Published in final edited form as:

Behav Brain Res. 2014 May 15; 265: 188–197. doi:10.1016/j.bbr.2014.02.029.

Role of the kappa-opioid receptor system in stress-induced reinstatement of nicotine seeking in rats

Stephanie L Grella^{a,b}, Douglas Funk^{a,*}, Kathy Coen^a, Zhaoxia Li^a, and A.D. Lê^{a,b,c}

^aNeurobiology of Alcohol Laboratory, Centre for Addiction and Mental Health, 33 Russell St., Toronto, Ontario M5S 2S1

^bDepartment of Pharmacology & Toxicology, University of Toronto Medical Sciences Building, Rm 4207, 1 King's College Circle, Toronto, Ontario M5S 1A8

^cDepartment of Psychiatry, University of Toronto, 250 College Street, 8th floor, Toronto, Ontario M5T 1R8

Abstract

Rationale—The correlation between stress and smoking is well established. The mechanisms that underlie this relationship are, however, unclear. Recent data suggest the kappa-opioid system is involved in the mediation of negative affective states associated with stress thereby promoting drug addiction and relapse. Pharmacological treatments targeting the kappa opioid system and this mechanism may prove to be useful therapeutics for nicotine addiction in the future.

Objectives—We sought to determine whether there was a stress-specific role of the kappa opioid system in nicotine seeking behavior.

Method—Groups of male Long Evans rats were trained to self-administer nicotine intravenously; their operant responding for nicotine was extinguished prior to tests of reinstatement. Pretreatment with systemic injections of the kappa opioid receptor (KOR) antagonist nor-binaltorphimine (nor-BNI) was given prior to tests of stress (systemic injections of yohimbine (YOH)) or cue-induced reinstatement of nicotine seeking. Systemic injections of the KOR agonist U50,488 were also given in a test for reinstatement of nicotine seeking.

Results—Nor-BNI pretreatment at 1 hr and 24 hrs prior to testing was able to block YOH-induced, but not cue-induced reinstatement of nicotine seeking. U50,488 reinstated nicotine seeking behavior in a dose-dependent manner.

Conclusions—These findings support the hypothesis that the kappa opioid system is involved in relapse to nicotine seeking induced by stress, but not by conditioned cues. KOR antagonists such

© 2014 Elsevier B.V. All rights reserved.

*Corresponding Author: Douglas Funk, Neurobiology of Alcohol Laboratory, Centre for Addiction and Mental Health, 33 Russell St. Toronto, ON, M5S 2S1, Phone: [416]535-8501, Ext 6751, FAX: [416]595-6922, douglas.funk@camh.ca.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

as nor-BNI may therefore be useful novel therapeutic agents for decreasing the risk of stress-induced drug relapse.

Keywords

nicotine; reinstatement; stress; yohimbine; nor-binaltorphimine; U50, 488

1. Introduction

1.1. Nicotine addiction and stress

A principal difficulty with smoking is the difficulty in remaining abstinent, with relapse rates as high as 97% within 6 months after quitting smoking (Benowitz, 2009). It is well established that stress is a risk factor for the development and maintenance of nicotine addiction (Finkelstein et al., 2006; Schepis and Rao, 2005), and also contributes significantly to relapse (Brown et al., 1995; Daughton et al., 1990; McKee et al., 2011; Pomerleau and Pomerleau, 1991). Individuals exposed to high levels of stress are more likely to relapse (Bruijnzeel and Gold, 2005). This is supported by the results of studies in laboratory animals showing that exposure to stress can reinstate nicotine seeking following extinction of operant responding (Buczek et al., 1999; Martin-García et al., 2009; Yamada and Bruijnzeel, 2011; Zislin et al., 2007). Animals trained to self-administer nicotine also display long-lasting increases in their responsivity to stress (Chen et al., 2008).

1.2. The κ -opioid system and stress

Recently, data suggest κ -opioid receptor (KOR) activation by endogenous dynorphins (DYNs) may participate in the mediation of negative affective states associated with stress thereby promoting drug addiction and relapse risk (Shippenberg et al., 2007). Growing evidence suggests activation of KORs may potentiate, or mimic the response to stress, and in this way may modulate the appetitive properties of nicotine (Smith et al., 2012).

A number of studies strongly implicate KORs in the rewarding properties of drugs. KOR antagonists have been shown to alleviate somatic withdrawal symptoms (Jackson et al., 2010) and proDYN (precursor to DYN) knockout mice show diminished aversive responses to nicotine withdrawal (Galeote et al., 2009). Moreover, in mice, the KOR agonist U50,488 potentiated the development of (McLaughlin et al., 2006; Schindler et al., 2010) and significantly reinstated (Redila and Chavkin, 2008) cocaine conditioned place preference (CPP) after extinction, effects which were blocked with the selective KOR antagonist norbinaltorphimine (nor-BNI) (McLaughlin et al., 2006; Redila and Chavkin, 2008; Schindler et al., 2010). Nor-BNI did not, however, block cocaine prime-induced reinstatement of cocaine seeking (Redila and Chavkin, 2008). Redila and Chavkin (2008) also found that KOR and proDYN knockout mice failed to demonstrate foot shock- and forced swim stress (FSS)-induced reinstatement. Similar effects were found in rats where pretreatment with the KOR antagonist JDTC (Beardsley et al., 2005) or Aroclor (Carey et al., 2007) was able to block stress-induced cocaine seeking but not cocaine prime-induced reinstatement. This suggests a stress specific role for these receptors although it should be noted that Schank et al. (2012) found no effect of pretreatment with JDTC on stress-induced reinstatement of alcohol seeking.

To date, the only studies examining the role of KOR system in nicotine seeking were conducted in mice using the CPP procedure. KOR activation significantly potentiated nicotine CPP which was blocked by nor-BNI given systemically or directly into the amygdala (Smith et al., 2012). Interestingly, Al-Hasani et al. (2013) found that mice given chronic mild stress prior showed a greatly reduced degree of U50,488-induced reinstatement of CPP to nicotine. This absence of reinstatement produced by prior exposure to stress was not seen in reinstatement of CPP primed by nicotine. To further demonstrate the specific role of KORs in stress related increases in drug taking, it was shown that pretreatment with KOR antagonists nor-BNI (Jackson et al., 2013) and JD1c (Jackson et al., 2010) significantly attenuated FSS-induced reinstatement of nicotine CPP but had no effect on nicotine prime-induced reinstatement of CPP. Based on these data, we hypothesize the aversive and stress-like effects produced by stimulation of KORs contribute to the stress-induced increases in nicotine seeking using the reinstatement model. The present study addresses this by first testing whether systemic injections of nor-BNI would block reinstatement of nicotine-seeking induced by the α -2 adrenergic antagonist yohimbine (YOH), a commonly-used pharmacological stressor that reliably reinstates the seeking of a number of drugs of abuse, including nicotine (Banna et al., 2010; Cippitelli et al., 2010; Funk et al., 2014; Shepard et al., 2004; Zhou et al., 2013). Secondly, the role of KORs in the reinstatement of nicotine seeking was further examined by determining whether systemic injections of the selective KOR agonist, U50,488 would reinstate nicotine seeking behavior. And finally, we expanded on previous work showing that the KOR system plays a stress specific role by investigating whether nor-BNI blocks reinstatement of nicotine seeking induced by conditioned cues associated with nicotine delivery. We hypothesized that stimulation of KORs with the agonist would promote nicotine seeking behavior while antagonism of KORs would attenuate stress-induced, but not cue-induced, reinstatement of nicotine seeking, demonstrating a stress specific role for the KOR system in relapse to nicotine seeking behavior.

2. Materials and Methods

2.1. Subjects

Male Long Evans rats (70–75 days old), purchased from Charles River Laboratories (Quebec, Canada) were maintained on a reverse light-dark cycle (lights on at 7:00 pm, lights off at 7:00am). Thirty-one rats were used for Exp. 1, 15 rats for Exp. 2, and 19 rats for Exp. 3. Rats were individually housed in clear Plexiglas cages in a humidity and temperature regulated vivarium and given 5×4g pellets of standard rat chow per day and *ad libitum* access to water. All of the procedures carried out were in accordance with the Canadian Council on Animal Care and the CAMH Animal Care Committee.

2.2. Apparatus

Testing was conducted in operant conditioning chambers operated by a computer – controlled interface system (Med. Associates Inc., St Albans, VT, USA). Each chamber contained two response levers, a sucrose pellet dispenser, a speaker, and a stimulus light located above each lever. A syringe mounted on a motor driven pump (Razel) delivered nicotine infusions to the rat's catheter via Tygon tubing attached to a swivel located above

the test chamber. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. All boxes were controlled by a PC running Med-PC IV.

