JN-BC-2711-14 (revision 2)

Systemic administration of a brain-penetrant TrkB antagonist reduces behavioral measures of cocaine dependence

Abbreviated title:

Systemic TrkB antagonist reduces cocaine intake

Verheij MMM[1,2], Vendruscolo LF[2,3], Caffino L[4,5], Giannotti G[4,5], Cazorla M[6,7], Fumagalli F[4,5], Riva MA[4], Homberg JR[1], Koob GF[2,3] and Contet C[2]

[1]Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, the Netherlands, [2]Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute La Jolla, CA 92037 San Diego, USA, [3]Neurobiology of Addiction Section, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD21224, USA, [4]Department of Pharmacological and Biomolecular Sciences, 20133 Milan, Italy, [5]Collaborative Center of Department of Antidrug Policies, Presidency of the Council of Ministers, 00187 Rome, Italy, [6]University Grenoble Alpes, Grenoble Institute of Neuroscience, GIN F-38000, Grenoble, France, [7]INSERM U1216, F-38000, Grenoble, France.

Please address correspondence and reprint requests to: M.M.M. Verheij, Department of Cognitive Neuroscience (CNS), PO Box 9101, Radboud University Nijmegen Medical Centre (RUNMC), 6500 HB Nijmegen, The Netherlands, Tel: 31-24-3619565, Fax: 31-24-3540044, E-mail: M.Verheij@cns.umcn.nl, Michel.Verheij@radboudumc.nl.

Number of pages: 30, number of Tables: 1, number of Figures: 9, number of words for Abstract: 239, Introduction: 641 and Discussion: 1,370. Total number of words (Abstract, Introduction, Materials & Methods, Results, Discussion, References, Figure and Table Legends): 7,581.

Abstract

Cocaine exposure alters Brain-Derived Neurotrophic Factor (BDNF) expression in the brain. BDNF signaling through TrkB receptors differentially modulates cocaine self-administration depending on the brain regions involved. In the present study, we determined how brain-wide inhibition of TrkB signaling affects cocaine intake, the motivation for the drug, and reinstatement of drug taking after extinction. To overcome the inability of TrkB ligands to cross the blood-brain barrier, the TrkB antagonist cyclotraxin-B was fused to the non-toxic transduction domain of the tat protein from human immunodeficiency virus type 1 (tat-cyclotraxin-B). Intravenous injection of tat-cyclotraxin-B dose-dependently reduced cocaine intake in rats that had short and extended access to self-administration. In addition, it decreased cocaine intake under a progressive ratio schedule of reinforcement as well as reinstatement of cocaine taking. In contrast, the treatment did not affect operant responding for a glucose/saccharin solution, demonstrating that the effects of tat-cyclotraxin-B are specific for cocaine reward. Cocaine self-administration increased TrkB signaling and activated the downstream Akt pathway in the nucleus accumbens, and had opposite effects in the prefrontal cortex. Pretreatment with tat-cyclotraxin-B normalized protein levels in these two dopamine-innervated brain regions. Cocaine self-administration also increased TrkB signaling in the ventral tegmental area, where the dopaminergic projections originate, but pretreatment with tat-cyclotraxin-B did not alter this effect. Altogether, our data show that systemic administration of a brain-penetrant TrkB antagonist leads to brain region-specific effects and may be a potential pharmacological strategy for the treatment of cocaine addiction.

Significance Statement

Brain-Derived Neurotrophic Factor (BDNF) signaling through TrkB receptors plays a well-established role in cocaine reinforcement. However, local manipulation of BNDF signaling yields divergent effects depending on the brain region, thereby questioning the viability of systemic TrkB targeting for the treatment of cocaine use disorders. Our study provides first-time evidence that systemic administration of a brain-penetrant TrkB antagonist (tat-cyclotraxin-B) reduces several behavioral measures of cocaine dependence, without altering motor performance or reinforcement by a sweet palatable solution. In addition, although cocaine self-administration produces opposite effects on TrkB signaling in the nucleus accumbens and prefrontal cortex, tat-cyclotraxin-B administration normalized these cocaine-induced changes in both brain regions.

Introduction

Numerous studies have shown that Brain-Derived Neurotrophic Factor (BDNF) signaling through TrkB receptors plays a key role in different aspects of cocaine dependence (for review: Corominas et al., 2007; McGinty et al., 2010; McCarthy et al., 2012). An interaction between BDNF and the mesocorticolimbic dopamine system is believed to modulate the behavioral effects of cocaine (Altar et al., 1992; Martin-Iverson et al., 1994; Guillin et al., 2001; Goggi et al., 2003; Corominas et al., 2007; Lobo et al., 2010; McGinty et al., 2010; McCarthy et al., 2012). Accordingly, forced exposure to cocaine increases BDNF expression in the prefrontal cortex (Le Foll et al., 2005; Fumagalli et al., 2007; Fumagalli et al., 2009; Lu et al., 2010) and the nucleus accumbens (Filip et al., 2006; Graham et al., 2007; Huang et al., 2011). Cocaine self-administration increases BDNF expression not only in these cortical (McGinty et al., 2010; Sadri-Vakili et al., 2010; Fumagalli et al., 2013) and ventral striatal (Grimm et al., 2003; Graham et al., 2007; Fumagalli et al., 2013; Li et al., 2013) regions innervated by dopamine neurons, but also in the ventral tegmental area (Grimm et al., 2003; Pu et al., 2006; Graham et al., 2009; Schmidt et al., 2012) where these dopaminergic neurons originate.

Cocaine intake is modulated by BDNF signaling in a brain region-dependent manner. Local injections of BDNF into the nucleus accumbens or the ventral tegmental area enhance cocaine reward, cocaine self-administration, and reinstatement of cocaine seeking (Horger et al., 1999; Lu et al., 2004; Graham et al., 2007; Bahi et al., 2008; Lu et al., 2009), and intra-accumbens infusion of BDNF antiserum and virally-mediated TrkB silencing reduce various measures of cocaine dependence (Graham et al., 2007; Bahi et al., 2008; Graham et al., 2009; Li et al., 2013). In contrast, local injection of BDNF in the medial prefrontal cortex reduces cocaine seeking (Berglind et al., 2007; Berglind et al., 2009; Whitfield et al., 2011), and virally-mediated BDNF silencing in this brain region increases the motivation to take cocaine (Sadri-Vakili et al., 2010).

