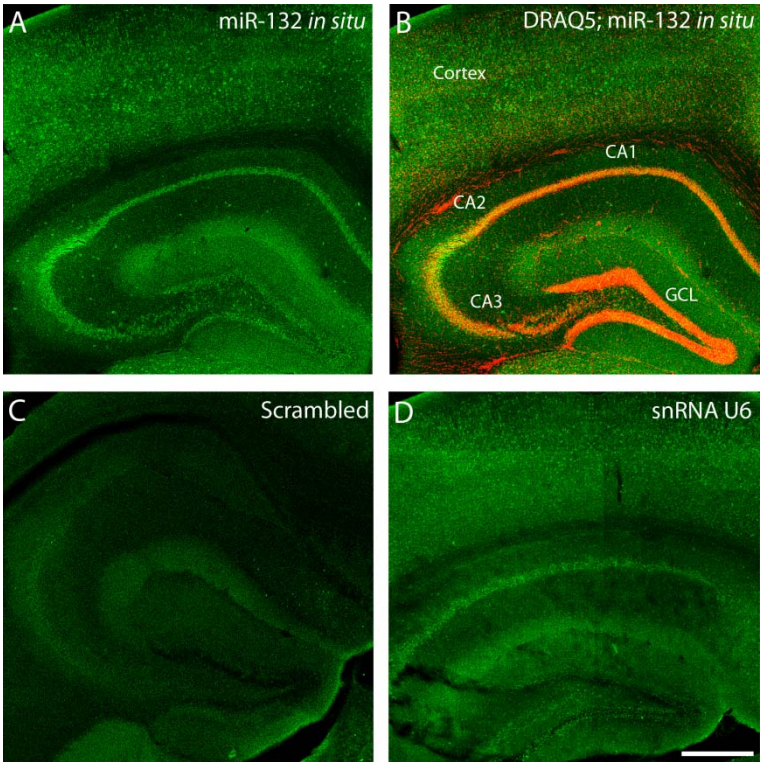


**Fig. 1 miR-132 expression in the hippocampus** (A) Fluorescent *in situ* hybridization (FISH) using an LNA-antisense probe directed against miR-132 reveals robust miR-132 expression throughout the cortex and hippocampus of wild-type animals. (B) Within the hippocampus, marked excitatory-layer-specific expression of miR-132 was confirmed by merging the miR-132 fluorescence micrograph in *A* with a fluorescence micrograph image of DRAQ5 labeling (red). (C) Representative FISH labeling using a scrambled miRNA probe: minimal labeling was detected. (D) As a positive control for the FISH labeling approach, hippocampal sections were incubated with an antisense probe against the snRNA U6. CA1-3, hippocampal subfields; GCL, granule cell layer. Scale bar: 400 µm.

