Down-regulated striatal gene expression for synaptic plasticity-associated proteins in addiction and relapse vulnerable animals

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Abstract
Reducing the likelihood of relapse represents one of the greatest obstacles in the successful treatment of cocaine addiction. Dysregulation of the synaptic plasticity processes long-term potentiation (LTP) and long-term depression (LTD) is thought to be associated with protracted relapse risk. To improve our understanding of the molecular mechanisms contributing to relapse vulnerability we trained rats (n = 52) to self-administer cocaine and phenotyped animals as relapse-vulnerable or relapse-resilient using procedures adapted from Deroche-Gamonet et al. (Science 2004, 305, 1014–1017). Gene expression analysis, targeted at synaptic plasticity-related genes, revealed significant transcript down-regulation in the ventral and dorsal striatum of relapse-vulnerable animals compared to relapse-resilient controls. This included reduced expression of genes encoding proteins implicated in the dendritic translation of synaptic plasticity-related transcripts, the dynamic regulation and trafficking of ionotropic glutamate receptors important for LTP and LTD, along with neuronal surface receptors that initiate downstream signalling pathways associated with synaptic plasticity. Together, our data are consistent with recent reports of an inability to evoke LTD in the striatum of addiction-vulnerable rats. To our knowledge, this is the first study to demonstrate down-regulated synaptic plasticity-associated gene expression not only in the ventral striatum, where the majority of addiction-related synaptic plasticity studies have been conducted, but also in the dorsal striatum of animals categorized as relapse-vulnerable. As these neural correlates were elucidated using an approach incorporating individual behavioural differences, they potentially provide more relevant insight into addiction and assist the development of novel pharmacotherapies to treat relapse.

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Introduction
It is thought that the optimal treatment strategy to reduce relapse to cocaine taking involves a combination of behaviour and pharmacotherapies (Penberthy et al. 2010). Unfortunately, however, efficacious pharmacotherapies for cocaine addiction are presently lacking. This paucity probably stems from an inadequate understanding of the cellular and molecular mechanisms that contribute to long-term relapse risk. While progress has been made towards elucidating the molecular changes that occur in response to acute and chronic cocaine exposure (Dietz et al. 2009; Kalivas & O’Brien, 2008), only a few studies have attempted to identify molecular changes after protracted withdrawal (Freeman et al. 2008, 2010; Grimm et al. 2003; Hearing et al. 2008a, b), and even fewer have assessed what changes might actually subserve individual differences in relapse and addiction vulnerability (Dalley et al. 2007). Such studies are fundamental to understanding the neurobiological mechanisms responsible for this disease given the small percentage of cocaine users who become addicts (Anthony et al. 1994). Therefore, in the present study we have employed an animal model that assesses individual differences in the development of addiction...
vulnerability to identify molecular mechanisms that underpin relapse following protracted abstinence.

Several leading hypotheses regarding protracted relapse vulnerability propose that the addiction process co-opts the brain’s natural reward-learning pathways (Hyman, 2005; Hyman et al. 2006). Neuroadaptations within these pathways are hypothesized to promote lasting relapse risk by facilitating the associative learning process between drug-taking behaviour, euphoria, and environmental contexts (Hyman, 2005; Hyman et al. 2006), and by reducing the capacity of these same processes to learn and evoke new strategies to overcome addiction. Consistent with this suggestion, clinical and preclinical studies show that re-exposure to drug-linked contextual cues triggers drug-seeking behaviour and relapse (see Kalivas & O’Brien, 2008; Weiss, 2005 for review).

