β-Adrenergic Receptor Mediation of Stress-Induced Reinstatement of Extinguished Cocaine-Induced Conditioned Place Preference in Mice: Roles for β₁ and β₂ Adrenergic Receptors

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Received February 23, 2012; accepted May 15, 2012

ABSTRACT

Stress can trigger the relapse of drug use in recovering cocaine addicts and reinstatement in rodent models through mechanisms that may involve norepinephrine release and β-adrenergic receptor activation. The present study examined the role of β-adrenergic receptor subtypes in the stressor-induced reinstatement of extinguished cocaine-induced (15 mg/kg i.p.) conditioned place preference in mice. Forced swim (6 min at 22°C) stress or activation of central noradrenergic neurotransmission via administration of the selective α₂ adrenergic receptor antagonist 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isindole (BRL-44,408) (10 mg/kg i.p.) induced reinstatement in wild-type, but not β₁ adrenergic receptor-deficient Adbrb1/Adbrb2 double-knockout, mice. In contrast, cocaine administration (15 mg/kg i.p.) resulted in reinstatement in both wild-type and β₁-adrenergic receptor knockout mice. Stress-induced reinstatement probably involved β₂ adrenergic receptors. The β₂ adrenergic receptor antagonist -(isopropylamino)-1-[(7-methyl-4-indanyl)oxy]butan-2-ol (ICI-118,551) (1 or 2 mg/kg i.p.) blocked reinstatement by forced swim or BRL-44,408, whereas administration of the nonselective β₂-adrenergic receptor agonist isoproterenol (2 or 4 mg/kg i.p.) or the β₂ adrenergic receptor-selective agonist clenbuterol (2 or 4 mg/kg i.p.) induced reinstatement. Forced swim-induced, but not BRL-44,408-induced, reinstatement was also blocked by a high (20 mg/kg) but not low (10 mg/kg) dose of the β₁ adrenergic receptor antagonist betaxolol, and isoproterenol-induced reinstatement was blocked by pretreatment with either ICI-118,551 or betaxolol, suggesting a potential cooperative role for β₁ and β₂ adrenergic receptors in stress-induced reinstatement. Overall, these findings suggest that targeting β₂-adrenergic receptors may represent a promising pharmacotherapeutic strategy for preventing drug relapse, particularly in cocaine addicts whose drug use is stress related.

Introduction

In many cocaine addicts, stress serves as a potent trigger for drug use. Reports that stressful stimuli can precipitate craving for cocaine in human subjects in a laboratory setting (Sinha et al., 2000) are paralleled by findings that stressors can induce reinstatement in rodent models of drug relapse (Shaham et al., 2000a). In abstinent cocaine addicts, the ability of stress to promote drug use is particularly problematic because, unlike many triggers for relapse (e.g., drug use and exposure to many drug-associated contexts and cues), the occurrence of stress is usually unavoidable. For this reason, understanding the receptor mechanisms through which stressors evoke drug-seeking behavior probably is important for the identification of better pharmacotherapeutic strategies for relapse prevention.

Noradrenergic signaling in the brain has been implicated in stress-induced relapse (Koob, 1999; Weinshenker and Schroeder, 2007). In rodent and nonhuman primate models, central delivery of norepinephrine (Brown et al., 2011) or activation of central noradrenergic neurotransmission via antagonism of α₂ adrenergic receptors (Lee et al., 2004; Feltenstein and See, 2006) reinstates extinguished cocaine seeking. Likewise, functional antagonism of noradrenergic neurotransmission through the administration of α₂ adrenergic receptor agonists blocks stress-induced reinstatement in preclinical models (Erb et al., 2000) and attenuates stress-induced craving in human cocaine addicts (Jobes et al., 2011; Fox et al., 2012).

ABBREVIATIONS: WT, wild type; KO, knockout; ANOVA, analysis of variance; BNST, bed nucleus of the stria terminalis; CRF, corticotrophin-releasing factor; VTA, ventral tegmental area; BRL-44,408, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isindole; ICI-118,551, -(isopropylamino)-1-[(7-methyl-4-indanyl)oxy]butan-2-ol.
The apparent involvement of noradrenergic systems in stress-induced drug use poses adrenergic receptors as potential medicinal targets for relapse prevention.

One method for the preclinical investigation of drug relapse is the conditioned place preference/reinstatement approach, in which the ability of stimuli to re-establish cocaine-induced conditioned place preference after extinction is used to model drug relapse (Mueller and Stewart, 2000). An advantage of this method is that it can be used to evaluate reinstatement in mice, thus permitting assessment in available genetically modified rodent models. We and others have reported that exposing mice to a variety of stressors, including forced swim in 22°C water (Kreibich and Blendy, 2004; Mantsch et al., 2010), social stress (Ribeiro Do Couto et al., 2006), and electric foot shock (Redila and Chavkin, 2008), reinstates extinguishing drug-induced conditioned place preference, suggesting that this procedure can be used to examine the neurobiological processes that contribute to stress-induced relapse.

Using the conditioned place preference/reinstatement approach, we reported previously that reinstatement by a stressor, forced swim, or by activation of central noradrenergic neurotransmission via administration of the α2 adrenergic receptor antagonist yohimbine, is blocked by pretreatment with the nonselective β-adrenergic receptor antagonist propranolol, but not the α1-adrenergic receptor antagonist prazosin, implicating β, but not α1, adrenergic receptors in stress-induced relapse (Mantsch et al., 2010). A preliminary experiment suggested the involvement of β2 adrenergic receptors in reinstatement in response to forced swim (Mantsch et al., 2010). It is noteworthy that although these findings are indicative of a selective role for β-adrenergic receptors in stress-induced reinstatement, it has been reported that prazosin can block yohimbine- or foot shock stress-induced reinstatement of extinguished food or alcohol seeking in rats (Lê et al., 2011). In the present study we further investigate the role of β-adrenergic receptors in stress-induced relapse by testing for reinstatement of extinguished cocaine-induced conditioned place preference in β-adrenergic receptor-deficient mice. In addition, we examine the role of β-adrenergic receptor subtypes in stress-induced reinstatement by testing for the abilities of β-adrenergic receptor agonists to induce reinstatement and β-adrenergic receptor antagonists to block reinstatement after forced swim in wild-type (WT) mice. Our data support previous findings demonstrating β-adrenergic receptor involvement in reinstatement in response to stress but not cocaine administration and suggest that activation of β2 adrenergic receptors is both necessary for stress-induced cocaine seeking and sufficient for reinstatement, although the precise role of β1 adrenergic receptors remains unclear.

