Published in final edited form as:

Neuropharmacology. 2013 October ; 73: 75–86. doi:10.1016/j.neuropharm.2013.05.016.

Neuronal Nicotinic Receptor Agonists Improve Gait and Balance in Olivocerebellar Ataxia

L. Wecker^{1,2,3}, M.E. Engberg¹, R.M. Philpot¹, C.S. Lambert², C.W. Kang⁴, J.C. Antilla⁴, P.C. Bickford^{2,5,6}, C. E. Hudson⁶, T. A. Zesiewicz³, and Peter P. Rowell⁷

¹Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Neurosciences, University of South Florida

²Department of Molecular Pharmacology and Physiology, University of South Florida

³Department of Neurology, University of South Florida

⁴Department of Chemistry, University of South Florida

⁵Department of Neurosurgery, University of South Florida

⁶James A. Haley Veterans Hospital, Tampa, FL

⁷Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY

Abstract

Clinical evidence indicates that the nicotinic receptor agonist varenicline improves axial symptoms in patients with spinocerebellar ataxia type 3, but pharmacological evidence in an animal model of olivocerebellar degeneration has not been demonstrated. This study investigated whether varenicline and nicotine were efficacious for attenuating ataxia in rats induced by chemical destruction of the olivocerebellar pathway. Rats were trained to maintain their balance on a rotating rod and walk across a stationary beam; rod and beam performance, locomotor activity, and gait were assessed prior to and after administration of the neurotoxin 3-acetylpyridine (3-AP). The administration of 3-AP led to an 85% loss of neurons in the inferior olive at one week after administration without evidence of recovery over the following 4 weeks. The lesion was accompanied by a 72% decrease in rotorod activity, a 3.1-fold increase in the time required to traverse a stationary beam, a 19% decrease in velocity and 31% decrease in distance moved in the open field, and alterations in both forepaw and hindpaw gait parameters, with a 19% increase in hindpaw stride width. The daily administration of nicotine (0.33 mg free base/kg) improved rotorod performance by 50%, an effect apparent following the first week of administration, and which did not improve further over time. Nicotine also normalized the increased hindpaw stride width induced by the lesion. The ability of nicotine to alleviate both rotorod and gait deficits induced by 3-AP were prevented by the administration of the nicotinic antagonist mecamylamine (0.8 mg free base/kg) prior to the daily administration of nicotine. The effects of varenicline were dose-related and doses of 1.0 and 3.0 mg free base/kg daily improved rotorod performance by approximately 50% following the first week of administration. Further, varenicline did not alter

© 2013 Elsevier Ltd. All rights reserved.

Corresponding author: Lynn Wecker, Department of Psychiatry and Behavioral, Neurosciences, University of South Florida Morsani College of Medicine, 3515 East, Fletcher Avenue, Tampa, FL 33613-4706; voice: (813) 974-5189; fax: (813) 974-2478; lwecker@health.usf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the time required for animals to traverse the stationary beam, but did improve the ability of rats to maintain their balance on the beam by increasing lateral tail movements following 3 weeks of administration at doses of 0.3 and 1.0 mg free base/kg daily. Further, doses of nicotine and varenicline that improved the impaired balance and gait did not affect any measure of locomotor activity in the open field. Results provide evidence that nicotinic agonists are of benefit for alleviating some of the behavioral deficits in olivocerebellar ataxia and warrant further studies to elucidate the specific mechanism(s) involved.

Keywords

Ataxia; neuronal nicotinic receptors; nicotine; olivocerebellar degeneration; varenicline

1. Introduction

Several recent clinical studies have indicated that varenicline, a partial agonist at neuronal nicotinic $\alpha 4\beta 2$ receptors used for smoking cessation (Rollema et al., 2007a; 2007b), improves symptoms in patients with ataxic neurodegenerative disorders of distinct pathogenic etiologies. Varenicline has been shown to improve: both ataxia and imbalance in a patient with fragile X-associated tremor/ataxia (Zesiewicz et al., 2009a); gait, balance, and depth perception in a patient with spinocerebellar ataxia type 3 (SCA3); balance and coordination in a patient with SCA14 (Zesiewicz and Sullivan, 2008); and proprioception and posture in 2 patients with Friedreich's ataxia (Zesiewicz et al., 2009b). Further, a recent double-blind, placebo-controlled, randomized study has shown that treatment with varenicline for 2 months improved axial symptoms and rapid alternating movements in adult patients with genetically confirmed SCA3 (Zesiewicz et al., 2012).

The idea that nicotinic receptor activation may be of therapeutic benefit for some types of ataxia is supported by several studies conducted during the late 1970s using both the cholinesterase inhibitor physostigmine that crosses the blood brain barrier and increases brain levels of acetylcholine, and choline, a precursor for acetylcholine and direct $\alpha 7$ nicotinic receptor agonist. Studies with physostigmine reported beneficial effects in patients with spinocerebellar degenerations and various inherited ataxias (Kark et al., 1977; Rodriguez-Budelli et al., 1978; Kark et al., 1981), but no benefit in patients with autosomal dominant and idiopathic cerebellar ataxia (Wessel et al., 1997). Similarly, beneficial effects of choline were reported in a patient with late-onset idiopathic cerebellar degeneration (Legg, 1978), in ataxic patients with multiple sclerosis (Blattel, 1979), and in some patients with sporadic cerebellar degeneration and atypical spinocerebellar degeneration (Livingstone and Mastaglia, 1979), but no improvement was noted for patients with cerebellar ataxia (Legg, 1979) or inherited ataxia of late onset (Philcox and Kies, 1979). Unfortunately, optimal doses of either physostigmine or choline were not determined and further studies were not pursued. However, these initial clinical findings support the idea that nicotinic receptor activation may be of benefit for ataxia, depending on the specific disorder manifest.

Preclinical studies have demonstrated that the administration of nicotine for 3 weeks improves motor activity, longevity and the survival of cerebellar Purkinje cells in the mutant Han Wistar rat, a model with an unidentified autosomal recessive gene conferring glutamate toxicity to cerebellar Purkinje cells and exhibiting spastic paresis characterized by ataxia, tremor and hind limb rigidity (Cohen et al., 1991; Hildebrandt et al., 2003). Further, a role for cerebellar nicotinic receptors in ataxia has been supported by animal studies demonstrating that the intracerebellar administration of nicotine and other nicotinic agonists attenuate ethanol-induced ataxia, an effect prevented by nicotinic antagonists (Dar et al.,

1994; Taslim et al., 2011). Thus, the objective of these studies was to determine whether nicotinic agonists were efficacious for improving ataxia in rats following lesions of the olivocerebellar pathway induced by the administration of 3-acetylpyridine (3-AP), which selectively destroys the inferior olivary complex, the source of climbing fiber input to Purkinje cells (Gasbarri et al., 2003). Results indicate that varenicline and nicotine, administered daily beginning 1 week after initiation of the lesion, specifically improve the impaired balance, an effect prevented by pretreatment with the nicotinic receptor antagonist mecamylamine. This study provides pharmacological evidence that in this animal model of human olivocerebellar degeneration, nicotinic receptor activation tempers the expression of cerebellar-mediated balance deficits, supporting the idea that nicotinic receptor agonists may have therapeutic benefit for the treatment of human movement disorders of similar etiology.

2. Materials and methods

2.1. Animals and chemicals

All studies were conducted using male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) with an initial weight of 220–224 g. Upon arrival at the University of South Florida Morsani College of Medicine vivarium, rats were housed 2 per cage and maintained on a 12 hour light/dark cycle with food and water available *ad libitum*; animals were acclimatized to these housing conditions for 7 days prior to experimentation. All experiments were conducted during the light phase, and the care and use of animals was in accordance with guidelines set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to use the least number of animals/experiment that were sufficient for statistical analyses.

Unless otherwise specified, chemicals and other reagents were obtained from Sigma-Aldrich, Co. (St. Louis, MO).

