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ORIGINAL INVESTIGATION

Resveratrol fails to affect cocaine conditioned place preference behavior, but alleviates anxiety-like behaviors in cocaine withdrawn rats

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Abstract
Rationale Resveratrol participates in regulating abnormal behaviors in psychostimulant-exposed animals.
Objectives To examine effects of resveratrol on relapse and anxiety-like behaviors in cocaine withdrawn rats and to investigate possible molecular mechanisms underlying resveratrol effects in hippocampus (HP) and prefrontal cortex (PFC).
Methods Conditioned place preference (CPP) assay and elevated plus maze (EPM) test were used to examine cocaine CPP behavior and anxiety-like behaviors in rats, respectively. Resveratrol was administrated to cocaine withdrawn rats. Levels of MDA, GSH and SOD were examined to evaluate oxidative status, and levels of IL-6, IL-1β and TNF α were measured to examine inflammatory status and levels of caspase-3 and BAX was examined to evaluate apoptotic status in HP and PFC. SIRT expression was also examined here.
Results Resveratrol did not affect cocaine CPP behaviors, but attenuated anxiety-like behaviors in cocaine withdrawn rats. Levels of MDA and TNFα in PFC, and levels of MDA, SOD, GSH, IL-6, IL-1β, TNFα, caspase-3 and BAX in HP, but not SIRT1 expression in both regions were significantly changed during cocaine withdrawal period. Except SOD, resveratrol reversed above neurochemical changes induced by cocaine withdrawal. Furthermore, RSV induced a greater upregulation of SIRT1 expression in PFC in cocaine withdrawn rats than that in saline controls.
Conclusions Current findings suggest that resveratrol may influence behaviors in cocaine withdrawn rats. Oxidative stress, inflammation, apoptosis, and SIRT1 signaling pathway in HP or PFC might be involved in mediating effects of RSV on behaviors in cocaine withdrawn rats.

Keywords Cocaine · Resveratrol · Withdrawal · Conditioned place preference · Anxiety · SIRT1

Abbreviations

CAS3	Caspase-3
CPP	Conditioned place preference
EPM	Elevated plus maze
GSH	Glutathione
HP	Hippocampus
IL-1β	Interleukin 1β
IL-6	Interleukin 6
MDA	Malondialdehyde
PFC	Prefrontal cortex
RSV	Resveratrol
SIRT1	Sirtunin1
SOD	Superoxide dismutase
TNF α	Tumor necrosis factor α

Introduction

Cocaine addiction represents a chronic mental disorder that is characterized by a loss of control over drug intake and compulsive drug taking despite negative consequences. Treatment of cocaine addiction has been challenged by the high rate of

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relapse. Cocaine withdrawal symptoms greatly increase the risk of cocaine relapse (Gawin 1991; Gawin and Kleber 1986; Sinha 2001). Unlike heroin or alcohol, physical withdrawal syndromes are uncommon during cocaine withdrawal period (Gawin and Kleber 1986; Naifeh et al. 2012; Perrine et al. 2008). However, psychological symptoms, such as depression, fear, anxiety, malaise, and agitation, are intense and considered as characteristic cocaine withdrawal symptoms (El Hage et al. 2012; O'Leary et al. 2000; Perrine et al. 2008). In order to avoid these psychological withdrawal problems and to gain "euphoria" feeling, individuals often seek for cocaine intake. Unfortunately, there has been a lack of therapeutic drugs in clinic that can effectively alleviate cocaine withdrawal symptoms and reduce the relapse to cocaine abuse.

Resveratrol (RSV) is a natural phytoalexin found in a variety of plants including grapes, peanuts, and berries (Bhat et al. 2001). RSV can pass blood-brain barrier and may be a potent neuroprotective compound against neurodegenerative diseases, including stroke, Alzheimer's disease, Parkinson's disease, and epilepsy (Shetty 2011; Sun et al. 2010; Wight et al. 2012; Zhao et al. 2013). Furthermore, RSV administration has been reported to exert anxiolytic-like effects and enhance cortex- and hippocampus-dependent memories in rodents (Ali et al. 2015; Damián et al. 2014; Zhao et al. 2013). Recent studies suggested that repeated RSV may attenuate hyperactivity induced by methamphetamine (Miller et al. 2013), but acute RSV administration could enhance cocaine-increased locomotive behaviors (Shuto et al. 2013). These RSV effects on drugs of abuse are presumably via mediating neurotransmitter signaling pathway in the brain, such as dopamine (Miller et al. 2013; Shuto et al. 2013). However, most previous studies focus on the effects of RSV treatment on the changes in rewarding and locomotive behaviors induced by drugs exposure. Few animal studies explore the effects of RSV treatment on behaviors and neurochemical changes in cocaine withdrawn rats.

In the current study, we aim to examine the effects of repeated RSV treatment on cocaine conditioned place preference (CPP) behaviors and anxiety-like behaviors in cocaine withdrawn rats and to investigate its possible molecular mechanisms in brain. The acquisition, extinction, and reinstatement of cocaine CPP assay in rats were used to mimic different phases of cocaine abuse in humans, including chronic exposure, abstinence, and relapse periods. Elevated plus maze (EPM) was used to assess anxiety-like behaviors in animals. Hippocampus (HP) and prefrontal cortex (PFC) are two important brain regions that are closely associated with cocaine addiction and also with emotional processes such as anxiety (El Hage et al. 2012; Valzachi et al. 2013; Peters et al. 2009; Perry et al. 2011; Rogers and See 2007). RSV is thought to exert its beneficial effects against neurodegenerative diseases through its anti-inflammatory, anti-apoptotic, and antioxidant properties (Liu et al. 2012; Shetty 2011). Also, RSV serves as an activator of SIRT1 (Zhao et al. 2013), a class III histone deacetylase, which participates in the rewarding effects of cocaine and morphine (Ferguson et al. 2013, 2015; Renthal et al. 2009). Accordingly, we subsequently examined the effects of repeated RSV treatment on changes in oxidative stress, inflammatory, apoptotic status, and SIRT1 expression in HP and PFC in cocaine withdrawn rats.

Materials and methods

Animals Male Sprague-Dawley rats (280–300 g) were used in this study. Rats were maintained on a reverse light/dark cycle with food and water available ad libitum. All rats were allowed to acclimate for 7 days before receiving any experimental manipulation. All experiments were performed in accordance with the Nanjing Medical University's Guide for the Care and Use of Laboratory Animals, China and were approved by the Nanjing Medical University Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and to minimize their suffering.