Materials and Methods

Subjects. A total of 103 male mice were used. All mice were 8 to 9 weeks old at the start of the study. Mice were housed singly in a temperature- and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility under a 12-h light/dark cycle (lights on at 7:00 AM). They had access to food and water at all times, except when in the experimental chambers. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) as adopted and promulgated by the National Institutes of Health.

The role of β-adrenergic receptors in place conditioning, extinction, and reinstatement was initially examined by using β-adrenergic receptor-deficient Adrb1/Adrb2 double-knockout (KO) mice purchased from The Jackson Laboratory (Bar Harbor, ME). These mice were created by Rohrer et al. (1999) by mating Adrb1 homozygous knockout mice with Adrb2 homozygous knockout mice to generate compound heterozygotes, the offspring of which were then mated to obtain compound homozygotes. Backcrossed mice (seven generations) were maintained by The Jackson Laboratory by mating double homozygote null mice (eight generations). These mice have no gross physical or behavioral abnormalities and are eudic in basal metabolic rate but display attenuated effects of exercise and lack the tachycardia and hypotensive responses to the β-adrenergic receptor agonist isoproterenol (Rohrer et al., 1999). A number of strains have contributed to the background (C57BL/6J, DBA2, 129, FVB/N, and CD-1) of these mice. Because of the lack of availability of hybrid controls, C57BL/6J mice (Harlan, Indianapolis, IN) were used as wild-type control mice in the experiments conducted with the double-knockout mice. C57BL/6J mice were also used for the remainder of the experiments in the study.

Drugs. Cocaine HCl was acquired from the National Institute on Drug Abuse (Bethesda, MD) through its drug supply program. The selective β1 adrenergic receptor antagonist betaxolol HCl, the selective β2 adrenergic receptor antagonist -isopropylamino)-1-[(7-methyl-4-indanyl)oxy]butan-2-ol (ICI-118551) HCl, and the selective β2 adrenergic receptor agonist clenbuterol HCl were purchased from Sigma-Aldrich (St. Louis, MO). The nonselective β-adrenergic receptor agonist isoproterenol hydrochloride and the highly selective (relative to yohimbine) α2 adrenergic receptor antagonist 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1H-isoindole (BRL-44,408) maleate were acquired from Tocris Bioscience (Ellisville, MO). All drugs were dissolved in saline (0.9% NaCl solution) and administered intraperitoneally in a volume of 0.1 ml/20 g body mass.

Equipment. Behavioral testing was conducted by using six ENV-3013 mouse place preference chambers from MED Associates (St. Albans, VT). The stainless-steel and polivinyl chloride chambers consisted of three distinct compartments separated by 5 cm wide × 5.9 cm high manual guillotine doors. The two 46.5 × 12.7 × 12.7-cm side compartments consisted of a white compartment with a 6.35 × 6.35-mm stainless-steel mesh floor and a black compartment with a stainless-steel grid rod floor consisting of 3.2-mm rods spaced 7.9 mm apart. The side compartments were attached via a gray-colored 7.2-cm-long center compartment with a smooth floor. The clear tops of the compartments were hinged to permit placement and removal of the mice. Ceiling lights were attached to each top. To balance unconditioned side preference, only the light in the black compartment was illuminated during training/testing. Automated data collection was accomplished by using photobeams (six beams for the white and black test areas and two beams for the center gray area) that were evenly spaced across the length of the chamber and interfaced with a computer containing MED-PC software (MED Associates). Using this automated photobeam system, entry into a side compartment was defined as consecutive breaks of the first two photocell beams in that compartment located adjacent to the door separating that compartment from the center compartment. Exiting of a side compartment (and entry into the center compartment) was indicated by occlusion of the beams in the center compartment.

Cocaine-Induced Conditioned Place Preference. Cocaine-induced conditioned place preference was established by using a design in which one of the side compartments was randomly designated as the cocaine compartment and the other as the saline compartment as reported previously (Mantsch et al., 2010). On the first day of the procedure, mice were placed into the center compartment of the chamber and provided free access to both side compartments for 30 min in the absence of saline or cocaine pretreatment to determine preconditioning preference. During the 8-day conditioning phase of the experiment, mice received cocaine (15 mg/kg i.p.) and saline.
injections on alternating days after which time they were confined to
the randomly designated treatment-appropriate compartment for 30
min. After the final conditioning session, mice were tested for the
expression of cocaine-induced conditioned place preference by once
again placing them into the center compartment of the chamber
and providing them with free access to the side compartments for 30 min.
Conditioned place preference was defined as the change in time spent
(seconds) within the cocaine-paired compartment after condi-
tioning compared with the initial preconditioning session.

Extinction. After conditioning, daily extinction training was con-
ducted. During the extinction sessions, mice were placed into the
center compartment and once again provided with free access to the
side compartments for 30 min. Mice underwent daily extinction
training until the preference for the cocaine-paired compartment
during the session (i.e., change in time spent in the cocaine side
relative to preconditioning values) was reduced by at least 50%
compared with the postconditioning test session, at which time re-
instatement testing was conducted.

Reinstatement Testing. The reinstatement sessions were iden-
tical to the extinction sessions except that mice underwent forced
swim and/or received drug injections before the session. Reinstate-
ment was defined according to the time spent in the compartment
previously paired with cocaine. In most cases, mice were tested more
than once for reinstatement. In these cases, the sequence of re-
instatement test conditions were counterbalanced to address concerns
related to altered responsiveness with repeated testing. Reinstate-
ment sessions were separated by additional extinction sessions. Mice
were required to reach the extinction criterion once again before the
next reinstatement test was conducted. To confirm that alterations
in locomotor activity secondary to receptor deficiency or drug admin-
istration did not contribute to the observed effects by increasing or
decreasing exploration of the paired compartments in the three-
chamber apparatus, the total combined amount of time spent in the
compartments previously paired with cocaine or saline was also
examined during reinstatement.

Experiment 1: Stress and Cocaine-Induced Reinstatement
in β-Adrenergic Receptor-Deficient and Wild-Type Control
Mice. We previously reported that pharmacological blockade of β-ad-
renergic receptors prevents stress-, but not cocaine-, induced rein-
statement of extinguished conditioned place preference in mice
(Mantsch et al., 2010). To further investigate the role of β-adrenergic
receptors in stress-induced relapse, reinstatement after stress
(forced swim), administration of a priming injection of cocaine, or
activation of central adrenergic neurotransmission via delivery of the
selective α2 adrenergic receptor antagonist BRL-44,408 was tested in
β-adrenergic receptor-deficient Adrb1/Adrb2 double-knock-
out mice (n = 11) and C57BL/6J wild-type control mice (n = 9). These
mice underwent cocaine-induced place conditioning and extinction
before testing for reinstatement. In most cases, mice were tested for
reinstatement by all three stimuli in a counterbalanced sequence.

