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Nicotine-like discriminative stimulus effects of acetylcholinesterase inhibitors and a muscarinic receptor agonist in rhesus monkeys

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Abstract

Acetylcholinesterase (AChE) inhibitors and positive allosteric nicotinic acetylcholine receptor (nAChR) modulators are potential pharmacotherapies for nicotine dependence. Because some smoking cessation aids (e.g., varenicline) appear to work by mimicking the effects of nicotine, we used drug discrimination to examine whether AChE inhibitors and nAChR allosteric modulators mimic the effects of nicotine. Rhesus monkeys discriminated 1.78 mg/kg of nicotine s.c. under an FR5 schedule of stimulus-shock termination. Nicotine and the AChE inhibitors donepezil and galantamine dose-dependently increased responding on the nicotine-appropriate lever with ED₅₀ values of 0.35, 0.22, and 0.77 mg/kg, respectively. Donepezil (0.56 mg/kg) produced nicotine-like effects for at least 6 h, whereas the duration of action of galantamine (1.78 mg/kg) was less than 3 h. The positive allosteric nAChR modulator PNU-120596 (up to 10 mg/kg) and midazolam (up to 1.0 mg/kg) produced no more than 22% nicotine-lever responding. Oxotremorine, a muscarinic acetylcholine receptor agonist that was used to explore the extent to which muscarinic receptor agonism might contribute to the effects of AChE inhibitors, produced 94% nicotine-lever responding (ED₅₀ value 0.013 mg/kg). The muscarinic antagonist atropine significantly antagonized the effects of both oxotremorine and nicotine; however, the dose of atropine antagonizing oxotremorine was smaller than the dose required to antagonize nicotine. Collectively, these results suggest that AChE inhibitors can mimic the effects of nicotine by indirectly stimulating both nicotinic and muscarinic receptors. Inasmuch as some smoking cessation aids work by exerting nicotine-like effects, the current results are consistent with the potential use of AChE inhibitors as novel smoking cessation aids.

Introduction

Despite a decline in tobacco use over the last 50 years, it is still estimated that one in every five deaths in the United States each year can be attributed to cigarette smoking [1]. National Health Interview Survey data for 2010 reported that 68.8% of current smokers wanted to quit and 52.4% had made one or more attempts to do so in the past year; however, only 6.2%

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Disclosure statement

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were successful. Three pharmacotherapies for smoking cessation are currently approved by the U.S. Food and Drug Administration (FDA): nicotine replacement therapy, bupropion (Zyban®), and varenicline (Chantix®). However, despite increasing numbers of people utilizing pharmacological interventions, success rates remain inadequate. Thus, there is a need to develop new pharmacotherapies for smoking cessation, both to improve public health and to diminish the economic burden of cigarette smoking. However, a considerable amount of time and money are invested in a novel compound before it ever reaches the market. One way to circumvent this process is to consider repurposing drugs that have already been approved by the FDA for other indications.

Galantamine is one of the most widely prescribed drugs for Alzheimer's disease, but it is also being examined for utility as a smoking cessation pharmacotherapy. Galantamine is an acetylcholinesterase (AChE) inhibitor, and there is also evidence to suggest that galantamine is a positive allosteric modulator of nicotinic acetylcholine (ACh) receptors (nAChRs) [2, 3]. However, evidence that galantamine is an allosteric nAChR modulator is not unanimous [4]. Galantamine has been shown to attenuate nicotine self-administration in rats [5, 6], and to reduce cigarette smoking among alcohol-dependent patients [7]. It is unclear which mechanism of galantamine, AChE inhibition or allosteric modulation, might be responsible for producing this effect. However, if increasing ACh tone can produce nicotine-like effects, then one might predict other AChE inhibitors to produce similar results when applied to smoking cessation. Donepezil, another widely prescribed pharmacotherapy for Alzheimer's disease, differs from galantamine in that it is an AChE inhibitor that appears to lack activity as an allosteric modulator of nAChRs [8, 9]. Furthermore, another compound, PNU-120596, although not currently approved by the FDA had previously been shown to have cognitive-enhancing effects in monkeys [10]. As opposed to donepezil, the only known mechanism of PNU-120596 is as a positive allosteric modulator that is selective for $\alpha 7$ nAChRs [11]. The $\alpha 7$ nAChR subtype has been targeted for the development of analgesics [12], cognitive enhancing drugs [13] and smoking cessation aids [14].