The divergent effects of local TrkB signaling manipulation prompted us to investigate the net effect of brain-wide TrkB antagonism on measures of cocaine self-administration that reflect motivation to work for the drug and relapse-like behavior associated with compulsive cocaine seeking. However, BDNF and TrkB ligands do not undergo significant transport across the blood-brain barrier. To allow direct delivery to the brain, the non-toxic transduction domain of the tat protein from human immunodeficiency virus type 1 (HIV-1) was fused to the TrkB antagonist cyclotraxin-B (for details: Cazorla et al., 2010), resulting in tat-cyclotraxin-B (TC). Intravenous administration of TC following a double-injection procedure was previously shown to inhibit the phosphorylation of forebrain TrkB receptors by approximately 50% and to reduce anxiety-related behavior without affecting depression-like behavior or motor performance (Cazorla et al., 2010). In the present study, the behavioral effects of TC were tested in rats given limited (1 h/day) or extended (6 h/day) access to cocaine self-administration (for details: Ahmed and Koob, 1998; Orio et al., 2009; Wee et al., 2012). The stable short access (ShA) cocaine intake is believed to reflect non-dependent drug intake, whereas the long access (LgA)-induced escalation of cocaine intake is thought to reflect the transition to compulsive-like drug taking that is observed in drug dependence (for review: Koob, 2009; Koob and Volkow, 2010).

In addition, we analyzed TrkB signaling in the nucleus accumbens and prefrontal cortex to identify the role of these two dopamine-innervated brain regions in the modulation of cocaine self-administration by TC. Specifically, we measured the effect of cocaine self-administration and TC treatment on the total and phosphorylated levels of TrkB, as well as Akt and ERK, two major downstream effectors of TrkB (for review: Duman and Voleti, 2012). We also examined the effect of cocaine self-administration and TC pretreatment on TrkB levels in the ventral tegmental area.

Materials & methods

Animals. 22 Male Wistar rats (Charles River, Hollister, CA), weighing 350 ± 5 g at the beginning of the experiment were housed in groups of 2 or 3 in Macrolon type III cages under a 12h:12h reversed day/night cycle (lights off at 8:00 AM). Food and water were available *ad libitum* except during behavioral testing. All procedures described below were approved by The Scripps Research Institute Animal Care and Use Committee and were in accordance with national and international laws and guidelines for the care and use of laboratory animals.

Surgery. Rats were implanted with a micro Renathane catheter (0.3 mm i.d. × 0.64 mm o.d.; MRE037, Braintec scientific Inc, Braintree, MA) into the right external jugular vein according to previously reported procedures (Wee et al., 2007). This aseptic surgery procedure was performed under isoflurane anesthesia (2-3%). After surgery, rats were given analgesic (Flunixin®, 2.5 mg/kg, s.c., Sigma-Aldrich, St. Louis, MO) and antibiotic (Cefazolin®, 0.033 mg, i.v., Sagent Pharmaceuticals, Schaumburg, IL) treatment for at least one week. The catheter was flushed twice daily with heparinized saline (30 USP/ml, Hospira, Lake Forest, IL) during the entire experiment.

Self-administration chambers. Cocaine self-administration was performed in standard operant chambers (28 x 26 x 20 cm, Med Associates Inc., St Albans, VT) that were placed in ventilated, light- and sound-attenuating cubicles. The cocaine self-administration chambers were equipped with a swivel system allowing rats to move freely during self-administration sessions, whereas self-administration chambers for the glucose/saccharin solution were equipped with an acrylic drinking cup in the center of the wall between two levers. In both cages, drugs were delivered by a 15 r.p.m. syringe pump (Razel Scientific Instruments, Georgia, VT). The start of a session was signaled by the presentation of 2 retractable levers into the self-administration chamber. Pressing the right lever was programmed to deliver cocaine (volume: 0.1 ml in 4s) or the glucose/saccharin solution (volume: 0.1 ml in 0.5 s), whereas pressing the left lever had no programmed consequences. During drug administration, a stimulus light above the active lever was illuminated for 20 s for the cocaine

solution and 30 s for the glucose/saccharin solution, both indicating a timeout period when additional lever presses did not result in fluid delivery.

Cocaine training. One week after surgery, rats were trained to self-administer cocaine (0.5 mg/kg/infusion) for 12 days under a fixed ratio 1 (FR1) schedule of reinforcement (one lever press resulted in one drug injection) for 1 h per day. The first day of cocaine training was labeled experimental day 1 (see Figure 1).

Escalation of cocaine intake. After training, rats were divided into 2 groups matched by their number of infusions during the final training session. One group of rats continued to self-administer cocaine (0.5 mg/kg/infusion) in daily 1-h sessions (ShA), whereas the other group of rats self-administered the same cocaine dose in daily 6-h sessions (LgA) for 22 days (Figure 1). 22 Rats were initially exposed to cocaine self-administration (ShA: n=11, LgA: n=11). Over time, 3 rats had to be excluded because of occlusion of the intravenous catheter (ShA: n=2, LgA: n=1). The number of rats included in each experiment is indicated in the Figure legends.

Effects of TC on cocaine self-administration in ShA and LgA rats. After cocaine intake escalation reached a stable level, the effect of intravenous injection of various doses (2.5, 5.0, 7.5 and 10.0 mg/kg/0.5 ml) of the TrkB inhibitor cyclotraxin-B was tested on cocaine self-administration under both ShA and LgA conditions. To allow penetration into the brain, the non-toxic transduction domain of the tat protein from HIV-1 (Gump and Dowdy, 2007) was fused to cyclotraxin-B (for details: Cazorla et al., 2010), resulting in tat-cyclotraxin-B (TC, custom made by Neo peptide, Cambridge, MA). The ineffective tat peptide lacking the cyclotraxin-B sequence (tat empty: TE) served as control (see also: Cazorla et al., 2010). TC and TE were given in a within-subjects Latin-square design, in which 8 test days were separated by drug-free (no tat: NT) days (Figure 1). As previously reported, rats received two intravenous injections of TC or TE 60 min

apart (Cazorla et al., 2010), and were placed in the self-administration chambers immediately after the second administration.