Stress-Induced Reinstatement. Stress-induced reinstatement
was tested by using a forced swim protocol as reported previously
(Kreibich and Blendy, 2004; Mantsch et al., 2010). Mice were placed
into a 30 cm high × 20 cm deep cylindrical polypropylene container
filled with water (20–25°C) for 6 min. After forced swim, mice were
placed back into their home cages for 3 to 4 min before introduction
into the center compartment of the place conditioning chamber with
free access to both of the side compartments for reinstatement test-
ing, as described above. In cases where reinstatement was measured,
swimming behavior was videotaped, and the amount of time spent
immobile (versus actively swimming) was determined.

Cocaine- and BRL-44,408-Induced Reinstatement. Cocaine-
induced reinstatement was tested by injecting mice with 15 mg/kg
i.p. cocaine and then immediately placing them into the chamber for
reinstatement testing. Reinstatement by the α2 adrenergic receptor
antagonist BRL-44,408 (10 mg/kg i.p.) was tested 30 min after ad-
ministration. BRL-44,408 is more selective for α2 adrenergic recep-
tors than other commonly used antagonists (e.g., yohimbine) and has
been reported to increase central norepinephrine levels in rats (Dwy-
er et al., 2010). We have reported previously that this dose of
BRL-44,408 is optimal for reinstatement (Mantsch et al., 2010).

Experiment 2: Reinstatement of Conditioned Place Prefer-
ence by Isoproterenol. To determine whether the activation of
β-adrenergic receptors is sufficient for the reinstatement of extin-
guished cocaine-induced conditioned place preference, mice (n = 11)
were tested for reinstatement after administration of the nonselective
β-adrenergic receptor agonist isoproterenol. Mice received injec-
tions of isoproterenol (1, 2, or 4 mg/kg i.p.) or vehicle 30 min before
placement into the chambers and testing for reinstatement. Each
mouse was tested with each dose of isoproterenol in a counterbal-
anced sequence.

Experiment 3: Effects of β-Adrenergic Receptor Antago-
nists on Reinstatement. To determine the role of β1 and β2 adre-
nergic receptors in stress-induced reinstatement, mice were tested for
reinstatement of extinguished place preference in response to forced
swim or BRL-44,408 (10 mg/kg i.p.), as described in experiment 1,
after pretreatment with the selective β1 adrenergic receptor antag-
ontoxalol or the selective β2 adrenergic receptor antagonist
ICI-118,551. Seven mice were tested for the effects of betaxolol (10 or
20 mg/kg i.p.; 30-min pretreatment) and vehicle on reinstatement after
forced swim in a counterbalanced sequence. All seven mice were
tested for the effects of 10 mg/kg betaxolol and vehicle. Six of
these mice were also tested for the effects of 20 mg/kg betaxolol. Eight
mice were tested for the effects of ICI-118,551 (1 or 2 mg/kg i.p.;
30-min pretreatment) on swim-induced reinstatement. All eight
mice were tested for the effects of 1 mg/kg ICI-118,551 and vehicle.
Seven of the mice were also tested for the effects of 2 mg/kg ICI-
118,551. Seven mice were tested for the effects of betaxolol (10
mg/kg) or ICI-118,551 (1 mg/kg) and vehicle on reinstatement after
administration of BRL-44,408 in a counterbalanced sequence. Five
of these mice were tested for the effects of betaxolol, ICI-118,551, and
vehicle. One mouse was tested only for the effects of betaxolol and
vehicle, and one mouse was tested only for the effects of ICI-118,551
and vehicle. In addition, six mice were tested for the effects of 20
mg/kg betaxolol, 2 mg/kg ICI-118,551, and vehicle on BRL-44,408-
induced reinstatement in a counterbalanced sequence. Each of these
data was tested under each pretreatment condition.

Finally, 11 mice were tested for the effects of betaxolol (10 mg/kg
only) or ICI-118,551 (1 mg/kg only) and vehicle on reinstatement
after administration of isoproterenol (4 mg/kg i.p.). Ten of these
mice were tested for the effects of betaxolol, ICI-118,551, and vehicle.
One mouse was tested only for the effects of betaxolol on isoproterenol-
induced reinstatement.

Experiment 4: Reinstatement of Conditioned Place Prefer-
ence by Clenbuterol. Because reinstatement by stress and BRL-
44,408 was blocked by the β2 adrenergic receptor antagonist
ICI-118,551, mice (n = 7) were tested for reinstatement after
administration of the selective β2 adrenergic receptor agonist clen-
buterol to determine whether the activation of β2 adrenergic recep-
tors is sufficient for reinstatement. These mice received injections
of clenbuterol (1, 2, or 4 mg/kg i.p.) or vehicle 30 min before placement
into the chambers and testing for reinstatement. Each mouse was
tested with each dose of clenbuterol in a counterbalanced sequence.
In addition, 11 β-adrenergic receptor knockout and five C57BL/6J
wild-type control mice were tested for reinstatement by 4 mg/kg
clenbuterol to confirm that clenbuterol was producing reinstatement
through actions at β-adrenergic receptors. The knockout mice were
also used to test reinstatement in response to stress, cocaine, and
BRL-44,408 in experiment 1.

Locomotor Testing. To assist with dose selection, the effects of
various doses of betaxolol, ICI-118,551, clenbuterol, and isoproter-
on on locomotor activity were tested in 26 mice by using an auto-
mated AccuScan activity system (AccuScan Instruments, Inc., Co-
lumbus, OH) consisting of a frame containing photocells (eight per
cage) in which clear Plexiglas 29.5 cm long × 19 cm wide × 12.7 cm
high cages were placed. Activity was measured as total photobeam
breaks. During the week before locomotor testing, mice were habituated to the test environment during two 2-h sessions. On the test day, mice were placed into the chambers for 2 h before drug administration after which activity was measured over a 2-h period. The effects of betaxolol (0, 5, 10, and 20 mg/kg i.p.), clenbuterol (0, 1, 2, and 4 mg/kg i.p.), ICI-118,551 (0, 0.5, 1, and 2 mg/kg i.p.), and isoproterenol (0, 1, 2, and 4 mg/kg i.p.) were tested in separate groups of mice.

