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Acute and chronic effects of the M₁/M₄-preferring muscarinic agonist xanomeline on cocaine vs. food choice in rats

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Abstract

Rationale—We previously showed that the M₁/M₄-preferring muscarinic agonist xanomeline can attenuate or eliminate cocaine self-administration in mice acutely.

Objective—Medications used to treat addictions will arguably be administered in (sub)chronic or repeated regimens. Tests of acute effects often fail to predict chronic effects, highlighting the need for chronic testing of candidate medications.

Methods—Rats were trained to lever press under a concurrent FR5 FR5 schedule of intravenous cocaine and food reinforcement. Once baseline behavior stabilized, the effects of 7 days once-daily injections of xanomeline were evaluated.

Results—Xanomeline pretreatment dose-dependently (1.8–10 mg/kg/day) shifted the dose-effect curve for cocaine rightward (up to 5.6-fold increase in A50), with reallocation of behavior to the food-reinforced lever. There was no indication of tolerance, rather effects grew over days. The suppression of cocaine choice appeared surmountable at high cocaine doses, and xanomeline treatment did not significantly decrease total-session cocaine or food intake.

Conclusions—In terms of xanomeline's potential for promoting abstinence from cocaine in humans, the findings were mixed. Xanomeline did produce reallocation of behavior from cocaine to food with a robust increase in food reinforcers earned at some cocaine/xanomeline dose combinations. However, effects appeared surmountable, and food-maintained behavior was also decreased at some xanomeline/cocaine dose combinations, suggesting clinical usefulness may be limited. These data nevertheless support the notion that chronic muscarinic receptor stimulation can reduce cocaine self-administration. Future studies should show whether ligands with higher selectivity for M₁ or M₁/M₄ subtypes would be less limited by undesired effects and can achieve higher efficacy.

Introduction

Addiction to cocaine and other stimulants remains a considerable public health problem for which there is no widely effective treatment. Evidence implicates brain cholinergic muscarinic systems in drug addictions, including in the abuse-related effects of cocaine (for review, Williams & Adinoff 2008; Sofuoglu & Mooney 2009). Muscarinic systems are therefore being scrutinized as potential targets for addiction medications. Because subtype-selective muscarinic receptor agonists are only now becoming available, studies, particularly in humans, have largely relied on acetylcholinesterase (AChE) inhibitors (e.g., donepezil, galantamine, tacrine) that increase synaptic levels of acetylcholine, increasing stimulation of both nicotinic and muscarinic receptors. While AChE inhibitors have shown some promise in laboratory animals (Hikida et al. 2003; Takamatsu et al. 2006; Andersen et al. 2007; Grasing et al. 2008, 2009) they had mixed results in humans (Winhusen et al. 2005; De La Garza et al. 2008a,b, 2011; Grasing et al. 2010). The clinical usefulness of AChE inhibitors

may be limited by opposing effects at different receptors and by adverse effects that prevent effective doses from being used.

Acetylcholine exerts its effects through two different classes of receptors, the nicotinic ligand-gated ion channels, and the G-protein coupled muscarinic receptors. Five muscarinic acetylcholine receptor subtypes have been cloned, M₁-M₅, of which M₁, M₃ and M₅ subtypes couple to G_{q/11} proteins while M₂ and M₄ couple to G_{i/o} proteins (for review see Wess et al. 2007). M₁, M₄ and M₅ receptors are most abundant in the central nervous system, while M₂ and M₃ receptors are widely distributed in both central and peripheral tissues (Wess et al. 2007). Part of the limitations of AChE inhibitors may be due to the opposing modulation exerted by different muscarinic receptor populations on rewarded behaviors generally, and on behavioral effects of cocaine specifically. Briefly, pharmacological and lesion studies in rodents indicate that muscarinic receptors in the ventral tegmental area (VTA) and pedunculopontine tegmental nucleus, which are predominantly or solely of the M₅ subtype, facilitate drug reward (See Thomsen et al. 2010a for details and references). In contrast, activity of muscarinic receptors in striatal areas appears to oppose abuse-related effects of cocaine (Hikida et al. 2001, 2003; Smith et al. 2004; Mark et al. 2006). The muscarinic receptors in the striatum are predominantly the M₁, M₄, and M₂ subtypes, the latter being mostly presynaptic inhibitory autoreceptors (Weiner et al. 1990; Bernard et al. 1992; Hersch et al. 1994; Smiley et al. 1999). Further, muscarinic receptors within the striatum, both dorsal and nucleus accumbens, colocalize with dopamine receptors and modulate neuronal responses to dopamine receptor activation. Specifically, M₄ and D₁ receptors exert directly opposing effects on cyclic AMP synthesis, whereas M₁ receptors oppose the effects of D₂ receptors (Di Chiara et al., 1994; Onali and Olanas, 2002). Therefore, we previously hypothesized that subtype-selective muscarinic M₁ or M₄ agonists could attenuate the abuse-related effects of cocaine, with greater effectiveness and fewer and/or less severe adverse effects than non-selective agonists or AChE inhibitors. Indeed, we found that M₁-selective agonists and the M₁/M₄-preferring agonist xanomeline could attenuate cocaine's discriminative stimulus effects and essentially abolish cocaine self-administration behavior in mice (Thomsen et al. 2010a, 2012).

Xanomeline binds to all five muscarinic receptors but shows functional selectivity for the M₁ and M₄ receptors, at which it functions as a full agonist (Bymaster et al. 1994, 1997; Shannon et al. 1994). While xanomeline has low potency and efficacy at M₂ and M₅ receptors, the reported selectivity over M₃ receptors varies greatly, perhaps due to the apparent involvement of both orthosteric (competitive) and allosteric modes of action of xanomeline at several subtypes (De Lorme et al. 2007; Machova et al. 2007, Langmead et al. 2008, Heinrich et al. 2009). Xanomeline has much lower binding affinity for nicotinic cholinergic and non-cholinergic sites, with the exception that agonist or antagonist effects at various 5-HT receptor subtypes have been observed (Shannon et al. 1994; Watson et al. 1998; for review see Mirza et al. 2003). Acute and chronic xanomeline administration inhibited A10 VTA dopaminergic cell firing, with little effect on A9 Substantia nigra cells (Shannon et al. 2000). Behaviorally, xanomeline showed antipsychotic-like effects in laboratory animals (Shannon et al. 2000; Stanhope et al. 2001; Andersen et al. 2003), an effect likely mediated through M₄ receptor stimulation (Woolley et al. 2009; Thomsen et al. 2010b; Dencker et al. 2011). Xanomeline similarly showed some promise with antipsychotic and cognitive enhancing effects in clinical trials, but its use was hampered by side effects, mainly gastrointestinal and generally attributed to M₃ receptor stimulation (Bodick et al. 1997; Shekhar et al. 2008).

