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Social isolation and enrichment induce unique microRNA signatures in the prefrontal cortex and behavioral changes in young and middle-aged mice

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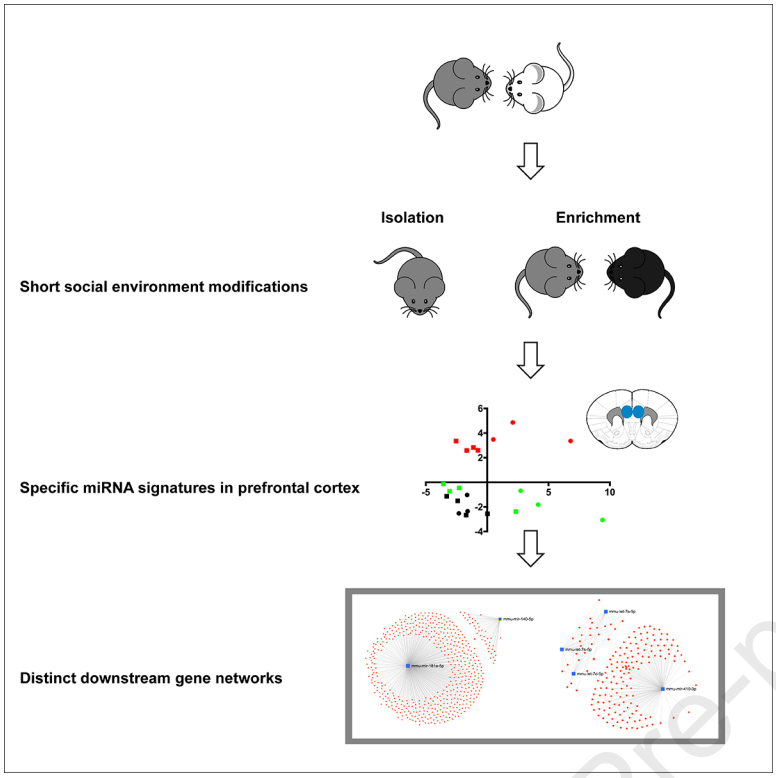
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Running title: Social environment and miRNAs

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Summary

An extensive body of evidence supports the notion that exposure to an enriched/impoverished 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.

Keywords: social behavior, aging, miRNA, social environment, mice

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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 Schratt, 2020; Roberson, 2006).

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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 and social interactions (Klippel, 1978; Gutwein and Fishbein, 1980; Cummins et al., 1982; Engellenner 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.

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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 (Fig. 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 (Fig. 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 (2-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 (2-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 ($PI_{control}=27.8\pm6.30$; $PI_{enriched}=53.6\pm12.4$ for 3-month-old mice and $PI_{control}=39.8\pm6.77$; $PI_{enriched}=22.8\pm3.51$ for 9-month-old mice ; 2-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 ($PI_{control}$=27.8±6.30; $PI_{isolated}$=13.5±6.85 for 3-month-old mice and $PI_{control}$=39.9±6.77; $PI_{isolated}$=6.81±5.12 for 9-month-old mice ; 2-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 (2-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 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 (Fig. 1B). However, 3-month-old mice exposed to the enriched environment show a significant increase in the time spent investigating the unfamiliar subject (Fig. 1B; $Time_{control}$=20.4±4.25; $Time_{enriched}$=35.5±5.40; 2-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): p=0.043) as well as in the number of interactions (Not shown; $Interactions_{control}$=18.3±3.79; $Interactions_{enriched}$=27.9±3.92; 2-way ANOVA, adjusted p for multiple comparisons (Dunnett's test): p=0.0034) whereas this effect is not present in older animals (Fig. 1B; $Time_{control}$=20.3±3.61; $Time_{enriched}$=23.9±3.70; 2-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 (Fig. 1C; 2-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 (Suppl. Fig. 1A). 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 (2-way ANOVA, Factor age, $F(2, 40)=8.55$, $p=0.0057$). However, social environment does not affect neither locomotion (2-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 (2-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;