Effects of TC on progressive ratio responding. In order to test the effects of TC on the motivation to work for cocaine, rats were allowed to self-administer cocaine under a progressive ratio (PR) schedule of reinforcement (Hodos, 1961). The number of lever presses required to obtain the next infusion of cocaine exponentially increased according to the following equation: number of responses per infusion = $(5 \times e^{(\text{injection number} \times 0.2)}) - 5$ (Richardson and Roberts, 1996). When a rat failed to achieve the response requirement within a period of 30 min, the PR session ended and breakpoints were recorded. We chose to test the effect of TC 7.5 mg/kg on PR responding based on the effect of this dose on FR1 responding in ShA and LgA rats.

Effects of TC on glucose/saccharin self-administration. To analyze whether TC also altered the self-administration of a non-drug reinforcer, rats were briefly trained (4 sessions) to press for the highly palatable reinforcer 'supersac' (3% w/v glucose and 0.125% w/v saccharin) (Martin-Fardon and Weiss, 2014). Given that the above-mentioned experiments revealed that 7.5 mg/kg TC reduced cocaine self-administration for a period of 90 min (see Table 1), the 30 min lasting glucose/saccharin test was performed between 30 and 60 min after the second 7.5 mg/kg TC injection in order to ensure the best match between behavioral testing and TC's pharmacokinetic profile.

Re-escalation and extinction. Following self-administration of the glucose/saccharin solution, cocaine intake was reduced by ~ 20% (see results). Rats were re-exposed to ShA and LgA of cocaine until cocaine intake during these re-escalation sessions no longer differed from cocaine intake observed on experimental day 35 (8 days, Figure 1). Cocaine was then replaced with saline

and extinction sessions of 4 h were performed daily until the rats received no more than 3 saline infusions per h.

Effects of TC on reinstatement of cocaine intake. Following extinction, rats were again given access to cocaine (0.5 mg/kg/inf) self-administration for 4 sessions of 1-h during which reinstatement of cocaine intake was triggered by a single manual i.v. infusion of cocaine. The effect of TC or TE (7.5 mg/kg) pretreatment was tested.

Effects of TC on BDNF system associated proteins. A separate cohort of 25 rats was subjected to 12 1-h sessions of cocaine self-administration followed by 22 6-h sessions, as described above. An additional cocaine self-administration session was conducted, before which rats were pretreated with TC (n=13) or TE (n=12) as described above. Another control group of rats (n=10) underwent surgery, but had no access to cocaine, and was pretreated with TE prior to sacrifice. These animals were not exposed to saline self-administration because rats do not lever press for this non-rewarding solution. However, they were handled on a daily basis for catheter flushing. All rats were sacrificed by decapitation 60 min after the start of the final self-administration session, i.e. 60 min after the second TC/TE injection. Bilateral punches of nucleus accumbens, ventral tegmental area and a medial punch of prefrontal cortex were collected from freshly dissected brain sections of 2 mm using a 1.2-mm diameter needle (Verheij et al., 2008). Western blot analysis was conducted to measure levels of total and phosphorylated TrkB, Akt and ERK, as previously described by Giannotti et al. (2014). Briefly, punched brain regions were homogenized by sonication using a cold buffer containing 0.32 M sucrose, 1 mM HEPES solution, 0.1 mM EGTA, 0.1 mM PMSF (pH=7.4), in presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Equal amounts of protein were measured under reducing conditions on the criterion TGX precast gels (Bio-Rad Laboratories, Milan, Italy) and then electrophoretically transferred onto polyvinylidene difluoride membranes (GE Healthcare, Milan, Italy). Blots were blocked one hour at

room temperature with 10% non-fat dry milk in TBS + 0.1% Tween-20 buffer, incubated with antibodies against the phosphorylated forms of the proteins and then stripped and reprobed with the antibodies against corresponding total proteins. The conditions of the primary antibodies were the following: anti phospho-TrkB Y706 (1:1000, Santa Cruz Biotechnology, USA); anti-TrkB (1:750, Santa Cruz Biotechnology, USA); anti phospho-ERK1 T202/Y204 (1:1000, Cell Signaling Technology, USA); anti total ERK1 (1:5000, Santa Cruz Biotechnology, USA); anti phospho-Akt S473 (1:1000, Cell Signaling Technology, USA); anti total Akt (1:1000, Cell Signaling Technology, USA); and anti β-Actin (1:10000, Sigma-Aldrich, Italy). Results were standardized using β-actin as the control protein, which was detected by evaluating the band density at 43kDa. Immunocomplexes were visualized by chemiluminescence using the Chemidoc MP Imaging System (Bio-Rad Laboratories).

Data analysis. Data are expressed mean ± standard error of the mean (SEM). The effects of TC on cocaine or glucose/saccharin self-administration were analyzed using a two-way or one-way analysis of variance (ANOVA) with a correction for repeated measures when required. The effects of TC on cocaine-induced protein regulation were analyzed using a one-way ANOVA. *Post-hoc* comparisons were performed by means of a Student's t-test or LSD depending on the number of experimental groups to be compared. Prism 6.0 (GraphPad, San Diego, CA) was used to analyze all the data.

Results

Systemic injection of a brain-penetrant TrkB antagonist reduces responding and motivation for cocaine self-administration.

After training, rats responded 12.3 ± 1.6 times per h for cocaine (Figure 2A; session 12). LgA, but not ShA, cocaine self-administration resulted in an escalation of the drug intake over sessions (Figure 2A; access x session interaction (two-way ANOVA for repeated measures): $F_{(22,440)}$=25.4, P<0.001; session effect LgA (one-way ANOVA for repeated measures): $F_{(22,220)}$=10.74, P<0.001, session effect ShA (one-way ANOVA for repeated measures): n.s.). In contrast to ShA cocaine self-administration, LgA cocaine self-administration resulted in an escalation of the drug intake during the first h (Figure 2B; access x session interaction (two-way ANOVA): $F_{(1,40)}$=10.5, P=0.002; session effect LgA (one-way ANOVA): $F_{(1,20)}$=19.91, P<0.001, session effect ShA (one-way ANOVA): n.s.). In addition to the differences in cocaine intake under a FR1 schedule of reinforcement (Figures 2A-B), cocaine intake under a PR schedule of reinforcement was also found to be larger in LgA than ShA rats (Figure 2C; access effect (one-way ANOVA): $F_{(1,20)}$=4.75, P=0.042).

