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Title: Cell type- and region-specific modulation of cocaine seeking by micro-RNA-1 in striatal projection neurons

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33 **ABSTRACT**

34 The persistent and experience-dependent nature of drug addiction may result in part from epigenetic
35 alterations, including non-coding micro-RNAs (miRNAs), which are both critical for neuronal function
36 and modulated by cocaine in the striatum. Two major striatal cell populations, the striato-nigral and
37 striato-pallidal projection neurons, express respectively the D1 (D1-SPNs) and D2 (D2-SPNs)
38 dopamine receptor, and display distinct but complementary functions in drug-evoked responses.
39 However, a cell-type-specific role for miRNAs action has yet to be clarified. Here, we evaluated the
40 expression of a subset of miRNAs proposed to modulate cocaine effects in the nucleus accumbens
41 (NAc) and dorsal striatum (DS) upon sustained cocaine exposure in mice and showed that these
42 selected miRNAs were preferentially up-regulated in the NAc. We focused on miR-1 considering the
43 important role of some of its predicted mRNA targets, *Fosb* and *Npas4*, in the effects of cocaine. We
44 validated these targets *in vitro* and *in vivo*. We explored the potential of miR-1 to regulate cocaine-
45 induced behavior by overexpressing it in specific striatal cell populations. In DS D1-SPNs miR-1
46 overexpression downregulated *Fosb* and *Npas4* and reduced cocaine-induced CPP reinstatement, but
47 increased cue-induced cocaine-seeking. In DS D2-SPNs miR-1 overexpression reduced the motivation
48 to self-administer cocaine. Our results indicate a role of miR1 and its target genes, *Fosb* and *Npas4*, in
49 these behaviors and highlight a precise cell-type- and region-specific modulatory role of miR-1,
50 illustrating the importance of cell-specific investigations.

51 **INTRODUCTION**

52 Drug addiction is a clinically devastating neuropsychiatric disorder thought to result from neural
53 adaptations at the molecular, cellular, and circuit levels, following repeated drug exposure [1]. Drugs
54 of abuse initially affect neuronal plasticity in the mesocorticolimbic system corresponding to dopamine
55 (DA) neurons of the ventral tegmental area (VTA) projecting onto the ventral part of the striatum, the
56 *nucleus accumbens* (NAc), and the prefrontal cortex [2,3]. The striatum plays crucial roles in diverse
57 behavioral processes including movement control and reward-dependent learning [4-6]. While the NAc
58 underlies the primary reinforcing effects of drugs of abuse such as cocaine, the emergence of
59 compulsive cocaine seeking habits, a hallmark of drug addiction, is associated with a shift to the dorsal
60 striatal (DS) control over behavior [7,8].

61 In the DS, and to a lesser extent in the NAc, striatal projection neurons (SPNs) are comprised of
62 two segregated GABAergic neuronal populations: the SPNs expressing the DA-D1 receptor subtype
63 (D1-SPNs) that project directly to the internal segment of the *globus pallidus* (GPi) and the *substantia*
64 *nigra pars reticulata* (SNr), and the SPNs expressing the DA-D2 receptor subtype (D2-SPNs), which
65 project mainly to the external segment of the *globus pallidus* (GPe). Furthermore, these two
66 subpopulations exert distinct, albeit complementary, functions. In the DS the D1-SPNs represent the
67 “direct pathway”, which activation facilitates the initiation and execution of movement, while the
68 activation of D2-SPNs of the “indirect pathway” inhibit movement [9,10]. However, both pathways are
69 necessary for action initiation [11]. The direct and indirect pathways are also thought to work in
70 opposing manners in reward-based learning, with the activation of the direct pathway promoting
71 reinforcement and the indirect pathway mediating punishment and aversion [5,12], although such a
72 dichotomy has been recently challenged [13-15]. Several studies investigated the participation of D1-
73 and D2-SPNs in behaviors related to cocaine exposure [16]. For example, activation of D1-SPNs in the
74 NAc increases the formation of cocaine reward-context associations, while activation of D2-SPNs in
75 the same structure decreases cocaine reward and self-administration (SA) [17,18]. D1-SPNs in the

76 NAc encode information related to the association between cocaine and contextual cues and drive
77 cocaine seeking induced by those cues [19]. While most of the studies investigating the role of D1- and
78 D2-SPNs in the behavioral effects of cocaine focused on the NAc, a recent work showed a very
79 specific role of D1-SPNs of the dorsomedial striatum in cocaine responses, where their inhibition had
80 no impact on loss of control over cocaine intake, motivation to obtain cocaine or compulsive cocaine
81 self-administration, but specifically reduced cue-induced cocaine-seeking in high-risk addiction groups
82 of rats [20].

83 The persistent and experience-dependent aspects of the addicted phenotype have suggested a
84 role for epigenetic modifications in drug-induced gene expression [21] as epigenetic mechanisms can
85 register and durably maintain transcriptional states [22]. Among various epigenetic processes, attention
86 has recently been focused on miRNAs, a subcategory of 21-25-nucleotides non-coding RNAs that
87 modulate gene expression by binding to complementary sequences in the 3' untranslated regions
88 (3'UTR) of many, up to hundreds, target mRNA transcripts per miRNA, thereby inducing mRNA
89 destabilization and subsequent degradation and/or repression of mRNA translation [23]. Changes in
90 miRNAs expression levels have been shown to play a role in multiple neuronal functions and are
91 altered in several psychiatric conditions [24], including addiction [25]. Several studies have provided
92 evidence that miRNA-mediated gene regulation plays an important role in cocaine-induced changes in
93 neurotransmission and behavior [26], but there is so far no direct evidence regarding cell-type-
94 specificity of cocaine-induced miRNA in the striatum, and more specifically in D1-SPNs versus D2-
95 SPNs. One study began to broadly address this aspect by specific ablation in D2-SPNs of Ago2 [27], a
96 key protein in the processing and production of miRNAs [28-30]. These mice showed loss of
97 motivation to self-administer cocaine, along with a decrease of around one third of miRNAs induced
98 by acute cocaine, thus indicating that miRNA induction occurs in both D1- and D2-SPNs. However,
99 the question of whether and how miRNAs regulation in D1-SPNs versus D2-SPNs controls gene
100 expression and behaviors induced by cocaine, remains to be addressed.

101 Here we aimed to identify the regional pattern, i.e. DS *versus* NAc, of expression of a set of
102 selected miRNAs, proposed to play a key role in cocaine action or neuronal plasticity after sub-chronic
103 cocaine administrations [24,25,31]. We observed an increase in the expression of several miRNAs,
104 including miR-1, in the Nacc but not the DS. MiR-1 targets included 2 mRNAs involved in addiction
105 processes: *fosb* and *npas4*. Interestingly, *fosb* and *npas4* mRNA levels were up-regulated by cocaine in
106 the DS but not the NAcc, leading us to hypothesize that the absence of miR-1 up-regulation in the DS
107 might allow their induction in that area, while their induction was blunted by miR-1 in the NAc. In line
108 with this homeostatic hypothesis, we next investigated the effect of miR-1 overexpression in D1- or
109 D2-SPNs of the NAc or DS in multiple behaviors related to cocaine addiction by using a validated
110 viral strategy [32-34]. We found a key role of miR-1 overexpression in D1-SPNs of the DS in cocaine-
111 and cue-induced relapse to cocaine seeking, with a reduction of cocaine-induced reinstatement of
112 cocaine seeking in conditioned place preference (CPP) paradigm, and an increase in cue-induced
113 reinstatement of cocaine SA. We also observed a reduction of motivation for cocaine SA after miR-1
114 overexpression in D2-SPNs of the DS. Altogether our data unveil a complex cell-type- and region-
115 specific regulatory role for miR-1 in different relapse behaviors, and the importance of cell-specific
116 investigations.