2.2. Production and verification of olivocerebellar lesions

The primary afferent pathway innervating cerebellar Purkinje cells was lesioned by the intraperitoneal (i.p.) administration of 3-AP (70 mg/kg) followed at 3.5 hours by the i.p. administration of nicotinamide (300 mg/kg), a protocol that has been reported to selectively destroy neurons in the inferior olive (Llinas et al., 1975; Sotelo et al., 1975).

The integrity of inferior olivary neurons was assessed by immunohistochemical (IHC) staining for NeuN. Rats were deeply anesthetized with pentobarbital (175–200 mg/kg) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Following perfusion, brains were post-fixed in 20% sucrose, transferred into 30% sucrose and stored at 4°C. The cerebellum and brain stem were embedded in gelatin containing 10% sucrose, incubated until firm, and placed in 30% sucrose. Embedded tissues were sectioned sagittally at 40µm using a Microm cryostat (Richard-Allan Scientific, Kalamazoo, MI) and stored in cryoprotectant at –20°C until processed. Every 6th section of the inferior olive was washed 3x in 0.1 M phosphate-buffered saline (PBS), incubated in 40% methanol and 2% H₂O₂ for 15 minutes, washed and blocked in 10% normal horse serum (NHS, Lampire Biological Laboratories, Pipersville, PA) and 0.3% Triton X-100 for 1 hour. Sections were incubated overnight at 4°C with mouse monoclonal anti-NeuN antibody (clone 60, 1:10,000; EMD Millipore, Billerica, MA) in 0.1 M PBS containing 3% NHS and 0.1% Triton X-100. Sections were washed with 0.1 M PBS containing 3% NHS, incubated for 1 hour in 0.1 M PBS containing 3% NHS, 0.1% Triton X-100 and biotinylated horse anti-mouse IgG antibody (1:300; Vector Laboratories, Inc., Burlingame, CA) and again washed in 0.1 M PBS. Sections were stained using the Vectastain Elite ABC kit (Vector Laboratories, Inc.) and SIGMAFAST™ 3, 3' diaminobenzidine tablets according to manufacturer's instructions. Sections were placed in 0.1 M PBS, stored at 4°C and mounted on slides

(Fisherbrand Superfrost Plus, Thermo Fisher Scientific, Waltham, MA) within 2–3 days. Mounted sections were dried overnight and rehydrated immediately prior to counterstaining with Vector® Hematoxylin QS (Vector Laboratories, Inc.).

The number of NeuN(+) cells in the inferior olive was quantified using Stereo Investigator software (MicroBrightField, Colchester, VT) and a Nikon Eclipse 600 microscope (Nikon Inc., Melville, NY). The optical fractionator method of stereological cell counting was utilized; the entire olive was outlined at 4x magnification and quantified at 40x magnification. The optical disectors were 100 × 100 and the grid size was 900 × 900. These parameters provided a minimum of 200 NeuN(+) cells counted per brain with an error coefficient of 0.07. Representative images depicted in Fig. 1 were captured on a Leica DM2500 microscope using Leica Application Suite v4.0 (Leica Microsystems, Switzerland).

2.3. Drug preparation and treatment

Rats received subcutaneous (s.c.) injections of PBS (0.3 ml/kg, pH 7.4), varenicline (0.3–3.0 mg free base/kg/day equivalent to 1.4–14.2 μmoles/kg/day) or nicotine hydrogen bitartrate (0.33 mg free base/kg/day equivalent to 2 μmoles/kg/day); all drug solutions had a pH ranging between 7.0–7.4. Doses of nicotine and varenicline were chosen based on studies in the literature reported to improve L-dopa-induced dyskinesias in laboratory animals (Bordia et al., 2010; Huang et al., 2011a). For antagonist studies, rats received s.c. injections of mecamylamine HCl (0.82 mg free base/kg/day equivalent to 4.9 μmoles/kg/day) 30 minutes prior to nicotine administration.

For time-course studies, varenicline could not be obtained from the manufacturer due to intellectual property issues, and was unavailable commercially at the time. Thus, these studies were conducted using varenicline dihydrochloride prepared from the commercial Chantix™ preparation. All other studies were conducted using varenicline bitartrate (Tocris Bioscience, Minneapolis, MN).

To prepare varenicline dihydrochloride, 224 tablets of commercial Chantix™ (each tablet containing 1.71 mg varenicline tartrate or 1.0 mg varenicline free base) were purchased from a local pharmacy and dissolved in distilled water (300 ml), methanol (300 ml) and sodium carbonate (560 mg). The mixture was stirred for 24 hours at 75°C followed by cooling at room temperature. The precipitate was allowed to settle and the supernatant was decanted. An additional volume of methanol (300 ml) was added to the precipitate, the mixture was stirred 5 minutes, and the supernatant was decanted again. Remaining methanol was removed *in vacuo*, and the residue was extracted 3 times with ethylacetate (200 ml). The organic layer was dried with sodium sulfate and concentrated *in vacuo*. Purification by silica gel column chromatography (ethylacetate:methanol:ammonium hydroxide, 25:75:2.5, v/v) yielded 154 mg varenicline free base (with some impurities), which was dissolved in methanol (10 ml). The solution was treated with 10 ml HCl/ethylacetate (HCl gas was generated from H₂SO₄ and NaCl bubbled in ethylacetate until the pH reached 1), concentrated, methanol (2 × 10 ml) was added, and the mixture was distilled by azeotrope to yield solid material. The solids were dissolved in methanol (20 ml) and filtered through charcoal on a pad of celite for decolorization. The filtrate was recrystallized from methanol/diethylether to yield the dihydrochloride salt as a white solid (96 mg, 0.34 mmoles, 32% yield). Analysis by nuclear magnetic resonance spectroscopy and mass spectrometry indicated: ¹H NMR (400 MHz, CD₃OD) δ 8.92 (s, 2H), 8.13 (s, 2H), 3.72 (s, 2H), 3.57 (d, *J* = 12 Hz, 2H), 3.38 (d, *J* = 12.4 Hz, 2H), 2.52 (m, 1H), 2.32 (d, *J* = 11.6, 1H); ¹³C NMR (62.9 MHz, CD₃OD) δ 147.07, 146.14, 144.82, 125.06, 41.65, 40.06; HRMS- TOF: *m/z* [M + H]⁺ calculated for C₁₃H₁₃N₃: 212.1182, measured 212.1203.

2.4. Rotorod assessment

A Rotamex-5 (Columbus Instruments, Columbus, Ohio) equipped with a spindle diameter of 7.0 cm was used to assess the ability of animals to maintain coordination and balance. To accomplish this, rats were trained over a 3-day period to maintain their position on the rotating rod for 3 minutes. On day 1, animals were placed on the spindle at rest with the acceleration set to increase at a rate of 0.2 revolutions/sec to a maximum rotational speed of 20 revolutions/min (rpm); on day 2, the maximum speed was set to 30 rpm, and on day 3, the maximum speed was set to 40 rpm. Animals received 3 training sessions per day with a maximal duration of 3 minutes each. The rotorod was wiped with a 70% ethanol solution between all trials to eliminate olfactory influences on behavior. On the days of testing, rats were placed on the rod rotating at a constant speed of 20 rpm. The criterion for this task was defined as being able to maintain balance on the rod for 3 minutes for at least 3/6 trials; animals who failed to meet criterion were not used for further study. Assessments consisted of 3 trials/day for 2 consecutive days following training (baseline), on days 6 and 7 following the administration of 3-AP, and weekly for up to 4 weeks following drug administration. For the latter, behavior was determined a minimum of 18 hours after drug administration.