Cocaine exposure evokes the synaptic plasticity processes long-term potentiation (LTP) and depression (LTD) in ventral tegmental area (VTA) dopamine neurons (Fu et al. 2007; Ungless et al. 2001). Furthermore, persistent potentiation of VTA dopamine neurons that occurs after multiple cocaine exposures results in drug-related synaptic plasticity changes in the nucleus accumbens (NAc) (Kourrich et al. 2007; Mameli et al. 2009). It is thought that these processes may result in an over-learning of drug-cue associations. However, after prolonged cocaine exposure dysregulation of glutamate homeostasis, particularly between corticostriatal circuits, is thought to impair the ability to evoke LTP and LTD in NAc (Anderson et al. 2008; Kalivas, 2009; Mameli et al. 2009; Moussawi et al. 2009; Wolf & Ferrario, 2010) – effects that are hypothesized to impede the learning of new strategies to overcome addiction by producing behavioural inflexibility (Martin et al. 2006). Of particular relevance to the present study is that an enduring impairment in the ability to induce synaptic plasticity was recently found to occur within the NAc of animals behaviourally phenotyped as addiction-vulnerable but not addiction-resilient, despite both groups consuming similar amounts of cocaine (Kasanetz et al. 2010). To date no studies have used the behavioural approach taken by Kasanetz et al. (2010) to identify dysregulated gene expression potentially responsible for protracted relapse vulnerability (Belin et al. 2009; Deroche-Gamonet et al. 2004; Kasanetz et al. 2010). Therefore, we have employed a modified version of this model to phenotype animals as either addiction and relapse-vulnerable or addiction and relapse-resilient. Importantly in these previous reports, phenotyped groups displayed indistinguishable levels of cocaine consumption allowing an evaluation of neuroadaptations independent of the direct effects of cocaine exposure. We then used quantitative PCR (qPCR) to examine mRNA transcript changes within the dorsal and ventral striatum, brain regions considered important in the development and maintenance of addiction and relapse (Everitt & Robbins, 2005). Our main focus was centred on genes that encode proteins implicated in synaptic plasticity and cocaine-induced neuroadaptations including: D₁ and D₂ dopamine receptors – activation of dopamine receptors in the striatum has been shown to induce LTP and LTD (Calabresi et al. 1999; Centonze et al. 2001); ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors – not only crucial to the processes of LTP and LTD (Contractor & Heinemann, 2002), as there are now a significant number of studies demonstrating that relapse-like behaviour can be modified through altered subunit composition and localization of these receptors (Anderson et al. 2008; Conrad et al. 2008; Cornish & Kalivas, 2000; Sutton et al. 2003; Zavala et al. 2007); and group I metabotropic glutamate receptors – which have a known role in cocaine-induced synaptic changes (Moussawi et al. 2009). The expression of genes for the mammalian target of rapamycin (mTOR) and activity-regulated cytoskeletal (Arc) protein were also assessed – both are involved dendritic protein translation, including regulation of AMPA receptor (AMPAR) subunit expression – as well as protein kinase C (PKC), and CGMP-dependent protein kinase II (Bramham et al. 2010; Hoefffer & Klann, 2010; Luu & Malenko, 2008; Serulle et al. 2007). In addition, changes in the expression of the transcript encoding the upstream regulator of mTOR, phosphatidylinositol 3-kinase (PI3K), was also determined (Dietz et al. 2009). We hypothesized that, compared to relapse-resilient controls, a significant down-regulation of synaptic plasticity-associated gene transcripts would be seen in the striatum of animals categorized as relapse-vulnerable.

Materials and methods

Subjects

Male Sprague-Dawley rats (University of Newcastle, Australia; weighing 200–250 g upon arrival) were housed two per cage in a temperature- and humidity-controlled environment under a reversed 12-h light/dark cycle (lights off 07:00 hours) with food and water available ad libitum. All procedures were performed in strict accordance with protocols approved by the University of Newcastle Animal Care and Ethics
Committee, New South Wales Animal Research Act and Regulations, and the Australian Code of Practice for care and use of animals for scientific purposes.

**Drugs**

For cocaine self-administration (SA), cocaine hydrochloride (Johnson Matthey, UK) was dissolved in sterile physiological saline (2.5 mg/ml) according to a previous study (Martin-Fardon et al. 2009).