Drug discrimination is a behavioral assay that is used to examine the extent to which a test compound has qualitatively similar effects with a training drug such as nicotine [15]. Animals are trained to make a "correct" choice depending on the presence versus the absence of a selected nicotine training dose. During training, animals are presented with a choice between two alternative and mutually exclusive responses (e.g., pressing a left lever versus a right lever). The correct response results in delivery of a reinforcer such as food or avoidance of shock delivery. The correct response is determined by administration of nicotine or saline by the experimenter non-contingently beforehand, i.e., independent of any response made by the animal. After administration of the training dose of nicotine, the animal can only obtain reinforcers by responding on one of the two levers (e.g., right lever) and the same lever is always paired with the nicotine training dose. The other lever (e.g., left lever) is always the correct lever following saline administration. Incorrect responses do not result in delivery of the reinforcer. Training proceeds until animals reliably make the correct choice, as evidenced by responding on the drug-associated lever after drug administration and the vehicle-lever after vehicle administration. Once the discrimination is successfully learned according to pre-determined performance criteria, the animals are tested. Tests are the same as training sessions except that any dose of any drug can be administered.

Allocation of responses among the nicotine- versus saline-paired levers during tests provides information on the extent to which the test compound is similar qualitatively to nicotine, which also provides insight into how similar the CNS mechanism(s) of action of the test compound and training drug are. Drugs that substitute for nicotine could be useful substitution therapies (i.e., varenicline; [16]).

The goal of the current study was to examine the extent to which galantamine shares discriminative stimulus effects with nicotine. To further examine the potential contribution of AChE inhibition versus positive nAChR allosteric modulation to the effects of galantamine, donepezil and PNU-120596 were also tested. Because the initial studies reported here demonstrated that both galantamine and donepezil substituted for the discriminative stimulus effects of nicotine (1.78 mg/kg, s.c.), it was assumed that increases in ACh tone could involve actions at nicotinic and muscarinic ACh receptors (mAChRs). In order to examine the contribution that mAChRs might have in producing nicotine-like discriminative stimulus effects, further tests were conducted with the mAChR agonist oxotremorine [17] and the mAChR antagonist atropine [18].

Materials and Methods

Subjects

Two male and three female adult rhesus monkeys (*Macaca mulatta*) discriminated 1.78 mg/kg nicotine (s.c.) from saline. These monkeys had previously been trained to discriminate nicotine and had received both nicotinic and non-nicotinic drugs as described [16, 19]. Monkeys weighed 9.0 (range: 6.7–12.5) kg and were fed primate chow (Harlan Teklad High Protein Monkey Diet; Madison, WI), fresh fruit and peanuts daily. Each monkey was housed individually in a stainless steel cage in a room with controlled temperature and humidity on a 14/10-h light-dark cycle. Water was available continuously in the home cages. The maintenance and experimental use of animals was carried out in accordance with the 2011 Guide for Care and Use of Laboratory Animals, and all experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas Health Science Center at San Antonio.

Apparatus

When outside of the home cages, monkeys were seated in commercially available chairs (Model R001; Primate Products, Miami, FL). Feet were placed into shoes mounted on the front of the chair. Shoes were fitted with brass electrodes to which a brief electric shock (3 mA, 250 ms) could be delivered from an a/c generator (Coulbourn Instruments, Allentown, PA). Training and testing were conducted in sound-attenuating, ventilated operant chambers containing an operant panel below which was mounted a food receptacle. The operant panel consisted of a row of three lights and a second row of two levers; levers were mounted beneath the leftmost light and the rightmost light. All operant behavior was controlled and recorded by a computer connected to a commercially available interface and Med-PC software (MedAssociates, Inc., St. Albans, VT).

