Pharmacology, Biochemistry and Behavior 92 (2009) 603–607

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Tetrodotoxin reduces cue-induced drug craving and anxiety in abstinent heroin addicts

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ARTICLE INFO

Article history:
Received 18 November 2008
Received in revised form 14 February 2009
Accepted 24 February 2009
Available online 5 March 2009

Keywords:
Drug cues
Heroin dependence
Tetrodotoxin
Craving

ABSTRACT

Background: Tetrodotoxin (TTX) is a neurotoxin found in puffer fish and other marine animals. New clinical studies suggest that low-dose TTX can safely relieve severe, treatment-resistant cancer pain. The therapeutic potential of TTX in addiction is supported by studies in laboratory animals. The purpose of this double-blind, placebo-controlled study was to assess the effect of a single intramuscular dose of TTX on cue-induced craving and anxiety in abstinent heroin addicts.
Methods: Forty-five abstinent heroin addicts were randomly assigned to three treatment groups: placebo, 5 μg TTX, or 10 μg TTX. Participants were exposed to a neutral video or a heroin-related video. Craving, anxiety, blood pressure, and heart rate were measured pre- and post-exposure.
Results: Heroin-related cues increased both craving and anxiety and had no effect on blood pressure and heart rate. A single dose of TTX dose-dependently attenuated the increases in craving and anxiety while having no effect on blood pressure or heart rate.
Conclusion: The results suggest that low-dose TTX is acutely effective in reducing cue-induced increases in heroin craving and associated anxiety.

1. Introduction

Successful treatment of heroin dependence is deterred by the high rate of relapse. Even after long periods of abstinence, heroin-dependent individuals are vulnerable to impulsive drug use when in the presence of stimuli related to previous episodes of use (Carter and Tiffany, 1999), and relapse frequently occurs (O'Brien, 1997). Thus, prevention and attenuation of relapse is a high priority for clinicians. Numerous preclinical and clinical studies have shown that relapse to drug seeking can be induced after extended periods of abstinence by exposure to drug-associated environmental cues (Carter and Tiffany, 1999) or exposure to stressors (Kosten et al., 1986; Sinha et al., 1999; Shaham et al., 2000; Lu et al., 2003). These findings correspond with the clinical observation that drug-dependent individuals appear to use drug or relapse more frequently in environments associated with prior drug use.

Sensitivity to cues in humans has frequently been studied in terms of physiological and psychological responses in the cue-reactivity paradigm (Carter and Tiffany, 1999; Sinha et al., 1999). Drug-associated cues tested in this paradigm can include the sight of drug paraphernalia (Yu et al., 2007), imagery of craving (Weinstein et al., 1997), drug-related pictures (Waters et al., 2003), or drug-related words, sentences, and videos (Ooteman et al., 2006; Ren et al., 2009, in press; Shi et al., 2008). Such cues tend to induce changes in physiological measures such as heart rate, blood pressure, and withdrawal signs (Carter and Tiffany, 1999), and in psychological measures such as craving and mood (Fox et al., 2005). In daily life, these responses may contribute to relapse to drug abuse. Thus, identifying treatments that diminish such responses is greatly important (Sinha et al., 1999).

One seemingly unlikely candidate for the prevention of relapse is tetrodotoxin (TTX), a neurotoxin found in puffer fish and other marine animals (Narahashi et al., 1994). TTX inhibits the generation of electrical impulses in neurons by blocking voltage-dependent sodium channels (Narahashi, 1972). Until recently, TTX has been used mainly as a tool in physiological and pharmacological studies. However, new clinical studies suggest that TTX in low doses can safely relieve severe, treatment-resistant cancer pain (Hagen et al., 2007, 2008).

