MicroRNAs (miRNAs) are rapidly emerging as central regulators of gene expression in the postnatal mammalian brain. Initial studies mostly focused on the function of specific miRNAs during the development of neuronal connectivity in culture, using classical gain- and loss-of-function approaches. More recently, first examples have documented important roles of miRNAs in plastic processes in intact neural circuits in the rodent brain related to higher cognitive abilities and neuropsychiatric disease. At the same time, evidence is accumulating that miRNA function itself is subjected to sophisticated control mechanisms engaged by the activity of neural circuits. In this review, we attempt to pay tribute to this mutual relationship between miRNAs and synaptic plasticity. In particular, in the first part, we summarize how neuronal activity influences each step in the lifetime of miRNAs, including the regulation of transcription, maturation, gene regulatory function and turnover in mammals. In the second part, we discuss recent examples of miRNA function in synaptic plasticity in rodent models and their implications for higher cognitive function and neurological disorders, with a special emphasis on epilepsy as a disorder of abnormal nerve cell activity.

1. The miRNA biogenesis pathway

The transcription of a microRNA (miRNA) gene is usually exerted by RNA polymerase II, resulting in a primary transcript (pri-miRNA) with a typical hairpin-like structure [1]. In the nucleus, the core of the microprocessor complex, formed by the RNAseIII enzyme Drosha and the dsRNA-binding protein DGCR8, excises the hairpin from the primary transcript, liberating a so-called precursor miRNA (pre-miRNA). After nuclear export, the RNAseIII enzyme Dicer, with the aid of the dsRNA-binding protein TRBP, excises the loop of the hairpin from the pre-miRNA. TRBP also assists in the recruitment of the Argonaute (AGO) protein AGO2 to Dicer, resulting in the formation of the miRNA RNA-induced silencing complex (RISC)-loading complex (miRLC) [2]. Within miRLC, AGO proteins unwind the miRNA duplex and usually load only the strand with the less thermodynamically stable pairing at the 5' region, the so-called guide strand. Finally, AGO2 forms the nucleus for the RISC, which in addition consists of GW182 proteins (in mammals TNRC6 A/B/C) and a set of auxiliary proteins (e.g. MOV10, FMRP) often involved in translational repression [3]. The RISC further exerts its gene regulatory function by exploiting the direct binding of the loaded miRNA (nucleotides 2–8 in the miRNA sequence) to complementary sequences often conserved in the 3'UTR of mRNAs [4]. Depending on the degree of complementarity between the miRNA and the mRNA sequences, the target can be either endonucleolytically cleaved or prevented from being effectively translated. The latter mechanism involves a block in translational initiation, decapping and/or deadenylation and is more frequent in animals because of the usually imperfect base pairing of the miRNA to its targets [5]. Finally, several factors affecting miRNA stability have also been described in mammalian cells, such as direct post-transcriptional modifications of precursor and mature miRNA sequences, regulation of the availability/stability of miRNA biogenesis factors and miRNA degrading enzymes [6,7]. Importantly, several sequence-specific RNA-binding proteins (RBPs) have been shown to...
modulate miRNA function at any level of the miRNA pathway [8] (figure 1a–d, left panel).

2. Regulation of miRNAs by neuronal activity

(a) Activity-dependent transcription of miRNAs

In differentiated neurons, the transcription of specific brain-enriched miRNA genes is controlled by changes in neuronal activity, which is a result of experience or other plasticity-inducing cues. Originally, this mechanism was discovered in studies on the miR-132 gene, which is induced by a plethora of stimuli that can be associated with neuronal activity (membrane depolarization, neurotrophins, etc.). The activation of the related signalling pathways impinges on the activation of the transcription factor cAMP response element-binding protein (CREB), which binds two CRE responsive elements in the promoter of the pri-miR-132 gene and induces miR-132 transcription in cultured neurons. Like for early-response genes, this CREB-mediated transcription rapidly peaks at 30 min–1 h, and is followed by a more modest, persistent increase in mature miR-132 levels [9,10]. Pharmacology further points to an exquisite role of NMDA receptor activation and the downstream calcium/calmodulin-dependent kinase (CaMK) and MAPK–ERK pathways in pri-miR-132 expression [9–12].

Another miRNA whose expression is tightly controlled by activity is miR-134, a prototypical dendritic miRNA. In cultured rat neurons, treatments with brain-derived neurotrophic factor (BDNF) and KCl increase the transcription of a transcript probably spanning the entire miR379–410 cluster, which includes miR-134 and other miRNAs. In the case of miR-134, the transcription is induced by the activity-regulated transcription factor Me2, and peaks slightly later (2 h) compared with miR-132. This already indicated that activity-dependent expression of miR-132 and miR-134 could fulfill different purposes. Importantly, activity-regulated transcription of several miRNAs from this cluster was shown to regulate dendritic outgrowth, in particular, in the case of miR-134, through repression of Pumilio2 [13]. In another work, Gao et al. validated two binding motifs for the transcription factor yin yang 1 (YY1) upstream of the miR-134 locus. Together in a repressive complex, YY1 and the histone deacetylase Sirt1 repress miR-134 gene expression [14]. Interestingly, Sirt1 is also involved in the repression of other miRNA transcripts, e.g. pri-miR-138-1, and is itself a target of miR-138 [15] and miR-34 [16]. This suggests the existence of sophisticated feedback systems involving miRNAs and transcription factors as already described for developing neurons [17].

