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ORIGINAL ARTICLE

Distinct miRNA expression in dorsal striatal subregions is associated with risk for addiction in rats

RK Quinn[1,2,3], AL Brown[1,2,3], BJ Goldie[1,2,3], EM Levi[1,2,3], PW Dickson[1,2,3], DW Smith[1,2,3], MJ Cairns[1,2,3] and CV Dayas[1,2,3]

Recently, we published data using an animal model that allowed us to characterize animals into two groups, addiction vulnerable and addiction resilient, where we identified that addiction/relapse vulnerability was associated with deficits in synaptic plasticity-associated gene expression in the dorsal striatum (DS). Notable was the strong reduction in expression for activity-regulated cytoskeleton-associated protein (*Arc*) considered a master regulator of synaptic plasticity. In the present study, we confirmed that *Arc* messenger RNA was significantly decreased in the DS, but importantly, we identified that this reduction was restricted to the dorsomedial (DMS) and not dorsolateral striatum (DLS). There is recent evidence of microRNA (miRNA)-associated posttranscriptional suppression of *Arc* and animal models of addiction have identified a key role for miRNA in the regulation of addiction-relevant genes. In further support of this link, we identified several differentially expressed miRNA with the potential to influence addiction-relevant plasticity genes, including *Arc*. A key study recently reported that miR-212 expression is protective against compulsive cocaine-seeking. Supporting this hypothesis, we found that miR-212 expression was significantly reduced in the DMS but not DLS of addiction-vulnerable animals. Together, our data provide strong evidence that miRNA promote ongoing plasticity deficits in the DS of addiction-vulnerable animals.

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INTRODUCTION

A key feature of addiction is the loss of behavioral control over drug taking.[1] A prominent hypothesis that has been proposed to explain this phenomenon is devolution of control from brain areas involved in goal-directed decision making to those involved in habitual behaviors.[1] One of the brain regions strongly linked with the development and expression of habits is the dorsal striatum (DS).[2–5] However, the cellular and molecular adaptations that occur within the DS have received far less attention than those that occur in the nucleus accumbens (NAc) of the ventral striatum. Accordingly, we recently investigated gene expression profiles for key synaptic plasticity molecules in the DS of animals trained to self-administer cocaine and screened for expression of compulsive drug-seeking traits, including reinstatement—a rodent analog of human relapse.[6] This model allowed us to characterize animals into two groups at opposite ends of the addition vulnerability spectrum.[6,7] Using this approach, we observed decreased expression of synaptic plasticity-associated genes in the DS,[6] including the activity-regulated cytoskeleton-associated protein (*Arc*). These changes are consistent with reports that chronic cocaine-taking leads to a loss of plasticity at excitatory synapses in the striatum, albeit in the NAc,[8–10] and the role for *Arc* as a master regulator of synaptic plasticity.[11]

Importantly, while the DS is a key region involved in the formation of habits, this role appears to be restricted to the dorsolateral division (DLS).[4,12] In fact, a clear functional segregation exists between the DLS and dorsomedial striatum (DMS), which is implicated in goal-directed decision making.[5,13] This functional heterogeneity has significant implications for understanding how decision-making processes become disrupted in neuropsychiatric conditions. However, few studies have assessed how addiction-relevant changes in gene expression in the DS might be sustained. Such information may be relevant for explaining the behavioral switch that appears to drive compulsive drug seeking in addiction.[1,14–17]

One level of molecular control responsible for sustaining addiction-relevant reductions in synaptic plasticity gene expression are microRNA (miRNA), short, noncoding RNA molecules that posttranscriptionally regulate messenger RNA (mRNA).[18] Several miRNA have been implicated in promoting addiction-relevant behaviors. For example, altered expression of miR-181a, let-7a and miR-124 are implicated in the regulation of cocaine-induced conditioned place preference.[19] In a key study, Kenny and colleagues showed that the expression of miR-212 was significantly increased in the DS of rats that self-administer cocaine over an extended period of drug access.[16] Importantly, overexpression of miR-212 significantly reduced cocaine-taking, whereas its knockdown had the opposite effect.[16] These findings suggest that the expression of miR-212 is increased following protracted drug taking and possibly acts as a homeostatic control to protect against further cocaine-induced plasticity.[16] Importantly, in these previous studies, manipulation of miRNA expression was not restricted to DS subregions. Furthermore, our model allows us to behaviorally separate addiction-vulnerable from resilient animals, despite consuming similar levels of cocaine. On the basis of the evidence above, we predict that miR-212 expression should be decreased in the DMS in vulnerable versus resilient animals owing to the importance of this region in the regulation of goal-directed behavior. Critically, the animals used in this study have been denied access to drug for a period of up to 8 weeks. As such, any