The therapeutic potential of TTX in addiction is supported by studies in laboratory animals. Most of these studies have involved microinjections of TTX into specific brain regions. For example, TTX injected bilaterally into the basolateral amygdala and prefrontal cortex in rats selectively decreased conditioned responding for cocaine after extinction, whereas TTX inactivation of the nucleus accumbens significantly blocked the primary rewarding effects of cocaine after extinction (Grimm and See, 2000; Shaham et al., 2000; McLaughlin and See, 2003). In another such study, TTX injected into the

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doi:10.1016/j.pbb.2009.02.013

Table 1
Schedule for laboratory session.

Time	Event / activity
– 65: 00 min	Participant arrival; measures of heart rate, blood pressure, craving, anxiety.
– 60: 00 min	Tetrodotoxin/placebo administration.
Neutral-cue video	
0: 00 min	Baseline period, online measurement of heart rate, blood pressure, craving, anxiety.
0: 05 min	Neutral-cue video shown.
0: 10 min	Online measurement of heart rate, blood pressure, craving, anxiety.
0: 15 min	10 min relaxation period.
Heroin-cue video	
0: 25 min	Baseline period, online measurement of heart rate, blood pressure, craving, anxiety.
0: 30 min	Heroin-cue video shown.
0: 35 min	Online measurement of heart rate, blood pressure, craving, anxiety.

basolateral amygdala in rats abolished the ability of heroin-paired stimuli and heroin priming to reinstate responding for heroin (Fuchs and See, 2002). However, a finding of more direct clinical relevance is that *systemic* administration of TTX significantly inhibited morphine withdrawal symptoms in rats and mice (Chen et al., 2001). In this study, it is demonstrated that intramuscular TTX pretreatment significantly reduced the naloxone-precipitated withdrawal symptoms including jump in morphine-dependent mice and weight loss both in morphine-dependent rats and mice, an effect similar to clonidine pretreatment. Moreover, this study also indicated that the same dose of TTX in attenuation of withdrawal symptoms did not change the heart rate, blood pressure, and breathe rate in anesthetic rats (Chen et al., 2001). Although little is known about the mechanisms by which this occurred, it suggests that TTX may be useful in the treatment of opiate dependence.

We conducted a double-blind, placebo-controlled study to assess the effect of single doses of intramuscular TTX on cue-induced craving and anxiety in abstinent heroin addicts. Given the pharmacological action of TTX on the cardiovascular system, we also evaluated the cardiovascular effects of the doses administered.

2. Materials and methods

2.1. Participants

Participants were recruited from an inpatient treatment center of the Yichang Addiction Rehabilitation Center, Yichang, China. Inclusion criteria included the following: (1) men or nonpregnant/nursing women 18–45 years old, (2) heroin dependence as assessed by the Structured Clinical Interview for DSM-IV (SCID), but opiate-free for at least 1 month, and (3) no prior or current use of cocaine or other illicit drugs. Exclusion criteria included the following: (1) current or past cardiovascular disease, (2) history of allergy (food, medicine), (3) current or past psychiatric illness, (4) neurological signs and/or history of neurological disease, (5) current medical illness, and (6) participation in other clinical trials of medications within the past 3 months. All participants gave written informed consent. The study was approved by the Human Investigation Committee of Peking University Health Center.

2.2. Study design

Fifty participants provided informed consent and underwent baseline assessments. Five were excluded due to abnormal laboratory tests ($n=3$) or heart problems ($n=2$). The remaining 45 participants were randomly assigned to three groups: 5 μg TTX, 10 μg TTX, and placebo. The placebo used was 0.21 μg solution of citric acid, which was the vehicle for TTX. The TTX and placebo were administered by intramuscular injection, because previous studies have shown that intramuscular TTX is well tolerated at doses from 7.5 mg b.i.d. to 30 mg b.i.d. (Hagen et al., 2007; 2008).