Conditioned place preference (CPP) and drug treatment The experimental timeline for CPP procedure and drug treatment is shown in Fig. 1. Thirty-two adult male rats ($n = 8$ per group) were subjected to CPP assay. CPP was conducted in an apparatus constructed of three chambers (72 × 25 × 32 cm, Zhenghua Biologic Apparatus, China). The two larger side chambers (30.5× 25 × 32 cm each) differ in their walls (black or black with white stripes) and floors (stainless-steel mesh or stainless-steel bars). The smaller middle chamber (11 × 25 × 32 cm) has gray walls with a smooth PVC floor. The three distinct chambers are separated by removable guillotine doors. Time spent in each chamber was recorded by cameras connected to a computer. On D0 and D1, some rats were free to explore the three chambers for 15 min. Time spent in each chamber was calculated by computer and recorded as baseline data. Rats that spent more than 500 s in one chamber were dismissed from testing. From D2 to D7, CPP training was performed for six consecutive days with twice daily injections. The first injection was performed in the morning with administration of cocaine (10 mg/kg, i.p., C group) or saline (1 ml/kg, i.p., S group), and the rats were confined to one non-preferred conditioning side chamber (drug-paired chamber, biased procedure) for 30 min. Cocaine hydrochloride (Qinghai Pharmaceutical, China) was dissolved in sterile saline. The second injection was performed in the afternoon with administration of saline (1 ml/kg, i.p.), and the rats were confined to the other side chamber (drug-unpaired chamber) for 30 min. In subsequent post-conditioning tests, rats moved freely throughout the apparatus for 15 min and time spent in drug-paired chamber were recorded and defined as CPP

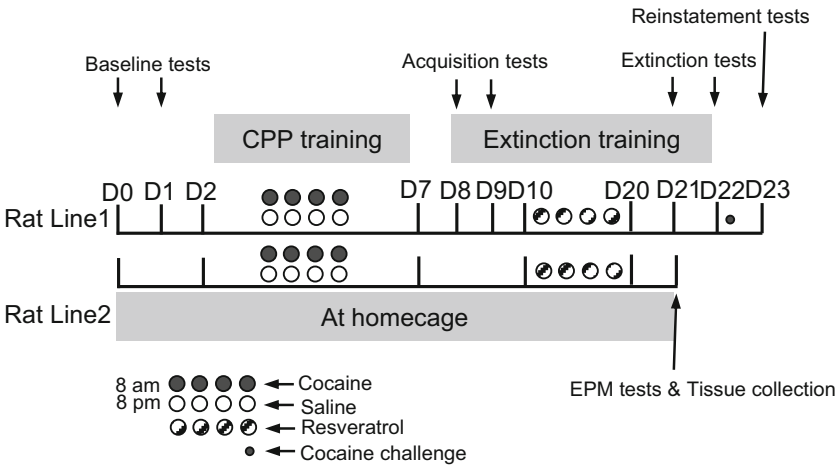

Fig. 1 Scheme of experimental design. *CPP* conditioned place preference, *D* day, *EPM* elevated plus maze

scores. The ratio of time spent in drug-paired chamber to that in drug-unpaired chamber is calculated and defined as CPP ratio. Tests for acquisition of CPP behavior were carried out on D8 and D9. Subsequently, rats were subjected to extinction training of cocaine conditioning during which all rats can freely access three chambers for 15 min each day without any injection. On D21 and D22, tests for extinction of CPP behavior were performed. On D23, rats received a small dose of cocaine (5 mg/kg, i.p.) injection and were subsequently subjected to test reinstatement behavior of cocaine CPP.

CPP is considered to be acquired when CPP score or CPP ratio is significantly higher than baseline. CPP is considered to be extinct when there are no differences in CPP scores and CPP ratios between obtained on D22 and on D1. CPP is considered to be reinstated when CPP scores and CPP ratios obtained after cocaine challenge on D23 are higher than those on D22.

In previous reports, dosages of 20 to 100 mg/kg of systemic resveratrol (RSV) administration were often used in rodents to investigate its protective roles in brain (Blanchet et al. 2008; Della-Morte et al. 2009; Gupta et al. 2001; Singleton et al. 2010; Shuto et al. 2013). In our preliminary experiments, we found that higher doses (90 and 110 mg/kg) of RSV, but not lower and medium doses (20, 40, and 60 mg/kg) of RSV were able to significantly increase central time of open field tests in repeated cocaine-exposed rats, suggesting its possible anxiolytic effects in rats (data not shown here). All these dosages of RSV failed to influence cocaine-primed reinstatement of cocaine CPP behaviors in rats. However, repeated RSV administration with dose of 110 mg/kg resulted in diarrhea in rats. Thus, we used 90 mg/kg dose of RSV in the current study. From D10 to D20, rats were injected with vehicle (1 ml/kg, s.c.) or RSV (90 mg/kg, s.c.) once daily which was prepared in a DMSO-containing stock and diluted by water vehicle. RSV was injected at 30 min prior to CPP extinction trainings.

Elevated plus maze behavior (EPM) and tissue preparation The other line of rats ($N=32$, $n=8$ per group) without experiencing CPP procedure was used to perform EPM. From D2 to D7, these rats were injected with cocaine (10 mg/kg, i.p.) or saline (1 ml/kg, i.p., controls) once daily. On D0, D1, and D8–D22, they were kept in their home cages without cocaine or saline administration. Thus, D8–D22 is considered as withdrawal period from cocaine treatment for these rats. From D10 to D20, rats were injected with vehicle (1 ml/kg, s.c.) or resveratrol (RSV, 90 mg/kg, s.c.) once daily. On P22, EPM was conducted to assess anxiety-like behavior in cocaine withdrawn rats.

The EPM apparatus consists of four elevated arms (70 cm above the floor) arranged in a cross pattern. It has two open arms bordered by clear plastic ledges (0.5 cm tall) and two closed arms bordered by black opaque walls (40 cm tall). Rats were placed in the center portion of the EPM facing an open arm and allowed to explore the maze for 5 min. Distance traveled, the number of entries into open arms, and the time spent in each arm were recorded and calculated by computer.

Rats were divided into four groups: saline withdrawn rats that received vehicle (S-vehicle), saline withdrawn rats that received 90 mg/kg of RSV (S-RSV), cocaine withdrawn rats with vehicle (C-vehicle), and cocaine withdrawn rat that received 90 mg/kg of RSV (C-RSV). At the end of EPM experiments, rats were immediately anesthetized and decapitated and their brains were quickly removed. Hippocampus (HP) and prefrontal cortex (PFC) tissues were dissected and frozen in liquid nitrogen and stored at 80 °C for subsequent oxidative assays and western blots. The experimental timeline for EPM procedure and tissue collection is shown in Fig. 1.