The control peptide of TC, labeled TE, did not alter cocaine self-administration under both ShA (Figure 3A; treatment effect and treatment x session interaction (two-way ANOVA for repeated measures): n.s.) and LgA (Figure 3B; treatment effect and treatment x session interaction (two-way ANOVA for repeated measures): n.s.) conditions. The TrkB inhibitor TC dose-dependently reduced cocaine intake under both ShA (Figure 3C; dose effect (one-way ANOVA for repeated measures): $F_{(4,40)}$=23.5, P<0.001) and LgA (Figure 3D; dose effect: $F_{(4,40)}$=7.29, P<0.001, dose x time interaction (two-way ANOVA for repeated measures): $F_{(20,200)}$=2.88, P<0.001) conditions. The intermediate dose of 7.5 mg/kg TC decreased PR responding in both ShA (Figure 3E; treatment effect (one-way ANOVA): $F_{(1,18)}$=17.60, P<0.001) and LgA (Figure 3F; treatment effect (one-way ANOVA): $F_{(1,20)}$=7.33, P=0.014) animals. By setting the maximum duration to reach the next PR criterion to 30 min (see: Materials & methods), TC-treated rats reached their breakpoints within the 90-min interval the TrkB antagonist was effective (ShA: 57 ± 8 min and LgA: 71 ± 8 min).

Systemic injection of a brain-penetrant TrkB antagonist does not impact responding for a natural reinforcer.

After the cocaine self-administration experiments, rats were briefly trained to press for a glucose/saccharin solution (Figure 4A; session effect (two-way ANOVA for repeated measures): $F_{(3,57)}$=26.1, P<0.001). The oral consumption of this highly palatable reinforcer was not different between rats with a ShA and LgA history of cocaine (Figure 4A; access effect and access x session interaction (two-way ANOVA for repeated measures): n.s.). TC did not change responding for glucose/saccharin (Figure 4B; treatment effect and treatment x access interaction (two-way ANOVA): n.s.).

Systemic injection of a brain-penetrant TrkB antagonist reduces cocaine-induced reinstatement of drug taking behavior.

To analyze the effects of TC on drug-induced reinstatement of cocaine intake, rats were first re-escalated to previous cocaine intake levels (Figure 5A; session effect: $F_{(7,119)}$=5.56, P<0.001; access x session interaction (two-way ANOVA for repeated measures): $F_{(7,119)}$=6.50, P<0.001), followed by an extinction period. LgA rats returned to baseline levels of lever pressing later than ShA rats (Figure 5B; session effect: $F_{(12,204)}$=12.2, P<0.001, access x session interaction (two-way ANOVA for repeated measures): $F_{(12,204)}$ = 4.19, P<0.001).

As expected, cocaine-induced reinstatement of drug taking was larger in LgA than in ShA rats (Figure 6A; reinstatement x access interaction (two-way ANOVA): $F_{(1,34)}$=4.31, P=0.046, reinstatement effect ShA (one-way ANOVA): $F_{(1,16)}$=6.71, P=0.020, reinstatement effect LgA (one-way ANOVA): $F_{(1,18)}$=17.82, P=0.002). The intermediate dose of 7.5 mg/kg TC reduced the

reinstatement of cocaine intake in both ShA (Figure 6B; treatment effect (one-way ANOVA): $F_{(1,16)}$=7.01, P=0.018) and LgA (Figure 6C; treatment effect (one-way ANOVA): $F_{(1,18)}$=5.39, P=0.032) rats. The latency to the first cocaine self-infusion was shorter in rats primed with cocaine than in rats primed with saline (Figure 6D; reinstatement effect (two-way ANOVA): F(1,34)=58.60, P<0.001, reinstatement x access interaction (two-way ANOVA): n.s.) and the latency of first self-infusion post-cocaine prime was shorter in LgA than in ShA rats (Figure 6D; access effect (one-way ANOVA): F(1,17)=6.29, P=0.023). Interestingly, TC increased this latency (Figure 6E (ShA); treatment effect (one-way ANOVA): F(1,16)=47.86, P < 0.001 and figure 6F (LgA); treatment effect (one-way ANOVA): F(1,18)=5.20, P = 0.035) more strongly in LgA than ShA rats (figures 6E vs 6F; treatment x access interaction (two-way ANOVA): F(1,34)= 3.90, P = 0.054).

Effects of brain-penetrant TrkB antagonist on active and inactive lever presses and timeout responses.

The above-mentioned effect of TC on cocaine self-administration under FR1 and PR in both ShA and LgA rats was driven by a selective decrease in active lever presses (Table 1 (A): left column), which was not accompanied by changes in timeout responding (Table 1 (A): middle column) or inactive lever presses (Table 1 (A): right column). TC did not change active (Table 1 (B): left column), timeout (Table 1 (B): middle column) or inactive (Table 1 (B): right column) responses for glucose/saccharin in either group of animals. However, TC reduced the number of active (Table 1 (C): left column) and timeout (Table 1 (C): middle column) presses during reinstatement, which was not accompanied by a change in inactive lever presses (Table 1 (C): right column).

Systemic injection of a brain-penetrant TrkB antagonist reverses cocaine self-administration-induced changes in TrkB signaling in the nucleus accumbens and prefrontal cortex.

A separate cohort of rats subjected to LgA cocaine self-administration and TC pretreatment was used to analyze TrkB signaling in the nucleus accumbens, medial prefrontal cortex, and ventral tegmental area. Cocaine intake in this second cohort was comparable to the intake of the first cohort used for behavioral characterization (1-h intake: 28 ± 5.5 infusions (second cohort) versus 29 ± 1.5 infusions (first cohort) respectively, cohort effect (one-way ANOVA): n.s.). In addition, systemic administration of 7.5 mg/kg TC reduced cocaine intake in this second cohort (treatment effect (one-way ANOVA): $F(1,23) = 23.44$, $P<0.001$) to the same extent as in the first cohort (reduction during first h of session: 60 ± 7.0 % (second cohort) versus 40 ± 10.6 % (first cohort) respectively, cohort effect (one-way ANOVA): n.s.). This TC-induced reduction in cocaine self-administration was again driven by a selective decrease in active lever presses (treatment effect (one-way ANOVA): $F(1,23) = 9.63$, $P=0.005$), whereas TC did not affect timeout responding or inactive lever pressing (treatment effect (one-way ANOVA): n.s.).