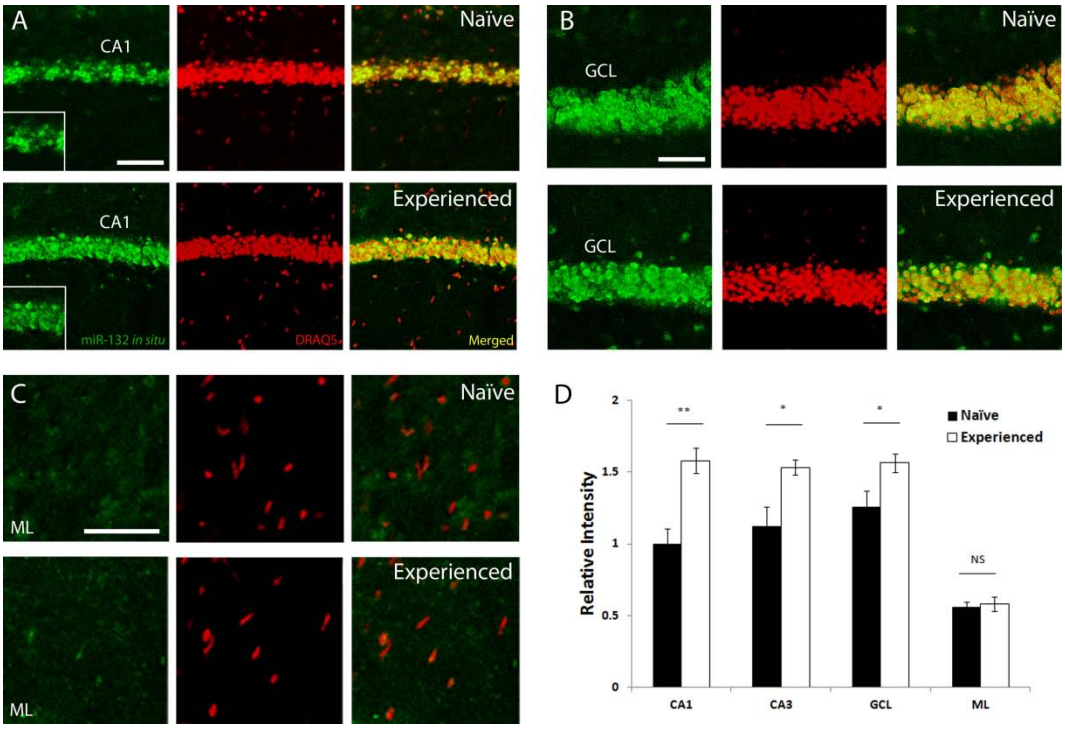


**Fig. 2 miR-132 expression is induced in response to the Barnes maze learning paradigm**

Hippocampal miR-132 expression was examined by FISH in control animals, and in animals that experienced two days of Barnes maze learning. (A-C) In animals expo sed to the learning paradigm, expression of miR-132 (green) was increased in the CA1 (A), CA3 (not shown), and GCL (B) excitatory cell layers relative to control animals, but not in the molecular layer (ML: C). Tissue was also labeled with the DNA stain DRAQ5 (red). Scale bar: 100 µm. (D) Quantification of relative fluorescent intensity ± SEM in CA1, CA3, GCL and ML; NS, not significant. *$p<0.05$, **$p<0.01$, two-tailed t-test, n=6 animals for each group.

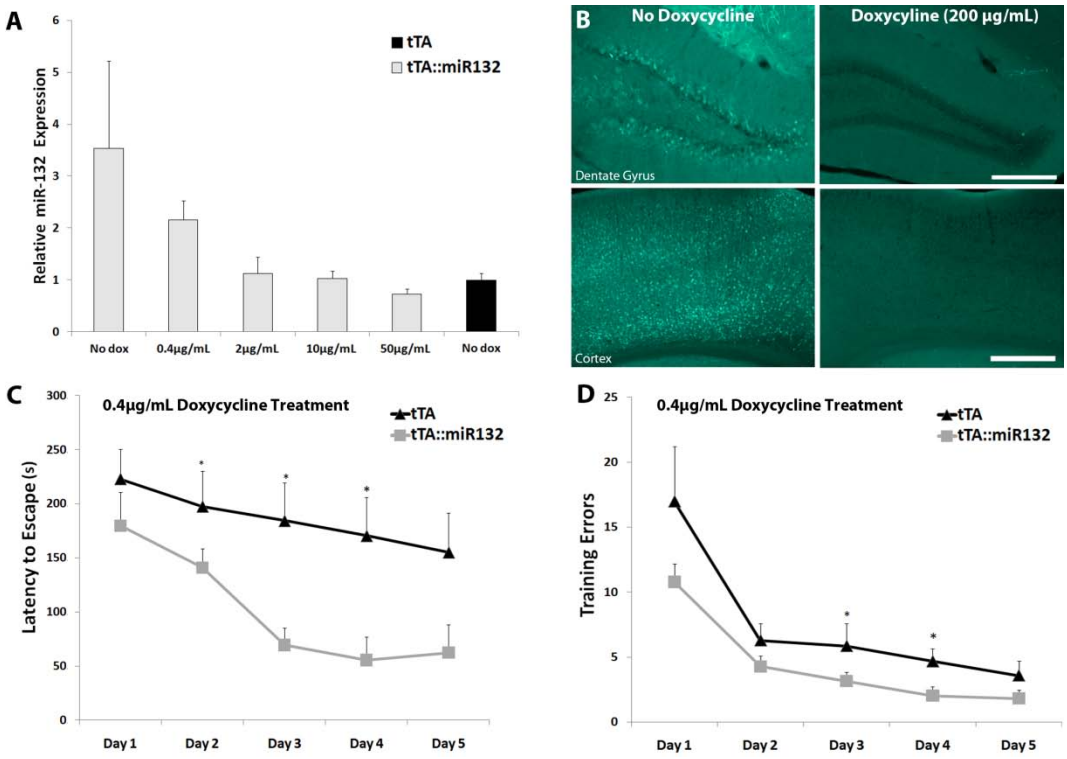


**Fig. 3 Limited expression of transgenic miR-132 enhances Barnes maze performance** (A) tTA::miR132 animals exhibited robust expression of miR-132 in the hippocampus by qPCR in the absences of doxycycline, while the addition of doxycycline administration to the drinking water for two weeks led to a dose-dependent reduction in miR-132 expression. From 2-10 µg/mL of doxycycline, miR-132 levels in tTA::miR132 mice were not significantly different from levels in monotransgenic animals (tTA). Total hippocampal RNA was isolated, reverse-transcribed, and miR-132 cDNA was profiled via real-time PCR. Mature miR-132 expression was normalized to RNU6B_2 cDNA levels and then presented as mean ± SEM (n=3 per group) expression relative to the tTA (No dox) condition, which was set equal to a value of 1. (B) Immunofluorescent analysis of cyan fluorescent protein (CFP) expression in tTA::miR132 mice confirms robust transgene expression in the absence of doxycycline administration, and potent suppression of transgene expression following two weeks of doxycycline administration (200 µg/mL). Scale bars: 200 µm. (C,D) Animals (tTA and tTA::miR132) were administered doxycycline (0.4