Oxidative stress assays All samples were homogenized individually. The homogenate was centrifuged and the supernatant used for the oxidative stress assay. We used commercial detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to measure activity of superoxide dismutase (SOD), the levels of glutathione (GSH), and malondialdehyde (MDA) according to the manufacturer's instructions. Six rats in each group were used for oxidative stress analysis.

Western blot Total protein was extracted from each sample using RIPA lysis buffer (Beyotime Institute of Biotechnology, China). Protein was separated by 8–10 % SDS-PAGE and electrophoretically transferred onto PVDF membranes. The transferred membranes were incubated with one of the following target primary anti-mouse antibodies at 4 °C overnight: caspase-3 (CAS3, Boster Biotechnology, China), BAX (Boster Biotechnology, China), interleukin 6 (IL-6, Boster Biotechnology, China), interleukin 1β (IL-1β, Boster Biotechnology, China), tumor necrosis factor α (TNFα, Boster Biotechnology, China), and sirtunin1 (SIRT1, Santa Cruz Biotechnology, USA); the membranes were incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, USA) at room temperature for 1 h. The blots were visualized by an ECL kit (Beyotime Institute of Biotechnology, China). β-actin (anti-rabbit, Santa Cruz Biotechnology, USA) was used as a loading control. Stripping buffer (Beyotime Institute of Biotechnology, China) was used to remove the above target antibodies from the blotted membranes in order to re-probe for the loading control on the same membranes. Values for protein levels were calculated using Image J software (NIH, USA) and normalized to β-actin. Fold changes against values in S-vehicle group were calculated and used to perform statistical analysis. Four rats in each group were used for western blot analysis.

Statistical analysis GraphPad Prism 6 (GraphPad Software, USA) was used to perform statistical analysis. All results are expressed as mean ± standard deviation. CPP data among groups were analyzed by one-way ANOVA with a Student-Newman-Keuls multiple comparisons test. EPM data, oxidative status data, and western blot data were analyzed by two-way ANOVA and Bonferroni post hoc test. Drug models (cocaine vs. saline) served as "A factor"; Dose treatment (vehicle vs. 90 mg/kg RSV) served as "B factor". $p < 0.05$ was considered to be significant in all tests.

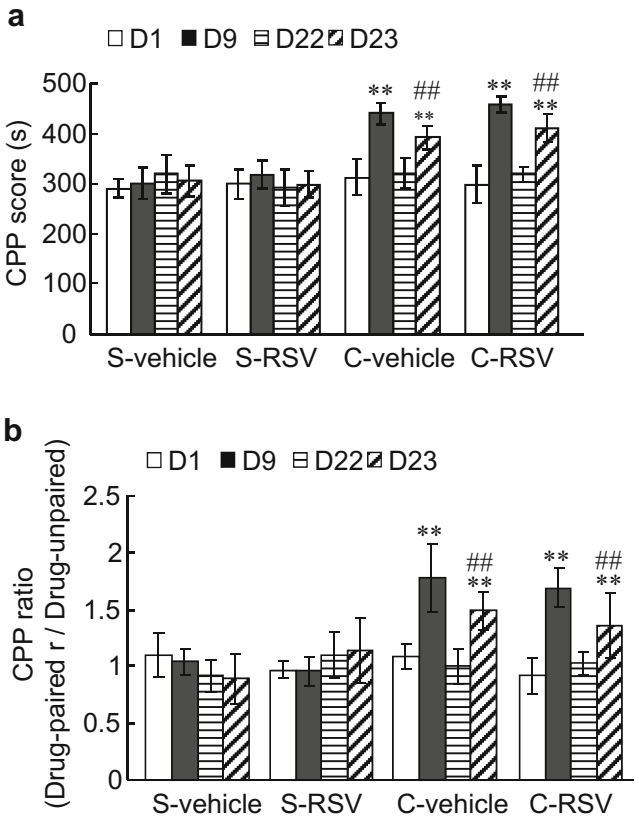

Fig. 2 Effects of resveratrol on conditioned place preference (*CPP*) behaviors in rats. **a** Cocaine scores. **b** CPP ratio of time spent in drug-paired chamber to that in drug-unpaired chamber. Baseline data were recorded on day 1 (*D1*). Acquisition tests of cocaine CPP were performed on D9. Extinction tests of cocaine CPP were performed on D22. Reinstatement tests of cocaine CPP were performed on D23. Resveratrol (90 mg/kg) or vehicle was given from D10 to D20 once daily. $^{**}p < 0.01$ vs. baseline on D1, $^{\#\#}p < 0.01$ vs. D9, $n = 8$ per group. *S-vehicle*, saline withdrawn rats with 11-day administrations of vehicle. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. *C-vehicle*, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

Results

Resveratrol did not affect cocaine CPP behavior in cocaine withdrawn rats

Following 6-day cocaine exposure during CPP training phase, rats showed higher CPP scores and CPP ratios on D9 than the baseline ($F_{(3,28)} = 33.77$, $p < 0.01$ vs. D1, Fig. 2a, b), suggesting an acquisition of cocaine CPP. Following 14-day extinction training, a CPP extinct behavior was obtained in cocaine withdrawn rats, as indicated by a similar CPP scores and CPP ratios between obtained on D22 and on D1 ($p > 0.05$). As shown in Fig. 2, RSV treatment (90 mg/kg once daily) during withdrawal period had no influence on CPP extinction ($F_{(3,28)} = 85.72$, $p > 0.05$ vs. corresponding vehicle group). There were no significant differences in CPP scores and CPP ratios on D23 between vehicle and RSV90 groups ($p > 0.05$), suggesting that RSV treatment did not affect the reinstatement of cocaine CPP.