Acute drug effects can provide key information on potency, time course and receptor pharmacology of candidate medications, but drugs used to treat addictions will arguably need to be administered in a chronic or repeated pattern. Not least in the field of stimulant

abuse, acute effects have often failed to predict effects of chronic administration, both in laboratory animals and in humans, highlighting the need for chronic testing. For instance, the dopamine D₂-like partial agonist aripiprazole showed promise as acute administration but actually increased cocaine taking when tested in chronic or subchronic regimens, in both rats and humans (Thomsen et al. 2008; Haney et al. 2011). A similar acute vs. chronic discrepancy was observed in human testing of the D₁ antagonist ecopipam (Haney et al. 2001). Conversely, d-amphetamine shifts the cocaine self-administration curve to the left as acute pretreatment, but decreased cocaine taking as a chronic regimen, both in rats, monkeys, and humans (Grabowski et al., 2001; Negus, 2003; Negus & Mello, 2003; Rush et al. 2009; Thomsen et al. 2013). To the extent that such comparisons are available, drugs that have shown promise at reducing cocaine taking in clinical trials have also shown sustained or enhanced effects when administered in repeated daily or chronic regimens in laboratory animals, such as the GABA agonist baclofen and the stimulant modafinil (Shoaib et al. 1998; Karila et al. 2008; Haney & Spealman 2008; Newman et al. 2010).

Assays of intravenous drug self-administration, especially those allowing long-term drug availability, are useful procedures for evaluating candidate treatments for drug abuse and dependence. Of the many experimental designs available, choice procedures are gaining popularity for use both in laboratory animals and especially in human studies (Rush et al. 2009; Greenwald et al. 2010; Haney et al. 2011), with various proposed advantages over the more classic single-reinforcer assays (Ahmed 2010; Negus & Banks 2011). The relative reinforcing effect of cocaine is compared to that of a palatable food, based on lever selection rather than response rate, making the assay less vulnerable to general performance decrements. This assay also models a hallmark of human drug addiction: the allocation of behavior to drug taking at the expense of other activities. Because choice procedures are increasingly used in human studies, their use in preclinical research with laboratory animals may also simplify comparisons between species. Finally, and of particular importance to this investigation, we found the procedure to be particularly suited for assessing chronic drug effects, because it produced stable baseline cocaine dose-effect curves, assessed daily within-session (Thomsen et al. 2008, 2013). Previously, we showed this choice procedure to yield orderly effects of parametric manipulations such as availability and magnitude of the reinforcers, or response requirement (Thomsen et al. 2013). So far, we have also found pharmacological effects to be consistent with studies in humans, e.g., using aripiprazole or the monoamine releaser d-amphetamine (Thomsen et al. 2008, 2013; Greenwald et al. 2010; Haney et al. 2011).

In this study, we examined the effects of daily xanomeline administration on cocaine vs. food choice in rats. The main goal was to extend our previous findings from acute to chronic effects of xanomeline pretreatment. Additionally, these studies extend our findings from mice to rats and from independent schedules of cocaine- and food-maintained responding to a concurrent choice schedule of cocaine and food availability.

Methods

Animals

Male Sprague-Dawley rats were acquired at 8 weeks of age (Charles River, Wilmington, MA) and acclimated to the laboratory for at least a week before training began. Rats were housed individually with free access to water in a temperature- and humidity-controlled facility maintained on a 12-h light/dark cycle (lights on at 07:00). Rats were fed $\approx$ 17g standard rat chow daily (Rat Diet 5001; PMI Feeds, Inc., St. Louis, MO), maintaining 400–500g bodyweight. For enrichment, treats were provided once or twice weekly (e.g., peanuts, “Yogurt Drops”, sunflower seeds; Bio-Serv, Frenchtown, NJ). Behavioral testing was conducted during the light phase. Husbandry and testing complied with the guidelines of the

National Institutes of Health Committee on Laboratory Animal Resources, and all protocols were approved by the McLean Hospital Institutional Animal Care and Use Committee.

Catheter implantation and maintenance

Rats were anesthetized with an isoflurane/oxygen vapor mixture and implanted with chronic indwelling i.v. catheters, exiting at the midscapular region (see Thomsen & Caine 2005). A single dose of analgesic (ketoprofen 5 mg/kg) and antibiotic (amikacin 10 mg/kg) were administered s.c. immediately before surgery. Rats were allowed at least 7 days recovery before being given access to i.v. cocaine. During this period, a prophylactic dose of cefazolin (30–40 mg/kg) was delivered daily through the catheter. Thereafter, catheters were flushed daily with sterile saline containing heparin (3 USP U/0.1 ml). If blood could not be withdrawn through the catheter, catheter patency was tested by administering 0.05–0.1 ml of a ketamine-midazolam mixture (15 + 0.75 mg/ml) through the catheter. Catheter patency was verified by prominent signs of sedation within 3 s. of infusion. Only data collected with demonstrated patent catheters were used.

Apparatus

Operant conditioning chambers (21 cm × 29.5 cm × 24.5 cm) and associated hardware from MED Associates Inc. (Georgia, VT) were used, placed within sound-attenuating cubicles equipped with a house light and an exhaust fan. Each chamber contained three retractable response levers (ENV-112CM), two “reinforcer” levers (referred to as the “left” and “right” levers) on one wall and a third “observer” lever centered on the opposite wall. A steel cup between the reinforcer levers served as a receptacle for the delivery and consumption of liquid food reinforcers. A three-light array, red, yellow, and green (ENV-222M), located above the right lever was illuminated to signify the availability food, an identical array with one additional yellow light, above the left lever, was used to signal the cocaine dose available. A white light was (ENV-229M) located above the observer lever. Each cubicle also contained two syringe pumps (3.3 rpm, model PHM-100), for the delivery of liquid food and i.v. cocaine, respectively, through Tygon tubing. Cocaine was delivered using a single channel fluid swivel (MS-1, Lomir Biomedical, Malone, NY) mounted on a balance arm, which allowed rats free movement.

Operant conditions

Details of the procedure were as described previously (Thomsen et al. 2008), with the exception that all three levers retracted after the completion of a response requirement. Rats were trained/tested in daily sessions Monday-Friday. Under the terminal schedule of reinforcement, daily sessions consisted of five 20-min components separated by 2-min timeout periods. Responding was reinforced under concurrent FR5 FR5 schedules of reinforcement: responses on the right lever were reinforced with liquid food (75 µl of 32% vanilla flavor Ensure® nutrition drink in water, Abbott Laboratories, Abbott, IL), responding on the left, with intravenous cocaine infusions of increasing dose for each component: 0, 0.06, 0.18, 0.56, 1.0 mg/kg/infusion. Cocaine doses were achieved by varying the infusion time, adjusted individually to bodyweight. Each component started with one response-independent automated delivery of each reinforcer (cocaine, then food) that would be available in the component, and the illumination of the observer lever’s cue light to signify that responses had scheduled consequences. One response on the observer lever retracted the observer lever, turned off the associated cue light, extended the left and right levers, and turned on the cue lights associated with the left and right levers to signify reinforcer availability. Specifically, illumination of triple cue light above the right lever signaled food availability, while the light array over the left lever signaled the cocaine unit dose available: no light for 0, green for 0.06, green+yellow for 0.18, green+yellow+red for 0.56, and green+yellow+red+yellow for 1.0 mg/kg/infusion. Responding on one reinforcer lever reset the

ratio requirement on the other. When a reinforcer was earned, left and right levers retracted and their associated cue lights were turned off. After a 20-s timeout period (including the infusion time), during which responses had no scheduled consequences, the observer lever extended and its associated cue light was illuminated, starting a new trial. Per component, 15 total reinforcers were available (completion of the response requirement on the left lever during availability of the zero cocaine dose counted as one reinforcer). If all 15 reinforcers were earned in less than 20 min, the component was terminated and the 2-min timeout started.