μg/mL) for three weeks prior to Barnes maze testing. Under this condition, tTA::miR132 animals exhibited faster escape latency (C) and fewer training errors (D) than monotransgenic littermates. All groups improved significantly across five days of acquisition: Repeated Measures Mixed-Model ANOVA, $F(4,80)=6.685$, $P<.001$ (Latency), $F(4,80)=17.723$, $P<.001$ (Training Errors). Differences within day: one-way ANOVA, $*p<0.05$. n=11 animals per group.

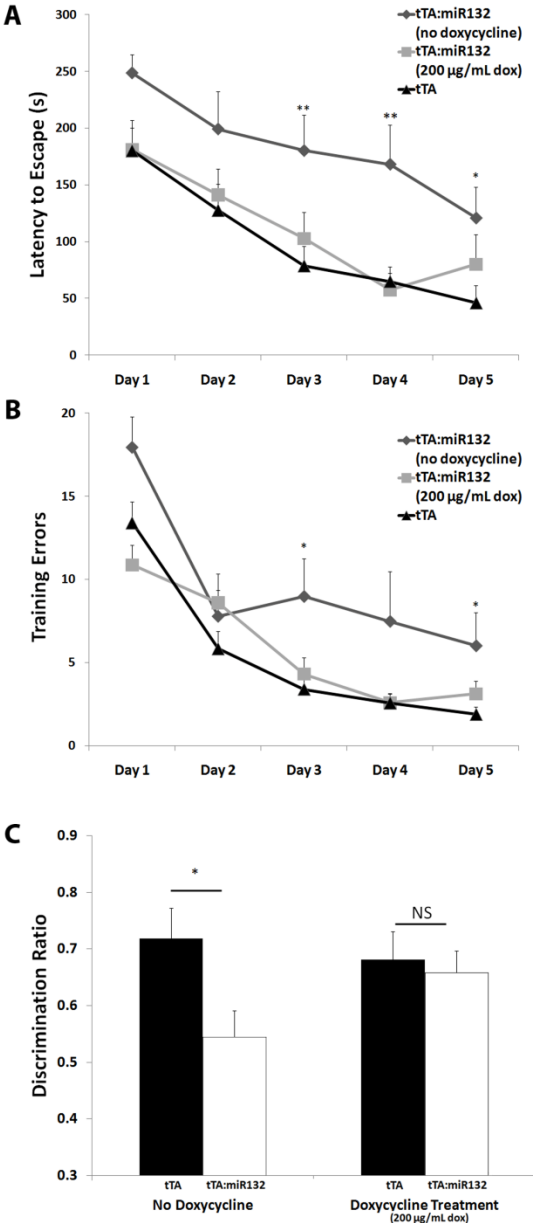


**Figure 4. tTA::miR132 animals under conditions of robust transgenic miR-132 show cognitive impairment.** (A,B) In the absence of doxycycline treatment, tTA::miR132 animals undergoing Barnes maze testing showed increased latency to escape (A), as well as increased training errors (B) relative to monotransgenic littermates (tTA). In a separate group of tTA::miR132 mice, doxycycline treatment (200 mg/mL: three weeks) suppressed the cognitive impairment observed in the tTA::miR132 animals that were not treated with doxycycline. tTA animals were also treated with doxycycline, but showed no differences from untreated controls (data not shown). All experimental groups improved significantly across five days of acquisition: Repeated Measures Mixed-Model ANOVA $F(4,160)=33.429$, $P<.001$ (Latency), $F(4,156)=45.65$, $P<.001$ (Training Errors).

Differences within day: one-way ANOVA, *p<0.05, **p<0.01. n=12 animals per group. (C) In the absence of doxycycline, tTA::miR132 animals showed significant impairment in novel object recognition memory. Hence, tTA::miR132 mice did not exhibit greater exploratory bias towards the novel versus the familiar object. In contrast, blocking transgenic miR-132 expression via doxycycline administration (200 µg/mL: three weeks) suppressed the novel object recognition impairment of tTA::miR132 animals. Hence, doxycycline-treated tTA::miR132 mice exhibited significantly greater exploration time with the novel object than with the familiar one, paralleling the behavioral phenotype of monotransgenic littermates (tTA). Data are presented as mean discrimination ratio ± SEM, *p<0.05, two-tailed t-test, n=5 animals per group. NS, not significant.