Resveratrol decreased anxiety-like behavior in cocaine withdrawn rats

To examine anxiety-like behavior during cocaine withdrawal period, rats were subjected to EPM tests. As shown in Fig. 3a, there are no differences in distance traveled by rat among groups in EPM tests, indicating that both chronic cocaine exposure and RSV treatment had no effect on locomotive behaviors in cocaine withdrawn rats ($F_{(1,28)} = 1.36$ for A factor, $F_{(1,28)} = 0.01$ for B factor, $F_{(1,28)} = 0.21$ for interaction, $p > 0.05$). Cocaine withdrawn rats spent less time in the open

arms ($F_{(1,28)}$ = 125.40 for A factor, $F_{(1,28)}$ = 7.25 for B factor, $F_{(1,28)}$ = 8.07 for interaction, p < 0.01 vs. S-vehicle) and more time in the close arms ($F_{(1,28)}$ = 90.76 for A factor, $F_{(1,28)}$ = 5.30 for B factor, $F_{(1,28)}$ = 3.53 for interaction) than those in saline withdrawn rats (p < 0.01 vs. S-vehicle, Fig. 3b). Furthermore, rats in C-vehicle group enter into open arms at few times than that in saline control group ($F_{(1,28)}$ = 43.28 for A factor, $F_{(1,28)}$ = 7.02 for B factor, $F_{(1,28)}$ = 6.14 for interaction, p < 0.01 vs. S-vehicle, Fig. 3c). These results indicated that an increased level of anxiety occurred in cocaine withdrawn rats. Repeated RSV treatment at the doses of 90 mg/kg significantly increased the time in the open arm (p < 0.01 vs. C-vehicle) and the number of entries into open arms (p < 0.01 vs. C-vehicle) in cocaine withdrawn rats, suggesting an alleviated effects of RSV on increased anxiety-like behavior in cocaine withdrawn rats.

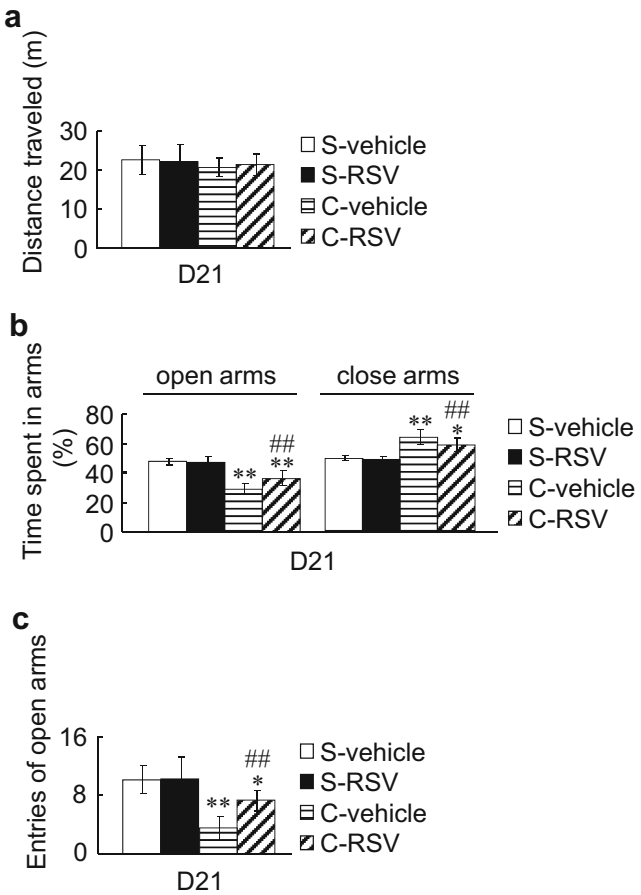

Fig. 3 Effects of resveratrol on elevated plus maze (*EPM*) tests in rats. **a** Distances traveled by rat. **b** Time spent in open arms and close arms of EPM apparatus. **c** Entries into open arms. **p < 0.01 vs. S-vehicle, ##p < 0.01 vs. C-RSV, n = 8 per group. *S-vehicle*, saline withdrawn rats with 11-day administrations of vehicle. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. C-vehicle, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

Effect of resveratrol on oxidative and antioxidant status in HP and PFC of cocaine withdrawn rats

The status of lipid oxidation was determined by measuring levels of MDA. As shown in Fig. 4a, MDA levels were significantly increased in both HP ($F_{(1,20)}$ = 57.10 for A factor, $F_{(1,20)}$ = 8.93 for B factor, $F_{(1,20)}$ = 7.73 for interaction) and PFC ($F_{(1,20)}$ = 66.01 for A factor, $F_{(1,20)}$ = 9.12 for B factor, $F_{(1,20)}$ = 9.41 for interaction) of cocaine withdrawn rats, as compared to saline withdrawn controls (p < 0.01). Repetitive RSV treatment partially reduced this elevation in MDA level in HP (p < 0.01 vs. C-vehicle), but did not affect that in PFC (p > 0.05), in cocaine withdrawn rats.

The antioxidant capacity was determined by measuring the levels of GSH and activities of SOD (Fig. 4b, c). In PFC, there were no differences in SOD activities among groups ($F_{(1,20)}$ = 0.08 for A factor, $F_{(1,20)}$ = 0.02 for B factor, $F_{(1,20)}$ = 0.01 for interaction, p > 0.05). In HP, there was an increased SOD activity ($F_{(1,20)}$ = 64.89 for A factor, $F_{(1,20)}$ = 0.21 for B factor, $F_{(1,20)}$ = 0.12 for interaction), but a decreased GSH level ($F_{(1,20)}$ = 4.33 for A factor, $F_{(1,20)}$ = 2.41 for

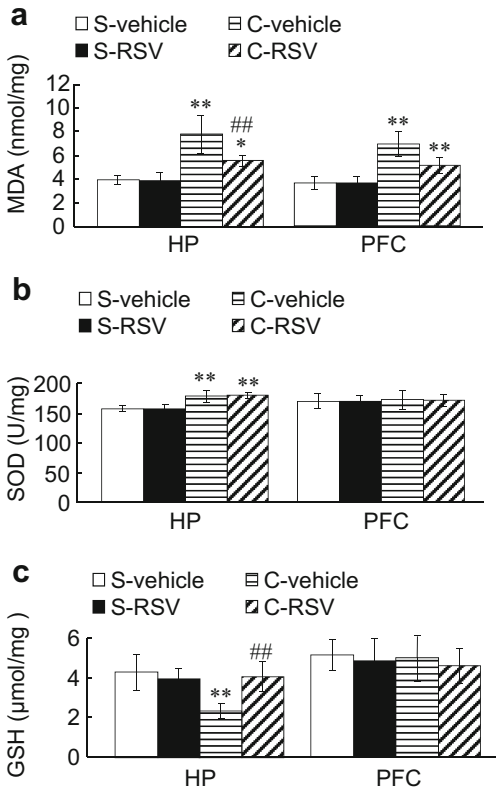

Fig. 4 Effects of resveratrol on oxidative and antioxidant status in hippocampus (*HP*) and prefrontal cortex (*PFC*) in rats. **a** Levels of malondialdehyde (*MDA*). **b** Activities of superoxide dismutase (*SOD*). **c** Levels of glutathione (*GSH*). *p < 0.05, **p < 0.01 vs. S-vehicle, ##p < 0.01 vs. C-vehicle, n = 6 per group. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. *C-vehicle*, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

B factor, $F_{(1,20)}=5.79$ for interaction) in cocaine withdrawn rats, as compared to that in saline withdrawn rats ($p<0.01$). RSV treatment did not affect the increased SOD activity ($p>0.05$ vs. C-vehicle), but normalized the elevated GSH level in HP induced by cocaine withdrawal ($p<0.01$ vs. C-vehicle). In saline withdrawn rats, RSV treatment did not affect the physiological level of MDA and GSH, and the activity of SOD in HP and PFC ($p>0.05$ vs. S-vehicle).