In the nucleus accumbens, cocaine self-administration increased the protein levels of TrkB (Figure 7A; treatment effect (one-way ANOVA): $F_{(2,32)}=6.99$, $P=0.003$, cocaine effect (t-test): $P=0.006$) and pTrkB (Figure 7B; treatment effect (one-way ANOVA): $F_{(2,32)}=3.93$, $P=0.030$, cocaine effect (t-test): $P=0.030$). It also increased the total and phosphorylated levels of Akt (Figure 7C (Akt); treatment effect (one-way ANOVA): $F_{(2,32)}=11.08$, $P<0.001$, cocaine effect (t-test): $P<0.001$ and Figure 7D (pAkt); treatment effect (one-way ANOVA): $F_{(2,32)}=4.38$, $P=0.021$, cocaine effect (t-test): $P=0.014$), but did not alter ERK (Figure 7E) and pERK (Figure 7F) levels (treatment effect (one-way ANOVA): n.s.). Conversely, in the prefrontal cortex, cocaine self-administration reduced pTrkB (Figure 8B; treatment effect (one-way ANOVA): $F_{(2,32)}=3.64$, $P=0.038$, cocaine effect (t-test): $P=0.009$) and pAkt (Figure 8D; treatment effect (one-way ANOVA): $F_{(2,32)}=8.49$, $P=0.001$, cocaine effect (t-test): $P=0.003$), without affecting TrkB (Figure 8A: treatment effect (one-way ANOVA): $F_{(2,32)}=3.30$, $P=0.050$, cocaine effect (t-test): n.s.) and Akt (Figure 8C;

treatment effect (one-way ANOVA): n.s.) total protein levels. pERK and ERK were also unaltered in the prefrontal cortex (Figures 8E-F: treatment effect (one-way ANOVA): n.s.).

TC reversed the effects of cocaine self-administration on TrkB, pTrkB, Akt and pAkt in the nucleus accumbens (TC effect (t-test): TrkB (Figure 7A); P=0.002, pTrkB (Figure 7B); P=0.016, Akt (Figure 7C); P<0.001, pAkt (Figure 7D); P=0.017), as well as on pTrkB and pAkt in the prefrontal cortex (TC effect (t-test): pTrkB (Figure 8B); P=0.012, pAkt (Figure 8D); P<0.001).

Systemic injection of a brain-penetrant TrkB antagonist does not affect cocaine self-administration-induced TrkB activation in the ventral tegmental area.

In the ventral tegmental area, cocaine self-administration increased the protein levels of TrkB (Figure 9A; treatment effect (one-way ANOVA): $F(2,32)=4.23$, P=0.025, cocaine effect (t-test): P=0.016) and pTrkB (Figure 9B; treatment effect (one-way ANOVA): $F(2,32)=7.59$, P=0.003, cocaine effect (t-test): P=0.005), and these effects were not altered by pretreatment with TC (TC effect (t-test): TrkB (Figure 9A) and pTrkB (Figure 9B); n.s.).

Discussion

Cocaine self-administration behavior.

Consistent with previous studies, cocaine self-administration under LgA, but not ShA, conditions resulted in an escalation of drug intake over time (see also: Ahmed and Koob, 1998; Orio et al., 2009; Wee et al., 2012). Compared to ShA animals, LgA animals showed increased motivation for cocaine intake, as indicated by increased breakpoints during a PR schedule of reinforcement. After 10 days of abstinence, the intake of cocaine re-escalated only in animals that

had LgA to cocaine. During extinction, responding diminished more slowly in LgA than in ShA rats. During reinstatement, the latency to first self-infusion was shorter and subsequent cocaine intake was larger in rats that had LgA to the drug. The finding that ShA and LgA rats did not differ in their glucose/saccharin intake corroborates the notion that the mechanisms mediating self-administration of palatable solutions and cocaine are not identical (see also: Vendruscolo et al., 2010).

Tat-cyclotraxin-B reduces cocaine self-administration behavior.

Systemic administration of the brain-penetrant TrkB antagonist tat-cyclotraxin-B (TC) dose-dependently decreased cocaine self-administration in both ShA and LgA rats. TC also effectively reduced the motivation to work for cocaine as well as the drug-induced reinstatement of cocaine taking in both groups of animals. In contrast to repeated post-session intra-accumbal infusions of BDNF antiserum that produced a long-lasting reduction in various measures of cocaine dependence (Graham et al., 2007), the acute pre-session systemic injections of TC decreased cocaine self-administration for less than 2 hours. Differential half-lives of systemically-administered TC and locally-infused BNDF antiserum may explain this discrepancy. In addition, an opposing action of TC outside of the nucleus accumbens may also shorten the duration of its inhibitory effect on cocaine self-administration. Chronic treatment with TC, before or after the cocaine self-administration sessions, may lead to longer-lasting behavioral effects. The finding that rats repeatedly treated with the control peptide tat-empty (TE) showed cocaine self-administration levels similar to non-tat-treated (NT) rats suggests that the fusion peptide does not produce major side-effects on operant behavior. In addition, TC did not alter the intake of a highly rewarding sweet solution, demonstrating that the effect of TC on self-administration is specific for cocaine reward. These results support the hypothesis that BDNF signaling in the mesolimbic dopamine pathway is not recruited by sweet reinforcers (Grimm et al., 2003; Graham et al., 2009), and further indicate

indicates that TC-induced inhibition of cocaine self-administration cannot be attributed to a reduction of motor performance. Altogether, the data suggest that BDNF-TrkB signaling is not engaged upon moderate activation of the brain reward system by a palatable sweet drink, but gets recruited upon excessive activity of this system by cocaine. In addition, our data suggest that BDNF-TrkB signaling is activated by cocaine prior to the transition into dependence since we observed similar effects of TC in ShA and LgA rats.

Brain region-specific changes in TrkB signaling after cocaine self-administration.

At the molecular level, the increases in TrkB signaling we observed in the nucleus accumbens and ventral tegmental area of cocaine self-administering rats are consistent with the increases in BDNF protein levels previously reported in these brain regions following cocaine self-administration (Grimm et al., 2003; Pu et al., 2006; Graham et al., 2007; Graham et al., 2009; Schmidt et al., 2012; Fumagalli et al., 2013; Li et al., 2013). The decrease in TrkB signaling we observed in the prefrontal cortex is more surprising given that BNDF protein level in this brain region was previously reported to be increased by cocaine (Fumagalli et al., 2007; Lu et al., 2010; McGinty et al., 2010; Sadri-Vakili et al., 2010; Fumagalli et al., 2013). However, in these previous studies, BDNF upregulation in the prefrontal cortex was detected several hours or days after last cocaine exposure, while we collected brain samples immediately after the last cocaine self-administration session. In addition, the decrease in TrkB signaling we observed could represent a postsynaptic adaptation independent of BNDF synthesis and release.