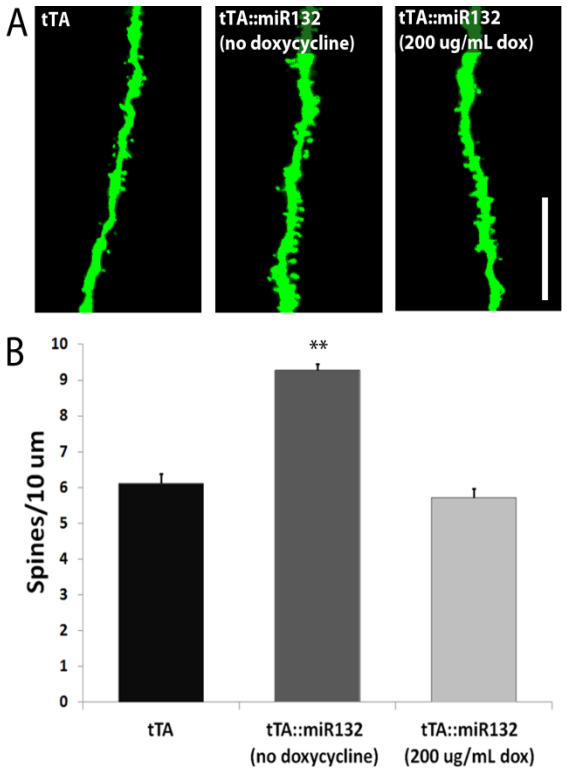


**Fig. 5 Transgenic miR-132-induced increases in spine density are suppressed by administration of doxycycline** A *Thy-1* driven GFP transgenic marker was used to examine spine density in tTA::miR132 mice. (A) Confocal images reveal an increase in spine density under conditions of robust miR-132 expression [tTA::miR132 (no doxycycline)] that is absent upon suppression of the transgene with doxycycline [tTA::miR132 (200 µg/mL dox)]. Scale bar: 10 µm. (B) Graphical representation of the mean ± SEM spine density. One-way ANOVA, **P < 0.01, F(2,10)= 83.563, n=5 animals for each group.

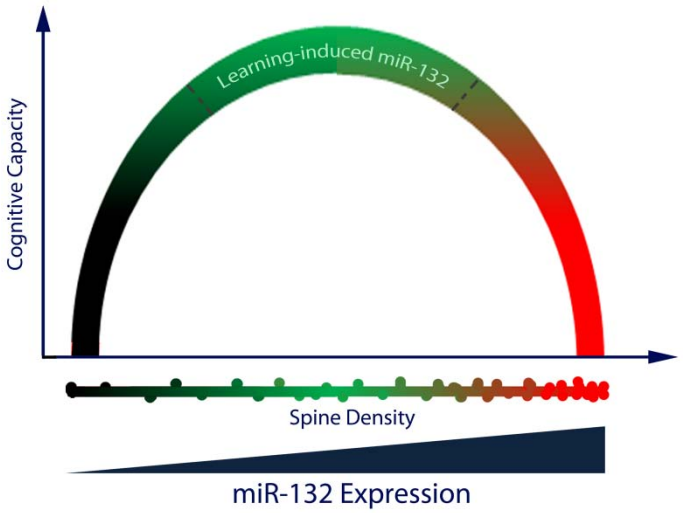


**Fig. 6 Model depicting the proposed relationships between miR-132, neuronal morphology and cognition** In this model, we propose that there is an optimal, and rather narrow, range of miR-132 expression which enhances cognitive capacity. Supra-physiological expression of miR-132 leads to excessive spine density and cognitive deficits that compromise learning and memory formation. Further, limited basal and/or damped inducible expression of miR-132 is hypothesized to inhibit cognition, possibly via a paucity of spine formation. Hence, deviation from this optimized miR-132 expression level (either excessive induction or too little expression) may lead to the severe learning deficits that are characteristic of a variety of neurocognitive disorders.