Effects of resveratrol on inflammatory status in HP and PFC of cocaine withdrawn rats

To examine the inflammatory status, protein levels of IL-6, IL-1β, and TNFa were measured in HP and PFC (Fig. 5a–d). Levels of IL-6 ($F_{(1,12)}=330.10$ for A factor, $F_{(1,12)}=47.59$ for B factor, $F_{(1,12)}=49.78$ for interaction), IL-1β ($F_{(1,12)}=529.70$ for A factor, $F_{(1,12)}=44.25$ for B factor, $F_{(1,12)}=39.1$ for interaction), and TNFa ($F_{(1,12)}=333.80$ for A factor, $F_{(1,12)}=64.97$ for B factor, $F_{(1,12)}=67.89$ for interaction) were significantly increased in HP of cocaine withdrawn rats, as compared to saline control ($p<0.01$). Repetitive RSV treatment partially reduced cocaine withdrawal-induced increase of IL-6, IL-1β, and TNFa expression in HP ($p<0.01$ vs. C-vehicle). In PFC, the level of TNFa ($F_{(1,12)}=189.3$ for A factor, $F_{(1,12)}=50.75$ for B factor, $F_{(1,12)}=48.01$ for interaction), but not IL-6 ($F_{(1,12)}=0.002$ for A factor, $F_{(1,12)}=0.002$ for B factor, $F_{(1,12)}=0.002$ for interaction, $p>0.05$) and IL-1β ($F_{(1,12)}=0.09$ for A factor, $F_{(1,12)}=0.0008$ for B factor, $F_{(1,12)}=0.001$ for interaction, $p>0.05$), was increased in rats after cocaine withdrawal, compared to saline controls ($p<0.01$). RSV treatment also partially reduced the increased TNFa level in PFC of cocaine withdrawn rats ($p<0.01$ vs. C-vehicle). There were no differences in the levels of IL-6, IL-1β, and TNFa between saline withdrawn groups that received vehicle and RSV administration ($p>0.05$).

Effects of resveratrol on apoptotic status in HP and PFC of cocaine withdrawn rats

To investigate apoptotic status in HP and PFC, we examined the protein levels of CAS3 and BAX (Fig. 6a–c). In HP, levels of CAS3 ($F_{(1,12)}=164.60$ for A factor, $F_{(1,12)}=34.30$ for B factor, $F_{(1,12)}=34.83$ for interaction) and Bax ($F_{(1,12)}=14.84$ for A factor, $F_{(1,12)}=11.22$ for B factor, $F_{(1,12)}=21.74$ for interaction) were significantly increased after cocaine withdrawal, compared to that in saline withdrawn control ($p<0.01$). Repetitive RSV treatment partially attenuated the upregulated CAS3 level, and completely normalized the increased BAX level in HP of cocaine withdrawn rats ($p<0.01$ vs. C-vehicle). In PFC, there were no differences in CAS3 ($F_{(1,12)}=0.01$ for A factor, $F_{(1,12)}=0.03$ for B factor, $F_{(1,12)}=0.11$ for interaction) and BAX ($F_{(1,12)}=2.10$ for A factor, $F_{(1,12)}=0.17$ for B factor, $F_{(1,12)}=0.5$ for interaction) levels among groups ($p>0.05$).

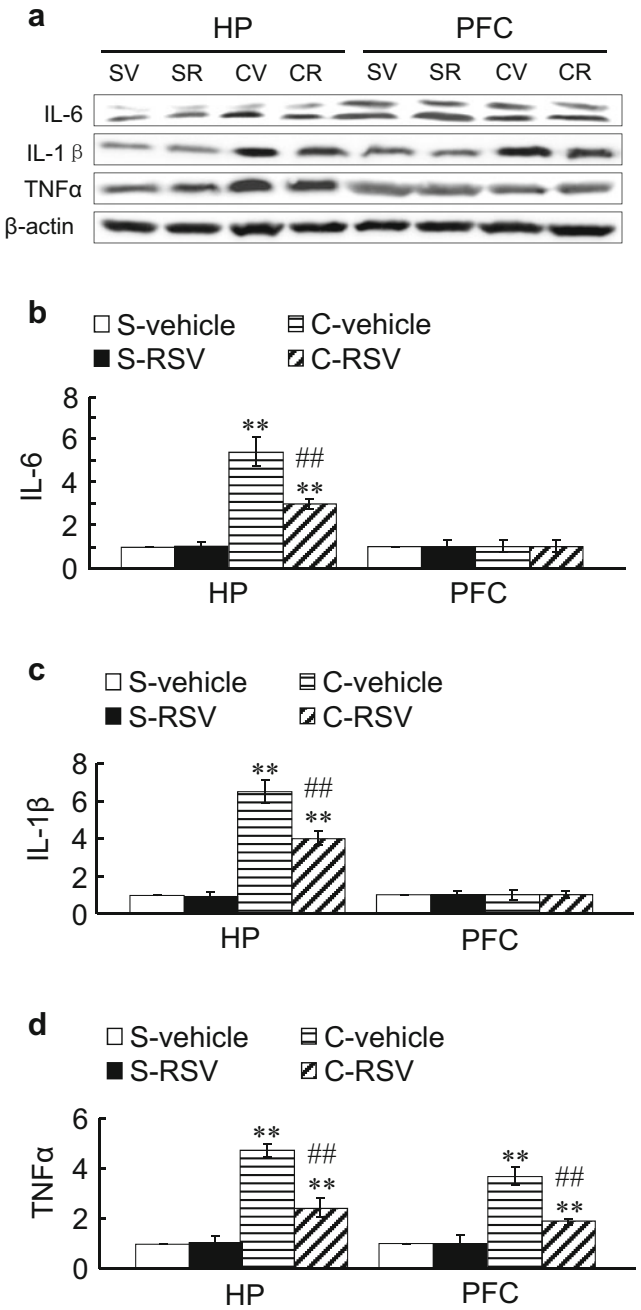

Fig. 5 Effects of resveratrol on inflammatory status in hippocampus (*HP*) and prefrontal cortex (*PFC*) in rats. **a** Representative protein bands. **b** Fold changes of interleukin 6 (IL-6). **c** Fold changes of interleukin 1β (*IL-1β*). **d** Fold changes of tumor necrosis factor α (*TNFα*). $^{**}p<0.01$ vs. S-vehicle, $^{\#\#}p<0.01$ vs. C-RSV, $n=4$ per group. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. *C-vehicle*, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