117

118 **MATERIALS AND METHODS**

119 More details on the materials and methods used are given in Supplementary Methods. Statistical
120 details are given in Supplementary Materials.

121 **Animals and drug treatments**

122 All animal treatments and procedures were conducted in accordance with local and European
123 directives for the care and use of laboratory animals (86/609/EEC) and approved by the institutional

124 French Animal Care Committee (agreement N°2010/63) and by the Spanish local ethical committee
125 (*Comitè Ètic d'Experimentació Animal-Parc de Recerca Biomèdica de Barcelona*, CEEA-PRBB).

126 **Viral vectors**

127 For the overexpression of miR-1 in D1-SPNs or D2-SPNs of the striatum, we used a combination of
128 AAVs (AAV9-*PPTA*-Cre or AAV9-*PPE*-Cre, respectively; titers: 1×10^{12} in combination with AAV9-
129 miR-1^{flox} or AAV9-miR-Scr^{flox} titers: 1×10^{12} genomic copies/mL). AAV-injected mice were used for
130 experiments after at least three weeks for the full expression of viral-mediated miRNA expression.

131 **Stereotaxic injections**

132 Mice were anesthetized using ketamine and xylazine (5:1 in amount; 0.1 mL/10 g), placed in a
133 stereotaxic frame (David Kopf, Tujunga, CA), and 1 μ l in the NAc or 2 μ l in the DS was injected at a
134 constant rate of 0.20 μ l/min by using a microinfusion pump (Harvard Apparatus, Holliston, MA). We
135 used the following coordinates to perform bilateral injections according to the mouse brain atlas of
136 Paxinos and Franklin: NAc coordinates: AP +1.4 mm; ML \pm 0.7 mm; DV -4.4 mm;
137 DS coordinates: AP +0.98 mm; ML \pm 1.75 mm; DV -3.5 mm.

138 **Quantitative PCR analysis (qPCR) of miRNA and mRNA levels**

139 Punches of NAc and DS were performed from frozen tissues and total RNA was isolated using
140 miRNeasy MiniKit (Qiagen) according to the manufacturer's instructions. For mRNA levels analysis,
141 total RNA (100 ng) isolated from each brain sample was reverse-transcribed with oligodT primers
142 using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher). For miRNA levels analysis, total
143 RNA (100 ng) was reverse-transcribed using the same kit with a combination of specific RT-primers
144 for each miRNA of interest (0.5 pmol of each RT-primer), as previously described [35], allowing high
145 specificity, sensitivity and homogeneity. qPCR was performed with a LightCycler 480 device (Roche)
146 using LightCycler SYBR Green I Master Mix (Roche) and specific couples of primers; samples were

147 run in triplicates. The primers sequences for miRNAs and mRNAs are presented in supplementary
148 **Figure 1a and b**, respectively.

149

150 **Luciferase assay to assess the interaction between miR-1 and the 3'UTR of *Bdnf*, *FosB* and**
151 ***Npas4* in HEK cells**

152 The pmirGLO-3'UTR_*Bdnf*, pmirGLO-3'UTR_*FosB* and pmirGLO-3'UTR_*Npas4* plasmids were
153 purchased from Creative Biogene and the mimick-miR-1a-3p and mimick-miR-negative control from
154 Qiagen (miScript miRNA Mimics). The protocol used is described in DOI: [10.21769/BioProtoc.420](https://doi.org/10.21769/BioProtoc.420).
155 The mean ratio of luminescence from the experimental reporter (firefly) to luminescence from the
156 control reporter (Renilla) was calculated for each triplicate and normalized to the ratio of control wells
157 (miRNA negative control which does not target the 3'UTR of mRNA of interest).

158

159 **Cocaine-induced behavioral responses**

160 The *locomotor activity* and its sensitization were evaluated using a circular corridor (actimeter) [36].
161 The *cocaine-induced conditioned place preference* (CPP), its extinction and reinstatement were
162 evaluated in a plexiglas Y-shaped apparatus (Immetronic) with one arm blocked, located in a
163 soundproof testing room with low luminosity using an unbiased procedure (see supplementary
164 methods for more details). Two of the three chambers were used for the conditioning and distinguished
165 by different floor textures and wall patterns. The device was connected to an electronic interface for
166 data collection. Entries and time spent in each chamber were measured, as well as the locomotor
167 activity of the mice during the experiments.

168 The operant self-administration of cocaine experiments were conducted in mouse operant chambers
169 (Model ENV307A-CT; Med Associates Inc., Georgia, VT, USA) as previously described [37]. Nose
170 pokes were the operant action that led to delivery of a cocaine infusion.

171 **Immunocytochemical studies**

172 The procedures for Cre-recombinase (anti-Cre-recombinase antibody; 1:500, mouse, MAB3120,
173 Merck Millipore) is detailed in **supplementary methods**.

174

175 **Statistical analysis**

176 All statistical analyses were carried out using GraphPad Prism and Statview softwares. For all
177 experiments, ANOVAs were performed followed, when appropriate, by post hoc test Bonferroni for
178 multiple comparisons, except when data did not pass the normality test and/or when the number of
179 individuals was too low. In those cases, non-parametric tests, such as Mann-Whitney (for 2 groups) or
180 Kruskal-Wallis followed by post-hoc Dunn's test (for more than two groups) were used. The null
181 hypothesis was rejected at $p < 0.05$. All the statistical results for principal figures are reported in **Table**
182 **1**.

183

184 RESULTS

185 Ten day cocaine exposure differentially regulates a subset of plasticity-related miRNAs in the 186 NAc and DS

187 Several miRNAs have been shown to respond to 1-week cocaine exposure in a broad miRNA
188 sequencing study [31]. Among those, we selected a subset of 14 miRNAs (miR-1, -16, -29b, -31, -32, -
189 124, -125a, -128b, -132, -181a, -212, -221, -223 and Let7) based on their association either with
190 cocaine addiction [25] or with other related brain disorders [24]. We measured the expression levels of
191 these 14 miRNAs in the NAc and DS by RT-qPCR, after a one-week repeated treatment with cocaine
192 mimicking a behavioral sensitization procedure (**Figure 1a**) known to induce modifications in
193 miRNAs expression [31]. The sequences of all miRNA primers used are presented in **Supplementary**
194 **Figure 1a**. Interestingly, the repeated exposure to cocaine did not significantly alter miRNA levels in
195 the DS but induced an up-regulation of 11 of these 14 miRNAs in the NAc (**Figure 1b and c, left**
196 **panels**).