**References**


Abdelmohsen K, Hutchison ER, Lee EK, Kuwano Y, Kim MM, Masuda K, Srikantan S, Subaran SS, Marasa BS, Mattson MP, Gorospe M (2010) miR-375 inhibits differentiation of neurites by lowering HuD levels. Mol Cell Biol 30 (17):4197-4210. doi:MCB.00316-10 [pii]10.1128/MCB.00316-10

Ambros V (2004) The functions of animal microRNAs. Nature 431 (7006):350-355. doi:nature02871 [pii]10.1038/nature02871

Barnes CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol 93 (1):74-104

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2):281-297. doi:S0092867404000455 [pii]

Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136 (2):215-233. doi:S0092-8674(09)00008-7 [pii]10.1016/j.cell.2009.01.002

Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc 1 (3):1306-1311. doi:nprot.2006.205 [pii]10.1038/nprot.2006.205

Bourtchouladze R, Patterson SL, Kelly MP, Kreibich A, Kandel ER, Abel T (2006) Chronically increased Gsalpha signaling disrupts associative and spatial learning. Learn Mem 13 (6):745-752. doi:13/6/745 [pii]10.1101/lm.354106

Cao X, Yeo G, Muotri AR, Kuwabara T, Gage FH (2006) Noncoding RNAs in the mammalian central nervous system. Annu Rev Neurosci 29:77-103. doi:10.1146/annurev.neuro.29.051605.112839

Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. Cell 136 (4):642-655. doi:S0092-8674(09)00083-X [pii]10.1016/j.cell.2009.01.035

Cheng HY, Papp JW, Varlamova O, Dziema H, Russell B, Curfman JP, Nakazawa T, Shimizu K, Okamura H, Impey S, Obrietan K (2007) microRNA modulation of circadian-clock period and entrainment. Neuron 54 (5):813-829. doi:S0896-6273(07)00374-1 [pii]10.1016/j.neuron.2007.05.017

Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, Cannon B, Kelnar K, Kemppainen J, Brown D, Chen C, Prinjha RK, Richardson JC, Saunders AM, Roses AD, Richards CA (2008) Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J Alzheimers Dis 14 (1):27-41

Cohen JE, Lee PR, Chen S, Li W, Fields RD (2011) MicroRNA regulation of homeostatic synaptic plasticity. Proc Natl Acad Sci U S A 108 (28):11650-11655. doi:1017576108 [pii]10.1073/pnas.1017576108

Colombo PJ, Brightwell JJ, Countryman RA (2003) Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. J Neurosci 23 (8):3547-3554. doi:23/8/3547 [pii]

Croll SD, Suri C, Compton DL, Simmons MV, Yancopoulos GD, Lindsay RM, Wiegand SJ, Rudge JS, Scharfman HE (1999) Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 93 (4):1491-1506. doi:S0306-4522(99)00296-1 [pii]

Cuellar TL, Davis TH, Nelson PT, Loeb GB, Harfe BD, Ullian E, McManus MT (2008) Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the

absence of neurodegeneration. Proc Natl Acad Sci U S A 105 (14):5614-5619. doi:0801689105 [pii]10.1073/pnas.0801689105
Eulalio A, Huntzinger E, Izaurralde E (2008) Getting to the root of miRNA-mediated gene silencing. Cell 132 (1):9-14. doi:S0092-8674(07)01697-2 [pii]10.1016/j.cell.2007.12.024
Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR (2000) Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 28 (1):41-51. doi:S0896-6273(00)00084-2 [pii]
Fiore R, Khudayberdiev S, Christensen M, Siegel G, Flavell SW, Kim TK, Greenberg ME, Schratt G (2009) Mef2-mediated transcription of the miR379-410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. EMBO J 28 (6):697-710. doi:emboj200910 [pii]10.1038/emboj.2009.10
Gao J, Wang WY, Mao YW, Gräff J, Guan JS, Pan L, Mak G, Kim D, Su SC, Tsai LH (2010) A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466 (7310):1105-1109. doi:nature09271 [pii]10.1038/nature09271
Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF (2005) MicroRNAs regulate brain morphogenesis in zebrafish. Science 308 (5723):833-838. doi:1109020 [pii]10.1126/science.1109020
Goodman RH, Smolik S (2000) CBP/p300 in cell growth, transformation, and development. Genes Dev 14 (13):1553-1577
Hall J, Thomas KL, Everitt BJ (2001) Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala. Eur J Neurosci 13 (7):1453-1458. doi:ejn1531 [pii]
Hansen JC, Ghosh RP, Woodcock CL (2010a) Binding of the Rett syndrome protein, MeCP2, to methylated and unmethylated DNA and chromatin. IUBMB Life 62 (10):732-738. doi:10.1002/iub.386
Hansen KF, Sakamoto K, Obrietan K (2011) MicroRNAs: a potential interface between the circadian clock and human health. Genome Med 3 (2):10. doi:gm224 [pii]10.1186/gm224
Hansen KF, Sakamoto K, Wayman GA, Impey S, Obrietan K (2010b) Transgenic miR-132 alters neuronal spine density and impairs novel object recognition memory. PLoS One 5 (11):e15497. doi:10.1371/journal.pone.0015497
Hutsler JJ, Zhang H (2010) Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res 1309:83-94. doi:S0006-8993(09)02311-7 [pii]10.1016/j.brainres.2009.09.120
Im HI, Hollander JA, Bali P, Kenny PJ (2010) MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat Neurosci 13 (9):1120-1127. doi:nn.2615 [pii]10.1038/nn.2615
Impey S, Davare M, Lasiek A, Fortin D, Ando H, Varlamova O, Obrietan K, Soderling TR, Goodman RH, Wayman GA (2010) An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. Mol Cell Neurosci 43 (1):146-156. doi:S1044-7431(09)00223-1 [pii]10.1016/j.mcn.2009.10.005
Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR (1998) Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. Nat Neurosci 1 (7):595-601. doi:10.1038/2830
Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler IJ, Greenough WT (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X

