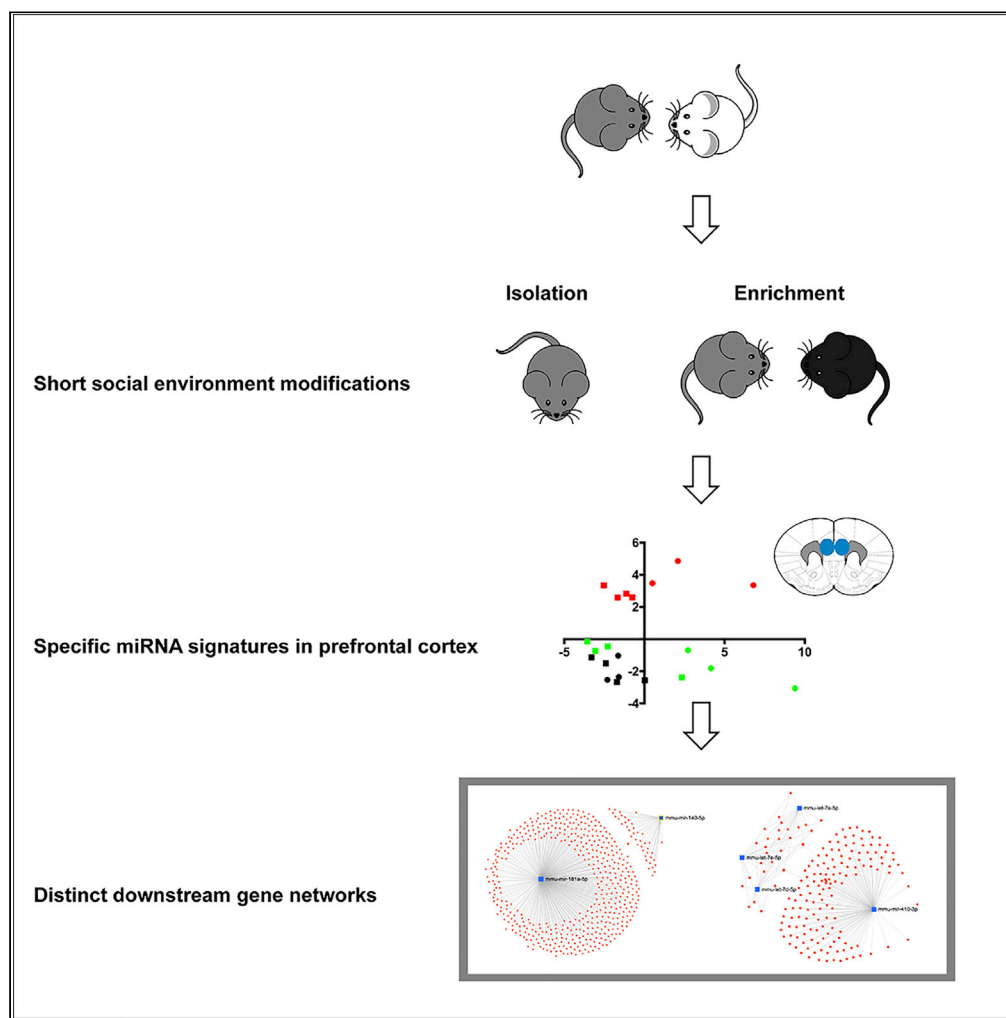


Article

Social Isolation and Enrichment Induce Unique miRNA Signatures in the Prefrontal Cortex and Behavioral Changes in Mice



Natalia Popa,
Flora Boyer,
Florence Jaouen,
Raoul Belzeaux,
Eduardo Gascon

eduardo.gascon-gonzalo@
univ-amu.fr

HIGHLIGHTS

Short modifications of social context alter performance in socially related tasks

The effects of social environment are age-dependent

Social context result in specific miRNA signatures in the medial prefrontal cortex

miRNAs altered by social isolation and enrichment target distinct cellular pathways

Popa et al., iScience 23,
101790
December 18, 2020 © 2020
The Authors.
<https://doi.org/10.1016/j.isci.2020.101790>

Article

Social Isolation and Enrichment Induce Unique miRNA Signatures in the Prefrontal Cortex and Behavioral Changes in Mice

Natalia Popa,^{1,4} Flora Boyer,^{1,4} Florence Jaouen,^{1,2,4} Raoul Belzeaux,^{1,3} and Eduardo Gascon^{1,5,*}

SUMMARY

An extensive body of evidence supports the notion that exposure to an enriched/impooverished environment alters brain functions via epigenetic changes. However, how specific modifications of social environment modulate brain functions remains poorly understood. To address this issue, we investigate the molecular and behavioral consequences of briefly manipulating social settings in young and middle-aged wild-type mice. We observe that, modifications of the social context, only affect the performance in socially related tasks. Social enrichment increases sociability whereas isolation leads to the opposite effect. Our work also pointed out specific miRNA signatures associated to each social environment. These miRNA alterations are reversible and found selectively in the medial prefrontal cortex. Finally, we show that miRNA modifications linked to social enrichment or isolation might target rather different intracellular pathways. Together, these observations suggest that the prefrontal cortex may be a key brain area integrating social information via the modification of precise miRNA networks.

INTRODUCTION

It has been long known that the environment sculpts brain function. This essential property of the brain, known as plasticity, ensures individuals adaptation to novel contexts/conditions and therefore survival in a changing environment. At the molecular level, epigenetic mechanisms are known to play a key role in environmental adaptations in both rodents and humans (Del Blanco and Barco, 2018). Among them, microRNAs (miRNAs), a class of short (~18–24 nucleotides) non-coding regulatory RNAs, have emerged as attractive candidates. miRNAs silence gene expression by recognizing short sequences (6–8 nucleotides) in target mRNAs and inducing their destabilization and/or inhibiting their translation. Since binding sequences are short enough to be found in thousands of transcripts, miRNA networks have been proposed to play a pivotal role in fine-tuning gene expression (Huntzinger and Izaurralde, 2011). Operating in almost any biological activity, miRNAs have been especially involved in adapting gene expression to environmental changes (Inui et al., 2010; John et al., 2004; McNeill and Van Vactor, 2012; Pauli et al., 2011). Indeed, because of their mechanism of action, miRNAs are ideally placed to act as molecular hubs capable of integrating environmental inputs and adjust gene expression accordingly (Hornstein and Shomron, 2006). In this regard, miRNA repertoire and expression levels are particularly high in the brain, the organ conveying and processing most of the environmental information (Salta and De Strooper, 2012). In the same line, miRNAs are profoundly deregulated in human conditions affecting the ability to provide an adapted behavioral outcome to the social context such as autism spectrum disorder or frontotemporal dementia (Narayanan and Schrott, 2020; Roberson, 2006).

In rodents, classical experiments carefully evaluated brain plasticity in the context of sensory deprivation (i.e. animals reared in the dark) and uncovered the existence of critical periods, defined as the time-window in which brain structure is mostly influenced by environmentally driven activity. Subsequent work mainly focused on environmental enrichment. It has been consistently shown that, in rodents, physical enrichment (i.e. running wheels and toys) improves performance in learning/memory tasks and alleviates functional deficits under multiple pathological conditions (Sale et al., 2009; Kempermann, 2019). In contrast, little is known about how modifications in the social settings influence brain function, an issue extremely relevant for socially organized animals such as humans. Early work in mice and rats revealed profound effects of extended social isolation on multiple features of rodent behavior including cognitive functions, sleep cycle

¹Aix-Marseille Université, CNRS, INT, Inst Neurosci Timone, UMR7289, 27, Boulevard Jean Moulin, 13005 Marseille, France

²NeuroBioTools Facility (NeuroVir), Aix Marseille Université, CNRS, INT, Inst Neurosci Timone, Marseille, France

³Assistance Publique Hôpitaux de Marseille, Sainte Marguerite Hospital, Pôle de Psychiatrie Universitaire Solaris, Marseille, France

⁴These authors contributed equally

⁵Lead Contact

*Correspondence: eduardo.gascon-gonzalo@univ-amu.fr

<https://doi.org/10.1016/j.isci.2020.101790>



and social interactions (Klippel, 1978; Gutwein and Fishbein, 1980; Cummins et al., 1982; Engellener et al., 1982). Similarly, the deleterious effects of maternal deprivation during the perinatal period has been consistently demonstrated (Der-Avakian and Markou, 2010; Gracia-Rubio et al., 2016; Huot et al., 2001; Llorente-Berzal et al., 2012). Nonetheless, experiments using long-term social isolation represent a crude experimental approach and their results are often difficult to interpret in a physiological context. More recently, enrichment protocols have included housing animals not only with toys but in large groups where social interactions are significantly increased (Holgate et al., 2017). Although a clear robust beneficial effect of this complex enrichment has been reported, the specific contribution of social cues remains difficult to assess independently of other variables (increase in activity or exploration, physical enrichment ...).

Here, we seek to assess the effects of specifically modifying the social environment and how aging influences brain ability to deal with such environmental changes. To restrict as much as possible our manipulations to the social context, we decided to house our mice in a rather poor social condition (1 male with a non-reproductive female) and then challenge them with either a short (1 week) social isolation or enrichment. At the behavioral level, we observe that such brief environmental modifications did not influence any but socially related tasks. Moreover, our data suggest that behavioral responses to social changes are age-dependent. Focusing on miRNAs, we find that the prefrontal cortex is the brain area exhibiting most robust changes in miRNA levels in the environment-challenged animals. Importantly, our results suggest that social isolation and enrichment led to specific miRNA signatures potentially modulating different genetic networks. Together, these observations support the notion that prefrontal areas are implicated in the detection and processing of social information via changes in precise miRNAs and that environmental modulations have differential behavioral outcomes depending on the age of the animal.

RESULTS

Short Modifications of Social Environment Lead to Subtle Changes in Socially Related Behaviors but Not in Cognitive Tasks

To assess the specific consequences of changes in social context, young and middle-aged mice were subjected to 1 week of either social deprivation (isolation in the home cage) or enrichment (exposure to different conspecifics every two days) and compared to a control group in which social settings remain unchanged (the male to be tested and a non-reproductive female). We first assessed behavioral changes in social tasks reasoning that, if any, social experience might primarily impinge on social functions.

In the three chambers social task, we found a similar behavior during the exploration phase (Figure 1A, upper panel) with no preference (preference index close to 0) in the investigation of the empty restrainers present on each of the side chambers. In the social phase, a conspecific is introduced in one of the restrainers (social), while the other remains empty (control). As expected, both young and aged mice exposed to social enrichment spend more time in the side chamber containing the social partner (Figure 1A, lower panel, positive preference index). In agreement with our previous observations (Boyer et al., 2019), we find that age does not significantly alter this preferential exploration (two-way ANOVA, factor age, $F(2, 40) = 1.90$, $p = 0.175$). Conversely, social environment has a significant effect on the preference index during the social phase (two-way ANOVA, factor social environment, $F(2, 40) = 8.36$, $p = 0.009$). Remarkably, young but not middle-aged mice having experienced a social enrichment display a more pronounced preference for the conspecific side ($PI_{\text{control}} = 27.8 \pm 6.30$; $PI_{\text{enriched}} = 53.6 \pm 12.4$ for 3-month-old mice and $PI_{\text{control}} = 39.8 \pm 6.77$; $PI_{\text{enriched}} = 22.8 \pm 3.51$ for 9-month-old mice; two-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): $p = 0.0313$ and $p = 0.230$ for 3- and 9-month-old animals). In sharp contrast, social isolation leads to a reduction in social exploration though this decrease is only significant in middle-aged animals ($PI_{\text{control}} = 27.8 \pm 6.30$; $PI_{\text{isolated}} = 13.5 \pm 6.85$ for 3-month-old mice and $PI_{\text{control}} = 39.9 \pm 6.77$; $PI_{\text{isolated}} = 6.81 \pm 5.12$ for 9-month-old mice; two-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): $p = 0.296$ and $p = 0.0104$ for 3- and 9-month-old animals). Similar results are obtained when the total time of interaction with the social partner (rather than the preference index) are analyzed (data not shown). In contrast, other parameters such as the time spent in each side-chamber or the total distance are not altered (not shown).

