Selective Norepinephrine Reuptake Inhibition by Atomoxetine Prevents Cue-Induced Heroin and Cocaine Seeking

Daina Economidou, Jeffrey W. Dalley, and Barry J. Everitt

Background: Preventing relapse to drug use is a major challenge for drug addiction treatment. We have recently shown that impulsivity predating drug-taking increases the susceptibility to relapse to cocaine seeking and that treatment with the anti-impulsivity drug atomoxetine (ATO), a selective norepinephrine re-uptake inhibitor (norepinephrine transporter), prevents relapse. Here, we investigated further the effects of ATO on cue-maintained heroin and cocaine seeking and relapse and compared these effects with those of the anti-impulsivity stimulant drug methylphenidate (MPH).

Methods: Rats were trained to seek and self-administer cocaine or heroin under a second-order schedule of reinforcement. After acquisition of stable responding, groups of rats ($n = 10–12$) were treated, in a within-subject design, with either ATO or MPH (.3–3.0 mg/kg IP), and the effects on cocaine and heroin seeking were measured. The effects of ATO (.3–1.0 mg/kg) on cue-induced relapse to cocaine seeking after a 1-week period of abstinence were also studied.

Results: Atomoxetine significantly decreased both cue-controlled cocaine and heroin seeking, whereas MPH had no significant effect. Atomoxetine also significantly attenuated cue-induced relapse to cocaine seeking after abstinence. The effects of ATO were selective for cue-controlled drug-seeking, because it did not affect responding in the absence of the drug-paired cue; nor did it alter responding for oral sucrose, except minimally at the highest dose, or locomotor activity.

Conclusions: Selective norepinephrine transporter inhibition by ATO might be an effective treatment for the prevention of relapse to both stimulant and opiate addiction.

Key Words: Addiction, atomoxetine, impulsivity, methylphenidate, relapse, second-order

Relapse to drug use after days or even years of abstinence is both a characteristic of drug addiction and a challenge to successful treatment (1). A key goal for the development of novel behavioral and pharmacotherapies, therefore, is to understand the neural and psychological mechanisms underlying the susceptibility to relapse. Among the factors that influence relapse is impulsivity, which is also a risk factor for stimulant addiction (2–5). We previously demonstrated that high impulsive behavior predating drug use predisposed animals to relapse to cocaine seeking with a novel procedure in which intermittent punishment of cocaine-seeking responses resulted in abstinence (6); this increased propensity to relapse was especially evident in rats with an extended history of cocaine self-administration (6). We also showed that treatment with the selective norepinephrine (NE) reuptake inhibitor atomoxetine (ATO), a drug used clinically to treat impulsivity (e.g., attention-deficit/hyperactivity disorder [ADHD]) (7–9), was highly effective in preventing the reinstatement of cocaine seeking (6). However, it was further observed that ATO reduced the propensity to relapse in rats with low levels of impulsivity, regardless of the duration of their cocaine-taking history (6), suggesting that the anti-relapse properties of ATO might not be restricted to high-impulsive individuals that are vulnerable to addiction and relapse.

From the Behavioral and Clinical Neuroscience Institute and Department of Experimental Psychology (DE, JWD, BJE), and the Department of Psychiatry (JWD), University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom.

Address correspondence to Daina Economidou, Ph.D., Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK; E-mail: de244@cam.ac.uk.

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In the present study, therefore, we investigated further the behavioral and pharmacological specificity of ATO in other animal models of drug seeking and relapse that particularly emphasize the role played by drug-associated, conditioned stimuli (CS), which are known to induce craving and relapse in addicted individuals (10–12). Under a second-order schedule of reinforcement, cocaine or heroin seeking is maintained over long time periods by contingent presentations of drug-associated CS, which act as conditioned reinforcers (13–17). This paradigm also has the advantage of allowing drug seeking to be measured independently of the response-rate–altering effects of cocaine or heroin (16). To study relapse, we adopted the procedure in which, after self-administration training, drug-seeking responses are reinforced solely by a drug-paired CS but in the absence of drug reinforcement (i.e., in extinction) after a period of enforced abstinence (18,19).