2.5. Stationary beam assessment

These studies used a wood beam (2 cm wide x 1 m long) supported 66 cm above a 12 cm thick foam pad with a start platform (30 cm²) at one end and a goal box (18 cm wide x 33 cm deep x 24 cm high) at the other. The box had a wide opening (10 cm high x 18 cm wide) and a black interior. Both the beam and platform were treated with Plasti-Dip to prevent fluid absorption (U.S. Plastic Corporation, Lima, Ohio, USA). The experiments were conducted in a bright fluorescently-lit procedure room. A tripod-mounted video camera level with the beam was used to record each trial. Animals were placed on the start platform with their forepaws on the beam and allowed to progress into the goal box. The time to cross the beam was measured from the time the last hind paw left the platform to when the nose of the animal entered the goal box. Balance corrections were assessed by counting lateral movements of the tail as the animal attempted to maintain or regain balance on the beam. Measurements were performed by an individual blinded to the treatment groups using MediaImpressions HD video software (ArcSoft Inc., Fremont, CA). Assessments consisted of 5 trials/day for 2 consecutive days following 3 days habituation (baseline), on days 6 and 7 following the administration of 3-AP, and weekly for up to 4 weeks following drug administration. For the latter, behavior was determined a minimum of 18 hours after drug administration. The last 3 of 5 trials/day were quantified.

2.6. Open field activity

Rats were acclimatized to an open field for 3 minutes/day for 3 days prior to baseline determinations. The field consisted of a black plastic circular platform 100 cm in diameter (perimeter = 314 cm and area = 7854 cm²), 70 cm above the ground; a white plastic barrier (30 cm high) enclosed the arena (Philpot and Wecker, 2008). A video camera was suspended directly over the arena to automatically record activity based on the center of body mass (defined as crown to rump length/2) of the animals (EthoVision, Noldus Information Technology, Leesburg, VA). On each day of testing, each animal was placed in 1 of 4 randomly selected zones in the open field and activity was assessed for 1.5 minutes under moderate levels of illumination. The arena was wiped with a 70% ethanol solution between all trials to eliminate olfactory influences on behavior. The velocity during movement (cm/sec), total time moving (sec), total distance moved (cm), and 'wobble' or stagger (degrees of rotation/cm) were ascertained. Assessments consisted of 1 trial/day for 2 consecutive days following familiarization, on days 6 and 7 following the administration of

3-AP, and on days 6 and 7 following drug administration. For the latter, behavior was determined a minimum of 18 hours after drug administration.

2.7. Footprint analysis

Alterations in gait were determined by obtaining paw prints from rats who traversed a runway consisting of a walled (30 cm high) corridor (18 cm wide x 66 cm long) leading to a dark box with a removable white recording sheet on its base. The forepaws and hindpaws of each animal were dipped into different colors of non-toxic washable paint, and the animal was placed on the paper and allowed to run freely into the darkened goal box. Following capture of 3 to 5 full stride lengths, each rat was removed from the apparatus, its feet washed with water, and the animal returned to its home cage. Rats were familiarized with the runway for 3 training runs prior to testing.

The forepaw and hindpaw stride lengths were determined by measuring the distance (cm) between the centers of the respective paw prints to the center of the successive ipsilateral prints. Forepaw and hindpaw stride widths were determined by measuring the distance between the centers of the respective paw prints to the corresponding contralateral stride length measurements at a right angle. For each step parameter, 2–3 values were measured from each run, excluding footprints at the beginning and end of each run when the animals were initiating and completing movement, respectively. The means of each set of values were used for analysis. Each animal was assessed once for 2 consecutive days following training (baseline), on days 6 and 7 following the administration of 3-AP, and on days 6 and 7 following drug administration. For the latter, behavior was determined a minimum of 18 hours after drug administration.

2.8. Data analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Significant differences between determinations prior to and after the administration of 3-AP were determined using the Student's t-test. Time-course and dose-response data were analyzed by a 2-way ANOVA (treatment x time and dose x time, respectively), and individual group differences determined by Tukey's test or Dunnett's test for multiple comparisons. All other significant differences were determined by analysis of variance (ANOVA) followed by Tukey's test or Dunnett's test. The criterion for significance was $p < 0.05$ for all comparisons.

3. Results

3.1 The administration of 3-AP leads to cell loss in the inferior olive and deficits in rotarod activity, performance on a stationary beam, and gait abnormalities

The administration of 3-AP followed by nicotinamide led to a rapid and progressive loss of NeuN(+) neurons in the inferior olive. At 3 days following 3-AP administration, the number of NeuN(+) cells was 54% of that in control animals. By one week, the number of cells continued to decline to 15% of that in controls; there was no recovery during the following 4 weeks (Fig. 1). Further, the cell loss appeared primarily in the rostral medial accessory olive, with surviving or 3-AP resistant cells in the caudal region (noted by the arrow in the micrograph from 35 days), in agreement with Seoane et al. (2005).

The effects of the lesion on motor coordination and balance, locomotor activity, and gait are shown in Figs. 2–4. These figures contain data from all animals studied to illustrate the distribution of these behaviors within the population at baseline before the administration of 3-AP and at 1 week post 3-AP. Fig. 2[A] demonstrates that following 3 days of training, animals could maintain their balance on the rotarod (rotating at 20 rpm) for an average of

159 $\pm$ 2 seconds; after the lesion, time on the rod decreased significantly ($p < 0.05$) by 72% to 45 $\pm$ 4 seconds ($n = 144$). The data also illustrate that: the variation prior to the lesion was 50% of the variation after the lesion; 2% of the population did not exhibit any deficit on the rotorod (they remained on the rod for the maximum 180 seconds); 20% were unable to stay on the rod at all; and 20% maintained their balance for less than 25 seconds. Thus, although all animals received the same doses of 3-AP and nicotinamide and were housed and handled identically, their behavioral response on the rotorod following the administration of 3-AP differed markedly. Further, there was no apparent relationship between behavioral performance on the rotorod and the severity of the lesion. Animals that either did not exhibit any deficit or exhibited a total deficit on rotorod performance were excluded from further study.

The effects of 3-AP on the performance of rats on the stationary beam are shown in Fig. 2[B] and indicate that the lesion increased both the time to cross the beam and the number of balance corrections (lateral tail movements). Following 3 days of familiarization on the beam, rats rapidly traversed the beam prior to the lesion in 1.6 $\pm$ 0.07 seconds, a time that increased significantly ($p < 0.05$) by 3.1-fold (to 5.0 $\pm$ 0.3 seconds) after 3-AP ($n = 40$). Similarly, the number of balance corrections increased significantly ($p < 0.05$) by nearly 4-fold (from 1.08 $\pm$ 0.07 to 4.30 $\pm$ 0.26). Results also demonstrate that the variation within the population following the administration of 3-AP for both behavioral measures on the beam increased 3.5-fold relative to baseline. Thus, similar to results on the rotorod, the degree of impairment on the stationary beam was clearly different within the population, despite identical treatment, and there was no apparent relationship between behavioral performance and the severity of the lesion. Those animals that did not exhibit any deficits on, or were unable to cross the beam were not used for further study.

Animals were also tested in an open field following 3 days of acclimatization to the apparatus to ascertain whether 3-AP affected locomotor activity. Results (Fig. 3) indicate that all measures of ambulation were significantly ($p < 0.05$) affected by the administration of 3-AP. Movement velocity decreased 19% from 23.0 $\pm$ 0.5 to 18.7 $\pm$ 0.4 cm/sec and total time moving decreased 12% from 48.1 $\pm$ 1.4 to 42.3 $\pm$ 1.7 seconds, resulting in a 31% decreased distance moved from 1139 $\pm$ 42 to 787 $\pm$ 34 cm ($n = 44$). Further, wobble (degrees of deviation from a linear trajectory) increased 35% from 4.26 $\pm$ 0.12 to 5.75 $\pm$ 0.22 degrees, indicating that the animals were unable to sustain a linear pattern of movement. Interestingly, the variation within the population for velocity, time moving and distance moved did not differ prior to and after the administration of 3-AP. However, the variation for wobble increased nearly 2-fold following the administration of 3-AP, perhaps indicative of differences in the abilities of animals to compensate for the induced alterations.

