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## Amitifadine, a triple monoamine re-uptake inhibitor, reduces nicotine self-administration in female rats

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### Abstract

A wider diversity of drug treatments to aid smoking cessation is needed to help tailor the most efficacious treatment for different types of smokers. This study was conducted to determine whether amitifadine, which inhibits re-uptake of dopamine, norepinephrine and serotonin, would decrease nicotine self-administration at doses that do not cause adverse side effects. Adult female Sprague-Dawley rats were trained to self-administer nicotine intravenous (IV) and were given acute doses of amitifadine in a repeated measures counterbalanced design. Effects of amitifadine on locomotor activity and food motivated responding were also evaluated. Chronic amitifadine effects were also examined. The 30 mg/kg amitifadine dose significantly reduced nicotine self-administration. The 5 and 10 mg/kg doses reduced nicotine self-administration during the first 15 min. of the session when the greatest amount of nicotine was self-administered. The 30 mg/kg amitifadine dose, but not the lower doses caused a significant reduction in locomotor activity averaged over the 1-hour session and reduced food motivated responding. The 10 mg/kg dose caused hypoactivity at the beginning of the session, but 5 mg/kg did not cause any hypoactivity. The effects of chronic amitifadine treatment (10 mg/kg) over the course of 15 sessions was also determined. Amitifadine caused a significant reduction in nicotine self-administration, which was not seen to diminish over two consecutive weeks of treatment and a week after enforced abstinence. Amitifadine significantly reduced nicotine self-administration. This prompts further research to determine if amitifadine might be an effective treatment for smoking cessation.

### Keywords

Amitifadine; Self-administration; Nicotine; Treatment; Dopamine; Serotonin; Norepinephrine

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## 1. Introduction

Tobacco addiction is responsible for millions of premature deaths each year (Hatsukami et al., 2008). The current drug treatments which include nicotine replacement, as well as varenicline and bupropion are more effective than placebo, but fall short of being effective for the majority of tobacco users (Frishman et al., 2006). The variety of tobacco users may benefit from different types of treatments, which would entail having available a greater variety of treatments.

Nicotine potentiates the release of a variety of neurotransmitters, including the dopamine, norepinephrine and serotonin (Li et al., 1998) which are important for a variety of important neurobehavioral functions, including addiction. Dopaminergic innervation from the ventral tegmental area to the nucleus accumbens has been shown in many studies to be critically involved in the neural basis of addiction (Di Chiara et al., 2004). There is also evidence for involvement of norepinephrine and serotonin (Benowitz and Peng, 2000; Dudas and George, 2005; Frishman, 2007; Rezvani et al., 1990), which interact with each other and with dopamine. We have shown that the serotonin 5HT<sub>2c</sub> agonist lorcaserin reduces nicotine self-administration (SA) in rats (Levin et al., 2011a). The noradrenergic  $\alpha_2$  agonist clonidine has been shown to improve smoking cessation pharmacotherapy (Glassman et al., 1988). Nortriptyline, which blocks the re-uptake of norepinephrine and to a lesser degree dopamine, has been found to improve smoking cessation pharmacotherapy (Hughes et al., 2005). Selective serotonin re-uptake inhibitors have not been found to be effective (Hughes et al., 2005) in general, although they may be efficacious in helping depressed smokers (Hitsman et al., 1999).

This study evaluated the efficacy of acute doses of amitifadine (EB-1010, DOV 21947), a serotonin-preferring triple re-uptake inhibitor of serotonin, norepinephrine, and dopamine, with K<sub>i</sub> values for inhibition of uptake of 96, 23 and 12 nM for dopamine, norepinephrine, and serotonin, respectively (Skolnick et al., 2003). Amitifadine in the dose-range selected for this study was found by Skolnick et al. to reduce anhedonia in rats as measured by reduction in the duration of immobility in the forced swim test (Skolnick et al., 2003). Amitifadine has been found to have no appreciable direct effect on neurotransmitter receptors, including nicotinic receptors (Bymaster, unpublished observation). *In vivo*, amitifadine was active with minimal effective doses of 5 mg/kg in the rat forced swim test and mouse tail suspension antidepressant models (Golembiowska et al., 2012; Skolnick et al., 2003). Consistent with triple re-uptake blockade, amitifadine inhibited *ex vivo* binding to dopamine, norepinephrine and serotonin transporters and robustly increased these transmitters in prefrontal cortex and dopamine in the striatum of rats (Golembiowska et al., 2012; Lengyel et al., 2008). Recently, amitifadine was found in humans to have robust antidepressant activity and be well tolerated (Tran et al., 2012).

This study examined the effects of both acute and chronic administration of amitifadine on nicotine self-administration (SA). Effects of amitifadine were also assessed on food-motivated responding and locomotor activity to assess ancillary effects. It was hypothesized that acute and chronic administration of amitifadine would significantly reduce nicotine SA in rats.

