Dopamine receptors are implicated in the reinforcing effects of food and drug reinforcement. The purpose of this study was to evaluate whether blocking D₂ dopamine receptors during extinction (secondary reinforcement) would affect reacquisition of responding for food pellets (primary reinforcement). Food-restricted rats self-administered (FR1) food pellets in 1-h daily sessions for 7 days. For the next 7 days rats responded in extinction phase, rats were allowed to reacquire food pellet self-administration in seven daily sessions, and received saline or eticlopride before each session. Four treatment groups were represented: saline extinction, saline reacquisition; eticlopride extinction, saline reacquisition; saline extinction, eticlopride reacquisition; and eticlopride extinction, eticlopride reacquisition. Locomotor activity did not differ between eticlopride-treated and saline-treated groups during extinction (secondary reinforcement) or reacquisition. Extinction was accelerated in eticlopride-treated rats. Eticlopride also delayed reacquisition of food self-administration compared with saline-treated rats. Rats administered eticlopride during extinction showed delayed reacquisition and a decreased response rate for food during the reacquisition phase. Indirectly reducing the value of a reinforcer in this way may provide a novel approach for reducing addiction-related food or drug self-administration behaviors. *Behavioural Pharmacology* 00:000–000 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Given the severity of the obesity epidemic (WHO, 2013), further evaluation of factors that maintain feeding behaviors, such as the role of specific neurotransmitters, is warranted. Dopamine (DA) is important in food reinforcement (Ettenberg, 1989; Wise, 2004). Depending on the schedule of reinforcement, DA antagonists decrease responding in the presence of food or in the presence of food-paired cues (Wise *et al.*, 1978a; Gray and Wise, 1980; Mason *et al.*, 1980; Spyraki *et al.*, 1982; Ettenberg and Camp, 1986; Wise and Raptis, 1986; Beninger *et al.*, 1987; Asin and Wirtshafter, 1990; Duarte *et al.*, 2003).

Exemplifying these findings are data from Wise *et al.* (1978a), who found that the DA receptor blocker pimozide reduced the ‘reward quality’ of food without impairing the animals’ physical ability to lever press. Rats were first trained to press a lever for food and were then either administered pimozide before operant conditioning sessions or put through several sessions of extinction. Rats that received pimozide before food-reinforced responding had reduced response rates for food in a manner similar to that of rats responding in extinction. Wise *et al.* (1978a) suggested that, in both cases, rats reduced responding because they were no longer experiencing the reinforcing effects of the food. That being said, it has been debated whether extinction of responding in the absence of food is equivalent to food-reinforced responding diminished by a DA antagonist (Gray and Wise, 1980; Mason *et al.*, 1980; Salamone, 1986). For example, giving rats either pimozide or the DA receptor antagonist haloperidol during extinction trials can accelerate extinction if rats had been trained on a continuous reinforcement schedule (Phillips and Fibiger, 1979; Feldon and Weiner, 1991). Acceleration of extinction would not be predicted if DA antagonism was expected only to block primary reinforcement, as extinction responding is technically extinction of secondary reinforcement (Phillips and Fibiger, 1979), the secondary reinforcer(s) being the click of the lever and perhaps the act of lever pressing itself (Bindra, 1972; Grimm *et al.*, 2000). One explanation for this apparent discrepancy is that DA antagonism reduces both primary and secondary reinforcement by reducing their incentive values (Wise, 2004).