**Surgery**

Rats ($n=52$, 250–300 g) were anaesthetized with isofluorane (1–3% with a flow rate of 2 l/min) and, using aseptic procedures, a Silastic catheter was surgically implanted into the right jugular vein as described in detail previously (Caine et al. 1992). Prior to surgery, rats were injected intramuscularly with 0.3 ml of a broad spectrum antibiotic (150 mg/ml procaine penicillin, 112.5 mg/ml benzathine penicillin; Norbrook Laboratories, UK) and with 0.2 ml of a 50 mg/ml solution of Carprofen (Norbrook Laboratories, UK), subcutaneously. Post-surgery, catheter lines were flushed with 0.3 ml of 50 mg/ml Cephazolin (Mayne Pharma, Australia). Rats were allowed 7 d recovery before the commencement of cocaine SA training. To ensure catheter patency catheters were flushed daily with 0.2 ml of 50 U heparinized saline.

**Behavioural testing equipment**

Behavioural procedures were conducted in standard operant conditioning chambers located inside sound-attenuating, ventilated cubicles (Med Associates, USA). Chambers were equipped with two retractable levers (6 cm above the floor), two white cue-lights (one above each lever), two speakers to deliver auditory stimuli and a house-light located at the top of the chamber wall opposing the levers. The auditory stimuli were produced by a white-noise generator/speaker adjacent to the house-light (Med Associates) that produced a 70-dB white noise. A syringe pump (5 rpm motor, Med Associates) located on the outside of the sound-attenuating cubicle delivered the intravenous (i.v.) cocaine. Data acquisition and behavioural testing equipment were controlled by a Windows-based PC running MED-PC IV (Med Associates).

**SA training and procedures**

Seven days after surgery, rats were trained to intravenously SA cocaine (3 h/d, 5 d/wk). Responding on the active (right) lever resulted in an i.v. infusion of cocaine at a volume of 0.1 ml over 4 s and activation of a white cue-light above the active lever that remained illuminated for 20 s signalling a time-out period. The inactive (left) lever was extended during training; however, pressing on this lever did not result in cocaine infusion or cue-light illumination. Initially, a 20 reward maximum was set to prevent overdose during the training phase. Training was continued until stable responding for cocaine was achieved (animals were required to obtain the maximum reward per session, $\pm 10\%$, over three sessions). Once stable responding was established, the 3-h daily sessions were divided into alternating ‘drug-available’ (DA) and ‘non-drug-available’ (NDA) periods (Deroche-Gamonet et al. 2004). Both periods were signalled by distinct discriminative stimuli. For the 40-min DA period, this involved a constant 70-dB white noise. The 20-min NDA period was signalled by constant illumination of the operant box by the white house-light. Intravenous infusions of cocaine were only possible during DA periods, while lever presses during NDA periods were recorded but had no scheduled consequence. A fixed ratio 1 (FR1) was employed during the initial DA sessions and then increased to FR5 for the remainder of the experiment with unrestricted access to drug. Persistence in drug-seeking during the NDA period was assessed over the five FR5 sessions preceding and one FR5 session following progressive-ratio (PR) testing [i.e. NDA-seeking was assessed during week 4 and 1 d after the final PR session, the latter (PR) being conducted during week 5 of cocaine SA, see Fig. 1]. Of the 52 animals initially trained to SA cocaine, seven animals developed failure of catheter patency assessed by ketamine challenge (10 mg/kg) and were excluded from the analysis.

**PR protocol**

Motivation to consume drug was assessed by PR testing. Cocaine was available for the entire session under DA cue conditions. The PR schedule was as follows: 5, 10, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, 268, 345, 445, 573, 737, 947, 1218, 1566, 2012, 2585. The protocol was performed for 5 h; however if a scheduled reward was not received within 45 min of the previous reward the session was programmed to shut down. Data was collected over five PR sessions assessed during week 5 of cocaine SA (Fig. 1).

**Extinction training and cue-induced reinstatement**

Lever responding was extinguished in 1-h extinction sessions (once per day) until responding was reduced
to baseline levels (active lever presses > 6 per session for three consecutive sessions). Extinction training commenced after the final FR5 session that followed PR testing. During extinction, DA stimuli were withheld along with i.v. infusions. Cues previously paired with drug availability during SA were reintroduced (on average, after 8 wk of extinction, see Fig. 1) to test for reinstatement of drug-seeking following extinction (1 h reinstatement tests).