## 2. Materials and Methods

### 2.1. Subjects

Young adult 3–5 month old female (Acute Study N=10; Chronic Study N=10 Controls and N=12 amitifadine treated) Sprague-Dawley rats (Taconic Lab, Germantown, NY, USA) were used in the present study. Female rats were used in order to compare the data from the current studies with our previous work (Levin et al., 2011a; Levin et al., 2010; Levin et al., 2008; Levin et al., 2011b; Levin et al., 2011c) in which we documented the effectiveness of treatments on related transmitter systems for reducing nicotine SA in female rats. Animals were individually housed in a temperature controlled vivarium room located adjacent to the nicotine SA testing room. Animals were maintained on a 12:12 reverse light-dark cycle so that experimental sessions occurred during the active part of the rats' diurnal cycle. Animals were given *ad lib* access to water at all times excluding experimental sessions, and were fed approximately 10–15 g of rat chow daily 20–30 min after the completion of their experimental session to keep the rats at a lean healthy weight. This study was conducted under a protocol approved by the Duke University Institutional Animal Care and Use Committee in accordance with USDA regulations.

### 2.2. Nicotinic Receptor Binding

To determine the possibility that amitifadine may have effects mediated via direct actions on nicotinic receptors we determined its affinity for  $\alpha 7$  and  $\alpha 4\beta 2$  nicotinic receptors in radioligand binding assays. Inhibition of binding to  $\alpha 4\beta 2$  nicotinic receptors in SH-SY5Y cells was determined using the radioligand [ $^3\text{H}$ ]cytosine at 0.6 nM concentration according to the method of Gopalakrishnan et al. (Gopalakrishnan et al., 1996). Incubation was for 120 min at 4°C and non-specific binding was determined with 10  $\mu\text{M}$  nicotine. Inhibition of binding to  $\alpha 7$  nicotinic receptors in SH-SY5Y cells was determined using [ $^{125}\text{I}$ ]  $\alpha$ -bungarotoxin (0.05 nM) according to the method of Sharples et al. (Sharples et al., 2000). Incubation was 120 min at 37°C and non-specific binding was determined using  $\alpha$ -bungarotoxin (1  $\mu\text{M}$ ). Specific binding of the radioligands was determined by scintillation spectrometry technology. The assessment was conducted by Cerep, Inc. (Poitiers, France)

### 2.3. Drug Treatments

Nicotine bitartrate solutions were prepared in isotonic sterile saline. The dose used for SA (0.03 mg/kg/infusion) was calculated as a function of the nicotine free base weight. The pH of the nicotine solution was adjusted to 7.0 using NaOH and the solution was filtered in a Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. Between sessions all nicotine was kept in a dark refrigerator. Amitifadine ((1*R*,5*S*)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0] hexane hydrochloride) was provided by Euthymics Bioscience, Inc., Cambridge, MA, USA.

Amitifadine solutions were prepared in sterile water for doses of 5, 10 and 30 mg/kg (p.o.). Water vehicle was used as the control. The volume of oral gavage was 4 ml/kg given 30 min before testing.

## 2.4. Acute Dose Study

In the acute amitifadine dose effect study, three doses of amitifadine along with vehicle (sterile water) were tested in a counterbalanced order in rats (N=10) with at least two days between consecutive injections. The entire dose-effect function was run twice (phase 1 and phase 2). The test of acute amitifadine on locomotor activity was conducted between the two phases, and the test of food motivated responding was conducted after phase 2. Both the locomotor activity and food motivated responding tests were conducted with the same dosing parameters as with the tests on nicotine SA.

## 2.5. Chronic Dosing Study

In a second set of rats trained in the same way as the rats in the first study, chronic effects of amitifadine were tested using a between-subjects design with control (N=10) and 10 mg/kg amitifadine (N=12) groups. The two treatment groups were matched for similar predrug rates of nicotine self-administration. Rats were administered 10 mg/kg of amitifadine by gavage 30 min. before testing for ten sessions over a period of two weeks with a two-day break between the first and second five sessions. To test for efficacy of amitifadine in attenuating resumption of nicotine SA, an additional five sessions after a nine-day period of enforced abstinence. This phase was conducted to model treatment efficacy in attenuation relapse after a cessation attempt. The nine-day break in nicotine access modeled an initially successful attempt at smoking cessation. Controls received sterile water vehicle. Amitifadine or vehicle administration only occurred on days when the animals were tested.

## 2.6. Behavioral Procedures

Before the start of nicotine SA sessions, all animals were trained to lever press in a standard dual-lever operant chamber (Med Associates, St. Albans, VT, USA) for food reinforcement. All rats had an equivalent three sessions of food training. All rats averaged more than 50 food reinforcements per session. Each chamber was equipped with two levers, two cue lights located directly above each lever, a house light, and a tone generator. After lever pressing was established, animals experienced three sessions of lever pressing for food under a fixed ratio (FR) 1 schedule of reinforcement. Following the completion of their final training session with food reinforcement, animals were anesthetized with a mixture of ketamine (60 mg/kg) and dormitor (15 mg/kg) and a catheter (Strategic Application Inc., Libertyville, IL, USA) was implanted into their jugular vein. The jugular catheter was attached to a harness that could be tethered to the infusion pump during experimental sessions.

Following recovery from surgery, all animals experienced five experimental sessions where a correct lever press on the same side rewarded as the correct side for the food training resulted in the delivery of a nicotine infusion (0.03 mg/kg/infusion in a volume of 50  $\mu$ l) on a fixed ratio (FR) 1 schedule of reinforcement, and the activation of a feedback tone for 0.5 s. Each infusion was followed by a one-min period where the cue lights went out, the house light, which was out during the access period, came on and correct responses were recorded but not reinforced. The sessions lasted for 45-min. After the initial 5 sessions of baseline nicotine SA, the rats were tested for effects of acute amitifadine on nicotine SA in a repeated measures counterbalanced design with all of the rats receiving all of the doses two times in separate phases. The sessions were 45-min. long.