Therefore, in the present study we examined whether extinction with DA receptor antagonism, compared with extinction alone, would lead to a change in the responding for food when it again became available. If DA antagonism reduces the incentive value of lever responding in the self-administration context, it would be expected that such an effect would be apparent even when primary reinforcement is again provided.
Rats were first allowed to respond for food pellets on a continuous reinforcement schedule for 7 days. They were then split into two groups and allowed to respond in extinction conditions for 7 days. One group was pretreated daily with saline, whereas the other was pretreated with the specific DA \( D_2 \) (DAD\(_2\)) receptor antagonist eticlopride. Studies have shown the \( D_2 \) receptor subtype to mediate food consumption (Beninger et al., 1987; Baldo et al., 2002; Duarte et al., 2003; Johnson and Kenny, 2010). After seven extinction sessions, the two extinction groups were further sub-divided. From the saline-treated extinction group, one subgroup continued to receive daily saline pretreatments, whereas the other received daily eticlopride pretreatments. From the eticlopride-treated extinction group, one subgroup continued to receive daily eticlopride pretreatments, whereas the other received daily saline pretreatments. Rats were again allowed to respond for food pellets for 7 days. The effects of DA antagonism on food self-administration were evaluated to confirm a role of DAD\(_2\) receptors in food reinforcement. Inactive lever responses and photobeam breaks (a locomotor activity measure) were recorded in all sessions, as a means of identifying any response generalization (inactive lever) or motor-related (inactive lever and locomotor activity) effects of DA antagonist treatment.

**Methods**

**Subjects**

Forty-three male Long-Evans rats (\(~4\) months old, 452.6±0.2 g at start of study), bred in the Western Washington University vivarium, were used for this experiment. Rats were housed individually under a 12-h reverse day/night cycle with lights off at 07:00 h. Rats were food restricted (20 g/day postsession, Mazuri Rodent Pellets; Purina Mills Inc., Saint Louis, Missouri, USA) and their weights were recorded every Monday, Wednesday, and Friday for the duration of the study. Food restriction began 48 h before the first day of training. All procedures followed the guidelines outlined in the ‘Principles of laboratory animal care’ (NIH Publication No. 85-23) and were approved by the Western Washington University Institutional Animal Care and Use Committee.

**Apparatus**

Operant procedures took place in operant conditioning chambers (30 × 20 × 24 cm; Med Associates, St Albans, Vermont, USA) equipped with two retractable levers on either side of the tray where food pellets were dispensed. Each chamber was also equipped with four infrared photobeams (Med Associates) that crossed the chamber. The operant conditioning chambers were enclosed in sound-attenuating chambers equipped with fans to provide air flow and white noise, and included a red house light on the wall opposite the levers that was illuminated during operant sessions.

**Materials**

Subjects’ active lever presses were reinforced with 45 mg food pellets (Rodent Tablets AIN-76A; Test Diet, St Louis, Missouri, USA). During the extinction and reacquisition phases, rats were injected with either saline (1 ml/kg) or the dopamine DAD\(_2\) receptor antagonist \(-\)-eticlopride hydrochloride (0.03 mg/kg in 1 ml/kg saline, subcutaneously; Sigma-Aldrich, Saint Louis, MO, USA). Injections were given 15 min before the start of a session. Rats were injected and remained in home cages until immediately before a session. Eticlopride was used as it has a higher affinity for the \( D_2 \) receptor (\( K_i \) DAD\(_2\) = 0.06 nmol/l) compared with compounds that have often been used to examine a role of DA in food-maintained responding, including pimozide (\( K_i \) DAD\(_2\) = 2.5 nmol/l) and haloperidol (\( K_i \) DAD\(_2\) = 0.5 nmol/l) \( [K_i \] values derived from \([\text{H}]\)spiperone competition assays (Tang et al., 1994; Roth et al., 1995)). Eticlopride is also more specific for DAD\(_2\) receptors as compared with pimozide or haloperidol. For example, pimozide has affinity for 5-HT\(_7\) receptors (\( K_i = 0.5 \text{ nmol/l} \) (Roth et al., 1994) and haloperidol inhibits NMDA receptors (Lynch and Gallagher, 1996). The dose of eticlopride was chosen based upon previous reports in which the drug altered operant behavior without obvious motor side effects (Barrett et al., 2004; Botly et al., 2008).