Discrimination training

Monkeys were trained once per day at 8:00 am, 7 days per week, to discriminate 1.78 mg/kg nicotine from saline (s.c.). Two monkeys (one male and one female) were chosen at random for which the left lever was set as the nicotine lever; in the other three monkeys (one male and two females), the right lever was set as the nicotine-appropriate lever. Two training conditions were possible: when monkeys received an injection of the training dose of nicotine, only responses on the nicotine-appropriate lever (i.e., correct lever) were reinforced; when monkeys received an injection of saline, only responses on the saline-appropriate lever (i.e., correct lever) were reinforced. The method of reinforcement was stimulus-shock termination (SST) under a fixed ratio 5 (FR5) schedule. Following injection was a 10-min experimental interval spent in the operant chamber during which no lights were illuminated and pressing the levers had no scheduled consequences. Following the experimental interval, red lights were illuminated above each lever and an electric shock was scheduled to occur every 10 s. Completion of the FR on the correct lever turned off the lights and postponed the shock schedule for 30 s. Responses on the incorrect lever reset the FR requirement. During the 30-s timeout period, lever pressing had no scheduled consequence. After the timeout period, the red lights were re-illuminated, the levers became active, and the shock schedule resumed (i.e., a shock scheduled to occur every 10 s). The duration of SST responding was 10 min, and sessions consisted of one or two consecutive cycles. Each cycle lasted 20 min, including a 10-min pretreatment followed by 10 min of responding under the FR5 schedule of SST. There were three training conditions: saline administered in the first cycle followed by saline in a second cycle, saline administered in the first cycle followed by nicotine in a second cycle, or nicotine administered at the beginning of one cycle. Only responses on the correct lever for a given cycle were reinforced for the duration of that cycle. Nicotine and saline training alternated daily or every other day; a training condition (i.e. nicotine or saline) was not repeated for more than two days consecutively. Monkeys had previously satisfied the criteria for testing, defined as five consecutive training sessions in which greater than 80% of the total session responses were made on the correct lever and less than five responses were made on the incorrect lever prior to delivery of the first reinforcer for three consecutive days.

Discrimination testing

Test days were scheduled so that approximately half followed a training day on which the monkey received nicotine and half followed a training day on which the monkey received only saline. Completion of the FR5 on either lever was reinforced. During inter-test sessions, monkeys were required to meet the discrimination training criteria (i.e., greater than 80% responses made on the correct lever and fewer than five responses made on the incorrect lever prior to delivery of the first reinforcer) for three consecutive days. The inter-test sessions included at least one training day on which the monkey received nicotine and one training day on which the monkey received only saline.

To examine the effects of a dose of a test drug over time, test sessions included up to six consecutive cycles. If this was insufficient to capture the duration of action of the test compound, additional tests were scheduled for which the drug was given as a pretreatment;

the duration of the pretreatment was timed such that the time point previously obtained in the sixth cycle corresponded to the same time in the first cycle on the second test day.

Dose-response functions for nicotine, donepezil, galantamine and oxotremorine were determined by administering a dose at the beginning of each cycle. The dose-response function for midazolam was determined by administering a dose at the beginning of the first cycle. For antagonism tests, atropine was administered as a 30-min pretreatment; in other words, the dose of antagonist was administered at the beginning of the first cycle followed by a dose of nicotine, donepezil, galantamine or oxotremorine at the beginning of the second cycle. Dose-response functions included ineffective doses, i.e., a dose producing less than 20% of the maximum effect, up to a dose producing greater than 80% of the maximum effect; the dose-response functions for nicotine included doses up to and including the training dose.