Statistical Analyses. Place preference during testing, extinction, and reinstatement was defined as the time spent (seconds) in the designated cocaine compartment. In the experiments comparing conditioning, extinction, and reinstatement between wild-type and β-adrenergic-receptor-deficient mice, the significance of differences was determined by using two-way ANOVA with genotype as a between-subjects factor and time spent in the cocaine side during conditioning (before conditioning vs. after conditioning), extinction (day 1 versus day 2), or reinstatement (extinction versus reinstatement session) as a repeated measure followed by post hoc testing using two-tailed Student's t tests. The significance of the effects of drug pretreatment (betaxolol or ICI-118,551) on reinstatement by each of the stimuli was determined by using two-way repeated-measures ANOVA followed by post hoc testing using two-tailed Student's t tests. The abilities of various doses of isoproterenol or clenbuterol to induce reinstatement and the dose-dependent effects of various drugs on locomotor activity were determined by using one-way repeated measures ANOVA followed by post hoc testing using the Dunnett's test. For all analyses, significance was defined as p < 0.05.

Results

Experiment 1: Stress and Cocaine-Induced Reinstatement in β-Adrenergic Receptor-Deficient and Wild-Type Control Mice

Place Conditioning and Extinction in β-Adrenergic Receptor-Deficient Mice. To examine the role of β-adrenergic receptors in stress-induced relapse, a total of 11 β-adrenergic receptor-deficient Adb1/Adrb2 double-knockout mice and nine wild-type mice underwent place conditioning and extinction and were tested for reinstatement. Both wild-type and double-knockout mice developed conditioned place preference, and no difference in the magnitude of cocaine-induced place preference was observed between genotypes (Fig. 1A). A two-way place conditioning (time spent in cocaine side preconditioning versus postconditioning; repeated measure) × genotype ANOVA showed that, overall, conditioned place preference was observed (significant main effect of conditioning; F_{1,18} = 14.812; p = 0.001). However, no effect of genotype or conditioning × genotype interaction was found.

Extinction of conditioned place preference in β-adrenergic receptor-deficient and wild-type mice is shown in Fig. 1, B and C. β-Adrenergic receptor knockout mice extinguished more quickly than wild-type controls (Fig. 1B). Wild-type mice reached the extinction criteria in 5.0 ± 0.68 sessions compared with 3.1 ± 0.23 for knockout mice (t_{14} = 2.167; p < 0.01). This observation is consistent with reports that pharmacological inhibition of β-adrenergic receptors produces postretrieval disruption of conditioned place preference, thereby reducing subsequent time spent in the cocaine-paired environment (Bernardi et al., 2009; Otis and Mueller, 2011). To further examine this possibility, we compared time spent in the cocaine compartment between genotypes on the 2 days after testing for the expression of place preference (i.e., on postconditioning days 2 and 3). A two-way ANOVA examining differences in time spent on the cocaine paired side on these days between genotypes showed significant main effects of genotype (F_{1,18} = 5.665; p < 0.05) and session/day (repeated measure F_{1,18} = 4.772; p < 0.05) and a non-significant trend toward a genotype × session/day interaction (p = 0.065). Despite the lack of a significant interaction, planned comparisons examining the amount of time spent in the cocaine-paired compartment on days 2 and 3 postconditioning in each genotype were performed. A significant reduction in the time spent in the cocaine-paired compartment on day 3 versus day 2 was observed only in knockout mice (t_{10} = 2.697; p < 0.05), and on day 3 postconditioning knockout mice spent less time in the cocaine-paired compartment compared with wild-type controls (t_{18} = 3.149; p < 0.01).

Stress- and Cocaine-Induced Reinstatement in β-Adrenergic Receptor-Deficient Mice. The ability of various stimuli to reinstate extinguished cocaine-induced conditioned place preference in β-adrenergic receptor-deficient and wild-type mice as well as the mean number of extinction sessions before reaching the extinction criterion (C). A, both wild-type and knockout mice developed conditioned place preference. *, p < 0.05 versus preconditioning. B and C, knockout mice showed reduced preference on postconditioning day 3 (B; **, p < 0.05 versus WT) and extinguished more rapidly (C; ***, p < 0.05 versus WT) than wild-type mice.
Type mice is shown in Fig. 2. Reinstatement of extinguished cocaine-induced place preference by a stressor, forced swim, was tested in 10 β-adrenergic receptor knockout mice and five wild-type mice (Fig. 2A). Swim-induced reinstatement was observed in wild-type, but not knockout, mice. A two-way ANOVA showed a significant interaction between genotype and reinstatement condition ($F_{1,13} = 10.815; p < 0.01$). Post hoc testing showed that swim stress increased the time spent in the cocaine compartment relative to extinction in wild-type mice (paired $t_4 = 4.722; p < 0.01$) but not in knockout mice. In addition, the amount of time spent in the cocaine compartment after forced swim was significantly increased in wild-type versus knockout mice (unpaired $t_{13} = 3.493; p < 0.01$). There was no significant difference between genotypes during the preceding extinction session. Immobility time during the 6-min swim sessions did not significantly differ between wild-type and β-adrenergic receptor-deficient mice (Table 1).

Reinstatement in response to the administration of cocaine (15 mg/kg i.p.) was determined in 11 knockout and six wild-type mice (Fig. 2B). In contrast to swim stress, cocaine administration reinstated extinguished place preference in both knockout and wild-type mice. Two-way ANOVA showed a significant overall reinstatement effect (reinstatement versus extinction; $F_{1,15} = 13.184; p < 0.01$) but no main effect of genotype or reinstatement × genotype interaction.

Because we reported previously that the activation of central noradrenergic neurotransmission through the administration of the α2 adrenergic receptor antagonist BRL-44,408 reinstates extinguished place preference (Mantsch et al., 2010), we determined the ability of 10 mg/kg i.p. BRL-44,408 to induce reinstatement in eight wild-type and 10 β-adrenergic receptor knockout mice (Fig. 2C). Similar to stress, reinstatement after BRL-44,408 administration was observed in wild-type, but not knockout, mice. A two-way ANOVA showed a significant interaction between genotype and reinstatement condition ($F_{1,16} = 5.409; p < 0.05$). Post hoc testing revealed that BRL-44,408 increased the amount of time spent in the cocaine-paired compartment in wild-type, but not knockout, mice (paired $t_7 = 2.768; p < 0.05$) during reinstatement testing compared with the preceding extinction session. In addition, time spent in the cocaine compartment after BRL-44,408 was significantly increased in wild-type versus knockout mice (unpaired $t_{16} = 3.050; p < 0.01$). It is noteworthy that post hoc testing also showed that the time spent in the cocaine compartment during the extinction sessions before testing for BRL-44,408-induced reinstatement was reduced in knockout mice ($p < 0.05$).