[1]Neurobiology of Addiction Laboratory, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia; [2]The Centre for Translational Neuroscience and Mental Health Research, University of Newcastle, Newcastle, NSW, Australia and [3]Hunter Medical Research Institute, Newcastle, NSW, Australia. Correspondence: Dr CV Dayas, School of Biomedical Sciences and Pharmacy, University of Newcastle and the Hunter Medical Research Institute, Newcastle, NSW 2308, Australia. E-mail: Christopher.Dayas@newcastle.edu.au

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changes we observed likely reflect long-term neuroadaptions that may contribute to an increased propensity to relapse.

Accordingly, the primary purpose of the present study was to assess the possible contribution of miRNA to the altered expression of *Arc* detected in our previous study in the DMS and DLS subregions of the DS.[6] We also assessed changes in miRNA identified using pathway analysis as having an involvement in long-term depression (LTD) and potentiation (LTP) signaling pathways.[20,21] Finally, we addressed the hypothesis that addiction/relapse-vulnerable animals display deficits in miR-212 expression in the DMS.

MATERIALS AND METHODS

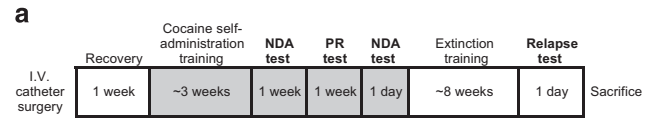
Tissue samples

Tissue samples ($n = 6$ per group) were obtained from animals previously phenotyped as addiction/relapse-vulnerable or resilient as described in detail in Brown *et al*.[6] Briefly, the animals were trained to self-administer cocaine (0.25 mg per 0.1 ml intravenously) for 3 h per day for ~5 weeks, during which time they were tested for three addiction-relevant behaviors: inability to refrain from drug seeking during a period of non-drug availability, motivation to consume drug using repeated progressive ratio tests and cue-induced reinstatement of drug-seeking (Figure 1). The animals were killed 24 h after reinstatement testing. Animals scoring in the top 40% of the distribution for reinstatement as well as the top 30% of the distribution for the remaining behaviors were phenotyped as addiction vulnerable, whereas those in the bottom of the distribution were phenotyped as addiction resilient.

Tissue dissection

The current study was performed on fresh dissections (opposite hemisphere) of DMS and DLS of rats previously phenotyped as vulnerable versus resilient.[6] Tissue for quantitative PCR and western blot analyses were macrodissected from 100 µm or 400 µm coronal sections, respectively, made on a Cryostat (Leica Biosystems CM1900, North Ryde, NSW, Australia) using 0.8–1 mm^2 diameter tissue punches (bregma levels 2.52 to 0.96).

a



b

Behavior	Vulnerable (mean ±SEM)	Resilient (mean ±SEM)	*P* value
NDA responding	150.1 ±47.88	8.1 ±1.26	0.008
PR breakpoint	222.9 ±18.63	46.9 ±7.76	<0.001
Reinstatement	122.7 ±9.29	35 ±8.65	<0.001

Figure 1. Experimental timeline for phenotyping of addiction vulnerable and resilient groups. (**a**) To phenotype animals into addiction vulnerable or resilient groups, animals were first implanted with an intravenous (i.v.) catheter and trained to self-administer cocaine (0.25 mg per 0.1 ml intravenously) on an FR1 schedule of responding which progressed to an FR3 and finally an FR5 schedule of responding. Animals were then tested for inability to refrain from drug seeking during periods of signalled drug non-availability (NDA) for 5 days. Following this, animals were tested for motivation to consume drug using a progressive ratio test, followed by a final FR5 cocaine session. Drug-seeking behavior was then extinguished by exposing animals to the operant chamber. Drug was absent and lever pressing did not result in a drug reward. Once responding returned to baseline levels, animals were re-exposed to cues associated with drug availability in a reinstatement test and killed 24 h later. Animals that scored in the top third of the distribution for each behavioral test were deemed to be addiction vulnerable, whereas those in the bottom third of the distribution were identified as addiction resilient. For detailed description of behavioral training and phenotyping, see Brown *et al*. (2010). (**b**) Animals phenotyped as addiction vulnerable showed significantly higher NDA responding, PR breakpoint and reinstatement scores that animals phenotyped as addiction resilient.

