Extinguishing cocaine-associated discriminative stimuli attenuates cocaine vs food choice in male rhesus monkeys

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ABSTRACT

Background: In drug addiction, relapse can be triggered by cues that function as discriminative stimuli to signal contingencies of drug availability and promote drug-taking behavior. Extinction procedures can weaken the association between drug-associated cues and drug use and may reduce the probability of relapse. This study evaluated effects of a regimen of extinction training on cocaine self-administration maintained in rhesus monkeys under a cocaine-vs.-food choice procedure that has been used previously to evaluate effectiveness of other candidate treatments for cocaine abuse.

Methods: Behavior was initially maintained under a concurrent schedule of food delivery (1-g food pellets; fixed-ratio 100 schedule) and cocaine injections (0-0.1 mg/kg/injection; fixed-ratio 10 schedule) during daily 2-h choice sessions in male rhesus monkeys (n=4). Subsequently, choice sessions were supplemented by daily 20-h extinction sessions for 14 consecutive days. During extinction sessions, cocaine-associated discriminative stimuli were presented, but responding did not produce cocaine injections. Cocaine continued to be available during choice sessions.

Results: Prior to extinction, cocaine maintained a dose-dependent increase in cocaine vs. food choice. Responding during extinction sessions declined to low levels by the fifth day. Exposure to extinction sessions produced a more gradual but significant decline in cocaine choice and a complementary increase in food choice during choice sessions.

Conclusions: These preclinical results support the effectiveness of extinguishing cocaine-associated discriminative stimuli as a non-pharmacological treatment strategy for reducing cocaine choice. Moreover, these results also support the construct validity of preclinical drug vs. food choice procedures as a tool for candidate treatment evaluation for cocaine addiction.

Keywords: Cocaine; Choice; Extinction; Cue; Discriminative stimulus; Rhesus monkey

1. Introduction

One drug addiction theory conceptualizes it as a chronic relapsing disease (Leshner, 1997; Volkow et al., 2016). Relapse can be triggered by cues (e.g. the sight of drug paraphernalia) that are known in instrumental conditioning terms as “discriminative stimuli” and that signal drug availability contingencies to promote drug use. Human laboratory research (O'Brien et al., 1998; Volkow et al., 2003) has suggested one obstacle to the successful cocaine addiction treatment is the persistent effectiveness of discriminative stimuli to promote drug-taking behavior even after protracted drug abstinence periods. Modest decreases in cocaine use have been achieved with extinction training behavioral therapy to blunt the association between cocaine-associated discriminative stimuli and cocaine availability by engaging inhibitory learning processes (Bouton, 1993; O'Brien et al., 1990). In these extinction-training procedures, subjects are brought to a laboratory environment and exposed to cues that may function as discriminative stimuli in the natural environment, but the subjects are then denied the opportunity to use cocaine. Such extinction training has been shown to reduce both physiological and subjective responses to cocaine-associated discriminative stimuli and produce at least a transient decrease in relapse probability when subjects return to the natural environment (O'Brien et al., 1990). However, a significant clinical challenge is to identify all cues that function as discriminative stimuli in human addicts.

These challenges are more manageable in preclinical research environments, where investigators have more precise control over the discriminative stimuli identity and ample opportunity to manipulate extinction-training parameters. Accordingly, the study goal was to determine effects of an extinction-training regimen on cocaine self-administration in rhesus monkeys under conditions that have been used previously to evaluate other pharmacological and non-pharmacological treatment strategies (Banks et al., 2013a; Banks et al., 2013c; Nader and Woolverton, 1992; Negus, 2005). Specifically, rhesus monkeys were initially trained to self-administer cocaine during a 2-hr “choice” session conducted daily from 0900-1100 h. During

these sessions, food pellets and increasing unit cocaine doses were concurrently available, and subjects could earn either food pellets by responding on one response key illuminated with red stimulus lights or cocaine injections by responding on a separate response key illuminated with green stimulus lights. Under these conditions, cocaine key illumination with green stimulus lights served as the cocaine-associated discriminative stimulus. Once choice behavior was stable, daily choice sessions continued, and 20-hr extinction sessions were introduced from 1200-0800 h each day for 14 consecutive days. During extinction sessions, the cocaine key was illuminated green, but responding produced saline injections. At the conclusion of this 14-day extinction-training regimen, the extinction sessions were terminated, and daily choice sessions continued for three additional days. We hypothesized that extinguished responding on the cocaine-associated key during extinction sessions would also decrease active cocaine self-administration during choice sessions. Confirmation of this hypothesis would provide an empirical basis for subsequent studies to manipulate extinction-training parameters and evaluate additional manipulations (e.g. drug treatments) that might enhance extinction effects.