The catheters were flushed daily before the experimental sessions with a 100U/ml heparinized saline solution. After the completion of each test session, nicotine remaining in the port was removed and a 0.3 ml sterile lock solution containing 500U/ml of heparinized saline and 8 mg/ml of gentamicin (American Pharmaceutical Partners, Schaumburg, IL, USA) was infused.

## 2.7. Locomotor Activity

Between the two phases of testing for amitifadine effects on nicotine SA, its effects on locomotor activity were tested in a figure-8 maze over the course of a one-hour session. The mazes had continuous enclosed alleys 10×10 cm in the shape of a figure-8. This apparatus has been used to measure locomotor activity and its habituation in a variety of studies of drug, toxin and toxicant studies as well as the effects of brain lesions (Crofton et al., 1995; Icenogle et al., 2004; Levin et al., 2006; Rezvani et al., 2008; St Omer et al., 1991; Vorhees and Minck, 1989; Wolansky et al., 2006). The overall dimensions of the apparatus were 70 cm long and 42 cm wide, with a 21×16 cm central arena, a 20-cm high ceiling and two blind alleys extending 20 cm from either side. Eight infrared photobeams, which crossed the alleys, indexed locomotor activity. One photobeam was located on each of the two blind alleys and three were located on each of two loops of the figure-8. The number of photobeam breaks was tallied during the one-hour session. The linear and quadratic trends across twelve five-min blocks in each session were calculated to determine the habituation of locomotor activity over the course of the session.

## 2.8. Food-Motivated Responding

Subsequent to the nicotine SA sessions, the rats were tested to assess amitifadine effects on responding for food reinforcement. The doses of 5, 10 and 30 mg/kg of amitifadine (p.o.) as well as the saline control were administered in a repeated measures counterbalanced order. The behavioral paradigm used FR1, with activation of a feedback tone for 0.5 s after reinforcement. Cue lights were on throughout the session with no house light illumination and no time out after reinforcement. The rewards were 45-mg food pellets. As with nicotine SA, the sessions for food SA were 45-min. long.

## 2.9. Data Analysis

The data were evaluated with a repeated measures analysis of variance. Amitifadine dose was the principal within-subjects factor. Fifteen-min. blocks within session and repeated testing sessions were also within-subjects factors. An alpha level of  $P < 0.05$  was used to determine statistical significance. Significant interactions were followed by tests of the simple main effects. Planned comparisons were used to assess the significance each of the amitifadine dose to the control vehicle. Dunnett's tests were used for comparisons of dose condition back to control.

# 3. Results

## 3.1. Nicotinic Receptor Binding

There was no appreciable effect of amitifadine at concentrations from 100 to 10,000 nM on binding of radioligands to either  $\alpha 7$  or  $\alpha 4\beta 2$  nicotinic receptors (Table 1).

### 3.2. Nicotine Self-Administration after Acute Amitifadine

The main effect of acutely administered amitifadine was significant ( $F(3,27)=13.74$ ,  $P<0.0005$ ) for reducing nicotine SA. As shown in Fig. 1, panel A, according to the Dunnett's two-tailed tests the 10 mg/kg ( $P<0.05$ ) and the 30 mg/kg ( $P<0.01$ ) doses of amitifadine each significantly reduced nicotine SA relative to control treatment averaged over the 45-min sessions.

Interestingly, there was a significant ( $F(6,54)=4.05$ ,  $P<0.005$ ) interaction of amitifadine and 15-min time block within the 45-min session (Fig. 1, panel B). Tests of the simple main effects of the amitifadine dose effects at each time block showed that the high 30 mg/kg dose caused significant reductions during the first ( $P<0.01$ ), second ( $P<0.05$ ) and third ( $P<0.05$ ) 15-min time blocks of the session. Both the 5 mg/kg ( $P<0.05$ ) and 10 mg/kg ( $P<0.01$ ) doses caused significant decreases in nicotine SA during the first 15-min block, but not during the later parts of the session as the control rates of nicotine SA decreased relative to the first 15-min period.

Amitifadine was administered in a repeated measures counterbalanced design twice. There was a significant interaction of amitifadine and test phase ( $F(3,27)=4.62$ ,  $P<0.01$ ). As shown in figure 1, panel C, during the first phase the 10 and 30 mg/kg amitifadine doses significantly ( $P<0.01$ ) reduced nicotine self-administration relative to the Phase 1 vehicle control condition. The effect with 10 mg/kg of amitifadine was not seen during the second phase. This appeared to be due to a lower rate of nicotine self-administration with the control condition during the second test phase nicotine. Nicotine self-administration for the 0 ( $P<0.05$ ) and 5 mg/kg ( $P<0.01$ ) conditions were significantly lower than during the first test phase. Nicotine SA with the 10 and 30 mg/kg conditions did not differ between the two test phases. The high dose of 30 mg/kg of amitifadine significantly decreased nicotine self-administration in the second phase ( $P<0.01$ ) as well as the first.