RNA extraction

Total RNA was extracted using QIAGEN miRNeasy Mini Kit (QIAGEN, Venlo, Netherlands) according to the manufacturer's instructions. Concentrations of RNA were determined using a Nanophotometer (Implen, Munich, Germany).

Bioinformatics analysis of miRNA interactions

The computational algorithms miRanda (http://www.microrna.org/) and TargetScan (http://www.targetscan.org/) were used to identify miRNA regulators of previously identified dysregulated synaptic plasticity genes. To identify candidate miRNA involved in LTD and LTP, we used Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA, USA).[22]

Reverse transcription and quantitative PCR

For mRNA expression analysis, 150–450 ng total mRNA was reverse transcribed using Superscript III reverse transcriptase and $oligo_{(dT)}$ primers according to manufacturer's instructions. miRNA reverse transcription was performed on 150 ng of RNA treated with DNase-1 (Invitrogen, Mulgrave, VIC, Australia). Reactions were performed using Superscript II with miRNA-specific primers in a pooled reverse transcription mix as previously described.[23,24]

Quantitative PCR reactions were performed essentially as described.[6] mRNA expression was analyzed with respect to the geometric mean of GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and 18S. Relative miRNA expression was compared with the housekeeper β-actin (ΔC_t). $\Delta\Delta C_t$ method was used to compare expression between addiction resilient and vulnerable cohorts.

Protein extraction

Following macrodissection, tissue was stored at −80 °C until required. 100 µl of homogenizing buffer (50 mM Tris/HCl pH 7.5, 1 mM EGTA, 1 × complete protease inhibitor cocktail tablet, 1 mM DTT, 80 µM ammonium molybate, 1 mM sodium pyrophosphate, 5 mM β-glycerophosphate, 1 mM sodium orthovandate, 2 µM microcystin, final concentration) was added and tissue sonicated for 3 × 10 s pulses at 4 °C using a microsonicator (UP50H, Hielscher Ultrasonics GmbH, Teltow, Germany). 10% SDS was added to a final concentration of 2.5% and the samples were boiled for 5 min, then centrifuged at 15 000 r.p.m. for 10 min at 25 °C. Supernatants were collected and the protein concentration determined using Pierce BCA assay (Thermo Fisher Scientific, Scoresby, VIC, Australia) according to the manufacturer's instructions. The samples were stored at −80 °C until required.

Western blot

Western blotting was performed essentially as previously described.[25] 15µg of protein sample was mixed with sample buffer (1% SDS, 10% glycerol, 0.5% DTT, 0.1% bromphenol blue, final concentration) and subjected to SDS–polyacrylamide gel electrophoresis before being transferred to nitrocellulose (Hybond ECL, GE Healthcare, Rydalmere, NSW, Australia). Nitrocellulose membranes were stained with Ponceau S (0.5% Ponceau in 1% acetic acid) to assess the efficacy of the transfer. The membranes were then washed in TBST (Tris-buffered saline with Tween) (150 mM sodium chloride, 10 mM Tris, 0.075% Tween-20, pH 7.5) and blocked in 5% skimmed milk powder in TBST for 1 h at 25 °C. The membranes were washed in TBST and incubated with anti-Arc (1:2000 Synaptic Systems #156002), overnight at 4 °C. The membranes were washed in TBST and incubated with horse-radish peroxidase-linked anti-IgG secondary specific antibodies for 1 h at 25 °C. The membranes were visualized on Fujifilm Las-3000 imaging system (Fuji, Stamford, CT, USA) using Luminata Forte Western HRP substrate (Millipore, Billerica, MA, USA). The density of bands was measured using a MultiGauge V3.0 (Fuji). *Arc* protein levels were normalized to β-actin. All the results are expressed as a fold change relative to the addiction/relapse resilient group.

Luciferase reporter assay

To validate miR-431 regulation of *Arc*, the miRNA-recognition element from 3′-UTR of *Arc* was cloned into pMIR-REPORT Luciferase miRNA Reporter

Vector (Ambion, Mulgrave, VIC, Australia) according to manufacturer's instructions. Reporter gene transfections and assays were performed essentially as described.[26,27] Briefly, HEK-293 cells were co-transfected with 4 ng of reporter construct, 20 ng of pRL-TK renilla luciferase construct and 100 nM chemically modified antisense (AS) inhibitor. The Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to measure luciferase activity on a BioTek Synergy 2 plate reader. The ability of each AS inhibitor to bind to the miRNA and thus prevent repression of reporter miRNA-recognition element was determined using the ratio of firefly luciferase activity to Renilla luciferase activity (transfection control). The data were normalized to negative controls.