We next examined the performances of our group of animals in another social task, the interactions with a juvenile stranger in a neutral arena. In this test, neither the age nor the social environment showed any effect on the time of interactions (two-way ANOVA, factor social environment, $F(2, 40) = 2.30$, $p = 0.114$; factor age, $F(2, 40) = 1.15$, $p = 0.291$). Nonetheless, when the impact of social context was analyzed within each

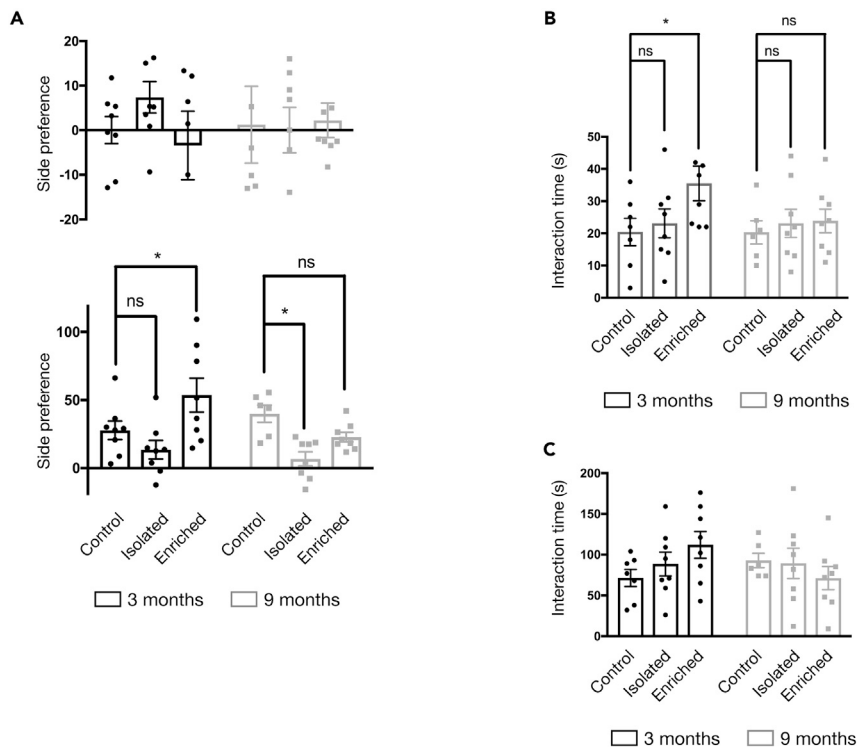


Figure 1. Mice Exposed to Different Social Environments Exhibit Subtle Behavioral Changes in Socially Related Tasks

(A). Three-chamber test. No differences are found during the exploration phase (Upper panel) among all groups. In the social phase (Lower panel), 3-month-old-enriched mice showed a significant increase in their preference for exploring the social partner, whereas 9-month-old mice having been isolated exhibited the opposite effect (* $p < 0.05$).

(B). Interactions in neutral arena. Young mice exposed to the social enrichment protocol show an increase in the interaction time with a juvenile mouse in the neutral arena. All other social environments or age groups do not modify interaction time (* $p < 0.05$).

(C). Intruder test. No differences in exploration time are observed when a juvenile conspecific is introduced in the home cage of young and middle-aged mice exposed to different social environments. All values represent mean \pm sem

age-group, 3- and 9-month-old mice showed a different outcome. Social isolation does not modify the exploration of a conspecific neither in young nor in old animals (Figure 1B). However, 3-month-old mice exposed to the enriched environment show a significant increase in the time spent investigating the unfamiliar subject (Figure 1B; $\text{Time}_{\text{control}} = 20.4 \pm 4.25$; $\text{Time}_{\text{enriched}} = 35.5 \pm 5.40$; two-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): $p = 0.043$) as well as in the number of interactions (Not shown; $\text{Interactions}_{\text{control}} = 18.3 \pm 3.79$; $\text{Interactions}_{\text{enriched}} = 27.9 \pm 3.92$; two-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): $p = 0.0034$) whereas this effect is not present in older animals (Figure 1B; $\text{Time}_{\text{control}} = 20.3 \pm 3.61$; $\text{Time}_{\text{enriched}} = 23.9 \pm 3.70$; two-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): $p = 0.99$). Similar to the 3 chambers test, these observations indicate a differential effect of social environment changes in 3- and 9-month-old mice.

Finally, in the intruder test, a task measuring dominance and aggressive behavior, we observed no significant effect of the social environment in the number of interactions (not shown) or in their duration (Figure 1C; two-way ANOVA, factor social environment, $F(2, 40) = 0.82$, $p = 0.114$). Together, these observations indicate that social drive/interest is finely tuned in response to changes in social conditions in an age-dependent manner.

To rule out that these behavioral changes are secondary to defects in locomotion or exploration, we quantified different behaviors in the open field (Figure S1A). As previously described (Boyer et al., 2019), middle-aged mice show a decrease in locomotor activity, evaluated by the total distance traveled during the test (two-way ANOVA, factor age, $F(2, 40) = 8.55$, $p = 0.0057$). However, social environment does not

affect neither locomotion (two-way ANOVA, factor social environment, $F(2, 40) = 1.52$, $p = 0.230$) nor the exploratory behavior measured by the time spent next to the walls or in the center of the arena (two-way ANOVA, factor social environment, $F(2, 40) = 0.186$, $p = 0.669$). These findings suggest that social settings do not profoundly influence basic locomotory/exploratory behavior.

We next investigated the effects of social environment on cognitive tasks using two well-established independent paradigms, the novel object recognition (NOR) and the Morris water maze (MWM). To accurately describe the exploratory behavior of mice in the NOR, a recognition index (RI) was calculated (see [Methods](#)). As expected, in the first phase, animals from all ages and social groups were similarly interested by both objects and spent roughly the same time exploring both objects (RI around 0.5; [Figure S1B](#), upper panel). During the second phase, animals showed a clear preference for the novel object and investigated it longer than the known object ($RI > 0.5$; [Figure S1B](#), lower panel). Social environment does not significantly alter RI in any phase. In the MWM ([Figure S1C](#)), during the training phase, escape latency gradually decreased over the days in all group of animals. Importantly, the learning curves are similar (two-way ANOVA for repeated measures, age 3 months, factor social environment, $F(2, 21) = 0.567$, $p = 0.578$; two-way ANOVA for repeated measures, age 9 months, factor social environment, $F(2, 19) = 0.402$, $p = 0.675$) suggesting that mice learned equally well how to use spatial information to navigate in this task independently of exposure to different social contexts. Similarly, in the probe trial, animals subjected to social isolation/enrichment did not differ in the latency times ([Figure S1D](#)) and platform crossovers (not shown) compared to their controls counterparts of the same age (one-way ANOVA, 3 months, latency, $F(2, 21) = 0.138$, $p = 0.872$; 1-way ANOVA, 3 months, frequency, $F(2, 21) = 2.55$, $p = 0.102$; 1-way ANOVA, 9 months, latency, $F(2, 19) = 0.139$, $p = 0.871$; 1-way ANOVA, 9 months, frequency, $F(2, 19) = 0.728$, $p = 0.497$), arguing again against any alteration of cognitive functions.

Overall, results of our behavioral experiments suggest that brief modifications of social settings might modulate specific aspects of social behavior without openly affecting cognitive tasks, locomotion, or exploration.

Social Environment Leads to Specific miRNA Alterations in the Prefrontal Cortex

miRNAs are thought to transduce environmental changes and adapt gene expression accordingly ([Hernandez-Rapp et al., 2017](#); [Hollins and Cairns, 2016](#)). We hypothesized that different social environments may result in specific miRNA changes and in defined miRNA signatures. To address this issue, we used an exploratory cohort of mice ($n = 21$, 9 young mice, and 12 middle-aged mice) submitted to the same environmental paradigm. We screened the expression of 48 miRNAs in five different brain regions: (i) two areas directly linked to social behaviors, the medial prefrontal cortex (mPFC) and the amygdala; (ii) two control regions, the motor cortex and the dorsal striatum, mainly involved in motor control; and (iii) the cerebellum as a region processing and integrating inputs from multiple modalities including social cues. miRNAs ([Table 1](#)) have been selected according to their level of expression in different brain regions and cell types ([He et al., 2012](#)).

We used multivariate analysis to determine whether particular miRNA signatures are associated to the social environment. We first performed a discriminant analysis, a statistical tool generally used to determine whether a combination of variables (in this case, miRNAs) can be used to discriminate between two or more naturally occurring groups (in this case, the different social environments) and therefore predict group membership ([Press and Wilson, 1978](#)). When miRNA levels from all regions and animals were considered together, animals being exposed to the same social environment clustered together with little overlap between groups ([Figure S2A](#)). Lambda Wilks test ($p < 0.0001$) suggests that, at least, one social condition might be different from the others. Previous findings have reported significant alterations of miRNAs along with aging ([Hébert et al., 2013](#); [Inukai et al., 2012](#); [Van den Hove et al., 2014](#)). To circumvent the potential confounding effects of aging, we repeated the analysis in young and middle-aged animals independently. As shown in [Figure 2A](#), the three groups cluster more sharply and the Lambda Wilks test p values are considerably lower ($p_{\text{young}} < 10^{-9}$; $p_{\text{middle-aged}} < 10^{-10}$) confirming the differential effects of social environment on miRNA contents.

To further analyze such changes, we performed a principal component analysis to investigate whether social environment drives specific miRNA signatures and, if so, in which brain regions. As illustrated in [Figure 2B](#), in the mPFC, control animals cluster together independently of their age suggesting that they show very similar miRNA profiles. In contrast, the first two principal components (PC1 and PC2) enable a clear discrimination of the isolated and enriched animals arguing for a significant and distinct effect of

miR-24-3p	miR-9-3p
miR-29a-3p	miR-9-5p
miR-34a-3p	miR-22-3p
miR-125a-5p	miR-26a-5p
miR-125b-5p	miR-29b-3p
miR-126a-5p	miR-30a-5p
miR-127-3p	miR-34a-5p
miR-139-3p	miR-124-3p
miR-143-3p	miR-128-3p
miR-148b-3p	miR-132-3p
miR-150-5p	miR-134-5p
miR-151-3p	miR-137-3p
miR-152-3p	miR-140-5p
miR-219a-5p	miR-181a-5p
miR-298-5p	miR-191-5p
miR-323-3p	miR-212-3p
miR-342-5p	miR-223-3p
miR-375-3p	miR-375-5p
miR-379-5p	miR-410-3p
miR-433-5p	miR-1197-3p
miR-543-3p	let-7a-5p
Let-7b-5p	let-7c-5p
Let-7d-5p	let-7e-5p
miR-16-5p	miR-101a-3p

Table 1. miRNAs Selected in This Study

social environment on miRNA contents in this brain region. Interestingly, differences between those groups of animals showing behavioral phenotypes and their control counterparts are well correlated to one of the identified components. Thus, differences between young control and enriched animals are mainly connected to PC1, whereas those from middle-aged control and isolated mice depend on the PC2. In other brain regions, animals from different ages and conditions show no obvious (amygdala and striatum) or modest clustering (cerebellum and motor cortex) (Figures 2B and S2B). These results indicate that: (i) social environment impinges primarily on mPFC; (ii) the most robust changes in miRNAs are observed in those animals showing behavioral changes; and (iii) that the effects of different social contexts on miRNA contents may not be identical.

