

Cocaine Modulation of Frontostriatal Expression of Zif268, D2, and 5-HT2c Receptors in High and Low Impulsive Rats

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Impulsivity shares high comorbidity with substance abuse in humans, and high impulsivity (HI) in rats has been identified as a predictive factor for cocaine addiction-like behavior. Despite the evidence that high impulsivity is associated with altered function of corticostriatal networks, the specific neural substrates underlying the increased vulnerability of impulsive individuals to develop cocaine addiction remain unknown. We therefore investigated specific neural correlates of HI within the corticostriatal circuitry and determined how they interact with a protracted history of cocaine self-administration. We used *in situ* hybridization to map brain expression of two major genes implicated in impulsivity, encoding the dopamine D2 receptor (DA D2R) and the 5-HT2c receptor (5-HT2cR), and an immediate early gene associated with neuronal plasticity, *zif268*, in groups of rats selected for HI and low impulsivity (LI) on a 5-choice serial reaction time task (5-CSRTT) immediately after 5-CSRTT training, and following 10 or 50 days of cocaine self-administration. HI rats exhibited decreased DA D2R mRNA in the mesolimbic pathway, and increased 5-HT2cR mRNA in the orbitofrontal cortex compared with LI rats. HI rats also showed decreased *zif268* mRNA in the ventral and dorsomedial striatum. Cocaine exposure decreased striatal D2R mRNA in both HI and LI rats, decreased 5-HT2cR mRNA differentially in striatal and prefrontal areas between HI and LI rats, and selectively decreased *zif268* mRNA in the orbitofrontal and infralimbic cortices of HI animals. These findings implicate novel markers underlying the vulnerability of impulsive rats to cocaine addiction that localize to the OFC, infralimbic cortex, and striatum.

Neuropsychopharmacology (2013) **38**, 1963–1973; doi:10.1038/npp.2013.95; published online 19 June 2013

Keywords: cocaine; corticostriatal network; gene expression; impulsivity; monoamines; zif268

INTRODUCTION

High impulsivity is an important feature of a number of psychiatric disorders including drug addiction (Verdejo-Garcia *et al*, 2008). Although the psychological and neurobiological underpinnings of impulsivity are not yet fully understood, accumulating evidence suggests that impulsivity is a major characteristic predisposing individuals to addiction (Verdejo-Garcia *et al*, 2008). Thus, ADHD patients and individuals with response inhibition deficits display increased vulnerability to develop addiction to nicotine, alcohol, and cocaine (Lee *et al*, 2011). Consistent with this evidence, high impulsive rats (HI) on the 5-choice serial reaction time task (5-CSRTT), a rodent

analog of the continuous performance task in humans (Robbins 2002), show an increased motivation to self-administer nicotine (Diergaarde *et al*, 2008) and higher propensity to escalate cocaine self-administration (Dalley *et al*, 2007) than low impulsive rats. HI rats also display a greater vulnerability both to develop compulsive cocaine self-administration (Belin *et al*, 2008) and relapse to cocaine seeking after abstinence (Economidou *et al*, 2008).

The transition from controlled to uncontrolled, compulsive, cocaine seeking and taking occurs only after a prolonged history of access to the drug (Belin *et al*, 2009; Deroche-Gamonet *et al*, 2004; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004) and HI rats display a greater propensity than low impulsive rats to undergo the transition to compulsive cocaine self-administration (Belin *et al*, 2008).

However, even though high impulsivity is known to be associated with altered corticostriatal function, the neurobiological mechanisms governing the emergence of compulsive drug seeking and taking in HI rats are poorly understood. Candidate mechanisms include neurobiological adaptations within frontostriatal circuitry, especially involving DA D2R and 5-HT2cR. Thus, not only have DA D2R

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been reported to be downregulated in both drug addicts (Volkow *et al*, 2004) and animals following chronic exposure to addictive drugs (Nader *et al*, 2006), but their availability is also reduced in the ventral but not dorsal striatum of HI rats before any drug exposure (Dalley *et al*, 2007). 5-HT₂cRs are highly expressed in the frontal cortex and the striatum (Clemett *et al*, 2000) where they are involved in modulating DA neurotransmission (Berg *et al*, 2008). 5-HT₂cRs are also linked to impulsivity (Dalley and Roiser, 2012; Robinson *et al*, 2008; Xu *et al*, 2009) and influence the neurochemical and behavioral effects of drugs of abuse, notably by modulating the effects of cocaine and other psychostimulants within mesocorticolimbic circuitry (Bubar *et al*, 2008; Higgins and Fletcher, 2003). Thus, both D2R and 5-HT₂cR are jointly implicated in impulse control and addiction; however, little is known about the way in which these receptors interact with cocaine history in high and low impulsive individuals to modulate neural plasticity and thereby the transition to compulsive cocaine intake (Belin *et al*, 2008).

In this study, we therefore investigated DA D2R and 5-HT₂cR gene expression in rats identified as HI or LI in the 5-CSRTT both before and following short- or long-term cocaine self-administration. On the basis of our previous work (Deroche-Gamonet *et al*, 2004; Belin *et al*, 2008), we hypothesized that neuroadaptive changes occurring after prolonged cocaine self-administration would be most relevant to the emergence of compulsive behavior in HI rats (Belin *et al*, 2008). We measured DA D2R and 5-HT₂cR mRNA levels using *in situ* hybridization in subregions of the prefrontal cortex, as well as in the striatum and its main source of dopaminergic afferents, the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc).

In addition, the long-lasting neurobiological effects of cocaine include the activation of zif268 (Valjent *et al*, 2006) under the regulation of DA and serotonin (5-HT) transmission (Bath and Baraban, 1993), and are associated with altered synaptic plasticity (Knapska and Kaczmarek, 2004). We therefore also investigated whether the duration of cocaine self-administration differentially recruited this marker of neural plasticity within the corticostriatal networks (Hearing *et al*, 2008; Unal *et al*, 2009) of HI and LI rats.

MATERIALS AND METHODS

Animals

One hundred and forty-four male Lister hooded rats (Charles River, Kent, UK), weighing approximately 300 g and about 7 weeks old at the start of the experiments were maintained at 85% of their free feeding weight during 5-CSRTT training, and housed 4 per cage under humidity- and temperature-controlled conditions and an alternating light/dark cycle (red lights on from 07:30 to 19:30 hours; white lights on from 19:30 to 07:30 hours). After surgery and during the self-administration experiments, rats were housed individually and fed 20 g per day, a quantity sufficient to maintain their growth. Water was provided *ad libitum*. All experimental procedures were conducted in accordance to the UK Animals (Scientific procedures) Act of 1986.