Statistical analyses

Two-tailed independent sample *t*-tests were used to analyze miRNA and luciferase assay data. The Mann–Whitney nonparametric *U*-test was conducted for data that violated the assumptions. An alpha value of 0.05 was adopted for all the tests. Statistics were conducted using IBM SPSS v19 (IBM, Armonk, NY, USA).

RESULTS

Behavioral phenotyping of addiction vulnerable versus resilient

Behavioral data from the animals used in this study have been published previously.[6] Briefly, animals were trained to self-administer cocaine and tested for three addiction-relevant behaviors: non-drug availability responding, progressive ratio breakpoint and cue-induced reinstatement of drug-seeking (Figure 1). Animals that scored in the top or bottom 40% of the distribution for reinstatement, and the top or bottom 30% for the two remaining behaviors were phenotyped as addiction/relapse-vulnerable or resilient, respectively.[6]

Analysis of Arc mRNA and protein in the DMS and DLS in addiction-vulnerable versus resilient animals

We have previously shown that the synaptic plasticity related gene *Arc* is significantly downregulated in the DS of addiction-vulnerable versus resilient rats.[6] To extend these findings, we examined the expression of *Arc* within the DMS and DLS. We found that *Arc* mRNA was significantly decreased in the DMS of animals phenotyped as addiction vulnerable versus resilient ($t_9 = 3.845$, $P = 0.004$, Figure 2), with no significant change detected in the DLS ($P > 0.05$). Interestingly, *Arc* protein was significantly decreased in both the DMS ($t_{10} = 3.295$, $P = 0.008$) and DLS ($t_{10} = 2.88$, $P = 0.01$).

Identification of candidate miRNA targeting Arc and synaptic plasticity-associated genes

To identify miRNA with the potential to regulate *Arc*[21,28] we used the miRNA-target prediction algorithms miRanda and TargetScan. This approach identified miR-431 and miR-221 as potential regulators of *Arc* mRNA.

In our previous work, we identified a general pattern of downregulated gene expression consistent with deficits in the ability to evoke synaptic plasticity. Further, dysregulated striatal LTP and LTD is thought to be a hallmark of addiction in experimental models.[8,29] Therefore, we used Ingenuity Pathway Analysis to identify candidate miRNA involved in regulation of genes in the LTP and LTD signaling pathways. Using this approach, we identified several miRNA, including miR-181a, miR-212, miR-132, miR-101b, miR-222, miR-342-5p, miR-382, miR-495, miR-7a, miR-708 and miR-99a, which are putative regulators of genes within the LTP and LTD pathways (Figure 3).

Analysis of Arc-relevant miRNA expression in the DMS and DLS of addiction-vulnerable versus resilient animals

After predicting a potential relationship between *Arc* transcript and the expression of miR-431 and miR-221, we investigated their expression in both the DLS and DMS subregion dissections. Interestingly, miR-431 expression was significantly increased in both the DMS ($t_{10} = 2.168$, $P = 0.05$) and DLS ($t_{10} = 2.71$, $P = 0.02$) of addiction-vulnerable compared with resilient rats (Figure 4). No changes in miR-221 expression were observed in the DMS ($P = 0.07$) or DLS ($P > 0.05$) between the addiction vulnerability groups.

To validate a potential functional interaction between miR-431 and *Arc,* we used a luciferase reporter assay. Relative luciferase activity from the construct containing the *Arc* miRNA-recognition element was increased by 17% when transfected with AS-431 compared with AS control ($t_{14} = 3.539$, $P = 0.003$, Figure 5). These data suggest that miR-431 has the capacity to regulate its cognate recognition elements in *Arc*.

Analysis of LTD- and LTP-associated miRNA expression in addiction-vulnerable versus resilient animals

Ingenuity Pathway Analysis was used to identify candidate miRNA within LTD and LTP pathways. We then used the quantitative PCR to analyze the expression of selected miRNAs (Figure 4) in the DMS and DLS of addiction-vulnerable versus resilient animals. miR-101b expression was significantly increased in the DMS (Mann–Whitney *U*-test = 6.00, $P = 0.05$) and DLS (Mann–Whitney *U*-test = 2.00, $P = 0.01$) of addiction-vulnerable animals. miR-181a was