We have also compared the effects of methylphenidate (MPH) on drug seeking, because this drug is used widely in the treatment of impulse control disorders, including ADHD (7–9), as well as having the effect of reducing impulsivity in animals (20–23). Atomoxetine and MPH are pharmacologically distinct: MPH is a psychostimulant that inhibits the dopaminergic transporter (DAT), but it also has substantial affinity for the norepinephrine transporter (NET); ATO, by contrast, is a selective NET inhibitor (24–29). We show that ATO but not MPH had the marked effect of reducing both cocaine and heroin seeking as well as relapse to cocaine seeking but did not affect general locomotor activity or responding for a high-incentive ingestive reward at the lower effective dose. The results suggest the clinical potential for ATO to reduce relapse in both cocaine and heroin addicts.

Methods and Materials

Animals

Outbred male Lister Hooded rats (Charles River, Margate, United Kingdom) weighing 300–330 g at the beginning of the experi-

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ments were used. Rats were housed individually, under temperature- and humidity-controlled conditions and a reversed 12-hour light/dark cycle (lights off at 7:00 AM). Water was available ad libitum, and food was given at the end of each day's testing. All experimental procedures conformed to the UK (1986) Animal (Scientific Procedures) Act (Project license 80/2234).

Drugs

Atomoxetine hydrochloride (a gift from Eli Lilly, Basingstoke, United Kingdom) was dissolved in .01 mol/L phosphate-buffered saline. Methylphenidate hydrochloride (Sigma, Cambridge, United Kingdom) was dissolved in physiological sterile saline. Both drugs were given by IP injection in a dose volume of 1 mL/kg. Doses were based on previous studies (20,30). Cocaine hydrochloride and heroin hydrochloride (McFarlan-Smith, Edinburgh, United Kingdom) were dissolved in sterile physiological saline and were infused intravenously at the rate of .1 mL/5 sec.

IV Surgery

Rats were anesthetized IP with ketamine hydrochloride (100 mg/kg; Ketaset, Fort Dodge Animal Health LTD, Southampton, United Kingdom) and xylazine (9 mg/kg; Rompun, Bayer, Newbury, Germany), supplemented with ketamine as needed (approximately 20 mg/kg) and received implantation of a single catheter in the right jugular vein. Catheters made from 22-gauge stainless steel cannulae and Silastic tubing were located subcutaneously between the scapulae (5). Rats were treated after surgery with 10 mg/kg Baytril (Bayer) to prevent postoperative infection.

Procedures

Cocaine or Heroin Self-Administration Under a Second-Order Schedule of Reinforcement. Daily experimental testing in the operant self-administration chambers (Med Associates, St. Albans, Vermont) began 7–10 days after IV surgery. Rats were trained daily (2-hour sessions) to self-administer cocaine (.25 mg/infusion) or heroin (.04 mg/infusion) under a fixed-ratio (FR1) schedule such that each active lever press resulted in a drug infusion, illumination of a CS light above the lever for 20 sec, retraction of both levers, and the extinction of the house-light for 20 sec. After this 20-sec time-out (TO), the house-light was again illuminated, the CS was extinguished, and the two levers were again inserted into the chamber. Active and inactive levers were counterbalanced between left and right sides for individual animals. Responses on the inactive lever had no programmed consequences but were recorded to assess discriminated responding and general levels of motor activity. Rats were limited to a maximum of 30 infusions during each 2-hour session. After the acquisition of cocaine or heroin self-administration (3–5 days), a fixed-interval (FI) schedule of reinforcement was introduced that was increased daily from FI1 min to FI2, FI4, FI8, and FI10 min, before stabilizing at FI15 min for three consecutive sessions. Subsequently, a second-order schedule was introduced, in which every 10th active lever press resulted in a short CS presentation for 1 sec (FI15[FR10:S]). On completion of the first 10 responses after the FI15 min had elapsed, cocaine or heroin was infused, and the CS was presented for 20 sec. Sessions terminated after either five infusions or 2 hours, whichever criterion was met first.