Tat-cyclotraxin-B normalizes cocaine self-administration-induced changes in TrkB signaling.

We found that pretreatment with TC reverses the effects of cocaine self-administration on TrkB signaling in the nucleus accumbens and prefrontal cortex. Cyclotraxin-B is a potent and

specific inhibitor of TrkB and the paradigm of TC administration we used in the present study was previously shown to reduce the phosphorylation of central TrkB receptors by approximately 50% (Cazorla et al., 2010). Combining our results with the existing literature on the effects of BDNF system modulation in the mesocorticolimbic dopaminergic pathway (Horger et al., 1999; Lu et al., 2004; Graham et al., 2007; Bahi et al., 2008; Graham et al., 2009; Lu et al., 2009; Li et al., 2013), we propose that the reduction in TrkB signaling induced by TC in the nucleus accumbens drives the reduction in cocaine self-administration. We further hypothesize that the reversal of cocaine-induced decrease in cortical TrkB signaling by TC indirectly results from the blunting of cocaine-induced TrkB activation in the nucleus accumbens. According to this framework, the inhibitory effect of LgA cocaine self-administration on TrkB signaling in the prefrontal cortex would occur secondarily to the increased signaling observed in the nucleus accumbens. Such antagonistic feedback from the nucleus accumbens to the prefrontal cortex could be relayed via the ventral tegmental area, which receives inhibitory GABAergic inputs from the nucleus accumbens and sends dopaminergic projections to the prefrontal cortex (see Russo and Nestler (2013) or via cortico-striatal-pallidal-thalamic loops that provide a basal ganglia- cortical feedback (Haber SN, Fudge JL, McFarland NR. J Neurosci, 2000, 20:2369-2382). Reduction of cocaine self-administration by TC may ultimately result from the combined reversal of cocaine effects on TrkB signaling in the nucleus accumbens and prefrontal cortex, since both reduced TrkB signaling in the nucleus accumbens and increased TrkB signaling in the prefrontal cortex have previously been found to reduce cocaine seeking (Horger et al., 1999; Lu et al., 2004; Berglind et al., 2007; Graham et al., 2007; Bahi et al., 2008; Berglind et al., 2009; Graham et al., 2009; Lu et al., 2009; Sadri-Vakili et al., 2010; Whitfield et al., 2011; Li et al., 2013). In both brain regions, the observed changes in TrkB signaling were accompanied by similar changes in Akt, but not in ERK, indicating that the behavioral effects of cocaine and TC are intracellular pathway-specific.

An alternative interpretation of our molecular data would be that TC-induced changes in TrkB/Akt signaling result from, rather than drive, the reduced cocaine intake. However, the fact that TC did not affect TrkB and pTrkB levels in the ventral tegmental area of cocaine self-administering rats does not support this interpretation. The latter finding suggests that the effects of TC on TrkB signaling result from a brain region-specific action of TC, rather than being an indirect consequence of lower levels of cocaine exposure throughout the brain. Although the mechanism underlying this regional specificity of TC activity remains to be determined, it is worth noting that ANA-12, another brain-penetrant TrkB antagonist that, similar to TC, displays anxiolytic-like properties, exerts more potent TrkB inhibition in striatal than cortical areas (Cazorla et al., 2011).

Perspectives.

Given that dopaminergic signaling in the nucleus accumbens and prefrontal cortex plays a key role in the positively reinforcing effects of cocaine (Everitt and Robbins, 2005; Koob and Volkow, 2010; George et al., 2012), the inhibitory effect of TC on the cocaine intake of ShA rats probably results from a disruption of the complex interplay between BDNF and dopamine signaling in these dopamine-innervated regions. In addition, an action of TC on the glutamatergic neurons regulating accumbal dopamine release may also contribute (Berglind et al., 2009). The inhibitory effect of TC on the cocaine intake of LgA rats raises the possibility that incentive salience driven by negative reinforcement also involves BDNF signaling. Accordingly, genetic deletion of BDNF in ventral tegmental area dopaminergic neurons reduces social aversion elicited by repeated aggression (Berton et al., 2006) and systemic administration of the brain-penetrant TrkB inhibitors TC and ANA-12 exerts anxiolytic-like and anti-depressant-like properties in naïve mice (Cazorla et al., 2010; Cazorla et al., 2011). These findings therefore suggest that the effects of TC on inhibiting the negative emotional state associated with withdrawal may also contribute to the reduction in

cocaine intake in LgA rats. Altogether our study validates systemic TrkB antagonism as a potential new strategy to curb cocaine use disorders.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

The authors like to thank the Zardi-Gori Foundation for providing funding to FF and NIH grants AA020913 and AA024198 for supporting CC. MV, JH, GK and CC were also supported by a joint program of the Netherlands Organization for Scientific Research (zonMW) and the USA National Institute for Drug Abuse (NIDA), project no. 31180005. MV was also supported by an ECNP research grant for young scientists and a NIDA INVEST Drug Abuse Research Fellowship. The NIDA Intramural Research Program supported LF and GK. We also wish to thank Kiki Rink and Famke Ouwerkerk (department of Cognitive Neuroscience, Radboud University Nijmegen, the Netherlands) for technical assistance.

References

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Altar CA, Boylan CB, Jackson C, Hershenson S, Miller J, Wiegand SJ, Lindsay RM, Hyman C (1992) Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. Proc Natl Acad Sci 89:11347-11351.

Bahi A, Boyer F, Chandrasekar V, Dreyer JL (2008) Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. Psychopharmacology (Berl) 199:169-182.

Berglind WJ, Whitfield TW, Jr., LaLumiere RT, Kalivas PW, McGinty JF (2009) A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. J Neurosci 29:3715-3719.

Berglind WJ, See RE, Fuchs RA, Ghee SM, Whitfield TW, Jr., Miller SW, McGinty JF (2007) A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. Eur J Neurosci 26:757-766.

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864-868.

Cazorla M, Premont J, Mann A, Girard N, Kellendonk C, Rognan D (2011) Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. J Clin Invest 121:1846-1857.

Cazorla M, Jouvenceau A, Rose C, Guilloux JP, Pilon C, Dranovsky A, Premont J (2010) Cyclotraxin-B, the first highly potent and selective TrkB inhibitor, has anxiolytic properties in mice. PLoS One 5:e9777.