Choice training continued until behavior stabilized: three consecutive sessions with 5 reinforcers/component earned in components 1–4 and 1 reinforcer earned in component 5, and with the dose of cocaine producing 80% cocaine choice on any given day remaining within one-half log unit of the prior 3-day mean.

Testing

Once training was completed, we tested the effects of xanomeline (1.8, 3.2, 5.6 and 10.0 mg/kg) administered once daily for 7 consecutive days, starting on a Friday. Rats were injected but not tested on Saturday and Sunday (treatment day 2 and 3), while choice sessions were conducted on days 1, and 4 through 7. Xanomeline was administered s.c., 5 min before placing the rat in the operant box and starting the session. Repeated administration was used rather than continuous infusion (e.g., osmotic minipumps) because pilot studies in rats and mice indicated xanomeline was not stable in solution (i.e., solution prepared 3 days in advance was ineffective). Xanomeline treatment was discontinued after 7 days because visual inspection of the data indicated effects stabilized after day 5. Although we were not able to test all doses in all rats, most rats received more than one dose: five rats were tested with 1.8, 3.2 and 5.6 mg/kg/day, in a non-systematic order, with additional rats making up the rest of the group sizes including 10 mg/kg xanomeline. After each dose, rats had to again meet criteria for stable baseline behavior in order to test again, allowing at least one week between doses.

Data Analysis

The primary dependent variables recorded for each component were: (1) number of cocaine injections earned, (2) number of food reinforcers earned, and (3) percent cocaine choice, calculated as (number of ratios completed on the cocaine-associated lever ÷ total number of ratios completed) × 100. Total response rate and response rate on the reinforcer levers alone were also recorded and analyzed, but are not shown because they showed the same effects as total reinforcers earned. Total cocaine intake per session (mg/kg), total food reinforcers earned per session, and total reinforcers per component were also calculated for each rat. The percent cocaine choice data were used to calculate A_{50} values (potency), defined as the dose of cocaine that produced 50% cocaine choice in each rat, and determined by interpolation from two adjacent points spanning 50% cocaine choice. In one case where cocaine choice was 53% at the lowest dose, extrapolation was used. In two cases where cocaine choice was >60% at the lowest dose, a value of 0.032 mg/kg/injection was used as a conservative estimate for inclusion in statistical analyses (i.e., quarter-log below the lowest cocaine dose tested). Group means and 95% confidence intervals were calculated from the log(10) of individual A_{50} values, but are reported transformed to linear values for ease of reading.

Repeated measures ANOVA was used to analyze the effects of xanomeline and cocaine dose on percent cocaine choice, cocaine reinforcers, food reinforcers, and total reinforcers earned per component. Factors were cocaine dose and treatment day (baseline, day 1, day 7). Significant effects of treatment or treatment by cocaine dose interactions were scrutinized

post-hoc by Bonferroni post-test vs. baseline. Because all xanomeline doses could not always be tested in each rat (within-subject), each xanomeline dose was analyzed separately, so that xanomeline treatment vs. baseline could be analyzed within-subjects. Cocaine dose was always highly significant ($p < 0.0001$) and thus not reported beyond the baseline data.

Total cocaine and total food per session, and log-transformed A_{50} values, were each compared by 2-way ANOVA with treatment dose (including baseline, averaged over xanomeline doses for each rat) and xanomeline treatment day (day 1 and 7, post-xanomeline re-baseline) as variables. Significant effects or interactions were then examined by repeated-measures one-way ANOVA for each dose followed by Dunnett's multiple comparisons test vs. baseline. Significance level was set at $p < 0.05$.

Drugs

Cocaine hydrochloride was provided by the National Institute on Drug Abuse, National Institutes of Health (Bethesda, MD). Xanomeline hydrochloride was synthesized following published methods (Kane et al. 2008). Both drugs were dissolved in sterile 0.9% saline; xanomeline solutions were prepared fresh daily. Doses refer to the salt form of the drugs.

Results

Baseline data

Baseline data showed orderly relationships between cocaine dose and behavioral measures, with no significant differences between dose groups or across the three baseline days (data not shown per day/group). Pre-xanomeline baseline data were analyzed by one-way ANOVA with cocaine dose as a repeated measures factor. Percent cocaine choice increased with cocaine dose [$F(4,44)=408$, $p < 0.0001$]; all cocaine doses generated higher allocation of behavior to the cocaine lever relative to the no-cocaine condition ($p < 0.01$, Dunnett's). Numbers of cocaine injections earned were related to cocaine dose in an inverted U-shaped curve [$F(4,44)=121$, $p < 0.0001$]; rats earned more reinforcers for all cocaine doses relative to no-cocaine (all $p < 0.01$, Dunnett's except 1.0 mg/kg/injection, $p < 0.05$). Numbers of food reinforcers earned decreased with cocaine dose [$F(4,44)=281$, $p < 0.0001$], with all doses significantly lower than the no-cocaine condition ($p < 0.01$). Total reinforcers earned also related to cocaine dose [$F(4,44)=374$, $p < 0.0001$], being lower for 0.18 mg/kg/injection cocaine and above ($p < 0.01$ vs. no cocaine).

Rats showed good stability also beyond the initial three-day baseline, typically returning to pre-xanomeline baselines within one or two sessions after xanomeline treatment ended (Figure 1 "pre-xanomeline" vs. "post-xanomeline"). There were no significant effects of pre- vs. post-xanomeline determination on any recorded measures. For the larger xanomeline doses especially, cocaine reinforcers tended to remain lower for the first post-xanomeline session (24hr after the last xanomeline administration, see Table 2 "post day 1" for total intake data). By the second session, data were not significantly different from pre-xanomeline baseline values (i.e., typically day 4 after xanomeline, due to weekend). We did not observe evidence of increased cocaine choice at any time after termination of xanomeline treatment. Calculated A_{50} values for cocaine choice also showed no difference between pre- and post-xanomeline baselines (see Table 1).

Xanomeline effects

Figure 2 shows acute (day 1) and chronic (1-week) effects of xanomeline treatment on cocaine choice, cocaine and food reinforcers earned, and total reinforcers earned. Effects on intervening days were intermediate, and are not shown for brevity and clarity. Xanomeline treatment produced rightward shifts in the cocaine choice curve, more so after repeated

administration than on the first exposure (Figure 2, top row). At 10 mg/kg/day xanomeline, behavior was suppressed in some rats at some cocaine doses, and ANOVA could not be performed on percent cocaine choice due to missing values. Main effects of treatment day were significant at 1.8 mg/kg xanomeline [$F(2,60)=5.92$, $p<0.05$] and at 5.6 mg/kg xanomeline [$F(2,60)=10.0$, $p<0.01$], while there were significant treatment by cocaine dose interactions for all three doses analyzed (1.8 mg/kg [$F(8,60)=3.69$, $p<0.05$], 3.2 mg/kg [$F(8,60)=3.36$, $p<0.01$], 5.6 mg/kg [$F(8,60)=3.86$, $p=0.001$]). Post-hoc, cocaine choice was significantly decreased on day 7 at 0.18 mg/kg/injection cocaine for all three xanomeline doses ($p<0.001$ vs. baseline, Bonferroni post-test). Choice of 0.018 mg/kg/injection cocaine was similarly decreased on day 1, for the 3.2 mg/kg xanomeline dose only ($p<0.01$). Calculated A_{50} values confirmed a significant rightward shift in cocaine choice on day 7, for all xanomeline doses (Table 1). The largest shift was seen at 5.6 mg/kg/day xanomeline, with an average 5.6-fold shift relative to baseline.