Focusing on the visual system, several groups demonstrated that light-dependent transcriptional activation of miRNA genes also occurs in vivo. For example, Król et al. [18] show that the primary transcripts of the miR-183/96/182 cluster and the independent intergenic transcripts of miR-204 and miR-211 are restored upon light re-exposure of dark-adapted retinas. Other studies performed in the mouse visual cortex report an increase in histone marks at the CRE loci present in the miR-132/212 promoter in response to visual experience, which is paralleled by a pronounced increase in miR-132 transcription. Conversely, in the visual cortex of mice grown under dark rearing or deprived of an eye, transcription of miR-132 and the other cluster member, miR-212, is reduced. Interestingly, pri-miR-132 reaccumulation is achieved upon re-exposure to light [19,20]. Also in the suprachiasmatic nucleus, light pulses induce levels of pre-miR-132 via CREB and independent of the circadian phase. Conversely, transcription of miR-219 is regulated by the circadian clock in vivo, but is unaffected by light stimulation (or other CREB-activating stimuli), although an upstream CRE-binding site was identified [21] (figure 1a).

miRNA changes in response to plasticity-inducing stimuli that are also physiologically relevant in the context of plasticity, learning and memory will be discussed in §3.

(b) Activity-dependent regulation of miRNA biogenesis

Post-transcriptional regulation of miRNA expression by modulation of critical components of the miRNA biogenesis pathway was recently shown in several biological contexts [22,23]. Generally speaking, activity-dependent regulation of miRNA biogenesis in neurons could be achieved by post-translational modifications (PTMs)/degradation of the core biogenesis factors or associated RBPs that have modulatory effects. However, specific examples derived from the neuronal system are still sparse.

Stimulus-dependent regulation of the microprocessor complex has been most extensively studied in non-neuronal systems. For example, it was recently demonstrated that DGCR8 is multi-phosphorylated by the MEK/ERK pathway in vivo, leading to DGCR8 stabilization and to a concomitant upregulation of miRNA expression [24]. DGCR8 was also shown to stabilize Drosha levels via protein–protein interactions, and in a negative loop to be processed by Drosha itself at the mRNA level [25,26]. Furthermore, increasing concentration of pri-miRNA (e.g. upon neuronal stimulation) resulted in increased levels/activity of Drosha/DGCR8 mRNA and protein levels [27] (figure 1b, right panel). Together, this suggests the existence of intricate regulatory mechanisms that ensure miRNA homeostasis in response to environmental signals. In contrast, little is known regarding activity-dependent regulation of the microprocessor in neurons. NMDA and bicuculline treatment in hippocampal cultures seem to increase DGCR8 mRNA and protein levels and slightly downregulate Drosha and Dicer protein levels [28]. However, in the retina, light and dark adaptation has no effect on Drosha protein levels [18].

Despite this initial evidence, a better understanding on how neuronal activity directly regulates the microprocessor complex in the context of extracellular stimulation is needed.

Recent studies point to an important function of PTMs of the Dicer/TRBP complex in the regulation of neuronal miRNA activity. Initially, it was found that Dicer is activated in response to activity by limited proteolysis due to cleavage by the calcium-dependent protease calpain. This resulted in increased RNAse III activity of Dicer on dsRNA substrates [29]. In a more recent study, BDNF-mediated stimulation of the ERK pathway was shown to stabilize Dicer protein levels via phosphorylation of TRBP [30]. The authors presented a working model whereby BDNF-induced Dicer activation promoted the biogenesis of a selected set of premiRNAs. In the same study, Huang et al. further found that BDNF stimulation led to the accumulation of the RBP LIN28. This in turn caused a specific decrease in LIN28-regulated miRNAs and a corresponding upregulation in the translation of their target miRNAs [30]. Similarly, expression