The effects of 3-AP on hindpaw and forepaw stride lengths and widths are shown in Fig. 4 and indicate that all gait measures were altered significantly ($p < 0.05$) by 3-AP. Forepaw and hindpaw stride lengths decreased by 7% (from 14.22 $\pm$ 0.14 to 13.22 $\pm$ 0.11 cm) and 6% (from 13.90 $\pm$ 0.14 to 13.07 $\pm$ 0.11 cm), respectively, whereas forepaw and hindpaw stride widths increased by 9% (from 2.43 $\pm$ 0.05 to 2.64 $\pm$ 0.06 cm) and 19% (from 3.63 $\pm$ 0.06 to 4.31 $\pm$ 0.07 cm), respectively ($n = 76$). As observed for other behavioral measures, some animals did not exhibit changes in gait parameters following the administration of 3-AP, as illustrated in the bottom scatter graph depicting changes in stride lengths and widths induced by 3-AP. Animals that did not exhibit any deficits (Δ cm = 0) in hindpaw stride width were excluded from further study.

3.2 Initial evidence that nicotinic agonists improve impaired behaviors

To determine whether varenicline or nicotine improved the ability of lesioned rats to maintain their balance on the rotating rod, animals received daily injections of saline or the

nicotinic agonists for up to 4 weeks beginning 1 week after the administration of 3-AP, and performance was assessed at weekly intervals. For time course experiments, nicotine and varenicline were administered at doses reported to improve L-dopa-induced dyskinesias in laboratory animals (Bordia et al., 2010; Huang et al., 2011a), viz., 0.75 mg varenicline free base/kg/day or 0.33 mg nicotine free base/kg/day. Results (Fig. 5) demonstrate that for saline-injected animals, the 3-AP induced rotorod impairment was relatively constant throughout the 4 weeks of saline administration. The administration of nicotine significantly ($p<0.05$) improved performance by 50%, an effect that was apparent following the first week of administration, and which did not improve further over time. The administration of varenicline also led to improvements, but these effects were not evident until 2 weeks of drug administration, at which time there was a significant ($p<0.05$) increase in rotorod performance relative to the saline control group, and this improvement was maintained, but not increased, with further daily drug administration.

Although rotorod performance was assessed 18 hours following the administration of the nicotinic agonists, because these compounds can increase motor activity, it was possible that the enhanced performance on the rotorod was due to a stimulant effect. To investigate this possibility, locomotor activity in the open field was determined 18 hours after 1 week daily drug administration (Table 1). Results indicate that locomotor behavior in the open field did not differ among animals who received injections of saline, nicotine or varenicline. Thus, the dose of nicotine that improved performance on the rotorod following 1 week of administration could not be attributed to a stimulant effect because it did not alter any measure of locomotor activity.

To determine whether 1 week of nicotine administration affected gait, rats received injections of saline or 0.33 mg nicotine free base/kg/day beginning 1 week following the administration of 3-AP, and gait parameters were evaluated. The administration of saline did not affect the 3-AP-induced alterations in gait. Further, the administration of nicotine did not affect either forepaw stride length or width or hindpaw stride length (Fig. 6). However, nicotine administration significantly ($p<0.05$) attenuated the 19% increase in hindpaw stride width induced by 3-AP. Hindpaw stride width following the administration of nicotine (3.65 ± 0.21 cm) did not differ from baseline (3.27 ± 0.18 cm) and was significantly ($p<0.05$) less than that induced by 3-AP (4.46 ± 0.12 cm).

To ascertain whether the ability of nicotine to improve behavior in 3-AP lesioned rats could be attributed to the nicotine-induced activation of neuronal nicotinic receptors, the ability of mecamylamine to block the effects of nicotine was determined. For these experiments, rats received injections of saline or mecamylamine 30 minutes prior to injections of saline or nicotine daily for 1 week beginning 1 week after the administration of 3-AP, and both behavior on the rotating rod and hindpaw stride width were measured. Results from these experiments are shown in Fig. 7 and demonstrate that: 1) neither saline nor mecamylamine affected the 3-AP-induced deficits on either the ability of rats to maintain balance on the rotating rod or hindpaw stride width; 2) nicotine significantly ($p<0.05$) improved both measures; and 3) mecamylamine, when administered prior to nicotine, prevented the ability of nicotine to improve both impairments. Thus, results indicate that the ability of nicotine to improve the balance and coordination and the increased hindpaw stride width of ataxic rats represents a nicotinic receptor-mediated action.

3.3 Dose-related effects of varenicline

Because beneficial effects of varenicline have been demonstrated in patients with SCA3 (Zesiewicz et al., 2012), when this compound became available commercially, dose-response studies were initiated to ascertain effects on rotorod performance in 3-AP lesioned animals. For this study, rats received daily injections of saline or 0.3, 1 or 3 mg varenicline

free base/kg for up to 2 weeks beginning 1 week after the administration of 3-AP, and rotorod performance evaluated weekly. Results (Fig. 8) indicate that the administration of the two higher doses of varenicline for 1 week significantly ($p<0.05$) improved performance on the rotating rod with a doubling of the time that animals could maintain their balance. Following 2 weeks daily administration of varenicline, the lowest dose studied, 0.3 mg free base/kg, increased the time on the rod by 50%, although this increase was not significant due to the large variance within the group. Further, administration of 1 or 3 mg free base varenicline for 2 weeks did not improve performance above that determined following drug administration for 1 week. Thus, results indicate that a maximal effect of varenicline was achieved following the daily administration of 1–3 mg varenicline free base/kg/day for 1 week.

Based on studies in patients with SCA3 indicating that varenicline improved some, but not all impaired functions (Zesiewicz, 2012), the stationary beam was also used to assess the effects of varenicline. The dose-response effects of varenicline administered daily for 4 weeks on stationary beam performance measured weekly are shown in Fig. 9. Both the time to cross the beam and balance corrections, defined as the number of lateral tail movements to either maintain or regain balance, were quantified. The time required for rats to traverse the beam improved in all groups throughout the 4 week period, and the performance of rats who received varenicline did not differ from the performance of rats who received saline (Fig. 9[A]). Although the time to cross the beam did not differ among groups, there was a significant ($p<0.05$) effect of varenicline (0.3 and 1.0 mg free base/kg) on balance corrections following 3 and 4 weeks of drug administration (Fig. 9[B]). Thus, results suggest that following the prolonged administration of these doses of varenicline, although animals do not move faster across a stationary beam, they do have an increased ability to maintain or regain their balance.

4. Discussion

The objective of these studies was to establish pharmacological evidence for the use of nicotinic agonists for the treatment of ataxia using rats rendered ataxic by olivocerebellar lesions. Results indicate that nicotine and varenicline could partially restore 3-AP-induced deficits in coordination, balance and gait, and that these actions could be attributed to a nicotinic receptor-mediated effect unrelated to possible stimulant properties of these compounds. This study is the first demonstration of the anti-ataxic effects of nicotine agonists administered peripherally in a laboratory animal model involving olivocerebellar degeneration.

The administration of 3-AP to rats has been shown to alter a variety of motor behaviors, with the severity of such varying from task to task and study to study (Sukin et al., 1987; Gasbarri et al., 2003; Seoane et al., 2005; Saxon and White, 2006). These findings underscored the necessity of characterizing impairments in a rat population using several tasks. Results indicate that all measures of balance/coordination, locomotor activity and gait were affected by the lesion. Further, the nicotinic agonists did not have a generalized effect, but rather, improved only balance/coordination on the rotating rod and the increased hindpaw stride width; locomotor activity, performance on the stationary beam, and other gait parameters were unaffected. The finding that only selective measures were improved is similar to clinical evidence indicating that varenicline improved only gait, stance and a timed 25-foot walk in patients with SCA, and did not affect other impairments (Zesiewicz et al., 2012). Further, recent studies in rats analyzing both dynamic and static parameters of gait indicate a beneficial effect of varenicline on many, but not all gait measures (Lambert et al., in preparation). Thus, the nicotinic agonists do not lead to a general improvement, but rather are specific for selective measures of gait and balance/coordination.