197 The mRNA expression levels of the key players in miRNA processing, such as *Drosha*, *Dicer*, *Tar*
198 *RNA Binding Protein (TRBP)* and *Ago2*, were not modulated by cocaine (**Figure 1b and c, right**
199 **panels**), indicating that the observed miRNA regulation did not result from alterations in the general
200 miRNA processing machinery at the mRNA level.

201 In order to predict cocaine-related target genes of the selected miRNAs, we used a combination
202 of databases, including miRBase, Miranda, TargetScan and Tarbase, crossed with literature data on
203 cocaine addiction. We identified 9 potential interesting targets: *Bdnf*, *Fosb*, *Npas4*, *Gria2*, *Grin2b*,
204 *Gria3*, *Pi3k*, *Drd1* and *Mef2c* (**Figure 1d**) and studied expression levels of these mRNAs in the DS
205 and NAc after our protocol of sustained cocaine administration. Among these mRNAs, we only
206 observed a significant increase in the expression levels of *FosB*, *Npas4* and *Pi3k* mRNAs in the DS,
207 but not in the NAc where an increase in *Bdnf* expression was found (**Figure 1e**).

208

209 **Validation of *FosB* and *Npas4* mRNAs as targets of miR-1**

210 In most cases, miRNAs interact with the 3'UTR of target mRNAs to induce mRNA degradation or
211 translational repression [38]. We hypothesized that the absence of detectable up-regulation of *FosB*
212 and *Npas4* mRNAs by cocaine in the NAc might be due to cocaine-induced up-regulation of
213 miRNA(s) targeting these mRNAs in this region. Among the selected miRNAs, we focused on miR-1
214 because the “in silico” prediction indicated that it potentially targeted both *fosB* and *npas4* mRNAs
215 (**Figure 1d**), which are key players in cocaine-induced long-term adaptations [41,42]. We
216 experimentally tested whether these potential targets were directly bound by miR-1 (**Figure 2**) using a
217 luciferase reporter assay with constructs containing either *FosB*, *Npas4* mRNA 3'UTRs (**Figure 2a left**
218 **panel**), or the *Bdnf* 3'UTR as a positive control [39]. We observed a significant decrease in luciferase
219 activity in HEK cells expressing the pmiRGlo plasmid encoding the 3'UTR of *Bdnf*, *FosB* or *Npas4* in
220 the presence of a mimick-miR-1 when compared to the negative miR-control (**Figure 2a, right**
221 **panels**). We thus validated *in vitro* that the 3'UTR of *Bdnf*, *FosB* and *Npas4* are directly targeted by
222 miR-1.

223 We further aimed to assess whether miR-1 could repress *Bdnf*, *Fosb* and *Npas4* mRNA levels
224 *in vivo*. To this end, we used an AAV-mediated cell-type specific strategy allowing to overexpress
225 miR-1 in either D1R-SPNs or D2R-SPNs. We used a combination of two AAVs, with one expressing
226 miR-1 in a Cre recombinase-dependent manner and the other one expressing Cre under a cell type-
227 specific promoter (PPE-*Cre* and PPTA-*Cre*, expressed in D1R-SPNs and D2R-SPNs respectively) as
228 previously validated [32-34] (**Figures 2 b and e, see also supplementary Figure 2a-b**). As compared
229 to the control group (injected with an AAV expressing scrambled RNA, Scr) we found a marked
230 increase in miR-1 levels after overexpression of miR-1 in D1R- or D2R-SPNs in either the DS
231 (**Figures 2c and f**) or the NAc (**Figures 2d and g**), with a concomitant decrease in the expression of
232 *Fosb* and *Npas4* mRNAs, which was significant in several cases (**Figure 2c-g**). We thus confirmed the

233 ability of miR-1 to repress *Fosb* and *Npas4* mRNAs expression *in vivo*. Consistent with the preferential
234 expression of *Bdnf* mRNA in axons from afferent excitatory cortical neurons [40], and their low levels
235 in SPNs we failed to detect a significant effect of miR-1 overexpression on *Bdnf* mRNA levels in any
236 conditions. miR-1 overexpression did not impact on the expression levels of mRNAs involved in
237 miRNA processing, nor levels of miR-206 a miRNA from the same family than miR-1
238 **(Supplementary Figure 3a and b).**

239

240 **Effects of miR-1 overexpression in D1- and D2R-SPNs of the NAc and DS on cocaine-induced** 241 **locomotor sensitization**

242 Having established the inhibitory role of miR-1 on *Fosb* and *Npas4* mRNA expression levels, we
243 investigated the potential role of miR-1 overexpression on behavioral adaptations to cocaine. We
244 specifically overexpressed miR-1 in D1- or D2-SPNs in regions where it is induced by cocaine (NAc)
245 or not (DS). We first studied the consequences of this overexpression on cocaine-induced locomotor
246 sensitization (15 mg/kg) **(Supplementary Figure 4a-e)**. The overexpression of miR-1 in either D1-
247 SPNs or in D2-SPNs of the NAc or DS did not modify cocaine-induced locomotor activity or its
248 sensitization **(Supplementary Figure 4b-e)**, indicating that miR-1 and its targets are not critical for
249 the sensitizing effects of cocaine.

250

251 **Cell type- and region-specific effects of miR-1 in cocaine-induced conditioned place preference**

252 We then measured the effects of cell-type-specific overexpression of miR-1 on the rewarding
253 properties of cocaine using the cocaine-induced conditioned place preference (CPP) paradigm, which
254 can be used as a proxy for the rewarding properties of cocaine. Overexpression of miR-1 in D1-SPNs
255 of the DS did not alter cocaine-induced CPP **(Figure 3a, left panel)**. We then extinguished the CPP by
256 freely exposing the mice to cocaine-paired and unpaired environments after saline injection and
257 observed that miR-1 overexpression in D1-SPNs of the DS did not modify CPP extinction **(Figure 3a,**

258 **middle panel**). In contrast, the reinstatement of cocaine CPP induced by an acute injection of cocaine
259 after the extinction period (15 mg/kg) was prevented by miR-1 overexpression in DS D1-SPNs
260 **(Figure 3a, right panel)**.

261 We measured the levels of miR-1 and its target genes in the DS of these mice 1 h after the start
262 of the reinstatement test. As expected we found, high levels of miR-1 in both saline and cocaine miR-
263 1-injected groups, while miR-1 levels were not altered in control groups **(Figure 3b, left panel)**. miR-
264 1 overexpression in D1-SPNs blunted *Fosb* and *Npas4* mRNA up-regulation observed in the control
265 scramble group after cocaine-induced reinstatement of CPP **(Figure 3b, right panel)**. miR-1
266 overexpression in D1-SPNs of the NAc did not alter the development of cocaine-induced CPP, nor its
267 extinction and cocaine-induced reinstatement **(Figure 3c)**. As shown in **Figure 4a and b**, the
268 overexpression of miR-1 in D2-SPNs of the DS or NAc had no consequence on cocaine-induced CPP,
269 extinction or cocaine-induced reinstatement. These results indicate that miR1-overexpression in DS
270 D1-SPNs may specifically alter cocaine-induced CPP reinstatement, indicating a role of miR-1
271 targeting these genes in this behavior.