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Figure 1. Regulation of microRNA biogenesis by neuronal activity. (a) Activity-dependent miRNA transcription. Under basal conditions, the transcription of miRNA genes (blue) is under control of transcriptional activators (TA) or transcriptional repressors (TR) that bind to their respective regulatory sequences (left panel). Upon neuronal stimulation, specific TAs (e.g. CREB, Mef2) become active, resulting in an enhancement of pri-miRNA production (upper-right panel). This is accompanied also by histone modifications within the promoter region of activity-regulated miRNAs that favour transcription of miRNA genes (lower-right panel). (b) Most of the pri-miRNAs undergo the first enzymatic cleavage by the microprocessor complex, resulting in pre-miRNAs. This step can be regulated by RNA-binding proteins (RBP; left panel). It should be noted that post-translational modifications (PTMs, yellow circles) of Drosha and DGCR8 that facilitate pri-miRNA processing were only reported in non-neuronal cells so far, so the indicated regulation in neurons remain speculative (right panel). (c) After cytoplasmic export, pre-miRNAs undergo the second processing step by the Dicer–TRBP complex, resulting in the formation of miRNA duplexes. This step can be aided by RBPs such as FMRP (left panel). Upon neuronal stimulation, Dicer enzymatic activity increases following limited proteolysis or protein stabilization upon TRBP phosphorylation. In addition, activity-driven regulation of RBP protein levels (e.g. LIN28) can modulate the processing of specific pre-miRNAs in a bidirectional manner. (d) In the final steps of maturation, miRNA duplexes are unwound and the resulting single-stranded mature miRNAs are loaded in the RISC complex, which in turns targets complementary sequences within 3′ UTRs of mRNAs. The RISC complex includes AGO2, GW182 and several auxiliary proteins (e.g. MOV10, FMRP), serves as platform for the recruitment of translational repressors (pale yellow) and possibly interferes with ribosomal function at different stages of translation (ribosome in grey). Upon neuronal activity, examples of both a reduction and enhancement of miRNA-mediated silencing have been described (see also figure 2).
of the synaptic miR-138 also appears to be regulated at the level of precursor processing. According to Obermosterer et al. [31], non-neuronal cells possess a specific inhibitor that blocks processing of pre-miR-138, thereby preventing expression of mature miR-138 in non-neuronal cells. The identity of this repressor molecule and whether it is subjected to activity regulation in neurons is, however, unknown [31]. Moreover, it was recently shown that the DEAH-box protein DHX36, which mediates the transport of pre-miR-134 to dendrites, protects the pre-miR-134 from Dicer processing by binding to the terminal loop structure [32] (figure 1c, right panel). As reported in §1, AGO proteins also participate in miRNA biogenesis, but evidence about this aspect of AGO function in neurons has still not been provided. In conclusion, several examples now demonstrate activity-dependent regulation of miRNA biogenesis also in neurons, but the detailed mechanisms and their physiological ramifications are mostly elusive.

(c) Activity-dependent regulation of miRNA turnover

 Originally, miRNAs were considered to be extremely stable molecules in comparison with the bulk of cellular mRNAs, but this view is increasingly challenged [7]. Regulated miRNA turnover appears to be particularly relevant in neuronal cells, which might be attributed to their constant need for experience-dependent adaptations.

In a seminal study on miRNA turnover performed in the retina, Krol et al. [18] showed that general miRNA expression levels physiologically change upon light and dark adaptation. In particular, the levels of the miR182/96/183 cluster and miR-204 and miR-201 are reduced by 50% after 30 min of adaptation to the dark. miRNA levels are restored to basal levels within 30 min of light re-adaptation, demonstrating the fast kinetics of this regulation. Importantly, regulated turnover upon dark adaptation occurs specifically in neurons and apparently does not involve alterations in Drosha or Dicer protein levels, suggesting that activity directly affects enzymes responsible for miRNA degradation or stability [18]. In the same study, this concept has been also extended to cultured neurons, where expression of the plasticity-relevant miR-124, miR-128, miR-134 and miR-138 was significantly reduced after 30 min of transcriptional blockade. This effect was prevented by the application of tetrodotoxin, an inhibitor of neuronal firing, providing first evidence that neuronal network activity is involved in the regulation of miRNA levels. Consistently, stimulation of glutamate receptors further accelerated the downregulation of the same miRNAs. Like in the retina, this mechanism was a particular feature of fully mature neurons and not observed in other cell types [18]. Interestingly, the turnover of miR-132 displays inverted dynamics compared with the other plasticity-relevant miRNAs. In the adult mouse visual cortex, as discussed before, miR-132 levels are stable, whereas the drop in miR-132 under dark rearing condition seems to be a consequence of transcriptional inhibition rather than of activity-regulated turnover [19,20].

A number of other studies have recently interrogated miRNA expression in cultured neurons in the context of various activity-related paradigms. In particular, hippocampal neurons were induced with chemical long-term potentiation (LTP) and long-term depression (LTD) protocols, and chronic treatments inducing homeostatic adaptations. As a result, more than threefold bidirectional changes in the miRNA expression levels were detected with specific time kinetics related to the kind of stimulus [33,34]. Studies conducted in the cortex and hippocampus in vivo mostly confirmed the previously presented data from in vitro experiments, although a comparative analysis is missing. For example, induction of NMDA-dependent LTP in the dentate gyrus (DG) led to bidirectional changes in the expression of specific miRNAs [35]. Interestingly, a common and persistent (24 h) modulation of miRNAs in the hippocampus was also reported from mice subjected to specific learning paradigms (e.g. fear-conditioning). Interestingly, a subgroup of the upregulated miRNAs in this study preferentially targeted inhibitors of the mTOR pathway [28], suggesting that this pathway could be a common target of plasticity-regulated miRNAs.