2.3. Training the operant response

In a separate set of operant chambers, rats were initially trained to receive 50 mg sucrose pellets via lever presses on an FR1 schedule of reinforcement with *ad libitum* access to water, prior to surgery. Rats were given one *day* session for 8 hrs and one *night* session for 16 hours in counterbalanced order with 24 hrs separating the sessions. The house light remained illuminated throughout pellet training but no stimulus light or tone was presented. Post-recovery from surgery, rats were given a consolidation session which terminated after 100 sucrose reinforcements or 1 hr, whichever came first. This was to confirm that the prior learning of lever pressing behaviour had not been disrupted by surgery.

2.4. Intravenous catheter surgery

Rats were surgically implanted using aseptic procedures, with an IV cannula in the right jugular vein externalized above the scapulae. For Exps. 1 and 2, rats were anesthetized under an isoflurane/oxygen gas mixture (4–5% induction, 2.5% maintenance) and for Exp.3, rats were given a ketamine (75mg/kg)/xylazine (10mg/kg) mixture intraperitoneally (i.p.). For all rats, the antibiotic Derapen was administered subcutaneously (s.c.) (0.1 ml of 300000u/ml solution). Rats were also given Marcaine (0.125%), a local anaesthetic, 0.1 ml was applied at each incision site. Ketoprofen (5mg/ml; 1ml/kg s.c.) was administered during surgery preparation to prevent post-operative discomfort and rats were kept warm and supervised until full recovery from anesthesia. Rats were given 3ml of saline (s.c.) to replace fluid loss during surgery. All rats were given 1 week to recover. Three days after surgery, cannulae were flushed with 0.03ml of a heparin (500u/ml)/dextrose (25%) solution, and beginning 3 d later, cannulae were flushed daily with 0.1 ml of 50 U/ml heparin in sterile saline solution until the end of the study to maintain cannula patency. Animals were administered sodium thiopental dissolved in sterile water to test catheter patency (0.2ml of 20mg/ml solution) 2 hrs after the last self-administration session.

2.5. Nicotine self-administration training

After recovery from surgery rats were trained to self-administer nicotine IV in 1 hr daily sessions in operant chambers. Presses on the active lever activated the infusion pump (Med Associates) and resulted in the delivery of 0.03mg of nicotine/kg/0.025 ml sterile saline through the IV catheter. The visual (cue light, 30sec) and auditory (2900 Hz, 1 sec) stimuli were turned on when an infusion of nicotine was obtained. A time out period of 40 sec was imposed to prevent overdose, during which the house light was turned off, the cue light above the lever was illuminated, and active lever responses were recorded but no drug was delivered. Responses on the inactive lever were also recorded but had no programmed consequences. Rats were initially trained on an FR1 schedule of reinforcement (5 days), graduated to an FR2 schedule (3–5 days), and eventually reached an FR3 schedule of reinforcement (5–7 days or until a criterion was met where total inactive lever responses were less than 30% of the total active lever responses). Seven animals in Exp. 1 were

removed due to high inactive lever responding. For Exp. 2, animals were trained for 5 additional days on an FR5 schedule of reinforcement after completion of the FR3 schedule to address this issue; therefore for Exp. 2, all animals remained in the study except for one rat which did not extinguish (see below). Animals in Exp. 3 were trained to an FR3 schedule of reinforcement and no animals were excluded.

2.6. Extinction of nicotine self-administration

For Exps. 1 and 2, during the daily 1 hr extinction sessions, conditions were identical to those during the final days of training, (including the presence of visual and auditory cues) with the exception that nicotine was replaced with saline. Tests for reinstatement commenced after approximately 10–28 daily extinction sessions, when the rats reached the extinction criterion (fewer than 15 presses on active lever per 1 hr for 2 consecutive sessions). For Exp. 3, extinction sessions were given as daily 1 hr sessions for 6 days after which two 1 hr sessions were given daily for an additional 6 days. Unlike Exps. 1 and 2, cues (visual and auditory) were not present during extinction. During the extinction phase in all of the experiments, rats were injected 3–4 times with saline in order to habituate them to the injection procedures used in the reinstatement tests (see below).

2.7. Drugs

Nicotine bitartrate (Sigma-Aldrich, Oakville, ON) was dissolved in sterile physiological saline and prepared fresh daily (pH 7). Yohimbine HCl (17-hydroxy-yohimban-16-carboxylic acid methyl ester hydrochloride; Sigma St. Louis, MO) (YOH), was dissolved in distilled water (1.25 mg/kg i.p.). U50,488 (2- (3,4-dichlorophenyl)-N-methyl-N-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl] acetamide) and norbinaltorphimine (nor-BNI)-HCl were provided by the National Institute on Drug Abuse Drug Supply Program. U50,488 was dissolved in sterile saline (0, 1, 2.5, 5 mg/kg) and nor-BNI in sterile water (10 mg/kg). All drug doses were calculated as the free base and drugs or vehicle were injected at a volume of 1 ml/kg.

2.8. Statistical analyses

Two-way repeated measures ANOVAs were conducted using Sigma Plot version 11 for all data unless otherwise stated. Separate analyses were conducted on active lever presses and inactive lever presses. Post hoc analyses were conducted using Tukey's Honestly Significant Difference test where appropriate.

2.9. Experiment 1. Does KOR blockade attenuate stress-induced reinstatement of nicotine seeking?

Three groups of rats ($n=7-9$ /group) were pretreated with either vehicle (sterile water) 1 hr prior, or nor-BNI 1 hr or 24 hrs prior to the extinction tests with YOH vehicle or YOH. These pretreatment times were chosen based on previous studies which have found nor-BNI to have long-lasting effects with more selective antagonism at KORs 24 hrs after administration compared to 1 hr after administration when it has modest affinity for the μ -opioid receptor as well (Broadbent et al., 1994). YOH vehicle or YOH (1.25 mg/kg ip) was given 30 min prior to the test session.

2.10. Experiment 2. Does KOR activation reinstate nicotine seeking?

Using a balanced within latin square design, the ability of various doses (0, 1, 2.5, 5 mg/kg, i.p.) of the KOR agonist U50,488 to reinstate extinguished nicotine seeking was examined (n=15). Rats received one of the doses of U50,488 or vehicle (saline), injected 30 min prior to the reinstatement test. Four reinstatement tests were conducted each separated by a minimum of 2 extinction sessions until the extinction criterion was again met.

2.11. Experiment 3. Does KOR blockade attenuate cue-induced reinstatement of nicotine seeking?

The ability of re-exposure to a cue previously associated with nicotine delivery in rats pretreated with either nor-BNI or vehicle, was examined. Once the extinction criteria was met, rats received either nor-BNI (10mg/kg, i.p.) or vehicle (sterile water) 30 min after the last baseline extinction session. They were given a cue-induced reinstatement test 24 hrs later (n=8–11/group).

3. Results

3.1. Nicotine self-administration

The self-administration data of the animals, prior to drug testing in each of the three experiments are displayed in Table 1.

3.2. Exp. 1: KOR blockade attenuates YOH-induced reinstatement of nicotine seeking

3.2.1. Active lever—Figure 1a illustrates that YOH significantly reinstated nicotine seeking in rats pretreated with vehicle. This effect was attenuated in rats pretreated with nor-BNI at 1 hr, and 24 hrs prior to testing. There was a significant group by session interaction [$F(2, 42) = 7.642, p = 0.001$]. Pairwise comparisons revealed no group differences during the last two extinction sessions (mean of last two extinction sessions prior to any drug treatment) and showed the significant interaction was attributed to group differences found during the YOH-induced reinstatement test. Both groups given nor-BNI pretreatment at 1 hr [$q(3) = 7.193, p < 0.001$] and 24 hrs [$q(3) = 5.087, p = 0.002$] were significantly different from the vehicle group. Although a significant difference was not found between each of the nor-BNI pretreatment groups there was a trend [$q(3) = 3.152, p = 0.078$] suggesting that pretreatment at 1 hr was more effective than 24 hrs.