**General procedures**

**Training phase**

Rats underwent 7 daily 1-h sessions (days 1–7) wherein they learned to press the active lever for a food pellet. Active lever presses (left lever) were reinforced under a fixed-ratio 1 schedule. No cues (e.g. light + tone) were associated with active lever presses and there was no time-out between food pellet delivery opportunities. Inactive lever presses elicited no response and were recorded as a control for discriminated responding and motor activity. On the last 2 days of training the rats were administered saline handling injections (1 ml/kg, subcutaneously) in the vivarium \(~1\) h after the session.

**Extinction phase**

Following the final day of training, rats were randomly assigned to one of four groups: Saline–Saline (SAL/SAL), Saline–Eticlopride (SAL/ETIC), Eticlopride–Saline (ETIC/SAL), and Eticlopride–Eticlopride (ETIC/ETIC). Group designations indicated whether rats would receive saline or eticlopride before extinction or reacquisition sessions. For example, ETIC/SAL rats received eticlopride before each extinction session and saline before each reacquisition session.

Starting the next day, rats underwent 7 daily 1-h extinction sessions (days 8–14) during which active presses no longer elicited food pellets. The pellet dispenser was activated by lever presses, but it was empty. Rats were pretreated with saline (1 ml/kg, subcutaneously) or eticlopride (0.03 mg/kg,
subcutaneously) 15 min before sessions, according to their
group designations.

**Reacquisition phase**
Starting the day following the last day of extinction, rats
underwent seven daily 1-h reacquisition sessions (days
15–21). During these sessions, active presses were once
again reinforced with food pellets. The precession
injections continued according to the group designations
defined above.

**Statistical analyses**
Active (equivalent to number of pellets earned) and
inactive lever presses, and photobeam breaks, were
analyzed separately. Training, extinction, and reacquisition
data were also analyzed separately. Body weight data were
analyzed across the entire study. Data totals were analyzed
using mixed-model repeated-measures analysis of variance
(RM ANOVA). The repeated measure was Time (TIME)
and the between-groups variables were Extinction Drug
(EXTDRUG) and Reacquisition Drug (REACQDRUG).
To identify whether any overall differences in responding
on the first day of extinction and the first day of
reacquisition occurred at the start of these sessions rather
than appearing later, ANOVA was conducted on the first
2 min bins of active lever responding on these days, using
the variables EXTDRUG and REACQDRUG. Post-hoc
comparisons for all ANOVAs were made using Fisher’s
least significant difference tests with a Bonferroni-
corrected z. Other than these corrected z’s, the criterion
for statistical significance was P value less than 0.05. For
brevity, in most instances only statistics for significant
main effects and interactions are noted in the text.
Means±SEM are indicated in the text and in the figures.

**Results**

**Body weight**
Body weights increased over the course of the study, as
indicated by a significant effect of TIME [F(8,312) = 8.9,
P < 0.001]. There were no significant effects for EX-
TDRUG or REACQDRUG or interactions between these
variables and TIME, indicating that groups did not differ
a priori, and neither did the weights of the rats in the
various conditions differ over the course of the study.
After visual inspection of the weight increase over TIME,
a curve fit was conducted in SPSS using the averaged
weights for each day. As expected from the RM ANOVA
result, the increase in weight was described by a linear
function [F(1,8) = 8.6, P < 0.05, R² = 0.5]. It was also
described by a cubic function [F(3,8) = 6.3, P < 0.05,
R³ = 0.8]. These findings indicate that rats gained weight
over the training phase (day 6: 469.8±7.1 g), lost weight
over the extinction phase (day 13: 461.5±6.5 g), and then
again gained weight over the reacquisition phase (day 20:
476.7±6.5 g). As rats were maintained on the same daily
amount of food in the home cage over the course of the
study (20 g/day), these results indicate that the brief
access to food pellets during self-administration (training
and reacquisition) had a small impact on body weight.