Although all this evidence points towards an activity-dependent functional modulation of miRNA turnover, the molecular explanation underlying this effect is still lacking. miRNA decay could be directly affected by the regulation of specific exonucleases, post-transcriptional modifications of the miRNA sequence (e.g. trimming, tailing, editing) or the stability of the RISC complex itself. Alternatively, upregulated miRNA levels could be a result of activity-dependent transcription or accelerated biogenesis. Clearly, more work is needed to fully understand the molecular mechanisms of activity-dependent miRNA turnover in neurons, in particular with regard to the spatio-temporal dynamics in the context of synaptic plasticity.

Finally, impairments in transcriptional supply of specific pri-miRNAs, such as for miR-132, or in their proper maturation, such as for the let-7 miRNA family, can affect miRNA decay or expression, adding yet another layer of complexity at the level of miRNA biogenesis.

(d) Activity-dependent regulation of the repressive function of miRNAs within miRISC

In neurons, reversible miRNA-mediated repression at the level of miRISC seems to be particularly suited for the regulation of local protein synthesis upon neuronal activation (figures 1d and 2). Actually, one of the first pieces of evidence for such a dynamic mechanism was described in primary hippocampal neurons, where the repressive effect of miR-134 on Link1 local translation could be reversed upon BDNF-mediated activation of the mTOR kinase pathway [36]. Functionally, miR-134 is a negative regulator of spine growth, but whether this effect is also reversed upon BDNF treatment remains unknown. In a more recent study, dynamic control of miRNA activity was also shown in the context of neuronal homeostasis, again involving mTOR. Specifically, activated mTOR was shown to promote the miR-129-mediated repression of the voltage-gated potassium channel Kv1.1 mRNA. Conversely, under conditions of inactive mTOR, association of the RBP HuD with AU-rich sequences on Kv1.1 mRNA was facilitated, resulting in an activation of Kv1.1 local translation. It was further suggested that the positive effect of HuD on Kv1.1 translation is the final result of the titration of other high affinity HuD targets [37] (figure 2a). In a different study, it was shown that the RISC-associated protein MOV10 is rapidly ubiquitinated and degraded upon membrane depolarization or activation of NMDA channels. This resulted in a de-repression of several miRNA target mRNAs, including CaMKIIα, the miR-138 target APTI and the miR-134 target Limk1 at synapses [38]. Interestingly, miR-138 was shown to negatively regulate
spine growth by targeting APT1, suggesting that activity-dependent miR-138 inactivation could be a mechanism of synaptic potentiation [39]. Muddashetty et al. [40] investigated a possible function of FMRP in the dynamic control of miRNA function. They found that in its phosphorylated state, FRMP forms a complex with AGO2-bound miR-125 to repress local PSD95 mRNA translation. Activation of metabotropic glutamate receptors (mGluRs) promoted the dephosphorylation of FMRP and proteasomal degradation of MOV10 lead to a release of RISC from target miRNAs. Examples of AGO2 PTMs induced by extracellular stimuli were only described in non-neuronal cells and therefore remain speculative in neurons. (c) Endogenous miRNA ‘sponges’, e.g. ciRS-7, are circular RNAs that contain several binding sites for miRNAs and are able to sequester the RISC away from other natural targets, thereby leading to a derepression. Whether circRNAs are subjected to activity-dependent regulation is unknown.

Figure 2. Dynamic regulation of microRNA function in neurons. Neuronal activity and other factors working in trans can influence miRNA-mediated repression bidirectionally. (a) Specific RBPs can compete with miRNAs for binding to target sites in the 3’UTRs in an activity-dependent manner, as shown for example for the HuD protein. (b) Activity-induced PTMs (yellow circles) of the RISC complex modulate the repressive function of miRNAs. For example, activity-dependent dephosphorylation of FMRP and proteasomal degradation of MOV10 lead to a release of RISC from target miRNAs. Examples of AGO2 PTMs induced by extracellular stimuli were only described in non-neuronal cells and therefore remain speculative in neurons. (c) Endogenous miRNA ‘sponges’, e.g. ciRS-7, are circular RNAs that contain several binding sites for miRNAs and are able to sequester the RISC away from other natural targets, thereby leading to a derepression. Whether circRNAs are subjected to activity-dependent regulation is unknown.

repression on its target MAP1B can be relieved in a similar manner via the activation of mGluRs. This dynamic regulation is involved in mGluR-dependent AMPA receptor internalization associated with LTD [42]. In contrast to these multiple examples of miRNAs that negatively regulate synaptic strength, miR-132 promotes dendritic outgrowth and spine enlargement in cultured neurons [10,41,43]. It remains to be shown whether this miRNA is also subjected to activity-dependent regulation at the level of the miRISC.