3.2.2. Inactive lever—There was a significant group by session interaction [$F(2, 42) = 4.724, p = 0.014$] for responses made during the last two extinction sessions and the YOH reinstatement test as depicted in figure 1b. Pairwise comparisons showed the significant interaction was attributed to an increase in responding on the inactive lever after YOH administration in rats pretreated with vehicle as well as group differences found during the reinstatement test. Both groups given nor-BNI pretreatment at 1 hr [$q(3) = 6.531, p < 0.001$] and 24 hrs [$q(3) = 3.451, p = 0.049$] were significantly different from the vehicle group. Although a significant difference was not found between each of the nor-BNI pretreatment groups there was a trend [$q(3) = 3.378, p = 0.055$].

3.2.3. Effect of nor-BNI on extinction—Rats assigned to the group pretreated with nor-BNI 24 hrs before the YOH challenge were given an extinction session 1 hr following the administration of nor-BNI. We compared responding during this session to the previous extinction session and did not observe any significant effect of session or lever with nor-BNI on either active or inactive lever responding (Figure 2).

3.3. Exp. 2: KOR agonist U50,488 reinstates nicotine seeking

3.3.1. Active Lever—Figure 3 illustrates the KOR agonist U50,488 significantly reinstated nicotine seeking. There was a significant effect of dose [$F(3, 40) = 4.95, p = 0.005$] on responding. Post hoc comparisons showed this effect was attributed to a significant difference between vehicle and each of the two higher doses of U50,488: 2.5mg/kg [$q(4) = 5.022, p = 0.005$] and 5 mg/kg [$q(4) = 4.024, p = 0.034$], but not the lower dose 1mg/kg [$q(4) = 2.039, ns$].

3.3.2. Inactive Lever—No significant effect of group or dose on responding was found.

3.4. Exp. 3: KOR blockade does not attenuate cue-induced reinstatement of nicotine seeking

Active lever—Figure 4a illustrates that re-exposure to the nicotine-paired cues (light and tone) significantly reinstated nicotine seeking in rats given vehicle pretreatment. This effect was not blocked by pretreatment with nor-BNI 24 hrs prior to testing. For active lever responding, there was a significant effect of session [$F(1, 17) = 12.378, p = 0.003$] but no effect of group.

Inactive lever—No significant effects were observed (Figure 4b).

4. Discussion

Few studies have examined the role of KORs in nicotine dependence and relapse (Wee & Koob, 2010) despite the involvement of the KOR system in stress-related processes and the interaction between stress and relapse to nicotine. The principal findings in this study are that the KOR antagonist, nor-BNI, was able to block YOH stress-induced, but not cue-induced reinstatement of nicotine seeking. Furthermore, KOR activation with U50,488 was able to reinstate nicotine seeking in a dose-dependent manner. Taken together with previous reports, the present results suggest that antagonists such as nor-BNI may be considered as pharmacotherapies for smoking cessation.

4.1. The KOR/DYN system and nicotine seeking

The present findings offer support for previous studies which demonstrated a specific role for the KOR system in drug seeking, particularly, nicotine seeking (Al-Hasani et al., 2013; Ismayilova and Shoaib, 2010; Jackson et al., 2010, 2013; Smith et al., 2012). We found that activation of KORs with U50,488 dose dependently reinstated nicotine seeking, an effect which was attenuated by the KOR antagonist nor-BNI. This finding parallels the results of Smith et al., (2012) who found that U50,488 reinstated nicotine CPP in mice, an effect blocked with systemic injections of nor-BNI. Our results are also in accordance with those

studies which have found that KOR stimulation promotes reinstatement (Redila & Chavkin, 2008) or potentiation of CPP to cocaine in mice (McLaughlin et al., 2006), or reinstatement of cocaine seeking (lever pressing) in squirrel monkeys (Valdez et al., 2007).

At moderate to high doses, activation of KORs generates behavioral phenomena associated with relapse e.g. anxiety- and depressive-like behavior (Bals-Kubik et al., 1993; Bruchas et al., 2009; Carlezon et al., 2006; Mague et al., 2003; Motta et al., 1995; Nobre et al., 2000; Smith et al., 2012; Wittmann et al., 2009). Bruchas et al. (2009) and Smith et al. (2012) both demonstrated systemic injections of the KOR agonist U50,488 significantly decreased open arm exploration in the elevated plus maze, indicative of increased anxiety, while Wittmann et al. (2009) found DYN knock-out mice showed significantly increased open arm exploration. Mague et al. (2003) found systemic injections of the KOR agonist U-69593 produced dose dependent increases in immobility while Carlezon et al. (2006) found similar results with systemic administration of the KOR agonist salvinorin A in animals subject to forced swim stress (FSS) (Porsolt et al., 1977; Schwarzer, 2009). KOR agonists can also produce conditioned place aversion (CPA) (Bals-Kubik et al., 1993; Land et al., 2008; 2009; McLaughlin et al., 2006). Consistent with these data from animal studies, KOR activation typically produces feelings of anxiety and dysphoria in humans (Land et al., 2008; Pfeiffer et al., 1986). Therefore, our findings that the KOR system has a facilitatory role in nicotine seeking are consistent with these stress-related effects reported following KOR activation.

4.2. The KOR/DYN system and stress-induced reinstatement of nicotine seeking

Stress causes the release of endogenous DYN neuropeptides, which selectively bind to KORs (Bruchas et al., 2007; Bruchas et al., 2010; Chavkin et al., 1982; Land et al., 2008; McLaughlin et al., 2003; Nabeshima et al., 1992; Smith and Lee, 1988). FSS administered repeatedly causes activation of KORs in the nucleus accumbens (NAC) (Bruchas et al., 2008) and increases DYN in the hippocampus (Shirayama et al., 2004). In addition, phosphorylated KOR immunoreactivity is observed in several key brain structures involved in stress responses following FSS (Bruchas et al., 2007). Likewise, immobilization stress induces DYN immunoreactivity in the hippocampus and NAC (Shirayama et al., 2004). These data suggest a role for KOR in mediating stress responses.

In this study, we demonstrated that nor-BNI was able to block YOH stress-, but not cue-induced nicotine seeking. Although previous studies have reported that KOR antagonists block stress-, but not drug prime-induced reinstatement (Beardsley et al., 2005; Carey et al., 2007; Jackson et al., 2010; Redila and Chavkin, 2008), few have examined the role of the KOR system on reinstatement induced by cues. We recently found that nor-BNI blocked cue-induced reinstatement of alcohol seeking when given 2 hrs prior to test (Funk et al., 2014). These results are in accordance with those of Shank et al. (2012) showing that the KOR antagonist, JDTic, blocked cue-induced reinstatement of alcohol seeking using a similar pretreatment interval. Taken together with the present results, these findings indicate a role for the KOR system in reinstatement induced by alcohol-associated cues but not nicotine-related cues.

We found that nor-BNI possessed a greater ability to block stress-induced reinstatement of nicotine seeking at the 1 hr pretreatment timepoint compared to 24 hrs. However, other work

has shown that nor-BNI has very long-lasting effects (weeks) (Broadbear et al., 1994; Endoh et al., 1992; Jones and Holtzman, 1992). One possible explanation for this discrepancy is that nor-BNI impairs motoric performance 1hr after administration, but not after 24hrs. We did observe motor effects of nor-BNI in that rats exhibited less mobility and appeared uncoordinated in the first few minutes after administration of the drug. The possibility that a carryover of this effect may explain why nor-BNI possessed a greater ability to block stress-induced reinstatement of nicotine seeking at 1 hr pretreatment compared to 24 hrs is unlikely since McLaughlin et al. (2003) found systemic injections of nor-BNI actually decreased immobility 1 hr after administration of FSS. Moreover, we observed no effect of nor-BNI on lever responding under extinction conditions 1 h after injection. An alternative hypothesis to explain this is related to a potential time-dependency of the relative affinity of nor-BNI for KOR vs. μ -opioid receptors. It has been reported that nor-BNI has a greater affinity for μ -opioid receptors 1 hr after administration compared to 24 hrs. Broadbear et al., (1994) found that the ability of nor-BNI to shift the dose-effect curve of morphine (μ -agonist) in a writhing assay in mice was much less 24 hrs after administration compared to 1 hr. Interestingly, our results are consistent with those of Schank et al. (2012) who showed nor-BNI was more effective at suppressing alcohol self-administration 2hrs following administration compared to 24hrs, which is consistent with recent findings from our lab showing that nor-BNI blocked U50,488-induced reinstatement of alcohol seeking when administered 2hrs, but not 24 hrs, prior to test (Funk et al., 2014). Neither of these studies reported any motoric effects of nor-BNI. Future studies should investigate these time-dependent effects of nor-BNI on motor performance to disambiguate these differences.