Each participant underwent a screening interview for demographic and heroin-abuse characteristics and administration of the Hamilton Anxiety Scale (HAMA) and Beck Depression Inventory (BDI). On the morning of the experimental day, participants were intramuscularly administered TTX or placebo 1 h before cue exposure. Heart rate and blood pressure were monitored online using a 9062D monitor (Baozhong Biotechnology Company, Beijing, China). Side effects were recorded as they occurred and were treated as necessary. Cue exposure occurred over two sessions, with a neutral videotape first and a heroin-related videotape second. This fixed order was chosen to prevent a "carry-over" effect from drug-related cues to neutral cues (Weinstein et al., 1997). Baseline psychological measures (including craving and anxiety ratings) and physiological measures (including blood pressure and heart rate) were obtained 5 min before each session and also obtained immediately after cue presentation. A 10 min interval occurred between the two sessions. Participants were allowed to leave when their physiological measures had returned to baseline levels. The schedule of assessments is shown in Table 1.

2.3. Psychological and physiologic measurements

The HAMA and BDI were used to assess anxiety and depression. Craving and anxiety before and after cue exposure were assessed with a 10-point visual analog scale (VAS; (Sinha et al., 1999), in which participants marked from 1 ("not at all") to 10 ("extremely high") their response to the question, "How much do you feel an urge to use heroin?" Two kinds of videotapes (neutral and heroin-related), each 5 min in length, were used as cues. The neutral videotape involved scenes that were non-emotional in content, such as birds, flowers, or trees. The heroin-related videotape included heroin-use scenes (Shi et al., 2007; Yu et al., 2007; Ren et al., 2009, in press).

2.4. Data analysis

Demographic and clinical characteristics of the three groups were assessed using one-way analysis of variance (ANOVA) and *post hoc t*-test if the omnibus F was significant. One-way ANOVA was used to analyze baseline scores on craving and anxiety measures. Repeated-measures ANOVAs with the within-subjects factors Stimulus Type (heroin cue and neutral cue) and Exposure Time (pre- and post-cue exposure) and the between-subjects factor Group (placebo, 5 and 10 μg TTX) were used to compare scores on craving and anxiety measures. Change scores were analyzed by two-way ANOVA with the between-group factor Treatment (placebo, 5 and 10 μg TTX) and the within-group factor Stimulus Type (heroin cue and neutral cue). *Post hoc* Fisher LSD tests were used when appropriate. P values less than or

Table 2
Demographic and clinical characteristics of participants.

	Placebo	TTX (5 μg)	TTX (10 μg)	p
	$n=15$	$n=15$	$n=15$	
Age (years)	31.53 ± 5.95	31.80 ± 5.17	31.20 ± 5.65	0.96
Male/female (n)	10/5	10/5	10/5	
Education (years)	10.73 ± 2.76	10.40 ± 1.84	10.33 ± 1.50	0.86
Duration of heroin use (years)	5.97 ± 3.18	5.53 ± 2.97	5.90 ± 1.61	0.89
Average dose abused (g/day)	0.55 ± 0.32	0.50 ± 0.23	0.57 ± 0.26	0.79
Duration of abstinence (months)	5.08 ± 0.76	5.51 ± 1.78	5.42 ± 1.46	0.68
HAMA score	5.80 ± 0.76	5.87 ± 0.80	6.07 ± 0.71	0.96
BDI score	11.27 ± 1.08	11.13 ± 1.10	11.67 ± 1.22	0.94

Values are expressed as mean ± SD. No significant differences were found on any demographic measures.