2. Methods

2.1. Animals

Four adult male rhesus monkeys (*Macaca mulatta*) of Chinese- or Indonesian-origin weresurgically implanted with a double-lumen catheter (STI Flow, Raleigh, NC) inserted into a major vein and with prior cocaine self-administration histories (Banks et al., 2014; Banks et al., 2013a; Banks et al., 2013c; Banks et al., 2015; Hutsell et al., 2016) served as subjects. Monkeys weighed 9-13 kg and were maintained on a fresh fruit and food biscuits diet (Lab Diet Monkey Biscuits no. 5045; PMI Nutrition, St. Louis, MO). Water was continuously available via an automatic system. A 12-h light-dark cycle was in effect (lights on from 0600 to 1800 h). Animal research and maintenance were conducted according to the Guide for the Care and Use of Laboratory Animals (Council, 2011) and reported according to the ARRIVE guidelines

(Kilkenny et al., 2010). Animal facilities were licensed by the United States Department of Agriculture and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The Institutional Animal Care and Use Committee approved the research and enrichment protocols. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Operant procedures and foraging devices were provided for environmental manipulation and enrichment. Videos were played for additional enrichment.

2.2. Apparatus and catheter maintenance

Monkeys were housed individually in well-ventilated, stainless steel chambers that also served as experimental chambers (Banks et al., 2013b). Each chamber was equipped with a custom operant panel that contained two horizontally arranged response keys, a pellet dispenser (Model ENV-203-1000; Med Associates, St Albans, VT), and two syringe pumps (Model PHM-108; Med Associates), one for each lumen of the double lumen catheter. One syringe pump (the “self-administration” pump) delivered response-contingent cocaine injections during choice sessions, and the second syringe pump (the “treatment” pump) delivered both response-contingent saline injections during extinction sessions and non-contingent saline injections at a programmed rate of 0.1 mLs every 20 min. The intravenous (i.v.) catheter was protected by a tether and jacket system (Lomir Biomedical, Malone, NY). Catheter patency (loss of muscle tone within 10 s) was periodically evaluated by ketamine (3 mg/kg, i.v.) administration and after a rightward shift in the cocaine choice dose effect function produced by an experimental manipulation.

2.3. Behavioral Procedure

Initially, monkeys responded during daily 2-hr choice sessions (0900 to 1100 h) that consisted of a five-component concurrent schedule of food pellet and i.v. cocaine availability (Banks et al., 2013b). During each component, responses on the left key were reinforced with

food (1-g banana-flavored pellet; Test Diets, Richmond, IN) according to a fixed-ratio (FR) 100 schedule, and responses on the right key were reinforced with i.v. cocaine (0–0.1 mg/kg/injection) according to an FR 10 schedule. A response on one key reset the ratio requirement on the alternative key. Each reinforcer delivery was followed by a 3-s timeout during which all stimulus lights were extinguished, and responding had no programmed consequences. During each component, the food and cocaine keys were transilluminated with red and green stimulus lights, respectively. Additionally, the stimulus lights for the cocaine key flashed on and off in 3 s cycles, and longer flashes were associated with larger cocaine doses. The cocaine key was continuously illuminated green during availability of the highest cocaine dose (0.1 mg/kg/injection). Furthermore, response requirement completion on the food key always produced delivery of a single food pellet, whereas response requirement completion on the cocaine key produced the available unit cocaine dose (0, 0.0032, 0.01, 0.032, and 0.1 mg/kg/injection during components 1–5, respectively) by manipulating the injection volume (0, 0.01, 0.03, 0.1, and 0.3ml/injection, respectively). Each component was in effect until 10 total reinforcers were earned or 20 min elapsed, whichever occurred first.

Once cocaine vs. food choice was stable, daily extinction-training sessions were introduced to provide conditions during which cocaine-associated discriminative stimuli that were previously associated with 0.1 mg/kg/injection cocaine availability were now associated with i.v. saline injections for 20 hr each day (1200 to 0800 h). During extinction-training sessions, the same right response key associated with cocaine injections during the choice session was transilluminated green, and response requirement (FR10) completion extinguished the green light, activated the treatment pump to deliver saline (0.3 mL/injection), and initiated a 15-min timeout. At the end of the 14-day experimental period, the 20-hr extinction sessions were terminated, and choice behavior was monitored for an additional 3 days.