5-CSRTT Training

Three groups of 48 rats were trained in operant 5-CSRTT chambers controlled by the WhiskerServer software (version 2.8) and FiveChoice client (version 2.6). Each daily session consisted of 100 discrete trials with stable performance being achieved after about 40 sessions. Animals were trained to enter a food magazine to initiate a trial. After an intertrial interval (ITI) of 5 s had elapsed, a brief light stimulus (0.5 s) was pseudorandomly presented in one of five apertures. Following a nose-poke in this aperture ('correct' response), animals were rewarded with the delivery of one food pellet (45 mg Noyes dustless pellets) in the magazine. A nose-poke response in any of the adjacent apertures ('incorrect response'), or a failure to respond within 5 s after the onset of the stimulus ('omission'), resulted in no food delivery and a time-out period with the house light extinguished for 5 s. Nose-pokes made during the ITI, that is, before the onset of the stimulus (or 'premature responses') were recorded as a measure of impulsivity, and resulted in a 5 s time-out and no food reward.

Screening for High and Low Impulsivity

Following acquisition of the 5-CSRTT, rats were challenged with three long (7 s) ITI sessions presented at weekly intervals to encourage impulsive responding, as described previously (Belin *et al*, 2008; Besson *et al*, 2010; Dalley *et al*, 2007; Economidou *et al*, 2008). Subjects were ranked according to their level of impulsivity throughout the 3-week screening procedure and the highest and lowest seven subjects selected as HI and LI rats, respectively.

One group of HI and LI rats was used for *in situ* hybridization (drug-naive group), whereas the other two groups of HI and LI rats were trained to self-administer cocaine (see below) before subsequent *in situ* hybridization experiments (Supplementary Figure S1 and Supplementary Table 1).

Surgery

Rats were deeply anesthetized with ketamine (Ketazet, 90 mg/kg, intraperitoneally; Fort Dodge Animal Health Ltd, Southampton, UK) and xylazine (Rompun, 6.7 mg/kg, intraperitoneally; Bayer, Wuppertal, Germany), and implanted with a catheter (Brian Fromant, Cowley Road, Cambridge, UK) in the right jugular vein (Belin and Everitt, 2008). Rats were given 7 days to recover from surgery before the start of the behavioral experiment.

Self-Administration

Animals were placed for 2 h per day in operant chambers different from those used for 5-CSRTT training and in which they were allowed to self-administer a set dose of cocaine (250 µg/injection) under a fixed-ratio 1 schedule of reinforcement. The maximum number of infusions was set to 50 to prevent any differential cocaine intake between the experimental groups. One group of HI and LI rats self-administered cocaine daily for 11 sessions (short-term cocaine group), whereas another group of HI and LI rats

self-administered cocaine daily for 44 sessions (long-term cocaine group) (Supplementary Figure S2). These cocaine access periods were based on our earlier research showing that compulsive cocaine taking emerges after at least 40 daily sessions of cocaine self-administration (Belin *et al*, 2009, 2011; Deroche-Gamonet *et al*, 2004; Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004) specifically in HI rats (Belin *et al*, 2008). We were interested in the interactions between endophenotypes of impulsivity and the duration of cocaine exposure that subserve the transition to, and not the expression of, compulsivity in HI rats. We therefore did not probe addiction-like behaviors (Belin *et al*, 2008) in self-administering rats to avoid the differential behavioral responses of HI and LI rats in the test associated with the measure of compulsivity (resistance to punishment) that could have uncontrolled effects on the molecular markers we studied here.

In Situ Hybridization

Animals from the short- and long-term cocaine groups were decapitated 60 min after the last cocaine self-administration session, whereas animals from the drug-naive group were killed after the end of the screening for high and low impulsivity. The *in situ* hybridization procedure was performed on brain sections with validated oligonucleotide probes for the DA D2R, the 5-HT2cR, and *zif268* (Belin *et al*, 2007). Sections were then exposed at room temperature to Kodak Biomax MR films for different times. Images were captured and analyzed using an image analyzer system for densitometry (Interfocus Imaging, MCID Digital Densitometry System).

Statistical Analysis

Baseline differences in mRNA levels between HI and LI rats were analyzed with Student's *t*-tests and repeated-measures ANOVAs with group (HI vs LI) as between-subject factor and brain area as within-subject factor (performed separately for the striatum, cortex, and midbrain). For the HI and LI phenotypes, and for each brain area or clusters of brain areas, the comparison between mRNA levels in drug-naive, short- and long-term cocaine rats were analyzed using one-way ANOVAs, with drug treatment as the between-subject factor. Repeated-measures ANOVAs were used to compare mRNA levels in clusters of brain areas between HI and LI rats. All significant main effects ($p < 0.05$) were further analyzed using Student–Newman–Keuls *post hoc* test. Dimensional inter-relationships were investigated using Pearson's parametric correlation analyses. See detailed methods in Supplementary Information.

RESULTS

In Situ Hybridization

A highly specific and homogeneous macroscopic signal was obtained with each probe for every region of interest (Supplementary Figures S3–5). All the optical densities were within the range of linearity of the X-ray films.

Short- and Long-Term Cocaine Self-Administration in HI and LI Rats

In both self-administration experiments, that is, short- (Supplementary Figure S2a) and long- (Supplementary Figure S2b) term cocaine exposure, HI rats differed from LI rats neither in their acquisition of instrumental responding for cocaine nor in their total amount of cocaine intake per session. By the end of the self-administration sessions, rats had been exposed to a cumulative dose of self-infused cocaine of 109 ± 8 and 98 ± 4 mg in short-term HI and LI groups, respectively (Supplementary Figure S2c), and of 462 ± 20 and 475 ± 34 mg in long-term HI and LI groups, respectively (Supplementary Figure S2d).