Drugs

Nicotine hydrogen tartrate salt, oxotremorine and atropine (Sigma-Aldrich, St. Louis, MO), as well as midazolam (Ben Venue Labs, Bedford, OH), were dissolved in physiological saline. Donepezil and galantamine (Sigma) were dissolved in a vehicle containing 2-parts emulphor (Alkamuls EL-620, Solvay, Princeton, NJ), 1-part ethanol and 7-parts saline. PNU-120596 (National Institute on Drug Abuse, Rockville, MD) was dissolved in a vehicle containing 1-part emulphor (Alkamuls EL-620), 1-part ethanol and 18-parts saline. Drugs were studied from ineffective doses up to doses that produced greater than 80% responding on the drug-associated lever or that were judged to be the largest safe dose based on known side effects (e.g., vomiting, seizure). The order of drug dosing was non-systematic when possible; it was systematic when the smallest or largest behaviorally active doses were unknown and doses were incremented by $\frac{1}{4}$ log units until the lowest behaviorally active dose or the maximum safe dose was reached. Donepezil and galantamine were administered intramuscularly in a volume of 0.1–1 ml/kg vehicle into the thigh muscle. All other drugs were administered subcutaneously in the mid-scapular region of the back in a volume of 0.1–1 ml/kg saline or 1:1:18 vehicle. Drug doses are expressed as the weight, in mg/kg, of the forms listed, except for nicotine, which is expressed as the weight of the base, in mg/kg.

Data analyses

Two primary measures were recorded for each subject: 1) the total number of responses made on the drug lever, divided by the total number of responses made on both levers, excluding responses during timeouts (discrimination data) and 2) the average rate of responding, excluding responses during timeouts, in responses/s (response rate). For each subject, the mean of the most recent five saline training sessions for which the subject met the training criteria were defined as the control response rate. Test data for response rate were then calculated as a percentage of their control response rate. These two measures were averaged across subjects ($n=4-5$ per dose) and expressed as a mean $\pm$ 1 standard error of the mean (S.E.M). These two measures were plotted as a function of dose or a function of time using GraphPad Prism (v. 5.0; GraphPad Scientific, San Diego, CA, USA). ED_{50} values were calculated using the linear regression of data from each subject in pooled analysis. The ED_{50} values were used to calculate potency ratios; ED_{50} values were considered

significantly different from one another when the 95% confidence limits of their potency ratio did not include 1. Effects of a drug on response rate as a function of dose were examined using one-way repeated measures analysis of variance (ANOVA) separately for each drug, followed by Dunnett's test to examine significant differences from the vehicle control ($p<0.05$). One-way ANOVA and Dunnett's test were also used to examine the effects of a dose as a function of time.

Results

The effects of nicotine, oxotremorine, donepezil and galantamine in monkeys discriminating 1.78 mg/kg nicotine: dose-response determinations

Nicotine dose-dependently increased nicotine-lever responding without significantly modifying response rate. Saline produced 3% responding on the nicotine lever (Fig. 1, VEH), whereas nicotine increased nicotine-lever responding to 100% at both 1 mg/kg as well as the training dose (1.78 mg/kg) (Fig. 1 top, circles). The ED₅₀ value (95% confidence limits) of nicotine to produce discriminative stimulus effects was 0.35 (0.17–0.74) mg/kg. Oxotremorine, donepezil and galantamine also dose-dependently increased responding on the nicotine-associated lever (Fig. 1 top). Oxotremorine produced a maximum of 94% responding on the nicotine lever at a dose of 0.032 mg/kg; the ED₅₀ value (95% confidence limits) of oxotremorine was 0.013 (0.0069–0.025) mg/kg. Donepezil produced a maximum of 89% responding on the nicotine-associated lever at a dose of 0.56 mg/kg; the ED₅₀ value of donepezil was 0.22 (0.14–0.34) mg/kg. Galantamine produced a maximum of 98% responding on the nicotine lever at a dose of 1.78 mg/kg; the ED₅₀ value of galantamine was 0.77 (0.38–1.6) mg/kg. The slopes of the dose-response functions for nicotine, oxotremorine, donepezil and galantamine were not significantly different from each other ($F_{3,43}=0.052$, $p=0.98$). Oxotremorine was 27-fold more potent than nicotine. The potencies of nicotine, donepezil and galantamine were not significantly different from one another. Oxotremorine, donepezil and galantamine did not significantly modify response rate at any of the doses tested (Fig. 1 bottom).