Relapse and ‘addiction’ phenotyping

Relapse and addiction scoring was performed in a similar manner to that reported in recent studies employing this phenotyping procedure (Belin et al. 2009; Deroche-Gamonet et al. 2004). Therefore, animals whose reinstatement scores were in the top 40% of the distribution were considered ‘high reinstators’, whereas those that scored in the bottom 40% were considered ‘low reinstators’. A retrospective analysis of the behavioural scores displayed by these animals for the two other addiction-like behaviours was then made. Thus, for an animal to be categorized as relapse-vulnerable they were also required to score in the top third of the distribution for these two behaviours whereas relapse-resilient animals were classified as those that scored in the bottom third for both of the addiction-like behaviours (Supplementary Table S1, Fig. S2).

Given that the relapse/addiction-resilient group has an identical training and cocaine consumption history to the vulnerable group, the former were used as the control or reference group for the comparison of synaptic plasticity-related gene expression changes with the relapse/addiction-vulnerable group.

Animal sacrifice and tissue harvesting

To avoid immediate early gene expression changes that are associated with acute reinstatement effects (including locomotor activity), animals were sacrificed 24 h after reinstatement testing. Brains were rapidly removed and cooled in ice-cold diethylpyrocarbonate (DEPC)-treated PBS. Brains were blocked into forebrain, midbrain and hindbrain regions on an ice-cold stage and the blocks were frozen in dry-ice chilled isopentane. Tissue was stored at −80 °C until required. Starting at the rostral extent of the corpus callosum 3 × 500 μm sections, from animals in the relapse-vulnerable (n = 6) and relapse-resilient (n = 6) groups, were cryosectioned from the forebrain block. The ventral striatum and dorsal striatum were then macrodissected (Supplementary Fig. S1) from the sections using a 2-mm diameter tissue punch and placed immediately in QIAzol Lysis Reagent (Qiagen, Australia) for disruption and homogenization.

RNA extraction

Total RNA was extracted and contaminating genomic DNA (gDNA) removed by on-column DNase-I digestion using Qiagen’s miRNeasy mini kit and DNase reagents, according to the manufacturer’s instructions. Concentrations of DNase-treated RNA were determined using Thermo Scientific’s NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA).
Reverse transcription and qPCR

Reverse transcription

Reverse transcription was performed using SuperScript III (Invitrogen, Australia), according to the manufacturer’s instructions. Briefly, 100–500 ng of total RNA, 2.5 μM oligo(dT)20 primers, 1 μl of 10 mM dNTP, and molecular biology grade water to 13 μl, were mixed and heated for 5 min at 65 °C, then chilled on ice for 1 min. Next, 4 μl 5× first-strand buffer, 1 μl of 0.1 M DTT, 1 μl RNaseOUT (40 U/μl) and 1 μl SuperScript III RT (200 U/μl) were added and the mixture incubated for 60 min at 50 °C, followed by 70 °C for 15 min. Reverse transcription without reverse transcriptase was also performed to assess gDNA contamination.

Real-time qPCR

All qPCR primers (Table 1) were designed using standard qPCR primer design criteria with the web-based software Primer 3. Primer pairs then were subjected to NCBI primer BLAST to ensure primer specificity. Reactions were performed in 12.5 μl volumes containing: 6.25 μl 2× SensiMixPlus SYBR (Quantace, UK); 200 nM each of forward and reverse primers; 2–20 ng cDNA; molecular biology grade water to 12.5 μl. After an initial 10 min, 95 °C enzyme activation step, 40 cycles of 95 °C for 30 s (step 1) followed by 60 °C for 30 s (step 2) were completed. Melt curves were generated to confirm the presence of a single PCR product. Primers were deemed specific if a single amplified product of appropriate size was detected by both melt curve analysis and gel electrophoresis. Reactions were performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA) and analysed using the Applied Biosystems 7500 Sequence Detection software (version 1.4). For each gene, the samples obtained from the six relapse-vulnerable and six relapse-resilient animals were run on the same plate in triplicate. Delta Ct (ΔCt, threshold cycle) was determined for each gene relative to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the well known ΔΔCt method (Schmittgen & Livak, 2008) was employed to enable a comparison between relapse-vulnerable and relapse-resilient groups. A representative qPCR amplification curve and quality control dissociation curves are provided in the Supplementary online material (Supplementary Figs S3, S4).