Our findings that nor-BNI reduced stress- but not cue-induced reinstatement of nicotine seeking in this study is in part consistent with the results of Redila and Chavkin (2008) who found KOR and proDYN knockout mice failed to demonstrate stress-induced reinstatement of cocaine seeking but were not impaired in cocaine prime-induced reinstatement. They are also consistent with the results of Beardsley et al. (2005) and Carey et al. (2007) who found KOR blockade with JDTic or the peptide aroDYN, respectively, blocked stress-induced but not cocaine prime-induced reinstatement of cocaine seeking. This suggests a stress-specific role for these receptors. However, Schank et al. (2012) found JDTic blocked cue-induced reinstatement of alcohol when administered 2hrs prior to the reinstatement test, which is supported by our observation that nor-BNI blocks cue-induced alcohol seeking when administered at the same pretreatment interval (Funk et al., 2014). The reason for this discrepancy between the present results and those of Schank et al. and Funk et al., are not known, although it could be speculated that the effects of KOR blockade on cue-induced reinstatement are specific to alcohol. We also mention here that while naltrexone can block contextual renewal (Marinelli et al., 2007) and discrete cue-induced reinstatement of alcohol seeking, our unpublished data shows no effect of similar doses of naltrexone on discrete or contextual cue induced reinstatement of nicotine seeking.

Negative affective states (e.g. anxiety, depression, & stress) have been linked to relapse. Blockade of KORs with nor-BNI has been shown in numerous studies to attenuate anxiety- and stress-like responses in the EPM (Knoll et al., 2007; Bruchas et al., 2009; Wittmann et al., 2009), depressive-like responses using FSS (Beardsley et al., 2005; Land et al., 2008;

Mague et al., 2003; McLaughlin et al., 2003), and foot shock-induced CPA (Land et al., 2008). Moreover, nor-BNI has been shown to block FSS- (Schindler et al., 2010) and U50,488-induced (McLaughlin et al., 2006) potentiation of cocaine-induced CPP, as well as, social defeat stress- (Land et al., 2009), FSS-, and U50,488-induced (Redila and Chavkin, 2008) reinstatement of cocaine-induced CPP. Furthermore, KOR activation is enhanced in limbic brain regions during nicotine withdrawal (Isola et al., 2008; McCarthy et al., 2010). This suggests that KORs may be a promising focus for the development of therapies to treat or prevent relapse.

4.3. Yohimbine

We found in the present study that nor-BNI was able to block reinstatement of nicotine seeking induced by the α -2 adrenoceptor antagonist YOH. These findings are consistent with previous work showing that KOR antagonists block stress-induced reinstatement of cocaine seeking (Beardsley et al., 2005; Carey et al., 2007; Land et al. 2009; Redila & Chavkin et al., 2008; Schindler et al., 2010) and stress-induced reinstatement of nicotine seeking using CPP (Jackson et al., 2010; 2013) and extend this to stress-induced nicotine seeking using the reinstatement procedure.

YOH is a traditional α -2 adrenoceptor antagonist that induces stress-like states in both humans and nonhuman primates (Bremmer et al., 1996; b; Holmberg and Gershon 1961; Lang and Gershon, 1963). We employed YOH for two main reasons. First is the translational utility of this stressor: YOH reliably reinstates drug seeking or conditioned drug effects in mice (Mantsch et al., 2010), rats (Richards et al., 2008; Shepard et al., 2004), and monkeys (Lee et al., 2004). It also provokes drug craving in detoxified alcoholics (Umhau et al., 2011) and methadone-maintained patients (Stine et al., 2002), and increases opiate-taking behavior in buprenorphine-maintained individuals (Greenwald et al., 2013). The effectiveness of YOH in humans and animals recommends it for use in animal experiments designed to help understand the clinical problem of relapse to stress-induced drug seeking. Second, YOH has potent and reliable effects on reinstatement of drug seeking across drug classes, species, labs, and procedures (Cippitelli et al., 2010; Feltenstein and See, 2006; Le et al., 2005; Richards et al., 2008; Shepard et al., 2004; Simms et al., 2011).

Besides its properties as an α -2 adrenoceptor antagonist, YOH acts on other receptors. It is an antagonist of D2-family dopamine receptors (Scatton et al., 1980) and α -1 adrenoceptors (Doxey et al., 1984), and an agonist of 5-HT_{1A} receptors (Millan et al., 2000; Winter and Rabin 1992). The critical receptor sub-type involved in YOH's effect on reinstatement of drug seeking is largely unknown. It is unlikely that D2 receptors or α -1 adrenoceptors play a role. D2-family receptor antagonists do not reinstate drug seeking (Shalev et al., 2002; Spealman et al., 1999) and YOH has an α -2/ α -1 selectivity ratio of about 40–45 (Doxey et al., 1984); we also found that YOH-induced reinstatement is blocked by the α -1 adrenoceptor antagonist prazosin (Le et al., 2011). On the other hand, both α -2 adrenoceptors and 5-HT_{1A} receptors contribute to yohimbine-induced reinstatement of alcohol seeking. We found that this reinstatement is attenuated by the α -2 adrenoceptor agonist clonidine and the 5-HT_{1A} antagonist WAY 100,635 (Le et al., 2009). It is unlikely, however that selective antagonism of α -2 adrenoceptor is critical for YOH's effect on

reinstatement, because the selective α -2 adrenoceptor antagonist RS79948 (Uhlen et al., 1998) does not reinstate alcohol or food seeking (Le et al., 2009; Nair et al., 2009). Finally, data from two recent studies on YOH-induced reinstatement of food seeking (Calu et al., 2013; Nair et al., 2011) suggest that activation of prefrontal dopamine by YOH (Tanda et al., 1996) is downstream of YOH-induced prefrontal noradrenaline release (Millan et al., 2009).

It is also unlikely that the reinstating effects of YOH mimicked the effects of a nicotine-primed injection in our study. In previous work nor-BNI had no effect on nicotine-primed reinstatement but did have an effect on stress-induced reinstatement (Al-Hasani et al., 2013; Jackson et al., 2010; 2013).

Although the effects of YOH are robust and consistent, there may be some disadvantages associated with the use of this drug in terms of lever selectivity. While several studies have shown YOH reinstated responding selectively on the lever previously associated with drug delivery, (Banna et al., 2010; Cippitelli et al., 2010; Feltenstein and See, 2006; Richards et al., 2009), several studies including ours, have found YOH increases responding during reinstatement for the inactive, as well as the active, lever (Funk et al., 2014, submitted; Grella and Leri, unpublished observations).

The explanation for this is not clear, but one hypothesis is that increases on the inactive lever may be a result of non-specific increases in activity although tests of YOH on locomotor activity have produced inconsistent results (Gentsch et al., 1989; Majczynski et al., 2006; Schroeder et al., 2003).

It is also possible that the increases observed on the inactive lever are not a result of increased behavioral activity but rather response generalization commonly observed during extinction (Catania, 1992). During self-administration, some of our rats showed high inactive lever responding as well which has been observed previously for both males and females self-administering the same dose of nicotine used in this experiment (Chaudhri et al., 2005). Chaudhri et al., (2005) found that inactive lever responding decreased as the dose of nicotine increased, and that females showed higher responding than males. It is unclear why higher levels of inactive lever responding appear to be common with nicotine self-administration, but one potential explanation comes from the observations of Chaudhri et al. (2005) who reported that animals pressed the inactive lever during the time out period when responses on the active lever did not yield nicotine infusions. Nonetheless, the large majority of the rats in our study demonstrated a significantly higher level of responding on the active lever compared to the inactive lever suggesting successful lever discrimination. During reinstatement, some rats also pressed at relatively high levels on the inactive lever following YOH treatment; whether this reflects response generalization or some other factor is not known. Nevertheless, significantly more responses were made on the active lever compared to the inactive lever suggesting YOH did precipitate drug seeking.

4.4. The CRF/DYN interaction

Corticotropin-releasing factor (CRF) is a primary neuropeptide involved in the coordination of stress responses and is released in the extended amygdala during drug withdrawal (Alheid and Heimer, 1988; Merlo-Pich et al., 1995). Central infusions of CRF reinstate drug seeking

(Lê et al., 2002) and CRF antagonists attenuate stress-induced reinstatement of drug seeking (Erb et al., 1998; Le et al., 2000; Shalev et al., 2006).