Basal Differences in mRNA Levels Between HI and LI Rats

HI rats exhibited higher *zif268* mRNA levels than LI rats in the nucleus accumbens shell (NAcbS) and core (NAcbC) ($t = 2.249$, $p = 0.048$, $df = 10$ and $t = 2.460$, $p = 0.034$, $df = 10$, respectively) and in the dorsomedial striatum (DMS) ($t = 4.046$, $p = 0.0023$, $df = 10$) (Figure 1a). The highly significant effect observed in the DMS was confirmed by repeated-measure ANOVAs (group effect: $F(1,10) = 8.12$, $p < 0.05$; brain area effect $F(3,30) = 54.07$, $p \leq 0.001$) and subsequent *post hoc* analysis ($p = 0.005$). This differential profile of *zif268* mRNA levels within the striatum between drug-naive HI and LI rats was accompanied by alterations in DA D2R (Figure 1b) and 5-HT2cR (Figure 1c) mRNA levels (Figure 5). Compared with LI rats, HI rats showed lower DA D2R mRNA levels in the NAcbS ($t = -2.313$, $p = 0.043$, $df = 10$) and the VTA ($t = -2.600$, $p = 0.026$, $df = 10$) but higher 5-HT2cR mRNA levels in the orbito-frontal cortex (OFC) ($t = 2.235$, $p = 0.049$, $df = 10$), which was confirmed by repeated-measure ANOVAs (brain area effect: $F(5,50) = 50.30$, $p \leq 0.001$; group \times brain area interaction: $F(5,50) = 4.95$, $p \leq 0.001$), and subsequent *post hoc* analysis ($p = 0.000187$). There was a significant correlation between DA D2R mRNA levels in the VTA and the SNc in LI rats ($R = 0.618$, $p \leq 0.05$), whereas this was not the case in HI rats ($R = 0.361$, NS). 5-HT2cR mRNA levels in the DMS and dorsolateral striatum (DLS) were correlated in HI rats, as they were between the different regions of the prefrontal cortex, except the OFC, which was not the case for LI animals, where 5-HT2cR mRNA levels were correlated between the NAcbS and NAcbC (Supplementary Table 2).

Effect of Short- and Long-Term Cocaine Self-Administration on *zif268* mRNA Levels in HI and LI Rats

Differential effects were observed on *zif268* mRNA levels in HI and LI rats after cocaine self-administration (Figure 2). Three-way repeated-measures ANOVAs performed for striatal areas showed a group \times drug treatment interaction ($F_{2,28} = 4.18$, $p < 0.05$) with a main effect of brain area ($F_{3,84} = 98.23$, $p \leq 0.001$), brain area \times drug treatment interaction ($F_{6,84} = 4.89$, $p < 0.001$), and brain area \times group \times drug treatment interaction ($F_{6,84} = 2.22$, $p < 0.05$). Newman–Keuls *post hoc* tests showed significant differences between drug-naive and short-term cocaine LI rats in the DMS ($p = 0.0014$). For cortical areas, three-way

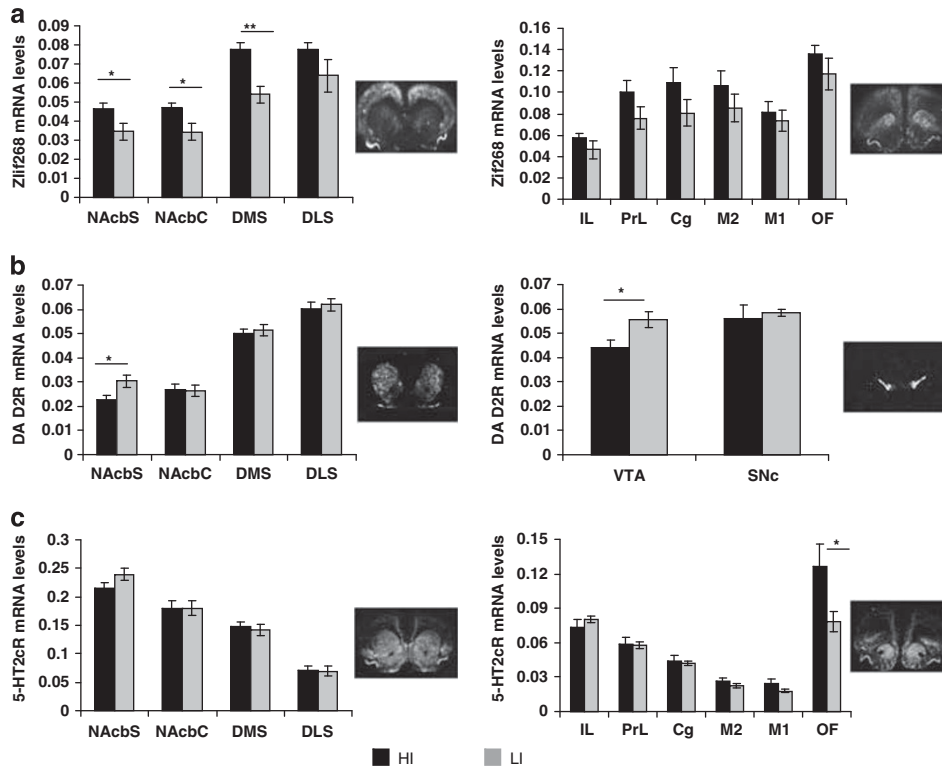


Figure 1 Differences in mRNA levels (expressed as mean \pm SEM) between high impulsive (HI) (black bars) and low impulsive (LI) (gray bars) rats for (a) zif268 (HI, $n = 6$; LI, $n = 6$), (b) dopamine D2 receptor (DA D2R) (HI, $n = 5$; LI, $n = 7$), and (c) 5-HT2c receptor (5-HT2cR) (HI, $n = 6$; LI, $n = 6$). The optical densities were measured in the nucleus accumbens shell (NAcbS), nucleus accumbens core (NAcbC), dorsomedial striatum (DMS), dorsolateral striatum (DLS) (for zif268, DA D2R, and 5-HT2cR); the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (for DA D2R); and the infralimbic (IL), prelimbic (PrL), cingulate (Cg), secondary motor (M2), primary motor (M1), and orbitofrontal (OF) cortices (for zif268 and 5-HT2cR) of HI and LI drug-naive animals. * $p \leq 0.05$, ** $p \leq 0.01$.

ANOVAs showed a significant main effect of brain area ($F_{5,150} = 104.39$, $p \leq 0.001$) and brain area \times drug treatment interaction ($F_{10,150} = 1.98$, $p < 0.05$). Upon confirmation of these main effects, analyses were then completed with ANOVAs performed separately in HI and LI groups.

In HI rats but not in LI rats, zif268 mRNA levels were decreased after cocaine self-administration in the cortex (drug exposure: $F_{2,15} = 4.203$, $p < 0.05$; cortical structure: $F_{2,5} = 66.392$, $p < 0.001$). This effect was observed after both short- ($p = 0.017$) and long-term cocaine exposure ($p = 0.014$). ANOVA restricted to each cortical area showed that this effect was limited to the infralimbic (IL) cortex ($F_{2,15} = 4.98$, $p < 0.05$) and OFC ($F_{2,15} = 5.66$, $p < 0.05$) of HI rats, regardless of the duration of cocaine exposure. *Post hoc* analyses confirmed that HI rats exposed to short- and long-term cocaine self-administration displayed decreased zif268 mRNA levels in the IL ($p = 0.037$ and 0.016 , respectively) and the OFC ($p = 0.018$ and 0.019 , respectively).