Suppl. Fig. 1B, 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; Suppl. Fig. 1B, lower panel). Social environment does not significantly alter RI in any phase. In the MWM (Suppl. Fig. 1C), during the training phase, escape latency gradually decreased over the days in all group of animals. Importantly, the learning curves are similar (2-way ANOVA for repeated measures, Age 3 months, Factor social environment, $F(2, 21) = 0.567$, $p=0.578$; 2-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 (Suppl. Fig. 1D) 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 (Suppl. Fig. 2A). 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 Fig. 2A, the three groups cluster more sharply and the Lambda Wilks test p values are considerably lower (p young$<10^{-9}$; p 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 (PCA) to investigate whether social environment drives specific miRNA signatures and, if so, in which brain regions. As illustrated in Fig. 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 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) (Fig. 2B and Suppl. Fig. 2B). 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) (Suppl. Fig. 2C). 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 Fig. 2C, taking into 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 Fig. 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 (Fig. 3A). Network visualization enables the identification of mRNA common targets (Tor1aip2, Atp2b2 and Nf2) for the different conditions.

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More importantly, when targets are submitted to KEGG enrichment analysis (Fig. 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 (Suppl. Fig. 3). These findings give further support to the hypothesis that different social environments drive specific molecular alterations.

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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.

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, 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 bi-directionally 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 (Fig. 2B and Suppl. Fig. 2B). 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 (Suppl. Fig. 2D) 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 (Ambrozkiewicz 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 (Fig. 3 and Suppl. Fig. 3) 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 (Fig. 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 (Kulesskaya 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 (Fig. 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; Leidinger

et al., 2013) and their understanding and manipulation would be extremely useful for clinical purposes.

Limitations of this 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.

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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.

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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 (http://dx.doi.org/10.17632/mpp5k9czr8.1).

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Author contributions

NP performed the molecular experiments. FB performed the behavioral tests. NP, FB and FJ analyzed the data. RB and EG supervised the project and wrote the manuscript**.**

Declaration of Interests

All authors declare having no conflict of interest

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Figure legends

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 show a significant increase in their preference for exploring the social partner whereas 9-month-old mice having been isolated exhibit 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±sem

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.

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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-months 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.

C. miRNAs significantly altered by social enrichment and isolation.

Upper panels: miRNAs significantly deregulated in 3-months enriched mice (One-way ANOVA, * $p<0.05$,) include miR-140-5p and miR-181a-5p. Lower panels: miRNAs significantly deregulated in 9-months 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-months 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-months 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±sem

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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).