Next, we sought to identify which miRNAs are modified in response to specific social contexts in the mPFC. Using ANOVA, we found 4 miRNAs specifically modified in 9-month isolated mice (let-7a-5p, let-7e-5p, miR-125a-5p, miR-375-3p). Four additional miRNAs showed significant changes only in the group of 3-month-enriched animals (miR-132-3p, miR-137-3p, miR-140-5p and miR-181a-5p) (Figure S2C). Remarkably, the 4 miRNAs identified from the enriched group of young animals contribute mostly to the PC1, whereas 3 of the 4 miRNAs in the isolated group come from the PC2.

In order to validate those miRNA changes, we performed the same analyses in a second independent group of mice (n = 15 animals, 6 young, and 9 middle-aged mice). As shown in Figure 2C, taking into

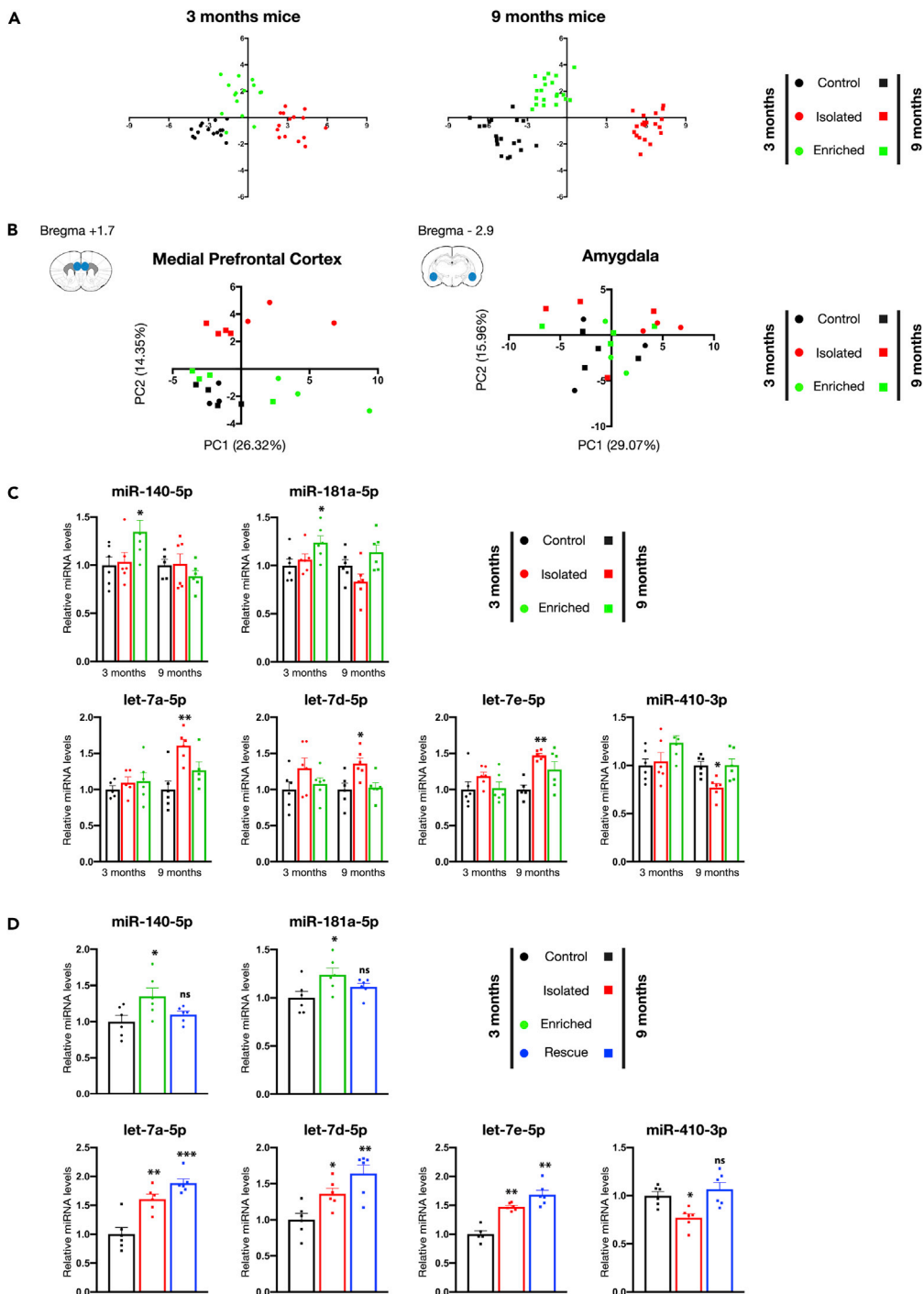


Figure 2. Short Modifications of Social Environment Drive Specific and Reversible miRNA Changes

(A) Results of discriminant analysis in 3- and 9-months mice exposed to different social environments. When the levels of 43 miRNAs in 5 different brain regions are considered, animals having been exposed to the same environment cluster in close proximity at both ages.

(B) Principal component analysis (PCA) of the miRNA levels (43 different miRNAs) in the prefrontal cortex and the amygdala. Using the first two components, this analysis shows a striking difference in the impact of social environment among brain regions. Animals clearly split into the four quadrants according to their age and social environment in the prefrontal cortex (control and 9-month-enriched mice are in the bottom left quadrant; isolated mice occupy the two upper quadrants and the young enriched cluster in the bottom right quadrant), whereas they are intermingled in the amygdala.

Figure 2. Continued

(C) miRNAs significantly altered by social enrichment and isolation. Upper panels: miRNAs significantly deregulated in 3-month-enriched mice (One-way ANOVA, $*p < 0.05$) include miR-140-5p and miR-181a-5p. Lower panels: miRNAs significantly deregulated in 9-month isolated mice (One-way ANOVA, $*p < 0.05$, $**p < 0.01$) include let-7a-5p, let-7d-5p, let-7e-5p, and miR-410-3p.

(D) Reversal of miRNA changes in mice re-exposed to a control social environment. Upper panels: miRNAs significantly deregulated in 3-month-enriched mice return to control levels after 1 week back to the control settings (One-way ANOVA, $*p < 0.05$). Lower panels: only levels of miR-410-3p are normalized in 9-month isolated mice having been submitted to the reversal protocol. let-7a-5p, let-7d-5p, and let-7e-5p levels remain significantly elevated (One-way ANOVA, $*p < 0.05$, $**p < 0.01$). All values represent mean \pm sem

account additional data from this validation cohort, we could confirm the significant changes of four of the eight previously identified miRNAs, miR-140-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0322$) and miR-181a-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0264$) for the young enriched mice; let-7a-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0008$) and let-7e-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0012$) for middle-aged isolated animals. Two additional miRNAs, let-7d-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0274$) and miR-410-3p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0237$), showing a clear trend in the first cohort, reached significance in the new dataset. These findings suggest that exposure to different social contexts affect a different set of miRNAs in the PFC.

Finally, to obtain further support to the specificity of these miRNA changes, we carried out a rescue experiment. We reasoned that, if driven by environmental changes, miRNA modifications should revert if animals are returned back to the control social settings. To test this hypothesis, an additional set of mice comprising young ($n = 6$) and middle-aged ($n = 6$) mice were exposed for one week to social enrichment or isolation, respectively. They were left with their original home mate (control social context) for an additional week and then sacrificed for analysis of miRNA expression. As shown in Figure 2D, we found that levels of miR-140-5p and miR-181a-5p (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.7757$ for miR-140-5p and $p = 0.4104$ for miR-181a-5p) are back to control levels in young enriched mice submitted to the reversal protocol. miRNA changes associated to social isolation (let-7a-5p, let-7d-5p, let-7e-5p, and miR-410-3p) are only partially reversed in those middle-aged mice being returned to control social context (one-way ANOVA, adjusted p for multiple comparisons [Dunnett's test]: $p = 0.0001$ for let-7a-5p and let-7e-5p, $p = 0.0002$ for let-7d-5p and $p = 0.7402$ for miR-410-3p). Overall, the results of rescue experiments give further support to the specificity of miRNA alterations driven by social environment.

miRNA Associated to Social Enrichment or Isolation Target Distinct Cellular Pathways

miRNAs networks are thought to fine-tune gene expression and modulate specific cellular signaling cascades. Since social enrichment and isolation result in modification of rather distinct miRNAs, we investigate whether the downstream targets regulated by different social contexts impinge on specific cellular pathways. Using miRNet 2.0, a web-based interface to integrate miRNA gene targets into functional networks (Chang et al., 2020), we identify >500 and >150 targets for the enriched (miR-140-5p and miR-181a-5p) and isolated (let-7a-5p, let-7d-5p, let-7e-5p, and miR-410-3p) miRNAs, respectively (Figure 3A). Network visualization enables the identification of mRNA common targets (Tor1aip2, Atp2b2, and Nf2) for the different conditions.

More importantly, when targets are submitted to KEGG enrichment analysis (Figure 3B), we observe that pathways modulated by social enrichment and isolation are rather distinct. Social enrichment preferentially targets genes related to synaptic function, especially glutamatergic transmission. In sharp contrast, miRNAs affected by social isolation affect specific molecular pathways, namely Hippo and Wnt signaling broadly involved in controlling cell proliferation and stemness. Similar results are obtained using the Reactome database (Figure S3). These findings give further support to the hypothesis that different social environments drive specific molecular alterations.

DISCUSSION

In this study, we characterized the effect of short modifications of social environment at the behavioral and miRNA level in wild-type young and middle-aged mice. Our main findings are the following: (i) social context alter behavioral performance in socially related tasks; (ii) the effects of social environment are age-dependent; (iii) mPFC is the brain region most sensitive to social changes; (iv) different social conditions result in specific miRNA signatures that target distinct signaling pathways.

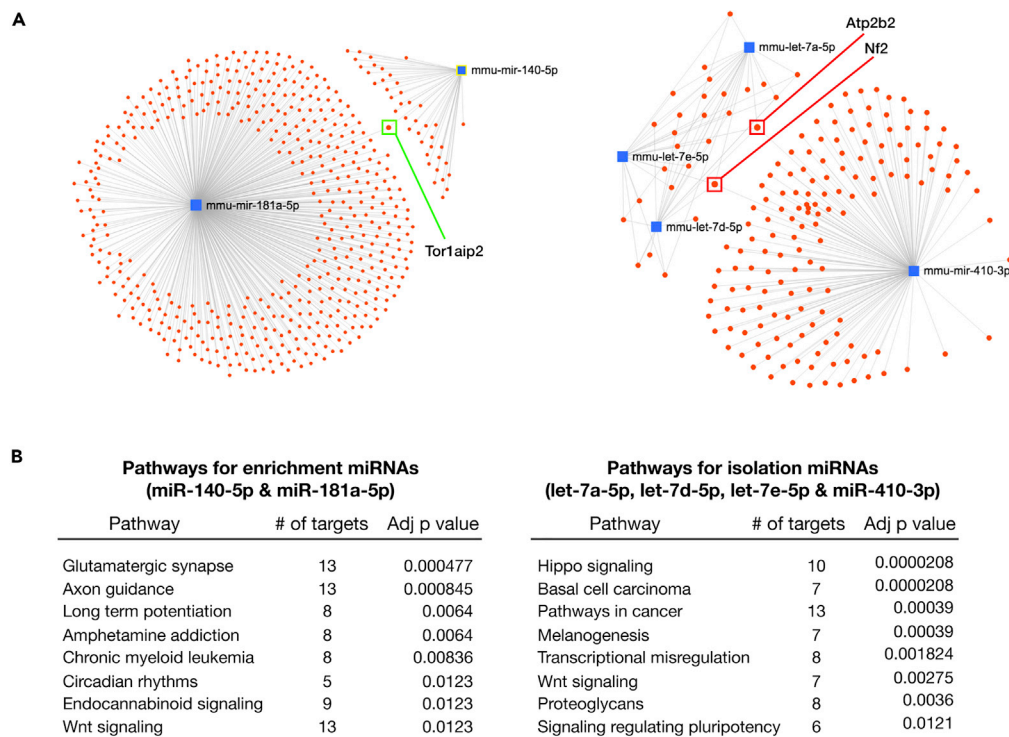


Figure 3. In Silico Analysis of Target Networks Downstream of miRNAs Altered by Social Enrichment and Isolation

(A). miRNA network visualization of social enrichment (left) or isolation (right). This analysis revealed a common target for social enrichment miRNAs, Tor1aip2, and two, Atp2b2 and Nf2, for the miRNAs altered by social isolation. (B). Pathway analysis of the target mRNAs in enriched and isolated animals using KEGG library. The table depicts the top 8 pathways showing a significant enrichment of mRNAs from each network. This analysis showed a sharp difference between social enrichment (mostly, associated to neuronal function) and isolation (mostly, linked to cell proliferation/cancer and specific cellular signaling pathways).