Xanomeline treatment produced rightward/downward shifts in the dose-effect curve for cocaine injections earned (Figure 2, second row). The main effect of treatment day reached significance at 5.6 mg/kg xanomeline [$F(2,60)=7.27$, $p<0.01$], while there were significant treatment by cocaine dose interactions for all but the 10 mg/kg/day dose (1.8 mg/kg [$F(8,60)=2.76$, $p<0.05$], 3.2 mg/kg [$F(8,60)=2.55$, $p<0.05$], 5.6 mg/kg [$F(8,60)=6.14$, $p<0.0001$]). Post-hoc, numbers of cocaine reinforcers were significantly decreased on day 7 at 0.18 mg/kg/injection cocaine for those three xanomeline doses ($p<0.01$ to $p<0.001$ vs. baseline) and at 0.06 mg/kg/injection cocaine at the 5.6 mg/kg xanomeline dose. Rats similarly earned fewer cocaine injections on day 1, at 0.18 mg/kg/injection cocaine for the 3.2 mg/kg xanomeline dose ($p<0.05$), and at 0.06 mg/kg/injection cocaine for the 5.6 mg/kg xanomeline dose ($p<0.05$).

Calculated as total cocaine intake per session, effects were moderate and variable. Acutely, xanomeline increased total cocaine intake at 1.8, 3.2 and 5.6 mg/kg (see Table 2). After chronic xanomeline treatment, daily cocaine intake was not affected in a consistent or statistically significant manner, except for an increase following the 3.2 mg/kg/day regimen. Interestingly, on the first day after xanomeline treatment, total cocaine intake was decreased significantly at 1.8 and 5.6 mg/kg xanomeline (Table 2).

Generally, xanomeline treatment decreased numbers of food reinforcers earned in the early components, and increased food reinforcers earned in the middle components (Figure 2, third row). Effects of treatment day reached significance at 10 mg/kg xanomeline [$F(2,48)=8.40$, $p<0.01$], with a significant treatment by cocaine interaction at all doses (1.8 mg/kg [$F(8,60)=2.59$, $p<0.05$], 3.2 mg/kg [$F(8,60)=4.02$, $p<0.001$], 5.6 mg/kg [$F(8,60)=14.3$, $p<0.0001$], and 10 mg/kg [$F(8,48)=4.40$, $p<0.001$]). On day 1, post hoc effects reached significance only for 3.2 mg/kg xanomeline, rats earning more food reinforcers when 0.18 mg/kg/injection cocaine was available ($p<0.05$ vs. baseline). On day 7, rats earned more food reinforcers when 0.18 mg/kg/injection cocaine was available after treatment with 1.8, 3.2 or 5.6 mg/kg/day xanomeline ($p<0.05$ to $p<0.001$), and fewer food reinforcers when no cocaine or 0.06 mg/kg/injection was available after treatment with 5.6 or 10 mg/kg/day xanomeline ($p<0.05$ to $p<0.001$). The food reinforcers earned were consumed, as no liquid food was found in the reservoir at the end of the session.

Calculated as total food reinforcers earned per session, food-maintained behavior was not significantly modified by xanomeline, acutely or chronically (see Table 2), though increases and decreases were observed in individual rats. The post-xanomeline decrease observed for cocaine intake was not accompanied by decreased food reinforcers.

Total numbers of reinforcers earned and rates of responding were modulated by xanomeline treatment, differentially as a function of time and cocaine dose. Figure 2, bottom panels show total reinforcers earned, but similar patterns were observed for rates of responding. 1.8 mg/kg xanomeline had no significant effect on total reinforcers earned. At 3.2 and 5.6 mg/kg, total reinforcers earned were decreased at no-cocaine or the low cocaine dose and increased at higher cocaine doses, with a significant treatment by cocaine interaction (3.2 mg/kg [F(8,60)=4.84, $p=0.0001$], 5.6 mg/kg [F(8,60)=11.6, $p<0.0001$]). Post-hoc, effects of 3.2 mg/kg xanomeline reached significance on day 7, with decreased numbers for reinforcers in the first two components ($p<0.05$ vs. baseline). For the 5.6 mg/kg dose, numbers of reinforcers were decreased significantly in the second component both on day 1 and day 7, and in the first component on day 7 (all $p<0.001$). For both doses, total reinforcers earned remained unchanged at 0.18 mg/kg/injection cocaine: behavior was reallocated from cocaine towards food with no net change in total responding. At 10 mg/kg xanomeline total numbers of reinforcers were decreased over the first three components, relating to treatment day [F(2,48)=6.30, $p<0.05$] with a treatment by cocaine interaction [F(8,48)=3.65, $p<0.01$]. Post-hoc, effects only reached significance on day 7, with decreased numbers for reinforcers in the first two components ($p<0.01$, $p<0.001$).

Discussion

The present investigation extends our previous findings that stimulation of muscarinic M_1 or M_4 receptors can attenuate the discriminative stimulus effects and reinforcing effects of cocaine in mice (Thomsen et al. 2010a, 2012). Here, we examine the effects of repeated daily xanomeline administration in rats trained to lever press for either intravenous cocaine or liquid food in a choice procedure. We found that xanomeline produced dose-dependent rightward shifts in the cocaine choice curve, with reallocation of behavior from the cocaine-reinforced lever towards the food-reinforced lever. Importantly, these effects showed no indication of tolerance, indeed shifts were larger after one week of treatment than on day 1. Cocaine self-administration remained reduced briefly after treatment cessation, then returned to baseline with no evidence of increased cocaine self-administration. Measured as total cocaine intake, repeated xanomeline treatment had little effect, as effects appeared surmountable when relatively high cocaine doses were available. Xanomeline also decreased responding for food when no or low cocaine doses were available, though total food intake was not decreased until 10 mg/kg xanomeline.

Baseline data were comparable to those we have obtained previously using this procedure (Thomsen et al. 2008 and unpublished observations). Also similar to earlier investigations, we found good repeatability of the baseline behavior across days, both pre-xanomeline (see Thomsen et al. 2013), and comparing pre- and post-xanomeline baselines. We determined earlier that vehicle administration, either as injections or as subcutaneously implanted osmotic minipumps, had no appreciable effect on the behavior (Thomsen et al. 2013; unpublished observations).