Other attractive targets for activity-dependent modification within miRISC are AGO proteins. However, in neurons, evidence so far is limited to a single study that described a morphine-induced decrease in AGO2 protein levels in dopaminergic neurons that could be reversed upon drug withdrawal [44].
In contrast, a number of AGO PTMs influencing its stability and function have been described in non-neuronal cells and could be potentially relevant in neurons (as reviewed in Meister et al. [3]). Finally, the stability of AGO2 is also affected by the levels of mature miRNAs and, indirectly, by modulatory events upstream in the miRNA pathway [45]. Taken together, future studies investigating activity-dependent AGO2 modifications and turnover in neurons are warranted.

(e) Regulation of miRNAs by sequestration
Finally, the recently discovered circular RNAs (circRNAs) could represent yet another layer of miRNA regulation (figure 2c). Similar to artificially designed miRNA sponge constructs, some of these circRNAs appear to work by sequestering endogenous targets, thereby relieving the repression of other physiological target miRNAs of the same miRNA. For example, ciRS-7 contains 73 binding sites for the neuronal miR-7 and colocalizes with miR-7 in the hippocampus. Inactivation of ciRS-7 leads to a brain developmental phenotype in zebrafish, but the function of this molecule in the mammalian brain, if any, as well as the (de)regulated miR-7 target miRNAs remain to be determined [46,47]. Interestingly, one of these studies also described a circRNA (an alternative transcript of the SRY gene) that works as a specific sponge for the plasticity-regulating miR-138. However, this circRNA was reported to be expressed specifically in the testes [48], making it unlikely that this regulation is involved in neuronal development or plasticity. In the future, it will be interesting to see how widespread miRNA regulation by endogenous sponges (circRNAs, ceRNAs, etc.) in neurons is and how the miRNA–target interactions can be modulated by neuronal activity. circRNAs are inactivated by enzymatic linearization, so this could be a potential target for activity-dependent pathways.

3. miRNA function in synaptic plasticity with implications for higher cognitive function and related neurological diseases

(a) Regulation of miRNAs by synaptic plasticity
The discovery of brain-specific/enriched miRNAs and their regulation by common activity paradigms (see §2) suggests the necessity of further research at the network level to discriminate the region-specific presence as well as function of miRNAs. General profiling by microarrays distinguished the differential expression of miRNAs in different regions of the rodent brain [49,50] with a specific emphasis on the cortex and hippocampus [51,52]. More recently, miRNA expression was also explored under conditions of elevated activity known to induce synaptic plasticity. For example, similar to behavioural activation, both chemically induced LTP [33,53] and LTD in the hippocampal CA1 region lead to a general increase in miRNA levels with a high degree of overlap [33]. Electrically induced LTP in the DG also increases the level of a number of primary-miRNAs and possibly boosts the turnover of mature miRNAs [35]. This further suggests specific roles for miRNAs in higher-order brain functions.

(b) miRNAs in synaptic plasticity, learning and memory
Considering the vital role of miRNAs in post-transcriptional regulation, their widespread expression in different brain regions and their regulation by neuronal activity, miRNAs are increasingly considered as central players in synaptic plasticity, a cellular mechanism that is thought to underlie many complex brain functions, including learning and memory. Different forms of synaptic plasticity are involved in the developing as well as the mature brain. For example, ocular dominance plasticity in the visual cortex occurs specifically during a critical period after eye opening. Another well-known type is the long-term synaptic plasticity at excitatory synapses of the cortex/hippocampus that is regarded to be an important mechanism involved in learning and memory formation.

Intuitively, alterations in miRNA levels might be indicative of their functional involvement; however, more detailed work is necessary to enlighten the molecular pathways in the intact brain. Advances in molecular biology techniques provide us with valuable tools in the field of miRNA research allowing sophisticated behavioural patterns to be explored. The classical approach of genomic modification has been fine-tuned to conditional/inducible gene alterations that have the advantage to circumvent developmental complications. Transgenic miR-132 overexpressing mice [54], conditional DGC8 [55] and Dicer1 [56] knockout mice are just a few of the examples previously employed in the miRNA field. Furthermore, direct delivery of miRNA sense or antisense oligonucleotides into the brain provides the necessary flexibility to overexpress or inhibit specific miRNAs in a temporally and spatially controlled manner [14,19,57,58].