272

273 **Cocaine self-administration, extinction and reinstatement after miR-1 overexpression in**

274 **D1- or D2-SPNs of the DS of mice**

275 We then investigated whether miR-1 overexpression in D1-SPNs of the DS altered cocaine SA. MiR-1
276 overexpression in DS D1-SPNs preserved the acquisition of SA, the motivation to self-administer
277 cocaine assessed in a progressive ratio (PR) schedule of reinforcement **(Figure 5a-b)**, and the
278 extinction of this behavior **(Figure 5c)**. By contrast, miR-1 overexpression in D1-SPNs of the DS
279 induced a significant increased cue-induced reinstatement of cocaine seeking **(Figure 5d)**. MiR-1
280 overexpression in D2-SPNs of the DS did not alter the acquisition of cocaine-SA **(Figure 5e)**.
281 Interestingly, it reduced the breaking point for cocaine-SA under PR schedule of reinforcement

282 (**Figure 5f**, showing a reduced motivation to self-administer cocaine-SA upon miR-1 overexpression
283 in D2-SPN of the DS. In contrast to what we observed in D1-SPNs, miR-1 overexpression in D2-SPNs
284 had no effect on SA extinction (**Figure 5g**) or cue-induced reinstatement of cocaine seeking (**Figure**
285 **h**). Altogether these results establish a cell-type specific function of miR-1 and its target genes in the
286 DS in controlling distinct components of cocaine-adaptative behaviors.

287 **DISCUSSION**

288 Our data show that miR-1 is up-regulated in the NAc, but not the DS, by a 10-day regimen of
289 cocaine and that at least three mRNAs encoding proteins associated with cocaine-induced
290 neuronal plasticity, FOSB, NPAS4 and BDNF are targeted by miR-1. We also demonstrate that
291 overexpression of miR-1 in D1-SPNs of the DS decreases cocaine-induced reinstatement of
292 cocaine seeking in a CPP paradigm, but increases cue-induced reinstatement of cocaine seeking
293 in a SA protocol. In addition, overexpression of miR-1 in D2-SPNs of the DS reduces the
294 breaking point for cocaine-SA under a progressive ratio schedule of reinforcement. These results
295 unveil the modulatory capacity of miR-1, and suggest that its target genes in DS neurons are
296 involved in mediating specific aspects of cocaine-induced behavior.

297 Multiple miRNAs were reported to be modulated by cocaine [26]. In a miRNA profiling
298 study, a panel of miRNAs was either up- or down-regulated by prolonged cocaine exposure [43].
299 Induction of miR-132 and miR-212 occurred in the DS after extended access to cocaine self-
300 administration, and overexpression of miR-212 reduced compulsive cocaine intake in rats
301 through CREB, MeCP2 and BDNF signaling [44,45]. *In silico* genome-wide sequencing
302 identified miR-495 among miRNAs whose targets are enriched in an addiction-related gene
303 (ARG) database. Lentiviral-mediated overexpression of miR-495 in the NAc suppressed the
304 motivation to self-administer and seek cocaine [46].

305 Deficiency of Ago2 in D2-SPNs greatly influenced a specific group of miRNAs, and
306 reduced approximately by half the expression of miR-1 [27], suggesting that miR-1 is expressed
307 in both types of SPNs in this structure. Similarly to our study, Hollander et al. (2010) showed
308 that overexpression of miR-1 in the DS, failed to alter cocaine intake, but cell-type-specificity
309 and reinstatement of drug seeking were not investigated [44]. Herein, we provide evidence that
310 miR-1 targets the mRNA encoding the transcription factor FosB, the spliced version of which,
311 Δ FosB, persistently accumulates in D1-SPN of the NAc after chronic psychostimulant exposure,

312 and plays a key role in cocaine addiction [47]. This D1-SPN cell-type specific Δ FosB expression
313 induced by psychostimulant was further confirmed in reporter lines [48,49] or using ribosomal
314 tagging approaches [50]. Furthermore, Δ FosB overexpression in D1-SPN increases locomotor
315 activity and rewarding effects of low doses of cocaine [51], as well as cocaine self-administration
316 [52]. Since miR-1 reduces the expression *fosb* mRNAs, which encodes both FosB and Δ FosB, it
317 was surprising to observe that miR-1 overexpression in D1-SPNs of the NAc failed to alter
318 cocaine-induced locomotor sensitization and CPP. However, the involvement of Δ FosB in
319 cocaine's effect seems highly dependent on the environment. For example, chronic cocaine
320 treatment elevates Δ FosB protein levels in the NAc of isolated rats, but not in enriched
321 conditions due to an already elevated basal accumulation of Δ FosB [53]. Furthermore, the
322 facilitating effects of Δ FosB overexpression in D1-SPN on cocaine-induced behaviors were seen
323 in individually housed animals [52]. In addition, overexpression of Δ FosB in the NAc shell of
324 pair-housed rats increases operant responding for sucrose when motivated by hunger, but
325 decreases responding in satiated animals, in cocaine self-administration and cocaine-induced
326 relapse. In our study, the mice were housed five per cage, and we did not observe a significant
327 increase in *fosB* mRNAs in the NAc upon sustained cocaine administration, similarly to previous
328 studies in rats [53]. The absence of effect of miR-1 overexpression in D1-SPNs of the NAc on
329 cocaine-induced locomotor sensitization and CPP might thus be due to our environmental
330 conditions. Alternatively, we cannot exclude that Δ FosB overexpression previously observed in
331 D1-SPNs of the NAc [47-49] results from an accumulation of the stable protein rather than from
332 de novo gene expression, or that a partial decrease induced by miR-1 overexpression may not be
333 sufficient to prevent the behavior.

334 Another validated target of miR-1 was *Bdnf* mRNA encoding brain-derived neurotrophic
335 factor (BDNF), which also plays a key role in cocaine-induced behaviors [54]. In particular, data

336 from several labs suggest a facilitating effect of BDNF in the NAc on cocaine-induced CPP and
337 cocaine-primed reinstatement [55,56]. However, the main source of BDNF within the striatum,
338 including the NAc, arises from prefrontal cortical neurons release [57], which mediates the
339 majority of BDNF effects on cocaine-related behaviors [58]. By contrast, *Bdnf* mRNA levels are
340 very low in the striatum under basal conditions, as observed in our RT-qPCR data. Altogether,
341 these observations can explain the lack of effect of miR-1 overexpression on *Bdnf* mRNA levels
342 *in vivo*, despite a clear demonstration that miR-1 targets its 3'UTR region *in vitro*. Further
343 experiments, which are out of the scope of our study, could include overexpression of miR-1 in
344 prefrontal cortical neurons.

345 Finally, we demonstrate that *Npas4* is a target of miR-1 both *in vitro* and *in vivo*. *Npas4*
346 encodes NPAS4, an activity-dependent transcription factor that plays a key role in neuronal
347 plasticity and is induced in the NAc by cocaine [42]. NPAS4 expression is higher in D1- than in
348 D2-SPN in the NAc after cocaine-induced CPP [59] and NPAS4 positive cells co-express cFos
349 [42], another activity-related immediate early gene, which is selectively induced by cocaine in
350 D1-SPN [60]. Conditional deletion of *Npas4* in the NAc significantly reduced cocaine-induced
351 CPP and delayed the acquisition of cocaine-SA, without affecting cue-induced reinstatement of
352 cocaine seeking [42]. In our study, miR-1 overexpression reduced but did not abolish *Npas4*
353 mRNA, a result that could explain its lack of effect in D1-SPN of the NAc on cocaine-induced
354 CPP.