Brain function needs to be adapted to a changing environment. Accumulating data provide compelling evidence that reversible epigenetic modifications enable such essential adjustments at the molecular level (Bale, 2015; Del Blanco and Barco, 2018). Thus, histone post-translational modifications (Neidl et al., 2016), DNA methylation (Irier et al., 2014; Zhang et al., 2018) and miRNAs (Barak et al., 2013), have been consistently shown to be altered in rodents exposed to an enriched physical environment. More recently, it has been shown that even a 1h exposure to a novel environment induce robust epigenetic changes in activated hippocampal mouse neurons (Fernandez-Albert et al., 2019). However, given the promiscuity of epigenetic pathways, it has not been possible to obtain direct causal link between environment, epigenetic modifications at discrete loci and behavioral changes until recently (Heller et al., 2014; Liu et al., 2018). Our findings support the contention that, similar to other environmental cues, social context might induce fine-tuned changes in miRNA contents in key social areas such as the prefrontal cortex and sculpts brain function accordingly.

Accumulating evidences indicate that social environment may significantly impact brain function (Allen and Dwivedi, 2019; Sweatt, 2016). Nonetheless, only the effect of social deprivation at early post-natal life has been investigated in rodents (Peña et al., 2017; Sabatini et al., 2007), monkeys (Baker et al., 2017; de Campo et al., 2017), and humans (Bos et al., 2010; Smyke et al., 2009; Zeanah et al., 2003). In contrast, very little studies have examined this issue in the adulthood (Brenes et al., 2016) and explored simultaneously social enrichment/impoverishment. Interestingly, we observed that young animals are 'sensitive' to a positive enriched environment that promoted sociality and, somehow, protected against the deleterious effects of isolation. In sharp contrast, a 9-month-old mice show the opposite responses with a decrease social interest in animals having been isolated and very little effect of social enrichment. These findings are in line with human data showing the decrease of social interest and social network size in older individuals (Bruine de

Bruin et al., 2020). In summary, our results indicate that social environment might bidirectionally modulate social interactions (promote or inhibit) and that the influence of social context on brain functions is dependent on aging.

An important observation of our study is the striking region-specificity of miRNA changes (Figures 2B and S2B). Our results support that miRNA are mostly altered in mPFC, whereas differences are less obvious in other areas known to participate in social behavior such as the amygdala (Lubin et al., 2003; Márquez et al., 2013; Schiffer et al., 2011) or the cerebellum (Carta et al., 2019). Our findings are consistent with previous reports in mice and rats indicating a prominent role of the prefrontal cortex in social processing/functions (Gascon et al., 2014; Ko, 2017; Levy et al., 2019). Very recent work has provided compelling evidence that specific prefrontal cortex neurons represent conspecifics during social interaction (Kingsbury et al., 2020). It will be therefore important to determine how miRNA changes associated to social context affect these neuronal subsets.

We also identified unique miRNAs significantly altered by social enrichment and isolation. Several lines of evidence argue for the specificity of these findings: (i) multiple miRNAs such as miR-124-3p having important roles in the PFC (Gascon et al., 2014; Gu et al., 2019; Kozuka et al., 2019) are not modified by social experience (Figure S2D) in agreement with recent data (Brenes et al., 2016); (ii) we have obtained consistent changes in miRNAs in two independent cohorts of mice; and (iii) our rescue experiments show that miRNA alterations are reversible. Nonetheless, one can argue that repeated modifications in social context might stress animals and result in miRNA changes. Although we can not formally rule out, this hypothesis is extremely unlikely as none of the miRNAs identified in this study has been previously shown to be deregulated neither in social stress (Du et al., 2019; Miao et al., 2018; Misiewicz et al., 2019; Sun et al., 2019) nor in unpredictable chronic mild stress paradigms (Fan et al., 2018; Si et al., 2018).

miR-140-5p and miR-181a-5p, the miRNAs modulated in an enriched context, are miRNAs expressed both in glia and neurons. Although previous work has implicated them in similar pathological processes such as brain ischemia (Casey et al., 2020; Moon et al., 2013; Han et al., 2018) and Alzheimer disease (Akhter et al., 2018; Ansari et al., 2019; Rodriguez-Ortiz et al., 2020), their physiological functions remain poorly explored. Increasing evidence suggest that both miR-140-5p (Ambrozkiwicz et al., 2018) and miR-181a-5p (Chandrasekar and Dreyer, 2009; Rodriguez-Ortiz et al., 2020; Saba et al., 2012) play important roles in neurons and are required for proper cognition (Gullett et al., 2020; Xu et al., 2018; Zhang et al., 2017). Remarkably, modulation of miR-140-5p expression by social context has been already reported in zebra finches (Shi et al., 2013). Regarding miRNAs linked to social isolation, let-7 family has consistently been involved in controlling neural stem cell proliferation and glial differentiation (Cimadamore et al., 2013; Patterson et al., 2014; Shenoy et al., 2015). In this line, let-7 miRNAs are abundantly expressed in astrocytes (Jovičić and Gitler, 2017). miR-410-3p has been mainly associated with cancer (Wen et al., 2018), but it has been reported that it can also promote astrocyte differentiation when expressed in neurospheres (Tsan et al., 2016).

miRNAs regulate gene expression and cellular behavior in response to environmental modifications, ultimately shaping neurobehavioral phenotypes. Our target network analysis (Figures 3 and S3) strongly indicate that isolation and enrichment affect rather distinct molecular pathways. They also support the notion that they impinge on different cell populations. On one hand, miRNAs altered in the enriched group (miR-140-5p and miR-181a-5p) affect neuronal functions such as glutamate transmission (e.g. Gria3) or synaptic plasticity (e.g. Grin2c). Such pathways are consistent with the physiological functions mentioned above for these miRNAs in neurons and open the intriguing possibility that a rich social environment might lead to synaptic reinforcement/remodeling in key brain areas such as the mPFC. On the other hand, miRNAs deregulated by isolation (let-7a-5p, let-7d-5p, let-7e-5p, and miR-410-3p) target mainly signaling cascades associated with cell proliferation/cancer and precise signaling pathways (i.e. hippo/Wnt). Such signaling events are likely to be active in glial cells and/or neural precursors further arguing for the existence of strong divergences in the cell types and molecular pathways affected by enrichment and isolation. Our work also pointed out some interesting mRNAs targets, potentially regulated by either social enrichment (Tor1aip2) or isolation (Atp2b2 and Nf2). Interestingly, known functions of these genes are consistent with our observations. Thus, Tor1aip2 is an activator of TorsinA, a ubiquitous nuclear envelope protein whose function is known to be crucial in neurons (Demircioglu et al., 2016; Goodchild et al., 2005). Nf2 is an important activator of Hippo signaling (Lavado et al., 2013; Litan et al., 2019), one of the cellular pathways we identified (Figure 3B). Further work would help to elucidate the functional role of these genes and also to obtain additional mechanistic insights into the responses to social environment changes.

Finally, our rescue experiments highlighted that miRNAs changes driven by short environmental modifications are mostly reversible as previously reported (Kuleshkaya et al., 2011; Neidl et al., 2016; Singh-Taylor et al., 2018). In this regard, it is important to note again that reversibility is also dependent on the social context. Returning animals for a week back to the previous social environment is sufficient to revert the expression of miRNAs altered by enrichment to control levels. In contrast, only one of the four miRNAs (miR-410-3p) altered upon isolation exhibit such reversibility (Figure 2D).

Overall, our work provide experimental evidence supporting the link between environmental modifications, behavioral changes and precise miRNAs profiles in the mouse PFC. Specific miRNA signatures have been consistently found in multiple brain conditions (Belzeaux et al., 2018; Burns et al., 2018; Friedman et al., 2019; Leiding et al., 2013), and their understanding and manipulation would be extremely useful for clinical purposes.

Limitations of the Study

Because of its sensitivity and specificity, quantitative PCR using TaqMan assays represents the gold standard for miRNA quantification (Chen et al., 2005; Tong et al., 2015). Nonetheless, it is a low-throughput technique that has precluded the assessment of many other potentially interesting miRNAs, especially miRNA clusters. One paradigmatic example illustrating such limitation is the miR-379 cluster. Two independent miR-379 knockout mice strains have been generated and show conflicting results regarding the effects of cluster deletion on social functions (Lackinger et al., 2019; Marty et al., 2016). Comprising 38 different miRNAs, careful quantification of levels of each miRNA in the cluster is an arduous task via quantitative PCR. In our work, we analyze several members of the cluster (miR-134-5p, miR-323-3p, miR-379-3p, miR-379-5p, miR-410-3p, and miR-543-3p) but only miR-410-3p turned out to be significantly altered by social isolation. It would have been extremely informative to evaluate how social experience modulates the expression of the remaining elements of the cluster. miRNA sequencing is a potential option to circumvent this limitation in future studies and provide a more detailed picture of miRNA signatures associated to social environments.

Another caveat of our study is the interpretation of the rescue experiments. Several reasons might account for the differences observed between isolated and enriched animals. Thus, social isolation might be a stronger social alteration leading to more sustained molecular changes. Alternatively, miRNA turnover might be very different and returning to control levels might require longer periods for particular miRNAs. Finally, the age of the animals (3 months versus 9 months) might contribute to the observed differences.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Eduardo Gascon (eduardo.gascon-gonzalo@univ-amu.fr)

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Source data for figures in the paper is available here (<https://doi.org/10.17632/mpp5k9czz8.1>).

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2020.101790>.

ACKNOWLEDGMENTS

This study was supported by grants from The French Research Agency (ANR15-CE16-0008-01), the Fondation pour la Recherche Médicale (FRM), Fondation de France and the Fondation NRJ to EG. We thank Ana Borges-Correia, Elodie Caccamo-Garcia, and Catherine Lepolard for technical assistance.

AUTHOR CONTRIBUTIONS

N.P. performed the molecular experiments. F.B. performed the behavioral tests. N.P., F.B., and F.J. analyzed the data. R.B. and E.G. supervised the project and wrote the manuscript.

DECLARATION OF INTERESTS

All authors declare having no conflict of interest.