syndrome: a quantitative examination. Am J Med Genet 98 (2):161-167. doi:10.1002/1096-8628(20010115)98:2<161::AID-AJMG1025>3.0.CO;2-B [pii]
Jimenez-Mateos EM, Bray I, Sanz-Rodriguez A, Engel T, McKiernan RC, Mouri G, Tanaka K, Sano T, Saugstad JA, Simon RP, Stallings RL, Henshall DC (2011) miRNA Expression Profile after Status Epilepticus and Hippocampal Neuroprotection by Targeting miR-132. Am J Pathol. doi:S0002-9440(11)00758-9 [pii]10.1016/j.ajpath.2011.07.036
Kaitsuka T, Li ST, Nakamura K, Takao K, Miyakawa T, Matsushita M (2011) Forebrain-specific constitutively active CaMKKα transgenic mice show deficits in hippocampus-dependent long-term memory. Neurobiol Learn Mem 96 (2):238-247. doi:S1074-7427(11)00091-8 [pii]10.1016/j.nlm.2011.04.017
Khudayberdiev S, Fiore R, Schratt G (2009) MicroRNA as modulators of neuronal responses. Commun Integr Biol 2 (5):411-413
Kim AH, Reimers M, Maher B, Williamson V, McMichael O, McClay JL, van den Oord EJ, Riley BP, Kendler KS, Vladimirov VI (2010) MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophr Res 124 (1-3):183-191. doi:S0920-9964(10)01381-2 [pii]10.1016/j.schres.2010.07.002
Kim IH, Park SK, Sun W, Kang Y, Kim HT, Kim H (2004a) Spatial learning enhances the expression of inositol 1,4,5-trisphosphate 3-kinase A in the hippocampal formation of rat. Brain Res Mol Brain Res 124 (1):12-19. doi:S0169328X04000282 [pii]10.1016/j.molbrainres.2003.12.016
Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G (2004b) Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proc Natl Acad Sci U S A 101 (1):360-365. doi:2333854100 [pii]10.1073/pnas.2333854100
Kleim JA, Swain RA, Armstrong KA, Napper RM, Jones TA, Greenough WT (1998) Selective synaptic plasticity within the cerebellar cortex following complex motor skill learning. Neurobiol Learn Mem 69 (3):274-289. doi:S1074-7427(98)93827-8 [pii]10.1006/nlme.1998.3827
Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH (2007) Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci 10 (12):1513-1514. doi:nn2010 [pii] 10.1038/nn2010
Konopka W, Kiryk A, Novak M, Herwerth M, Parkitna JR, Wawrzyniak M, Kowarsch A, Michaluk P, Dzwonek J, Arnsperger T, Wilczynski G, Merkenschlager M, Theis FJ, Köhr G, Kaczmarek L, Schütz G (2010) MicroRNA loss enhances learning and memory in mice. J Neurosci 30 (44):14835-14842. doi:30/44/14835 [pii]10.1523/JNEUROSCI.3030-10.2010
Konopka W, Schütz G, Kaczmarek L (2011) The MicroRNA Contribution to Learning and Memory. Neuroscientist. doi:1073858411411721 [pii]10.1177/1073858411411721
Kosik KS (2006) The neuronal microRNA system. Nat Rev Neurosci 7 (12):911-920. doi:nrn2037 [pii] 10.1038/nrn2037
Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T (2002) Identification of tissue-specific microRNAs from mouse. Curr Biol 12 (9):735-739. doi:S0960982202008096 [pii]

Lagos D, Pollara G, Henderson S, Gratrix F, Fabani M, Milne RS, Gotch F, Boshoff C (2010) miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nat Cell Biol 12 (5):513-519. doi:ncb2054 [pii]10.1038/ncb2054

Lambert TJ, Storm DR, Sullivan JM (2010) MicroRNA132 modulates short-term synaptic plasticity but not basal release probability in hippocampal neurons. PLoS One 5 (12):e15182. doi:10.1371/journal.pone.0015182

Leuner B, Falduto J, Shors TJ (2003) Associative memory formation increases the observation of dendritic spines in the hippocampus. J Neurosci 23 (2):659-665. doi:23/2/659 [pii]