To confirm that differences in locomotor activity between genotypes under extinction and reinstatement conditions did not significantly contribute to the observed effects of β-adrenergic receptor deficiency on reinstatement, the total combined amount of time spent in the cocaine and saline compartments of the three-chamber apparatus was also measured. In all cases (swim, cocaine, and BRL-44,408), two-way ANOVA failed to show significant overall effects of genotype or reinstatement condition or significant genotype × reinstatement condition interactions (data not shown), suggesting that the observed effects on reinstatement were not associated with differences in the amount of time that the mice spent outside of the center compartment in which they were placed at the start of the test session.

**Experiment 2: Reinstatement of Conditioned Place Preference by Isoproterenol**

To determine whether the activation of β-adrenergic receptors is sufficient to reinstate extinguished place preference, nine mice were tested with the nonselective β-adrenergic agonist isoproterenol. These mice showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$). Isoproterenol dose-dependently reinstated extinguished place preference (preconditioning time spent in the cocaine side = 698.70 ± 45.801 s; postconditioning time spent = 1012.99 ± 52.59 s; paired test; $t_8 = 4.581; p < 0.01$).

**Table 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Immobility Time (seconds ± S.E.) during a 6-min period of forced swim in β-adrenergic receptor-deficient Adrb1/Adrb2 double-knockout mice and wild-type C57BL/6J mice pretreated with vehicle, the β1 adrenergic receptor antagonist ICI-118,551 (1 or 2 mg/kg)</th>
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<tr>
<td>WT/vehicle</td>
<td>231.08 ± 22.28</td>
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<td>β-Adrenergic KO</td>
<td>195.25 ± 14.72</td>
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<td>Betaxolol, 10 mg/kg</td>
<td>176.55 ± 26.73</td>
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<td>Betaxolol, 20 mg/kg</td>
<td>218.92 ± 16.26</td>
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<td>ICI-118,551, 1 mg/kg</td>
<td>163.15 ± 20.41</td>
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<tr>
<td>ICI-118,551, 2 mg/kg</td>
<td>218.00 ± 18.65</td>
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</tbody>
</table>

Fig. 2. Reinstatement after forced swim (A), cocaine (15 mg/kg i.p.) administration (B), or delivery of the selective α2 adrenergic receptor antagonist BRL-44,408 (10 mg/kg i.p.; C) in β-adrenergic receptor-deficient Adrb1/Adrb2 double-KO and WT C57BL/6J control mice. Data represent the time spent in the cocaine-paired side (s ± S.E.) during reinstatement testing or the preceding extinction session (Ext). A and C, forced swim (A) and BRL-44,408 (C) induced reinstatement in WT but not KO mice. B, in contrast, cocaine induced reinstatement in both WT and KO mice. *, $p < 0.05$ versus extinction. **, $p < 0.05$ KO versus WT.
preference (Fig. 3). Two-way repeated-measures ANOVA showed a significant interaction (\( F_{3,24} = 4.922; p < 0.01 \)) between isoproterenol dose (0, 1, 2, and 4 mg/kg) and test condition (extinction versus reinstatement). Post hoc analysis showed that significant reinstatement was observed at the 2 mg/kg dose (\( t_8 = 2.382; p < 0.05 \) versus extinction) and 4 mg/kg dose (\( t_8 = 3.983; p < 0.01 \) versus extinction) but not after administration of vehicle or the lowest (i.e., 1 mg/kg) isoproterenol dose tested. Isoproterenol did not increase the total amount of time spent in the cocaine and saline compartments combined during reinstatement testing, suggesting the reinstatement was not an artifact of increased locomotor activity (data not shown).

Experiment 3: Effects of \( \beta \)-Adrenergic Receptor Antagonists on Reinstatement

Role of \( \beta_1 \) and \( \beta_2 \) Adrenergic Receptors in Stress-Induced Reinstatement. To determine which \( \beta \)-adrenergic receptor subtype mediates stress-induced reinstatement of extinguished cocaine-induced conditioned place preference, the effects of the selective \( \beta_1 \) receptor antagonist betaxolol (10 or 20 mg/kg) or the selective \( \beta_2 \) antagonist ICI-118,551 (1 mg/kg) on reinstatement after forced swim were tested (Fig. 4). For this experiment, mice were tested for stress-induced reinstatement multiple times in a counterbalanced sequence. Seven mice were tested for the effects of betaxolol on reinstatement. All of these mice were tested for the effects of 10 mg/kg betaxolol, and six of them were tested for the effects of the 20 mg/kg betaxolol dose. These mice showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 531.66 ± 95.89 s; postconditioning time spent = 828.36 ± 92.03 s; paired test; \( t_6 = 5.196; p < 0.01 \)). Reinstatement was not blocked by the 10 mg/kg betaxolol dose (Fig. 4A). A two-way repeated measures ANOVA showed that forced swim produced reinstatement (significant overall main effect of swim versus extinction; \( F_{1,5} = 10.729; p < 0.05 \)). However, no main effect of 10 mg/kg betaxolol dose on reinstatement was observed (Fig. 4B). ICI-118,551 blocked swim-induced reinstatement at both doses tested (significant overall main effect of ICI-118,551 versus extinction; \( F_{1,5} = 10.729; p < 0.05 \)).
betaxolol and no significant betaxolol × reinstatement interaction were observed. In contrast, a two-way ANOVA showed a significant interaction between betaxolol pretreatment and reinstatement condition in mice that received 20 mg/kg betaxolol ($F_{1,6} = 8.210; p < 0.05$; Fig. 4B). In this group, post hoc testing revealed significant reinstatement in mice pretreated with vehicle ($t_6 = 2.512; p < 0.05$) but not 20 mg/kg betaxolol.

In contrast to betaxolol, both doses of ICI-118,551 that were tested blocked swim-induced reinstatement. Eight mice were tested for the effects of ICI-118,551 on reinstatement. All eight of these mice were tested for the effects of the 1 mg/kg ICI-118,551 dose (Fig. 4C), whereas only seven were tested for the effects of the 2 mg/kg dose (Fig. 4D). These mice showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 535.22 ± 83.12 s; postconditioning time spent = 847.94 ± 82.07 s; paired test; $t_7 = 6.016; p = 0.001$). For both doses of ICI-118,551, significant interactions between ICI-118,551 treatment (drug versus vehicle) and reinstatement conditions (swim versus extinction) were observed ($F_{1,7} = 5.659$ for the 1 mg/kg dose; $F_{1,6} = 7.065$ for the 2 mg/kg dose; $p < 0.05$ for both). In both cases, post hoc testing revealed that forced swim produced reinstatement in mice pretreated with vehicle ($t_7 = 2.350, p = 0.05$ for the 1 mg/kg dose vehicle; paired $t_6 = 2.925, p < 0.05$ for the 2 mg/kg dose vehicle), but not ICI-118,551.