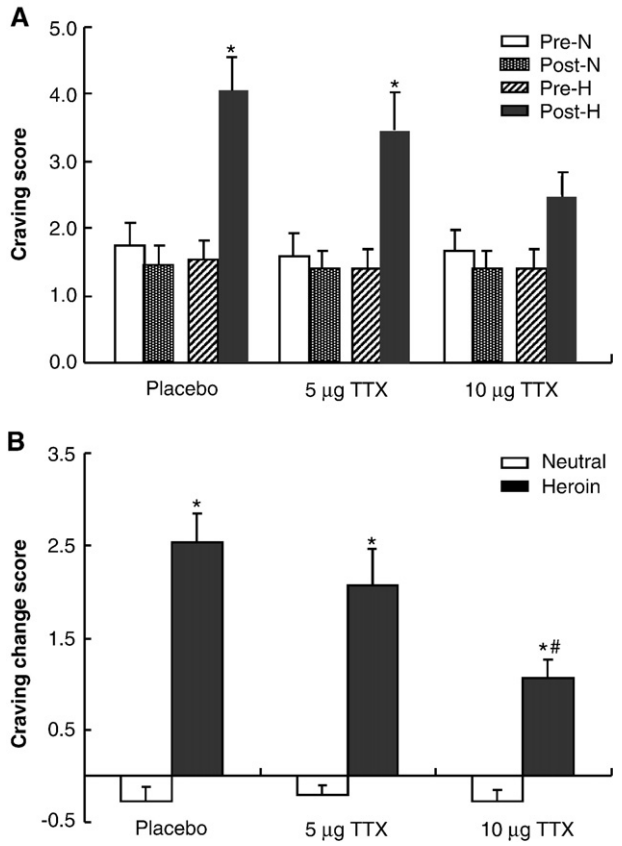

Fig. 1. Means (A) and mean change scores (B) for craving in response to neutral and heroin cues. Pre-N and Post N: before and after exposure to neutral cues. Pre-H and Post-H: before and after exposure to heroin cues. Drug condition (placebo, 5 and 10 μg TTX) differed across groups. Each group was exposed to both types of cues. (A) $^{*}p<0.05$, compared with Pre-N, Pre-H, and Post-H. (B) $^{*}p<0.05$, compared with neutral image stimuli within the same group. $^{\#}p<0.05$, compared with placebo and 5 μg TTX group.

equal to 0.05 were considered statistically significant. The analyses were performed with the GLM procedure in SAS 8.0.

3. Results

3.1. Demographics

Forty-five patients were enrolled and completed the study. Each group included 10 men and 5 women. Most of them had relapsed more than once. Based on their self-reports, the most common precipitant of relapse was environmental cues, and the second most common was stress or frustration. As shown in Table 2, no significant differences were observed among the three groups with regard to demographic and heroin-abuse characteristics. Levels of anxiety on the HAMA were within the normal range. However, the mean BDI score (>10) suggested moderate depression.

3.2. Drug craving

During baseline (shown in Fig. 1A as "Pre-N" and "Pre-H"), no significant group differences in craving scores were found prior to exposure to neutral cues or heroin cues. After cue exposure, craving increased more in response to heroin-related cues than neutral cues (Stimulus Type × Exposure Time: $F_{1,162}=29.60$, $p<0.01$; Fig. 1A). Post hoc Fisher LSD tests showed that craving scores were higher post-H exposure than pre-H, pre-N, and post-N exposure (all $p<0.05$) in both the placebo and 5 μg TTX treatment groups.

Craving change scores (i.e., craving score post-exposure minus craving score pre-exposure) are shown in Fig. 1B. ANOVA showed significant main effects of Treatment ($F_{3,56}=4.72$, $p=0.01$) and Stimulus Type ($F_{1,42}=112.19$, $p<0.0001$) and a significant Treatment × Stimulus Type interaction ($F_{2,42}=4.53$, $p=0.02$; Fig. 1B). *Post hoc* Fisher LSD tests showed that change scores were higher after heroin-related cue exposure than neutral cue exposure in each of the three groups (all $p<0.05$).

3.3. Anxiety

During baseline (shown in Fig. 2A as "Pre-N" and "Pre-H"), no significant group differences were observed in anxiety scores prior to exposure to neutral cues or heroin cues. After cue exposure, anxiety increased more after exposure to heroin-related cues (Stimulus Type × Exposure Time: $F_{1,162}=5.61$, $p<0.05$; Fig. 2A). *Post hoc* Fisher LSD tests showed that anxiety scores were higher post-H exposure than pre-H, pre-N, and post-N exposure.