While not systematically tested in the present set of experiments, it has been recently hypothesized that there is a functional interaction between the CRF and KOR/DYN systems and more specifically, that the dysphoric component of stress is encoded by CRF-induced activation of DYN (Bruchas et al., 2010; Land et al., 2008). Evidence for this comes from studies showing that activation of these two systems produces similar behavioral effects and the co-expression of CRF and DYN receptors in many brain areas involved in mediating stress responses (Fallon and Leslie, 1986; Khachaturian et al., 1982; Ma et al., 2003; Mansour et al., 1994; Marchant et al., 2007; Merchenthaler et al., 1997; Poulin et al., 2009; Reyes et al., 2008; Roth et al., 1983). Although converging evidence suggests there is a CRF-DYN interaction, its nature is poorly understood.

The mechanism by which nor-BNI was able to block YOH-induced reinstatement of nicotine seeking in this study likely involved effects on CRF. Administration of YOH increases CRF activity in the extended amygdala (Funk et al., 2006) which in turn stimulates the release of DYN (Bruchas et al., 2009; Nikolarakis et al., 1986; Sirinathsinghji et al., 1989). Furthermore, KOR antagonism /gene deletion has previously been shown to block CRF-induced anxiety-like behavior (Bruchas et al., 2009; Land et al., 2008). There is also evidence to suggest directionality of the CRF/DYN interaction in the reverse since KOR activation also stimulates the release of CRF (Cheng et al., 2000; Overton and Fisher, 1989) and KOR agonist-induced reinstatement of cocaine (Valdez et al., 2007) and alcohol (Funk et al., 2014) seeking can be blocked with a CRF antagonist. To help elucidate the nature of this relationship, future studies in our lab will investigate whether U50,488-induced reinstatement of nicotine seeking can be blocked with a CRF antagonist and conversely, whether CRF-induced reinstatement of nicotine seeking can be blocked with nor-BNI.

4.5. Conclusions

The results of our series of experiments provide clear evidence that KORs are involved in relapse to nicotine seeking behavior. Although the connection between stress and relapse to the seeking of nicotine is well established (Brown et al., 1995; Kosten et al., 1986; Pomerleau and Pomerleau, 1991) and endogenous DYN peptides have an important role in mediating stress responses (Knoll and Carlezon, 2010; Land et al., 2008) and a stress-DYN interaction plays a role in the seeking of alcohol and cocaine, the relationship between stress, KOR and nicotine-seeking has been little studied. We showed that KORs are critically involved in relapse to nicotine seeking induced by stress, and also established a role for KORs in nicotine seeking under non-stress conditions. This work supports the idea that KOR antagonists may be useful novel therapeutic agents for decreasing the risk of stress-induced drug relapse.

Acknowledgments

This work was supported by a grant from the NIAAA (AA13108) to A.D. Lê. We thank Kenner Rice of the Intramural Research Program, NIDA-NIH for the generous gift of U50,488 and nor-BNI.

References

- Al-Hasani R, McCall JG, Bruchas MR. Exposure to chronic mild stress prevents kappa opioid-mediated reinstatement of cocaine and nicotine place preference. *Front Pharmacol*. 2013; 4:96. [PubMed: 23964239]
- Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. *J Pharmacol Exp Ther*. 1993; 264(1):489–495. [PubMed: 8093731]
- Banna KM, Back SE, Do P, See RE. Yohimbine stress potentiates conditioned cue-induced reinstatement of heroin-seeking in rats. *Behav Brain Res*. 2010; 208:144–148. [PubMed: 19931573]
- Beardsley PM, Howard JL, Shelton KL, Carroll FI. Differential effects of the novel kappa opioid receptor antagonist, JD1c, on reinstatement of cocaine-seeking induced by foot shock stressors vs. cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berl)*. 2005; 183(1): 118–126. [PubMed: 16184376]
- Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol*. 2009; 49:57–71.
- Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: I. preclinical studies. *Synapse*. 1996a; 23:28–38. [PubMed: 8723133]
- Broadbent JH, Negus SS, Butelman ER, de Costa BR, Woods JH. Differential effects of systemically administered norbinaltorphimine (nor-BNI) on kappa-opioid agonists in the mouse writhing assay. *Psychopharmacology (Berl)*. 1994; 115(3):311–319. [PubMed: 7871070]
- Brown SA, Vik PW, Patterson TL, Grant I, Schuckit MA. Stress, vulnerability and adult alcohol relapse. *J Stud Alcohol*. 1995; 56(5):538–545. [PubMed: 7475034]
- Brown ZJ, Tribe E, D'souza NA, Erb S. Interaction between noradrenaline and corticotrophin-releasing factor in the reinstatement of cocaine seeking in the rat. *Psychopharmacology (Berl)*. 2009; 203(1):121–130. [PubMed: 18985323]
- Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, Chavkin C. Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. *J Neurosci*. 2007; 27(43):11614–11623. [PubMed: 17959804]
- Bruchas MR, Xu M, Chavkin C. Repeated swim stress induces kappa opioid-mediated activation of extracellular signal-regulated kinase 1/2. *Neuroreport*. 2008; 19(14):1417–1422. [PubMed: 18766023]
- Bruchas MR, Land BB, Lemos JC, Chavkin C. CRF1-R activation of the dynorphin/kappa opioid system in the mouse basolateral amygdala mediates anxiety-like behavior. *PLoS One*. 2009; 4(12):e8528. [PubMed: 20052275]
- Bruchas MR, Land BB, Chavkin C. The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. *Brain Res*. 2010; 1314:44–55. [PubMed: 19716811]
- Bruijnzeel AW, Gold MS. The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence. *Brain Res Brain Res Rev*. 2005; 49(3):505–528. [PubMed: 16269317]
- Buczek Y, Lê AD, Wang A, Stewart J, Shaham Y. Stress reinstates nicotine seeking but not sucrose solution seeking in rats. *Psychopharmacology (Berl)*. 1999; 144(2):183–188. [PubMed: 10395000]
- Calu DJ, Kawa AB, Marchant NJ, Navarre BM, Henderson MJ, Chen B, Yau HJ, Bossert JM, Schoenbaum G, Deisseroth K, Harvey BK, Hope BT, Shaham Y. Optogenetic inhibition of dorsal medial prefrontal cortex attenuates stress-induced reinstatement of palatable food seeking in female rats. *J Neurosci*. 2013; 33:214–226. [PubMed: 23283335]
- Carey AN, Borozny K, Aldrich JV, McLaughlin JP. Reinstatement of cocaine place-conditioning prevented by the peptide kappa-opioid receptor antagonist, Aroclon. *Eur J Pharmacol*. 2007; 569(1–2):84–89.
- Carlezon WA Jr, Béguin C, Dinieri JA, Baumann MH, Richards MR, Todtenkopf MS, Rothman RB, Ma Z, Lee DY, Cohen BM. Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J Pharmacol Exp Ther*. 2006; 316(1):440–447. [PubMed: 16223871]
- Carr GV, Bangasser DA, Bethea T, Young M, Valentino RJ, Lucki I. Anti-depressive like effects of kappa opioid antagonists in Wistar Kyoto rats. *Neuropsychopharmacol*. 2009; 35(3):752–763.