Received: February 14, 2020

Revised: September 14, 2020

Accepted: November 6, 2020

Published: December 18, 2020

REFERENCES

- Akhter, R., Shao, Y., Shaw, M., Formica, S., Khrestian, M., Leverenz, J.B., and Bekris, L.M. (2018). Regulation of ADAM10 by miR-140-5p and potential relevance for Alzheimer's disease. *Neurobiol. Aging* 63, 110–119.
- Allen, L., and Dwivedi, Y. (2019). MicroRNA mediators of early life stress vulnerability to depression and suicidal behavior. *Mol. Psychiatry* 25, 308–320.
- Ambrozkiwicz, M.C., Schwark, M., Kishimoto-Suga, M., Borisova, E., Hori, K., Salazar-Lázaro, A., Rusanova, A., Altas, B., Piepkorn, L., Bessa, P., et al. (2018). Polarity acquisition in cortical neurons is driven by synergistic action of sox9-regulated Wwp1 and Wwp2 E3 ubiquitin ligases and intronic miR-140. *Neuron* 100, 1097–1115.e15.
- Ansari, A., Maffioletti, E., Milanese, E., Marizzoni, M., Frisoni, G.B., Blin, O., Richardson, J.C., Bordet, R., Forloni, G., Gennarelli, M., et al. (2019). miR-146a and miR-181a are involved in the progression of mild cognitive impairment to Alzheimer's disease. *Neurobiol. Aging* 82, 102–109.
- Baker, M., Lindell, S.G., Driscoll, C.A., Zhou, Z., Yuan, Q., Schwandt, M.L., Miller-Crews, I., Simpson, E.A., Paukner, A., Ferrari, P.F., et al. (2017). Early rearing history influences oxytocin receptor epigenetic regulation in rhesus macaques. *Proc. Natl. Acad. Sci. U S A* 114, 11769–11774.
- Bale, T.L. (2015). Epigenetic and transgenerational reprogramming of brain development. *Nat. Rev. Neurosci.* 16, 332–344.
- Barak, B., Shvarts-Serebro, I., Modai, S., Gilam, A., Okun, E., Michaelson, D.M., Mattson, M.P., Shomron, N., and Ashery, U. (2013). Opposing actions of environmental enrichment and Alzheimer's disease on the expression of hippocampal microRNAs in mouse models. *Transl. Psychiatry* 3, e304.
- Belzeaux, R., Lin, R., Ju, C., Chay, M.A., Fiori, L.M., Lutz, P.E., and Turecki, G. (2018). Transcriptomic and epigenomic biomarkers of antidepressant response. *J. Affect. Disord.* 233, 36–44.
- Bos, K.J., Zeanah, C.H., Smyke, A.T., Fox, N.A., and Nelson, C.A. (2010). Stereotypies in children with a history of early institutional care. *Arch. Pediatr. Adolesc. Med.* 164, 406–411.
- Boyer, F., Jaouen, F., Ibrahim, E.C., and Gascon, E. (2019). Deficits in social behavior precede cognitive decline in middle-aged mice. *Front. Behav. Neurosci.* 13, 55.
- Brenes, J.C., Lackinger, M., Höglinger, G.U., Schratz, G., Schwarting, R.K., and Wöhr, M. (2016). Differential effects of social and physical environmental enrichment on brain plasticity, cognition, and ultrasonic communication in rats. *J. Comp. Neurol.* 524, 1586–1607.
- Bruine de Bruin, W., Parker, A.M., and Strough, J. (2020). Age differences in reported social networks and well-being. *Psychol. Aging* 35, 159–168.
- Burns, S.B., Szyszkwicz, J.K., Luheshi, G.N., Lutz, P.E., and Turecki, G. (2018). Plasticity of the epigenome during early-life stress. *Semin. Cell Dev. Biol.* 77, 115–132.
- Carta, I., Chen, C.H., Schott, A.L., Dorizan, S., and Khodakhah, K. (2019). Cerebellar modulation of the reward circuitry and social behavior. *Science* 363, eaav0581.
- Casey, S., Goasdoue, K., Miller, S.M., Brennan, G.P., Cowin, G., O'Mahony, A.G., Burke, C., Hallberg, B., Boylan, G.B., Sullivan, A.M., et al. (2020). Temporally altered miRNA expression in a piglet model of hypoxic ischemic brain injury. *Mol. Neurobiol.* 57, 4322–4344.
- Chandrasekar, V., and Dreyer, J.L. (2009). microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Mol. Cell. Neurosci.* 42, 350–362.
- Chang, L., Zhou, G., Soufan, O., and Xia, J. (2020). miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Res.* 48, W244–W251.
- Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., et al. (2005). Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* 33, e179.
- Cimadamore, F., Amador-Arjona, A., Chen, C., Huang, C.T., and Terskikh, A.V. (2013). SOX2-LIN28/let-7 pathway regulates proliferation and neurogenesis in neural precursors. *Proc. Natl. Acad. Sci. U S A* 110, E3017–E3026.
- Cummins, R.A., Livesey, P.J., and Bell, J.A. (1982). Cortical depth changes in enriched and isolated mice. *Dev. Psychobiol.* 15, 187–195.
- de Campo, D.M., Cameron, J.L., Miano, J.M., Lewis, D.A., Mirnics, K., and Fudge, J.L. (2017). Maternal deprivation alters expression of neural maturation gene *tbr1* in the amygdala paralamina nucleus in infant female macaques. *Dev. Psychobiol.* 59, 235–249.
- Del Blanco, B., and Barco, A. (2018). Impact of environmental conditions and chemicals on the neuronal epigenome. *Curr. Opin. Chem. Biol.* 45, 157–165.
- Demircioglu, F.E., Sosa, B.A., Ingram, J., Ploegh, H.L., and Schwartz, T.U. (2016). Structures of TorsinA and its disease-mutant complexed with an activator reveal the molecular basis for primary dystonia. *Elife* 5, e17983.
- Der-Avakian, A., and Markou, A. (2010). Neonatal maternal separation exacerbates the reward-enhancing effect of acute amphetamine administration and the anhedonic effect of repeated social defeat in adult rats. *Neuroscience* 170, 1189–1198.
- Du, K., Lu, W., Sun, Y., Feng, J., and Wang, J.H. (2019). mRNA and miRNA profiles in the nucleus accumbens are related to fear memory and anxiety induced by physical or psychological stress. *J. Psychiatr. Res.* 118, 44–65.
- Engellenner, W.J., Goodlett, C.R., Burrell, R.G., and Donovick, P.J. (1982). Environmental enrichment and restriction: effects on reactivity, exploration and maze learning in mice with septal lesions. *Physiol. Behav.* 29, 885–893.
- Fan, C., Zhu, X., Song, Q., Wang, P., Liu, Z., and Yu, S.Y. (2018). MiR-134 modulates chronic stress-induced structural plasticity and depression-like behaviors via downregulation of *Limk1/cofilin* signaling in rats. *Neuropharmacology* 131, 364–376.
- Fernandez-Albert, J., Lipinski, M., Lopez-Cascales, M.T., Rowley, M.J., Martin-Gonzalez, A.M., Del Blanco, B., Corces, V.G., and Barco, A. (2019). Immediate and deferred epigenomic signatures of in vivo neuronal activation in mouse hippocampus. *Nat. Neurosci.* 22, 1718–1730.
- Friedman, T.N., Yousuf, M.S., Catuneanu, A., Desai, M., Jużwik, C.A., Fournier, A.E., and Kerr, B.J. (2019). Profiling the microRNA signature of the peripheral sensory ganglia in experimental autoimmune encephalomyelitis (EAE). *J. Neuroinflammation* 16, 223.

- Gascon, E., Lynch, K., Ruan, H., Almeida, S., Verheyden, J.M., Seeley, W.W., Dickson, D.W., Petrucelli, L., Sun, D., Jiao, J., et al. (2014). Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat. Med.* **20**, 1444–1451.
- Goodchild, R.E., Kim, C.E., and Dauer, W.T. (2005). Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. *Neuron* **48**, 923–932.
- Gracia-Rubio, I., Moscoso-Castro, M., Pozo, O.J., Marcos, J., Nadal, R., and Valverde, O. (2016). Maternal separation induces neuroinflammation and long-lasting emotional alterations in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **65**, 104–117.
- Gu, Z., Pan, J., and Chen, L. (2019). MiR-124 suppression in the prefrontal cortex reduces depression-like behavior in mice. *Biosci. Rep.* **39**, BSR20190186.
- Gullett, J.M., Chen, Z., O’Shea, A., Akbar, M., Bian, J., Rani, A., Porges, E.C., Foster, T.C., Woods, A.J., Modave, F., and Cohen, R.A. (2020). MicroRNA predicts cognitive performance in healthy older adults. *Neurobiol. Aging* **95**, 186–194.
- Gutwein, B.M., and Fishbein, W. (1980). Paradoxical sleep and memory (I): selective alterations following enriched and impoverished environmental rearing. *Brain Res. Bull.* **5**, 9–12.
- Han, X.R., Wen, X., Wang, Y.J., Wang, S., Shen, M., Zhang, Z.F., Fan, S.H., Shan, Q., Wang, L., Li, M.Q., et al. (2018). MicroRNA-140-5p elevates cerebral protection of dexmedetomidine against hypoxic-ischaemic brain damage via the Wnt/ β -catenin signalling pathway. *J. Cell. Mol. Med.* **22**, 3167–3182.
- He, M., Liu, Y., Wang, X., Zhang, M.Q., Hannon, G.J., and Huang, Z.J. (2012). Cell-type-based analysis of microRNA profiles in the mouse brain. *Neuron* **73**, 35–48.
- Hébert, S.S., Wang, W.X., Zhu, Q., and Nelson, P.T. (2013). A study of small RNAs from cerebral neocortex of pathology-verified Alzheimer’s disease, dementia with lewy bodies, hippocampal sclerosis, frontotemporal lobar dementia, and non-demented human controls. *J. Alzheimers Dis.* **35**, 335–348.
- Heller, E.A., Cates, H.M., Peña, C.J., Sun, H., Shao, N., Feng, J., Golden, S.A., Herman, J.P., Walsh, J.J., Mazei-Robison, M., et al. (2014). Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nat. Neurosci.* **17**, 1720–1727.
- Hernandez-Rapp, J., Rainone, S., and Hébert, S.S. (2017). MicroRNAs underlying memory deficits in neurodegenerative disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **73**, 79–86.
- Holgate, J.Y., Garcia, H., Chatterjee, S., and Bartlett, S.E. (2017). Social and environmental enrichment has different effects on ethanol and sucrose consumption in mice. *Brain Behav.* **7**, e00767.
- Hollins, S.L., and Cairns, M.J. (2016). MicroRNA: small RNA mediators of the brains genomic response to environmental stress. *Prog. Neurobiol.* **143**, 61–81.
- Hornstein, E., and Shomron, N. (2006). Canalization of development by microRNAs. *Nat. Genet.* **38**, S20–S24.
- Huntzinger, E., and Izaurralde, E. (2011). Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* **12**, 99–110.
- Huot, R.L., Thirvikraman, K.V., Meaney, M.J., and Plotsky, P.M. (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl)* **158**, 366–373.
- Inui, M., Martello, G., and Piccolo, S. (2010). MicroRNA control of signal transduction. *Nat. Rev. Mol. Cell. Biol.* **11**, 252–263.
- Inukai, S., de Lencastre, A., Turner, M., and Slack, F. (2012). Novel microRNAs differentially expressed during aging in the mouse brain. *PLoS One* **7**, e40028.
- Irier, H., Street, R.C., Dave, R., Lin, L., Cai, C., Davis, T.H., Yao, B., Cheng, Y., and Jin, P. (2014). Environmental enrichment modulates 5-hydroxymethylcytosine dynamics in hippocampus. *Genomics* **104**, 376–382.
- John, B., Enright, A.J., Aravin, A., Tuschl, T., Sander, C., and Marks, D.S. (2004). Human MicroRNA targets. *PLoS Biol.* **2**, e363.
- Jovičić, A., and Gitler, A.D. (2017). Distinct repertoires of microRNAs present in mouse astrocytes compared to astrocyte-secreted exosomes. *PLoS One* **12**, e0171418.
- Kempermann, G. (2019). Environmental enrichment, new neurons and the neurobiology of individuality. *Nat. Rev. Neurosci.* **20**, 235–245.
- Kingsbury, L., Huang, S., Raam, T., Ye, L.S., Wei, D., Hu, R.K., Ye, L., and Hong, W. (2020). Cortical representations of conspecific sex shape social behavior. *Neuron* **107**, 941–953.e7.
- Klippel, J.A. (1978). Behavioral persistence following switchovers between environmental enrichment and impoverishment in mice. *Dev. Psychobiol.* **11**, 541–557.
- Ko, J. (2017). Neuroanatomical substrates of rodent social behavior: the medial prefrontal cortex and its projection patterns. *Front. Neural Circuits* **11**, 41.
- Kozuka, T., Omori, Y., Watanabe, S., Tarusawa, E., Yamamoto, H., Chaya, T., Furuhashi, M., Morita, M., Sato, T., Hirose, S., et al. (2019). miR-124 dosage regulates prefrontal cortex function by dopaminergic modulation. *Sci. Rep.* **9**, 3445.
- Kuleskaya, N., Rauvala, H., and Voikar, V. (2011). Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice. *PLoS One* **6**, e24755.
- Lackinger, M., Sungur, A.Ö., Daswani, R., Soutschek, M., Bicker, S., Stemmler, L., Wüst, T., Fiore, R., Dieterich, C., Schwarting, R.K., et al. (2019). A placental mammal-specific microRNA cluster acts as a natural brake for sociability in mice. *EMBO Rep.* **20**, e46429.
- Lavado, A., He, Y., Paré, J., Neale, G., Olson, E.N., Giovannini, M., and Cao, X. (2013). Tumor suppressor Nf2 limits expansion of the neural progenitor pool by inhibiting Yap/Taz transcriptional coactivators. *Development* **140**, 3323–3334.
- Leidinger, P., Backes, C., Deutscher, S., Schmitt, K., Mueller, S.C., Frese, K., Haas, J., Ruprecht, K., Paul, F., Stähler, C., et al. (2013). A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biol.* **14**, R78.
- Levy, D.R., Tamir, T., Kaufman, M., Parabucki, A., Weissbrod, A., Schneidman, E., and Yizhar, O. (2019). Dynamics of social representation in the mouse prefrontal cortex. *Nat. Neurosci.* **22**, 2013–2022.
- Litan, A., Li, Z., Tokhtaeva, E., Kelly, P., Vagin, O., and Langhans, S.A. (2019). A functional interaction between Na,K-ATPase β_2 -subunit/AMOG and NF2/merlin regulates growth factor signaling in cerebellar granule cells. *Mol. Neurobiol.* **56**, 7557–7571.
- Liu, X.S., Wu, H., Krzisch, M., Wu, X., Graef, J., Muffat, J., Hnisz, D., Li, C.H., Yuan, B., Xu, C., et al. (2018). Rescue of fragile X syndrome neurons by DNA methylation editing of the FMR1 gene. *Cell* **172**, 979–992.e6.
- Llorente-Berzal, A., Mela, V., Borcel, E., Valero, M., López-Gallardo, M., Viveros, M.P., and Marco, E.M. (2012). Neurobehavioral and metabolic long-term consequences of neonatal maternal deprivation stress and adolescent olanzapine treatment in male and female rats. *Neuropharmacology* **62**, 1332–1341.
- Lubin, D.A., Elliott, J.C., Black, M.C., and Johns, J.M. (2003). An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. *Behav. Neurosci.* **117**, 195–201.
- Márquez, C., Poirier, G.L., Cordero, M.I., Larsen, M.H., Groner, A., Marquis, J., Magistretti, P.J., Trono, D., and Sandi, C. (2013). Peripuberty stress leads to abnormal aggression, altered amygdala and orbitofrontal reactivity and increased prefrontal MAOA gene expression. *Transl. Psychiatry* **3**, e216.
- Marty, V., Labialle, S., Bortolin-Cavaillé, M.L., Ferreira De Medeiros, G., Moisan, M.P., Florian, C., and Cavaillé, J. (2016). Deletion of the miR-379/miR-410 gene cluster at the imprinted Dlk1-Dio3 locus enhances anxiety-related behaviour. *Hum. Mol. Genet.* **25**, 728–739.
- McNeill, E., and Van Vactor, D. (2012). MicroRNAs shape the neuronal landscape. *Neuron* **75**, 363–379.
- Miao, Z., Mao, F., Liang, J., Szyf, M., Wang, Y., and Sun, Z.S. (2018). Anxiety-related behaviours associated with microRNA-206-3p and BDNF expression in pregnant female mice following psychological social stress. *Mol. Neurobiol.* **55**, 1097–1111.
- Misiewicz, Z., Iurato, S., Kuleskaya, N., Salminen, L., Rodrigues, L., Maccarrone, G., Martins, J., Czamara, D., Laine, M.A., Sokolowska, E., et al. (2019). Multi-omics analysis identifies mitochondrial pathways associated with anxiety-related behavior. *PLoS Genet.* **15**, e1008358.