Despite a tendency to reduce immobility during forced swim testing at the lower doses, neither betaxolol (10 or 20 mg/kg) nor ICI-118,551 (1 or 2 mg/kg) significantly altered immobility time during the 6-min sessions relative to vehicle treatment (Table 1). In addition, neither betaxolol (10 or 20 mg/kg) nor ICI-118,551 (1 or 2 mg/kg) reduced the total amount of time spent in the cocaine and saline compartments combined during reinstatement testing, suggesting that the inhibition of reinstatement was not attributable to a general reduction of locomotor activity and decreased movement out of the center start compartment (data not shown).

**Role of $\beta_1$ and $\beta_2$ Adrenergic Receptors in Reinstate-ment by BRL-44,408.** The effects of betaxolol (10 and 20 mg/kg) and ICI-118,551 (1 and 2 mg/kg) on reinstatement by the selective $\beta_2$ adrenergic receptor antagonist BRL-44,408 are shown in Fig. 5. Mice tested for reinstatement in response to BRL-44,408 ($n = 13$) showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 650.66 ± 59.13 s; postconditioning time spent = 981.67 ± 645.118.00 s; paired test; $t_{12} = 5.170; p < 0.01$). Five of these mice were tested for the effects of 10 mg/kg betaxolol and 1 mg/kg ICI-118,551. One mouse was tested only for the effects of 10 mg/kg betaxolol, and one...
mouse was tested only for the effects of 1 mg/kgICI-118,551. An additional six mice were tested for the effects of both 20 mg/kg betaxolol and 2 mg/kgICI-118,551 on reinstatement by BRL-44,408.

Neither 10 mg/kg i.p. (Fig. 5A) nor 20 mg/kg i.p. (Fig. 5B) betaxolol blocked BRL-44,408-induced reinstatement. Two-way repeated measures ANOVA showed significant reinstating effects of BRL-44,408 (F1,15 = 11.196, p < 0.05, 10 mg/kg betaxolol group; F1,5 = 22.117, p = 0.005, 20 mg/kg betaxolol group) but no significant main effects of betaxolol (10 or 20 mg/kg) and no betaxolol × reinstatement condition interactions. In contrast, both 1 mg/kg i.p. (Fig. 5C) and 2 mg/kg i.p. (Fig. 5D) ICI-118,551 blocked reinstatement by BRL-44,408. At the 1 mg/kgICI-118,551 dose, a two-way repeated measures ANOVA showed a significant interaction between ICI-118,551 pretreatment and BRL-44,408-induced reinstatement (F1,10 = 25.175; p < 0.01). Post hoc testing revealed that BRL-44,408 reinstated place preference in mice after vehicle, but not ICI-118,551, pretreatment (BRL 44,408 versus extinction; paired t9 = 3.589; p < 0.05). At the 2 mg/kgICI-118,551 dose, a two-way repeated measures ANOVA showed a near-significant interaction between ICI-118,551 pretreatment and BRL-44,408-induced reinstatement (F1,5 = 6.115; p = 0.056). A planned comparison test revealed that BRL-44,408 reinstated place preference in mice after vehicle, but not ICI-118,551, pretreatment (BRL 44,408 versus extinction; paired t9 = 2.553; p = 0.05). As was the case with forced swim, neither betaxolol nor ICI-118,551 reduced the total amount of time spent in the cocaine and saline compartments combined during reinstatement testing, suggesting that general reductions in locomotor activity and decreased movement out of the center start compartment did not contribute to the effects on reinstatement (data not shown).

Role of β1 and β2 Adrenergic Receptors in Isoproterenol-Induced Reinstatement. To examine the role of β1 and β2 adrenergic receptor subtypes in reinstatement in response to the nonselective β-receptor agonist isoproterenol, mice were tested for reinstatement by 4 mg/kg isoproterenol after pretreatment with the β1 adrenergic receptor-selective antagonist betaxolol (10 mg/kg i.p.; Fig. 6A) or the β2 receptor-selective antagonist ICI-118,551 (1 mg/kg i.p.; Fig. 6B). These mice (n = 11) showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 579.75 ± 59.87 s; postconditioning time spent = 944.08 ± 39.28 s; paired test; t10 = 6.704; p < 0.001). We were surprised to find that isoproterenol-induced reinstatement was blocked by either betaxolol (n = 11) or ICI-118,551 (n = 10) pretreatment. A two-way repeated measures ANOVA examining the effects of betaxolol on isoproterenol-induced reinstatement showed a significant interaction between betaxolol pretreatment (10 mg/kg betaxolol versus vehicle) and reinstatement (F1,10 = 19.981; p < 0.01). Likewise, a two-way repeated measures ANOVA examining the effects of ICI-118,551 on reinstatement showed a significant interaction between ICI-118,551 pretreatment (1 mg/kg ICI-118,551 versus vehicle) and reinstatement (F1,9 = 5.420; p < 0.05). In both cases, significant reinstatement of place preference was observed after pretreatment with vehicle (t10 = 3.338, p < 0.01 for betaxolol experiment; t9 = 2.890, p < 0.05 for ICI-118,551 experiment) but not after administration of either β-adrenergic receptor antagonist. Effects on the total amount of time spent in the cocaine and saline compartments combined during reinstatement testing were not observed (data not shown).

Experiment 4: Reinstatement of Conditioned Place Preference by Clenbuterol

To determine whether β2 adrenergic receptor activation alone is sufficient to restate extinguished place preference, seven mice were tested for reinstatement after administration of the selective β2 receptor agonist clenbuterol. These mice showed significant cocaine-induced place preference (preconditioning time spent in the cocaine side = 603.03 ± 53.88 s; postconditioning time spent = 987.06 ± 35.72 s; paired test; t9 = 5.675; p < 0.01). Clenbuterol dose-dependently reinstated extinguished place preference (Fig. 7A). Two-way repeated-measures ANOVA showed a significant interaction (F1,18 = 5.206; p < 0.01) between clenbuterol dose (0, 1, 2, and 4 mg/kg) and test condition (extinction versus reinstatement). Post hoc analysis showed that significant reinstatement was observed at the 2 mg/kg dose (t9 = 3.18; p < 0.05 versus extinction) and 4 mg/kg dose (t9 = 4.677; p < 0.01 versus extinction) but not after administration of vehicle or the lowest (i.e., 1 mg/kg) clenbuterol dose. As was the case with isoproterenol, clenbuterol did not increase the total amount of time spent in the cocaine and saline compartments combined during reinstatement testing, suggesting that reinstatement was not an artifact of increased locomotor activity (data not shown). As expected, clenbuterol-induced reinstatement was not observed in β-adrenergic receptor-deficient mice (Fig. 7B). A two-way reinstatement × genotype ANOVA showed a significant interaction between genotype (knockout versus wild type) and clenbuterol-induced reinstatement.