355 From a behavioral point of view, we found effects of miR-1 overexpression in D1-SPN of
356 the DS on cocaine-induced reinstatement of cocaine CPP and cue-induced reinstatement of
357 cocaine seeking in a self-administration paradigm. Thus, while miR-1 overexpression in DS D1-
358 SPN blunted cocaine-induced reinstatement of CPP, it potentiated cue-induced reinstatement of
359 self-administration. These differential effects on cocaine-induced reinstatement of a cocaine-
360 induced CPP and on cue-induced reinstatement of cocaine-induced SA could be explained by

361 differences in cocaine doses and routes of administration used in the two protocols, the time
362 period of extinction and by the fact that the reinstatement of cocaine seeking is induced by a
363 priming dose of cocaine for CPP and by a cue previously associated with cocaine (thus in
364 cocaine-free state) in SA. This differential effect may be due to differences in neuronal circuits
365 involved in each behavior (see [61-63] for reviews). It would have been interesting to evaluate
366 the effect of miR-1 overexpression in D1-SPN on cocaine-induced reinstatement after SA and
367 extinction, but cocaine priming does not induce reliable reinstatement of cocaine seeking in
368 strain of mice (C57Bl6) [64] used in this study, contrasting with Swiss albino mice [65].

369 Finally, we found a decrease in breaking point for cocaine-SA under a PR schedule of
370 reinforcement induced by miR-1 overexpression in D2-SPNs of the DS, suggesting a reduction
371 in motivation for cocaine-SA. There are very few examples in the literature for specific reduction
372 of motivation for cocaine-SA. D2-SPNs of the DS are inhibitory neurons projecting to the
373 external part of the globus pallidus (GPe), which contains inhibitory neurons projecting to the
374 subthalamic nucleus (STN). The inhibition of D2-SPN activity thus results in an inhibition of
375 STN activity. Of interest, either lesion or inhibition of STN neurons can lead to a reduction in the
376 breaking-point for cocaine-SA without altering SA under a fixed ratio schedule [66] much like
377 what we observed in the present study.

378 In conclusion, our data show a specific and distinct role of miR-1 in cocaine- and cue-induced
379 relapses of two different cocaine-dependent behaviors after an extinction period and on
380 motivation for cocaine-SA. This contributes to the dissection of the mechanisms underlying
381 addictive behaviors. Our results show the cell type-specific and region-specific role of miR-1 and
382 underlines differences between cocaine- and cue-induced reinstatement in non-operant and
383 operant paradigms. Although the expression of miR-1 is not induced in the DS after cocaine
384 administration, our data provides valuable information about its role and the involvement of its
385 targets FOSB and NPAS4 in specific neurons of the striatum in behaviors related to cocaine

386 addiction and offers interesting therapeutic perspectives. Further studies investigating the effect
387 of miR-1 knock-down in the VS will be interesting to test the intrinsic function of miR-1 in this
388 structure. Altogether our results highlight the complexity of the control of addictive behaviors
389 and emphasizes the importance of regional cell-type specific investigations.

390

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415 experiments and participated in the writing of the manuscript. E.M.G E.S.M., and L.D.R.
416 performed the cocaine-self-administration experiment. V.K. M.M. and M.C.A participated in the
417 RT-qPCR and immunofluorescence experiments. P.P. performed most of the *in vitro*
418 experiments and participated in the RT-qPCR experiments. A.A performed viral infections and
419 immunofluorescence experiments for tracing D1 and D2 striatal pathways. R.M. designed the
420 cocaine self-administration experiments. P.V., J.A.G. and R.M. participated in experimental
421 design and writing of the manuscript.

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611 **FIGURE LEGENDS**

612 **Figure 1: A set of miRNAs is up-regulated in the ventral (NAc), but not the dorsal (DS),**
613 **striatum after sub-chronic treatment with cocaine.**

614 **a)** Schematic representation of the timing of cocaine (20mg/kg) or saline administrations and
615 brain extraction. **b-c)** Expression levels of miRNAs (**left panels**) and mRNAs involved in
616 miRNA processing (**right panels**) were analyzed in the DS (**b**) and the NAc (**c**) one hour after
617 the last injection of cocaine or saline on day 10. **d)** In silico analysis of cocaine-related targets of
618 miRNAs upregulated in the NAc, as indicated in (**c**). **e)** Expression of predicted mRNA targets
619 one hour after the last injection of cocaine or saline on day 10, in the DS (**upper panel**) or the
620 NAc (**lower panel**). **b) c) and e)** : Mean +/- SEM are represented. Mann-Whitney tests: *p<0.05,
621 **p<0.01 when comparing saline and cocaine groups.

622

623 **Figure 2: Validation of miR-1 targets.**

624 **a) Left panel:** schematic representation of the pmiR-Glo constructs used in HEK cells. **Right**
625 **Panel,** up: 3'UTR sequences of *bdnf*, *fosB* and *npas4* mRNAs. Bold and underlined nucleotides
626 represent complementarity with the seed sequence of miR-1; lower part: normalized
627 Luciferase/Renilla activity of pmiR-Glo plasmid with *bdnf*, *fosB* and *npas4* 3'UTRs mRNAs in
628 the presence of miRNA mimics for miR-1 (black bars) or miR- control (grey bars). **b-d)**
629 overexpression of miR-1 in D1-SPN. **b)** representation of the combination of AAVs to infect
630 D1-SPNs. **c-d)** normalized fold changes in expression of miR-1 (over miR-scr condition) (**left**
631 **panels**) and *bdnf*, *fos-B* or *npas4* mRNA (**right panels**) after viral injection of miR-1 or miR-scr
632 sequences in D1-SPNs of the DS (**c**) or the NAc (**d**) of mice. **e-g)** overexpression of miR-1 in
633 D2-SPNs. **e)** representation of the combination of AAVs to infect D2-SPNs. **f-g)** normalized fold
634 changes in expression of miR-1 (over miR-scr condition) (**left panels**) and *bdnf*, *fos-B* or *npas4*

635 mRNA (**right panels**) after viral injection of miR-1 or miR-scr sequences in D2-SPNs of the DS
636 (**f**) or the NAc (**g**) of mice. **a-g**) Mean +/- SEM are represented. Mann-Whitney tests: *: $p<0.05$;
637 **: $p<0.01$; ***: $p<0.001$.