Cue-Induced Relapse to Cocaine Seeking After Abstinence. After IV surgery, rats were trained to self-administer cocaine (.25 mg/infusion) under an FR1 schedule of reinforcement (daily 1-hour sessions). Each cocaine infusion was followed by a 20 sec TO accompanied by CS illumination, the retraction of both levers, and extinction of the house-light. After the termination of the TO, the house-light was again illuminated, the CS was extinguished, and the two levers were inserted into the chamber. Responses on the inactive lever were recorded but had no programmed consequences. Rats were limited to a maximum of 30 infusions during the first 2–3 sessions of acquisition. Animals were then allowed to self-administer cocaine for 6-hour daily sessions or until a maximum of 150 cocaine infusions were obtained (cocaine long-access) for 10 consecutive days. Rats were then withdrawn from cocaine self-administration and maintained in their home-cages for 1 week. At the termination of the abstinence period, animals were tested during a 1-hour extinction session, where responding on the active lever resulted in the presentation of the CS but no cocaine.

Experiment 1: Effects of ATO on Cocaine and Heroin Seeking Under a Second-Order Schedule of Reinforcement

Rats were trained to self-administer cocaine ($n = 12$) or heroin ($n = 12$) under a second-order schedule of reinforcement (14–16). After extended training (>1 month) during which habitual responding was consolidated (31), animals were administered ATO (.3, 1.0, and 3.0 mg/kg, IP) or its vehicle in a counterbalanced manner and tested for cocaine seeking 20 min later. Drug testing was repeated every fourth day. On the first day after drug treatment animals remained in their home-cages. On Day 2 and Day 3, rats were re-baselined under the second-order schedule.

Experiment 2: Effects of ATO on Cocaine Seeking Under an FI15-Min Schedule of Reinforcement

Rats ($n = 12$) were trained to respond for cocaine under an FI15-min schedule of reinforcement (i.e., with no response-contingent CS presentations during the 15-min interval). After stable responding, animals were treated in a within-subjects design with ATO (.3, 1.0, or 3.0 mg/kg, IP) or its vehicle 20 min before the initiation of the session. Drug testing was again performed every fourth day (see Experiment 1).

Experiment 3: Effects of MPH on Cocaine and Heroin Seeking Under a Second-Order Schedule of Reinforcement

Rats were trained to self-administer cocaine ($n = 10$) or heroin ($n = 12$) under a second-order schedule of reinforcement. After extensive second-order training (> 1 month) animals received, in a within-subjects design, MPH at the doses of .3, 1.0, and 3.0 mg/kg IP or its vehicle 30 min before the initiation of each session. Drug testing was conducted every fourth day (see Experiment 1).

Experiment 4: Effects of ATO on Cue-Induced Relapse to Cocaine Seeking After Abstinence

After long-access exposure to cocaine (10 sessions, 6 hours/session), rats were divided into three groups ($n = 6$–7/group) with similar baseline levels of cocaine self-administration and then tested for cue-induced relapse after 1 week of withdrawal. For the relapse test, one group of rats received injection IP with vehicle, whereas the other two groups received ATO at the doses of .3 and 1.0 mg/kg, respectively, 20 min before testing commenced.

Statistical Analysis

Active and inactive lever responses for the first (drug-free) and second (after drug infusion) 15-min intervals (Experiments 1–3) were analyzed with a one-way within-subject analysis of variance (ANOVA) (SPSS, Chicago, Illinois). For the cue-induced relapse experiment, data were analyzed by two-way ANOVA with one between-subjects factor (drug treatment) and one within-subject factor (relapse). Post hoc comparisons were performed with the Newman–Keuls test. Statistical significance was set at $p < .05$.