![Fig. 6. Effects of the selective β2 adrenergic receptor antagonist betaxolol (10 mg/kg i.p.; A) or the selective β2 adrenergic receptor antagonist ICI-118,551 (1 mg/kg i.p.; B) on reinstatement after administration of isoproterenol (4 mg/kg i.p.) in wild-type C57BL/6J mice. Data represent the amount of time spent in the cocaine (Coc)-paired side (± S.E.) during reinstatement testing after isoproterenol administration after antagonist or vehicle pretreatment (Veh) or during the preceding extinction session (Ext). Both ICI-118,551 and betaxolol blocked isoproterenol-induced reinstatement. * significant reinstatement, p < 0.05 versus extinction.](image-url)
FIG. 7. Dose-dependent reinstatement by the β2 adrenergic receptor agonist clenbuterol in WT C57BL/6J mice (A) and reinstatement after administration of 4 mg/kg clenbuterol in WT and β-adrenergic receptor KO mice (B). Data represent time spent in the cocaine-paired side (s ± S.E.) during reinstatement testing after pretreatment with clenbuterol or during the preceding extinction session (Ext). Clenbuterol produced reinstatement at the 2 and 4 mg/kg doses but failed to produce reinstatement in KO mice. *p < 0.05 versus extinction. **p < 0.05 KO versus WT.

TABLE 2
Effects of adrenergic drugs on locomotor activity
Data represent the mean number of ambulatory counts (± S.E.) recorded over a 2-h period after intraperitoneal administration of various doses of betaxolol, ICI-118,551, isoproterenol, and clenbuterol.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Betaxolol</th>
<th>ICI-118,551</th>
<th>Isoproterenol</th>
<th>Clenbuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulatory Counts</td>
<td>Ambulatory Counts</td>
<td>Ambulatory Counts</td>
<td>Ambulatory Counts</td>
<td></td>
</tr>
<tr>
<td>0 mg/kg</td>
<td>3501.60 ± 385.63</td>
<td>0</td>
<td>2455.76 ± 248.19</td>
<td>0</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>1927.60 ± 368.37*</td>
<td>0.5</td>
<td>2408.40 ± 346.86</td>
<td>0.5</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>1519.40 ± 304.82*</td>
<td>1.0</td>
<td>1316.20 ± 152.39*</td>
<td>1.0</td>
</tr>
<tr>
<td>20.0 mg/kg</td>
<td>279.80 ± 84.53*</td>
<td>2.0</td>
<td>806.60 ± 110.01*</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* p < 0.05 versus 0 mg/kg dose.

(F1,14 = 15.930; p < 0.01). Clenbuterol (4 mg/kg i.p.) reinstated place preference in wild-type mice (t9 = 6.474; p < 0.05; n = 5) but not Adrb1/Adrb2 double-knockout mice (n = 11).

**Discussion**

We reported previously that disinhibition of central noradrenergic neurotransmission via α1 adrenergic receptor antagonism using yohimbine or the more selective antagonist BRL-44,408 is sufficient to reinstate extinguished cocaine-induced conditioned place preference, whereas functional antagonism of noradrenergic activity via administration of the α2 adrenergic receptor agonist clonidine prevents reinstatement by a stressor, forced swim, in mice (Mantsch et al., 2010). Furthermore, we reported that stress- and yohimbine-induced reinstatement is prevented by propranolol, but not the α1 adrenergic receptor antagonist prazosin (Mantsch et al., 2010). In this study, we confirm the role of β-adrenergic receptors in stress-induced reinstatement by demonstrating that reinstatement by forced swim or BRL-44,408 is not observed in β-receptor-deficient Adrb1/Adrb2 double-knockout mice, and that β-receptor activation using isoproterenol, a nonselective agonist, is sufficient to induce reinstatement in wild-type mice. Although suggestive of a role for β-adrenergic receptors, the absence of reinstatement in knockout mice should be interpreted with caution because of the lack of hybrid background strain controls. However, it should be noted that stress-induced reinstatement of cocaine-induced place preference has been observed in a number of mouse strains, including other hybrid background strains (Kreibich and Blendy, 2004; Redila and Chavkin, 2008; Land et al., 2009; Briand et al., 2010; Mantsch et al., 2010; Bruchas et al., 2011).

Our findings suggest that β2 adrenergic receptor activation is both necessary for stress-induced reinstatement and sufficient to reinstate and that stress-induced reinstatement may also involve β1 receptors. The selective β2 receptor antagonist ICI-118,551 blocked reinstatement by forced swim or BRL-44,408 at all doses tested, whereas the selective β2 receptor agonist clenbuterol dose-dependently reinstated. Because the β1 receptor antagonist betaxolol also interfered with swim-induced (but not BRL-44,408-induced) reinstatement at a high dose, we can not rule out a contribution of β1 receptors to stress-induced reinstatement. In contrast to ICI-118,551, which has a 300-fold higher affinity for β2 relative to β1 receptors, the selectivity for betaxolol for β1 versus β2 receptors is much lower, approximately a 35-fold affinity difference (Wellstein et al., 1986). Thus, it is possible that at the higher betaxolol dose there was some β2 receptor antagonism. However, considering that it has been reported that the same betaxolol dose can alter other stress-induced behaviors in mice on which ICI-118,551 has no effect (Stone et al., 1996), coordinated roles for both receptors are possible. Accordingly, reinstatement by isoproterenol was blocked by either betaxolol or ICI-188,551. Because activation of β2 receptors alone is sufficient for reinstatement (clenbuterol re-
instates), the requirement for β₁ receptors for reinstatement is intriguing and may imply that tonic levels of β₁ receptor activation, possibly by basal levels of norepinephrine, are necessary for the activation of β₂ receptors and/or their effects on cocaine seeking.

Both ICI-118,551 and betaxolol inhibited locomotor activity at doses tested for effects on reinstatement. Because reduced locomotor activity could produce effects that resemble decreased reinstatement in the three-compartment apparatus caused by potential reductions in the overall time spent outside of the start chamber, we also examined the total time spent in the saline and cocaine compartments combined during reinstatement testing. In all cases, antagonist-induced reductions in the time spent in the cocaine compartment were accompanied by increases in time spent in the saline compartment such that the total time spent outside of the start chamber was no different from vehicle control conditions. Thus, we consider it unlikely that locomotor-suppressing effects contributed to the reductions in reinstatement.