By contrast, cocaine produced different effects on zif268 mRNA levels in the striatum of LI rats (drug exposure: $F_{2,14} = 4.15$, $p < 0.05$; drug exposure \times striatal structure interaction: $F_{2,6} = 6.15$, $p < 0.001$) (Figure 2b). Thus in LI rats, an increase in zif268 mRNA levels was observed after cocaine self-administration in the DMS ($F_{2,15} = 12.94$, $p < 0.001$) after short- ($p = 0.0001$) but not long-term ($p = 0.2521$) cocaine exposure. No quantitative differences

in zif268 mRNA levels were observed in the IL and OFC between drug-naive and cocaine-exposed LI rats.

These differential changes in zif268 mRNA levels between HI and LI rats were further emphasized by repeated-measures ANOVAs showing that zif268 mRNA levels were significantly decreased following cocaine self-administration in the IL and OFC of HI rats compared with LI rats (group effect: $F_{1,22} = 4.84$, $p < 0.05$) (Figure 5).

Effect of Short- and Long-Term Cocaine Self-Administration on DA D2R mRNA Levels in HI and LI Rats

HI and LI rats displayed different neurobiological adaptations to the duration of cocaine self-administration with regard to D2 mRNA levels. Three-way repeated-measures ANOVAs for striatal areas revealed a group \times drug treatment interaction ($F_{2,28} = 10.62$, $p < 0.01$), a brain area effect ($F_{3,84} = 372.73$, $p \leq 0.001$), a brain area \times drug treatment interaction ($F_{6,84} = 4.18$, $p < 0.001$), as well as a brain area \times group \times drug treatment ($F_{6,84} = 2.38$, $p = 0.0357$). Newman-Keuls *post hoc* tests showed significant differences between drug-naive and short-term cocaine LI rats in the DMS ($p = 0.002$) and DLS ($p = 0.0002$). For midbrain areas (VTA and SNc), global ANOVAs showed a group effect ($F_{1,32} = 6.95$, $p < 0.05$) and a brain area effect ($F_{1,32} = 8.09$,

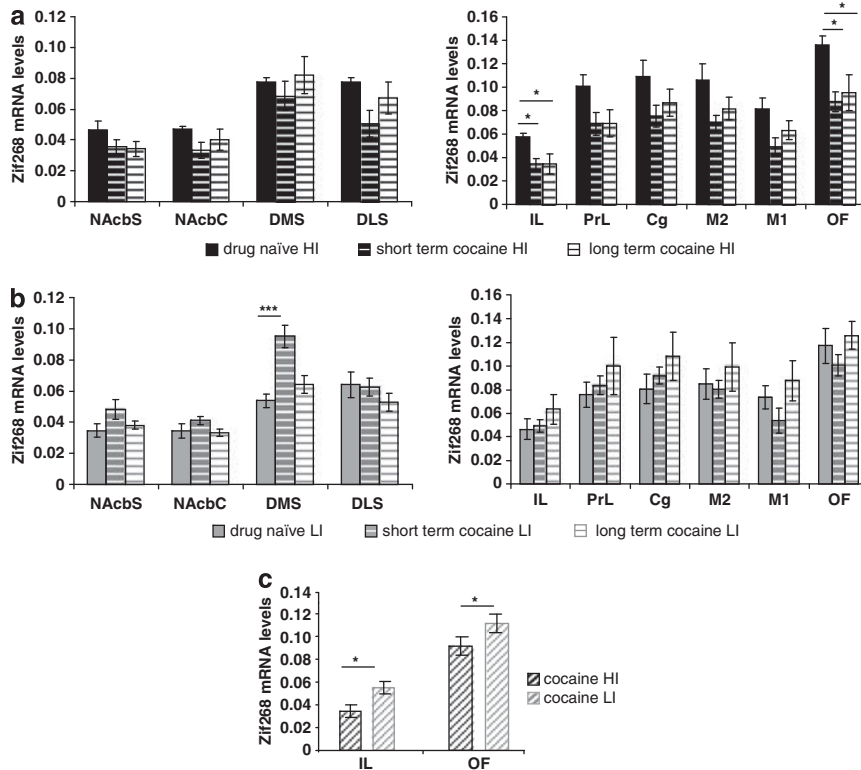


Figure 2 Effects of short- and long-term cocaine self-administration on zif268 mRNA levels (expressed as mean \pm SEM) in high impulsive (HI) and low impulsive (LI) rats. (a) Zif268 mRNA levels in drug-naïve (black plain bars, $n = 6$), short-term cocaine (black bars with white stripes, $n = 6$), and long-term cocaine (white bars with black stripes, $n = 7$) HI rats; (b) zif268 mRNA in drug-naïve (gray plain bars, $n = 6$), short-term cocaine (gray bars with white stripes, $n = 7$), and long-term cocaine (white bars with gray stripes, $n = 5$) LI rats; (c) zif268 mRNA in pooled short- and long-term cocaine HI (black bars with white diagonals, $n = 13$) and LI (gray bars with gray diagonals, $n = 12$) rats. The optical densities were measured in the nucleus accumbens shell (NAcbS), nucleus accumbens core (NAcbC), dorsomedial striatum (DMS), dorsolateral striatum (DLS), and in the infralimbic (IL), prelimbic (PrL), cingulate (Cg), secondary motor (M2), primary motor (M1), and orbitofrontal (OF) cortices. * $p \leq 0.05$, *** $p \leq 0.001$.