638

639 **Figure 3: Cocaine-induced conditioned place preference (CPP) and relapse in mice after**
640 **miR-1 over-expression in D1-SPNs of the DS or NAc.**

641 **a-b)** overexpression of miR-1 in D1-SPNs of the dorsal striatum. **a) Left panel:** CPP after miR-1
642 over-expression in DS D1-SPNs. The CPP index is the difference of CPP score between the
643 pretest and the test. The 2-way ANOVA indicated a significant effect of treatment, no effect of
644 AAV and no interaction. **Middle panel:** extinction sessions for cocaine/miR-Scr and
645 cocaine/miR-1 groups. The 2-way ANOVA indicated a significant effect of session but not of
646 AAV and no interaction. **Right panel:** Cocaine-induced reinstatement of cocaine seeking after
647 extinction in mice with miR-1 over-expression in D1-SPN of the dorsal striatum. The 2-way
648 ANOVA indicated a significant effect of treatment, no effect of AAV and no interaction. Post
649 hoc tests : Bonferroni's multiple comparison tests: * $p<0.05$; ** $p<0.01$. **b) Left panel:**
650 expression of miR-1 after cocaine-induced reinstatement of cocaine seeking in mice with miR-1
651 or miR-Scr over-expression in D1-SPN of the DS. **Right panel:** normalized fold changes of
652 expression levels of *npas4* and *fosB* mRNA after cocaine-induced reinstatement of cocaine
653 seeking in mice with miR-1 over-expression in D1SPN of the DS; The Kruskal-Wallis test on
654 *npas4* and *fosB* mRNAs expression levels indicated a significant group effect. The Post-hoc
655 analysis (Dunn's multiple comparison test) indicated significant differences between the
656 saline/miR-Scr and the cocaine/miR-Scr groups for both *npas4* and *fosB* mRNAs: * $p<0.05$ and
657 between the cocaine/miR-Scr and the cocaine/miR-1 groups for both *npas4* and *fosB* mRNAs:
658 # $p<0.05$; ## $p<0.01$. **c)** overexpression of miR-1 in D1-SPNs of the NAc. **Left panel:** CPP after

659 miR-1 over-expression in D1-SPN of the NAc of mice. The CPP index is the difference of CPP
660 score between the pretest and the test. **Middle panel:** extinction sessions for cocaine/miR-Scr
661 and cocaine/miR-1 groups. **Right panel:** Cocaine-induced reinstatement of cocaine seeking after
662 extinction in mice with miR-1 over-expression in D1-SPN of the NAc. Bonferroni's multiple
663 comparison tests: * $p < 0.05$. **a-c)** Mean and SEM are represented.

664

665 **Figure 4: Cocaine-induced Conditioned Place Preference and relapse after miR-1**
666 **overexpression in D2-SPN of the dorsal striatum or NAc of mice.**

667 a) miR-1 overexpression in D2-SPN of the dorsal striatum (DS) of mice. **Left panel:** CPP. The
668 2-way ANOVA performed on the CPP index indicated a significant effect of treatment but not of
669 AAV and no interaction. **Middle panel:** extinction sessions for coc/ Scr and coc/miR-1 groups.
670 The 2way ANOVA indicated a significant effect of session but not of AAV and no interaction.
671 **Right panel:** Cocaine-induced reinstatement of cocaine seeking after extinction. The two-way
672 ANOVA performed on the reinstatement index (difference of CPP score between the extinction
673 and the reinstatement test) indicated a significant effect of treatment but not of AAV and no
674 interaction. b) miR-1 overexpression in D2-SPN of the NAc of mice. **Left panel:** CPP. The
675 two-way ANOVA indicated a significant effect of treatment but not of AAV and no interaction.
676 **Middle panel:** extinction sessions for coc/Scr and coc/miR-1 groups. The 2-way ANOVA
677 indicated a significant effect of session but not of AAV and no interaction. **Right panel:**
678 Cocaine-induced reinstatement of cocaine seeking after extinction. The two-way ANOVA
679 indicated a significant effect of treatment but not of AAV and no interaction. **a-b)** Mean +/-
680 SEM are represented. Post-hoc Bonferroni's multiple comparison tests * $p < 0.05$; ** $p < 0.01$;
681 *** $p < 0.001$.

682 **Figure 5: Cocaine Self-Administration and relapse after extinction in mice with miR-1**
683 **overexpression in D1- or D2-SPNs of the dorsal striatum. a-e)** miR-1 overexpression in D1-
684 SPNs of the dorsal striatum. **a)** Cocaine Self-Administration. **Left panel:** active nose-pokes
685 during the acquisition of cocaine (0.5 mg/kg/infusion)-induced self-administration in mice with
686 (n=11) or without (n=11, scr) miR-1 overexpression in the D1-SPNs of the DS. The 2-way
687 ANOVA indicated no effect of miR-1 overexpression, a significant effect of session, and no
688 interaction. **Right panel:** number of cocaine infusions received during the acquisition of cocaine
689 -induced self-administration. The 2-way ANOVA indicated no effect of miR-1 overexpression),
690 a significant effect of session and no interaction. **b)** Breaking point for cocaine
691 (0.5mg/kg/infusion)-induced self-administration under a progressive ratio. The Mann-Whitney
692 test indicated no AAV effect. **c)** Active nose-pokes during the extinction sessions. The 2-way
693 ANOVA indicated no effect of miR-1 overexpression, a significant effect of session and no
694 interaction. **d)** Cue-induced reinstatement of cocaine seeking after extinction (miR-1
695 overexpression in the D1-SPNs of the DS). The 2-way ANOVA indicated a significant effect of
696 miR-1 overexpression, a significant effect of cue-reinstatement and a significant interaction.
697 Post-hoc Bonferroni's multiple comparison test ***: $p < 0.01$ (extinction vs cue-reinstatement); #:
698 $p < 0.05$ (scr vs miR-1). **e-h)** miR-1 overexpression in D2- SPNs of the dorsal striatum. **e)**
699 Cocaine Self-Administration. **Left panel:** active nose-pokes during the acquisition of cocaine
700 (0.5 mg/kg/infusion)-induced self-administration in mice with (n=14) or without (n=11, scr)
701 miR-1 overexpression in the D2-SPNs of the DS. The 2-way ANOVA indicated no effect of
702 miR-1 overexpression, a significant effect of session, and no interaction). **Right panel:** number
703 of cocaine infusions received during the acquisition of cocaine -induced self-administration. The
704 2-way ANOVA indicated no effect of miR-1 overexpression, a significant effect of session and
705 no interaction. **f)** Breaking point for cocaine (0.5mg/kg/infusion)-induced self-administration
706 under a progressive ratio (miR-1 overexpression in the D2-SPNs of the DS). The Mann-Whitney

707 test indicated a significant effect of miR-1 overexpression ($p=0.018$). **g)** Active nose-pokes
708 during the extinction sessions after miR-1 overexpression in the D2-SPNs of the DS. The 2-way
709 ANOVA indicated no effect of miR-1 overexpression, a significant effect of session and no
710 interaction. **h)** Cue-induced reinstatement of cocaine seeking after extinction. The 2-way
711 ANOVA indicated no effect of miR-1 overexpression, a significant effect of cue-reinstatement
712 and no interaction. Post-hoc Bonferroni's multiple comparison test ***: $p<0.01$ (extinction vs
713 cue-reinstatement). **a-h)** Mean \pm SEM are represented.

Figure 1, Forget et al.