The effects of donepezil and galantamine: time courses

The nicotine-like discriminative stimulus effects of donepezil varied significantly as a function of time after administration ($F_{6,24}=15.9$, $p<0.01$). The onset to the maximum effect of 0.56 mg/kg donepezil was less than 10 min and the duration of the effect was greater than 6 h, but less than 24 h (Fig. 2 top left). The nicotine-like discriminative stimulus effects of galantamine also varied significantly as a function of time after administration ($F_{8,36}=6.14$, $p<0.0001$). The onset to the maximum effect of 1.78 mg/kg galantamine was less than 10 min and the duration of the effect was shorter, i.e., 2 h (Fig. 2 top right), as compared with the duration of the nicotine-like discriminative stimulus effects of 0.56 mg/kg donepezil. Neither donepezil nor galantamine significantly modified response rate as a function of time.

The effects of PNU-120596 and midazolam

PNU-120596 and midazolam resulted in predominantly saline-lever responding at all of the doses tested. PNU-120596 produced 0% and 21% responding on the nicotine lever at doses of 3.2 and 10 mg/kg, respectively (Fig. 3 top, squares). At 10 mg/kg, one of the five

monkeys responded 100% on the nicotine-appropriate lever, whereas the other four monkeys responded 5%, 0%, 0% and 0% on the nicotine-appropriate lever. Response rate was 101% of control at 3.2 mg/kg PNU-120596 and 107% of control at 10 mg/kg PNU-120596; these rates were not significantly different from the response rate of a saline injection ($F_{2,8}=0.55$, $p=0.51$). Midazolam, tested at doses of 0.32, 1.0 and 3.2 mg/kg, produced 0%, 1% and 0% nicotine-lever responding, respectively (Fig. 3 top, circles). At the same doses, midazolam significantly decreased response rate to 79%, 49% and 30% of control ($F_{3,9}=22.3$, $p<0.01$); the ED_{50} value (95% confidence limits) of midazolam to decrease response rate was 0.36 (0.18–0.81) mg/kg.

Atropine in combination with oxotremorine and nicotine

Atropine alone at doses of 0.1 and 0.32 mg/kg produced 0% and 1% responding on the nicotine lever, respectively, and did not significantly modify response rate (Fig. 4, VEH). When the dose-response function of oxotremorine was re-determined in the presence of 0.1 mg/kg atropine, there was a rightward shift (Fig. 4 top left). The ED_{50} value (95% confidence limits) of oxotremorine was significantly increased to 0.079 (0.029–0.21) mg/kg, as evidenced by a dose ratio (95% confidence limits) of 6.1 (1.8–20). In contrast, 0.1 mg/kg atropine did not significantly modify nicotine-appropriate responding produced by 0.56 mg/kg nicotine in any of the three monkeys tested with this combination ($t_2=1.49$, $p=0.28$). A larger dose of atropine (0.32 mg/kg) was needed to produce significant antagonism of the discriminative stimulus effects of nicotine (Fig. 4 top right); thus, in the presence of 0.32 mg/kg atropine, the nicotine dose-response function was shifted 3.8-fold to the right (95% confidence limits 1.8–8.1). Rate of responding was not significantly modified by atropine alone or in combination with any dose of nicotine or oxotremorine tested ($p>0.05$) (Fig. 4 bottom)