Statistical analysis

Distribution of behavioural data was determined following a Shapiro–Wilk test for normality. Data was
then analysed accordingly using parametric unpaired Student’s t tests, or non-parametric, Wilcoxon Mann–Whitney U tests. Gene expression data was statistically analysed using unpaired Student’s t tests. An alpha level of 0.05 was adopted for all statistical tests.

**Results**

**Behavioural phenotyping**

Forty-five of the 52 animals initially trained to SA cocaine completed the behavioural phenotyping protocol. Compared to the relapse-resilient control group (n = 7), vulnerable animals (n = 6) had 3-fold higher reinstatement scores (Fig. 2a, t₁₇ = 6.90, p < 0.001), 15-fold higher NDA lever-pressing scores (Fig. 2b, W = 28, Z = −3.0, p = 0.001), and 5-fold higher PR break-point scores (Fig. 2c, t₁₇ = 9.22, p < 0.001) (see also Supplementary Fig. S2). These behavioural indices demonstrate the relapse-vulnerable animals were also less able to refrain from drug-seeking in the absence of drug, and had an increased motivation to acquire the drug at earlier time-points in the training protocol. Importantly, the development of these behaviours was independent of cocaine consumption as no significant difference in consumption was observed between the two groups in either the initial training phase [average consumption (±S.E.M.): relapse-resilient = 65.0 ± 6.0 mg/kg; relapse-vulnerable = 55.0 ± 11.0 mg/kg; t₁₇ = 0.82, p = 0.43] or throughout the complete protocol (relapse-resilient = 278.0 ± 27.08 mg/kg; relapse-vulnerable = 341.1 ± 17.92 mg/kg; t₁₇ = 1.87, p = 0.09).

Furthermore, relapse-vulnerable and relapse-resilient animals did not display a significant difference in the number of days of training or the number of days on which cocaine was consumed (see also Supplementary Fig. S2). Of note, the percentage of animals behaviourally phenotyped as relapse-vulnerable that also displayed other addiction-like behaviours was about 13%, similar to that reported by Deroche-Gamonet et al. (2004) and consistent with human cocaine addiction risk.

**Molecular analysis**

In the ventral striatum (NAc) of relapse-vulnerable animals, an independent-samples t test showed significant decreases in transcript expression (Fig. 3a). This included reduced expression in: the gene encoding the activity-regulated cytoskeletal protein, Arc (t₁₈ = 2.45, p < 0.05); the dopamine receptors, Drd1a (t₁₈ = 2.97, p < 0.01) and Drd2 (t₁₈ = 3.99, p < 0.01); mammalian target of rapamycin, mTOR (t₁₈ = 2.89, p < 0.01); phosphatidylinositol-3-kinase, catalytic, beta polypeptide, PIK3CB (t₁₈ = 2.15, p < 0.05); protein kinase C, beta 1, PRKCB1 (t₁₈ = 2.30, p < 0.05); and eGMP-dependent protein kinase II, PRKG2 (t₁₈ = 2.61, p < 0.05).