The finding that nicotinic agonists improve these aspects of motor behavior supports the idea that these compounds may have therapeutic benefit, consistent with evidence that nicotine administration is efficacious for reducing L-dopa-induced dyskinesias in a rat parkinsonian model (Bordia et al., 2008), for improving motor behavior in rats after a focal stroke (Gonzalez et al., 2006), and for improving sensorimotor performance in mice after intracerebral hemorrhage (Hijioka et al., 2012). It is likely that nicotinic agonist-induced alterations in cellular processes lead to either the rescue of dying cells, or alternatively, to the facilitation of compensatory behavioral/physiological mechanisms that can temper the primary insult. In support of the former idea, studies have shown that climbing fibers that survive following the administration of 3-AP can sprout and form new arborizations that reinnervate denervated Purkinje cells (Rossi et al., 1991a; 1991b). Further, evidence indicates that impaired balance and coordination in adult rats who were exposed to ethanol postnatally can be improved by motor task learning (Klintsova et al., 1998), and may be attributed to enhanced cerebellar synaptogenesis (Klintsova et al., 2002). Indeed, the plasticity of the olivocerebellar pathway in the mature brain is well documented (for reviews see Dusart et al., 2005; Cesa and Strata, 2009). Thus, nicotinic agonists may lead to behavioral improvements by increasing the survival and connectivity of olivocerebellar neurons. This idea is supported by studies indicating that varenicline reduced L-dopa-induced dyskinesias only in rats with partial, but not complete lesions of the striatum (Huang et al., 2011a).

Alternatively, nicotinic agonists may improve behaviors through compensatory mechanisms involving the recruitment and alterations in the activity of motor circuits not involved in gait and balance in normal or unlesioned rats. Indeed, studies have documented that the circuitry regulating motor behavior in the brain is capable of adaptation (for review see Doyon and Benali, 2005). Laboratory animal studies have demonstrated that both the cerebellar thalamocortical and basal ganglia thalamocortical circuits reorganize functionally and demonstrate alterations in neural activation following exercise training (Holschneider et al., 2007). The idea that nicotinic agonists may enhance such adaptive mechanisms is supported by studies indicating that the administration of the reversible cholinesterase inhibitor physostigmine following cortical impact injury, improved rotorod performance (Holschneider et al., 2011) and increased functional activation of the cerebellar vermis and enhanced cerebral blood flow of the cerebellar thalamocortical circuit (Holschneider et al., 2013). The idea that compensation may be involved in the improvement of motor behaviors following cerebellar insult is supported by a recent voxel-based morphometry study in humans indicating that physical training, which improved balance, led to increased grey matter volume in the dorsal premotor cortex, a non-affected region of the cerebellar-cortical circuit, in individuals with cerebellar degeneration (Burciu et al., 2013).

The ability of mecamylamine to block the nicotinic agonist-induced improvements in the present study supports the involvement of nicotinic receptors. It is likely that effects reflect a differential or combination of actions at different subtypes of neuronal nicotinic receptors. Varenicline was developed as a partial agonist at nicotinic heteromeric $\alpha 4\beta 2$ receptors (Rollema et al., 2007a; 2007b). However, it has differential actions at diverse nicotinic receptors in the brain. In addition to its action as a partial agonist at $\alpha 4\beta 2$ receptors, varenicline has full agonist activity at homomeric $\alpha 7$ receptors, and weak partial agonist activity at $\alpha 3\beta 2$ and $\alpha 6$ -containing receptors (Mihalak et al., 2006). Further, although initial investigations characterized varenicline as a partial agonist at $\alpha 3\beta 4$ receptors (Mihalak et al., 2006; Rollema et al., 2007a), a recent study has indicated full agonist activity at these receptors (Chatterjee et al., 2011).

Several subtypes of neuronal nicotinic receptors have been reported to be expressed in rat cerebellum including, in decreasing order of abundance, $\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 3\beta 2\beta 4 > \alpha 3\alpha 4\beta 2$

= $\alpha 4\beta 2\beta 4$ (Turner and Kellar, 2005), and recent studies have indicated that most of these receptors are located in cells within the cerebellum including granule cells, Purkinje cells and interneurons, rather than on afferents (Turner et al., 2011). Studies have also indicated the presence of $\alpha 7$ subunits in mostly perisynaptic regions in the granule cell layer, albeit in very low abundance (Caruncho et al., 1997). Thus, the action of nicotinic agonists may occur within the cerebellum. It is also possible that the nicotinic agonists affect receptors in other brain regions involved in regulating motor behavior. Nicotinic receptors composed of different subunits are also abundant in the thalamus and include $\alpha 4\beta 2$, $\alpha 4\beta 2\alpha 5$, $\alpha 6\alpha 3\beta 2$, and $\alpha 3\beta 4$ receptors (Nguyen et al., 2003; Gotti et al., 2008; Kassam et al., 2008; Mao et al., 2008). Further, nicotine has been shown to regulate the activity of the central medial thalamus (Newman and Mair, 2007) and activate the thalamocortical pathway (Lambe et al., 2005; Kawai et al., 2007). Indeed, Huang et al. (2011a) demonstrated that both varenicline and the agonist 5-iodo-A-85380 (A-85380), which selectively activates $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ receptors (Mukhin et al., 2000; Kulak et al., 2002; Mogg et al., 2004), improved L-dopa-induced dyskinesias in the parkinsonian rat model, and postulated that the anti-dyskinetic actions of these agonists could be attributed to actions on presynaptic $\alpha 4\beta 2^*$ or $\alpha 6\beta 2^*$ receptors, an idea supported by the lack of effect in $\beta 2(-/-)$ mice (Huang et al., 2011b). Further, based on studies demonstrating that both nicotine and mecamylamine ameliorated the induced abnormal involuntary movements in these rats to a similar extent, Bordia et al. (2010) suggested that the beneficial effects of nicotine may involve desensitization of neuronal nicotinic receptors. The present results, indicating that rotorod performance in ataxic rats improved with the agonists and was unaltered by mecamylamine, suggest that the anti-ataxic effect of the agonists is mediated by receptor activation, rather than the inactivation that may be apparent following the chronic administration of nicotinic agonists. Thus, it is likely that nicotine agonist activation of one or more of these receptors directly alters cellular activity of one or several structures within the cerebellar thalamo-cortical pathway, leading to the anti-ataxic effects.

In sum, this study provides proof-of-principle for the use of nicotinic agonists for olivocerebellar ataxia. Results demonstrating that these compounds temper the coordination and gait deficits manifest following lesions of the primary afferent input to Purkinje cells have important clinical implications because there is no current efficacious pharmacological therapy to alleviate the imbalance and lack of voluntary muscle coordination manifest by individuals with ataxia. Results from the current study are the first to demonstrate the utility of nicotinic agonists for the treatment of ataxias, and warrant further studies to elucidate the specific mechanism(s) mediating this effect.

Acknowledgments

These studies were supported by grants from the Florida Center of Excellence for Biomolecular Identification and Targeted Therapeutics, the USF Neuroscience Collaborative, and the National Institutes of Health National Institute of Neurological Disorders and Stroke (grant #NS072114). Preliminary results from this work were presented at the 2011 Annual Meeting for the Society for Neuroscience [Wecker, L., Engberg, M.E., Philpot, R.M., Lambert, C.S., Bickford, P.C., and Hudson, C. (2011) Neuronal nicotinic receptor agonists ameliorate 3-acetylpyridine-induced ataxia. Program #/Poster# 746.04. *Neuroscience Meeting Planner*. Washington, DC: Society for Neuroscience, Online], a Satellite to the 2011 Meeting of the Society for Neuroscience entitled "Nicotinic Acetylcholine Receptors as Therapeutic Targets: Emerging Frontiers in Basic Research and Clinical Science [Wecker, L., Engberg, M.E., Philpot, R.M., Lambert, C.S., Bickford, P.C., and Hudson, C. (2011) Neuronal nicotinic receptor agonists ameliorate 3-acetylpyridine-induced ataxia. *Biochem. Pharmacol.* 82: 1041] and the 2012 Experimental Biology Meetings [Lambert, C.S., Philpot, R.M., Engberg, M.E., and Wecker, L. (2012) Neuronal nicotinic acetylcholine receptors (nAChRs) are involved in nicotine-mediated reversal of ataxia in an animal model. *EB '12 Abst.* Program No. 1044.3.]