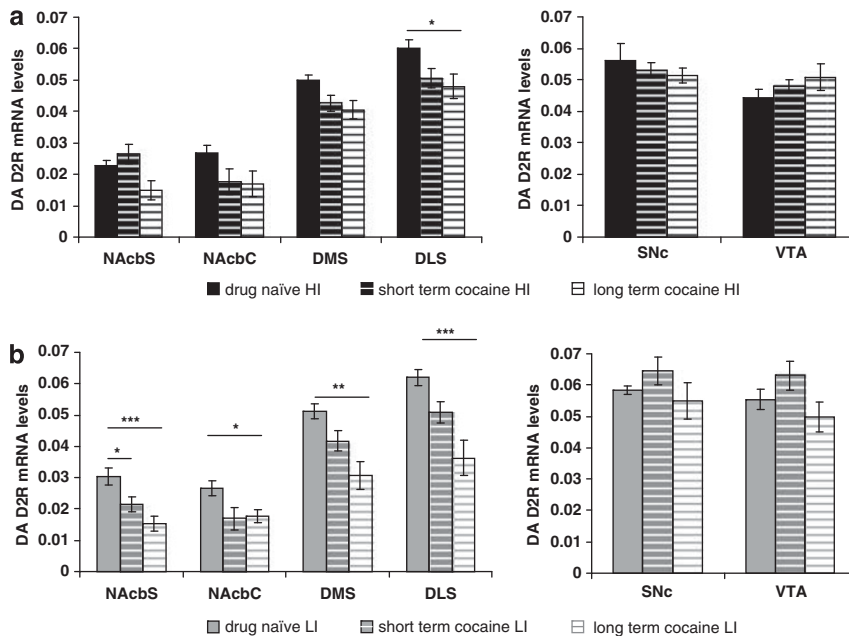


Figure 3 Effects of short- and long-term cocaine self-administration on dopamine D2 receptor (DA D2R) mRNA levels (expressed as mean \pm SEM) in high impulsive (HI) and low impulsive (LI) rats. (a) DA D2R mRNA levels in drug-naïve (black plain bars, $n = 5$), short-term cocaine (black bars with white stripes, $n = 5$), and long-term cocaine (white bars with black stripes, $n = 6$) HI rats; (b) DA D2R mRNA levels in drug-naïve (gray plain bars, $n = 7$), short-term cocaine (gray bars with white stripes, $n = 7$), and long-term cocaine (white bars with gray stripes, $n = 5$) LI rats. The optical densities were measured in the nucleus accumbens shell (NAcbS), nucleus accumbens core (NAcbC), dorsomedial striatum (DMS), dorsolateral striatum (DLS), ventral tegmental area (VTA), and substantia nigra pars compacta (SNc). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

$p < 0.01$). Analyses were then completed with ANOVAs performed separately in HI and LI groups.

In HI rats, cocaine self-administration resulted in decreased DA D2R mRNA levels in the striatum (drug \times structure interaction: $F_{2,6} = 3.537$, $p < 0.01$) (Figure 3a), an effect mainly attributed to the DLS ($F_{2,12} = 3.45$, $p < 0.05$) and the NAcS ($F_{2,13} = 4.60$, $p < 0.05$), where DA D2R were lower in HI rats exposed to long-term cocaine self-administration than in drug-naive HI rats ($p = 0.045$ and 0.074 , respectively) (Figure 5).

In LI rats, cocaine self-administration was associated with decreased DA D2R mRNA levels in the dorsal and ventral striatum (drug \times structure interaction: $F_{2,6} = 3.83$, $p < 0.05$) (Figure 3b). This effect was significant in each striatal structure, namely the NAcS ($F_{2,17} = 9.44$, $p < 0.01$), NAcC ($F_{2,17} = 3.74$, $p < 0.05$), DMS ($F_{2,17} = 8.93$, $p < 0.01$), and DLS ($F_{2,17} = 11.06$, $p < 0.01$). *Post hoc* analyses revealed that this effect was significant after both short- ($n = 7$) and long-term ($n = 5$) cocaine self-administration in the NAcS ($p = 0.019$ and 0.001 , respectively), and after long-term cocaine self-administration in the NAcC ($p = 0.039$), the DMS ($p = 0.002$), and the DLS ($p = 0.0006$) (Figure 5).

Effect of Short- and Long-Term Cocaine Self-Administration on 5-HT₂cR mRNA Levels in HI and LI Rats

The history of cocaine self-administration differentially altered 5-HT₂cR mRNA levels in the corticostriatal circuitry of HI and LI rats. Three-way repeated-measures ANOVAs performed for striatal areas showed an effect of drug treatment ($F_{2,30} = 4.97$, $p < 0.05$) and brain area ($F_{3,90} = 393.90$, $p \leq 0.001$). For cortical areas, global ANOVAs showed a brain area effect ($F_{5,150} = 0.13$, $p \leq 0.001$) and a brain area \times group \times drug treatment ($F_{10,150} = 2.75$, $p < 0.01$). Analyses were then completed with ANOVAs performed separately in HI and LI groups.

5-HT₂cR mRNA levels in HI rats were significantly decreased after cocaine self-administration in the cingulate (Cg) cortex and the NAcS ($F_{2,16} = 3.95$, $p < 0.05$; $F_{2,15} = 4.95$, $p < 0.05$, respectively) (Figure 4a). *Post hoc* analyses revealed that this decrease was observed after long- ($p = 0.019$) but not short-term ($p = 0.307$) cocaine exposure in the NAcS, opposite to that observed in the Cg cortex ($p = 0.007$ and $p = 0.060$ for short- and long-term cocaine self-administration, respectively) (Figure 5).

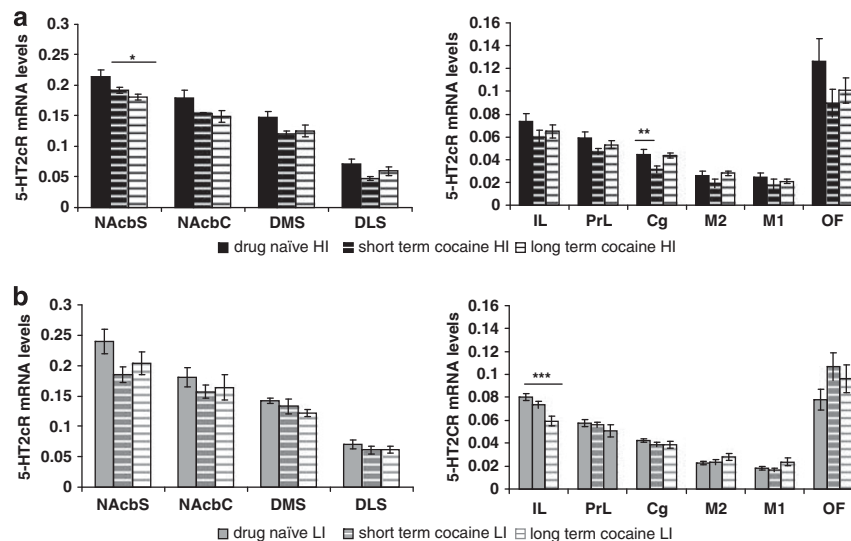


Figure 4 Effects of short- and long-term cocaine self-administration on 5-HT₂c receptor (5-HT₂cR) mRNA levels (expressed as mean \pm SEM) in high impulsive (HI) and low impulsive (LI) rats. (a) 5-HT₂cR mRNA levels in drug-naive (black plain bars, $n = 6$), short-term cocaine (black bars with white stripes, $n = 6$), and long-term cocaine (white bars with black stripes, $n = 6$) HI rats; (b) 5-HT₂cR mRNA in drug-naive (gray plain bars, $n = 6$), short-term cocaine (gray bars with white stripes, $n = 7$), and long-term cocaine (white bars with gray stripes, $n = 5$) LI rats. The optical densities were measured in the nucleus accumbens shell (NAcS), nucleus accumbens core (NAcC), dorsomedial striatum (DMS), dorsolateral striatum (DLS), and in the infralimbic (IL), prelimbic (PrL), cingulate (Cg), secondary motor (M2), primary motor (M1), and orbitofrontal (OF) cortices. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