Expression of these genes, with the exception of mTOR (t₁₈ = 1.73, p = 0.057), were also seen to be significantly reduced in the dorsal striatum (see Fig. 3b) [Arc (t₁₈ = 1.93, p < 0.05); Drd1a (t₁₈ = 5.11, p < 0.001); Drd2 (t₁₈ = 2.85, p < 0.01); PIK3CB (t₁₈ = 3.04, p < 0.01); PRKCB1 (t₁₈ = 2.31, p < 0.05); PRKG2 (t₁₈ = 3.16, p < 0.01)]. In addition, the transcript levels of the ionotropic glutamate receptor subunit 1, Grin1 (t₁₈ = 1.86,

![Fig. 2. Reinstatement and addiction-like behaviours. Animals were classified as relapse-vulnerable (n = 6) or relapse-resilient (n = 7) according to scores obtained during behavioural testing (see Materials and Methods section and Supplementary Table S1). (a) Relapse-like behaviour assessed by the number of lever presses during a cue-induced reinstatement test demonstrated vulnerable animals had a heightened propensity to relapse compared to resilient animals about 2 months after drug withdrawal. (b) Persistence in drug-seeking. Relapse-vulnerable animals displayed an inability to refrain from drug-seeking in the absence of drug as demonstrated by the significant increase in lever-pressing scores during non-drug-available periods. (c) Motivation for the drug was determined during progressive-ratio testing. Animals classified as relapse-vulnerable had significantly higher break-point scores, indicating increased motivation to acquire and consume drug. Data are means ± standard error of the mean (*** p < 0.001) (see also Supplementary Fig. S2).]
and Grm1 (t_{18} = 2.20, p < 0.05) were both significantly down-regulated in the dorsal striatum of relapse-vulnerable animals compared to relapse-resilient controls. Expression levels of the Gria2 and Grm5 transcripts were also assessed; however no significant differences between relapse-vulnerable and relapse-resilient animals in the ventral or dorsal striatum were observed (data not shown).

Discussion

In the present study we employed a recently developed and highly novel addiction phenotyping protocol to identify addiction and relapse-vulnerable and relapse-resilient groups. Changes in gene expression in brain regions thought to underpin addiction-like behaviours were then examined. We show that even after prolonged abstinence from cocaine, animals phenotyped as relapse-vulnerable displayed a generalized reduction in the expression of genes encoding synaptic plasticity-associated proteins within the striatum, such as those that regulate AMPAR subunit composition, e.g. mTOR and Arc. Importantly, the gene expression differences we observed were independent of large differences in cocaine consumption, indicating that addiction and relapse vulnerability are likely to be related to individual differences in the way brain circuitry maladapts to chronic drug use.

Of interest, in the context of the present study, is the recent report of Kasanetz et al. (2010) demonstrating that only animals phenotyped as addiction-vulnerable show persistent impairments in synaptic plasticity, specifically LTD. We believe that our findings are consistent with these functional data and point to the possibility that dysregulated transcriptional control of synaptic plasticity-related protein expression may be responsible for these changes observed in addiction-vulnerable animals. However, there are some methodological differences between our study and Kasanetz et al. (2010) that are important to highlight. First, whereas in our study animals underwent withdrawal, extinction training (~8 wk) followed by reinstatement testing, in the electrophysiological study of Kasanetz et al. (2010), animals were assessed for deficits in synaptic plasticity immediately after 30 d of daily cocaine SA. Therefore, it will be important for future studies to determine whether there are protracted changes in the potential for synaptic plasticity on a similar time-scale to the gene expression changes we observed here. In this regard, it is noteworthy that Moussawi et al. (2009) who also demonstrated deficits in the ability to evoke synaptic plasticity in the NAc of animals, performed their experiments on animals with a significant history of cocaine SA, extinction training and withdrawal (~6 wk) (Moussawi et al. 2009). While these authors did not compare addiction-vulnerable vs. addiction-resilient groups, they did demonstrate that the synaptic plasticity impairments were not related to extinction training after withdrawal, as rats exposed to
home-cage abstinence displayed similar deficits. In considering these two studies together with our data, we suggest that protracted changes in the potential to elicit synaptic plasticity occurred in addiction-vulnerable animals.