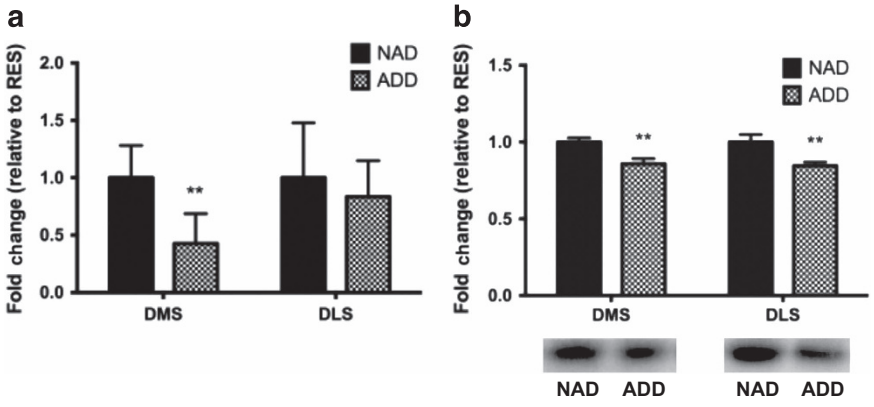


Figure 2. Changes in *Arc* expression in the DS subregions of addiction vulnerable animals. (**a**) Animals phenotyped as addiction vulnerable displayed altered Arc mRNA expression in the DMS but not DLS compared with addiction resilient controls. (**b**) *Arc* protein was significantly decreased in both the DMS and DLS subregions of addiction vulnerable versus resilient animals. *$P < 0.05$; **$P < 0.01$. n=6 per group. DLS, dorsolateral striatum; DMS, dorsomedial striatum; DS, dorsal striatum; mRNA, messenger RNA.

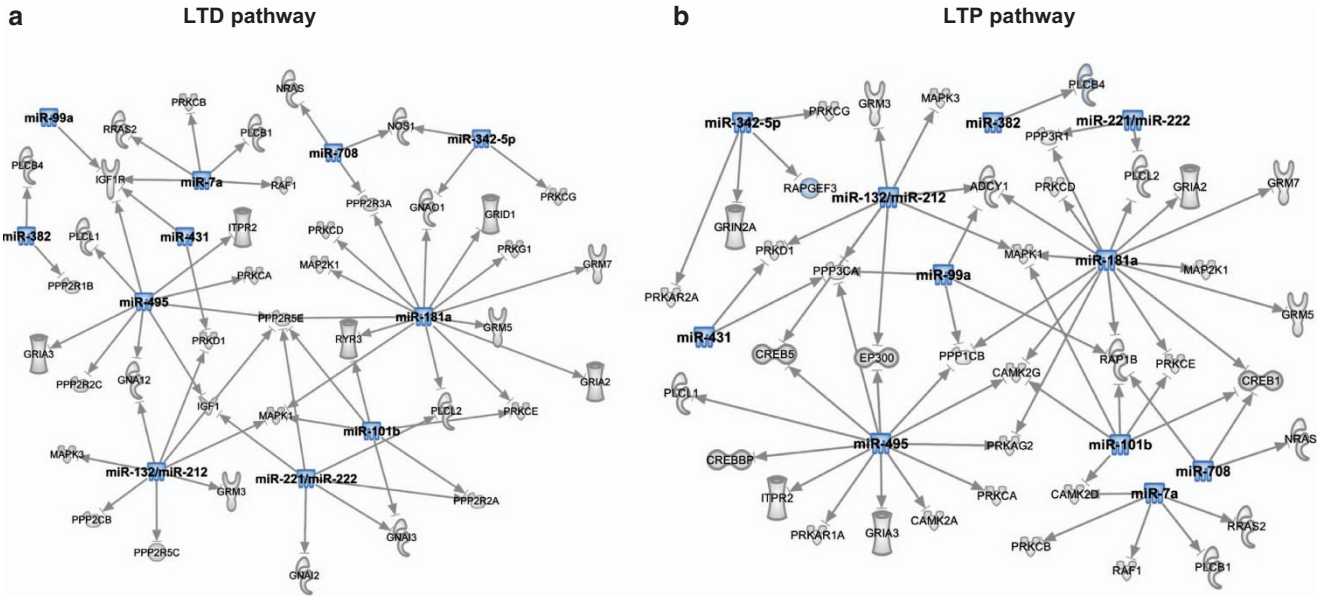


Figure 3. Predicted interactions between miRNA and mRNA targets within addiction relevant signaling pathways. IPA was used to identify miRNA involved in regulation of genes within synaptic plasticity-associated signaling pathways. Putative interactions are shown between miRNA and mRNA targets within the synaptic LTD (**a**) and synaptic LTP (**b**) pathways. IPA, ingenuity pathway analysis; LTD, long-term depression; LTP, long-term potentiation; mRNA, messenger RNA; miRNA, microRNA.

increased in the DLS ($t_{10} = 2.735$, $P = 0.02$) but not DMS of addiction-vulnerable animals. Interestingly, this miRNA has previously been shown to be altered in a number of brain regions, including the NAc, following cocaine exposure.[19,30] miR-708 was significantly increased in the DLS (Mann–Whitney *U*-test = 5.00, $P = 0.03$) but not DMS ($P > 0.05$) of addiction-vulnerable animals.