Discussion

Donepezil, galantamine and oxotremorine, but not PNU-120596 or midazolam, were able to substitute for the discriminative stimulus effects of nicotine (1.78 mg/kg, s.c.) in rhesus monkeys. The time course of the discriminative stimulus effects of both donepezil and galantamine were considerably longer (6 and 2 h, respectively) than what has been previously demonstrated for the current training dose of nicotine (1 h; [16]). Atropine antagonized both nicotine and the nicotine-like discriminative stimulus effects of oxotremorine, albeit the antagonism of nicotine required a larger dose of atropine than that of oxotremorine. These results suggest that AChE inhibition can mimic the subjective effects of nicotine through actions that potentially involve stimulation of both nicotinic and muscarinic ACh receptors. Because the training dose of nicotine used in the present study may be relatively large, it is noteworthy that donepezil, galantamine and oxotremorine were able to substitute for nicotine. Substitution of these same test compounds for nicotine would also be expected for smaller training doses of nicotine, as smaller training doses would be expected to be relatively more selective for nAChRs. However, this assumes the training dose of nicotine must be sufficiently large to generate a robust enough stimulus to create a pharmacologically selective discrimination, as training doses that are too small can result in discriminations that lack pharmacological selectivity, i.e., drugs with pharmacological

mechanisms different from the training drug, when the training dose is too small, can substitute for the training drug.

PNU-120596, up to 10 mg/kg, produced 100% nicotine-lever responding in one monkey and predominantly saline-lever responding in four other monkeys. Limitations of drug solubility prevented us from testing larger doses of PNU-120596, so it is unclear whether or not larger doses of this compound would have increased nicotine-appropriate responding further or modified response rate. In a separate study conducted in mice discriminating 1 mg/kg nicotine (s.c.), 32 mg/kg PNU-120596 decreased responding maintained by food delivery [20]. In the same study, PNU-120596 failed to substitute for the discriminative stimulus effects of nicotine or to modify the effects of nicotine. The failure of PNU-120596 to modify response rate in the current study could reflect the relative insensitivity of the current reinforcer (i.e., stimulus-shock termination) to the disruptive effects of drugs as compared with food delivery, or to studies of insufficiently large doses (i.e., greater than 10 mg/kg). While it is not clear whether or not PNU-120596 was administered in sufficiently large doses based on the current results, previous studies from another group demonstrated that 3 mg/kg of PNU-120596 administered p.o. was sufficient to modify performance in a delayed-match-to-sample task in rhesus monkeys [10]. Though drug sensitivity may depend on experimental procedures employed, it appears that 10 mg/kg in the present study is a behaviorally active dose. Thus, the lack of the nicotine-like discriminative stimulus effect of PNU-120596 in the present study suggest that allosteric modulation of nAChRs *per se* is insufficient to produce a the nicotine-like discriminative stimulus effect.

The nicotine-like discriminative stimulus effects of donepezil and galantamine, but not PNU-120596, suggest that AChE inhibition is sufficient to produce nicotine-like effects and is potentially the mechanism by which galantamine shares effects with nicotine. The current results differ from past discrimination experiments with nicotine as a training drug. For example, a prototypical AChE inhibitor physostigmine did not substitute for nicotine in rats [21]. However, there is evidence that at least some AChE inhibitors may partially generalize to the nicotine discriminative stimulus [22]. When physostigmine was trained as a discrimination stimulus, nicotine did not substitute for physostigmine and mecamylamine did not antagonize the discriminative stimulus effects of physostigmine [23]. However, oxotremorine and arecoline, both mAChR agonists, did generalize to the physostigmine discriminative stimulus [24]. AChE inhibitors can have actions at both nAChRs and mAChRs through increased endogenous ACh. This raised the possibility of a muscarinic component to the effects of some of the test drugs reported here. In fact, in the present study, the mAChR agonist oxotremorine fully substituted for the nicotine discriminative stimulus, implicating the involvement of mAChRs. Moreover, atropine antagonized the nicotine-like effects of oxotremorine as well as the discriminative stimulus effects of nicotine. Antagonism of the rate-decreasing effects of nicotine by atropine in rats has been reported previously [25]. Furthermore, atropine methylnitrate, a peripherally restricted mAChR antagonist, was not able to antagonize these effects, providing evidence for some effect of nicotine at a central muscarinic site. The dose of atropine needed to antagonize the nicotine-like discriminative stimulus effects of oxotremorine was smaller than that required to antagonize the discriminative stimulus effects of nicotine. Thus, while oxotremorine and nicotine have overlapping discriminative stimulus effects involving, at least to some extent