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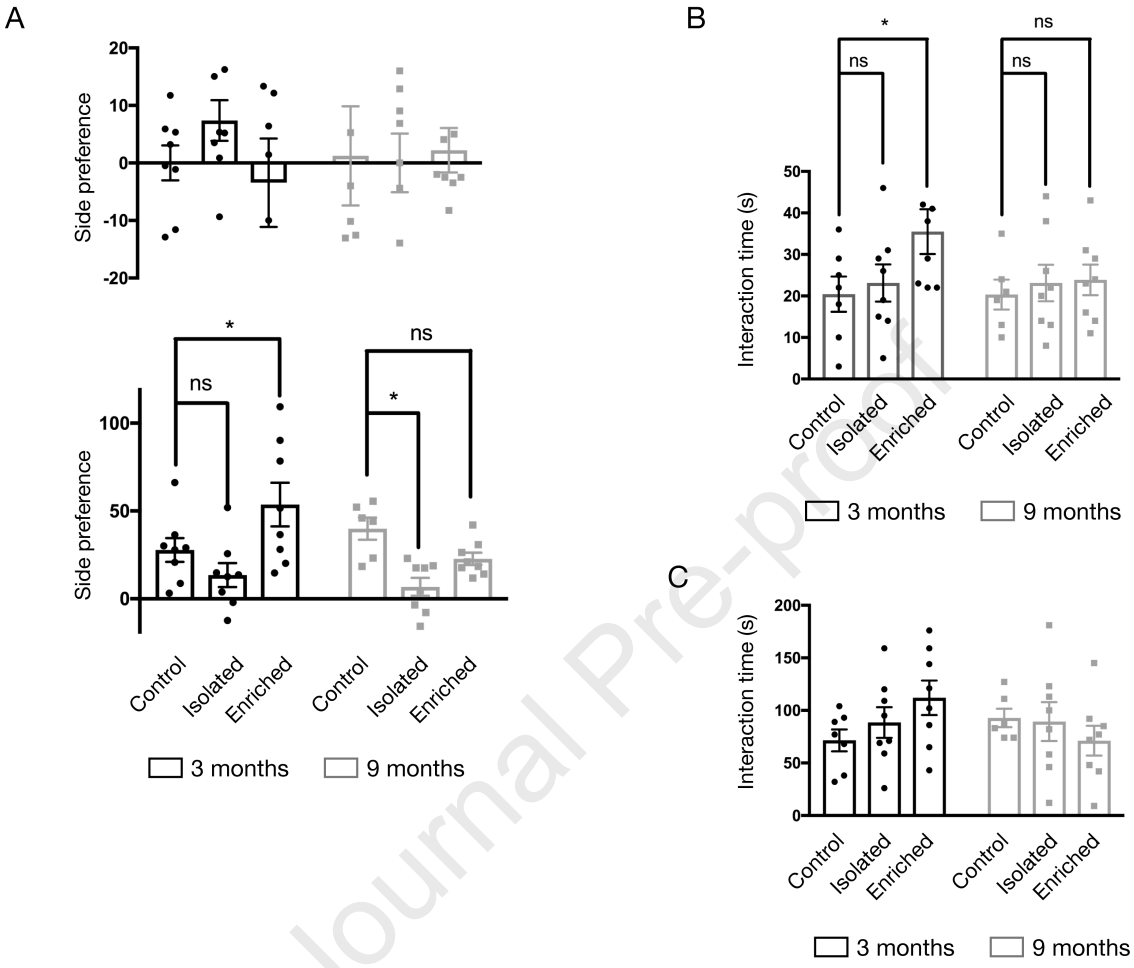
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Table 1. miRNAs selected in this study