In terms of the potential of xanomeline as a medication for promoting abstinence from cocaine use, the present findings are mixed. On the one hand, xanomeline failed to significantly decrease total cocaine intake over the session, because the decreased intake at intermediate cocaine doses was balanced by increased intake at the highest cocaine doses, consistent with a surmountable rightward shift, as is typically observed with dopamine antagonists (Bergman et al. 1990). It is, however, possible that the apparent surmountability is due in part to pharmacokinetic effects, i.e., diminishing effects of xanomeline in the last components of the session. Indeed, in previous studies in mice, the effects of xanomeline were largely dissipated by 2–2.5 hours after administration, a timeframe close to the present session duration of 108 min (pilot experiments and unpublished analyses of the data reported

in Thomsen et al. 2010a). The measured plasma half-life of xanomeline in rats after oral dosing was 0.54 h (Mirza et al. 2003). At the lower xanomeline doses, one week of treatment tended to increase total cocaine intake, but 5.6 mg/kg/day showed a trend towards decreased cocaine intake. It is difficult to translate these findings to a clinical situation, where a multitude of factors including availability and cost of cocaine, and availability and strength of alternative reinforcers, all affect drug-taking behavior. In the rat choice assay, manipulations of experimental parameters parallel to those factors powerfully modulated choice behavior (e.g., response requirement for cocaine and food, food concentration available; Thomsen et al. 2013). Thus xanomeline may decrease total cocaine intake under some experimental conditions, and, perhaps, likewise under some clinical conditions in humans. Nevertheless, the present data are not overwhelmingly encouraging for this particular drug. Doses of xanomeline that affected cocaine self-administration also reduced food-maintained behavior in the first components, although we observed no other adverse effects. In our previous studies, xanomeline similarly decreased rates of responding in food-maintained behaviors, limiting the doses that could be employed – but M_1 -selective stimulation did not (Thomsen et al. 2010a, 2012). Studies using knockout mice lacking M_1 , M_4 , or both subtypes, indicated that xanomeline's anti-cocaine effects are attributable to both M_1 and M_4 receptor stimulation (Thomsen et al. 2010a, 2012). Xanomeline has limited selectivity for M_1/M_4 receptors over other muscarinic (M_2 , M_3) and non-cholinergic receptors (Shannon et al. 1994; Heinrich et al. 2009), which may contribute to its effects on food-maintained behavior in our studies, and its adverse effects in human studies (Bodick et al. 1997; Shekhar et al. 2008). Future studies should show whether more selective M_1 and/or M_4 ligands, administered chronically in this model, can significantly decrease cocaine intake at doses not limited by undesired effects.

On the other hand, the present results are promising in that xanomeline did effect reallocation of behavior from cocaine to food with a robust increase in food reinforcers earned at some cocaine/xanomeline dose combinations. This is similar to our findings with chronic administration of d-amphetamine in this assay (Thomsen et al. 2013), and unlike any effects we have observed using other test compounds, e.g., direct dopamine receptor agonists, partial agonists, or antagonists (Thomsen et al. 2008; unpublished observations). In particular, dopamine ligands that decreased cocaine self-administration acutely, showed either profound attenuation or indeed reversal of their effects on cocaine self-administration (but not food-maintained behavior), upon chronic testing (Thomsen et al. 2008; unpublished observations). Those observations are in line with other preclinical and clinical data, showing the importance of chronic testing (Greenwald et al. 2010; Haney & Spealman 2008; Haney et al. 2011). The present effects of xanomeline were therefore qualitatively different, in that the shift from cocaine to food not only persisted, but grew with repeated administration. Admittedly, an important caveat to making that comparison is that the regimen of administration differed between the present experiment (repeated once-daily) and the previous investigations of dopamine receptor ligands (continuous). This difference could well account for the different results in terms of chronic effects, i.e., sensitization vs. tolerance (Samaha et al. 2008, but see Shannon et al. 2000). It remains that xanomeline effected robust reallocation of behavior from cocaine self-administration to food-taking, when a reinforcing dose of cocaine was available, a feature we did not observe with dopamine D_2 ligands, acutely or chronically (Thomsen et al. 2008; unpublished observations). Clearly, future goals for extending the present findings must include testing M_1/M_4 agonists in a more sustained administration procedure, be it through pharmacological advances (longer lasting or more stable ligands), or through methodological changes (e.g., achieving stable levels of the test ligand through IV administration, necessitating dual or double-lumen catheters).

Intriguingly, we did observe significant decreases in cocaine intake on the first day following cessation of xanomeline treatment, without similar decreases in food. While we have no substantiated explanation for this phenomenon (e.g., long-acting metabolites), we had noticed the same trend in some earlier experiments, e.g., a high incidence of failed cocaine training days (but not saline days) following xanomeline administration days in the drug discrimination procedure (unpublished observations from the studies reported in Thomsen et al. 2010a, 2012). Cognitive enhancers, including AChE inhibitors, have been suggested as a possible treatment strategy in stimulant abuse (Sofuoglu 2010), and cognitive enhancing effects of xanomeline have been reported in humans (Bodick et al. 1997; Friedman 2004; Shekhar et al. 2008). It is possible that these delayed effects on cocaine self-administration are attributable to cognitive enhancing effects of xanomeline, possibly by consolidating the memory of reduced reinforcing value of cocaine experienced during the xanomeline treatment.

In conclusion, xanomeline treatment shifted allocation of behavior from cocaine-taking towards food, both acutely and chronically. While the current findings were not uniformly positive in terms of obtaining selective effects on cocaine self-administration, they confirmed and extended our previous findings that M_1/M_4 muscarinic stimulation can curtail reinforcing effects of cocaine. In particular, the findings that the effects of xanomeline remained robust, possibly even increased, after a chronic regimen, and that cocaine self-administration did not increase upon cessation of xanomeline treatment, are encouraging. Together with our previous findings, these results suggest that selective M_1/M_4 receptor stimulation may provide a viable pharmacological treatment strategy in cocaine addiction. Thus, while xanomeline itself may or may not have clinical usefulness in psychostimulant addiction, further scrutiny of selective M_1 and M_4 receptor agonists is warranted.

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References

- Ahmed SH. Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews*. 2010; 35:172–184. [PubMed: 20417231]
- Andersen MB, Fink-Jensen A, Peacock L, Gerlach J, Bymaster F, Lundbaek JA, Werge T. The muscarinic M_1/M_4 receptor agonist xanomeline exhibits antipsychotic-like activity in Cebus apella monkeys. *Neuropsychopharmacology*. 2003; 28:1168–1175. [PubMed: 12700711]
- Andersen MB, Werge T, Fink-Jensen A. The acetylcholinesterase inhibitor galantamine inhibits d-amphetamine-induced psychotic-like behavior in Cebus monkeys. *J Pharmacol Exp Ther*. 2007; 321:1179–82. [PubMed: 17374745]
- Bernard V, Normand E, Bloch B. Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J Neurosci*. 1992; 12:3591–600. [PubMed: 1527598]
- Bergman J, Kamien JB, Spealman RD. Antagonism of cocaine self-administration by selective dopamine D_1 and D_2 antagonists. *Behav Pharmacol*. 1990; 1:355–363. [PubMed: 11175420]
- Bodick NC, Offen WW, Levey AI, Cutler NR, Gauthier SG, Satlin A, Shannon HE, Tollefson GD, Rasmussen K, Bymaster FP, Hurley DJ, Potter WZ, Paul SM. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in alzheimer disease. *Arch Neurol*. 1997; 54:465–473. [PubMed: 9109749]
- Bymaster FP, Wong DT, Mitch CH, Ward JS, Calligaro DO, Schoepp DD, Shannon HE, Sheardown MJ, Olesen PH, Suzdak PD, Swedberg MDB, Sauerberg P. Neurochemical Effects of the M_1