### 3.3. Locomotor Activity

Fig. 2 shows the effect of amitifadine on average locomotor activity over the course of the one-hour test session. There was a significant main effect of amitifadine ( $F(3,27)=6.45$ ,  $P<0.005$ ). Planned comparisons of each amitifadine dose to control showed that only the highest dose of 30 mg/kg caused a significant ( $P<0.01$ ) decrease in locomotor activity. Fig. 2, panel B shows the effect of amitifadine over the twelve 5-min blocks of the test session. There was a significant main effect of time block ( $F(11,297)=28.22$ ,  $P<0.0005$ ). There was also a significant interaction of time-block  $\times$  amitifadine ( $F(33,297)=1.84$ ,  $P<0.005$ ). Tests of the simple main effects showed 30 mg/kg decreased activity at block 1 ( $P<0.01$ ), block 2 ( $P<0.01$ ) and block 3 ( $P<0.05$ ). 10 mg/kg decreased activity at block 1 ( $P<0.05$ ).

### 3.4. Food-Motivated Responding

There was a significant ( $F(3,24)=8.53$ ,  $P<0.001$ ) main effect of amitifadine on responding on the food rewarded task. Amitifadine did not significantly affect food motivated responding at doses of 5 and 10 mg/kg (Fig. 3A). However, at the higher 30 mg/kg amitifadine dose there was a significant ( $P<0.01$ ) decrease in food motivated responding. There was a significant main effect of time over the three 15-min time blocks ( $F(2,16)=33.60$ ,  $p<0.0005$ ). There was



also a significant interaction of amitifadine  $\times$  time block ( $F(6,48)=13.93$ ,  $P<0.0005$ ) with substantial food earned in the first 15 minutes and much less in the latter time blocks (Fig. 3B). As shown in figure 3B, the 30 mg/kg amitifadine significantly ( $P<0.01$ ) decreased food-motivated responding for the first 15-min of the session but not thereafter. Neither the 5 and 10 mg/kg amitifadine doses significantly affected food-motivated responding.

### 3.5. Nicotine Self-Administration after Chronic Amitifadine

Over the 15 sessions of chronic amitifadine administration, rats showed a significant ( $F(1,20)=31.97$ ,  $P<0.0005$ ) reduction in nicotine SA relative to controls (Fig. 4). There was no indication of attenuation over the course of treatment from the first five days of treatment to the second five days of treatment with a two-day abstinence period between or to the week of treatment after a nine-day period of enforced abstinence. There was a significant ( $F(2,40)=6.89$ ,  $P<0.005$ ) interaction of amitifadine  $\times$  15-min. session block. Tests of the simple main effects of amitifadine at each session block showed significant (1–15 min  $P<0.0005$ ; 16–30 min.  $P<0.0005$ ; 31–45 min.  $P<0.005$ ) amitifadine effects at each 15-min. block. Fig. 4, panel B shows the infusions per 15-min. block averaged over all sessions. Fig. 4, panel C shows nicotine SA session by session throughout the period two weeks of chronic treatment and during the week of resumed access after the week-long enforced abstinence period. Amitifadine induced reduction in nicotine SA is seen starting with the acute first dose of the series and continued without sign of tolerance throughout the rest of treatment before and after the period of enforced abstinence.

## 4. Discussion

The triple monoamine re-uptake inhibitor amitifadine (EB-1010) significantly reduced nicotine SA at doses that were not seen to cause locomotor hypoactivity (5 mg/kg, for the initial part of the SA session) or not seen to decrease responding for food (5 or 10 mg/kg). Chronic treatment with 10 mg/kg of amitifadine caused a robust decrease in nicotine SA which did not show signs of attenuation over the course of three weeks of treatment. This effect did not appear to be due to direct effects of amitifadine on nicotinic receptors, as no appreciable effects of amitifadine at concentrations from 100 to 10,000 nM on binding of radioligands to either  $\alpha 7$  or  $\alpha 4\beta 2$  nicotinic receptors were found. This prompts further research to determine if this monoamine re-uptake inhibitor might effectively aid smoking cessation.

The effects of the dose range of amitifadine on nicotine SA need to be interpreted in light of its effects on other behavioral measures. The 30 mg/kg dose of amitifadine caused a substantial reduction in nicotine SA throughout the session, but also caused significant hypoactivity and reduction in food motivated responding. Amitifadine has previously been shown to reduce food intake in studies with rats (Tizzano et al., 2008) at doses of 20 or 40 mg/kg. Tizzano and others (2008) also found that with 6 mg/kg of amitifadine there was a decrease in ad lib food consumption however we did not see either 5 or 10 mg/kg (PO) of amitifadine to significantly reduce food motivated responding in a task similar to the nicotine SA task. In the current study, we found that a lower amitifadine dose (10 mg/kg) significantly reduced nicotine SA, an effect that lasted for two weeks of treatment and into

an additional week of treatment after a period of nicotine withdrawal. Interpretation of the suppression of nicotine SA with the 30 mg/kg amitifadine dose must be tempered by the documented hypoactivity it causes as well as the more general suppression in another type of motivated behavior. The lower dose of 10 mg/kg did not cause hypoactivity averaged across the 1-hour test session and did not reduce food motivated responding. The kinetics of amitifadine are important to consider for interpreting its duration of effect. The plasma half-life of amitifadine is 2.6 hours in rats, so the presence of the drug should have persisted through the 45-min session after the 30-min pre-session dosing interval though it would be declining over that period (Tizzano et al., 2008). Thus, the greater effect of lower doses of amitifadine in the first third of the session was likely more related to the higher rates of responding in the control condition that are typically seen, rather than pharmacokinetic effects since the kinetic profile of amitifadine outlasts the period of testing.