2.4. Data Analysis

The primary dependent measures for choice sessions were percent cocaine choice, defined as {(number of completed ratios on the cocaine-associated key ÷ total completed ratios) × 10} and the numbers of total, cocaine, and food reinforcers earned per session. Data for each of these variables was averaged within a monkey for the last 3 days of each 7-day treatment block, and then across monkeys. Due to equipment failure on extinction-training session day 7 in 3 out of 4 monkeys, results for the 7-day treatment block were expressed as the mean for days 5 and 6. Results indicated that extinction session exposure produced the greatest effects on choice between 0.032 mg/kg/injection cocaine and food (see Results). As a result, a subsequent time-course analysis evaluated the number of saline injections per day during extinction sessions, and the numbers of cocaine injections (0.032 mg/kg/injection) and food pellets per day during choice sessions, before, during and after exposure to extinctions sessions.

Cocaine choice results were analyzed using two-way repeated-measures ANOVA with treatment time (7 or 14 days) and cocaine dose as the fixed effects and significant effects were followed up by a Holm-Sidak test. Extinction time course results were analyzed using non-linear regression to determine the time required for behavior to decrease (saline injections and cocaine choices) or increase (food choices) by 50% ($T_{1/2}$) and an extra sum-of-squares *F*-test on rate parameters (Motulsky and Christopoulos, 2003). Statistical significance criterion was set at the 95% confidence level ($P<0.05$). Analyses were conducted using Prism 6.0 for Mac (GraphPad Software, La Jolla, CA).

2.5. Drugs

(–)-Cocaine HCl was provided by the National Institute on Drug Abuse (Bethesda, MD) Drug Supply Program. Cocaine was dissolved in sterile saline and passed through a sterile 0.22-μm filter (Millipore Corp, Billerica, MA) before i.v. delivery. Doses were calculated using the salt form.

3. Results

Figure 1A shows extinctions session effects on the cocaine-choice dose-effect curve during choice sessions. Under baseline conditions, cocaine maintained a dose-dependent increase in cocaine choice, and cocaine was chosen almost exclusively over food at the highest two cocaine doses (0.032 and 0.1 mg/kg/injection). Seven days of extinction-training sessions did not significantly alter the cocaine choice dose-effect curve; however, at 14 days, 0.032 mg/kg/injection cocaine choice was significantly attenuated (Fig. 1A; cocaine dose: $F(4,12)=66.99, P<0.0001$; cocaine dose x extinction: $F(8,24)=3.01, P=0.017$). Figure 1B shows extinction-training sessions did not significantly alter total, food, or cocaine choices during choice sessions, although there was a trend over time for increased food choices and decreased cocaine choices.

Initial data analysis shown in Figure 1 indicated that extinction-training session exposure produced its greatest effects on responding maintained by 0.032 mg/kg/injection during choice sessions. To determine the time course of this effect in greater detail, Figure 2 compared the time course of saline-maintained responding during extinction sessions (2A) with the time course of changes in cocaine (0.032 mg/kg/injection) and food choice during choice sessions (2B and 2C, respectively). Although there was a decrease over time in both saline-maintained responding during extinction sessions and 0.032 mg/kg/injection cocaine choices during choice sessions, saline-maintained responding declined (mean $T_{1/2} \pm$ 95% CL = 2.2±1.4 sessions) more quickly ($F(1,108)=8.28$, $P=0.0048$) than the 0.032 mg/kg/injection cocaine choice decline (mean $T_{1/2} \pm$ 95% CL = 10.2 ±10.0 sessions). Figure 2C shows that reductions in 0.032 mg/kg/injection cocaine choice were accompanied by a reciprocal increase in food choice. Finally, Figures 2B and 2C show the attenuation in 0.032 mg/kg/injection cocaine choice were still evident 3 days post termination in 2 out of 3 monkeys. The fourth monkey died following the extinction-training sessions due to non-experimental reasons.

4. Discussion

The study aim was to determine the effects of extinguishing cocaine-associated discriminative stimuli outside the cocaine self-administration session on cocaine vs. food choice. There were two main findings. First, saline-maintained responding during extinction sessions declined rapidly to stable low levels during 14 extinction session exposure days. Thus, extinction session introduction did produce evidence of behavioral extinction during presentation of cocaine-associated discriminative stimulus. Second, extinction session exposure also produced a more gradual, but significant decrease in cocaine choice and complementary increase in food choice during cocaine choice sessions. Furthermore, this effect was especially prominent during availability of 0.032 mg/kg/injection cocaine demonstrating an inhibitory learning effect was produced (Bouton, 1993). Taken together, these results provide preclinical evidence that extinction of responding occasioned by a cocaine-associated discriminative stimulus in one context can decrease cocaine self-administration in a different context. Additionally, these results provide an empirical foundation for future studies to manipulate extinction parameters and evaluate additional manipulations (e.g. drug treatments) that might enhance extinction therapeutic efficacy.