- Muscarinic Agonist Xanomeline (LY246708/NNC11-0232). *J Pharmacol Exp Ther.* 1994; 269:282–289. [PubMed: 7909558]
- Bymaster FP, Whitesitt CA, Shannon HE, DeLapp N, Ward JS, Calligaro DO, Shipley LA, Buelke-Sam JL, Bodick NC, Farde L, Sheardown MJ, Olesen PH, Hansen KT, Suzdak PD, Swedberg MDB, Sauerberg P, Mitch CH. Xanomeline: A Selective Muscarinic Agonist for the Treatment of Alzheimer's Disease. *Drug Dev Res.* 1997; 40:158–170.
- De La Garza R 2nd, Mahoney JJ 3rd, Culbertson C, Shoptaw S, Newton TF. The acetylcholinesterase inhibitor rivastigmine does not alter total choices for methamphetamine, but may reduce positive subjective effects, in a laboratory model of intravenous self-administration in human volunteers. *Pharmacol Biochem Behav.* 2008a; 89:200–8. [PubMed: 18207225]
- De La Garza R, Shoptaw S, Newton TF. Evaluation of the cardiovascular and subjective effects of rivastigmine in combination with methamphetamine in methamphetamine-dependent human volunteers. *Int J Neuropsychopharmacol.* 2008b; 11:729–41. [PubMed: 18248689]
- De La Garza R 2nd, Newton TF, Haile CN, Yoon JH, Nerumalla CS, Mahoney JJ 3rd, Aziziyeh A. Rivastigmine reduces “Likely to use methamphetamine” In methamphetamine-dependent volunteers. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011; 37:141–146. [PubMed: 22230648]
- De Lorme KC, Grant MKO, Noetzel MJ, Polson SB, El-Fakahany EE. Long-Term Changes in the Muscarinic M₁ Receptor Induced by Instantaneous Formation of Wash-Resistant Xanomeline-Receptor Complex. *J Pharmacol Exp Ther.* 2007; 323:868–876. [PubMed: 17855477]
- Dencker D, Wortwein G, Weikop P, Jeon J, Thomsen M, Sager TN, Mork A, Woldbye DP, Wess J, Fink-Jensen. A Involvement of a Subpopulation of Neuronal M₄ Muscarinic Acetylcholine Receptors in the Antipsychotic-like Effects of the M₁/M₄ Preferring Muscarinic Receptor Agonist Xanomeline. *J Neurosci.* 2011; 31:5905–5908. [PubMed: 21508215]
- Di Chiara G, Morelli M, Consolo S. Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. *Trends Neurosci.* 1994; 17:228–233. [PubMed: 7521083]
- Friedman JI. Cholinergic targets for cognitive enhancement in schizophrenia: Focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl).* 2004; 174:45–53. [PubMed: 15205878]
- Grabowski J, Rhoades H, Schmitz J, Stotts A, Daruzska LA, Creson D, Moeller FG. Dextroamphetamine for cocaine-dependence treatment: A double-blind randomized clinical trial. *Journal of Clinical Psychopharmacology.* 2001; 21:522–526. [PubMed: 11593078]
- Grasing K, He S, Yang Y. Dose-related effects of the acetylcholinesterase inhibitor tacrine on cocaine and food self-administration in rats. *Psychopharmacology (Berl).* 2008; 196:133–42. [PubMed: 17917719]
- Grasing K, He S, Yang Y. Long-lasting decreases in cocaine-reinforced behavior following treatment with the cholinesterase inhibitor tacrine in rats selectively bred for drug self-administration. *Pharmacol Biochem Behav.* 2009; 94:169–78. [PubMed: 19698738]
- Grasing K, Mathur D, Newton TF, DeSouza C. Donepezil treatment and the subjective effects of intravenous cocaine in dependent individuals. *Drug Alcohol Depend.* 2010; 107:69–75. [PubMed: 19836169]
- Greenwald MK, Lundahl LH, Steinmiller CL. Sustained release d-amphetamine reduces cocaine but not ‘speedball’-seeking in buprenorphine-maintained volunteers: A test of dual-agonist pharmacotherapy for cocaine/heroin polydrug abusers. *Neuropsychopharmacology.* 2010; 35:2624–2637. [PubMed: 20881947]
- Haney M, Rubin E, Foltin RW. Aripiprazole maintenance increases smoked cocaine self-administration in humans. *Psychopharmacology (Berl).* 2010; 216:379–387. [PubMed: 21373790]
- Haney M, Spealman R. Controversies in translational research: Drug self-administration. *Psychopharmacology (Berl).* 2008; 199:403–419. [PubMed: 18283437]
- Haney M, Ward AS, Foltin RW, Fischman MW. Effects of ecopipam, a selective dopamine d1 antagonist, on smoked cocaine self-administration by humans. *Psychopharmacology (Berl).* 2001; 155:330–337. [PubMed: 11441422]
- Heinrich JN, Butera JA, Carrick T, Kramer A, Kowal D, Lock T, Marquis KL, Pausch MH, Popiolek M, Sun SC, Tseng E, Uveges AJ, Mayer SC. Pharmacological comparison of muscarinic ligands:

- historical versus more recent muscarinic M1-preferring receptor agonists. *Eur J Pharmacol.* 2009; 605:53–6. [PubMed: 19168056]
- Hersch SM, Gutekunst CA, Rees HD, Heilman CJ, Levey AI. Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J Neurosci.* 1994; 14:3351–3363. [PubMed: 8182478]
- Hikida T, Kaneko S, Isobe T, Kitabatake Y, Watanabe D, Pastan I, Nakanishi S. Increased sensitivity to cocaine by cholinergic cell ablation in nucleus accumbens. *Proc Natl Acad Sci U S A.* 2001; 98:13351–13354. [PubMed: 11606786]
- Hikida T, Kitabatake Y, Pastan I, Nakanishi S. Acetylcholine enhancement in the nucleus accumbens prevents addictive behaviors of cocaine and morphine. *Proc Natl Acad Sci U S A.* 2003; 100:6169–73. [PubMed: 12721372]
- Kane BE, Grant MK, El-Fakahany EE, Ferguson DM. Synthesis and evaluation of xanomeline analogs--probing the wash-resistant phenomenon at the M1 muscarinic acetylcholine receptor. *Bioorg Med Chem.* 2008; 16:1376–92. [PubMed: 17977730]
- Karila L, Gorelick D, Weinstein A, Noble F, Benyamina A, Coscas S, Blecha L, Lowenstein W, Martinot JL, Reynaud M, Lepine JP. New treatments for cocaine dependence: a focused review. *Int J Neuropsychopharmacol.* 2008; 11:425–38. [PubMed: 17927843]
- Langmead CJ, Austin NE, Branch CL, Brown JT, Buchanan KA, Davies CH, Forbes IT, Fry VA, Hagan JJ, Herdon HJ, Jones GA, Jeggo R, Kew JN, Mazzali A, Melarange R, Patel N, Pardoe J, Randall AD, Roberts C, Roopun A, Starr KR, Teriakidis A, Wood MD, Whittington M, Wu Z, Watson J. Characterization of a CNS penetrant, selective M1 muscarinic receptor agonist, 77-LH-28-1. *Br J Pharmacol.* 2008a; 154:1104–1115. [PubMed: 18454168]
- Machova E, Jakubik J, El-Fakahany EE, Dolezal V. Wash-resistantly bound xanomeline inhibits acetylcholine release by persistent activation of presynaptic M2 and M4 muscarinic receptors in rat brain. *J Pharmacol Exp Ther.* 2007; 322:316–323. [PubMed: 17446301]
- Mark GP, Kinney AE, Grubb MC, Zhu X, Finn DA, Mader SL, Berger SP, Bechtholt AJ. Injection of oxotremorine in nucleus accumbens shell reduces cocaine but not food self-administration in rats. *Brain Res.* 2006; 1123:51–9. [PubMed: 17045970]
- Mirza NR, Peters D, Sparks RG. Xanomeline and the Antipsychotic Potential of Muscarinic Receptor Subtype Selective Agonists. *CNS Drug Reviews.* 2003; 9:159–186. [PubMed: 12847557]
- Negus SS. Rapid assessment of choice between cocaine and food in rhesus monkeys: Effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. *Neuropsychopharmacology.* 2003; 28:919–931. [PubMed: 12637948]
- Negus SS, Mello NK. Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys. *Psychopharmacology (Berl).* 2003b; 167:324–332. [PubMed: 12652348]
- Negus, SS.; Banks, ML. Making the right choice: Lessons from drug discrimination for research on drug reinforcement and drug self-administration. In: Glennon, RA.; Young, R., editors. *Drug Discrimination: Applications to Medicinal Chemistry and Drug Studies.* John Wiley and Sons; Hoboken, NJ: 2011. p. 361–388.
- Newman JL, Negus SS, Lozama A, Prisinzano TE, Mello NK. Behavioral evaluation of modafinil and the abuse-related effects of cocaine in rhesus monkeys. *Exp Clin Psychopharmacol.* 2010; 18:395–408. [PubMed: 20939643]
- Onali P, Olanas MC. Muscarinic M4 receptor inhibition of dopamine D1-like receptor signalling in rat nucleus accumbens. *Eur J Pharmacol.* 2002; 448:105–111. [PubMed: 12144929]
- Rush CR, Stoops WW, Hays LR. Cocaine effects during d-amphetamine maintenance: A human laboratory analysis of safety, tolerability and efficacy. *Drug Alcohol Depend.* 2009; 99:261–271. [PubMed: 18926645]
- Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S. Less is more: Antipsychotic drug effects are greater with transient rather than continuous delivery. *Biol Psychiatry.* 2008; 64:145–152. [PubMed: 18295747]
- Shannon HE, Bymaster FP, Calligaro DO, Greenwood B, Mitch CH, Sawyer BD, Ward JS, Wong DT, Olesen PH, Sheardown MJ, et al. Xanomeline: a novel muscarinic receptor agonist with functional selectivity for M1 receptors. *J Pharmacol Exp Ther.* 1994; 269:271–81. [PubMed: 7909557]

- Shannon HE, Rasmussen K, Bymaster FP, Hart JC, Peters SC, Swedberg MD, Jeppesen L, Sheardown MJ, Sauerberg P, Fink-Jensen A. Xanomeline, an m(1)/m(4) preferring muscarinic cholinergic receptor agonist, produces antipsychotic-like activity in rats and mice. *Schizophr Res.* 2000; 42:249–259. [PubMed: 10785583]
- Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dube S, Mallinckrodt C, Bymaster FP, McKinzie DL, Felder CC. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am J Psychiatry.* 2008; 165:1033–1039. [PubMed: 18593778]
- Shoaib M, Swanner LS, Beyer CE, Goldberg SR, Schindler CW. The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol.* 1998; 9:195–206. [PubMed: 9832934]
- Smiley JF, Levey AI, Mesulam MM. m2 muscarinic receptor immunolocalization in cholinergic cells of the monkey basal forebrain and striatum. *Neuroscience.* 1999; 90:803–14. [PubMed: 10218781]
- Smith JE, Co C, Yin X, Sizemore GM, Liguori A, Johnson WE 3rd, Martin TJ. Involvement of cholinergic neuronal systems in intravenous cocaine self-administration. *Neurosci Biobehav Rev.* 2004; 27:841–50. [PubMed: 15019433]
- Sofuoglu M. Cognitive enhancement as a pharmacotherapy target for stimulant addiction. *Addiction.* 2010; 105:38–48. [PubMed: 20078461]
- Sofuoglu M, Mooney M. Cholinergic functioning in stimulant addiction: implications for medications development. *CNS Drugs.* 2009; 23:939–52. [PubMed: 19845415]
- Stanhope KJ, Mirza NR, Bickerdike MJ, Bright JL, Harrington NR, Hesselink MB, Kennett GA, Lightowler S, Sheardown MJ, Syed R, Upton RL, Wadsworth G, Weiss SM, Wyatt A. The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. *J Pharmacol Exp Ther.* 2001; 299:782–792. [PubMed: 11602695]
- Takamatsu Y, Yamanishi Y, Hagino Y, Yamamoto H, Ikeda K. Differential effects of donepezil on methamphetamine and cocaine dependencies. *Ann N Y Acad Sci.* 2006; 1074:418–26. [PubMed: 17105940]
- Thomsen M, Caine SB. Chronic intravenous drug self-administration in rats and mice. *Curr Protoc Neurosci.* 2005; Chapter 9(Unit 9):20. [PubMed: 18428629]
- Thomsen M, Fink-Jensen A, Woldbye PD, Wortwein G, Sager TN, Holm R, Pepe LM, Caine SB. Effects of acute and chronic aripiprazole treatment on choice between cocaine self-administration and food under a concurrent schedule of reinforcement in rats. *Psychopharmacology (Berl).* 2008; 201:43–53. [PubMed: 18612628]
- Thomsen M, Conn PJ, Lindsley C, Wess J, Boon JY, Fulton BS, Fink-Jensen A, Caine SB. Attenuation of cocaine's reinforcing and discriminative stimulus effects via muscarinic M1 acetylcholine receptor stimulation. *J Pharmacol Exp Ther.* 2010a; 332:959–69. [PubMed: 19996296]
- Thomsen M, Wess J, Fulton B, Fink-Jensen A, Caine SB. Modulation of prepulse inhibition through both M₁ and M₄ muscarinic receptors in mice. *Psychopharmacology.* 2010b; 208:401–416. [PubMed: 20013114]
- Thomsen M, Lindsley CW, Conn PJ, Wessell JE, Fulton BS, Wess J, Caine SB. Contribution of both M₁ and M₄ receptors to muscarinic agonist-mediated attenuation of the cocaine discriminative stimulus in mice. *Psychopharmacology (Berl).* 2012; 220:673–685. [PubMed: 21964721]
- Thomsen M, Barrett AC, Negus SS, Caine SB. Cocaine versus food choice procedure in rats: Environmental manipulations and effects of amphetamine. *J Exp Anal Behav.* 2013; 99:211–233. [PubMed: 23319458]
- Watson J, Brough S, Coldwell MC, Gager T, Ho M, Hunter AJ, Jerman J, Middlemiss DN, Riley GJ, Brown AM. Functional effects of the muscarinic receptor agonist, xanomeline, at 5-HT₁ and 5-HT₂ receptors. *Br J Pharmacol.* 1998; 125:1413–1420. [PubMed: 9884068]
- Weiner DM, Levey AI, Brann MR. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc Natl Acad Sci U S A.* 1990; 87:7050–7054. [PubMed: 2402490]
- Wess J, Eglen RM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nat Rev Drug Discov.* 2007; 6:721–33. [PubMed: 17762886]
- Williams MJ, Adinoff B. The role of acetylcholine in cocaine addiction. *Neuropsychopharmacology.* 2008; 33:1779–1797. [PubMed: 17928814]