There is now substantial evidence that altered AMPAR subunit composition and localization modulates relapse-like behaviour (Anderson et al. 2008; Conrad et al. 2008; Cornish & Kalivas, 2000; Sutton et al. 2003; Zavala et al. 2007). While the precise nature of the changes that occur at the protein level remain to be fully elucidated, our data imply that altered transcriptional control over AMPAR subunit production in addiction vulnerable individuals may contribute to abnormal synaptic AMPAR subunit composition and neuronal excitability. Indeed, we found that a number of additional transcripts that encode proteins known to be intimately involved in the translational control of dendritic AMPAR subunits, were down-regulated in addiction and relapse-vulnerable animals, e.g. mTOR, Arc and Grm1 (for review see Luscher & Huber, 2010; Waung & Huber, 2009). It is particularly interesting that we found that Grm1 and Gria1 gene expression changes were restricted to the dorsal and not ventral striatum, considering the known role of the ventral region (i.e. the NAc) in addiction-related behaviours (Caine & Koob, 1994). Recent evidence indicates that the dorsal striatum assumes control over drug-taking once this behaviour transitions from goal-directed to habit-like responding (for review see Everitt & Robbins, 2005; Pierce & Vanderschuren, 2010), and the observed lack of NAc differences for glutamatergic neurotransmission-related genes may simply reflect that we collected brains after the ventral to dorsal transition had occurred (Belin et al. 2008; Deroche-Gamonet et al. 2004; Kasanetz et al. 2010). It will be important for future studies to determine the timeframe with which the putative transition of control takes place and whether similar electrophysiological changes reported to occur in the ventral striatum (i.e. NAc) are seen in the dorsal striatum as well. Such a proposal appears highly plausible in light of our present findings.

One of the more novel genes with reduced expression in the ventral striatum of addiction-vulnerable rats (and approached significance in the dorsal) was mTOR, a serine threonine kinase now recognized as an important regulator of dendritic protein translation, including AMPAR subunit expression (Hay & Sonenberg, 2004; Hoeffer & Klann, 2010; Laplante & Sabatini, 2009; Parsons et al. 2006; Slipeczuk et al. 2009). Mammeli et al. (2007) demonstrated that acute cocaine-induced potentiation of synaptic inputs onto VTA dopamine neurons was reversed by mGluR1 receptor-mediated LTD. This effect was shown to be dependent on mTOR activity and involved a replacement of GluR2-lacking (Ca\(^{2+}\) permeable) AMPARs with GluR2-containing (Ca\(^{2+}\) impermeable) AMPARs (Mameli et al. 2007). Luscher and Huber have recently suggested that, at least early in the addiction cycle, mGluR/mTOR-dependent LTD may oppose increased cocaine-induced excitability, with exhaustion of this mechanism in response to repeated exposure resulting in lasting synaptic alterations in the VTA (Luscher & Huber, 2010; Mameli et al. 2007). While it is not known whether mTOR plays a role in LTD within the striatum, NAc D\(_2\) receptor-mediated activation increases striatal expression of mTOR and stimulation of group 1 mGluRs results in striatal mTOR phosphorylation (Page et al. 2006; Santini et al. 2009). Importantly, our study also shows that the expression of the upstream regulator of mTOR, PI3KCB, was reduced in both striatal regions of vulnerable animals. PI3KCB is a constituent of the IRS-PI3K-AKT pathway which has been implicated in controlling psychostimulant-induced neuroadaptations (Dietz et al. 2009). For example, administration of the PI3K inhibitor, LY294002, has been shown to impede the behavioural expression of cocaine sensitization (Izzo et al. 2002). Further underscoring the potential importance of mTOR in drug-relevant behaviours is the demonstration that intra-NAc injections of rapamycin, a highly specific inhibitor of mTOR, prevented sensitization to methamphetamine (Narita et al. 2005) and suppressed cue-induced heroin-seeking in abstinent heroin addicts (Shi et al. 2009). Taken together, the present data indicate a possible role for mTOR in reduced striatal plasticity and addiction vulnerability.

Another factor that regulates AMPAR endocytosis from the plasma membrane is the activity-regulated cytoskeletal-associated protein, Arc (Bramham et al. 2008, 2010; Chowdhury et al. 2006; Guzowski et al. 2000). Thus, Arc is potentially a critical determinant of synaptic plasticity (Bramham et al. 2010), and we showed that its expression was reduced in both the ventral and dorsal striatum of relapse-vulnerable animals, compared to relapse-resilient controls. This finding is consistent with the reports of Freeman et al. (2008, 2010), who assessed gene expression changes in the PFC and NAc using targeted qPCR and microarray at multiple time-points after abstinence from cocaine (1, 10, 100 d).