**Training phase**
As acquisition of responding was highly variable over the first
4 days of training, RM ANOVAs were only conducted for
days 5–7. Rats responded on the active lever at stable rates
over these 3 days, as there was no significant effect of TIME.
There were also no significant effects or interactions for
EXTDRUG or REACQDRUG, indicating that rats that
were subsequently assigned to these conditions did not
differ in response rates beforehand. Also, there were no
significant effects or interactions for TIME, EXTDRUG, or
REACQDRUG for inactive lever responding or photobeam
breaks. Training data are depicted in Fig. 1.

**Extinction phase**
There were 21 rats assigned to the SAL condition and
22 rats assigned to the ETIC condition. For active
lever responding there were significant effects of TIME
[F(6,234) = 39.3] and EXTDRUG [F(1,39) = 25.3], and
a significant TIME × EXTDRUG interaction [F(6,234) =
7.6; all P’s < 0.001]. Ectiprolide accelerated extinction
responding across the 7 days of the extinction phase.
Active lever responses are shown in Fig. 2. There were
no significant effects or interaction for active lever
responding or photobeam breaks. Inactive presses in the
SAL and ETIC groups averaged 2.1±0.5 and 1.2±0.5
presses per session, respectively. Photobeam breaks in
the SAL and ETIC groups averaged 1552.2±152.4 and
1522.4±115.4 breaks per session, respectively. Active
lever responding in the first 2-min bin of the first day
of extinction was not significantly different across
conditions (SAL 15.0±1.4, ETIC 13.0±1.9 responses).

**Reacquisition phase**
Group sizes at the start of the reacquisition phase were
SAL/SAL (n = 10), SAL/ETIC (n = 11), ETIC/SAL
(n = 13), and ETIC/ETIC (n = 9). For active lever
responding there were significant effects of TIME
[F(6,234) = 5.8, P < 0.001], EXTDRUG [F(1,39) = 10.7,
P < 0.01], REACQDRUG [F(1,39) = 45.0, P < 0.001],
and significant TIME × EXTDRUG [F(6,234) = 2.8,
P < 0.05], and TIME × REACQDRUG [F(6,234) = 3.9,
P < 0.01] interactions. The three-way (TIME × EX-
TDRUG × REACQDRUG) interaction was not statisti-
cally significant [F(6,234) = 0.83, NS].

Figures 3 and 4 presents active lever response data
organized according to the significant two-way interaction
terms. Figure 3 presents the TIME × REACQDRUG
interaction. The effect of eticlopride to decrease
responding for food pellets was apparent on the first
day of reacquisition and continued for the seven daily
sessions. The effect was not immediate, however, as
active lever responding in the first 2-min bin of the first
day of extinction was not significantly different across conditions (SAL/SAL 9.5±3.8, SAL/ETIC 7.4±3.1, ETIC/SAL 4.7±1.7, and ETIC/ETIC 2.4±1.4 responses). Figure 4 presents the TIME/C2 EXTDRUG interaction. Rats with a history of eticlopride during extinction responded less for food pellets over the first 4 days of reacquisition sessions. This drug history effect was not explained by the response rates of rats on the last day of extinction. We found this to be the case according to two statistical tests. First, response rate on the last day of extinction was not significant when used as a covariate in the active lever RM ANOVA for reacquisition $[F(1,38) = 0.012, \text{NS}]$. Second, responding on the last day of extinction did not correlate with that on the first day of reacquisition $[P_r = 0.067, P = 0.67]$. To confirm the effects of drug and drug history, we performed planned comparisons for each day of reacquisition, first between the SAL/SAL and SAL/ETIC groups (drug effect) and then between the SAL/SAL and ETIC/SAL groups (drug history effect). Active lever responding for all conditions and the statistically significant comparisons are show in Fig. 5, where the same patterns of results from Figs 3 and 4 were observed when only considering rats from the complete control condition (SAL/SAL) versus either the test for the drug effect (SAL/ETIC) or the test for the drug history effect (ETIC/SAL). The general replication of findings indicates that the effects observed in Figs 3 and 4 were not because of collapsing groups across conditions; specifically, the ETIC/ETIC condition did not account for most of the reduced responding in Figs 3 and 4.