Abbreviations

3-AP	3-acetylpyridine
ANOVA	analysis of variance
SCA3	spinocerebellar ataxia type 3

References

- Blattel RA. Use of choline in the treatment of ataxia associated with multiple sclerosis. *Can Med Assoc J.* 1979; 121:1568. [PubMed: 534968]
- Bordia T, Campos C, Huang L, Quik M. Continuous and intermittent nicotine treatment reduces L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesias in a rat model of Parkinson's disease. *J Pharmacol Exp Ther.* 2008; 327:239–247. [PubMed: 18650244]
- Bordia T, Campos C, McIntosh JM, Quik M. Nicotinic receptor-mediated reduction in L-DOPA-induced dyskinesias may occur via desensitization. *J Pharmacol Exp Ther.* 2010; 333:929–938. [PubMed: 20200117]
- Burciu RG, Fritsche N, Granert O, Schmitz L, Sponemann N, Konczak J, Theysohn N, Gerwig M, van Eimeren T, Timmann D. Brain changes associated with postural training in patients with cerebellar degeneration: a voxel-based morphometry study. *J Neurosci.* 2013; 33:4594–4604. [PubMed: 23467375]
- Caruncho HJ, Guidotti A, Lindstrom J, Costa E, Pesold C. Subcellular localization of the $\alpha 7$ nicotinic receptor in rat cerebellar granule cell layer. *Neuroreport.* 1997; 8:1431–1433. [PubMed: 9172148]
- Cesa R, Strata P. Axonal competition in the synaptic wiring of the cerebellar cortex during development and in the mature cerebellum. *Neuroscience.* 2009; 162:624–632. [PubMed: 19272433]
- Chatterjee S, Steensland P, Simms JA, Holgate J, Coe JW, Hurst RS, Shaffer CL, Lowe J, Rollemma H, Bartlett SE. Partial agonists of the $\alpha 3\beta 4^*$ neuronal nicotinic acetylcholine receptor reduce ethanol consumption and seeking in rats. *Neuropsychopharmacology.* 2011; 36:603–615. [PubMed: 21048701]
- Cohen RW, Fisher RS, Duong T, Handley VW, Campagnoni AT, Hull CD, Buchwald NA, Levine MS. Altered excitatory amino acid function and morphology of the cerebellum of the spastic Han-Wistar rat. *Brain Res Mol Brain Res.* 1991; 11:27–36. [PubMed: 1685005]
- Dar MS, Bowman ER, Li C. Intracerebellar nicotinic-cholinergic participation in the cerebellar adenosinergic modulation of ethanol-induced motor incoordination in mice. *Brain Res.* 1994; 644:117–127. [PubMed: 7913399]
- Doyon J, Benali H. Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol.* 2005; 15:161–167. [PubMed: 15831397]
- Dusart I, Ghoumari A, Wehrle R, Morel MP, Bouslama-Oueghlani L, Camand E, Sotelo C. Cell death and axon regeneration of Purkinje cells after axotomy: challenges of classical hypotheses of axon regeneration. *Brain Res Brain Res Rev.* 2005; 49:300–316. [PubMed: 16111558]
- Gasbarri A, Pompili A, Pacitti C, Cicirata F. Comparative effects of lesions to the ponto-cerebellar and olivo-cerebellar pathways on motor and spatial learning in the rat. *Neuroscience.* 2003; 116:1131–1140. [PubMed: 12617954]
- Gonzalez CL, Gharbawie OA, Kolb B. Chronic low-dose administration of nicotine facilitates recovery and synaptic change after focal ischemia in rats. *Neuropharmacology.* 2006; 50:777–787. [PubMed: 16469338]
- Gotti C, Moretti M, Meinerz NM, Clementi F, Gaimarri A, Collins AC, Marks MJ. Partial deletion of the nicotinic cholinergic receptor $\alpha 4$ or $\beta 2$ subunit genes changes the acetylcholine sensitivity of receptor-mediated $^{86}\text{Rb}^+$ efflux in cortex and thalamus and alters relative expression of $\alpha 4$ and $\beta 2$ subunits. *Mol Pharmacol.* 2008; 73:1796–1807. [PubMed: 18337473]
- Hijioka M, Matsushita H, Ishibashi H, Hisatsune A, Isohama Y, Katsuki H. $\alpha 7$ Nicotinic acetylcholine receptor agonist attenuates neuropathological changes associated with intracerebral hemorrhage in mice. *Neuroscience.* 2012; 222:10–19. [PubMed: 22820264]

- Hildebrandt IJ, Termeer JL, Toledo D, Cohen RW. Characterization of chronic nicotine exposure on the survival of the spastic Han-Wistar rat. *Nicotine Tob Res.* 2003; 5:827–836. [PubMed: 14668067]
- Holschneider DP, Guo Y, Roch M, Norman KM, Scremin OU. Acetylcholinesterase inhibition and locomotor function after motor-sensory cortex impact injury. *J Neurotrauma.* 2011; 28:1909–1919. [PubMed: 21787180]
- Holschneider DP, Guo Y, Wang Z, Roch M, Scremin OU. Remote brain networks changes after unilateral cortical impact injury and their modulation by acetylcholinesterase inhibition. *J Neurotrauma.* 2013 Jan 23. [Epub ahead of print].
- Holschneider DP, Yang J, Guo Y, Maarek JM. Reorganization of functional brain maps after exercise training: Importance of cerebellar-thalamic-cortical pathway. *Brain Res.* 2007; 1184:96–107. [PubMed: 17964551]
- Huang LZ, Campos C, Ly J, Carroll FI, Quik M. Nicotinic receptor agonists decrease L-dopa-induced dyskinesias most effectively in partially lesioned parkinsonian rats. *Neuropharmacology.* 2011a; 60:861–868. [PubMed: 21232546]
- Huang LZ, Grady SR, Quik M. Nicotine reduces L-DOPA-induced dyskinesias by acting at $\beta 2^*$ nicotinic receptors. *J Pharmacol Exp Ther.* 2011b; 338:932–941. [PubMed: 21665941]
- Kark RA, Blass JP, Spence MA. Physostigmine in familial ataxias. *Neurology.* 1977; 27:70–72. [PubMed: 556821]
- Kark RA, Budelli MM, Wachsner R. Double-blind, triple-crossover trial of low doses of oral physostigmine in inherited ataxias. *Neurology.* 1981; 31:288–292. [PubMed: 7010207]
- Kassam SM, Herman PM, Goodfellow NM, Alves NC, Lambe EK. Developmental excitation of corticothalamic neurons by nicotinic acetylcholine receptors. *J Neurosci.* 2008; 28:8756–8764. [PubMed: 18753377]
- Kawai H, Lazar R, Metherate R. Nicotinic control of axon excitability regulates thalamocortical transmission. *Nat Neurosci.* 2007; 10:1168–1175. [PubMed: 17704774]
- Klintsova AY, Cowell RM, Swain RA, Napper RM, Goodlett CR, Greenough WT. Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats: I. Behavioral results. *Brain Res.* 1998; 800:48–61. [PubMed: 9685583]
- Klintsova AY, Scamra C, Hoffman M, Napper RM, Goodlett CR, Greenough WT. Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats: II. A quantitative stereological study of synaptic plasticity in female rat cerebellum. *Brain Res.* 2002; 937:83–93. [PubMed: 12020866]
- Kulak JM, Sum J, Musachio JL, McIntosh JM, Quik M. 5-Iodo-A-85380 binds to α -conotoxin MII-sensitive nicotinic acetylcholine receptors (nAChRs) as well as $\alpha 4\beta 2^*$ subtypes. *J Neurochem.* 2002; 81:403–406. [PubMed: 12064487]
- Lambe EK, Olausson P, Horst NK, Taylor JR, Aghajanian GK. Hypocretin and nicotine excite the same thalamocortical synapses in prefrontal cortex: correlation with improved attention in rat. *J Neurosci.* 2005; 25:5225–5229. [PubMed: 15917462]
- Legg N. Oral choline in cerebellar ataxia. *Br Med J.* 1979; 2:133. [PubMed: 380770]
- Legg NJ. Oral choline in cerebellar ataxia. *Br Med J.* 1978; 2:1403–1404. [PubMed: 719428]
- Livingstone IR, Mastaglia FL. Choline chloride in the treatment of ataxia. *Br Med J.* 1979; 2:939. [PubMed: 391326]
- Llinas R, Walton K, Hillman DE, Sotelo C. Inferior olive: its role in motor learning. *Science.* 1975; 190:1230–1231. [PubMed: 128123]
- Mao D, Perry DC, Yasuda RP, Wolfe BB, Kellar KJ. The $\alpha 4\beta 2\alpha 5$ nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine *in vivo*. *J Neurochem.* 2008; 104:446–456. [PubMed: 17961152]
- Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors. *Mol Pharmacol.* 2006; 70:801–805. [PubMed: 16766716]
- Mogg AJ, Jones FA, Pullar IA, Sharples CG, Wonnacott S. Functional responses and subunit composition of presynaptic nicotinic receptor subtypes explored using the novel agonist 5-iodo-A-85380. *Neuropharmacology.* 2004; 47:848–859. [PubMed: 15527819]