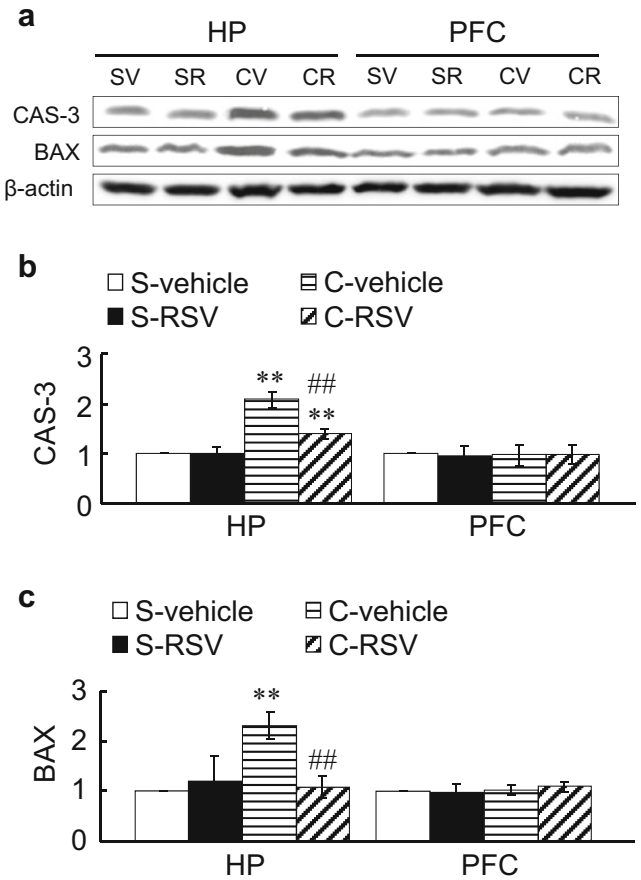

Fig. 6 Effects of resveratrol on apoptotic status in hippocampus (*HP*) and prefrontal cortex (*PFC*) in rats. **a** Representative protein bands. **b** Fold changes of caspase-3 (*CAS3*). **c** Fold changes of BAX. $^{**}p < 0.01$ vs. S-vehicle, $^{\#\#}p < 0.01$ vs. C-RSV, $n = 4$ per group. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. *C-vehicle*, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

Effects of resveratrol on SIRT1 expression in HP and PFC of cocaine withdrawn rats

RSV is an activator of SIRT1 protein (Zhao et al. 2013). Cocaine withdrawal alone (C-vehicle) did not change SIRT1 expression in HP and PFC, as compared to saline withdrawn controls (S-vehicle, $p < 0.01$, Fig. 7a, b). Repetitive RSV treatment significantly increased SIRT1 expressions in HP ($F_{(1,12)} = 0.1$ for A factor, $F_{(1,12)} = 116.60$ for B factor, $F_{(1,12)} = 1.17$ for interaction) and PFC ($F_{(1,12)} = 32.04$ for A factor, $F_{(1,12)} = 135.30$ for B factor, $F_{(1,12)} = 27.65$ for interaction) in both saline withdrawn and cocaine withdrawn rats, as compared to corresponding vehicle ($p < 0.01$). In addition, the levels of SIRT1 in PFC, but not in HP, from cocaine withdrawn rats that received RSV (C-RSV) were significantly higher than those in saline withdrawn rats received the same RSV treatment ($p < 0.01$ vs. S-RSV).

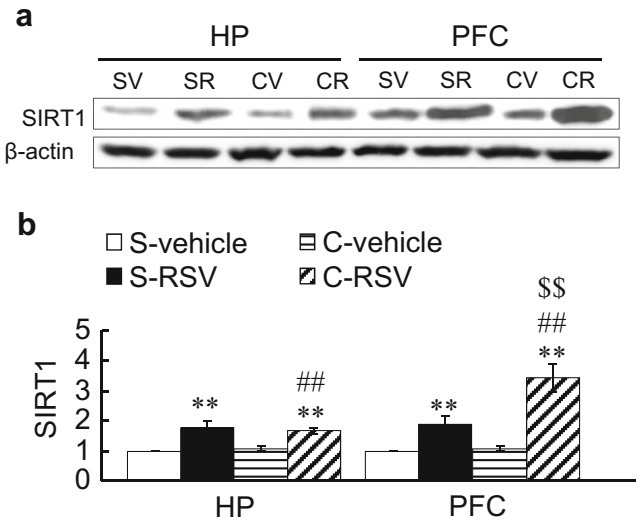

Fig. 7 Effects of resveratrol on Sirtunin1 (*SIRT1*) expressions in hippocampus (*HP*) and prefrontal cortex (*PFC*) in rats. **a** Representative protein bands. **b** Fold changes of SIRT1 protein. $^{**}p < 0.01$ vs. S-vehicle, $^{\#\#}p < 0.01$ vs. C-RSV, $\$\$p < 0.01$ vs. S-RSV, $n = 4$ per group. *S-RSV*, saline withdrawn rats with 11-day administrations of RSV. *C-vehicle*, cocaine withdrawn rats with 11-day administrations of vehicle. *C-RSV*, cocaine withdrawn rats with 11-day administrations of RSV

Discussion

Increasing amount of evidence suggested that RSV may be against neurodegenerative diseases, such as stroke, Alzheimer's disease, Parkinson's disease, and epilepsy (Shetty 2011; Sun et al. 2010; Wight et al. 2012; Zhao et al. 2013). Regarding psychostimulant addiction, recent study suggested that acute RSV treatment enhanced cocaine-induced hyperactivity in mice (Shuto et al. 2013); Repeated RSV treatments do not affect cocaine-related hyperactivity, but it attenuate methamphetamine-induced hyperactivity (Miller et al. 2013). Both studies demonstrate that the effects of RSV on behavioral effects of psychostimulants by regulating dopaminergic system (Miller et al. 2013; Shuto et al. 2013). Based on these findings, RSV maybe influences on cocaine CPP behaviors, such as extinction and reinstatement, in withdrawn rats. Unexpected, repeated RSV treatment at a dose of 90 mg/kg in cocaine withdrawn rats failed to affect the extinction and cocaine-primed reinstatement of cocaine CPP in this study. Accordingly, differences in drug treatment paradigm and in the timing of RSV administration may exert different effects on brain disorders induced by cocaine abuse.