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433 (7027):769-773. doi:nature03315 [pii]10.1038/nature03315

Lin Q, Wei W, Coelho CM, Li X, Baker-Andresen D, Dudley K, Ratnu VS, Boskovic Z, Kobor MS, Sun YE, Bredy TW (2011) The brain-specific microRNA miR-128b regulates the formation of fear-extinction memory. Nat Neurosci 14 (9):1115-1117. doi:nn.2891 [pii] 10.1038/nn.2891

Lopez de Armentia M, Jancic D, Olivares R, Alarcon JM, Kandel ER, Barco A (2007) cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons. J Neurosci 27 (50):13909-13918. doi:27/50/13909 [pii] 10.1523/JNEUROSCI.3850-07.2007

Magill ST, Cambronne XA, Luikart BW, Lioy DT, Leighton BH, Westbrook GL, Mandel G, Goodman RH (2010) microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. Proc Natl Acad Sci U S A 107 (47):20382-20387. doi:1015691107 [pii] 10.1073/pnas.1015691107

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER (1996) Control of memory formation through regulated expression of a CaMKII transgene. Science 274 (5293):1678-1683

Mellios N, Sugihara H, Castro J, Banerjee A, Le C, Kumar A, Crawford B, Strathmann J, Tropea D, Levine SS, Edbauer D, Sur M (2011) miR-132, an experience-dependent microRNA, is essential for visual cortex plasticity. Nat Neurosci 14 (10):1240-1242. doi:nn.2909 [pii] 10.1038/nn.2909

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature 396 (6710):433-439. doi:10.1038/24790

Mizuno M, Yamada K, Maekawa N, Saito K, Seishima M, Nabeshima T (2002) CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. Behav Brain Res 133 (2):135-141. doi:S0166432801004703 [pii]

Moon SY, Zang H, Zheng Y (2003) Characterization of a brain-specific Rho GTPase-activating protein, p200RhoGAP. J Biol Chem 278 (6):4151-4159. doi:M207789200 [pii] 10.1074/jbc.M207789200

Morris R (1985) Thy-1 in developing nervous tissue. Dev Neurosci 7 (3):133-160

Nakayama AY, Harms MB, Luo L (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. J Neurosci 20 (14):5329-5338. doi:20/14/5329 [pii]

Nudelman AS, DiRocco DP, Lambert TJ, Garelick MG, Le J, Nathanson NM, Storm DR (2010) Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. Hippocampus 20 (4):492-498. doi:10.1002/hipo.20646

Nuovo GJ (2010) In situ detection of microRNAs in paraffin embedded, formalin fixed tissues and the co-localization of their putative targets. Methods 52 (4):307-315. doi:S1046-2023(10)00210-0 [pii] 10.1016/j.ymeth.2010.08.009

Obernosterer G, Martinez J, Alenius M (2007) Locked nucleic acid-based in situ detection of microRNAs in mouse tissue sections. Nat Protoc 2 (6):1508-1514. doi:nprot.2007.153 [pii] 10.1038/nprot.2007.153

Packer AN, Xing Y, Harper SQ, Jones L, Davidson BL (2008) The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. J Neurosci 28 (53):14341-14346. doi:28/53/14341 [pii]10.1523/JNEUROSCI.2390-08.2008

Pietropaolo S, Paterna JC, Büeler H, Feldon J, Yee BK (2007) Bidirectional changes in water-maze learning following recombinant adenovirus-associated viral vector (rAAV)-mediated brain-derived neurotrophic factor expression in the rat hippocampus. Behav Pharmacol 18 (5-6):533-547. doi:00008877-200709000-00021 [pii] 10.1097/FBP.0b013e3282da0bf6

Pineda VV, Athos JI, Wang H, Celver J, Ippolito D, Boulay G, Birnbaumer L, Storm DR (2004) Removal of G(ialpha1) constraints on adenylyl cyclase in the hippocampus enhances LTP and impairs memory formation. Neuron 41 (1):153-163. doi:S0896627303008134 [pii]

Pollak DD, Herkner K, Hoeger H, Lubec G (2005) Behavioral testing upregulates pCaMKII, BDNF, PSD-95 and egr-1 in hippocampus of FVB/N mice. Behav Brain Res 163 (1):128-135. doi:S0166-4328(05)00165-8 [pii]10.1016/j.bbr.2005.04.010

Porte Y, Buhot MC, Mons NE (2008) Spatial memory in the Morris water maze and activation of cyclic AMP response element-binding (CREB) protein within the mouse hippocampus. Learn Mem 15 (12):885-894. doi:15/12/885 [pii]10.1101/lm.1094208