The effect of amitifadine in blunting the initial flurry of nicotine SA during the first 15-min of nicotine access complements the effects we have previously seen with the nicotinic receptor desensitization agent sazetidine-A, which has a more prominent effect on later asymptotic levels of nicotine SA (Johnson et al., 2012). It may be the case that different neural substrates subsume higher levels of nicotine use seen upon initial access vs. later maintenance levels, and that different treatments are needed to reduce each of these phases of nicotine use. This hypothesis could be investigated by administering drug using osmotic minipump with a zero order kinetic, so that differential effects across a test session could be more clearly differentiated from pharmacokinetic changes.

Post-deprivation nicotine use seems to be more effectively reduced by increasing monoamine transmitter availability as was induced by the triple monoamine re-uptake inhibitor amitifadine compared to nicotinic receptor desensitization with sazetidine-A. Certainly, another monoamine re-uptake inhibitor bupropion has been found to be effective in reducing withdrawal effects after smoking cessation (Shiffman et al., 2000). Which monoamine transmitter or transmitters are key to this effect remain to be determined. Maintenance levels of nicotine SA seem to be more effectively reduced by desensitization of nicotinic  $\alpha 4\beta 2$  receptors as induced by sazetidine-A (Levin et al., 2010; Rezvani et al., 2010). More effective reduction of both types of nicotine SA may be achieved through combined use of a monoamine re-uptake inhibitor and a nicotinic receptor desensitizing agent.

Nicotine SA was different during the first and second rounds of testing. During the second testing phase nicotine SA was lower in both control condition and the low amitifadine dose of 5 mg/kg. The apparently lessened effect in the second phase of the repeated measures acute dose effect study was mostly the result of significantly lower nicotine self-administration in the control condition. This led to the hypothesis that it was the carryover effect of intercurrent amitifadine administration that lowered the control condition in the second phase. This hypothesis was tested in the chronic study, which tested the effect of 10 mg/kg of amitifadine repeatedly in one set of rats vs. vehicle control administration in a separate set of rats that never received amitifadine. The study was important in several regards. First, it showed the clear efficacy of 10 mg/kg of amitifadine right from the first session. Second, the efficacy of this amitifadine dose did not diminish with repeated



administration. Finally, the rats in the control condition showed a relatively constant rate of nicotine self-administration with repeated testing, indicating that it likely was the intercurrent amitifadine administration that caused the decrease in nicotine self-administration in the control condition from the first to the second phase of the acute dose-effect study. Using the between-subjects design, there was a more robust amitifadine effect. Nicotine SA was significantly reduced over all three of the fifteen-min blocks within the 45-min. session. The amitifadine-induced decrease in nicotine SA was clearly seen starting with the first session. This is the same acute effect as was tested in the tests of food SA and locomotor activity where the same dose of 10 mg/kg did not significantly affect either food motivated responding or activity. This suppression of nicotine SA persisted without any apparent diminution through the next nine sessions of continued treatment with a 62% decrease in nicotine SA. The amitifadine-induced decrease in nicotine SA continued unabated during the five sessions of treatment after the period of enforced abstinence.

The current study was conducted with female rats. This was done to be confluent with our previous research with females (Levin et al., 2011a; Levin et al., 2010; Levin et al., 2008; Levin et al., 2011b; Levin et al., 2011c). Previously, we have found that nicotine SA in rats is not significantly correlated with estrus phase (Rezvani et al., 2008). It is likely that amitifadine would also be effective in reducing nicotine SA in males especially given the effectiveness of some other monoamine reuptake inhibitors in male rats as discussed below. Further research is needed to determine possible sex differences in response to amitifadine with regard to nicotine SA

The effects of several monoamine reuptake inhibitors on nicotine SA have been investigated previously. The norepinephrine reuptake inhibitor reboxetine dose-dependently acutely and with repeated administration markedly reduced nicotine SA in rats and had a much smaller effect on sucrose-maintained responding (Rauhut et al., 2002). However, the effect of reboxetine is confounded by potential direct interactions with nicotinic receptors (Rauhut et al., 2003). The selective norepinephrine reuptake inhibitor nisoxetine also reduced nicotine SA in rats (Coen et al., 2009). The NE/DA reuptake inhibitor bupropion blocks nicotine SA only at high doses (Paterson et al., 2008; Rauhut et al., 2003; Shoaib et al., 2003). Thus, the monoamine neuronal systems involved in the blocking of nicotine SA is complex, but may involve norepinephrine neurons.

