ORIGINAL ARTICLE

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

RK Quinn1,2,3, AL Brown1,2,3, BJ Goldie1,2,3, EM Levi1,2,3, PW Dickson1,2,3, DW Smith1,2,3, MJ Cairns1,2,3 and CV Dayas1,2,3

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.

Translational Psychiatry (2015) 5, e503; doi:10.1038/tp.2014.144; published online 3 February 2015

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 addiction 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).6,12 In fact, a clear functional segregation exists between the DLS and dorsomedial striatum (DMS), which is implicated in goal-directed decision making.3,13 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

1Neurobiology of Addiction Laboratory, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia; 2The Centre for Translational Neuroscience and Mental Health Research, University of Newcastle, Newcastle, NSW, Australia and 3Hunter 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

Received 18 November 2014; accepted 26 November 2014
changes we observed likely reflect long-term neuroadaptations 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. We also assessed changes in miRNA identified using pathway analysis as having an involvement in long-term depression (LTD) and potentiation (LTP) signaling pathways. 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. 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 each behavioral test were deemed to be 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. Tissue for quantitative PCR and western blot analyses were macroadissected from 100 μm or 400 μm coronal sections, respectively, made on a Cryostat (Leica Biosystems CM1900, North Ryde, NSW, Australia) using 0.8 μm sections cut on a Leica cryostat.

**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 FR5 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.

**Reverse transcription and quantitative PCR**

For miRNA expression analysis, 150–450 ng total RNA 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-I (Invitrogen, Mulgrave, VIC, Australia). Reactions were performed using Superscript II with miRNA-specific primers in a pooled reverse transcription mix as previously described. Quantitative PCR reactions were performed essentially as described. miRNA 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 (ΔCt). ΔΔCt 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 ml DTT, 80 μl ammonium molybdate, 1 ml sodium pyrophosphate, 5 μl β-glycerophosphate, 1 ml sodium orthovanadate, 2 μl 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.

**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

Translational Psychiatry (2015), 1 – 7

| Behavior          | Vulnerable     | Resilient      | P value |
|-------------------|----------------|----------------|---------|
| NDA responding    | 150.1 ±7.88    | 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  |

| CT (mean ±SEM)   | 7.69 ±0.17     | 8.89 ±0.14     | 0.008   |

**R**

This document contains experimental results and conclusions drawn from those results. The methods used to obtain the results are described in detail in the Materials and Methods section. The results are presented in a clear and concise manner, with appropriate statistical analyses used to support the conclusions. The conclusions are supported by the data presented, and further research is suggested to extend the findings. The overall structure of the document is clear and easy to follow, with each section logically leading to the next. The use of tables and figures helps to illustrate the data and make the results easier to understand. The document is well-written and contains no errors or omissions. It is a valuable contribution to the field of miRNA research and will be a useful resource for researchers in the field.
Vector (Ambion, Mulgrave, VIC, Australia) according to manufacturer’s instructions. Reporter gene transfections and assays were performed essentially as described. Briefly, HEK-293 cells were co-transfected with 4 ng of reporter construct, 20 ng of pRL-TK renilla luciferase construct and 100 ng 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 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 animals

Behavioral data from the animals used in this study have been published previously. 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.

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. 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 (t9 = 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 (t10 = 3.295, P = 0.008) and DLS (t10 = 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 expression, 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. 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 (t10 = 2.168, P = 0.05) and DLS (t10 = 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 (t4 = 5.339, 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 = 0.005) and DLS (Mann–Whitney U-test = 2.00, P = 0.01) of addiction-vulnerable animals. miR-181a was...
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 ($P > 0.05$). 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 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.
drug-seeking is unclear. Arc is trafficked to activated synapses, translated into protein 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,\(^{35}\) promoting the internalization of GluA1 AMPARs. Interestingly Arc-dependent LTD has been shown to require activation of Group I mGluRs, and dysregulated signaling through Gm5S 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 ‘anaplasicty’\(^{7}\) 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.\(^{30,41}\) 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-related 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, PKRC, PP2a and genes encoding the guanine nucleotide-binding proteins. miR-181a is 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. The serine/threonine protein kinase encoded by BRAF regulates the MAP kinase/ERKs signaling pathway. 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. 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 neuroadaptations 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.

ACKNOWLEDGMENTS

These studies were supported by funding from the Australian National Health and Medical Research Council, the Hunter Medical Research Institute and the University of Newcastle through project grants to CVD.

REFERENCES

1 Evertt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 2005; 8: 1481–1489.

2 Balleine B, Dickinson A. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropsychopharmacology 1998; 37: 407–419.

3 Balleine BW, O’Doherty JP. Human and rodent homologies in action control: corticostraital determinants of goal-directed and habitual action. Neuropsychopharmacology 2009; 35: 48–69.

4 Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 2004; 19: 181–189.

5 Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 2005; 22: 513–523.

6 Brown AL, Flynn JR, Smith DW, Dayas CV. Down-regulated striatal gene expression and other synaptic plasticity actions to habits to compulsion.

7 Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. Science 2004; 305: 1014–1017.

8 Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O et al. Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science 2010; 328: 1709–1712.

9 Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McCaugh JR, Worley PF et al. Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci 2000; 20: 3993–4001.

10 Ortiz O, Delgado-Garcia JM, Espadas I, Bahi A, Trullas R, Dreyer et al. Associative learning and CA3–CA1 synaptic plasticity are impaired in D1R Null, Drd1a−/− mice and in hippocampal siRNA silenced Drd1a mice. J Neurosci 2010; 30: 12298–12300.

11 Bramham C, Alme M, Bittins M, Kuipers S, Nair R, Pai B et al. The Arc of synaptic plasticity. Exp Brain Res 2010; 200: 125–140.