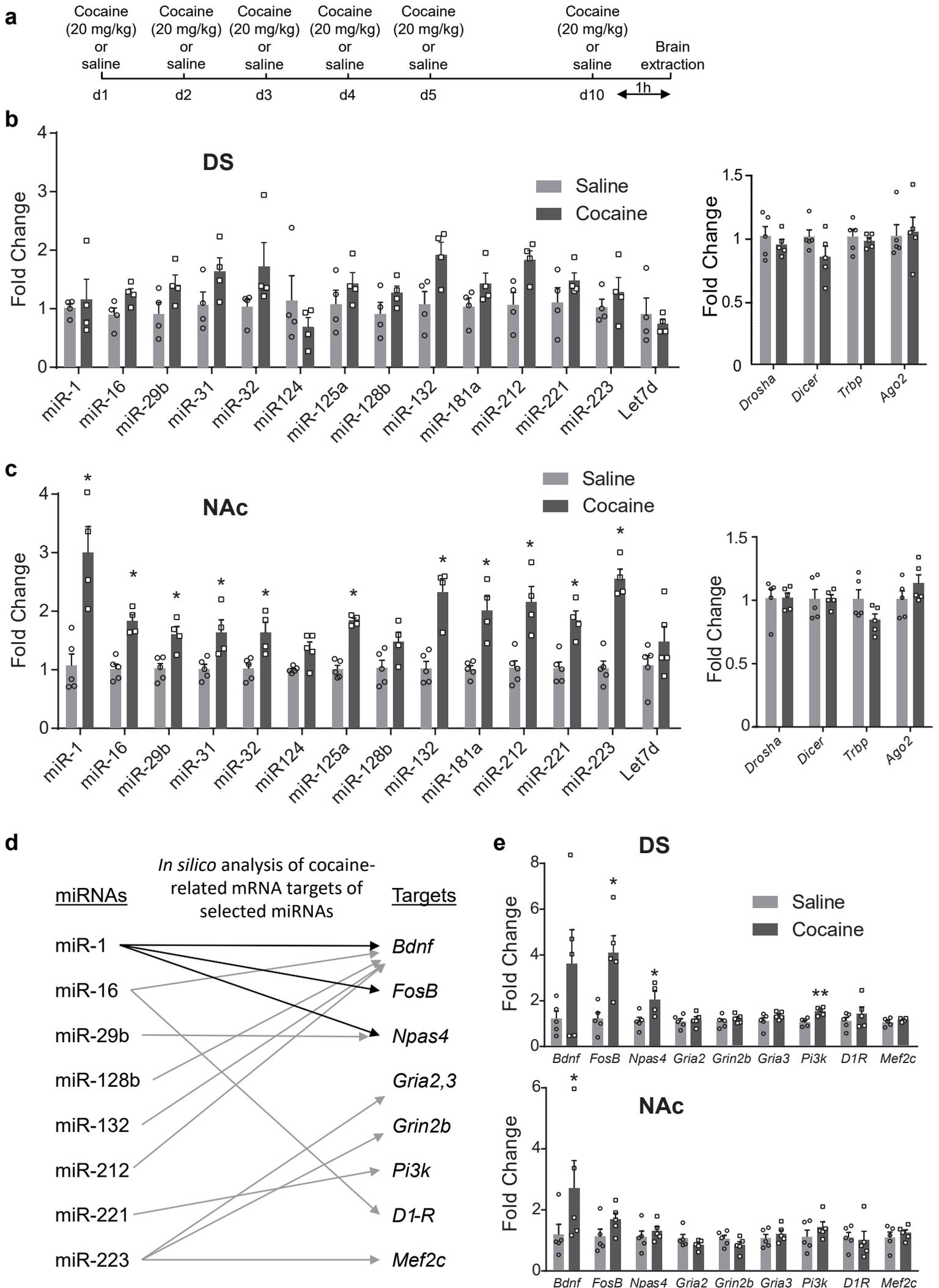
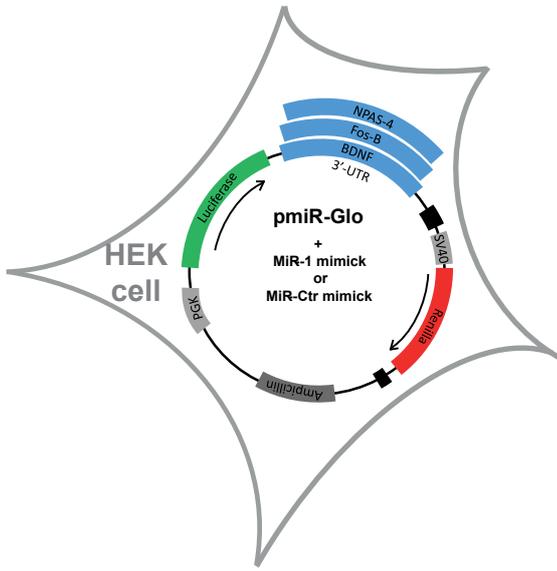


Figure 2, Forget et al.

a

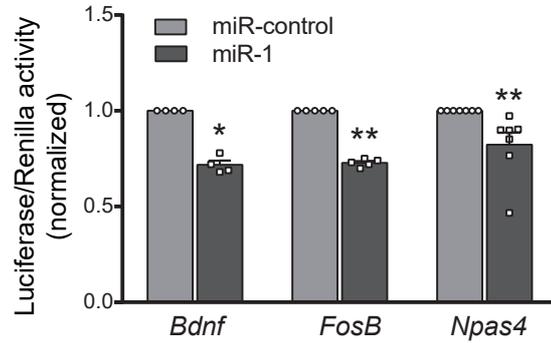


3'UTR sequence of miR-1 targets with complementarity with the seed sequence of miR-1

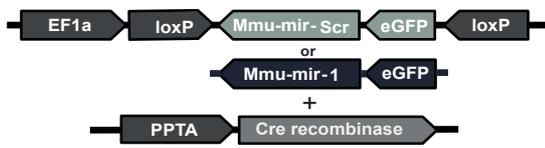
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FosB 5'...CCCCGUGCUGCAUGGAACAUAUCCAUACCCUGUCCUGGGCCCUAGGUUCCAACCUAAUCC...3'

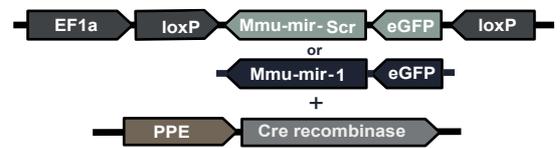
Npas4 5'...AGACGCUACAUAUCCUUUCCGAUGGAGGAAGGCAGAUUCGCCGUCACACGUG...3'