Dopaminergic systems have long been known to play a central role in the reinforcing effects of drugs, particularly the dopaminergic projection from the ventral tegmental area to the nucleus accumbens (Di Chiara et al., 2004). Noradrenergic and serotonergic systems have also been shown to play important roles in tobacco addiction (Benowitz and Peng, 2000; Dudas and George, 2005; Frishman, 2007; Levin et al., 2011a). A drug, which affects all three of these transmitters, may be effective in aiding smoking cessation. We have also seen in our previous studies with female rats that drugs affecting serotonin, histamine, glutamate, and nicotinic acetylcholine receptors effectively reduce nicotine SA (Levin et al., 2011a; Levin et al., 2010; Levin et al., 2008; Levin et al., 2011b; Levin et al., 2011c). The current studies add monoaminergic reuptake inhibition as an additional mechanism by which nicotine SA can be reduced. This variety of potential treatments could be useful for treating the diverse population of smokers who need assistance in smoking cessation.

Amitifadine is being developed as a treatment for depression (Tran et al., 2012) and the effects on mood may be beneficial for some of the effects of nicotine withdrawal including depression, anhedonia, anxiety, and somatic symptoms (Stoker et al., 2008). Given that negative affect is present both in depressed individuals, who smoke at higher levels than the general population, (Breslau, 1995) and non-depressed nicotine-abstinent individuals (Levin et al., 1994), there may be a double benefit of using an antidepressant drug to help reduce the reinforcing qualities of nicotine.

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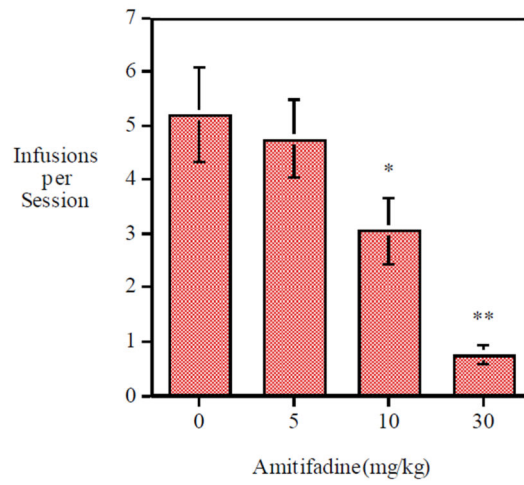
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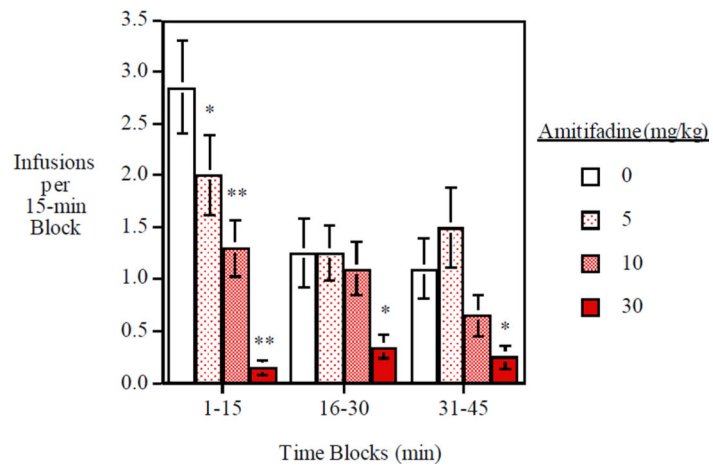
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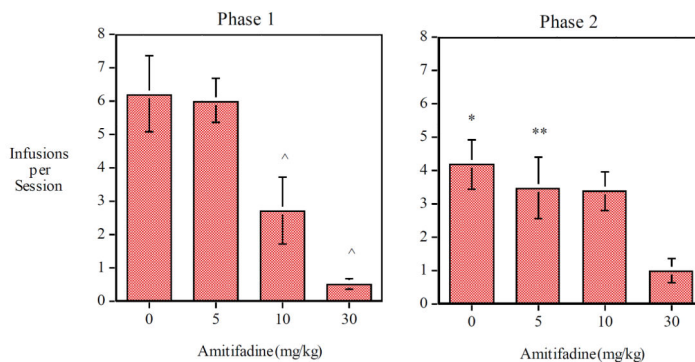
A.



B.



C.

**Fig. 1.**

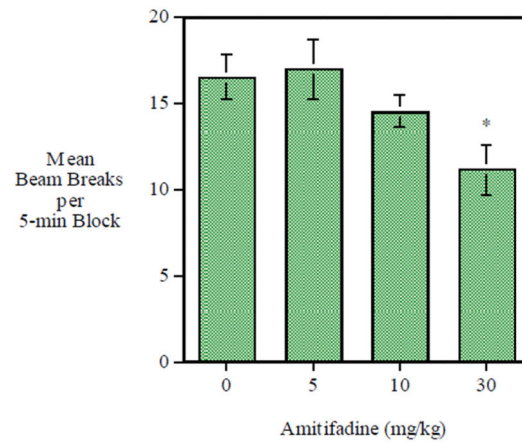
A. Amitifadine effects on nicotine self-administration averaged over the first and second test phases (mean±S.E.M.). Main effect of amitifadine  $P<0.0005$ , Dunnett's comparisons vs. control,  $*P<0.05$ ,  $**P<0.01$ ,  $N=10$ .