Anxiety change scores (i.e., anxiety score post-exposure minus anxiety score pre-exposure) are shown in Fig. 2B. ANOVA showed significant main effects of Treatment ($F_{2,42}=4.25$, $p=0.02$) and Stimulus Type ($F_{1,42}=100.107$, $p<0.0001$) and a significant Treatment × Stimulus Type interaction ($F_{2,42}=4.20$, $p=0.02$; Fig. 2B). *Post hoc* Fisher LSD tests showed that anxiety scores were higher after heroin-related cue exposure than neutral cue exposure in each of the three groups (all $p<0.05$).

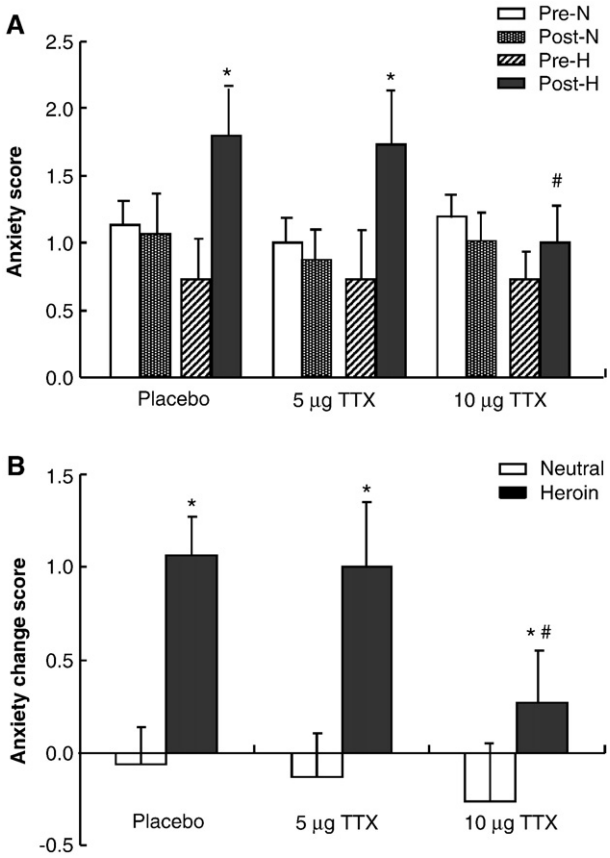

Fig. 2. Means (A) and mean change scores (B) for anxiety in response to neutral and heroin cues. (A) $^{*}p<0.05$, compared with Pre-N, Pre-H, and Post-H. (B) $^{*}p<0.05$, compared with neutral image stimuli within the same group. $^{\#}p<0.05$, compared with placebo and 5 μg TTX group.

3.4. Blood pressure and heart rate

Blood pressure and heart rate did not differ within groups at any time point tested or across groups at baseline (data not shown). No participants reported adverse effects during the study. Two patients reported mild headache the next day, which was considered unrelated to TTX administration. After explanations and overnight rest, the headache symptoms had resolved.

4. Discussion

The present study examined the effect of TTX on responses to heroin-related cues in heroin addicts who had been abstinent for at least 1 month. The main finding was that a single dose of 10 μg, but not 5 μg, TTX significantly inhibited cue-induced increases in craving and anxiety. Neither dose of TTX had any detectable effect on blood pressure or heart rate at any time point tested. These preliminary results suggest that TTX might be an effective and safe agent for relapse prevention in abstinent heroin addicts.