(i) miR-132
Until now, a few examples for miRNA function in specific forms of synaptic plasticity have been demonstrated in brain regions such as the hippocampal formation, specific cortical areas or the amygdala. Some elegant work points to an important role of miRNAs in the visual cortex. For example, visual cortex plasticity is regulated via miR-132 in a developmental and experience-dependent manner [19,20]. Rearing the animals in complete darkness or monocular deprivation reduces the developmental increase in pri-miR-132 and miR-132 [19,20], suggesting that transcriptional regulation via CREB, as shown in a number of in vitro studies (see §2), could be involved [20]. Light deprivation for only 3 days in the critical period of ocular dominance plasticity is enough to simulate the effect of dark rearing/monocular deprivation on the pri-miR-132 level and both could be rescued by light exposure [19,20]. Interestingly, increasing cortical miR-132 levels via synthetic mimics [20] or sequestration of miR-132 via a lentiviral sponge construct prior to the critical period leads to impairment in monocular deprivation-induced visual cortex plasticity [19]. At first glance, the results from these two studies arguing that both miR-132 expression and inhibition compromise visual cortex plasticity in vivo appear contradictory. However, given that miRNAs are well known to fine-tune protein levels of their targets within a narrow range around a physiological set-point, both increasing or decreasing miRNA activity might be detrimental (reviewed in [59]).

miR-132 is also studied in connection with learning and memory processes. Hippocampal miR-132 is increased by contextual- [60] and trace fear-conditioning [61]. The induction is rather rapid (within 30–45 min) and diminishes in 2 h [60,61], again consistent with a CREB-mediated transcriptional upregulation. miR-132 is furthermore required for memory
acquisition, because virus-mediated inhibition in vivo leads to an impairment in the storage of temporally associated information [61]. Mr-132 levels in the hippocampal formation are also increased by other behavioural tasks, such as the Barnes maze or novel object recognition [62], suggesting an involvement of miR-132 in spatial memory formation. However, mice expressing miR-132 above the physiologically relevant level unexpectedly performed worse in the novel object recognition task [54,62] and the Barnes maze [62]. Excessive spine growth might be one of the side-effects of transgenic miR-132 overexpression that might limit the functional working range of neurons [62]. Similarly, viral overexpression of miR-132 in the perirhinal cortex abolished novel object recognition memory when tested 20 min, but not 24 h after the training session [63]. Long-term plasticity, both LTP and LTD, in the perirhinal cortex is also reduced in the presence of abnormally high levels of miR-132 [63]. In line with a critical role for miR-132 dosage in plasticity, miR132/212 knockouts have impaired LTP in the somatosensory cortex [64]. However, LTP in the hippocampus is either not affected, when stimulated with high-frequency tetanus, or enhanced via theta burst stimulation [64]. Thus, reducing or increasing the level of miR-132 outside the physiological range results in similar impairments in learning and memory via different molecular and/or plasticity mechanisms, providing further support for the validity of the fine-tuning model of miRNA function in the brain in vivo.

(ii) Other specific miRNAs
Although miR-132 is arguably the best studied miRNA in the context of synaptic plasticity, other recent examples from the literature underscore the pivotal role that miRNAs play in this process. The abundant neuronal miR-34c was shown to increase with ageing and contribute to impairments in fear-conditioning memory that are usually observed with increased age. Importantly, this phenotype could be rescued by injection of miR-34c inhibitors into the hippocampus [16]. In this study, the miR-34c effect was attributed at least partially to decreased levels of the miR-34c target SIRT1 in the hippocampal formation [16]. These results are in accordance with the findings of another study that showed that mice lacking SIRT1 are impaired in contextual conditioning, object recognition as well as spatial learning [14]. Interestingly, another neuronal miRNA, miR-134, appears to be one of the critical downstream elements in the SIRT1 pathway. The authors elegantly show that loss-of-function of SIRT1 leads to increased levels of miR-134 that is followed by translational repression of target genes and leads to impaired LTP in the Sc–CA1 synapse [14]. Increasing hippocampal miR-134 levels by viral administration is sufficient to mimic the behavioural and electrophysiological phenotype of SIRT1-deficient mice [14]. Conversely, inhibition of hippocampal miR-134 rescues the memory impairment due to knockdown of SIRT1 and restores LTP in the hippocampal CA1 region [14]. A beneficial role for reduced levels of miR-134 and miR-124 is also supported by a more recent study. Resveratrol, a compound known for its anti-ageing effects, decreased miR-134 and miR-124 levels in cultured hippocampi and improved fear-conditioning and LTP induction at the Sc–CA1 synapse in ageing mice [65]. This effect was similarly SIRT1-dependent and might involve increases in CREB and BDNF protein levels because it was not observed in SIRT1-deficient mice [65]. Notably, reduction of miR-124 in the hippocampus and prefrontal cortex via injection of LNA-miR-124 also restored spatial memory, social interaction and LTP impairments in adult mice carrying a null mutation for the exchange protein directly activated by cAMP (EPAC) [66]. Conversely, overexpression of miR-124 mimics the behavioural and electrophysiological phenotype of EPAC-deficient mice [66]. The effect most probably is mediated through inadequate EPAC-Rap1 regulation on miR-124 levels which in turn represses translation of Zif268 [66]. The function of miR-124 in memory formation appears to be highly conserved, because miR-124 also regulates synaptic facilitation and memory formation in *Aplysia californica* [67].