Finally, there was a significant effect of REACQDRUG for inactive lever presses $[F(1,39) = 9.2, P < 0.01]$. Eticlopride reduced inactive lever responses from an across-sessions average of 1.5±0.2 (SAL) to 0.5±0.1
DOPAMINE D₂ RECEPTOR AND FOOD REINFORCEMENT

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The ability of DAD₂ antagonists to accelerate extinction responding and reduce operant responding for food is well documented (Wise et al., 1978a; Phillips and Fibiger, 1979; Beninger et al., 1987; Wise, 2004). DAD₂ receptor-preferring antagonists have also been found to decrease responding for other incentives, including water, saccharin, sucrose, brain stimulation, and drugs of abuse (Yokel and Wise, 1975; Wise et al., 1978b; Gerber et al., 1981; Wise, 2004; Smith and Smith, 2010).

Potential explanations for the response-decreasing effects of eticlopride on primary and secondary reinforcement include eticlopride-mediated motor impairment or changes in hunger. However, the acceleration of extinction responding by eticlopride was not attributable to motor impairment for three reasons. First, in the initial 2 min of the first day of extinction there was no difference in active lever responding between saline and drug-treated groups. Second, inactive lever responding did not differ between groups. And third, there was no difference in locomotor activity between groups. During reacquisition, the response-decreasing effect of eticlopride was also most likely not attributable to a motor-impairing effect of the drug: there was no difference across treatment conditions in the first 2 min of responding on the first day of reacquisition, nor were there group differences in locomotor activity across the reacquisition sessions. There was a significant effect of eticlopride on inactive lever responding, but the overall difference between SAL and ETIC groups was ~1 lever press/h. Given the already very low response rate on the inactive lever, the lack of effect of eticlopride on locomotor activity, and the lack of active lever group differences in the first 2 min of the first day of reacquisition, it is unlikely that the eticlopride-mediated decrease in active lever responding during reacquisition was because of motor impairment.

It is also unlikely that eticlopride reduced food-reinforced responding during reacquisition by reducing ‘hunger’. Although we did not directly examine this hypothesis in the present study, findings from other studies lead us to conclude that eticlopride is not an anorectic agent. Previous studies have demonstrated that DAD₂ antagonists reduce operant responding for food but are without effect on free feeding (Salamone et al., 2005; Randall et al., 2012). If DAD₂ antagonism reduced hunger, rats would be expected to reduce their intake of food in both operant and free-feeding conditions.

Motivation to respond in extinction or for food pellets must have been reduced by some other factor. We propose that eticlopride reduced responding by reducing primary and secondary reinforcement, at least within the context of the operant conditioning chamber. The acceleration of responding in extinction would therefore...
have been due to a decrease in the conditioned incentive value (Bindra and Palfai, 1967; Stewart and de Wit, 1987) of the operant conditioning chamber, including the lever that had previously been associated with food-pellet delivery. The reduction in food-reinforced responding in eticlopride-treated rats would then have been due to a drug-induced decrease in the incentive qualities of food pellets. This would require the rats to have some initial experience of responding for food under the effects of DAD₂ antagonism to ‘learn’ that the reinforcing efficacy of a food pellet was reduced. This appeared to be the case in the present study, as response rates in the first 2 min of the first reacquisition day were not different across treatment conditions. A similar effect has been reported previously by Wise et al. (1978a), who reported that the motivation of rats to respond for food pellets was not reduced until after experiencing response-dependent food pellets under the DA receptor blocking effects of pimozone. Similar patterns of learning where the incentive value of a reinforcer has been diminished by DA receptor blockage have been reported in runway procedures (Wise et al., 1978a; Ettenberg and Camp, 1986).