- Catania, CA. Learning. Prentice-Hall: Englewood Cliffs; 1992.
- Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib MA, Craven LA, Allen SS, Sved AF, Perkins KA. Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. *Psychopharmacology (Berl)*. 2005; 80(2):258–266. [PubMed: 15682294]
- Chavkin C, James IF, Goldstein A. Dynorphin is a specific endogenous ligand of the kappa opioid receptor. *Science*. 1982; 215(4531):413–415. [PubMed: 6120570]
- Chen H, Fu Y, Sharp BM. Chronic nicotine self-administration augments hypothalamic-pituitary-adrenal responses to mild acute stress. *Neuropsychopharmacol*. 2008; 33(4):721–730.
- Cheng PY, Birk AV, Gershengorn MC, Szeto HH. Dynorphin stimulates corticotropin release from mouse anterior pituitary AtT-20 cells through nonopioid mechanisms. *Neuroendocrinol*. 2000; 71:170–176.
- Cippitelli A, Damadzic R, Hansson AC, Singley E, Sommer WH, Eskay R, et al. Neuropeptide Y (NPY) suppresses yohimbine-induced reinstatement of alcohol seeking. *Psychopharmacology (Berl)*. 2010; 208:417–426. [PubMed: 20012021]
- Daughton DM, Roberts D, Patil KD, Rennard SI. Smoking cessation in the workplace: evaluation of relapse factors. *Prev Med*. 1990; 19(2):227–230. [PubMed: 2359744]
- Endoh T, Matsuura H, Tanaka C, Nagase H. Nor-binaltorphimine: a potent and selective kappa-opioid receptor antagonist with long-lasting activity in vivo. *Arch Int Pharmacodyn Ther*. 1992; 316:30–42. [PubMed: 1326932]
- Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. *J Neurosci*. 1998; 18(14):5529–5536. [PubMed: 9651233]
- Fallon JH, Leslie FM. Distribution of dynorphin and enkephalin peptides in the rat brain. *J Comp Neurol*. 1986; 249(3):293–336. [PubMed: 2874159]
- Feltenstein MW, See RE. Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. *Behav Brain Res*. 2006; 174:1–8. [PubMed: 16920204]
- Finkelstein DM, Kubzansky LD, Goodman E. Social status, stress, and adolescent smoking. *J Adolesc Health*. 2006; 39(5):678–685. [PubMed: 17046504]
- Funk D, Coen K, Lê AD. The role of kappa opioid receptors in stress-induced reinstatement of alcohol seeking in rats. *Brain and Behav*. 2014; 4(3):356–367.
- Funk D, Li Z, Lê AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. *Neuroscience*. 2006; 138(1):235–243. [PubMed: 16359808]
- Doxey JC, Lane AC, Roach AG, Virdee NK. Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781094), yohimbine, rauwolscine and corynanthine. *Naunyn Schmiedeberg Arch. Pharmacol*. 1984; 325:136–144. [PubMed: 6144048]
- Galeote L, Berrendero F, Bura SA, Zimmer A, Maldonado R. Prodynorphin gene disruption increases the sensitivity to nicotine self-administration in mice. *Int J Neuropsychopharmacol*. 2009; 12(5): 615–625. [PubMed: 18937881]
- Gentsch C, Lichteiner M, Feer H. Behavioral effects of yohimbine and chlordiazepoxide: dependence on the rat's previous familiarization with the test conditions. *Neuropsychobiol*. 1989; 22:101–107.
- Greenwald MK, Lundahl LH, Steinmiller CL. Yohimbine increases opioid-seeking behavior in heroin-dependent, buprenorphine-maintained individuals. *Psychopharmacology (Berl)*. 2013; 225:811–824. [PubMed: 23161001]
- Hanh B, Stolerman IP, Shoaib M. Kappa-opioid receptor modulation of nicotine-induced behaviour. *Neuropsychopharmacology*. 2000; 39:2848–2855.
- Holmberg G, Gershon S. Autonomic and psychic effects of yohimbine hydrochloride. *Psychopharmacologia*. 1961; 2:93–106. [PubMed: 13715444]
- Ise Y, Narita M, Nagase H, Suzuki T. Modulation of κ -opioidergic systems on mecamylamine-precipitated nicotine-withdrawal aversion in rats. *Neurosci Lett*. 2002; 323:164–166. [PubMed: 11950518]

- Ismayilova N, Shoaib M. Alteration of intravenous nicotine self-administration by opioid receptor agonist and antagonists in rats. *Psychopharmacology (Berl)*. 2010; 210(2):211–220. [PubMed: 20401605]
- Isola R, Zhang H, Teiwani GA, Neff NH, Hadjiconstantinou M. Dynorphin and Prodynorphin mRNA changes in the striatum during nicotine withdrawal. *Synapse*. 2008; 62:448–455. [PubMed: 18361441]
- Jackson KJ, Carroll FI, Negus SS, Damaj MI. Effect of the selective kappa-opioid receptor antagonist JDTC on nicotine antinociception, reward, and withdrawal in the mouse. *Psychopharmacology (Berl)*. 2010; 210(2):285–294. [PubMed: 20232057]
- Jackson KJ, McLaughlin JP, Carroll FI, Damaj MI. Effects of the kappa opioid receptor antagonist, norbinaltorphimine, on stress and drug-induced reinstatement of nicotine-conditioned place preference in mice. *Psychopharmacology (Berl)*. 2013; 226(4):763–768. [PubMed: 22526543]
- Jones DN, Holtzman SG. Long term kappa-opioid receptor blockade following norbinaltorphimine. *Eur J Pharmacol*. 1992; 215:345–348. [PubMed: 1327807]
- Khachataturian H, Watson SJ, Lewis ME, Coy D, Goldstein A, Akil H. Dynorphin immunocytochemistry in the rat central nervous system. *Peptides*. 1982; 3:941–954. [PubMed: 6132365]
- Knoll AT, Meloni EG, Thomas JB, Carroll FI, Carlezon WA Jr. Anxiolytic-like effects of kappa-opioid receptor antagonists in models of unlearned and learned fear in rats. *J Pharmacol Exp Ther*. 2007; 323(3):838–845. [PubMed: 17823306]
- Knoll AT, Carlezon WA Jr. Dynorphin, stress, and depression. *Brain Res*. 2010; 1314:56–73. [PubMed: 19782055]
- 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(8):733–738. [PubMed: 3729667]
- Land BB, Bruchas MR, Lemos JC, Xu M, Melief EJ, Chavkin C. The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. *J Neurosci*. 2008; 28(2):407–414. [PubMed: 18184783]
- Land BB, Bruchas MR, Schattauer S, Giardino WJ, Aita M, Messinger D, Hnasko TS, Palmiter RD, Chavkin C. Activation of the kappa opioid receptor in the dorsal raphe nucleus mediates the aversive effects of stress and reinstates drug seeking. *Proc Natl Acad Sci U.S.A.* 2009; 106(45):19168–19173. [PubMed: 19864633]
- Lang WJ, Gershon S. Effects of psychoactive drugs on yohimbine induced responses in conscious dogs. A proposed screening procedure for anti-anxiety agents. *Arch. Int. Pharmacodyn. Ther*. 1963; 142:457–472. [PubMed: 13928559]
- Lê AD, Harding S, Juzysch W, Watchus J, Shalev U, Shaham Y. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology (Berl)*. 2000; 150(3):317–324. [PubMed: 10923760]
- Lê AD, Harding S, Juzysch W, Fletcher PJ, Shaham Y. The role of corticotropin-releasing factor in the median raphe nucleus in relapse to alcohol. *J Neurosci*. 2002; 22(18):7844–7849. [PubMed: 12223536]
- Lê AD, Harding S, Juzysch W, Funk D, Shaham Y. Role of alpha-2-adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. *Psychopharmacology (Berl)*. 2005; 179(2):366–373. [PubMed: 15551068]
- Le AD, Funk D, Harding S, Juzysch W, Fletcher PJ. The role of noradrenaline and 5-hydroxytryptamine in yohimbine-induced increases in alcohol-seeking in rats. *Psychopharmacology (Berl)*. 2009; 204:477–488. [PubMed: 19229522]
- Le AD, Funk D, Juzysch W, Coen K, Navarre BM, Cifani C, Shaham Y. Effect of prazosin and guanfacine on stress-induced reinstatement of alcohol and food seeking in rats. *Psychopharmacology (Berl)*. 2011; 218:89–99. [PubMed: 21318567]
- Lê AD, Funk D, Coen K, Li Z, Shaham Y. Role of corticotropin-releasing factor in the median raphe nucleus in yohimbine-induced reinstatement of alcohol seeking in rats. *Addict Biol*. 2013; 18(3):448–451. [PubMed: 21967606]