Recent examples from the literature further lengthen the list of specific miRNAs thought to be involved in behavioural and/or plastic processes in the mammalian brain. Virus-mediated inhibition of miR-195 in the hippocampus resulted in impaired spatial memory in rats as assessed in the Morris water maze [68]. Overexpression of miR-195 in the hippocampus by itself has no enhancing effect on memory formation, but alleviates memory impairments induced by pathological conditions [68]. On the other hand, antagonizing miR-206 in the hippocampus of a learning deficient mouse model (Tg2576) rescues long-term plasticity, both LTP and LTD, in the hippocampus and prefrontal cortex via injection of LNA-miR-206 [69]. Interestingly, the achieved effects in this study are comparable between intracerebroventricular and intranasal delivery of the antagonir [69], suggesting that the latter form of delivery could be a valuable non-invasive route for effective miRNA inhibition in the intact human brain.

Beyond the hippocampus and visual cortex, miRNAs are also implicated in learning and memory paradigms in other brain regions. For example, auditory fear-conditioning is consolidated in the lateral amygdala by reducing miR-182 levels, whereas overexpression of miR-182 had the opposite effect [70]. Cortactin and Rac1, regulators of actin dynamics, were identified as the critical miR-182 targets involved in the regulation of structural plasticity in the amygdala required for long-term auditory memory formation [70]. On the other hand, fear-extinction memory in the infralimbic prefrontal cortex was blocked by inhibition of miR-128b [71].

It is easy to predict that many more miRNAs in addition to those mentioned here (table 1) are involved in shaping the sophisticated neural networks involved in cognitive or affective behaviours. The availability of sophisticated tools for miRNA expression screening (e.g. RNAseq) and manipulation will undoubtedly help us to identify the key miRNA players in the near future.

(iii) miRNA biogenesis genes
Animals with genetic modifications of miRNA biogenesis genes represent another valuable tool for the investigation of miRNA function in synaptic plasticity- and memory-related processes. Related studies have centred around the microprocessor component DGCR8 and the RNaseIII enzyme Dicer. A schizophrenia-related microdeletion at human 22q11.2 locus results in aberrant miRNA levels mostly owing to the haploinsufficiency of the DGCR8 gene, which resides within the deletion. Although the deletion itself contains several genes, recent results indicate that DGCR8 deficiency alone might account for some of the critical behavioural and morphological phenotypes associated with the schizophrenia-related microdeletion in mice [72]. Haploinsufficiency at the DGCR8 locus results in accumulation of pri-miRNAs and
Table 1. miRNAs related to behaviour and/or plasticity in rodents.

<table>
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<td>impaired LTP in neocortex; enhanced LTP in hippocampus</td>
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<td>↓</td>
<td>injection of inhibitor</td>
<td>reversal of 1g2576 phenotype; enhanced contextual fear-conditioning and Y-maze</td>
<td>[69]</td>
</tr>
<tr>
<td>miR-182</td>
<td>↑</td>
<td>injection of mimic</td>
<td>impaired auditory fear-conditioning</td>
<td>[70]</td>
</tr>
<tr>
<td>miR-128b</td>
<td>↓</td>
<td>viral inhibition/ overexpression</td>
<td>impaired fear-extinction memory/ enhanced fear-extinction memory</td>
<td>[71]</td>
</tr>
</tbody>
</table>

subsequent reduction in mature miRNA level. Although the gross anatomical features are retained, reduction in dendritic complexity, alteration in spine size and density [72] as well as selective decrease in inhibitory network [73] and deficient adult neurogenesis [74] has been reported in relation to mono-allelic Dgcr8 knockdown. These animals have normal motor activity and general sensory perception, but are impaired in tasks that require intact short-term memory [72,74]. In their prefrontal cortex, short-term depression is enhanced, whereas the initial phase of synaptic potentiation is reduced [75].

More recently, it was however suggested that a specific miRNA, miR-185, which is itself located within the 22q11.2 microdeletion (Df(16)), might play an important role in the abnormal dendritogenesis as well as synapse malformations [76]. Whether restoring miR-185 levels alone will also be sufficient to rescue the schizophrenia-related behavioural phenotypes associated with the microdeletion is a subject of further investigation.

Cleavage by Dicer is another vital step in miRNA biogenesis that is required for the generation of functional mature miRNAs. Conditional inactivation of Dicer in the forebrain leads to a decrease in the size of hippocampal and cortical regions as well as an increase in the size of lateral ventricles in mice [55,77]. Expectedly, the level of several miRNAs is reduced as a result of Dicer loss in the mouse forebrain [55,56].