Alterations in dopamine signalling within the striatum are often linked with addiction vulnerability. Our data point to a persistent reduction in D\(_2\)...
receptors, which is consistent with reductions in D2 receptor density and availability within the striatum of human cocaine addicts (Martinez et al. 2004; Volkow et al. 1993). Animal studies generally support this human data with a number of reports demonstrating a decrease in dopamine D2 receptor density within the ventral and dorsal striatum following extended cocaine SA exposure (Moore et al. 1998; Nader et al. 2002; Porrino et al. 2004). Further, mice lacking the D2 receptor display elevated rates of high-dose cocaine SA (Caine et al. 2002). Interestingly, a reduction in NAc D2 receptor transcripts and density is strongly associated with impulsivity (Hooks et al. 1994), and this behavioural phenotype is linked with compulsive cocaine taking (Dalley et al. 2007). Thus, these findings are in line with the hypothesis that dysregulated D2 receptor signalling is a predisposing factor for the development of addiction (Dalley et al. 2007). In the present study we also observed a reduction in D1 receptor transcript expression within both the dorsal and ventral striatum, a finding that is in keeping with reports of decreased D1 receptor numbers in the ventral striatum of cocaine SA rats (Graziella De Montis et al. 1998). In terms of the role of dopamine in synaptic plasticity, both LTP and LTD have been shown to be evoked through striatal dopamine receptor activation, although the vast majority of this evidence comes from studies on the dorsal striatum (Calabresi et al. 1999; Centonze et al. 2001). As outlined above, a potential role for mTOR in the development of addiction susceptibility has been highlighted by our studies and dopamine has been shown to alter mTOR expression in the striatum (Page et al. 2006; Santini et al. 2009). Whether dopamine receptor signalling works through a mTOR-dependent mechanism to alter the cell surface expression of GluR1-containing AMPARs and enhance relapse-like behaviour is yet to be tested.

The expression of a number of other synaptic plasticity-associated genes was also found to be down-regulated in the striatum of addiction-vulnerable animals. For example, we show reduced expression of PRKCB1 and PRKG2, transcripts encoding PKCβ1 and cGMP-dependent protein kinase II (cGKII) protein, respectively, in the ventral and dorsal striatum of relapse-vulnerable animals. PKC has been shown to be essential for spike-timing-dependent LTP in VTA dopamine neurons (Luu & Malenka, 2008), while cGKII has been shown to interact with GluR1 and is involved in regulating the trafficking of AMPARs (Serulle et al. 2007). Together these data support a generalized reduction in the expression of genes associated with synaptic plasticity.

Conclusions

To our knowledge this is the first attempt to assess prolonged changes in gene expression using the recently developed behavioural paradigm to phenotype animals as addiction-vulnerable vs. addiction-resilient (Deroche-Gamonet et al. 2004). While future studies will need to examine corresponding protein expression levels to determine the full functional implication of the present findings, our data are highly consistent with recent electrophysiological findings of synaptic plasticity deficits in addiction-vulnerable animals. The gene expression changes we observed occurred within both the dorsal and ventral striatum, suggesting that electrophysiological characterization of synaptic plasticity changes within addiction-vulnerable animals should also be assessed in the dorsal striatum. As it is now known that distinct components of the ventral striatum (NAc core and shell) and the dorsal striatum (medial and lateral) subserve different aspects of the addiction process, it will be important and informative for future studies to use the behavioural paradigm employed here to investigate gene and protein expression levels in these sub-regions. Nonetheless, the present study makes a significant contribution to our understanding of the molecular links between persistent changes in striatal synaptic plasticity potential and relapse vulnerability. Moreover, studies that incorporate individual behavioural differences in addiction and relapse vulnerability may provide more relevant mechanistic insight into this disease and improve translational outcomes.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest

None.
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