- Lee B, Tiefenbacher S, Platt DM, Spealman RD. Pharmacological blockade of alpha(2)-adrenoceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys. *Neuropsychopharmacol.* 2004; 29:686–693.
- Ma J, Ye N, Lange N, Cohen BM. Dynorphinergic GABA neurons are a target of both typical and atypical antipsychotic drugs in the nucleus accumbens shell, central amygdaloid nucleus, and thalamic central medial nucleus. *Neuroscience.* 2003; 121:991–998. [PubMed: 14580949]
- Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC Jr, Jones RM, Portoghesi PS, Carlezon WA Jr. Antidepressant-like effects of kappa-opioid receptor antagonists in the forced swim test in rats. *J Pharmacol Exp Ther.* 2003; 305(1):323–330. [PubMed: 12649385]
- Majczyk H, Cabaj A, Sławińska U, Górka T. Intrathecal administration of yohimbine impairs locomotion in intact rats. *Behav Brain Res.* 2006; 175(2):315–322. [PubMed: 17010450]
- Mansour A, Fox CA, Meng F, Akil H, Watson SJ. Kappa 1 receptor mRNA distribution in the rat CNS: comparison to kappa receptor binding and prodynorphin mRNA. *Mol Cell Neurosci.* 1994; 5:124–144. [PubMed: 8032682]
- Mantsch JR, Weyer A, Vranjkovic O, Beyer CE, Baker DA, Caretta H. Involvement of noradrenergic neurotransmission in the stress- but not cocaine-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: role for beta-2 adrenergic receptors. *Neuropsychopharmacol.* 2010; 35:2165–2178.
- Marchant NJ, Densmore VS, Osborne PB. Coexpression of prodynorphin and corticotropin-releasing hormone in rat central amygdala: evidence of two distinct endogenous opioid systems in the lateral division. *J Comp Neur.* 2007; 504:702–715. [PubMed: 17722034]
- Marinelli PW, Funk D, Juzysch W, Li Z, Lê AD. Effects of opioid receptor blockade on the renewal of alcohol seeking induced by context: relationship to c-fos mRNA expression. *Eur J Neurosci.* 2007; 26:2815–2823. [PubMed: 18001278]
- Martin-García E, Barbano MF, Galeote L, Maldonado R. New operant model of nicotine-seeking behavior in mice. *Int J Neuropsychopharmacol.* 2009; 12(3):343–356. [PubMed: 18752721]
- McCarthy MJ, Zhang H, Neff NH, Hadjiconstantinou M. *Psychopharmacology (Berl).* 2010; 210:221–229. [PubMed: 19806344]
- McKee SA, Sinha R, Weinberger AH, Sofuoglu M, Harrison EL, Lavery M, Wanzer J. Stress decreases the ability to resist smoking and potentiates smoking intensity and reward. *J Psychopharmacol.* 2011; 25(4):490–502. [PubMed: 20817750]
- McLaughlin JP, Marton-Popovici M, Chavkin C. Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J Neurosci.* 2003; 23(13):5674–5683. [PubMed: 12843270]
- McLaughlin JP, Land BB, Li S, Pintar JE, Chavkin C. Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. *Neuropsychopharmacol.* 2006; 31(4):787–794.
- Merchenthaler I, Maderdrut JL, Cianchetta P, Shughrue P, Bronstein D. In situ hybridization histochemical localization of prodynorphin messenger RNA in the central nervous system of the rat. *J Comp Neurol.* 1997; 384:211–232. [PubMed: 9215719]
- Merlo Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, Weiss F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci.* 1995; 15(8):5439–5447. [PubMed: 7643193]
- Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP, Coge F, Galizzi JP, Boutin JA, Rivet JM, Dekeyne A, Gobert A. Agonist and antagonist actions of yohimbine as compared to fluparoxan at alpha(2)-adrenergic receptors (AR)s, serotonin (5-HT)(1A-5-HT(1B-5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. *Synapse.* 2000; 35:79–95. [PubMed: 10611634]
- Motta V, Penha K, Brandão ML. Effects of microinjections of mu and kappa receptor agonists into the dorsal periaqueductal gray of rats submitted to the plus maze test. *Psychopharmacology (Berl).* 1995; 120(4):470–474. [PubMed: 8539329]

- Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology (Berl)*. 1985; 86(3):274–280. [PubMed: 2994144]
- Nabeshima T, Katoh A, Wada M, Kameyama T. Stress-induced changes in brain Met-enkephalin, Leu-enkephalin and dynorphin concentrations. *Life Sci*. 1992; 51(3):211–217. [PubMed: 1352028]
- Nair SG, Adams-Deutsch T, Epstein DH, Shaham Y. The neuropharmacology of relapse to food seeking: methodology, main findings, and comparison with relapse to drug seeking. *Prog Neurobiol*. 2009; 89:18–45. [PubMed: 19497349]
- Nair SG, Navarre BM, Cifani C, Pickens CL, Bossert JM, Shaham Y. Role of dorsal medial prefrontal cortex dopamine D1-family receptors in relapse to high-fat food seeking induced by the anxiogenic drug yohimbine. *Neuropsychopharmacol*. 2011; 36(2):497–510.
- Nikolarakis KE, Almeida OFX, Herz A. Stimulation of hypothalamic β -endorphin and dynorphin release by corticotropin-releasing factor (in vitro). *Brain Res*. 1986; 399:152–155. [PubMed: 2879612]
- Nobre MJ, Ribeiro dos Santos N, Aguiar MS, Brandao ML. Blockade of mu- and activation of kappa opioid receptors in the dorsal periaqueductal gray matter produce defensive behavior in rats tested in the elevated plus-maze. *Eur J Pharmacol*. 2000; 404:145–151. [PubMed: 10980273]
- Overton MJ, Fisher LA. Modulation of central nervous system actions of corticotropin-releasing factor by dynorphin-related peptides. *Brain Res*. 1989; 488:233–240. [PubMed: 2568150]
- Pfeiffer A, Brantl V, Herz A, Emrich HM. Psychotomimesis mediated by kappa opiate receptors. *Science*. 1986; 233(4765):774–776. [PubMed: 3016896]
- Pomerleau OF, Pomerleau CS. Research on stress and smoking: progress and problems. *Br J Addict*. 1991; 86(5):599–603. [PubMed: 1859926]
- Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977; 266(5604):730–732. [PubMed: 559941]
- Poulin JF, Arbour D, Laforest S, Drolet G. Neuroanatomical characterization of endogenous opioids in the bed nucleus of the stria terminalis. *Progr in Neuro-Psychopharmacol and Biol Psychiatry*. 2009; 33:1356–1365.
- Redila VA, Chavkin C. Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology (Berl)*. 2008; 200(1):59–70. [PubMed: 18575850]
- Reyes BAS, Drolet G, Van Bockstaele EJ. Dynorphin and stress-related peptides in rat locus coeruleus: contribution of amygdalar efferents. *J Comp Neur*. 2008; 508:663–675. [PubMed: 18381633]
- Richards JK, Simms JA, Steensland P, Taha SA, Borgland SL, Bonci A, Bartlett SE. Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long-Evans rats. *Psychopharmacology (Berl)*. 2008; 199:109–117. [PubMed: 18470506]
- Richards JK, Simms JA, Bartlett SE. Conditioned cues and yohimbine induce reinstatement of beer and near-beer seeking in Long-Evans rats. *Addict Biol*. 2009; 14:144–151. [PubMed: 19076928]
- Roth KA, Weber E, Barchas JD, Chang D, Chang JK. Immunoreactive dynorphin-(1–8) and corticotropin-releasing factor in subpopulation of hypothalamic neurons. *Science*. 1983; 219:189–191. [PubMed: 6129700]
- Scatton B, Zivkovic B, Dedek J. Antidopaminergic properties of yohimbine. *J. Pharmacol. Exp. Ther*. 1980; 215:494–499. [PubMed: 7192314]
- Schank JR, Goldstein AL, Rowe KE, King CE, Marusich JA, Wiley JL, Carroll FI, Thorsell A, Heilig M. The kappa opioid receptor antagonist JD1c attenuates alcohol seeking and withdrawal anxiety. *Addict Biol*. 2012; 17(3):634–647. [PubMed: 22515275]
- Schepis TS, Rao U. Epidemiology and etiology of adolescent smoking. *Curr Opin Pediatr*. 2005; 17(5):607–612.
- Schindler AG, Li S, Chavkin C. Behavioral stress may increase the rewarding valence of cocaine-associated cues through a dynorphin/kappa-opioid receptor-mediated mechanism without affecting associative learning or memory retrieval mechanisms. *Neuropsychopharmacol*. 2010; 35(9):1932–1942.