The expression of miR-222, miR-342-5p, miR-382, miR-495, miR-99a and miR-7a was not altered between phenotyped groups ($P > 0.05$) in either the DMS or DLS.

We also predicted that addiction-vulnerable animals would display reduced expression for miR-212 expression in the DMS compared with resilient animals, consistent with the protective role miR-212 has been shown to have in controlling cocaine consumption.[16,17] Consistent with this hypothesis, addiction-vulnerable animals displayed significantly reduced DMS miR-212 expression ($t_{10} = 2.876$, $P = 0.01$) but no significant changes were observed in the DLS ($P = 0.07$). Of note, the expression of the closely related miR-132 was significantly increased in the DLS ($t_{10} = 2.208$, $P = 0.05$), but not the DMS.

DISCUSSION

In this study, we examined the role of miRNA in the regulation of specific synaptic plasticity genes and signaling pathways associated with addiction/relapse vulnerability. We identified several miRNA in the DMS and DLS of addiction-vulnerable animals with the potential to regulate genes within LTP and LTD pathways including *Arc,* a 'master' regulator of plasticity. We also identified a pattern of miR-212 expression consistent with the hypothesis that loss of function of this miRNA in the DMS leads to compulsive drug-seeking and relapse risk.

miRNA control of Arc expression and relevance to addiction

In previous work, we observed that *Arc* expression was reduced in the DS of addiction-vulnerable animals,[6] however, when we investigated subregion-specific transcript changes here, this effect was restricted to the DMS. Importantly, we also observed a significant decrease in *Arc* protein in the DMS. Surprisingly, DLS *Arc* protein was also decreased. The discrepancy between *Arc* mRNA and protein in the DLS may indicate temporal differences in *Arc* recruitment, translational control and transcript degradation or stability between DS subregions.[11] The decrease in *Arc* mRNA detected was at baseline (that is, 24 h after reinstatement testing), and is likely to have persisted for many weeks after cocaine-taking had ceased. We hypothesized that the long-lasting decrease in *Arc* observed in our previous work would be associated with upregulated expression of miRNA that can bind to 3-prime end of this gene. Using bioinformatics, we identified miR-431 as a potential candidate for regulation of *Arc*. The expression of this miRNA was increased in the DMS and DLS of addiction-vulnerable animals. Further, we demonstrated using luciferase assays that miR-431 does regulate *Arc* expression *in vitro*. Thus, we predict that the dysregulation of *Arc* synthesis may have resulted in ongoing deficits in striatal plasticity and act as a molecular mediator of brain addiction processes.

Interestingly, other studies have also identified addiction-relevant changes in *Arc* expression. However, in contrast to the data presented here, Hearing *et al.*[31] found that *Arc* mRNA was increased in both the DMS and DLS of animals re-exposed to a cocaine-paired environment. These differences may be due to the different time point that brains were harvested or the use of forced abstinence model versus extinction of drug-seeking used in our model. In our study, we collected brains 24 h after reinstatement testing, whereas Hearing *et al.*[31] harvested tissue immediately after testing. Another possible factor is that our phenotyped groups did not differ in the levels of cocaine consumed, thereby controlling for the direct action of cocaine on *Arc* expression. Thus, the increase in *Arc* reported in previous studies could be due to pharmacological effects of cocaine. Interestingly, a subsequent study by the same group demonstrated that inhibition of *Arc* in the DLS did not alter drug-seeking during a context test. However, although the response of control animals decreased during subsequent extinction tests, inhibition of *Arc* in the DLS prevented this decrease in responding.[32] Despite these differences, both data sets implicate *Arc* recruitment and dysregulated signaling in the addiction process.