- Moon, J.M., Xu, L., and Giffard, R.G. (2013). Inhibition of microRNA-181 reduces forebrain ischemia-induced neuronal loss. *J. Cereb. Blood Flow Metab.* *33*, 1976–1982.
- Narayanan, R., and Schrott, G. (2020). miRNA regulation of social and anxiety-related behaviour. *Cell. Mol. Life Sci.* *77*, 4347–4364.
- Neidl, R., Schneider, A., Bousiges, O., Majchrzak, M., Barbelivien, A., de Vasconcelos, A.P., Dorgans, K., Doussau, F., Loeffler, J.P., Cassel, J.C., and Boutilier, A.L. (2016). Late-life environmental enrichment induces acetylation events and nuclear factor kb-dependent regulations in the Hippocampus of aged rats showing improved plasticity and learning. *J. Neurosci.* *36*, 4351–4361.
- Patterson, M., Gaeta, X., Loo, K., Edwards, M., Smale, S., Cinkornpumin, J., Xie, Y., Listgarten, J., Azghadi, S., Douglass, S.M., et al. (2014). let-7 miRNAs can act through notch to regulate human gliogenesis. *Stem Cell Rep.* *3*, 758–773.
- Pauli, A., Rinn, J.L., and Schier, A.F. (2011). Non-coding RNAs as regulators of embryogenesis. *Nat. Rev. Genet.* *12*, 136–149.
- Peña, C.J., Kronman, H.G., Walker, D.M., Cates, H.M., Bagot, R.C., Purushothaman, I., Issler, O., Loh, Y.E., Leong, T., Kiraly, D.D., et al. (2017). Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science* *356*, 1185–1188.
- Press, S.J., and Wilson, S. (1978). Choosing between logistic regression and discriminant analysis. *J. Am. Stat. Assoc.* *73*, 699–705.
- Roberson, E.D. (2006). Frontotemporal dementia. *Curr. Neurol. Neurosci. Rep.* *6*, 481–489.
- Rodriguez-Ortiz, C.J., Prieto, G.A., Martini, A.C., Forner, S., Trujillo-Estrada, L., LaFerla, F.M., Baglietto-Vargas, D., Cotman, C.W., and Kitazawa, M. (2020). miR-181a negatively modulates synaptic plasticity in hippocampal cultures and its inhibition rescues memory deficits in a mouse model of Alzheimer's disease. *Aging Cell* *19*, e13118.
- Saba, R., Störchel, P.H., Aksoy-Aksel, A., Kepura, F., Lippi, G., Plant, T.D., and Schrott, G.M. (2012). Dopamine-regulated microRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. *Mol. Cell Biol.* *32*, 619–632.
- Sabatini, M.J., Ebert, P., Lewis, D.A., Levitt, P., Cameron, J.L., and Mirnics, K. (2007). Amygdala gene expression correlates of social behavior in monkeys experiencing maternal separation. *J. Neurosci.* *27*, 3295–3304.
- Sale, A., Berardi, N., and Maffei, L. (2009). Enrich the environment to empower the brain. *Trends Neurosci.* *32*, 233–239.
- Salta, E., and De Strooper, B. (2012). Non-coding RNAs with essential roles in neurodegenerative disorders. *Lancet Neurol.* *11*, 189–200.
- Schiffer, B., Müller, B.W., Scherbaum, N., Hodgins, S., Forsting, M., Wiltfang, J., Gizewski, E.R., and Leygraf, N. (2011). Disentangling structural brain alterations associated with violent behavior from those associated with substance use disorders. *Arch. Gen. Psychiatry* *68*, 1039–1049.
- Shenoy, A., Danial, M., and Blesch, R.H. (2015). Let-7 and miR-125 cooperate to prime progenitors for astroglialogenesis. *EMBO J.* *34*, 1180–1194.
- Shi, Z., Luo, G., Fu, L., Fang, Z., Wang, X., and Li, X. (2013). miR-9 and miR-140-5p target FoxP2 and are regulated as a function of the social context of singing behavior in zebra finches. *J. Neurosci.* *33*, 16510–16521.
- Si, Y., Song, Z., Sun, X., and Wang, J.H. (2018). microRNA and mRNA profiles in nucleus accumbens underlying depression versus resilience in response to chronic stress. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* *177*, 563–579.
- Singh-Taylor, A., Molet, J., Jiang, S., Korosi, A., Bolton, J.L., Noam, Y., Simeone, K., Cope, J., Chen, Y., Mortazavi, A., and Baram, T.Z. (2018). NRSF-dependent epigenetic mechanisms contribute to programming of stress-sensitive neurons by neonatal experience, promoting resilience. *Mol. Psychiatry* *23*, 648–657.
- Smyke, A.T., Zeanah, C.H., Fox, N.A., and Nelson, C.A. (2009). A new model of foster care for young children: the Bucharest early intervention project. *Child Adolesc. Psychiatr. Clin. N. Am.* *18*, 721–734.
- Sun, Y., Lu, W., Du, K., and Wang, J.H. (2019). microRNA and mRNA profiles in the amygdala are relevant to fear memory induced by physical or psychological stress. *J. Neurophysiol.* *122*, 1002–1022.
- Sweatt, J.D. (2016). Neural plasticity and behavior - sixty years of conceptual advances. *J. Neurochem.* *139*, 179–199.
- Tong, L., Xue, H., Xiong, L., Xiao, J., and Zhou, Y. (2015). Improved RT-PCR assay to quantitate the pri-, pre-, and mature microRNAs with higher efficiency and accuracy. *Mol. Biotechnol.* *57*, 939–946.
- Tsan, Y.C., Morell, M.H., and O'Shea, K.S. (2016). miR-410 controls adult SVZ neurogenesis by targeting neurogenic genes. *Stem Cell Res.* *17*, 238–247.
- Van den Hove, D.L., Kompotis, K., Lardenoije, R., Kenis, G., Mill, J., Steinbusch, H.W., Lesch, K.P., Fitzsimons, C.P., De Strooper, B., and Rutten, B.P. (2014). Epigenetically regulated microRNAs in Alzheimer's disease. *Neurobiol. Aging* *35*, 731–745.
- Wen, R., Umeano, A.C., Essegian, D.J., Sabitaliyevich, U.Y., Wang, K., and Farooqi, A.A. (2018). Role of microRNA-410 in molecular oncology: a double edged sword. *J. Cell. Biochem.* *119*, 8737–8742.
- Xu, X.F., Jing, X., Ma, H.X., Yuan, R.R., Dong, Q., Dong, J.L., Han, X.F., Chen, Z.Y., Li, X.Z., and Wang, Y. (2018). miR-181a participates in contextual fear memory formation via activating mTOR signaling pathway. *Cereb. Cortex* *28*, 3309–3321.
- Zeanah, C.H., Nelson, C.A., Fox, N.A., Smyke, A.T., Marshall, P., Parker, S.W., and Koga, S. (2003). Designing research to study the effects of institutionalization on brain and behavioral development: the Bucharest Early Intervention Project. *Dev. Psychopathol.* *15*, 885–907.
- Zhang, S.F., Chen, J.C., Zhang, J., and Xu, J.G. (2017). miR-181a involves in the hippocampus-dependent memory formation via targeting PRKAA1. *Sci. Rep.* *7*, 8480.
- Zhang, T.Y., Keown, C.L., Wen, X., Li, J., Vousden, D.A., Anacker, C., Bhattacharyya, U., Ryan, R., Diorio, J., O'Toole, N., et al. (2018). Environmental enrichment increases transcriptional and epigenetic differentiation between mouse dorsal and ventral dentate gyrus. *Nat. Commun.* *9*, 298.