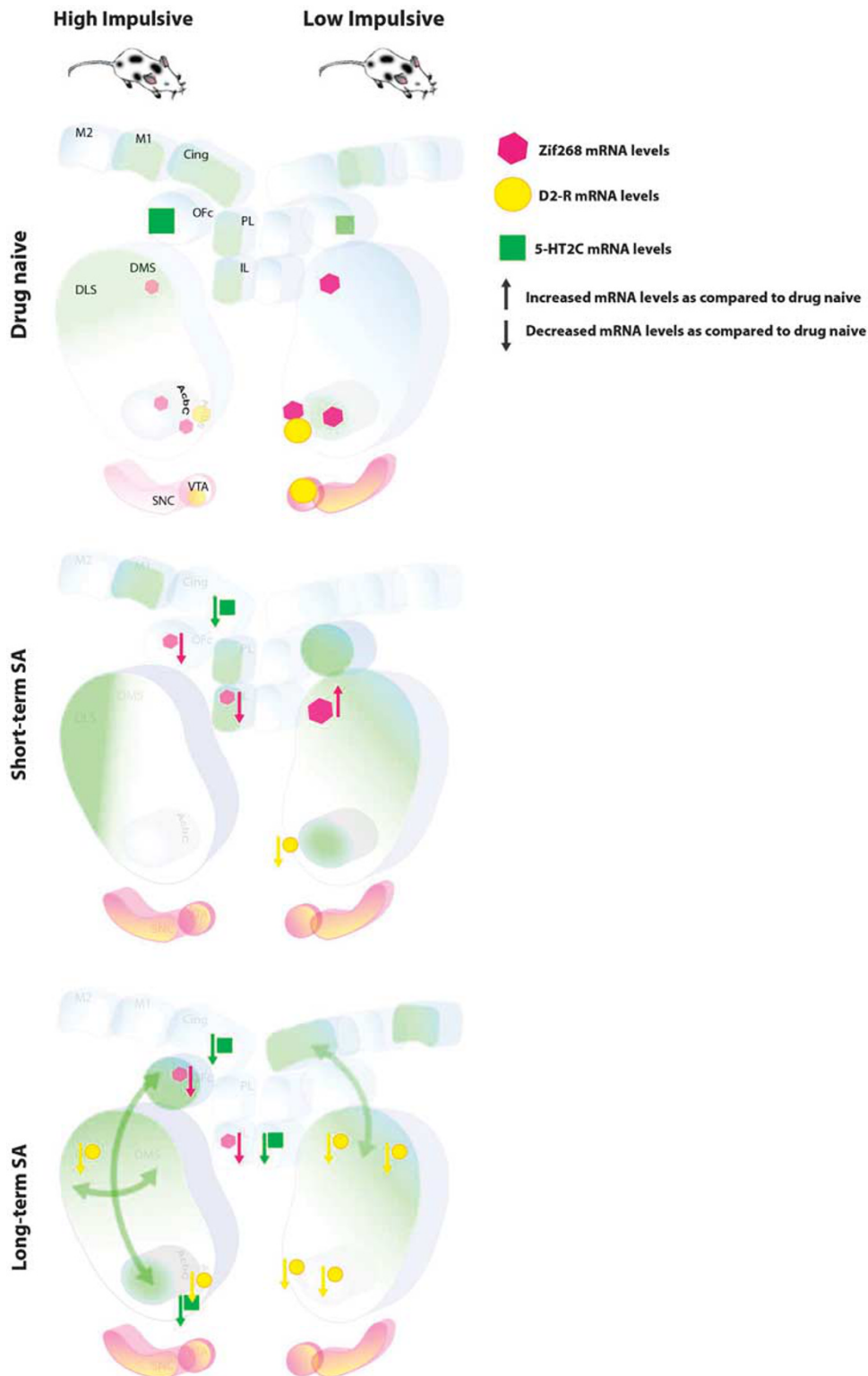
Figure 5 Schematic of the main differences observed between high impulsive (HI) and low impulsive (LI) rats in the course of cocaine self-administration history. HI rats were associated with a differential profile of expression, within the corticostriatal circuitry, of two important monoamine receptors involved in the regulation of impulse control and drug reinforcement, and of the plasticity-associated gene, *zif268*. Compared with LI rats, drug-naive HI rats displayed increased 5-HT₂c receptor (5-HT₂cR) mRNA levels in the orbitofrontal cortex (OFC) and lower D2R mRNA levels in the ventral tegmental area (VTA) and NAcS. HI rats had a higher level of *zif268* gene expression in the nucleus accumbens shell (NAcS), nucleus accumbens core (NAcC), and dorsomedial striatum (DMS) compared with LI rats. Cocaine self-administration resulted in different neuroadaptations within the corticostriatal circuitry of HI and LI rats. Cocaine exposure was associated with a decrease in 5-HT₂cR mRNA levels, specifically in the anterior cingulate (Cg) cortex and the NAcS of HI rats after short- and long-term self-administration, respectively, and in the infralimbic (IL) cortex of LI rats after long-term self-administration. This was paralleled by a decrease in dopamine D2 receptor (DA D2R) mRNA levels in the striatum of both HI and LI rats. This effect, which encompassed the entire striatum of LI rats, was more evident after long-term cocaine self-administration and mostly involved the DLS in HI rats. *Zif268* mRNA levels were decreased after short- and long-term cocaine self-administration in the IL and OF cortices of HI rats specifically. By contrast, in LI rats, *zif268* mRNA levels were increased in the DMS after short-term cocaine self-administration.

By contrast, cocaine self-administration resulted in a decrease in 5-HT_{2c}R mRNA levels in the IL cortex of LI rats ($F_{2,15} = 8.77$, $p < 0.05$), after long- ($p = 0.001$) but not short-term ($p = 0.176$) exposure to the drug (Figures 2b and 5).

Correlational analyses further showed that inter-regional patterns of 5-HT_{2c}R expression were also differentially

reorganized after cocaine exposure in HI and LI rats (see Supplementary Table 2).

In summary, as compared with LI animals, HI rats were characterized by decreased DA D₂R mRNA levels in the NAcb shell and the VTA, as well as increased 5-HT_{2c}R mRNAs level in the OFC. HI rats also displayed higher level of zif268 gene expression in the NAcbS, NAcbC, and DMS.



Zif268 mRNA levels were decreased after short- and long-term cocaine self-administration in the IL and OF cortices of HI rats, whereas in LI rats, zif268 mRNA levels were increased in the DMS after short-term cocaine.

DA D2R mRNA levels were decreased after cocaine exposure in the striatum of both HI and LI rats. This effect encompassed the entire striatum of LI rats, was more evident after long-term cocaine self-administration, and mostly involved the DLS in HI rats.