- Schroeder BE, Schiltz CA, Kelley AE. Neural activation profile elicited by cues associated with the anxiogenic drug yohimbine differs from that observed for reward-paired cues. *Neuropsychopharmacology*. 2003; 28:14–21. [PubMed: 12496936]
- Shalev U, Finnle PS, Quinn T, Tobin S, Wahi P. A role for corticotropin-releasing factor, but not corticosterone, in acute food-deprivation-induced reinstatement of heroin seeking in rats. *Psychopharmacology (Berl)*. 2006; 187(3):376–384. [PubMed: 16850287]
- Shalev U, Erb S, Shaham Y. Role of CRF and other neuropeptides in stress-induced reinstatement of drug-seeking. *Brain Res*. 2010; 314:15–28. [PubMed: 19631614]
- Shepard JD, Bossert JM, Liu SY, Shaham Y. The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biol. Psychiatry*. 2004; 55:1082–1089. [PubMed: 15158427]
- Shippenberg TS, Zapata A, Chefer VI. Dynorphin and the pathophysiology of drug addiction. *Pharmacol Ther*. 2007; 116(2):306–321. [PubMed: 17868902]
- Shirayama Y, Ishida H, Iwata M, Hazama GI, Kawahara R, Duman RS. Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant like effects. *J Neurochem*. 2004; 90:1258–1268. [PubMed: 15312181]
- Simms JA, Richards JK, Mill D, Kanholm I, Holgate JY, Bartlett SE. Induction of multiple reinstatements of ethanol- and sucrose-seeking behavior in Long-Evans rats by the α -2 adrenoreceptor antagonist yohimbine. *Psychopharmacology (Berl)*. 2011b; 218:101–110. [PubMed: 21863233]
- Sirinathsinghji DJS, Nikolarakis KE, Herz A. Corticotropin-releasing factor stimulates the release of methionine-enkephalin and dynorphin from the neostriatum and globus pallidus of the rat: in vitro and in vivo studies. *Brain Res*. 1989; 490:276–291. [PubMed: 2569903]
- Smith JS, Schindler AG, Martinelli E, Gustin RM, Bruchas MR, Chavkin C. Stress-induced activation of the dynorphin/ κ -opioid receptor system in the amygdala potentiates nicotine conditioned place preference. *J. Neurosci*. 2012; 32(4):1488–1495. [PubMed: 22279233]
- Spanagel R, Almeida OFX, Bartl C, Shippenberg TS. Endogenous κ -opioid systems in opiate withdrawal: role for aversion and accompanying changes in mesolimbic dopamine release. *Psychopharmacology (Berl)*. 1994; 115:121–127. [PubMed: 7862883]
- Spealman RD, Barrett-Larimore RL, Rowlett JK, Platt DM, Khroyan TV. Pharmacological and environmental determinants of relapse to cocaine-seeking behavior. *Pharmacol. Biochem. Behav*. 1999; 64:327–336. [PubMed: 10515309]
- Stine SM, Southwick SM, Petrakis IL, Kosten TR, Charney DS, Krystal JH. Yohimbine-induced withdrawal and anxiety symptoms in opioid-dependent patients. *Biol Psychiatry*. 2002; 51:642–651. [PubMed: 11955464]
- Tanda G, Bassareo V, Di Chiara G. Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. *Psychopharmacology (Berl)*. 1996; 123:127–130. [PubMed: 8741935]
- Uhlen S, Dambrova M, Nasman J, Schioth HB, Gu Y, Wikberg-Matsson A, Wikberg JE. [3H]RS79948-197 binding to human rat, guinea pig and pig α 2A-, α 2B- and α 2C-adrenoceptors. Comparison with MK912, RX821002, rauwolscine and yohimbine. *Eur. J. Pharmacol*. 1998; 343:93–101. [PubMed: 9551719]
- Umhau JC, Schwandt ML, Usala J, Geyer C, Singley E, George TD, Heilig M. Pharmacologically induced alcohol craving in treatment seeking alcoholics correlates with alcoholism severity, but is insensitive to acamprosate. *Neuropsychopharmacology (Berl)*. 2011; 60:303–311.
- Valdez GR, Platt DM, Rowlett JK, Ruedi-Bettschen D, Spealman RD. κ -agonist-induced reinstatement of cocaine seeking in squirrel monkeys: a role for opioid and stress-related mechanisms. *J Pharmacol Exp Ther*. 2007; 323(2):525–533. [PubMed: 17702903]
- Wee S, Koob GF. The role of the dynorphin- κ opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology (Berl)*. 2010; 210:121–135. [PubMed: 20352414]
- Wiley MD, Poveromo LB, Antapasis J, Herrera CM, Bolaños Guzmán CA. Kappa-opioid system regulates the long-lasting behavioral adaptations induced by early-life exposure to methylphenidate. *Neuropsychopharmacol*. 2009; 34(5):1339–1350.

- Winter JC, Rabin RA. Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination. *J. Pharmacol. Exp. Ther.* 1992; 263:682–689. [PubMed: 1359109]
- Wittmann W, Schunk E, Rosskoth I, Gaburro S, Singewald N, Herzog H, Schwarzer C. Prodynorphin-derived peptides are critical modulators of anxiety and regulate neurochemistry and corticosterone. *Neuropsychopharmacol.* 2009; 34(3):775–785.
- Yamada H, Bruijnzeel AW. Stimulation of α 2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. *Neuropharmacol.* 2011; 60(2–3):303–311.
- You ZB, Herrera-Marschitz M, Terenius L. Modulation of neurotransmitter release in the basal ganglia of the rat brain by dynorphin peptides. *J Pharmacol Exp Ther.* 1999; 290(3):1307–1315. [PubMed: 10454508]
- Zhou Y, Leri F, Grella SL, Aldrich JV, Kreek MJ. Involvement of dynorphin and kappa opioid receptor in yohimbine-induced reinstatement of heroin seeking in rats. *Synapse.* 2013; 67(6): 358–361. [PubMed: 23345075]
- Zislis G, Desai TV, Prado M, Shah HP, Bruijnzeel AW. Effects of the CRF receptor antagonist DPhe CRF(12–41) and the α 2-adrenergic receptor agonist clonidine on stress-induced reinstatement of nicotine-seeking behavior in rats. *Neuropharmacol.* 2007; 53(8):958–966.

Highlights

- We examine the role of the kappa opioid receptor(KOR) in nicotine (NIC) seeking.
- The KOR antagonist nor-BNI, blocked stress-induced reinstatement of NIC seeking.
- The KOR antagonist nor-BNI, did not block cue induced reinstatement of NIC seeking.
- The KOR agonist U50,488 reinstated NIC seeking in a dose-dependent manner.
- We report a stress-specific role for the KOR in NIC seeking behavior.

Fig. 1. Yohimbine-Induced Reinstatement Attenuated by KOR Antagonism

A) Mean ($\pm$ SEM) number of responses made on the last two days of extinction and on the day of the YOH reinstatement test by each group: VEH1hrYOH ($n=7$), NB1hrYOH ($n=8$), and NB24hrYOH ($n=9$). YOH significantly reinstated nicotine seeking in rats given vehicle pretreatment. This was attenuated in animals pretreated with nor-BNI, 1 hr and 24 hrs prior to testing. * Significant difference between extinction session and the test for reinstatement in the VEH1hrYOH group. + Significant difference between the VEH1hrYOH group and NB1hrYOH or NB24hrYOH groups during the test for reinstatement.

Effect of NB1hr on Extinction

Fig. 2. Effect of nor-BNI pretreatment (1hr) on Extinction Responding

Mean ($\pm$ SEM) numbers of active and inactive lever responses made during extinction (the day prior to the reinstatement test) 1 hr following an injection of nor-BNI ($n=9$). There were no significant effects.

Fig. 3. KOR Activation Reinstates Nicotine Seeking

Mean (+/- SEM) numbers of active and inactive lever responses made during reinstatement test in which rats received 4 different doses of U50,488 in counterbalanced order. N=15.

U50,488 significantly reinstated nicotine seeking when given at 2.5 and 5mg/kg. * =

Significant difference from vehicle on active lever responses.

Fig. 4. Cue-Induced Reinstatement is not Affected by KOR Antagonism

A) Mean ($\pm$ SEM) number of active lever responses made on the last two days of extinction and on the day of the cue-induced reinstatement test for each group: VEH24hrCUE (n=8), NB24hrCUE (n=11) for the active lever (A) and the inactive lever (B). Re-exposure to the nicotine-paired cues (light and tone) significantly reinstated nicotine seeking in rats given vehicle pretreatment. This was not affected by pretreatment with nor-BNI, 24 hrs prior to testing. * = Significant difference across sessions in VEH24hrCUE group for active lever responses.

Table 1

Nicotine Self Administration

Experiment	Active Lever Presses	Inactive Lever Presses	Reinforcements	FR Schedule	N
1	76.96 +/- 15.10	25.73 +/- 6.81	13.19 +/- 1.41	FR3	24
2	101.09 +/- 10.98	30.80 +/- 4.36	13.42 +/- 1.30	FR5	15
3	70.74 +/- 6.64	30.79 +/- 6.56	15.19 +/- 1.23	FR3	19

Values represent the mean (+/- SEM) number of active and inactive lever responses made, as well as the number of reinforcements obtained over the last 3 days of nicotine self-administration [0.03mg/kg/infusion (0.025ml)] in the three Expts. The schedule of reinforcement and the number of rats in each study is also reported.