iScience, Volume 23

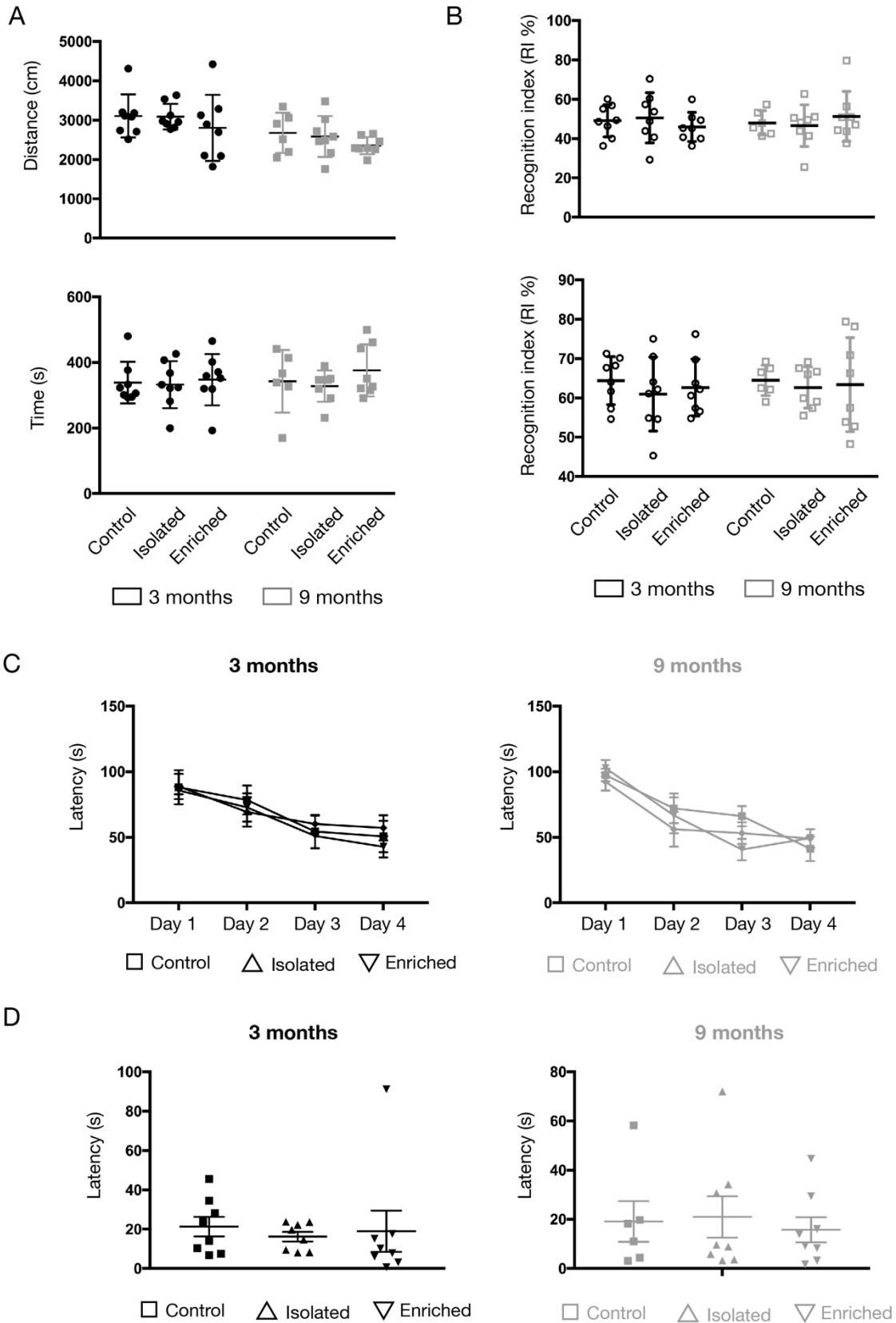
Supplemental Information

Social Isolation and Enrichment Induce Unique miRNA Signatures in the Prefrontal Cortex and Behavioral Changes in Mice

Natalia Popa, Flora Boyer, Florence Jaouen, Raoul Belzeaux, and Eduardo Gascon

Supplemental Figures and legends

Popa et al., Suppl Figure 1

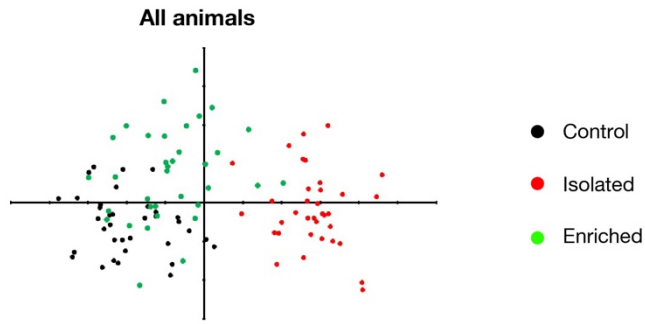


Supplementary Figure 1. Social environment does not modify exploration or cognitive performance in 3- and 9-month-old mice. Related to Figure 1

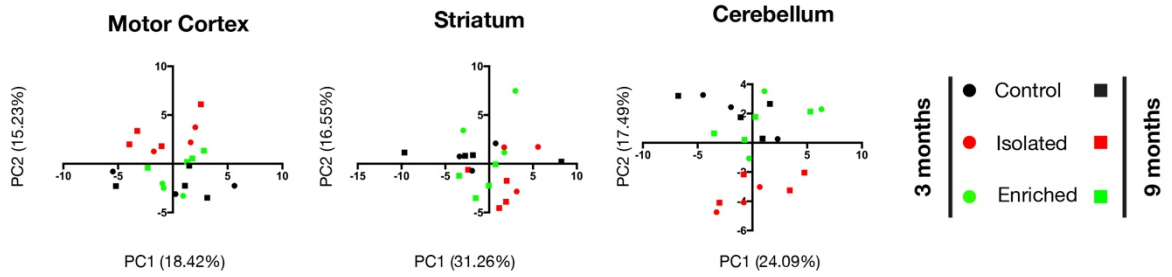
- A. Open field. Neither distance traveled (upper panel) nor time spent in close proximity to the walls (lower panel) are altered by social context.
- B. Novel object recognition. RI (see methods) during the exploration phase (around 50%) suggest similar interest by the two objects (upper panel). In the recognition phase (lower panel), RI increases as animals explore longer time the novel object. RI is not affected by aging or social conditions in any phase.
- C. Morris Water Maze. Learning curves over 4 consecutive days for young (left) and middle-aged (right) mice show no differences among social conditions.
- D. Morris Water Maze. The latency in the probe trial is not influenced by the social environment.

All values represent mean \pm sem

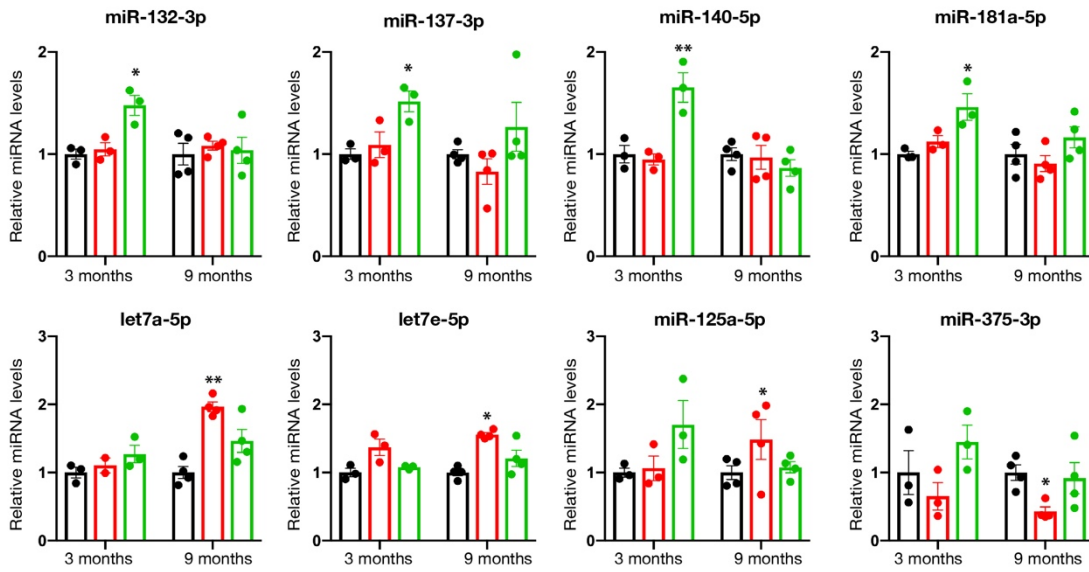
A



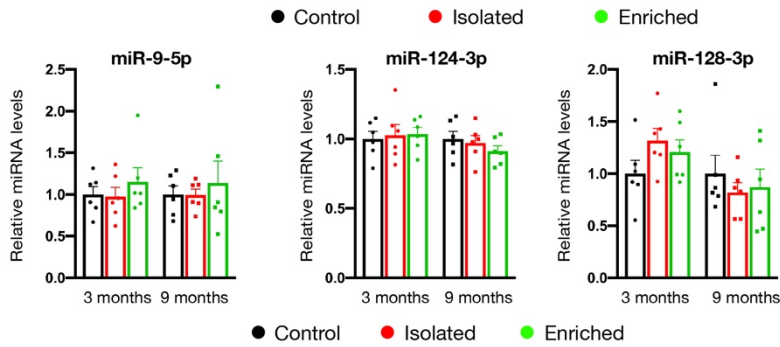
B



C



D



Supplementary Figure 2. Additional data on miRNA alterations associated to social environment changes. Related to Figure 2.

- A. Discriminant analysis of miRNA data in all animals of both ages. The social environment enables an overall satisfying classification of the miRNA data from the 5 brain regions analyzed.
- B. PCA analysis of the motor cortex, dorsal striatum and cerebellum show no or little correlation between miRNA levels in those regions and social environment.
- C. miRNAs deregulated by social enrichment (upper panels) and isolation (lower panels) in the exploratory cohort of mice (n=21).
- D. Highly abundant miRNAs with known functions in the brain such as miR-9-5p, miR-124-3p or miR-128-3p show no differences in the different environmental groups and ages.

All values represent mean \pm sem

Popa et al., Suppl Figure 3

Pathways for enrichment miRNAs (miR-140-5p & miR-181a-5p)			Pathways for isolation miRNAs (let-7a-5p, let-7d-5p, let-7e-5p & miR-410-3p)		
Pathway	# of targets	Adj p value	Pathway	# of targets	Adj p value
Neuronal system	19	0.004305	Wnt signaling (not beta-catenin)	6	0.0088
Transmission across synapses	15	0.004305	Platelet Ca homeostasis	3	0.0145
Glutamate Neurotransmitter	5	0.0061	Synthesis of IP3/IP4	3	0.0201
Neurotransmitter release	6	0.0162	Hemostasis	10	0.0201
Linoleic acid metabolism	3	0.0162	Wnt signaling (beta-catenin depend)	7	0.0201

Supplementary Figure 3. Pathway analysis of the target mRNAs in enriched and isolated animals using Reactome library. Related to Figure 3.

Transparent Methods

Animals

Mice were purchased from Charles Rivers Laboratories (France). Since in our experiments mice could be in physical contact, we decided to use only animals from the same sex (males) to circumvent potential confounding interactions (i.e. mating). A total of 96 C57Bl6J male mice were used in this study for the behavior and the miRNAs. Retired CD1 females (44) and juveniles (24) were also involved in the project (for housing or behavioral testing) although not included in any of the data. Half of the C57Bl6J were young adults (n=48, 10 weeks at the time of arrival) whereas the remaining animals were older adults (n=48, 9 months). Upon arrival, each male was housed with an elderly female presenting a low fertility (>12 months) for 2 weeks. This enables acclimation to the animal facility and provides a control social environment while limiting mating and fighting. The animals were kept under specific pathogen-free conditions with a regular 12-h light/dark cycle (light on at 8:00 am) and constant conditions ($21 \pm 1^\circ\text{C}$; 50% humidity). Food and water were supplied ad libitum. Mice also benefited from some environmental enrichment (a wooden stick and a piece of cotton with each cage change). We conducted behavioral experiments on 3 independent cohorts of 16 animals. miRNA profiling was performed on 3 independent groups of animals: i) an initial exploratory cohort of 21 animals (n=9 young and n=12 middle-aged mice); ii) a second confirmatory cohort of 15 animals (n=9 young and n=6 middle-aged mice); and) a third cohort of 12 animals for the rescue experiments. This latter cohort encompasses 6 young and 6 middle-aged animals.