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Legends for Figures

Figure 1: Extinction component effects on (A) cocaine vs food choice (B) total, food, and cocaine choices and (C) saline injections earned during the 20-h extinction component. (A) Ordinate: percent cocaine choice. Abscissa: unit dose cocaine in mg/kg/injection. (B) Ordinate: total choices summed across each component. Abscissa: total, food, or cocaine choices. All symbols and bars represent mean ± s.e.m. obtained during days 5-6 (7 day) and 12-14 (14 day) of the 14-day treatment period in four monkeys. Filled symbols indicate significantly different from baseline.

Figure 2: Time course of saline-maintained responding during extinction sessions, and choice for 0.032 mg/kg/injection cocaine and food during choice sessions, during 14 consecutive extinction-training exposure days. Abscissae (A-C): Extinction-training session exposure day. BL indicates baseline data before introduction of extinction sessions, and P1-P3 indicates data for the first three days after extinction session termination. Top ordinate: saline injections per day earned during extinction sessions. Middle ordinate: 0.032 mg/kg cocaine injections per day earned during choice sessions. Bottom ordinate: food pellets per day earned during concurrent availability of 0.032 mg/kg/injection cocaine of choice sessions. Symbols above baseline (BL) and session numbers 1-14 represent the mean ± s.e.m. from four monkeys. Symbols above days P1-P3 represent the mean ± s.e.m. from three monkeys.

Role of funding source

Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Numbers R01 DA026946 and T32 DA007027. The content is solely the authors' responsibility and does not necessarily represent the National Institutes of Health official views.

Contributors

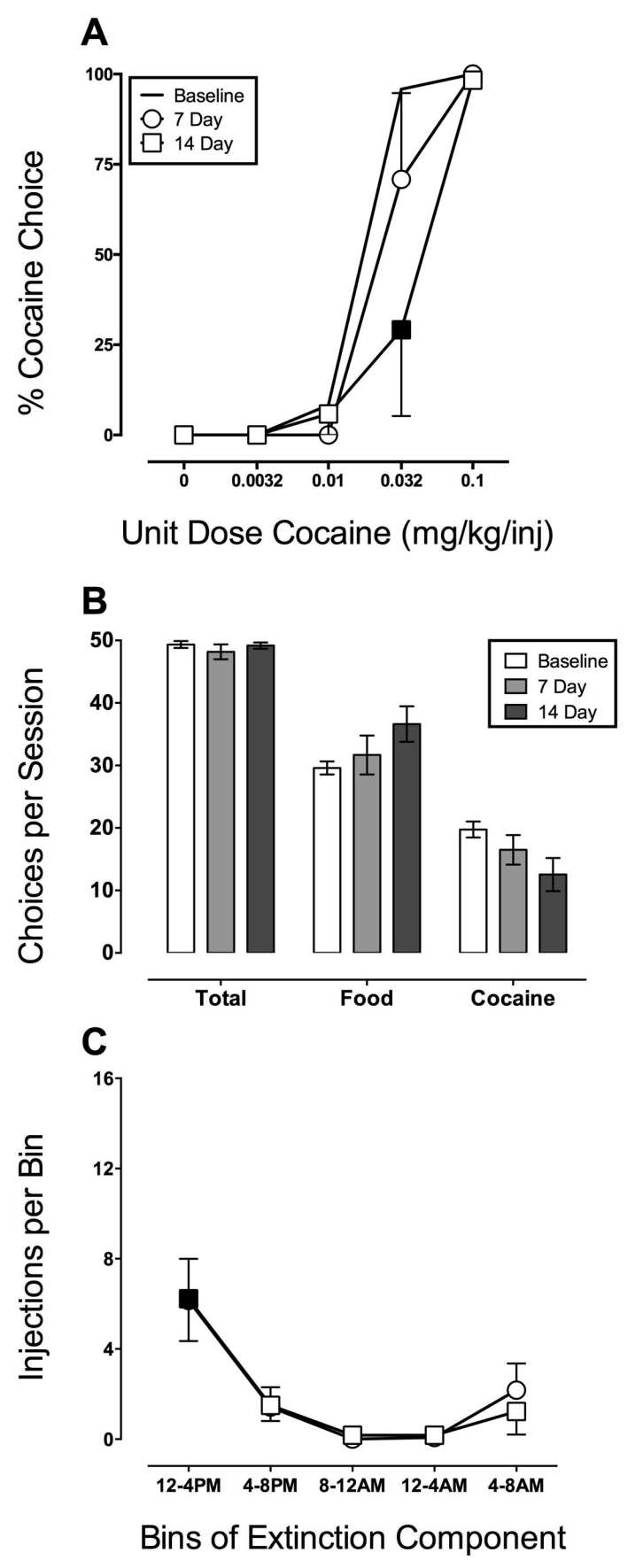
Banks and Negus designed the study. Banks and Hutsell drafted the manuscript. Hutsell performed the data analysis. Negus assisted with interpretation of findings and provided critical manuscript revision for intellectual content. All authors critically reviewed the content and approved the final version for publication.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

