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Analysis of gait in rats with olivocerebellar lesions and ability of the nicotinic acetylcholine receptor agonist varenicline to attenuate impairments

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Abstract

Studies have demonstrated that administration of the neuronal nicotinic receptor agonist varenicline to rats with olivocerebellar lesions attenuates balance deficits on a rotarod and balance beam, but the effects of this drug on gait deficits have not been investigated. To accomplish this, male Sprague-Dawley rats were trained to walk on a motorized treadmill at 25 and 35 cm/s and baseline performance determined; both temporal and spatial gait parameters were analyzed. A principal component analysis (PCA) was used to identify the key components of gait, and the cumulative gait index (CGI) was calculated, representing deviations from prototypical gait patterns. Subsequently, animals either remained as non-lesioned controls or received injections of 3-acetylpyridine (3-AP)/nicotinamide to destroy the climbing fibers innervating Purkinje cells. The gait of the non-lesioned group was assessed weekly to monitor changes in the normal population, while the gait of the lesioned group was assessed 1 week following 3-AP administration, and weekly following the daily administration of saline or varenicline (0.3, 1.0, or 3.0 mg free base/kg) for 2 weeks. Non-lesioned animals exhibited a 60–70% increased CGI over time due to increases in temporal gait measures, whereas lesioned animals exhibited a nearly 3-fold increased CGI as a consequence of increases in spatial measures. Following 2 weeks of treatment with the highest dose of varenicline (3.0 mg free base/kg), the swing duration of lesioned animals normalized, and stride duration, stride length and step angle in this population did not differ from the non-lesioned population. Thus, varenicline enabled animals to compensate for their impairments and rectify the timing of the gait cycle.

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Keywords

ataxia; varenicline; gait; principal component analysis; cumulative gait index

1. Introduction

Ataxia represents an array of symptoms including uncoordinated voluntary movements and deficits in balance, locomotion and gait. These characteristics typically manifest as a result of cerebellar dysfunction that can be inherited, idiopathic or result from a stroke, traumatic brain injury, lesion, or chemical insult to the brain [1]. Thus, the symptoms and deficits manifest can vary considerably among patients. Presently, there are no approved treatments for ataxia, although evidence suggests that modulation of the nicotinic cholinergic system within the central nervous system may provide therapeutic benefit.

Both preclinical and clinical studies have shown that nicotinic cholinergic agonists are effective in reducing motor abnormalities associated with several disorders. Nicotine has been shown to potentiate haloperidol-induced hypokinesia and catalepsy in rodent models of Tourette's syndrome [2, 3], and decreases motor tics in patients with this disorder [4–6]. Several nicotinic agonists have also been shown to decrease L-dopa-induced dyskinesias in both rodent and non-human primate models of Parkinson's disease [7–12], motor abnormalities in a rodent model of tardive dyskinesia [13], and ethanol-induced ataxia in rodents [14]. In addition, balance deficits in rats following traumatic brain injury are ameliorated by the administration of the cholinesterase inhibitor physostigmine, which increases extracellular levels of acetylcholine, thereby activating nicotinic receptors [15].

Clinical studies have shown that the nicotinic agonist varenicline improves gait and/or balance deficits in patients with spinocerebellar ataxia type 3 (SCA3) and type 14 (SCA14) [16], and a recent double-blind, placebo-controlled, randomized study demonstrated that treatment with varenicline for 2 months improved axial symptoms and rapid alternating movements in adult patients with genetically confirmed SCA3 [17]. Preclinical studies from our laboratory have shown that varenicline attenuates balance deficits on the rotarod and balance beam in animals with olivocerebellar lesions [18], but the effects of varenicline on lesion-induced gait deficits in laboratory animals have not been investigated.

Assessments of gait abnormalities in both ataxic laboratory animals and humans is typically confounded by inherent variability within a given population, as well as walking speed during the assessment [19, 20]. The variability confound has been addressed in the clinical literature by the use of principal component analysis (PCA) and the subsequent determination of a gait index to express gait deviations from a normal population [21–24]. A similar approach has been used in our laboratory for preclinical studies in rats following the administration of the neurotoxin 3-acetylpyridine (3-AP), which destroys the olivocerebellar pathway [25]. These studies demonstrated that ataxic animals exhibited a nearly 3-fold increase in deviation from normal gait compared to age-matched non-lesioned animals. This finding, in concert with alterations in specific gait variables in ataxic rats that parallel those reported in clinical studies, suggested that this approach may be translatable and used to investigate the potential therapeutic benefits of nicotinic receptor agonists for the treatment

of ataxia. Thus, the goals of this study were to: 1) use a PCA to isolate the key components of gait in animals walking on a motorized treadmill; 2) calculate a cumulative gait index (CGI) as a general measure of gait function relative to population norms; and 3) evaluate the dose-response effects of varenicline on 3-AP-induced gait impairments in laboratory rats following olivocerebellar lesions.