All procedures involving mice were approved by the local ethics committee (EU0488, #6357) and are in agreement with European regulations (Directive 2010/63/EU). A special effort was made in handling animals to minimize stress or anxiety.

Modification of social environment

Before behavioral testing or miRNA analysis, mice were randomly assigned to 3 different groups and submitted to one of these social environment conditions: i) in the control condition, animals remained in the same social context (1 aged female); ii) in the social isolation group, animals were housed individually for 1 week; and iii) in the social enriched condition, animals were exposed to novel social partner(s) every 2 days. Briefly, the first day of the enrichment the resident female was replaced by a non-familiar old female. On day 3, another old female was added to the cage. On day 5, one of the females was replaced by a novel female. Animals in isolated and enriched groups were returned to the control condition the day before starting the behavioral testing.

For rescue experiments, young mice (n=6) are first submitted to the enrichment and middle-aged mice (n=6) to isolation as described above. Then, animals are returned back with their initial home cage for an additional 1 week. After that, mice are sacrificed for miRNA quantitation.

Behavioral schedule and exclusion criteria

All behavioral experiments were conducted between 9 am and 7 pm. Animals were habituated to the behavioral room for at least 30 min before the start of the task. The 48 animals to be analyzed were divided into three cohorts that were tested

independently. For each cohort of animals, tests were conducted according to this schedule: 1) Three-chamber social task (Day 1); 2) Intruder test (Day 3); 3) Interactions in neutral arena (Day 5); 4) Openfield and Novel object recognition (Day 7); 5) Morris water maze (Day 9-13); 6) Olfactory behavior (Day 15). We randomized the order of animals on each test. All experiments were recorded using Ethovision software and with the experimenter out of the behavioral room.

One 9-month-old animal in the control group showing epileptic signs, was euthanized and therefore excluded from the study. For technical reasons during the preparation of the 3-chambers test, one additional 9-month-old animal had to be excluded from the analyses.

Openfield

The open field test was used to evaluate locomotion and exploratory behavior. The open field device consisted of a non-reflective opaque plexiglass box (40x40x40 cm) and a suspended digital camera. The animals were placed in facing an open field wall and their behavior was recorded for 10 min. The total distance traveled, the time spent in the center and near the walls are calculated.

Novel object recognition

For the novel object recognition task, we used the open field device and small plastic objects. In the first phase, the animals were placed facing a wall and two identical objects (green cylinders, 3.5 cm high, 4.5 cm in diameter) were placed in front of the opposite wall of the arena (10 cm from the wall and 5.5 cm apart from each other). The mice explored the arena for 10 min and were brought back to their cages for 10 min.

In the second phase, one of the objects was replaced by a novel object (yellow triangular prism, 3.5 cm high, 4.5 cm on each side). The time spent exploring each object (nose point within 2 cm from the object) was quantified and used to calculate a recognition Index (RI) as follows: $RI = \frac{\text{time exploring object 1}}{\text{time exploring object 1} + \text{time exploring object 2}}$

Morris water maze

The Morris Water Maze (MWM) was performed as previously described with minor modifications (LaSarge et al., 2007). Briefly, a round pool (120 cm in diameter) was filled with water (25°C) and tempera paint was added to the water until it becomes opaque. A hidden platform (10 cm in diameter) is placed about 1cm below the water surface. The spatial cues consisted of geometric figures of different colors located in privacy blinds surrounding the water tank. The subjects are monitored by a video tracking system directly above the water tank while swimming and the parameters are measured using Ethovision software in a computer.

During the training phase (4 days) each animal received 3 trials. In each trial, the animal is placed in the water tank facing to the wall and allowed to explore the maze until it reaches the platform. If the animal does not find the platform in 2 min, the experimenter guides it to the platform. In either case, the animal is left on the platform for 10 sec. Then it is dried and returned to the cage for 5 min until the next trial. Once all the animals have completed the training phase, they each perform one probe trial (2 min) on day 5, during which the platform is removed from the pool. The probe trial is performed to verify the animal's understanding of the platform location and to observe the exploratory strategy it follows when it discovers that the platform has been

removed. The latency to reach the platform and the frequency of crossings are evaluated.

Intruder test

The protocol was adapted from previously described methods (Gascon et al., 2014). To avoid any aggressive behaviors, a juvenile CD1 male (3-5 weeks) was introduced into the home cage of the test mouse and the interactions were recorded for 5 min. The time and the number of interactive and investigative behaviors (sniffing) initiated by the target mouse were quantified.

Interactions in neutral arena

In this task, the target mouse and a juvenile CD1 male (3-5 weeks) were simultaneously introduced into an open field arena (see before). The time and the number of interactive and investigative behaviors (sniffing) initiated by the target mouse during the 5 min of the test were assessed.

Three chambers social task

The three chambers apparatus is a rectangular arena (60x37.5x21 cm) made of transparent plexiglass plastic, divided into three compartments of the same size (18.5x37x21 cm). Two openings connect the center chamber with the two side chambers. In a corner of each side chamber, there is a cylindrical container (10 cm in diameter, 20 cm high). During the first trial (exploration), the target mouse is placed in the middle chamber facing a wall and allowed to explore. In the next trial (social), a stranger mouse is placed in one of the containers while the other is empty. Trials last 10 min and animals are left for 10 min in their home cages between trials. Strangers

are juvenile CD1 mice (3-5 weeks) that have been trained to be restrained in the container. For each mouse, the location of the stranger mice in the left or right side chamber is random. A social preference index is calculated as the subtraction of the time in close exploration (nose point within 2 cm of the container) from the social partner) and the containers. To quantify the behavior, only the first 5 min of each trial were used.

Device cleaning

Between trials or animals, behavioral devices were thoroughly cleaned with water and mild soap. After rinsing, surfaces were sprayed with a 70% ethanol solution and air-dried for 5 min.

RNA extraction

For molecular analysis, animals from each environmental group from an independent cohort of animals (n=3/group for 3 young mice, n=4/group middle-aged mice) were sacrificed by cervical dislocation. Brain was rapidly removed and dissected into ice-cold HBSS and coronally sliced (250-400 μm). Different antero-posterior levels were selected to isolate specific brain regions using a biopsy punch (500 μm diameter). Total RNA containing miRNAs and mRNAs was then extracted from these samples using NucleoSpin miRNA kit following manufacturer's protocol for RNA purification in combination with TRIzol lysis (Macherey-Nagel, Germany). RNA extraction protocol includes an on-column DNase treatment (30 min). Total RNA was quantified using a NanoDrop (Fisher Scientific).

miRNA reverse transcription and quantitative PCR

For quantification of miRNAs from specific brain regions, we first performed reverse transcription using TaqMan advanced miRNA cDNA synthesis kit and the protocol provided starting from 5 ng of total RNA (Applied Biosystems, France). Abundance of miRNAs in the samples was measured using custom TaqMan array Advanced miRNA cards (Applied Biosystems, USA) following manufacturer guidelines and a quantitative PCR thermocycler (QS7, Applied Biosystems, France). miRNAs that could be used as endogenous controls were determined as those giving the lower standard deviation of the ΔCrt values for all samples (ThermoFisher Scientific Digital Science online real-time qPCR software). From this analysis, we selected hsa-miR-16-5p and mmu-miR-101a-3p as the most stable across multiple brain regions in our experiments. These two miRNAs were therefore used as endogenous controls for the relative quantifications in all our subsequent analyses.

miRNA selection

Our array cards contained 48 miRNAs (46 target miRNAs and 2 control miRNAs, see table 1). We selected our target miRNAs according to some specific criteria:

- All miRNAs have been shown to be expressed in the mouse brain (He et al., 2012)
- We have chosen miRNAs enriched in different cell types (e.g. neurons: miR-124, miR-132; astrocytes: let-7a; oligodendrocytes: miR-219; microglia: miR-223) as well as others expressed across multiple cell types (e.g. miR-181a)
- We have selected miRNAs exhibiting different expression levels (high: miR-124, miR-9, miR-128; medium: miR-137, miR-181a; low: miR-219, miR-375) (He et al., 2012)

Although 48 miRNAs were initially included in the survey, we had to exclude 3 miRNAs from the analysis (let-7c-5p, miR-1197-3p and miR-375-5p) as they could not be consistently amplified in our samples.

Target network analysis

For the identification and analysis of downstream mRNA targets as well as the integration into functional networks, we used miRNet 2.0 (Chang et al., 2020) following the developers' guidelines. For the enrichment analysis, we used KEGG library and hypergeometric test. Network was visualized using the force atlas layout.

Data analysis and statistics

Behavioral data analysis. All behavioral tests were recorded and analyzed post-hoc. For tests involving two freely moving animals (intruder test and interactions in neutral arena), an investigator blind to the conditions manually scored the behaviors. For the other tests, automatic monitoring was carried out using dedicated software (Ethovision XT, Noldus, Netherlands). Data is presented as means \pm SEM unless indicated.

Determining adequate sample size. As we have no prior experience with the effect size relating to a postulated interaction between environment and age, we choose to conduct the behavioral experiments using small cohorts (8 mice per each of six experimental groups) arguing that, if significant, subtle changes in such small cohorts might represent bona fide alterations. To limit the potential confounding effects of inter-lot variability, we perform our tests from three independent cohorts of 16 animals. Sample sizes used for molecular studies are smaller (n=6 animals/group) and were obtained from 3 independent cohorts.

Outlier values. We perform an automatic outlier detection in our miRNA dataset using the ROUT method (Q=5%). 19 values were identified and eliminated from subsequent analysis (representing <0.15% of the dataset).

Between-group comparisons. We perform normality tests (Anderson-Darling, D'Agostino-Pearson, Shapiro-Wilk and Kolmogorov-Smirnov tests) on all our datasets that confirmed their normal distribution. For the detection of significant differences among groups in behavioral and molecular experiments, we used therefore ANOVA analysis followed by post-hoc tests (Dunnett-adjusted pairwise comparisons). Data is presented as means \pm SEM unless indicated.

The relative expression levels for each miRNA and animal are entered into a principal component analysis (PCA) and discriminant analysis (DA). In the PCA, the number of extracted components was determined by the parallel analysis with 95% percentile rule and confirmed with a scree plot. The assessment of individual miRNA influences on the obtained PCA component scores was done with general linear models.

Statistical analysis (ANOVA and post-hoc tests for multiple comparisons, outlier identification, normality tests) was performed using Prism GraphPad software (version 7) and XLStat (PCA and DA).

Supplemental references

Chang, L., Zhou, G., Soufan, O., and Xia, J. (2020). miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Res* 48, W244-W251.

Gascon, E., Lynch, K., Ruan, H., Almeida, S., Verheyden, J. M., Seeley, W. W., Dickson, D. W., Petrucelli, L., Sun, D., Jiao, J., Zhou, H., Jakovcevski, M., Akbarian, S., Yao, W. D., and Gao, F. B. (2014). Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat Med* 20, 1444-1451.

He, M., Liu, Y., Wang, X., Zhang, M. Q., Hannon, G. J., and Huang, Z. J. (2012). Cell-type-based analysis of microRNA profiles in the mouse brain. *Neuron* 73, 35-48.

LaSarge, C. L., Montgomery, K. S., Tucker, C., Slaton, G. S., Griffith, W. H., Setlow, B., and Bizon, J. L. (2007). Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Neurobiol Aging* 28, 928-936.