B. Amitifadine on nicotine self-administration per 15-min time block of the 45-min session (mean±S.E.M.) Amitifadine × block interaction,  $P<0.005$ , Dunnett's comparisons vs. control showed that the 30 mg/kg dose of amitifadine significantly ( $*P<0.05$ ,  $**P<0.01$ ) reduced nicotine self-administration during each time block. The 5 mg/kg ( $*P<0.025$ ) and 10 mg/kg ( $*P<0.05$ ,  $**P<0.01$ ) doses significantly reduced nicotine self-administration during the first 15-min time block, but not thereafter.  $N=10$ .

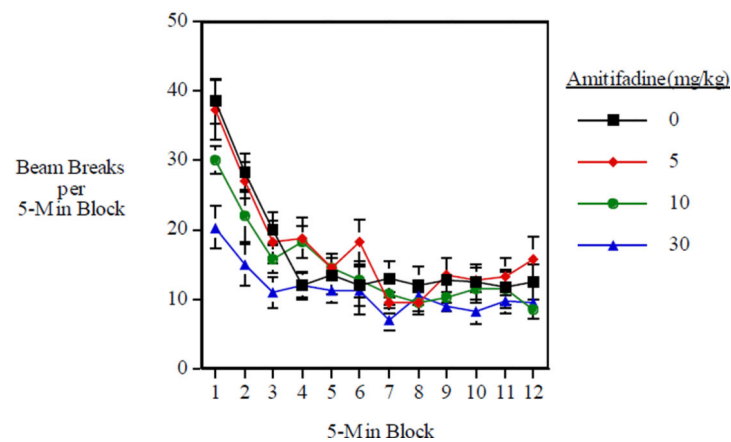
C. Amitifadine effects on nicotine self-administration in test phases 1 and 2 (mean±S.E.M.). The interaction of amitifadine × test phase was significant  $P<0.01$ . During Phase 2 there was significantly less nicotine-self-administration with 0 ( $*P<0.05$ ) and 5 mg/kg ( $*P<0.01$ ) than during Phase 1. Dunnett's comparisons showed that both 10 and 30 mg/kg amitifadine showed significant ( $^{\wedge}P<0.01$ ) decreases in nicotine relative to control during the first phase.  $N=10$ .



A.



B.

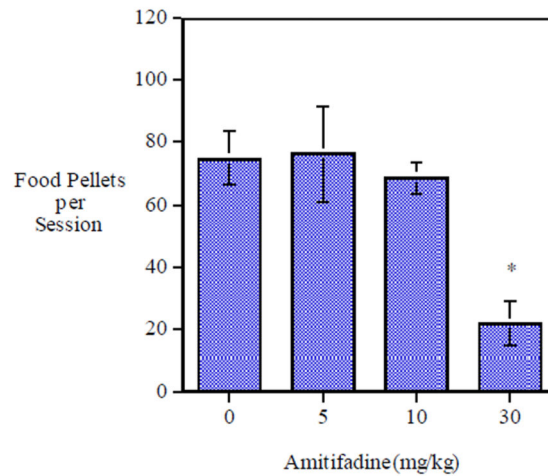
**Fig. 2.**

A. Amitifadine effects on locomotor activity in the figure-8 maze averaged over the one-hour test session (mean±S.E.M.). Dunnett's comparisons showed that the 30 mg/kg dose of amitifadine significantly (\* $P < 0.005$ ) reduced locomotor activity but the lower doses did not.  $N = 10$ .

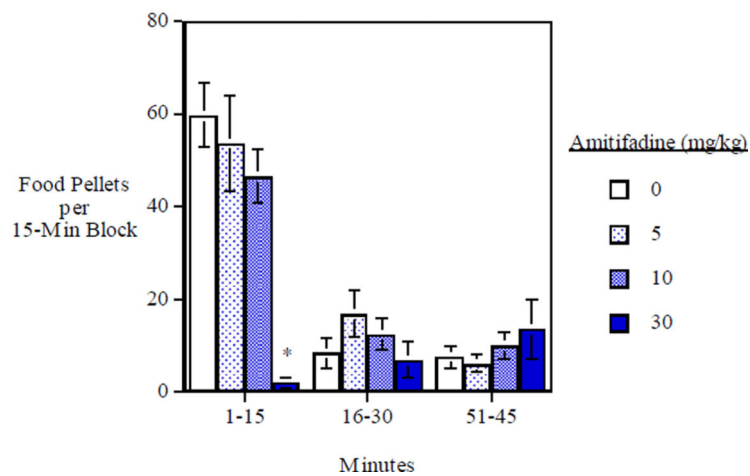
B. Amitifadine effects on locomotor activity in the figure-8 maze during each five-min block of the one-hour test session (mean±S.E.M.). There was a significant main effect of block ( $F(11,297) = 28.22$ ,  $P < 0.0005$ ). There was also a significant interaction of amitifadine × time

block ( $F(33,297)=1.84$ ,  $P<0.005$ ). Tests of the simple main effects comparing each dose to control at each block showed that 30 mg/kg decreased activity at blocks 1 ( $P<0.0005$ ), 2 ( $P<0.0005$ ), 3 ( $P<0.005$ ) and 7 ( $P<0.05$ ). The 10 mg/kg dose decreased activity at block 1 ( $P<0.005$ ) and block 2 ( $P<0.05$ ) and increased activity at block 4 ( $P<0.05$ ). The 5 mg/kg dose increased activity at block 4 ( $P<0.05$ ) and block 6 ( $P<0.05$ ).  $N=10$ .