Corominas M, Roncero C, Ribases M, Castells X, Casas M (2007) Brain-derived neurotrophic factor and its intracellular signaling pathways in cocaine addiction. Neuropsychobiology 55:2-13.

Duman RS, Voleti B (2012) Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends Neurosci 35:47-56.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481-1489.

Filip M, Faron-Gorecka A, Kusmider M, Golda A, Frankowska M, Dziedzicka-Wasylewska M (2006) Alterations in BDNF and trkB mRNAs following acute or sensitizing cocaine treatments and withdrawal. Brain Res 1071:218-225.

Fumagalli F, Caffino L, Racagni G, Riva MA (2009) Repeated stress prevents cocaine-induced activation of BDNF signaling in rat prefrontal cortex. Eur Neuropsychopharmacol 19:402-408.

Fumagalli F, Di Pasquale L, Caffino L, Racagni G, Riva MA (2007) Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex. Eur J Neurosci 26:2756-2763.

Fumagalli F, Moro F, Caffino L, Orru A, Cassina C, Giannotti G, Di Clemente A, Racagni G, Riva MA, Cervo L (2013) Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats. Int J Neuropsychopharmacol 16:913-918.

George O, Le Moal M, Koob GF (2012) Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems. Physiol Behav 106:58-64.

Giannotti G, Caffino L, Calabrese F, Racagni G, Riva MA, Fumagalli F (2014) Prolonged abstinence from developmental cocaine exposure dysregulates BDNF and its signaling network in the medial prefrontal cortex of adult rats. Int J Neuropsychopharmacol 17:625-634.

Goggi J, Pullar IA, Carney SL, Bradford HF (2003) Signalling pathways involved in the short-term potentiation of dopamine release by BDNF. Brain Res 968:156-161.

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

Graham DL, Krishnan V, Larson EB, Graham A, Edwards S, Bachtell RK, Simmons D, Gent LM, Berton O, Bolanos CA, DiLeone RJ, Parada LF, Nestler EJ, Self DW (2009) Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. Biol Psychiatry 65:696-701.

Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y (2003) Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742-747.

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P (2001) BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411:86-89.

Gump JM, Dowdy SF (2007) TAT transduction: the molecular mechanism and therapeutic prospects. Trends Mol Med 13:443-448.

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134:943-944.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR (1999) Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 19:4110-4122.

Huang CC, Yeh CM, Wu MY, Chang AY, Chan JY, Chan SH, Hsu KS (2011) Cocaine withdrawal impairs metabotropic glutamate receptor-dependent long-term depression in the nucleus accumbens. J Neurosci 31:4194-4203.

Koob GF (2009) Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology 56 Suppl 1:18-31.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217-238.

Kozorovitskiy Y, Saunders A, Johnson CA, Lowell BB, Sabatini BL (2012) Recurrent network activity drives striatal synaptogenesis. Nature 485:646-650.

Le Foll B, Diaz J, Sokoloff P (2005) A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. Neuroreport 16:175-178.

Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Meredith GE, Ford KA, Ferrario CR, Loweth JA, Wolf ME (2013) Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance. J Neurosci 33:1130-1142.

Lobo MK, Covington HE, 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ (2010) Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science 330:385-390.

Lu H, Cheng PL, Lim BK, Khoshnevisrad N, Poo MM (2010) Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition. Neuron 67:821-833.

Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y (2004) A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J Neurosci 24:1604-1611.

Lu L, Wang X, Wu P, Xu C, Zhao M, Morales M, Harvey BK, Hoffer BJ, Shaham Y (2009) Role of ventral tegmental area glial cell line-derived neurotrophic factor in incubation of cocaine craving. Biol Psychiatry 66:137-145.

Martin-Fardon R, Weiss F (2014) N-(2-methyl-6-benzoxazolyl)-N'-1,5-naphthyridin-4-yl urea (SB334867), a hypocretin receptor-1 antagonist, preferentially prevents ethanol seeking: comparison with natural reward seeking. Addict Biol 19:233–236.

Martin-Iverson MT, Todd KG, Altar CA (1994) Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine. J Neurosci 14:1262-1270.

McCarthy DM, Brown AN, Bhide PG (2012) Regulation of BDNF expression by cocaine. Yale J Biol Med 85:437-446.

McGinty JF, Whitfield TW, Jr., Berglind WJ (2010) Brain-derived neurotrophic factor and cocaine addiction. Brain Res 1314:183-193.

Orio L, Edwards S, George O, Parsons LH, Koob GF (2009) A role for the endocannabinoid system in the increased motivation for cocaine in extended-access conditions. J Neurosci 29:4846-4857.

Pu L, Liu QS, Poo MM (2006) BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. Nat Neurosci 9:605-607.

Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66:1-11.

Russo SJ, Nestler EJ (2013) The brain reward circuitry in mood disorders. Nat Rev Neurosci 14:609-625.

Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM, Overland RP, Xia E, Bass CE, Terwilliger EF, Pierce RC, Cha JH (2010) Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. J Neurosci 30:11735-11744.

Schmidt HD, Sangrey GR, Darnell SB, Schassburger RL, Cha JH, Pierce RC, Sadri-Vakili G (2012) Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon I-containing promoters. J Neurochem 120:202-209.

Vendruscolo LF, Gueye AB, Darnaudery M, Ahmed SH, Cador M (2010) Sugar overconsumption during adolescence selectively alters motivation and reward function in adult rats. PLoS One 5:e9296.

Verheij MM, de Mulder EL, De Leonibus E, van Loo KM, Cools AR (2008) Rats that differentially respond to cocaine differ in their dopaminergic storage capacity of the nucleus accumbens. J Neurochem 105:2122-2133.

Wee S, Specio SE, Koob GF (2007) Effects of dose and session duration on cocaine self-administration in rats. J Pharmacol Exp Ther 320:1134-1143.

Wee S, Vendruscolo LF, Misra KK, Schlosburg JE, Koob GF (2012) A combination of buprenorphine and naltrexone blocks compulsive cocaine intake in rodents without producing dependence. Sci Transl Med 4:146ra110.

Whitfield TW, Jr., Shi X, Sun WL, McGinty JF (2011) The suppressive effect of an intra-prefrontal cortical infusion of BDNF on cocaine-seeking is Trk receptor and extracellular signal-regulated protein kinase mitogen-activated protein kinase dependent. J Neurosci 31:834-842.