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

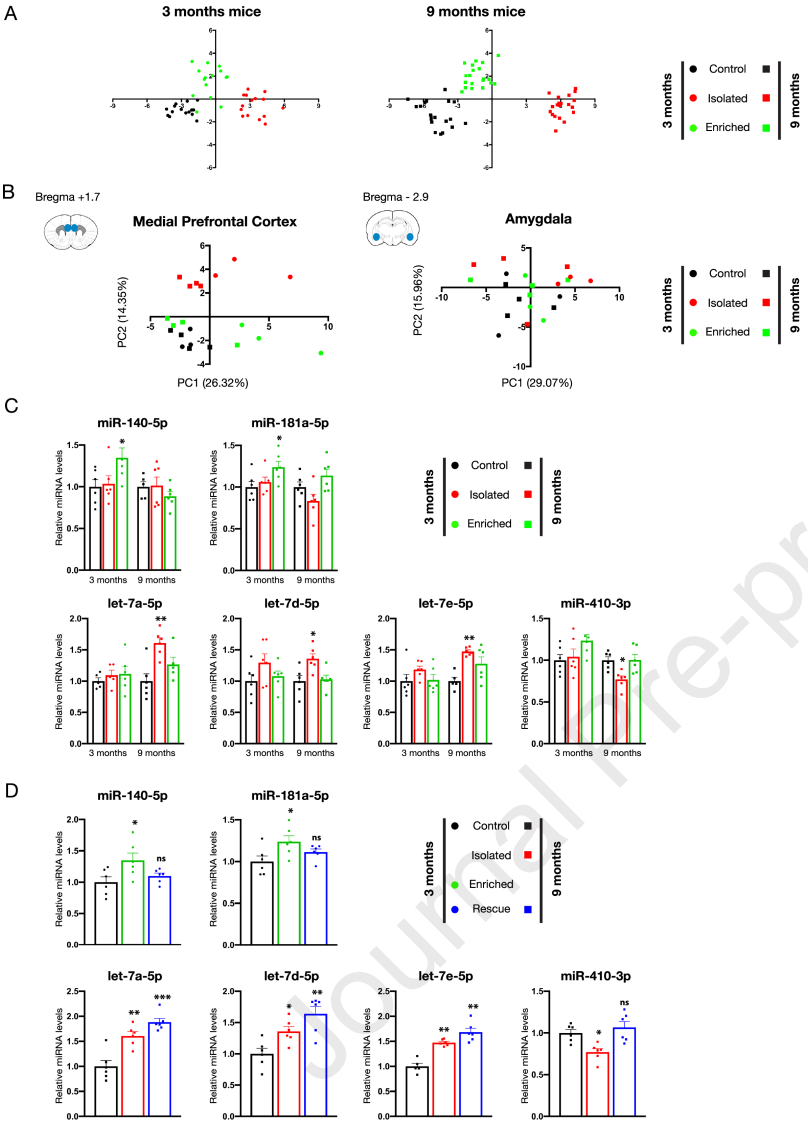
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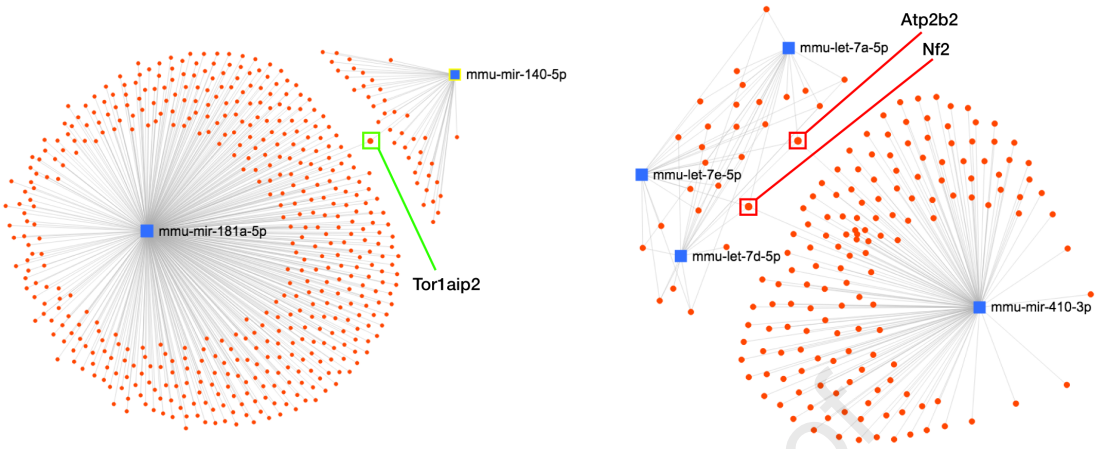
Figure 2. Short modifications of social environment drive specific and reversible miRNA changes



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Figure 3. In silico analysis of target networks downstream of miRNAs altered by social enrichment and isolation.

A



B

Pathways for enrichment miRNAs (miR-140-5p & miR-181a-5p)

Pathway	# of targets	Adj p value
Glutamatergic synapse	13	0.000477
Axon guidance	13	0.000845
Long term potentiation	8	0.0064
Amphetamine addiction	8	0.0064
Chronic myeloid leukemia	8	0.00836
Circadian rhythms	5	0.0123
Endocannabinoid signaling	9	0.0123
Wnt signaling	13	0.0123

Pathways for isolation miRNAs (let-7a-5p, let-7d-5p, let-7e-5p & miR-410-3p)

Pathway	# of targets	Adj p value
Hippo signaling	10	0.0000208
Basal cell carcinoma	7	0.0000208
Pathways in cancer	13	0.00039
Melanogenesis	7	0.00039
Transcriptional misregulation	8	0.001824
Wnt signaling	7	0.00275
Proteoglycans	8	0.0036
Signaling regulating pluripotency	6	0.0121

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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

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