To our knowledge, the present study is the first randomized, double-blind, placebo-controlled study of the therapeutic potential of TTX for this indication. Given that TTX at higher doses is a neurotoxin with suppressive effects on the respiratory and cardiovascular systems, its therapeutic use has been limited. To date, only one randomized, placebo-controlled clinical trial of TTX has been published, preceded by pilot safety studies (Hagen et al., 2008). The dose of TTX in the previous studies ranged from 7.5 to 30 μg, b.i.d., which is much higher than the dose that was effective in the present study. However, because participants in those studies were receiving a variety of concurrent medications for the treatment of cancer, the safety results may not be generalizable to other patient populations. As demonstrated in the present study, neither 5 nor 10 μg TTX altered blood pressure or heart rate before or after cue exposure in heroin addicts, suggesting that the 10 μg dose does not present a cardiovascular safety concern.

Although the mechanism by which TTX exerts its analgesic effects is not fully understood, it may involve actions on primary afferents and in the dorsal root ganglion (Omana-Zapata et al., 1997; Lyu et al., 2000). The mechanism by which TTX elicits anti-drug-craving effects in humans is even less clear and will require additional research. Studies in guinea pigs have shown that systemic administration of TTX can induce pharmacological effects within the central nervous system (Chang et al., 1990), but it is unclear whether systemically administered TTX has a selective effect on specific brain areas implicated in drug seeking, drug reward, or anxiety.

Previous studies indicated that TTX-induced inactivation of specific brain areas, when it occurs, is temporary, and we do not know what duration of treatment is needed to trigger neuroadaptations that might result in long-term benefits. Thus, although the present results support the acute efficacy of a single dose of TTX, the possibility of chronic administration needs to be investigated.

There are several limitations that need to be considered in interpreting the results of this study. First, we did not monitor skin temperature, breathing rate, or blood oxygen saturation. Although we found no adverse effects of TTX on respiration, future studies of TTX should include more thorough assessments. Second, we did not conduct cognitive assessments after TTX administration. In rats, TTX injection into the hippocampus induces cognitive disorganization (Wesierska et al., 2005; Olypher et al., 2006). No severe adverse effects on cognition were seen when patients with cancer pain were given therapeutic doses of TTX (Hagen et al., 2007; Hagen et al., 2008). Nonetheless, future studies of TTX in heroin addicts should incorporate cognitive function scales and electroencephalogram (EEG) recording. Finally, the present study did not assess the mechanism of the observed anti-drug-craving effects of TTX. More preclinical research is required to assess this.

In conclusion, this is the first study to report an attenuating effect of systemic TTX treatment on cue-induced craving and anxiety in heroin addicts during abstinence, an effect that occurred in the absence of adverse cardiovascular effects. These results support the potential use of TTX for relapse prevention in heroin addiction. Clinical trials with larger sample sizes and chronic administration may be warranted.

Acknowledgments

This work was supported in part by the National Basic Research Program of China (973 Program, 2007CB512302), the National High Technology Research and Development Program of China (863 Program, 2006AA02Z4D1), and the China–Canada Joint Health Research Program (No: 30611120528). The authors declare that they do not have any conflicts of interest (financial or otherwise) related to the data presented in this manuscript.