Table legends

Table 1: Effects of 7.5 mg/kg TC on the number of active and inactive lever presses, as well as active lever pressing during the timeout period. TC reduced the number of active lever presses to obtain cocaine (gray cells) without affecting either inactive lever pressing or the number of active lever presses to obtain glucose/saccharin solution (white cells). Values shown are mean number of lever presses ± SEM following either TE or TC pretreatment. F and P values were obtained using a one-way ANOVA with the factor treatment.

Figure legends

Figure 1: Schematic representation of the cocaine and glucose/saccharin self-administration experiments. The effects of TC and its control peptide (TE) were tested using a within-subjects Latin square design in which test days were separated by a drug free (no tat: NT) day. During these 'NT' days, rats had access to cocaine without drug treatment. Note: The animals of group A received TE followed by TC two experimental days later, whereas the animals of group B received TC followed by TE two experimental days later.

Figure 2: Cocaine self-administration in LgA, but not ShA rats increased over time (A). $^{\#}$Significant increase *vs.* session 14 (t-test: $P<0.05$). The initial drug loading (B) and the motivation to work for cocaine (C) were increased in LgA compared with ShA rats. ^Significant difference (one-way ANOVA: $P<0.05$) compared to ShA (B-C), *Significant difference (one-way ANOVA: $P<0.05$) compared to session 14 (B), n.s: no change compared to session 14 (B). ShA: n=11; LgA: n=11.

Figure 3: Intravenous administration of the control peptide TE did not alter cocaine self-administration under a fixed ratio 1 (FR1) schedule of reinforcement in both ShA and LgA conditions (A-B). Systemic administration of the TrkB antagonist TC dose-dependently reduced ShA (C) and LgA (D) cocaine intake. The dose of 7.5 mg/kg TC also reduced the motivation to work for cocaine under a progressive ratio (PR) schedule of reinforcement in both ShA (E) and LgA (F) rats. *Significant decrease (LSD: $P<0.05$) *vs.* TC 0.0 mg/kg (C-D) or significant decrease (one-way ANOVA: $P<0.05$) *vs.* the control peptide TE (E-F), n.s: no change compared to 'no tat' (NT) days (A-B) or TC 0.0 mg/kg (D). In C-F, values are normalized to preceding 'no tat' sessions. ShA: n=11 (A-C) or n=10 (E); LgA: n=11 (B, D and F).

Figure 4: Similar self-administration of a sweet solution in rats with a history of ShA and LgA to cocaine (A). #Significant increase *vs.* session 60 (t-test: $P<0.05$). Intravenous TC did not alter glucose/saccharin intake in these animals (B). n.s: no change *vs.* control peptide (TE). In B, values are normalized to preceding 'no tat' sessions. ShA: n=10, LgA: n=11.

Figure 5: Re-escalation of cocaine intake in rats with a history of LgA, but not in rats with a history of ShA, to cocaine (A). #Significant increase *vs.* session 70 (t-test: $P<0.05$). Slower extinction of responding in rats with a history of LgA cocaine self-administration (B). #Significant increase *vs.* baseline responding (t-test: $P<0.05$). ShA: n=9, LgA: n=10.

Figure 6: Greater drug-induced reinstatement of cocaine intake in LgA compared with ShA rats (A). Intravenous TC reduced drug-induced reinstatement of cocaine intake in both ShA (B) and LgA (C) rats. The latency to the first self-infusion was shorter in rats primed with a cocaine infusion than in rats primed with a saline infusion (D) and TC increased this latency more strongly in LgA than ShA rats (E vs F). *Significant difference (one-way ANOVA: $P<0.05$) compared to saline (SAL, A and D) or control peptide TE (B-C and E-F). ^Significant difference (one-way

ANOVA: P<0.05) compared to ShA (A and D). In B-C and E-F, values are normalized to preceding 'no tat' sessions. ShA: n=9, LgA: n=10. Note: cocaine intake remained stable throughout the four reinstatement sessions (B-C: TE bar is not different from 100% (one-sample t-test: n.s)), which suggest that the motivational drive for responding was not substantially affected by session repetition.

Figure 7: Cocaine-induced increase in TrkB (A), pTrkB Y706 (B), Akt (C) and pAkt S473 (D), but not in ERK1 (E) and pERK1 T202/Y204 (F) protein levels in the nucleus accumbens (N. Acc.). *Significant increase (t-test: P<0.05) *vs.* TE + no COC (A-D). TC prevented the cocaine-induced increase in protein levels. #Significant reduction (t-test: P<0.05) *vs.* TE + COC (A-D). n.s: no difference between TE + COC and TE + no COC or no difference between TC + COC and TE + COC (E-F). In each panel, values are normalized to 'TE + no COC' and pictures show representative Western blot bands of the corresponding proteins (left lane: TE + no COC, middle lane: TE + COC and right lane: TC + COC). TE + no COC: n=10, TE + COC: n=12, TC + COC: n=13.

Figure 8: Cocaine-induced reduction in pTrkB Y706 (B), and pAkt S473 (D), but not in TrkB (A), Akt (C), ERK1 (E) and pERK1 T202/Y204 (F) protein levels in the medial prefrontal cortex (mPFC). *Significant decrease (t-test: P<0.05) *vs.* TE + no COC (B and D). TC prevented the cocaine-induced decrease in protein levels. #Significant increase (t-test: P<0.05) *vs* TE + COC (B and D). n.s: no difference between TE + COC and TE + no COC or no difference between TC + COC and TE + COC (A, C, E and F). In each panel, values are normalized to 'TE + no COC' and pictures show representative Western blot bands of the corresponding proteins (left lane: TE + no COC, middle lane: TE + COC and right lane: TC + COC). TE + no COC: n=10, TE + COC: n=12, TC + COC: n=13.

Figure 9: No effect of TC on the cocaine-induced increase in TrkB (A) and pTrkB Y706 (B) protein levels in the ventral tegmental area (VTA). *Significant increase (t-test: $P<0.05$) *vs.* TE + no COC (A-B). n.s: no difference between TC + COC and TE + COC (A-B). In each panel, values are normalized to 'TE + no COC' and pictures show representative Western blot bands of the corresponding proteins (left lane: TE + no COC, middle lane: TE + COC and right lane: TC + COC). TE + no COC: n=10, TE + COC: n=12, TC + COC: n=13.