- Mukhin AG, Gundisch D, Horti AG, Koren AO, Tamagnan G, Kimes AS, Chambers J, Vaupel DB, King SL, Picciotto MR, Innis RB, London ED. 5-Iodo-A-85380, an $\alpha 4\beta 2$ subtype-selective ligand for nicotinic acetylcholine receptors. *Mol Pharmacol*. 2000; 57:642–649. [PubMed: 10692507]
- Newman LA, Mair RG. Cholinergic modulation of visuospatial responding in central thalamus. *Eur J Neurosci*. 2007; 26:3543–3552. [PubMed: 18088280]
- Nguyen HN, Rasmussen BA, Perry DC. Subtype-selective up-regulation by chronic nicotine of high-affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. *J Pharmacol Exp Ther*. 2003; 307:1090–1097. [PubMed: 14560040]
- Philcox DV, Kies B. Choline in hereditary ataxia. *Br Med J*. 1979; 2:613. [PubMed: 497742]
- Philpot RM, Wecker L. Dependence of adolescent novelty-seeking behavior on response phenotype and effects of apparatus scaling. *Behav Neurosci*. 2008; 122:861–875. [PubMed: 18729640]
- Rodriguez-Budelli MM, Kark RA, Blass JP, Spence MA. Action of physostigmine on inherited ataxias. *Adv Neurol*. 1978; 21:195–202. [PubMed: 735925]
- Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD 3rd, Williams KE. Pharmacological profile of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology*. 2007a; 52:985–994. [PubMed: 17157884]
- Rollema H, Coe JW, Chambers LK, Hurst RS, Stahl SM, Williams KE. Rationale, pharmacology and clinical efficacy of partial agonists of $\alpha 4\beta 2$ nACh receptors for smoking cessation. *Trends Pharmacol Sci*. 2007b; 28:316–325. [PubMed: 17573127]
- Rossi F, van der Want JJ, Wiklund L, Strata P. Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the inferior olive in the adult rat. II Synaptic organization on reinnervated Purkinje cells. *J Comp Neurol*. 1991a; 308:536–554. [PubMed: 1865016]
- Rossi F, Wiklund L, van der Want JJ, Strata P. Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the inferior olive in the adult rat. I Development of new collateral branches and terminal plexuses. *J Comp Neurol*. 1991b; 308:513–535. [PubMed: 1865015]
- Saxon DW, White G. Episodic vestibular disruption following ablation of the inferior olive in rats: behavioral correlates. *Behav Brain Res*. 2006; 175:128–138. [PubMed: 16979764]
- Seoane A, Apps R, Balbuena E, Herrero L, Llorens J. Differential effects of trans-crotononitrile and 3-acetylpyridine on inferior olive integrity and behavioural performance in the rat. *Eur J Neurosci*. 2005; 22:880–894. [PubMed: 16115211]
- Sotelo C, Hillman DE, Zamora AJ, Llinas R. Climbing fiber deafferentation: its action on Purkinje cell dendritic spines. *Brain Res*. 1975; 98:574–581. [PubMed: 1182538]
- Sukin D, Skedros DG, Beales M, Stratton SE, Lorden JF, Oltmans GA. Temporal sequence of motor disturbances and increased cerebellar glutamic acid decarboxylase activity following 3-acetylpyridine lesions in adult rats. *Brain Res*. 1987; 426:82–92. [PubMed: 3690321]
- Taslim N, Soderstrom K, Dar MS. Role of mouse cerebellar nicotinic acetylcholine receptor (nAChR) $\alpha 4\beta 2$ - and $\alpha 7$ subtypes in the behavioral cross-tolerance between nicotine and ethanol-induced ataxia. *Behav Brain Res*. 2011; 217:282–292. [PubMed: 20974182]
- Turner JR, Kellar KJ. Nicotinic cholinergic receptors in the rat cerebellum: multiple heteromeric subtypes. *J Neurosci*. 2005; 25:9258–9265. [PubMed: 16207885]
- Turner JR, Ortinski PI, Sherrard RM, Kellar KJ. Cerebellar nicotinic cholinergic receptors are intrinsic to the cerebellum: implications for diverse functional roles. *Cerebellum*. 2011; 10:748–757. [PubMed: 21562921]
- Wessel K, Langenberger K, Nitschke MF, Kompf D. Double-blind crossover study with physostigmine in patients with degenerative cerebellar diseases. *Arch Neurol*. 1997; 54:397–400. [PubMed: 9109740]
- Zesiewicz TA, Greenstein PE, Sullivan KL, Wecker L, Miller A, Jahan I, Chen R, Perlman SL. A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3. *Neurology*. 2012; 78:545–550. [PubMed: 22323747]
- Zesiewicz TA, Sullivan KL. Treatment of ataxia and imbalance with varenicline (Chantix): report of 2 patients with spinocerebellar ataxia (types 3 and 14). *Clin Neuropharmacol*. 2008; 31:363–365. [PubMed: 19050414]

- Zesiewicz TA, Sullivan KL, Freeman A, Juncos JL. Treatment of imbalance with varenicline (Chantix®): report of a patient with fragile X tremor/ataxia syndrome. *Acta Neurol Scand*. 2009a; 119:135–138. [PubMed: 18771524]
- Zesiewicz TA, Sullivan KL, Gooch CL, Lynch DR. Subjective improvement in proprioception in 2 patients with atypical Friedreich ataxia treated with varenicline (Chantix). *J Clin Neuromusc Dis*. 2009b; 10:191–193.

- 3-acetylpyridine administration decreased the number of inferior olivary neurons in rats by 85%
- 3-acetylpyridine administration impaired balance, coordination and gait of rats
- varenicline and nicotine improved several of the impaired behaviors
- nicotine effects were prevented by daily preadministration of the antagonist mecamylamine
- evidence indicates that nicotinic agonists improve deficits in olivocerebellar ataxia

Fig. 1.