Exactly how loss of *Arc* function might lead to the dysregulation of synaptic plasticity in the DMS and contribute to compulsive

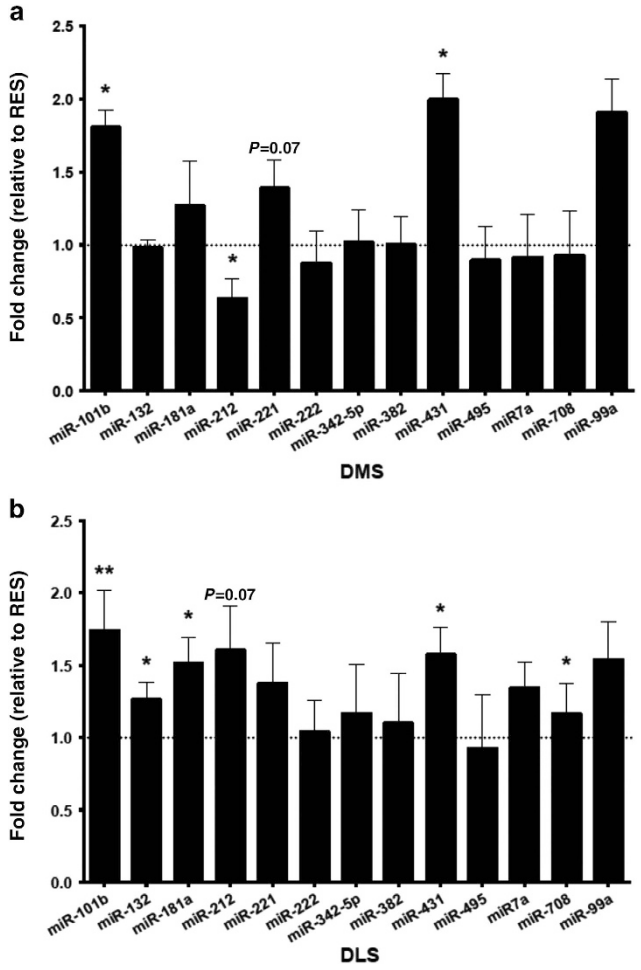


Figure 4. Changes in miRNA expression in addiction vulnerable versus resilient rats. Animals phenotyped as addiction vulnerable displayed altered miRNA expression profiles in both the DMS (**a**) and DLS (**b**) compared with addiction resilient controls. $^{*}P < 0.05$; $^{**}P < 0.01$. n=6 per group. DLS, dorsolateral striatum; DMS, dorsomedial striatum; miRNA, microRNA.

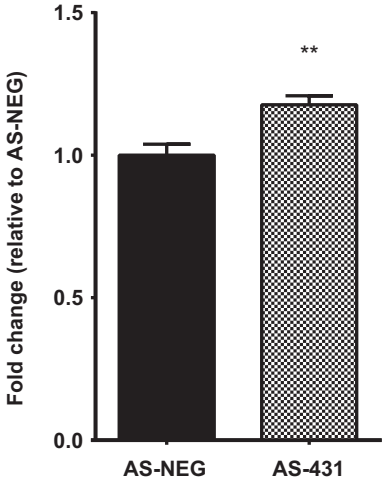


Figure 5. Luciferase reporter gene expression assay. AS-431 increased luciferase activity when co-transfected in HEK-293 cells with Arc MRE luciferase construct, suggesting miR-431 has the ability to regulate Arc expression *in vitro*. $^{*}P < 0.05$; $^{**}P < 0.01$.

drug-seeking is unclear. *Arc* is trafficked to activated synapses, translated into protein[33] and can promote both synaptic strengthening and weakening.[11] *Arc*-induced modulation of LTP is thought to occur through F-actin-mediated enhancement of AMPAR GluA trafficking, postsynaptic density remodeling and localization of translation machinery.[11,34] Modulation of LTP by *Arc* appears to be mediated by the recruitment of dynamin and endophilin 2/3,[34] promoting the internalization of *GluA1* AMPARs. Interestingly *Arc*-dependent LTD has been shown to require activation of Group I mGluRs, and dysregulated signaling through *Grm5* contributes to the expression of addiction-relevant behaviors.[35,36] Knockout of *Arc* expression in the hippocampus has been shown to impair memory formation.[37] Given the role of *Arc* in synaptic activation and memory formation, it is possible that suppressed *Arc* transcription in the DMS may negatively impact goal-directed decision-making process. By default, these changes may result in a predominance of habit-relevant neuroadaptations in the DLS, which manifests as a state of behavioral inflexibility. Given that *Arc* is an immediate early response gene, persistent deficits in *Arc* expression may alter the effects of novelty and impair the formation of new memories that allow the individual to adapt to changes in the value of the drug and contribute to the persistent nature of addiction.