Cocaine exposure was also associated with a decrease in 5-HT_{2c}R mRNA levels in the anterior Cg cortex and the NAcS of HI rats after short- and long-term self-administration, respectively, and in the IL cortex of LI rats after long-term self-administration.

The main results are summarized in Figure 5.

DISCUSSION

Increasing evidence suggests that impulse control deficits, as observed in highly impulsive subjects, may contribute to increased vulnerability to develop addiction (Verdejo-Garcia *et al*, 2008). Accordingly, high impulsivity on the 5-CSRTT has been shown to predict several hallmarks of cocaine addiction (Dalley *et al*, 2007; Belin *et al*, 2008). HI rats have been shown to be more vulnerable than LI rats to escalate cocaine self-administration when given extended and intermittent access to cocaine (Dalley *et al*, 2007) and to switch from controlled to compulsive cocaine seeking, that persists despite aversive foot-shocks, and which emerges after at least 40 days of cocaine self-administration (Belin *et al*, 2008). Furthermore, HI rats are more vulnerable to relapse after protracted abstinence (Economidou *et al*, 2008). However, HI did not differ from LI rats in their propensity to acquire cocaine self-administration (this study; Dalley *et al*, 2007; Belin *et al*, 2008; Economidou *et al*, 2008) nor in their average baseline intake during the 2 h daily self-administration sessions. Thus, the transition to compulsive drug seeking observed in HI rats after an extended cocaine self-administration history stems from the interaction between the drug and neurobiological substrates that are specific to those subjects (Everitt *et al*, 2008; Dalley *et al*, 2011). In this study, we used this lack of differential cocaine intake between HI and LI rats to identify neurobiological correlates of protracted cocaine self-administration that may contribute to the transition to compulsivity in vulnerable subjects. In doing so, we did not directly assess addiction-like criteria in HI and LI rats because this would have resulted in uncontrolled differential behaviors and differing quantities of cocaine intake between the two groups.

Previous studies have established that highly impulsive subjects have lower levels of DA D2/D3 receptor availability in the midbrain and ventral striatum but not in the nigrostriatal system (Buckholtz *et al*, 2010; Dalley *et al*, 2007; Lee *et al*, 2009). These findings, demonstrating that HI and LI rats display similar DA D2R mRNA levels in the nigrostriatal system but major differences in the mesolimbic system not only support these observations, but further specify the neurobiological locus of decreased DA D2R in the striatal system associated with the HI trait, namely the shell, but not the core, of the NAcB, and also the VTA. HI on

the 5-CSRTT has been associated *in vitro* with increased DA release in the NAcS (Diergaarde *et al*, 2008), whereas lower midbrain DA D2/3R availability in highly impulsive humans has also been proposed to be associated with enhanced DA activity (Buckholtz *et al*, 2010). DA D2R are expressed on GABA neurons within the ventral striatum, and also on VTA DA neurons where they are presynaptic and function as autoreceptors that regulate DA release associated with behavioral activation (Viggiano *et al*, 2003).

It is well documented that individuals addicted to cocaine and other drugs have lower DA D2R availability in the striatum when studied during abstinence (Volkow *et al*, 2004). Moreover, in monkeys with a history of cocaine self-administration, there is also a marked reduction in DA D2 receptors in the ventral striatum under short access conditions, which persists, and also progresses to the dorsal striatum after chronic self-administration (Porrino *et al*, 2004). In accord with these observations, we observed reduced DA D2R mRNA levels in the NAcB and the dorsal striatum of rats after cocaine self-administration, with a larger decrease in dorsal striatal areas after long-term self-administration. These reductions were found in both HI and LI rats, thereby suggesting that cocaine exposure interacts with other neurobiological vulnerability markers in impulsive individuals.

A considerable body of evidence links the 5-HT system with impulsivity (Dalley and Roiser, 2012), with a significant role for 5-HT_{2c}R (Dalley and Roiser, 2012; Robinson *et al*, 2008; Xu *et al*, 2009). Here we observed higher levels of 5-HT_{2c}R mRNA selectively in the OFC of HI rats. The OFC has an important role in response inhibitory control (Everitt *et al*, 2007) and damage to this area increases impulsivity in rodents (Eagle and Baunez, 2010). OFC 5-HT depletion decreases behavioral flexibility in monkeys (Clarke *et al*, 2007), which has been proposed to result from 5-HT_{2c}R supersensitivity (Boulougouris and Robbins, 2010). Conversely, 5-HT_{2c}R antagonism within the OFC improves cognitive flexibility in rats (Boulougouris and Robbins, 2010).

We further observed that 5-HT_{2c}R mRNA levels in the DMS and DLS were correlated in HI rats, as they were between different regions of the medial prefrontal cortex. This apparent convergent 5-HT_{2c}R regulation throughout the cortex and dorsal striatum in HI rats was in contrast to the lack of correlation between DA D2R mRNA levels in the VTA and SNc of these animals, which was instead apparent in LI rats. There is evidence that alteration in 5-HT activity influences impulsive behavior through a modulation of DA transmission (Harrison *et al*, 1997; Oades, 2008).

In this study, 5-HT_{2c}R mRNA levels were decreased in the NAcS of HI rats after long-term cocaine self-administration. Although previous studies have highlighted the role of 5-HT_{2c}R in psychostimulant action (Bubar and Cunningham, 2008), the consequences of drug exposure on these receptors has not been established to date (Filip *et al*, 2010). 5-HT_{2c}R control mesocorticolimbic DA activity (Di Matteo *et al*, 2002) and 5-HT_{2c}R expressed in the NAcS modulate NAcB DA outflow (Navailles *et al*, 2006). Drug-induced modifications in 5-HT_{2c}R gene expression may contribute to the changes in striatal DA activity that have been proposed to underlie the shift from impulsivity to

compulsive drug seeking occurring during the development of addiction (Everitt *et al*, 2008).

HI rats displayed a different profile of expression of the plasticity-related immediate-early gene *zif268* mRNA, the levels of which were increased in the ventral and dorsomedial striatum compared with LI subjects. This is in marked contrast to the differential response to cocaine in HI and LI rats in that, HI but not LI rats displayed a decrease in *zif268* mRNA levels selectively in the IL and OF cortices after short-term cocaine self-administration. This decrease persisted even after protracted exposure to the drug, suggesting altered function of these frontal areas after exposure to addictive drugs in vulnerable individuals.