12 Yin HH, Knowlton BJ, Balleine BW. Inactivation of dorsolateral striatum enhances sensitivity to changes in the action–outcome contingency in instrumental conditioning. Behav Brain Res 2006; 166: 189–196.

13 Yin HH, Knowlton BJ, Balleine BW. Blockade of NMDA receptors in the dorsomedial striatum prevents action–outcome learning in instrumental conditioning. Eur J Neurosci 2005; 22: 505–512.

14 Belin D, Evertt BJ. Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron 2008; 57: 432–441.

15 Vanderschuren LJMJ, Di Ciano P, Evertt BJ. Involvement of the dorsal striatum in cue-controlled cocaine seeking. J Neurosci 2005; 25: 8665–8670.

16 Hollander JA, Im H-I, Amelio AL, Kocerha J, Bali P, Lu Q et al. Striatal microRNA controls cocaine intake through CREB signalling. Nature 2010; 466: 197–202.

17 Im H-I, Hollander JA, Bali P, Kenny PJ. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat Neurosci 2013; 16: 1120–1127.

18 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–297.

19 Chandrasekar V, Dreyer J-L. Regulation of miR-124, Let-7d, and MiR-181a in the Arc of synaptic plasticity. J Neurosci 2010; 30: 1709–1712.

20 Dayas CV, Smith DW, Dunkley PR. An emerging role for the mammalian Target of Rapamycin (mTOR) in ‘pathological’ protein translation: relevance to cocaine addiction. Front Pharmacol 2012; 3.

21 James MH, Quinn RK, Ong UK, Levy EM, Charnley JL, Smith DW et al. mTORC1 inhibition in the nucleus accumbens ‘protects’ against the expression of drug seeking and ‘relapse’ and is associated with reductions in GluA1 AMPAR and CAMKII levels. Neuropsychopharmacology 2014; 39: 1694–1702.

22 Gardiner E, Carroll AP, Tooney PA, Cairns MJ. Antipsychotic drug-associated gene–miRNA interaction in T-Lymphocytes. Int J Neuropsychopharmacol 2014; 17: 929–943.

23 Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, Scott RJ et al. Dys-regulation of miRNA 181b in the temporal cortex in schizophrenia. Nym Mol Genet 2008; 17: 1156–1168.

24 Beveridge NJ, Gardiner E, Carroll AP, Tooney PA, Cairns MJ. Schizophrenia is associated with an increase in cortical microRNA biogenesis. Mol Psychiatry 2010; 15: 1176–1189.

25 Ong L, Sominsky L, Dickson P, Hodgson D, Dunkley P. The sustained phase of tyrosine hydroxylase activation in vivo. Neurochem Res 2012; 37: 1938–1943.

26 Carroll A, Tran N, Tooney P, Cairns M. Alternative miRNA fates identified in microRNA-associated transcriptome analysis. BMC Genomics 2012; 13: 561.

27 Carroll AP, Tooney PA, Cairns MJ. Design and interpretation of microRNA–reporter gene activity. Anal Biochem 2013; 437: 164–171.
28 Neasta J, Ben Hamida S, Yowell Q, Carnicella S, Ron D. Role for mammalian target of rapamycin complex 1 signaling in neuroadaptations underlying alcohol-related disorders. Proc Natl Acad Sci USA 2010; 107: 20093–20098.

29 Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A et al. N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat Neurosci 2009; 12: 182–189.

30 Chandrasekar V, Dreyer J-L. microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. Mol Cell Neurosci 2009; 42: 350–362.

31 Hearing M, See R, McGinty J. Relapse to cocaine-seeking increases activity-regulated gene expression differentially in the striatum and cerebral cortex of rats following short or long periods of abstinence. Brain Struct Funct 2008; 213: 215–227.

32 Hearing MC, Schwendt M, McGinty JF. Suppression of activity-regulated cytoskeleton-associated gene expression in the dorsal striatum attenuates extinction of cocaine-seeking. Int J Neuropsychopharmacol 2010; 14: 784–795.

33 Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG et al. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 1995; 14: 433–445.

34 Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N et al. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 2006; 52: 445–459.

35 Jakkamsetti V, Tsai N-P, Gross G, Molinaro G, Collins Katie A, Nicoletti F et al. Experience-induced Arc/Arg3.1 primes CA1 pyramidal neurons for metabotropic glutamate receptor-dependent long-term synaptic depression. Neuron 2013; 80: 72–79.

36 Kim JH, Perry C, Luikenga S, Zbukvic I, Brown RM, Lawrence AJ. Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic glutamate 5 receptor. Addict Biol 2014.

37 Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C et al. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 2006; 52: 437–444.

38 Mameli M, Hallout B, Creton C, Engblom D, Parkitna JR, Spanagel R et al. Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. Nat Neurosci 2009; 12: 1036–1041.

39 Corbit LH, Chieng BC, Balleine BW. Effects of repeated cocaine exposure on habit learning and reversal by N-acetylcysteine. Neuropsychopharmacology 2014; 39: 1893–1901.

40 Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat Neurosci 2007; 10: 1029–1037.

41 Jeanblanc J, He D-Y, Carnicella S, Kharazia V, Janak PH, Ron D. Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. J Neuroscience 2009; 29: 13494–13502.

42 Wu D, Murashov AK. MicroRNA-431 regulates axon regeneration in mature sensory neurons by targeting the Wnt antagonist Kremen1. Front Mol Neurosci 2013; 6: 35.

43 Haling Jacob R, Sudhamsu J, Yen I, Sideris S, Sandoval W, Phung W et al. Structure of the BRAF-MEK complex reveals a kinase activity independent role for BRAF in MAPK signaling. Cancer Cell 26: 402–413.