Importantly, vulnerable versus resilient animals did not exhibit differences in days to reach the extinction criterion,[6] suggesting that there were no major deficits in extinction learning. It is possible, however, that dysregulated striatal plasticity might still result in a more subtle failure to learn that cocaine cues no longer predict drug reward. Interestingly, a persistent state of 'anaplasticity' has been reported in the NAc of addiction-vulnerable animals, whereas in resilient cocaine-taking rats this plasticity recovered.[8] These findings are consistent with several other important studies reporting impairments in the ability to evoke LTD and LTP at excitatory synapses in the NAc of animals that have had a significant history of cocaine self-administration.[29,38] Interestingly, a recent study by Corbit *et al*.[39] demonstrated that cocaine exposure led to a more rapid shift in behavioral control from the DMS to DLS. This study found that animals exposed to cocaine that were subsequently trained to self-administer food rewards became insensitive to outcome devaluation more rapidly than saline controls. Further, they showed that cocaine exposure altered glutamatergic transmission only in the DMS with no effect seen in the DLS. These results align with the hypothesis that synaptic plasticity impairments in addiction-vulnerable animals lead to a premature shift in behavioral control from goal-directed to habitual.

Decreased miR-212 expression in the DMS of addiction-vulnerable versus resilient animals

miR-212 is the best characterized miRNA with respect to compulsive drug taking and addiction. Hollander *et al*.[16] showed that lenti-viral-mediated overexpression of miR-212 in the DS decreased cocaine consumption, whereas its knockdown had the opposite effect. Furthermore, they showed that miR-212 regulated compulsive cocaine consumption through a complex homeostatic interaction with *MeCP2* and *BDNF*.[17] Thus, overexpression of miR-212, which suppressed cocaine consumption, led to a decrease in *MeCP2* and *BDNF*.[17] These findings accord well with data demonstrating that increased striatal *BDNF* helps to promote drug-seeking behaviors.[40,41] Consistent with the hypothesis that miR-212 negatively regulates and is protective against cocaine-taking, we found that miR-212 expression was significantly decreased in the DMS of addiction-vulnerable animals but there was no significant change in DLS miR-212 expression.[16] This disparity is interesting given the functionally distinct roles of these subregions. We speculate that the decrease in miR-212 observed in the DMS may lead to a cascade of signaling changes that shifts

the balance of DS control over behavioral responding to the DLS. Together, these data support a role for miR-212 in addiction-relevant neuroplasticity but also identify a subregion or temporal specificity in the actions of miR-212 in the DS.

Expression of LTP- and LTD-associated miRNA in the DMS and DLS of addiction-vulnerable animals

Pathway analysis identified a number of candidate miRNAs with the potential to influence the expression of genes associated with LTP and LTD signaling pathways. For example, miR-101b, which was significantly increased in both the DLS and DMS, is predicted to target *MAPK1, PRKC, PP2a* and genes encoding the guanine nucleotide-binding proteins. miR-181a was significantly increased in the DLS of addiction-vulnerable animals and pathway analyses predict potential interactions with molecules linked with alterations in synaptic plasticity including the Group 1 metabotropic glutamate receptor *Grm5* and calcium impermeable AMPARs (*GluA2*). The expression of miR-431 was increased up to twofold in DS subregions and has been shown to decrease *BRAF* expression *in vitro*.[42] The serine/threonine protein kinase encoded by *BRAF* regulates the MAP kinase/ERKs signaling pathway.[43] miR-181a has previously been linked with cocaine-related addiction behavior. For example, silencing of miR-181a expression increased the rate of extinction of cocaine-induced conditioned place preference, effects that were accompanied by decreased dopamine receptor 3 and *MeCP2* expression in the NAc.[19] Notably, the homeostatic interaction between *MeCP2* and miR-212 is a key regulatory mechanism preventing runaway maladaptive changes in the striatum that can lead to compulsive drug-seeking.

CONCLUSIONS

A major clinical hurdle for addiction is the prolonged propensity for relapse, which can endure for many years. This suggests that underlying changes in brain circuits are also persistent and we have focused our attention on longer-term influences. It is important to reiterate that our measurements of gene and miRNA expression were made up to 8 weeks after drug exposure and extinction, and therefore likely reflect changes that underpin the more persistent aspects of addiction neurobiology. Using this approach, we have identified several addiction-relevant miRNA with the potential to regulate *Arc* and other synaptic plasticity genes, in the DMS and DLS of addiction/relapse-vulnerable animals. Importantly, we found subregion-specific changes in *Arc* expression focused in the DMS. We also provide new data to support the role for miR-212 in the neuroadapatations that promote addiction. Together our study has identified a number of miRNA that may contribute to the neuroadaptations that lead to the persistent risk of relapse associated with cocaine addiction. Our findings provide further support for proposals, which state that cocaine exposure promotes deficits in striatal synaptic plasticity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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