NeuN(+) immunohistochemistry in the inferior olive of control and 3-AP lesioned rats. Rats received injections of 3-AP (70 mg/kg, i.p.) followed at 3.5 hours by nicotinamide (300 mg/kg, i.p.), and were sacrificed after 3 days or 1, 3, or 5 weeks. Representative sections of the inferior olive were processed for NeuN(+) immunohistochemistry using a monoclonal anti-NeuN antibody, and the number of NeuN(+) cells was quantified using the optical fractionator method of stereological cell counting as described. Representative images shown were captured at 5x magnification. Sections depict the progressive loss of NeuN staining predominantly in the rostral portion of the inferior olive, with resistant cells remaining in the caudal region as shown by the arrow for the 5 week section. The graph represents means $\pm$ s.e.m. of determinations from 3 rats at each time point. All determinations following the administration of 3-AP were significantly ($p < 0.05$) different from controls as denoted by an * and determined by AVOVA followed by Dunnett's test for multiple comparisons.

Fig. 2.

3-AP impairs balance and coordination on the rotating rod and performance on a stationary beam. Rats were either trained over a 3-day period to maintain their balance on a rotating rod for 3 minutes or familiarized for 3 days to the stationary beam. Baseline performance was determined following training on the rod [A] or habituation to the beam [B], after which time rats received injections of 3-AP as for Fig. 1, and performance assessed 1 week later. Graphs depict means $\pm$ s.e.m., with individual data points shown to illustrate variations in performance of the animals prior to and following the administration of 3-AP. The number of rats assessed on the rotorod was 144; the number of rats assessed on the stationary beam was 38. The * denotes a significant ($p < 0.05$) difference between group values as determined by the Student's t-test.

Fig. 3.

3-AP decreases activity in the open field. Rats were familiarized to an open field for 3 minutes/day for 3 days after which baseline measures of velocity during movement (cm/sec), total time moving (sec), total distance moved (cm), and 'wobble' or stagger (degrees of rotation/cm) were recorded. Following establishment of baseline, rats received injections of 3-AP as for Fig. 1, and performance assessed 1 week later. Graphs depict means $\pm$ s.e.m. of determinations from 42 rats, with individual data points shown to illustrate variations in performance of the animals prior to and following the administration of 3-AP. The * denotes a significant ($p < 0.05$) difference between group values as determined by the Student's *t*-test.

Fig. 4.

3-AP alters all gait parameters. Rats were familiarized to a walled runway for 3 trials after which baseline measures were obtained. Following establishment of baseline, rats received injections of 3-AP as for Fig. 1, and performance assessed 1 week later. Graphs depict means $\pm$ s.e.m. of determinations from 76 rats, with individual data points shown to illustrate variations in performance of the animals prior to and following the administration of 3-AP. The * denotes a significant ($p < 0.05$) difference between group values as determined by the Student's t-test.

Fig. 5.

Nicotine and varenicline improve lesion-impaired performance on the rotating rod. Rats were trained and baseline performance assessed as described for Fig. 2. One week following the administration of 3-AP, rats were assigned to treatment groups using a matching procedure (animals with equal degrees of impairment were distributed evenly across treatment group). Rats received injections of saline, nicotine (0.33 mg free base/kg), or varenicline (0.75 mg varenicline free base/kg) daily for up to 4 weeks, and performance was determined at weekly intervals. To accommodate for individual differences in performance as a consequence of the administration of 3-AP, a performance ratio was calculated for each animal by dividing the time on the rotating rod after 'treatment' by the time determined at 1 week following 3-AP (see Fig. 2[A]). Each point represents the mean of determinations from 8–11 rats/group + s.e.m. Data were analyzed by a 2-way ANOVA (treatment x time or dose x time), and individual group differences determined by Tukey's test. The * denotes a significant ($p < 0.05$) difference from corresponding saline group values.

Fig. 6.

Nicotine improves lesion-induced increases in hindpaw stride width. Rats were habituated and tested as described for Fig. 4, and beginning at 1 week following the administration of 3-AP, rats were assigned to treatment groups using a matching procedure (based on the degree of impairment of hindpaw stride width). Rats received injections of saline or nicotine (0.33 mg free base/kg/day) for 1 week, and gait parameters evaluated. The performance ratio for each animal was calculated by dividing the length or width distance (cm) after 'treatment' to the distance determined at 1 week following 3-AP (see Fig. 4) to accommodate for individual differences in performance as a consequence of the administration of 3-AP. Columns represent the mean of determinations from 6–8 rats/group + s.e.m. The * denotes a significant ($p < 0.05$) difference between group values as determined by the Student's t-test.

Fig. 7.

The nicotinic antagonist mecamylamine prevents the ability of nicotine to improve performance. Rats were trained/habituated and tested on the rotorod or in the walled runway as described for Fig. 2[A] and Fig. 4, respectively. One week following the administration of 3-AP, animals were assigned to treatment groups using a matching procedure as described and received injections of saline (Sal) or mecamylamine (Mec, 0.82 mg free base/kg) followed 30 minutes later with injections of saline or nicotine (Nic, 0.33 mg free base/kg) daily for 1 week. Performance on the rotating rod [A] and hindpaw stride width [B] were determined. Columns represent the mean of determinations from 5–8 rats/group + s.e.m. The * denotes a significant ($p < 0.05$) difference from the saline/saline group as determined by ANOVA followed by Dunnett's test for multiple comparisons.

Fig. 8.

Varenicline improves lesion-impaired performance on the rotating rod. Rats were trained and baseline performance assessed as described for Fig. 2. One week following the administration of 3-AP, rats were assigned to treatment groups using a matching procedure as described. Rats received injections of saline or varenicline (0.3, 1 or 3 mg free base/kg) for 2 weeks and performance determined at weekly intervals. To accommodate for individual differences in performance as a consequence of the administration of 3-AP, a performance ratio was calculated for each animal by dividing the time on the rotating rod after 'treatment' by the time determined at 1 week following 3-AP (see Fig. 2[A]). Columns represent the mean of determinations from 5–9 rats/group + s.e.m. Data were analyzed by a 2-way ANOVA (treatment x time or dose x time), and individual group differences determined by Dunnett's test for multiple comparisons. The * denotes a significant ($p < 0.05$) difference from corresponding saline group values.

Fig. 9.

Effects of varenicline on performance on the stationary beam. Rats were familiarized to and tested on the stationary beam as described for Fig. 2[B]. One week following the administration of 3-AP, animals were assigned to treatment groups using a matching procedure as described. Rats received injections of saline or varenicline (0.3, 1 or 3 mg free base/kg) for up to 4 weeks and performance determined at weekly intervals. To accommodate for individual differences in performance as a consequence of the administration of 3-AP, a performance ratio was calculated for each animal by dividing the time on the rotating rod after 'treatment' by the time determined at 1 week following 3-AP (see Fig. 2[A]). Each point represents the mean of determinations from 6–11 rats/group + s.e.m. Data were analyzed by a 2-way ANOVA (treatment x time), and group differences

determined by Tukey's test. The * denotes a significant ($p < 0.05$) difference from corresponding saline group values.

Table 1

Effects of nicotine and varenicline administration on open field behavior of 3-AP impaired rats. At one week following the administration of 3-AP and measures of the induced impairment (see baseline and post 3-AP values in Fig.3), animals received daily injections of saline, nicotine (0.33 mg free base/kg) or varenicline (0.75 mg free base/kg) for 1 week. Locomotor activity and rotorod performance were assessed 18 hours after the final injection as described. Values are the mean of determinations from 14–15 animals/group $\pm$ s.e.m.

Measure	Saline	Nicotine	Varenicline
Velocity (cm/sec)	18.9 $\pm$ 0.78	18.7 $\pm$ 0.89	20.6 $\pm$ 0.89
Total time moving (sec)	46.3 $\pm$ 2.43	45.3 $\pm$ 3.40	48.1 $\pm$ 3.12
Total distance moved (cm)	899 $\pm$ 61.8	903 $\pm$ 73.1	994 $\pm$ 73.7
Wobble (degrees/cm)	6.36 $\pm$ 0.47	6.39 $\pm$ 0.43	5.82 $\pm$ 0.43