Abstinence from chronic cocaine intake (i.e., withdrawal) produces a variety of negative emotional problems, such as anxiety and depression-related behaviors (O'Leary et al. 2000). Severe anxiety is considered to contribute to relapse to cocaine (Gawin 1991; Sinha 2001). Consistent with previous findings in animals (Aujla et al. 2008; El Hage et al. 2012; Perrine et al. 2008), we found that cocaine withdrawn rats showed an increased level of anxiety behaviors, as indicated

by EPM tests. RSV has been reported to attenuate anxiety-like behaviors and has antidepressant properties in mice (Ali et al. 2015; Damián et al. 2014). In line with these findings, here, we found that repetitive daily RSV treatment during withdrawal period partially alleviated anxiety-like behaviors in cocaine withdrawn rats. Brain regions that are involved in dysfunctional aspects of anxiety processing in cocaine abusers are not well-understood. Both human and animal studies suggest that structural changes and functional deficits in HP and PFC may contribute to anxiety disorders produced by cocaine dependence and withdrawal (El Hage et al. 2012; Valzachi et al. 2013). Furthermore, RSV may exert its protective effects through its anti-inflammatory, anti-apoptotic, and antioxidant properties (Liu et al. 2012; Shetty 2011). Thus, we examined oxidant, inflammatory, and apoptotic status in HP and PFC to explore the possible neurochemical mechanisms that may underlie the effects of RSV on anxiety-like behavior in cocaine withdrawn rats.

In line with previous findings (Alvaro-Bartolomé et al. 2011; Pomierny-Chamioło et al. 2013; Valzachi et al. 2013), we found a higher level of oxidative stress and increased expression of pro-inflammatory factors in HP and PFC in cocaine withdrawn rats. Compared to PFC, HP may be more susceptible to neuronal damage by cocaine withdrawal, as indicated by increased levels of pro-apoptotic factors in HP, but not in PFC of cocaine withdrawn rats. As expected, here, we found that repetitive RSV treatment decreased oxidative stress, increased antioxidant capacity, and normalized the increased levels of pro-inflammatory and apoptotic factors in HP and PFC of cocaine withdrawn rats. Abnormal behaviors in cocaine abuser, including increased anxiety during cocaine withdrawal, are closely associated with impaired oxidative, inflammatory, and apoptotic statuses in brain (Alvaro-Bartolomé et al. 2011; Levandowski et al. 2014; Zaparte et al. 2015). Therefore, RSV might exert a protective effect against cocaine withdrawal-induced anxiety behavior via limiting these pathological changes in the HP and PFC.

RSV is an activator of sirtunin1 (SIRT1) (Zhao et al. 2013), a class III histone deacetylases that has been implicated in various processes in peripheral tissues, including growth, apoptosis, oxidative stress, cell morphology, and immunology (Bordo 2013; Herranz and Serrano 2010; Orozco-Solis et al. 2015). Recent studies suggest SIRT1 may also play an important role in brain functions, such as modulating synaptic plasticity and memory formation (Donmez and Outeiro 2013; Ma et al. 2014). More importantly, SIRT1 signaling pathway may be involved in drug addiction. For example, chronic cocaine exposure increased SIRT1 expression in the nucleus accumbens (NAc) in mice (Ferguson et al. 2013). Moreover, overexpression of SIRT1 in NAc enhances the rewarding effects of cocaine and morphine. In the present study, cocaine withdrawal did not change SIRT expression in HP and PFC. Thus, SIRT pathway may participate in cocaine addiction with a brain region-specific manner and in an addiction phase-specific pattern. Interestingly, repetitive RSV treatment significantly increased SIRT expression in HP and PFC in both cocaine withdrawn rats and saline withdrawn controls. Furthermore, the increased SIRT level by RSV treatment is much higher in PFC from cocaine withdrawn rats than that from saline withdrawn rat, suggesting an increased sensitivity to RSV during cocaine withdrawal. Future study is required to investigate the possible roles of SIRT pathway in response to RSV in cocaine withdrawn rats.

In conclusion, current study shows that RSV exerts anxiolytic effects in cocaine withdrawn rats, and this protective effect may be through its inhibition of oxidative stress, inflammation, apoptosis, and an increase of SIRT1 expression in HP and PFC. These findings may have important implications for future exploration of the neurobiology of RSV and SIRT1 on drug addiction and contribute to developing medications against cocaine addiction.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Ali SH, Madhana RM, Athira KV, Kasala ER, Bodduluru LN, Pitta S, Mahareddy JR, Lahkar M (2015) Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. Steroids 101:37–42

Alvaro-Bartolomé M, La Harpe R, Callado LF, Meana JJ, García-Sevilla JA (2011) Molecular adaptations of apoptotic pathways and signaling partners in the cerebral cortex of human cocaine addicts and cocaine-treated rats. Neuroscience 196:1–15

Aujla H, Martin-Fardon R, Weiss F (2008) Rats with extended access to cocaine exhibit increased stress reactivity and sensitivity to the anxiolytic-like effects of the mGluR 2/3 agonist LY379268 during abstinence. Neuropsychopharmacology 33:1818–1826

Bhat KPL, Kosmeder JW 2nd, Pezzuto JM (2001) Biological effects of resveratrol. Antioxid Redox Signal 3:1041–1064

Blanchet J, Longpré F, Bureau G, Morissette M, DiPaolo T, Bronchti G, Martinoli MG (2008) Resveratrol, a red wine polyphenol, protects dopaminergic neurons in MPTP-treated mice. Prog Neuropsychopharmacol Biol Psychiatry 32:1243–1250

Bordo D (2013) Structure and evolution of human sirtuins. Curr Drug Targets 14:662–665

Damián JP, Acosta V, Da Cuña M, Ramírez I, Oddone N, Zambrana A, Bervejillo V, Benech JC (2014) Effect of resveratrol on behavioral performance of streptozotocin-induced diabetic mice in anxiety tests. Exp Anim 63:277–287