A.



B.

**Fig. 3.**

A. Amitifadine effects on food-motivated responding averaged over the 45-min session. Amitifadine had a significant ( $P < 0.001$ ) main effect reducing food-motivated responding (mean  $\pm$  S.E.M.). Dunnett's comparisons showed that the 30 mg/kg dose of amitifadine significantly ( $*P < 0.01$ ) reduced food-motivated responding but the lower doses did not.  $N = 9$ .

B. Amitifadine effects on food-motivated responding during each 45-min time block. Amitifadine had a significant ( $P < 0.0005$ ) interaction with time reducing food-motivated

responding (mean±S.E.M.). Dunnett's comparisons showed that the 30 mg/kg dose of amitifadine significantly (\*P<0.01) reduced food-motivated responding during the first 15-min block but the lower doses did not. No amitifadine effects on food-motivated responding were seen later in the session. N=9.

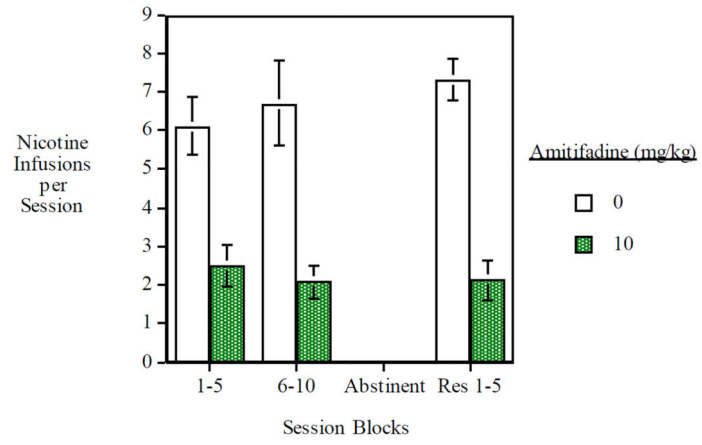
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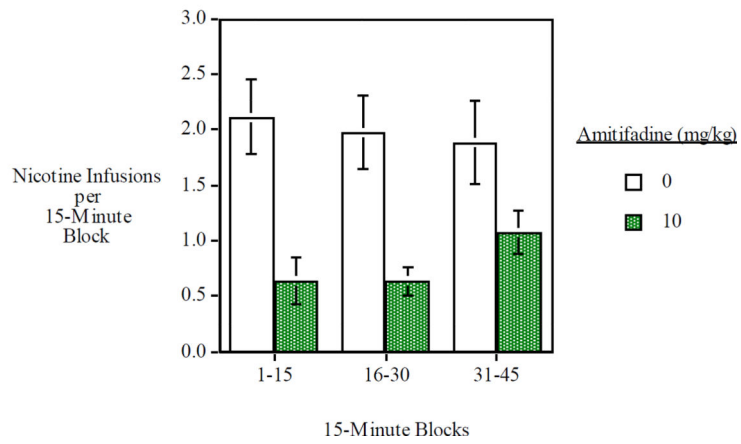
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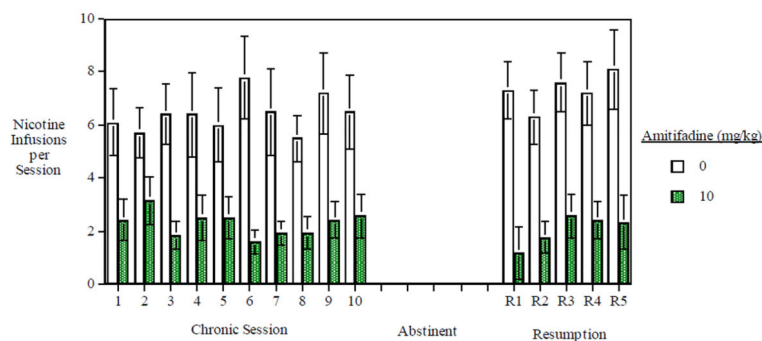
A,



B,



C.

**Fig. 4.**

A. Chronic amitifadine effects on nicotine self-administration over weeks. Chronic amitifadine had a significant ( $P < 0.0005$ ) main effect reducing nicotine self-administration (mean $\pm$ S.E.M.). There was no apparent diminution of effect from the first week of dosing to the second or to the week of resumed after a 9-day period of enforced abstinence. Control N=10, Amitifadine N=12.

B. Chronic amitifadine effects on nicotine self-administration over 15 min blocks. In the chronic study, amitifadine had continued efficacy over the three 15-min. blocks during the test session (mean $\pm$ S.E.M.) averaged over all sessions Amitifadine main effect ( $P < 0.0005$ ). Control N=10, Amitifadine N=12.

C. Chronic amitifadine effects on nicotine self-administration, session-by-session results. Amitifadine had continued efficacy (mean $\pm$ S.E.M.) over the 10 sessions of chronic treatment and 5 session of resumed access after enforced abstinence, amitifadine main effect ( $P < 0.0005$ ). Control N=10, Amitifadine N=12.



**Table 1**

Nicotinic receptor	Concentration of amitifadine, nM		
	100	1000	10000
	% Inhibition of binding		
$\alpha 4\beta 2$	17	14	13
$\alpha 7$	13	6	-9

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