References

Carter BL, Tiffany ST. Meta-analysis of cue-reactivity in addiction research. Addiction (Abingdon, England) 1999;94:327–40.
Chang FC, Benton BJ, Salyer JL, Foster RE, Franz DR. Respiratory and cardiovascular effects of tetrodotoxin in urethane-anesthetized guinea pigs. Brain Res 1990;528:259–68.
Chen S, Ren L, Huang Z. Effects of tetrodotoxin on naloxone-precipitated withdrawal syndrome in morphine-dependent rats and mice. Chin J Pharmcol Toxicol 2001;15:434–40.
Fox HC, Talih M, Malison R, Anderson GM, Kreek MJ, Sinha R. Frequency of recent cocaine and alcohol use affects drug craving and associated responses to stress and drug-related cues. Psychoneuroendocrinology 2005;30:880–91.
Fuchs RA, See RE. Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. Psychopharmacology (Berl) 2002;160:425–33.
Grimm JW, See RE. Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 2000;22:473–9.
Hagen NA, Fisher KM, Lapointe B, du Souich P, Chary S, Moulin D, et al. An open-label, multi-dose efficacy and safety study of intramuscular tetrodotoxin in patients with severe cancer-related pain. J Pain Symptom Manage 2007;34:171–82.
Hagen NA, du Souich P, Lapointe B, Ong-Lam M, Dubuc B, Walde D, et al. Tetrodotoxin for moderate to severe cancer pain: a randomized, double blind, parallel design multicenter study. J Pain Symptom Manage 2008;35:420–9.
Kosten TR, Rounsaville BJ, Kleber HD. A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. Arch Gen Psychiatry 1986;43:733–8.
Lu L, Shepard JD, Hall FS, Shaham Y. Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 2003;27:457–91.
Lyu YS, Park SK, Chung K, Chung JM. Low dose of tetrodotoxin reduces neuropathic pain behaviors in an animal model. Brain Res 2000;871:98-103.
McLaughlin J, See RE. Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl) 2003;168:57–65.
Narahashi T. Mechanism of action of tetrodotoxin and saxitoxin on excitable membranes. Fed Proc 1972;31:1124–32.
Narahashi T, Roy ML, Ginsburg KS. Recent advances in the study of mechanism of action of marine neurotoxins. Neurotoxicology 1994;15:545–54.
O'Brien CP. A range of research-based pharmacotherapies for addiction. Science 1997;278:66–70.
Olypher AV, Klement D, Fenton AA. Cognitive disorganization in hippocampus: a physiological model of the disorganization in psychosis. J Neurosci 2006;26:158–68.
Omana-Zapata I, Khabbaz MA, Hunter JC, Clarke DE, Bley KR. Tetrodotoxin inhibits neuropathic ectopic activity in neuromas, dorsal root ganglia and dorsal horn neurons. Pain 1997;72:41–9.
Ooteman W, Koeter MWJ, Vserheul R, Schippers GM, van den Brink W. Measuring craving: an attempt to connect subjective craving with cue reactivity. Alcohol Clin Exp Res 2006;30:57–69.
Ren ZY, Zhang XL, Liu Y, Zhao LY, Shi J, Bao Y, et al. Diurnal variation in cue-induced responses among protracted abstinent heroin users. Pharmacol Biochem Behav 2009;91:468–72.
Ren, Z., Shi, J., Epstein, D., Wang, J., Lu, L., Abnormal pain response in pain-intolerant opiate addicts predicts increased drug craving. Psychopharmacology (in press).
Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev 2000;33:13–33.
Shi J, Zhao LY, Epstein DH, Zhang XL, Lu L. Long-term methadone maintenance reduces protracted symptoms of heroin abstinence and cue-induced craving in Chinese heroin abusers. Pharmacol Biochem Behav 2007;87:141–5.
Shi J, Zhao LY, Copersino ML, Fang YX, Chen Y, Tian J, et al. PET imaging of dopamine transporter and drug craving during methadone maintenance treatment and after prolonged abstinence in heroin users. Eur J Pharmacol 2008;579:160–6.

Sinha R, Catapano D, O'Malley S. Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology (Berl) 1999;142:343–51.
Waters AJ, Shiffman S, Bradley BP, Mogg K. Attentional shifts to smoking cues in smokers. Addiction (Abingdon, England) 2003;98:1409–17.
Weinstein A, Wilson S, Bailey J, Myles J, Nutt D. Imagery of craving in opiate addicts undergoing detoxification. Drug Alcohol Depend 1997;48:25–31.
Wesierska M, Dockery C, Fenton AA. Beyond memory, navigation, and inhibition: behavioral evidence for hippocampus-dependent cognitive coordination in the rat. J Neurosci 2005;25:2413–9.
Yu J, Zhang S, Epstein DH, Fang Y, Shi J, Qin H, et al. Gender and stimulus difference in cue-induced responses in abstinent heroin users. Pharmacol Biochem Behav 2007;86:485–92.