Della-Morte D, Dave KR, DeFazio RA, Bao YC, Raval AP, Perez-Pinzon MA (2009) Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. Neuroscience 159:993–1002

Donmez G, Outeiro TF (2013) SIRT1 and SIRT2: emerging targets in neurodegeneration. EMBO Mol Med 5:344–352

El Hage C, Rappeneau V, Etievant A, Morel A-L, Scarna H, Zimmer L, Bérod A (2012) Enhanced anxiety observed in cocaine withdrawn rats is associated with altered reactivity of the dorsomedial prefrontal cortex. PLoS One 7, e43535

Ferguson D, Koo JW, Feng J, Heller E, Rabkin J, Heshmati M, Renthal W, Neve R, Liu X, Shao N, Sartorelli V, Shen L, Nestler EJ (2013) Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. J Neurosci 33:16088–16098

Ferguson D, Shao N, Heller E, Feng J, Neve R, Kim HD, Call T, Magazu S, Shen L, Nestler EJ (2015) SIRT1-FOXO3a regulate cocaine actions in the nucleus accumbens. J Neurosci 35:3100–3111

Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251:1580–1586

Gawin FH, Kleber HD (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers: clinical observations. Arch Gen Psychiatry 43:107–113

Gupta YK, Chaudhary G, Sinha K, Srivastava AK (2001) Protective effect of resveratrol against intracortical FeCl3-induced model of posttraumatic seizures in rats. Methods Find Exp Clin Pharmacol 23:241–244

Herranz D, Serrano M (2010) SIRT1: recent lessons from mouse models. Nat Rev Cancer 10:819–823

Levandowski ML, Viola TW, Wearick-Silva LE, Wieck A, Tractenberg SG, Brietzke E, Bauer ME, Teixeira AL, Grassi-Oliveira R (2014) Early life stress and tumor necrosis factor superfamily in crack cocaine withdrawal. J Psychiatr Res 53:180–186

Liu GS, Zhang ZS, Yang B, He W (2012) Resveratrol attenuates oxidative damage and ameliorates cognitive impairment in the brain of senescence-accelerated mice. Life Sci 91:872–877

Ma CY, Yao MJ, Zhai QW, Jiao JW, Yuan XB, Poo MM (2014) SIRT1 suppresses self-renewal of adult hippocampal neural stem cells. Development 141:4697–4709

Miller DK, Oelrichs CE, Sage AS, Sun GY, Simonyi A (2013) Repeated resveratrol treatment attenuates methamphetamine-induced hyperactivity and [3H]dopamine overflow in rodents. Neurosci Lett 554:53–58

Naifeh JA, Tull MT, Gratz KL (2012) Anxiety sensitivity, emotional avoidance, and PTSD symptom severity among crack/cocaine dependent patients in residential treatment. Cognit Ther Res 36:247–257

O'Leary TA, Rohsenow DJ, Martin R, Colby SM, Eaton CA, Monti PM (2000) The relationship between anxiety levels and outcome of cocaine abuse treatment. Am J Drug Alcohol Abuse 26:179–194

Orozco-Solis R, Ramadori G, Coppari R, Sassone-Corsi P (2015) SIRT1 relays nutritional inputs to the circadian clock through the Sf1 neurons of the ventromedial hypothalamus. Endocrinology 156:2174–2184

Perrine SA, Sheikh IS, Nwaneshiudu CA, Schroeder JA, Unterwald EM (2008) Withdrawal from chronic administration of cocaine decreases delta opioid receptor signaling and increases anxiety- and depression-like behaviors in the rat. Neuropharmacology 54:355–364

Perry JL, Joseph JE, Jiang Y, Zimmerman RS, Kelly TH, Darna M, Huettl P, Dwoskin LP, Bardo MT (2011) Prefrontal cortex and drug abuse vulnerability: translation to prevention and treatment interventions. Brain Res Rev 65:124–149

Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279–288

Pomierny-Chamioło L, Moniczewski A, Wydra K, Suder A, Filip M (2013) Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine. Neurotox Res 23:92–102

Renthal W, Kumar A, Xiao G, Wilkinson M, Covington HE 3rd, Maze I, Sikder D, Robison AJ, LaPlant Q, Dietz DM, Russo SJ, Vialou V, Chakravarty S, Kodadek TJ, Stack A, Kabbaj M, Nestler EJ (2009) Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 62:335–348

Rogers JL, See RE (2007) Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. Neurobiol Learn Mem 87:688–692

Shetty AK (2011) Promise of resveratrol for easing status epilepticus and epilepsy. Pharmacol Ther 131:269–286

Shuto T, Kuroiwa M, Koga Y, Kawahara Y, Sotogaku N, Toyomasu K, Nishi A (2013) Acute effects of resveratrol to enhance cocaine-induced dopamine neurotransmission in the striatum. Neurosci Lett 542:107–112

Singleton RH, Yan HQ, Fellows-Mayle W, Dixon CE (2010) Resveratrol attenuates behavioral impairments and reduces cortical and hippocampal loss in a rat controlled cortical impact model of traumatic brain injury. J Neurotrauma 27:1091–1099

Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl) 158:343–359

Sun AY, Wang Q, Simonyi A, Sun GY (2010) Resveratrol as a therapeutic agent for neurodegenerative diseases. Mol Neurobiol 41:375–383

Valzachi MC, Teodorov E, Marcourakis T, Bailey A, Camarini R (2013) Enhancement of behavioral sensitization, anxiety-like behavior, and Hippocampal and frontal cortical CREB levels following cocaine abstinence in mice exposed to cocaine during adolescence. PLoS One 8, e78317

Wight RD, Tull CA, Deel MW, Stroope BL, Eubanks AG, Chavis JA, Drew PD, Hensley LL (2012) Resveratrol effects on astrocyte function: relevance to neurodegenerative diseases. Biochem Biophys Res Commun 426:112–115

Zaparte A, Viola TW, Grassi-Oliveira R, da Silva Morrone M, Moreira JC, Bauer ME (2015) Early abstinence of crack-cocaine is effective to attenuate oxidative stress and to improve antioxidant defences. Psychopharmacology (Berl) 232:1405–1413

Zhao YN, Li WF, Li F, Zhang Z, Dai YD, Xu AL, Qi C, Gao JM, Gao J (2013) Resveratrol improves learning and memory in normally aged mice through microRNA-CREB pathway. Biochem Biophys Res Commun 435:597–602