Interestingly, although loss of Dicer severely compromises neuronal morphology, conditional Dicer knockout animals display an enhancement of memory in trace fear-conditioning as well as spatial learning [56]. These effects are also supported electrophysiologically by an increase in the post-tetanic potentiation at Sc–CA1 synapses. LTP or basal synaptic transmission is however not affected in these mice, suggesting that synaptic alterations might not be the only cause for the observed behavioural phenotypes [56].

It is worth mentioning that although deficiency of the Dgcr8 or Dicer gene results in a general decrease in a number of miRNAs and neuromorphological defects, effects on synaptic plasticity and memory formation are different or sometimes even opposite in these mice. The interpretation of the phenotypes is complicated by the fact that a detailed knowledge about the specific small RNAs that are affected, miRNAs and non-miRNAs, is still lacking. A future challenge will be to pinpoint the important phenotype-causing molecules in these valuable models.

(c) miRNAs in epilepsy

Not only the regular brain activity but also aberrant activity patterns such as epileptic seizures have recently been shown to affect miRNA function. Human temporal lobe epilepsy and experimentally induced epilepsy result in changes of the level of specific miRNAs in brain tissue in a region- and experimentally induced epilepsy result in changes of the level of specific miRNAs in brain tissue in a region- and animal species used, then a core set of four epilepsy-related miRNAs has emerged (miR-132, miR-134, miR-124, miR-34a) [82–85]. Pilocarpine-induced epilepsy results in increase of hippocampal pri-miR-132 [60] and miR-132 level [60,85]. miR-132 is also upregulated in kainic-acid-induced epilepsy (via injections into amygdala) only if the destructive stimulus is applied, but not if the animals are preconditioned to the seizures [81]. Moreover, in vivo inhibition of miR-132...
prior to the epileptic insult protects against the cellular damage in CA3, but has no effect on seizure occurrence [81]. Together, these results suggest a differential involvement of miR-132 in pathways controlling the physiological and structural alterations during epilepsy. Hippocampal miR-34a levels are also increased in the same epilepsy model, but its inhibition in vivo affects neither the seizure severity nor the hippocampal cellular damage [82]. These results contrast with another study, where epilepsy-induced cellular damage was reduced via miR-34a antagonist injection [84].

Whereas the functional significance of miR-132 and miR-34a in epilepsy is still unclear, a recent study unequivocally revealed a functional role for miR-134 in epilepsy [58]. Similar to miR-132 and miR-34a, the level of miR-134 and its incorporation into RISC is increased after intra-amgydalar kainic-acid-induced status epilepticus [58]. Moreover, intracerebroventricular injection of miR-134 antagonist prior to the seizure induction provides protective measures against recurrent seizures as well as the cellular damage in CA3 [58]. The effect of miR-134 in epilepsy could be mediated by changes in its target Limk1, because Limk1 protein levels in brain are reduced both in epileptic animals and temporal lobe epilepsy patients concomitant with miR-134 upregulation [58]. Because miR-134 upregulation by activity is important for dendritic growth in primary neurons, it is tempting to speculate that miR-134 upregulation during epilepsy represents a maladaptive excessive response due to an unphysiologically high stimulation of networks during seizures. If true, this would also raise some questions regarding the clinical use of miR-134 inhibitors, because those would be expected to compromise other homeostatic mechanisms vital for the functioning of the brain under physiological situations. Whether an artificial overexpression of miR-134 increases the susceptibility to epileptic triggers however is not clear. Brain-specific knockout of another miRNA, miR-128-2, causes hyperactivity and severe seizures that results in early death of the sufferers [86]. Hyperactivation of the ERK pathway in miR-128-deficient animals is most likely involved in this process. Conversely, overexpression of miR-128-2 has beneficial effect on the experimentally induced epileptic seizures and lowers ERK2 activation [86].

4. Conclusion
miRNA research over the past decade has mainly focused on the identification of miRNAs and their target genes that function in diverse aspects of nervous system development and plasticity. While this has led to a number of important discoveries, our knowledge of how miRNA function itself is controlled by neuronal activity is still in its infancy. Nevertheless, it is already becoming apparent that miRNAs are controlled at basically every step of biogenesis and within their gene regulatory complexes, involving transcriptional and post-transcriptional mechanisms.

Elucidating the mechanism underlying miRNA regulation by activity in turn is a prerequisite for understanding the role of miRNAs in synaptic plasticity and related higher cognitive functions. Manipulating miRNA levels under basal growth conditions will often not lead to discernible phenotypes. However, knowing when, where and how miRNAs are regulated by activity will provide researchers with a framework for miRNA intervention in the context of environmental challenges. This will be valuable not only for a better knowledge of experience-dependent development of neuronal circuits, but also for the development of novel therapeutic strategies in neurological disorders that have a high environmental component, such as stress-associated disorders (depression), drug abuse and epilepsy, to name just a few.

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