Based on the findings by Leri et al. (2002) that combined antagonism of β₁ and β₂ adrenergic receptors in the bed nucleus of the stria terminalis (BNST) or amygdala using a cocktail of betaxolol and ICI-118,551 blocks reinstatement of extinguished cocaine self-administration by foot shock stress in rats, we assume that our findings are attributable to the blockade of receptor function in the brain. However, a role for peripheral receptors cannot be ruled out, especially considering evidence that epinephrine released from the adrenal medulla can regulate a number of behavioral processes, despite its inability to cross the blood-brain barrier (see e.g., McGaugh and Roozendaal, 2002). Although our earlier finding that surgical adrenalectomy fails to prevent acute stress-induced reinstatement (Shaham et al., 2000b). Both β₁ and β₂ receptors are highly expressed in the mouse BNST (Lorton and Davis, 1987), and intra-BNST delivery of a cocktail of ICI-118,551 and betaxolol blocks stress-induced reinstatement in rats (Graf et al., 2011), the role of peripheral β₂ receptors remains to be determined.

β-Adrenergic receptor-mediated cocaine seeking probably involves the BNST. The ventral noradrenergic bundle, comprised of projections from medullary cell groups, heavily innervates the BNST and is critical for stress-induced reinstatement (Shaham et al., 2000b). Both β₁ and β₂ receptors are highly expressed in the mouse BNST (Lorton and Davis, 1987), and intra-BNST delivery of a cocktail of ICI-118,551 and betaxolol blocks stress-induced reinstatement in rats (Leri et al., 2002). In the BNST, norepinephrine enhances excitatory neurotransmission through actions at β receptors (Egli et al., 2005) via a mechanism that may involve corticotrophin-releasing factor (CRF) (Nobis et al., 2011), consistent with the finding that intra-BNST CRF delivery induces cocaine seeking, whereas CRF receptor antagonism in the BNST prevents stress-induced reinstatement (Erb and Stewart, 1999), and with reports that CRF is downstream from norepinephrine in the pathway that mediates stress-induced reinstatement (Brown et al., 2011). Adrenergic receptor-regulated GABAergic inputs to the ventral tegmental area (VTA) have been characterized (Dumont and Williams, 2004). Alternatively, β-adrenergic receptors may regulate efferents from the BNST that release CRF into the VTA (Rodaros et al., 2007). We and others have reported that delivery of CRF into the VTA can reinduce cocaine seeking in rats and VTA CRF receptor activation is necessary for stress-induced reinstatement (Wang et al., 2005; Blacktop et al., 2011). Determination of the ability of BNST β-adrenergic receptors to regulate CRF release into the VTA awaits further investigation.

β-Adrenergic receptors have also been implicated in anxiety-related responses associated with acute cocaine delivery (Schank et al., 2008) and cocaine withdrawal (Harris and Aston-Jones, 1993). In the case of withdrawal, these effects seem to be mediated by β₁ receptors, probably in the amygdala (Rudoy and Van Bockstaele, 2007). The relationship between withdrawal symptoms and stress-induced relapse is unclear. However, a study involving cocaine-dependent individuals undergoing outpatient treatment found that propranolol improved treatment outcomes only in subjects who displayed severe withdrawal symptoms (Kampman et al., 2001), suggesting that medications targeting β-adrenergic receptors may be more effective for relapse prevention in subpopulations of addicts whose use is stress related.

Despite preventing stress-induced reinstatement, β-adrenergic receptor deficiency and ICI-118,551 did not significantly alter immobility time during the forced swimming bouts. This finding was unexpected and may imply that there is a disconnection between stress-induced cocaine seeking and other stress-related behaviors. Surprisingly, it has been reported that clenbuterol decreases immobility during forced swim (Finnegan et al., 1987), whereas ICI-118,551 and propranolol increase it (Stone et al., 1995), suggesting that reduced drug seeking could be counterintuitively related to a depression phenotype.

β-Adrenergic receptor-deficient mice developed conditioned place preference and did not show reductions in its expression relative to wild-type controls, suggesting that β-adrenergic receptor activation is not necessary for cocaine’s rewarding effects or the retrieval of cocaine-associated memories. Cocaine-induced reinstatement was also unaffected in knockout mice, consistent with our earlier finding that pharmacological antagonism failed to alter reinstatement in response to cocaine (Mantsch et al., 2010). These findings are inconsistent with one report that β-receptor antagonism prevented the expression/retrieval of cocaine-induced conditioned place preference in rats (Otis and Mueller, 2011). Although conditioned place preference was not altered in knockout mice, differences relative to wild-type controls were observed during extinction, with no difference on the first test day after conditioning and decreased time spent in the cocaine compartment thereafter. Several groups have reported that antagonism of β receptors either before (Otis and Mueller, 2011) or after (Fricks-Gleason and Marshall, 2008; Bernardi et al., 2009) testing for place preference reduces subsequent preference, potentially because of interference with memory reconsolidation processes. Disruption of memory reconsolidation could explain the reductions in place preference with repeated testing during extinction in knockout mice. To the extent that reconsolidation of cocaine-associated memories was impaired, it did not affect subsequent cocaine-induced reinstatement, in contrast to a report by Fricks-Gleason and Marshall (2008) showing that pharmacological blockade of these receptors after retrieval produced deficits in later reinstatement by cocaine. Thus, it is unlikely that impaired memory reconsolidation contributed to the loss of stress-induced reinstatement in knockout mice. In support, acute pretreatment with ICI-118,551 selectively inhibited reinstatement by forced swim or BRL-44,408, an effect that
can not be attributed to earlier disruption of reconsolidation-related processes. To summarize, these data indicate that β-adrenergic receptor activation is both necessary for stress-induced reinstatement of cocaine seeking and sufficient to reinstate. Although our findings demonstrate a role for β2 receptors in this effect, they do not rule out a role for β1 receptors and, in fact, suggest that stress-induced cocaine seeking may involve coordination between the two receptor subtypes. Future studies will further examine the mechanism through which this potential coordination occurs as well as the brain regions and neurocircuity through which these receptors contribute to stress-induced relapse.

Acknowledgments
We thank Katia De La Cruz and Malia Thao for technical assistance.

Authorship Contributions
Participated in research design: Vranjkovic, Baker, and Mantsch. Conducted experiments: Vranjkovic, Hang, and Mantsch. Performed data analysis: Mantsch. Wrote or contributed to the writing of the manuscript: Mantsch.

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Egli RE, Kash TL, Silberman Y, and Winder DG (2011) β-Adrenergic receptors enhance excitatory transmission in the bed nucleus of the stria terminals through a corticotrophin-releasing factor receptor-dependent and cocaine-regulated mech-

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