- Winhusen TM, Somoza EC, Harrer JM, Mezinskis JP, Montgomery MA, Goldsmith RJ, Coleman FS, Bloch DA, Leiderman DB, Singal BM, Berger P, Elkashef A. A placebo-controlled screening trial of tiagabine, sertraline and donepezil as cocaine dependence treatments. *Addiction*. 2005; 100(Suppl 1):68–77. [PubMed: 15730351]
- Woolley ML, Carter HJ, Gartlon JE, Watson JM, Dawson LA. Attenuation of amphetamine-induced activity by the non-selective muscarinic receptor agonist, xanomeline, is absent in muscarinic M4 receptor knockout mice and attenuated in muscarinic M1 receptor knockout mice. *Eur J Pharmacol*. 2009; 603:147–149. [PubMed: 19111716]

Figure 1.

Baseline data from all rats included in the study (i.e., completing testing of one or more xanomeline doses), from sessions immediately preceding (pre-xanomeline) and following (post-xanomeline) xanomeline treatment days. Pre-xanomeline data represent the average of three daily baseline sessions immediately preceding xanomeline treatment days, post-xanomeline data represent the average of the first two sessions immediately following xanomeline treatment days. Data are percent cocaine choice (top), cocaine injections and food reinforcers earned per component (center, black and grey symbols, respectively), and total reinforcers earned per component (bottom), as groups means. Bars represent one standard error of the mean across subjects (s.e.m.). Abscissae: unit dose cocaine [mg/kg/injection]. N=12.

Figure 2.

Effects of xanomeline treatment on cocaine vs. food choice behavior after the first administrations (day 1) and after a week of daily treatment (day 7), relative to within-subjects baseline behavior. Data are percent cocaine choice (top), cocaine injections earned per component (second row), food reinforcers earned per component (third row), and total reinforcers earned per component (bottom), as groups means. Daily xanomeline dose is the same for each column and is indicated at the top of the graphs. Bars represent one standard error of the mean across subjects (s.e.m.). Abscissae: unit dose cocaine [mg/kg/injection]. 10 mg/kg xanomeline: N=5, all others: N=6.

Table 1

Xanomeline-induced shifts in cocaine vs. food choice curves

Xanomeline dose (mg/kg/day)	A ₅₀ [95% CI] (mg/kg/inf. cocaine)	Fold shift
1.8 mg/kg (N=6)		
baseline	0.09 [0.09–0.10]	
day 1	0.10 [0.09–0.11]	1.1
day 7	0.18 [0.11–0.30] **	2.4
re-baseline	0.10 [0.09–0.12]	1.1
3.2 mg/kg (N=6)		
baseline	0.09 [0.08–0.11]	
day 1	0.16 [0.09–0.30]	2.1
day 7	0.20 [0.11–0.36] *	2.5
re-baseline	0.08 [0.05–0.11]	0.9
5.6 mg/kg (N=6)		
baseline	0.08 [0.06–0.10]	
day 1	0.15 [0.09–0.24]	2.1
day 7	0.35 [0.19–0.63] **	5.6
re-baseline	0.07 [0.06–0.09]	1.0
10 mg/kg (N=5)		
baseline	0.07 [0.05–0.10]	
day 1	0.13 [0.08–0.28]	2.4
day 7	0.32 [0.32–0.32] **	4.9
re-baseline	0.10 [0.09–0.10]	1.4

* $p < 0.05$,** $p < 0.01$ higher than baseline, Dunnett's multiple comparisons test after ANOVA.Fold shift in A₅₀ was calculated in each rat as (treatment A₅₀)/(baseline A₅₀), then averaged across rats.

Table 2

Changes in total cocaine intake and total food reinforcers

Xanomeline dose (mg/kg/day)	Total cocaine intake (mg/kg/session)	Total food intake (reinforcers/session)
1.8 mg/kg (N=6)		
baseline	5.70 [5.11–6.28]	28.3 [26.8–29.8]
day 1	7.23 [6.67–7.78] [*]	29.7 [28.4–31.4]
day 7	6.01 [4.72–7.29]	32.8 [27.8–37.9]
post day 1	4.48 [3.87–5.10] [#]	32.2 [27.1–37.2]
re-baseline	5.30 [4.92–5.68]	28.8 [26.5–31.2]
3.2 mg/kg (N=6)		
baseline	5.64 [5.13–6.15]	28.3 [25.9–30.7]
day 1	7.36 [6.82–7.91] [*]	29.5 [16.5–42.5]
day 7	7.94 [6.10–9.77] ^{**}	25.7 [8.4–42.9]
post day 1	5.87 [5.42–6.32]	26.2 [20.4–32.0]
re-baseline	5.57 [5.09–6.05]	25.3 [19.5–31.1]
5.6 mg/kg (N=6)		
baseline	6.20 [5.19–7.21]	25.6 [22.1–29.1]
day 1	7.90 [6.14–9.66] [*]	21.7 [15.4–27.9]
day 7	5.23 [3.47–6.99]	22.0 [8.4–35.6]
post day 1	4.55 [3.43–5.66] [#]	24.5 [16.3–32.7]
re-baseline	6.02 [4.94–7.11]	21.2 [14.8–27.6]
10 mg/kg (N=5)		
baseline	6.36 [6.12–6.60]	23.7 [20.0–27.3]
day 1	6.53 [3.22–9.84]	23.6 [19.7–27.5]
day 7	7.14 [5.70–8.58]	11.2 [4.7–17.7]
post day 1	3.79 [1.67–5.92]	17.6 [9.2–26.0]
re-baseline	6.08 [4.60–7.57]	24.8 [17.0–32.6]

*
 $p < 0.05$,**
 $p < 0.01$ higher than baseline;#
 $p < 0.05$,##
 $p < 0.01$ lower than baseline, Dunnett's multiple comparisons test after ANOVA.