Remenyi J, Hunter CJ, Cole C, Ando H, Impey S, Monk CE, Martin KJ, Barton GJ, Hutvagner G, Arthur JS (2010) Regulation of the miR-212/132 locus by MSK1 and CREB in response to neurotrophins. Biochem J 428 (2):281-291. doi:BJ20100024 [pii] 10.1042/BJ20100024

Sakamoto K, Karelina K, Obrietan K (2011) CREB: a multifaceted regulator of neuronal plasticity and protection. J Neurochem 116 (1):1-9. doi:10.1111/j.1471-4159.2010.07080.x

Sanuki R, Onishi A, Koike C, Muramatsu R, Watanabe S, Muranishi Y, Irie S, Uneo S, Koyasu T, Matsui R, Chérasse Y, Urade Y, Watanabe D, Kondo M, Yamashita T, Furukawa T (2011) miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Nat Neurosci 14 (9):1125-1134. doi:nn.2897 [pii] 10.1038/nn.2897

Schmittgen TD, Lee EJ, Jiang J, Sarkar A, Yang L, Elton TS, Chen C (2008) Real-time PCR quantification of precursor and mature microRNA. Methods 44 (1):31-38. doi:S1046-2023(07)00171-5 [pii] 10.1016/j.ymeth.2007.09.006

Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME (2006) A brain-specific microRNA regulates dendritic spine development. Nature 439 (7074):283-289. doi:nature04367 [pii] 10.1038/nature04367

Sterner DE, Berger SL (2000) Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev 64 (2):435-459

Sunyer B, Lubec G, Höger H, Patil S (2007) Barnes maze, a useful task to assess spatial reference memory in the mice.

Suzuki A, Fukushima H, Mukawa T, Toyoda H, Wu LJ, Zhao MG, Xu H, Shang Y, Endoh K, Iwamoto T, Mamiya N, Okano E, Hasegawa S, Mercaldo V, Zhang Y, Maeda R, Ohta M, Josselyn SA, Zhuo M, Kida S (2011) Upregulation of CREB-mediated transcription enhances both short- and long-term memory. J Neurosci 31 (24):8786-8802. doi:31/24/8786 [pii]10.1523/JNEUROSCI.3257-10.2011

Tognini P, Putignano E, Coatti A, Pizzorusso T (2011) Experience-dependent expression of miR-132 regulates ocular dominance plasticity. Nat Neurosci 14 (10):1237-1239. doi:nn.2920 [pii]10.1038/nn.2920

Trommald M, Hulleberg G, Andersen P (1996) Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. Learn Mem 3 (2-3):218-228

Van Aelst L, Cline HT (2004) Rho GTPases and activity-dependent dendrite development. Curr Opin Neurobiol 14 (3):297-304. doi:S0959438804000790 [pii]10.1016/j.conb.2004.05.012

Vázquez SI, Vázquez A, Peña de Ortiz S (2000) Different hippocampal activity profiles for PKA and PKC in spatial discrimination learning. Behav Neurosci 114 (6):1109-1118

Viosca J, Malleret G, Bourtchouladze R, Benito E, Vronskava S, Kandel ER, Barco A (2009) Chronic enhancement of CREB activity in the hippocampus interferes with the retrieval of spatial information. Learn Mem 16 (3):198-209. doi:16/3/198 [pii]10.1101/lm.1220309

Vo N, Goodman RH (2001) CREB-binding protein and p300 in transcriptional regulation. J Biol Chem 276 (17):13505-13508. doi:R000025200 [pii]10.1074/jbc.R000025200

Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc Natl Acad Sci U S A 102 (45):16426-16431. doi:0508448102 [pii]10.1073/pnas.0508448102

Vuksic M, Del Turco D, Bas Orth C, Burbach GJ, Feng G, Müller CM, Schwarzacher SW, Deller T (2008) 3D-reconstruction and functional properties of GFP-positive and GFP-negative granule cells in the fascia dentata of the Thy1-GFP mouse. Hippocampus 18 (4):364-375. doi:10.1002/hipo.20398

Wayman GA, Davare M, Ando H, Fortin D, Varlamova O, Cheng HY, Marks D, Obrietan K, Soderling TR, Goodman RH, Impey S (2008) An activity-regulated microRNA controls dendritic plasticity by down-regulating p250GAP. Proc Natl Acad Sci U S A 105 (26):9093-9098. doi:0803072105 [pii] 10.1073/pnas.0803072105

Wibrand K, Panja D, Tiron A, Ofte ML, Skaftnesmo KO, Lee CS, Pena JT, Tuschl T, Bramham CR (2010) Differential regulation of mature and precursor microRNA expression by NMDA and metabotropic glutamate receptor activation during LTP in the adult dentate gyrus in vivo. Eur J Neurosci 31 (4):636-645. doi:EJN7112 [pii]