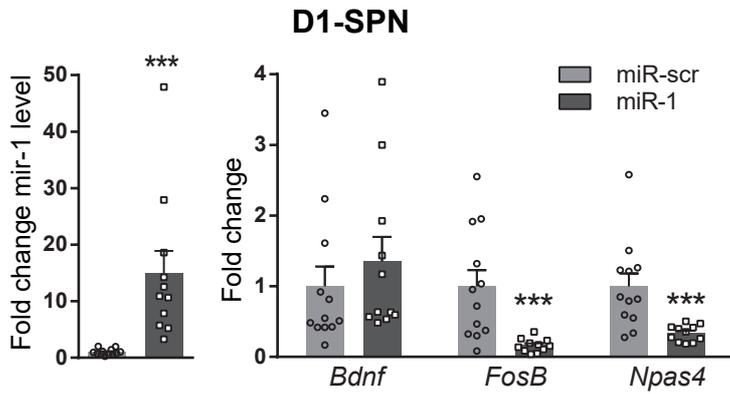
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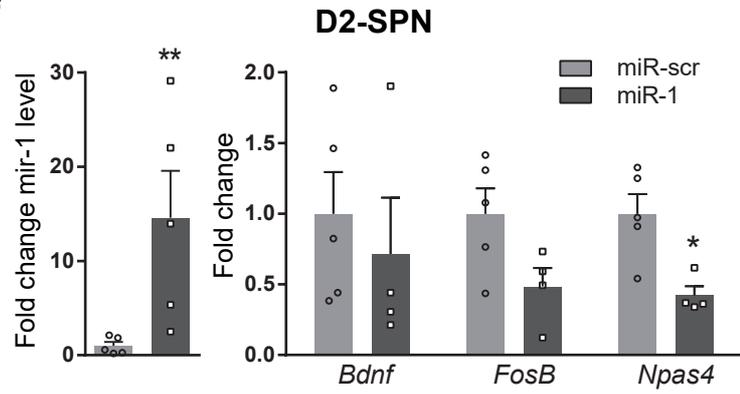
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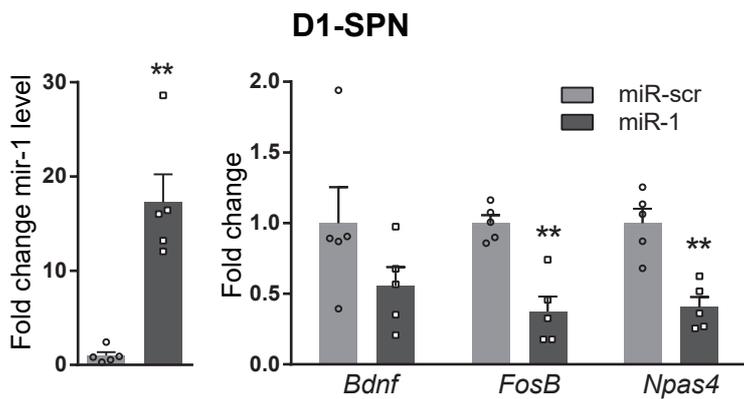
c



f



d



g

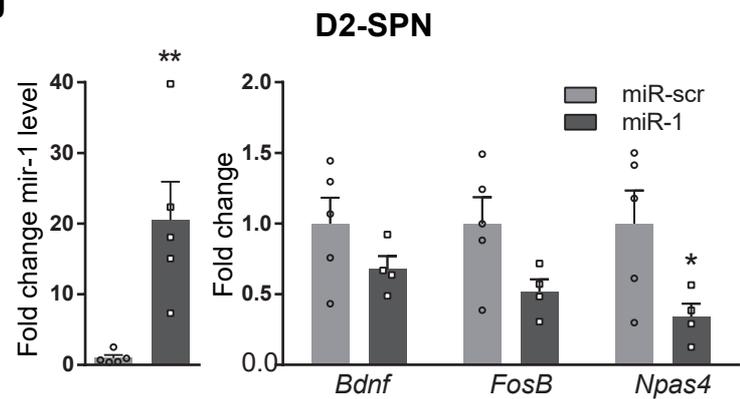


Figure 4, Forget et al.

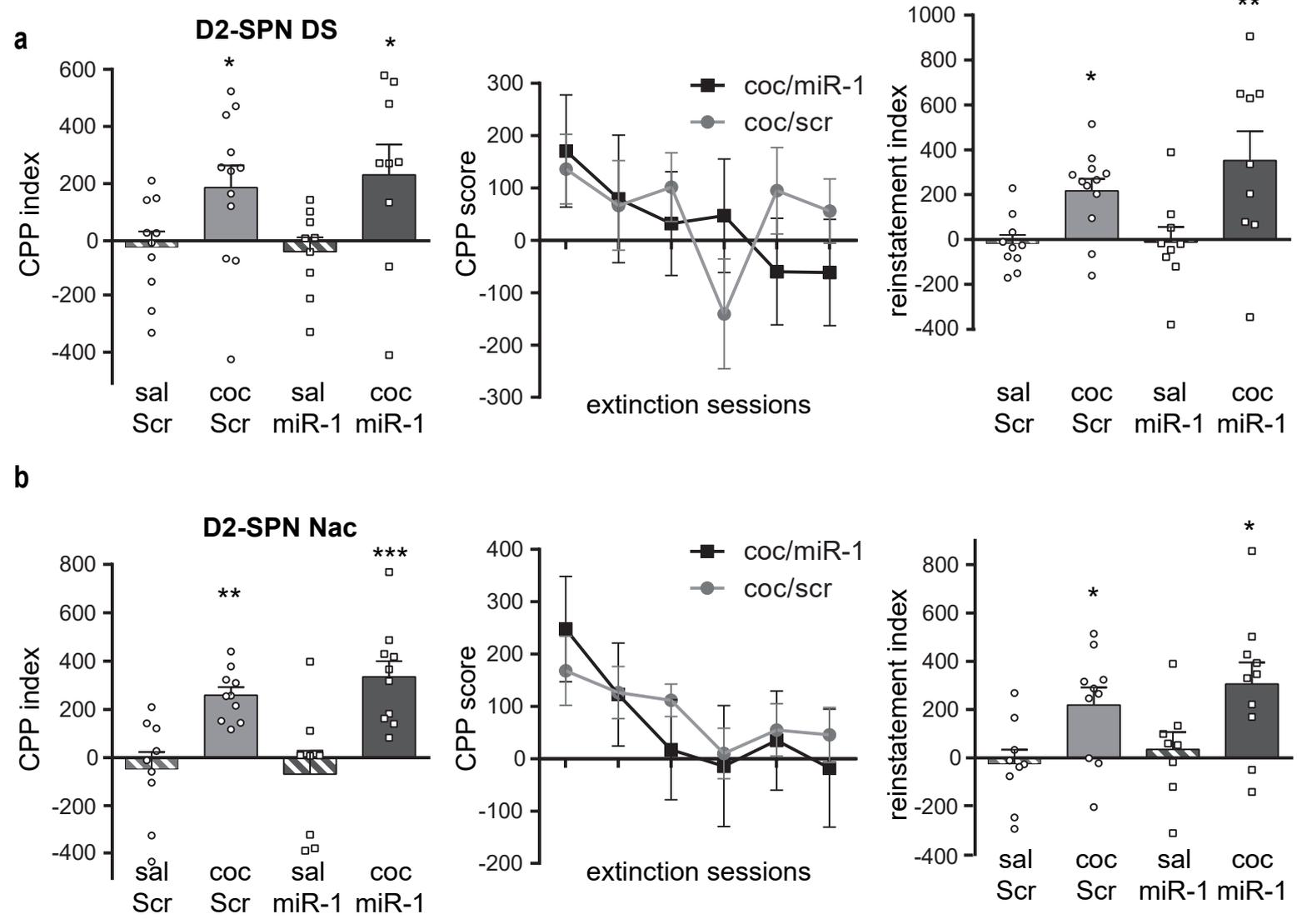


Figure 5, Forget et al.

SPN-subtype specific MiR-1 over-expression on cocaine -SA

D1-SPNs

D2-SPNs