for nicotine, actions at mAChR, differential antagonism by atropine (at least quantitatively) is consistent with different underlying receptor mechanisms, perhaps reflecting the involvement of different subtypes of nAChRs in the discriminative stimulus effects of nicotine and oxotremorine. Substitution of oxotremorine for the discriminative stimulus effects of nicotine might question the validity of the current assay as a pre-clinical model to identify smoking cessation aids. Alternatively, these data could suggest that mAChRs are a potential target for the development of smoking cessation aids.

Self-administration and drug discrimination are complimentary approaches for examining abuse liability and underlying pharmacological mechanisms. Known drugs of abuse can serve as training drugs, and then tests can be conducted with novel drugs. Overlapping discriminative stimulus effects suggests that there is the potential for overlapping abuse liability, and for the test substance to serve as a substitution therapy for abuse and/or dependence that develops to the abused drug that has been established as a training drug. Generating data in both self-administration and drug discrimination procedures is ideal and provides a more comprehensive pre-clinical profile than data collected with either procedure alone (e.g., [26]). In summary, galantamine likely produces nicotine-like discriminative stimulus effects in the current assay by increasing endogenous ACh tone via AChE inhibition. Furthermore, substitution with oxotremorine and antagonism with atropine are consistent with involvement of mAChRs, indicating that this increase in endogenous ACh neurotransmission may be happening, at least in part, through a muscarinic mechanism, which is consistent with the literature suggesting that muscarinic agonists, but not nicotinic agonists, are able to generalize to the physostigmine discriminative stimulus [23]. As multiple drugs which have as their primary mechanism of action AChE inhibition have been approved by the FDA, this suggests that these drugs could be repurposed for use in smoking cessation.

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Figure 1. Effects of nicotine (circles), oxotremorine (squares), donepezil (triangles) and galantamine (diamonds) in monkeys trained to discriminate 1.78 mg/kg nicotine (s.c.). Abscissae: Vehicle (VEH) or dose of oxotremorine, donepezil or galantamine in weight per kilogram body weight (mg/kg). Nicotine is expressed as free base weight per kilogram body weight (mg/kg). Ordinates: mean ($\pm$ S.E.M.) percentage of responding on the nicotine lever (top) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (bottom). All drugs were administered immediately prior to each session.

Figure 2. Discriminative stimulus effects of 0.56 mg/kg donepezil (left) or 1.78 mg/kg galantamine (right) over time in monkeys trained to discriminate 1.78 mg/kg nicotine (s.c.). Abscissae: time in hours. Ordinates: mean ($\pm$ S.E.M.) percentage of responding on the nicotine lever (top) or mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (bottom).

Figure 3. Effects midazolam (circles) and PNU-120596 (squares) in monkeys trained to discriminate 1.78 mg/kg nicotine (s.c.). Abscissae: dose of midazolam or PNU-120596 in weight per kilogram body weight (mg/kg). Ordinates: mean ($\pm$ S.E.M.) percentage of responding on the nicotine lever (top) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (bottom). Both drugs were administered immediately prior to each session.

Figure 4.

Effects of oxotremorine alone (open squares) and in combination with 0.1 mg/kg atropine (closed squares) (left) or nicotine alone (open circles) and in combination with 0.1 (closed squares) and 0.32 (closed circles) mg/kg atropine (right) in monkeys discriminating 1.78 mg/kg nicotine (s.c.). Abscissae: Vehicle (Veh) or dose of oxotremorine (left) or nicotine (right) in mg/kg body weight administered s.c. Nicotine doses are expressed as the weight of the free base. Ordinates: mean ($\pm$ S.E.M.) percentage of responding on the nicotine lever (top) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (bottom).