Acknowledgements

We acknowledge the technical assistance of Jennifer Gough and Kevin Costa for writing the original version of the behavioral programs that were modified for the present experiments.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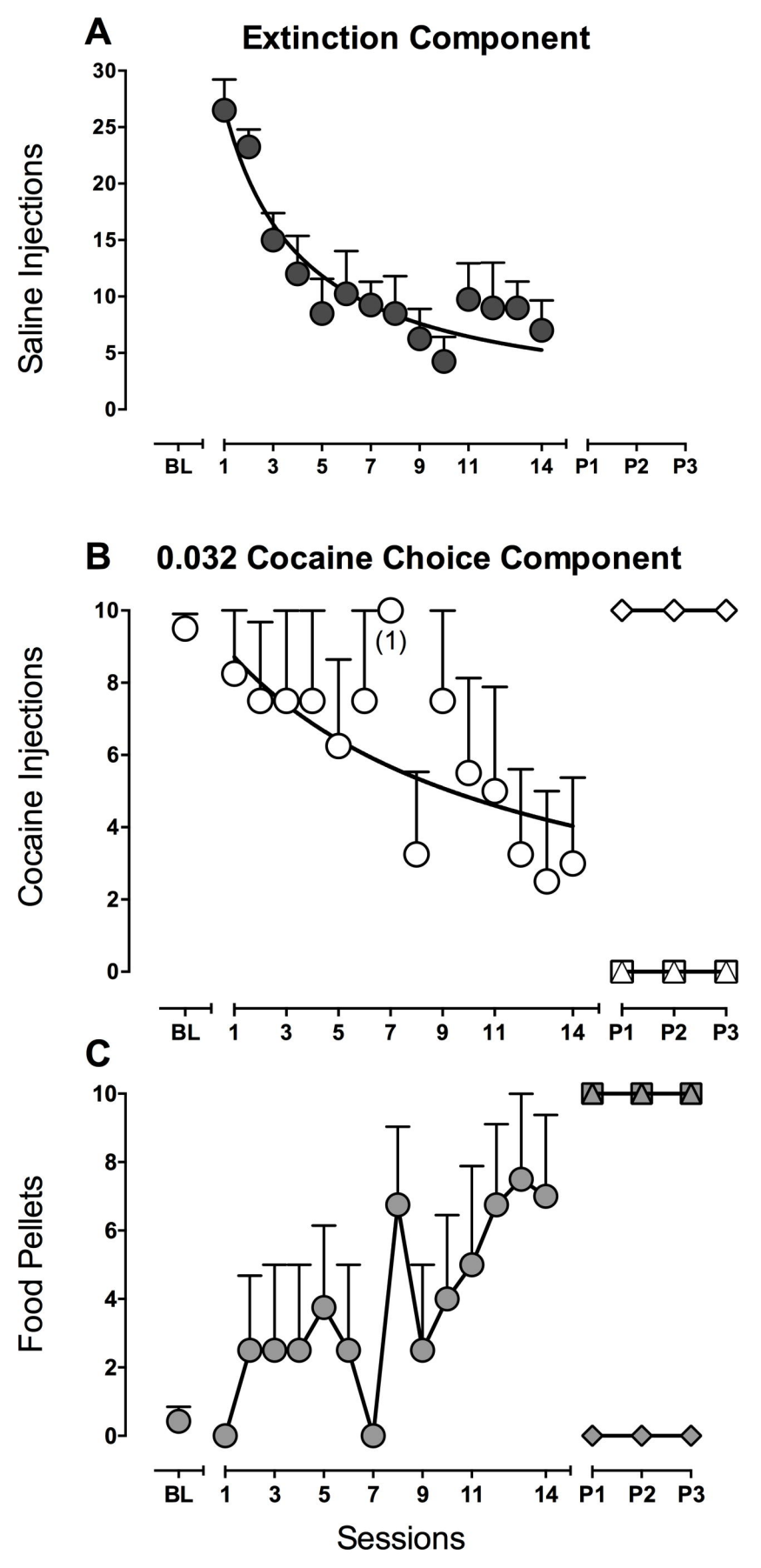
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