The most novel finding of the present study is that the apparent decrease in the incentive value of the self-administration context by eticlopride in extinction ‘transferred’ to a diminished ability of food pellets to reinforcer lever responding in reacquisition. Specifically, rats that received eticlopride during the extinction phase showed decreased responding for food pellets, compared with controls, during reacquisition (Figs 4 and 5). This was not explained by rate of responding on the last day of extinction (Fig. 2; see ANCOVA and correlation values in the Results section). In addition, this long-lasting effect (4 days) cannot be explained by a lingering effect of eticlopride itself, as the drug has a half-life of ~1 h (Norman et al., 2011).

We are not certain as to the prevailing cause of this ‘history’ effect, but we speculate that it may occur because DAD₂ antagonism changed the meaning of the self-administration context, including the active lever response, for the subjects. For example, eticlopride administration during extinction could have decreased or even negated the incentive value of the active lever, such that, in reacquisition, rats would need more time for the value of the lever to be re-established by contingent food delivery. This hypothesis fits with the pattern of reacquisition by rats with a history of eticlopride during extinction. This inhibitory signal hypothesis somewhat maps onto results obtained by other investigators utilizing the ‘renewal’ procedure where, typically, responding is first reinforcer in one context (A), extinguished in another (B), and then renewed upon return to the first context (A) (Bouton et al., 2012). In one especially relevant study utilizing this procedure, reacquisition of food self-administration was slower for rats that reacquired in the context in which their responding had been extinguished (ABB) compared with the reacquisition of rats in the context where extinction had not occurred (ABA) (Todd et al., 2012).

Future studies are needed to clarify whether the history effect is mediated by the changes in the incentive value and/or response-appropriate signaling properties of the self-administration context, including the response requirement and/or manipulandum. For example, a reacquisition comparison group could be to have rats reacquire self-administration using a novel response such as a nose poke. A comparison to investigate the role of the signaling properties of the context could be to have rats reacquire self-administration in a novel context. If the history effect were to be maintained in both of these comparisons, it might then be concluded that experience of DAD₂ antagonism during extinction literally transferred to food pellets, devaluing their incentive value. Such transfer effects between primary and secondary reinforcers have been reported previously (Harkness et al., 2010).

To better understand the effects observed in the present study, future studies could also explore the neuroanatomical loci of DAD₂ antagonism effects on primary and secondary reinforcement. Several studies have shown that the reinforcing effects of certain behaviors, including responding for food, are mediated by the nucleus accumbens (Kelley, 2004; Segovia et al., 2012; Nunes et al., 2013). For example, direct injections of DAD₁ and DAD₂ antagonists into the nucleus accumbens (both core and shell) of rats reduced responding for food reinforcement (Baldo et al., 2002). To determine the neuroanatomical locus of the eticlopride-mediated effects in the present study, it would be necessary to similarly direct intracranial injections of a DAD₂ antagonist. In addition, given the findings of Baldo et al. (2002) and our previous findings of reduced food (sucrose) cue reactivity following accumbal DAD₁ antagonist injections (Grimm et al., 2011), it would be of interest to compare the response-reducing effect of DAD₂ antagonists with those of DAD₁-specific antagonists. Given the results of the present study it would also be of interest to examine whether similar site-specific injections reduce extinction responding and, if so, whether such devaluation of the extinction context transfers over to reacquisition of responding for food. Further studies might also explore the generality of the effects observed in the present study by conducting similar studies with sucrose or a drug of abuse such as cocaine.
Conclusion
Eticlopride reduced the response rates of rats for food pellets during both the extinction and reacquisition phase. Also, eticlopride was able to indirectly reduce response rates in the reacquisition phase if had been administered during the extinction phase. Overall these results support a role for DAD2 receptors in both primary and secondary reinforcement. The eticlopride drug history effect may be particularly useful for the design of effective behavioral pharmacotherapies related to excessive food consumption (obesity) and, perhaps, drug addiction.

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Conflicts of interest
There are no conflicts of interest.

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