There is a close link between *zif268* gene expression and plasticity-linked neuronal activity (Knapska and Kaczmarek, 2004) and here we investigated *zif268* gene expression as a potential marker of plasticity engaged by DA and 5-HT transmission. ZIF268 protein acts as a transcription factor controlling the expression of downstream genes implicated in the regulation of neuronal responses to various stimuli. High levels of *zif268* mRNA in the ventral and dorsal striatum of HI animals suggest greater synaptic plasticity occurring in these neural loci after 5-CSRTT training only in animals with weak inhibitory control. However, the decrease in *zif268* mRNA levels in the IL and OFC of HI rats after short- and long-term cocaine self-administration, as compared with 5-CSRTT training, suggests a cocaine-induced loss of neuronal plasticity in these brain areas. Persistent OFC dysfunction has been demonstrated in humans addicted to cocaine and other drugs, as well as in rats even after relatively brief exposure to cocaine (Ersche *et al*, 2011; Schoenbaum and Shaham, 2008; Everitt *et al*, 2007). Dysfunction of this area leads to the emergence of inflexible and compulsive behavior, including compulsive drug seeking, a hallmark feature of drug addiction that is predicted by high impulsivity on the 5-CSRTT (Belin *et al*, 2008). In accordance with our present findings showing alterations in both OFC *zif268* and NAcS 5-HT2cR gene expression in HI after cocaine self-administration, it has been shown that OFC lesions both induce compulsive behavior in rats and cause a decrease in the content of striatal 5-HT (Schilman *et al*, 2010).

The IL cortex plays a role in the expression and consolidation of extinction of drug seeking (Millan *et al*, 2011) and inactivation of this area causes the reinstatement of previously extinguished drug seeking, whereas activation of the IL prevents reinstatement (Peters *et al*, 2008). The present data showing decreased *zif268* mRNA levels in this area after cocaine taking in HI rats are in accord with the increased susceptibility to relapse to cocaine seeking in these rats (Economidou *et al*, 2008). Decreased neuronal plasticity in the OFC and IL after cocaine self-administration may therefore also provide a mechanism for the emergence of compulsive drug taking in high impulsive individuals when exposed to specific conditions.

Zif268 gene expression has been shown to be increased in the ventral and dorsal striatum after acute cocaine administration and to return to pre-cocaine levels or be decreased after long-term cocaine exposure (Fumagalli *et al*, 2009; Hope *et al*, 1992). Similarly, we observed increased *zif268* mRNA levels in the DMS of LI rats after short- but

not long-term cocaine self-administration. By contrast, in HI rats, no changes in *zif268* mRNA levels were observed in striatal areas after cocaine taking, suggesting that LI individuals have a greater propensity than HI individuals to display neuronal plasticity in response to cocaine. This observation is consistent with the recent demonstration that rats vulnerable to addiction-like behavior for cocaine are characterized by a lack of metaplasticity, the so-called anaplasticity, which corresponds to an inability to counteract drug-induced impairments in synaptic plasticity, within the frontostriatal networks (Kasanetz *et al*, 2010, 2012).

In LI rats, 5-HT2cR mRNA levels were decreased after long access but not after short access to cocaine in the IL cortex. In contrast to HI animals, no other changes were observed selectively in LI rats after long-term cocaine self-administration. In LI rats, *zif268* mRNA levels were increased in the DMS after short- but not after long-term cocaine, indicating that neuroadaptations occur in the brain of these rats after long-term exposure to the drug. These data suggest that drug exposure has less persistent consequences for brain functioning in low impulsive rats compared with high impulsive rats.

The observed differences between HI and LI rats in the effects of cocaine self-administration on the DA D2R and 5-HT2cR mRNA levels illustrate at the gene expression level neurobiological adaptations to protracted drug exposure that occur differentially in vulnerable and non-vulnerable subjects, as also described by Kasanetz *et al* (2010, 2012) at the synaptic plasticity level. It also allowed us to rule out a potential confounding effect on gene expression of cocaine self-administered in the last session (but see Belin *et al*, 2007).

The differential drug-induced alterations in gene expression and plasticity observed in HI and LI rats could not be attributed to a differential effect of aging in these two subpopulations, not only because there is little if any evidence for a baseline shift in the expression of DA D2R and 5-HT2cRs in young adults over a period of 10 weeks, which is the larger window between the first long ITI and the last self-administration session of this study, but also because a potential differential effect of age on subpopulations of rats of the same strain has not been demonstrated previously. The effects observed here could, nevertheless, be attributable to various components of the self-administration history that are not directly related to overall drug exposure, namely instrumental training or protracted exposure to the self-administration context and associated Pavlovian stimuli that have been reported to modify DA D2R and DAT mRNA levels in sensitized animals (Belin *et al*, 2007).

The lack of assessment of compulsive aspects of addiction in this study, however, does not allow the direct conclusion that the adaptations observed here in HI rats subserve compulsive cocaine intake. Instead, they may reflect the development of a particular neurobiological state preceding the expression of compulsive drug use in vulnerable subjects. Little differential cocaine-induced plasticity on gene expression was observed in HI rats, suggesting that further modifications may be necessary to facilitate the emergence of compulsive cocaine self-administration.

In conclusion, we show that HI in rats is associated with regional differences in the expression of genes related to monoamine neurotransmission and neuronal plasticity in striatal and frontal areas, including decreased mesolimbic DA D2R mRNAs, increased OFC 5-HT_{2c} mRNAs, and decreased zif268 mRNAs in ventral and dorsal striatal areas. This study therefore suggests that cocaine exposure differentially alters 5-HT_{2c} and DA D2R mRNAs between HI and LI rats and that, when exposed to cocaine self-administration, the former display decreased zif268 mRNA levels in the IL and OF cortices, suggesting a dysfunction of these frontal areas after exposure to addictive drugs in vulnerable individuals. This may, in part, underlie the compulsive drug seeking and high rate of relapse observed in HI rats after protracted cocaine seeking (Belin *et al*, 2008; Economidou *et al*, 2008). In high impulsive individuals, basal or constitutive alterations in frontostriatal circuitry, especially regarding monoamine neurotransmission, might confer vulnerability to further drug-induced impairments in prefrontal cortical function, eventually favoring the development of cocaine addiction.

ACKNOWLEDGEMENTS

This study was supported by the MRC (G9536855, G1002231 and G0701500) and by a joint core award from the MRC and Wellcome Trust (MRC G1000183; WT 093875/Z/10/Z) in support of the Behavioural and Clinical Neuroscience Institute at Cambridge University. MB was supported by an EMBO fellowship. DB and ABR were supported by an INSERM AVENIR Grant, the AXA research fund, the fondation pour la recherche médicale (FRM), and the IREB. AL was supported by a Wellcome Trust Programme Grant (089589/Z/09/Z). We thank Mercedes Arroyo for technical assistance with the *in situ* hybridization experiments.

DISCLOSURE

The authors declare no conflict of interest.

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