2. Materials and Methods

2.1. Animals

Adult male Sprague-Dawley rats (225–250 g and 59–66 days old; $n = 63$; Harlan Industries, Indianapolis, IN) were housed two per cage (with enrichment) in a temperature and humidity-controlled vivarium on a 12:12 hour light-dark cycle (6 am:6 pm) with food and water available *ad libitum*. Animals were acclimated for 1 week prior to study and all behavioral evaluations were conducted between 10 am and 2 pm. The care and use of animals were approved in accordance with guidelines set by the University of South Florida Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

The DigiGait™ Imaging System and software (version 12.2; Mouse Specifics, Boston, MA) were used to generate temporal and spatial gait parameters as described [25]. Gait parameters, analyzed by a rater blinded to experimental condition, were generated from the hind limbs and included both temporal (swing duration, braking duration, propulsion duration, stance duration, stride duration and stride frequency) and spatial (stride length, stance width, paw angle, step angle, and paw area) measures, all of which are affected by the administration of 3-AP [25].

2.3. Training and baseline determinations

Animals were trained to walk on the treadmill at speeds of 25 and 35 cm/s for 2 minutes at each speed for 5 consecutive days, with the 35 cm/s trial run 1.5 hours after the 25 cm/s trial. The treadmill belt and enclosure were wiped with 70% ethanol between trials. At 2–3 days following completion of training, baseline performance was assessed for 2 minutes at each speed, and gait parameters were generated from a sequence consisting of at least 3 uninterrupted strides per paw, i.e., a total of 6 strides [25]. Animals that did not perform this sequence on a given trial were retested once at that speed. Of the 63 animals tested at baseline, 4 failed to meet this criterion and were removed from further study. For each animal, the number of sequences generated within the 2 minute trial at each speed was quantified, similar to a prior study [25].

A PCA was performed using the gait measures collected at baseline ($n = 59$) using both treadmill speeds. The factor loadings generated from this analysis were used to weight z-transformed gait variable data, which were then summed to produce deviation values for each identified factor. These values were summed to yield the CGI, an index representing overall deviations from prototypical gait patterns [25]. CGI values were calculated for each animal at each assessment time, and the group means determined.

2.4. Lesion induction and varenicline treatment

Following baseline gait assessments, animals were randomly assigned to either non-lesioned ($n = 11$) or lesioned ($n=48$) groups (Fig. 1). The non-lesioned group was included to ascertain whether multiple exposures to the apparatus over time affected gait. The non-lesioned animals were handled in a manner identical to the lesioned animals, but did not receive any injections. Animals in the lesioned group received intraperitoneal (i.p.) injections of 3-AP (70 mg/kg, Sigma-Aldrich Co., St. Louis, MO) followed by nicotinamide (300 mg/kg, i.p.) 3.5 hours later to selectively destroy the olivocerebellar pathway [18, 25]. At 1 week following baseline characterization, animals were reassessed on the treadmill for 2 minutes at 25 and 35 cm/s. Of the 48 animals who received 3-AP, 3 would not perform on the treadmill; thus, the remaining 45 animals were randomly assigned to 1 of 4 groups and received daily subcutaneous (s.c.) injections of either normal phosphate-buffered saline (PBS; 0.9%; 0.33 ml/kg) or varenicline tartrate (0.3, 1.0 or 3.0 mg free base/kg corresponding to 0.51, 1.71, and 5.13 mg tartrate salt/kg, respectively, from Tocris Bioscience, Bristol, UK or 0.43, 1.43, and 4.29 mg dihydrochloride salt/kg, respectively, kindly provided by Dr. F. Ivy Carroll, Research Triangle Institute, Research Triangle Park, N.C.) for 2 weeks. The doses of varenicline were selected based on studies demonstrating improvement of the performance of olivocerebellar-lesioned animals on the rotorod and balance beam, while the treatment duration was selected based on evidence that improvement on the rotorod plateaued following daily administration of varenicline for 2 weeks [18]. Stock solutions of varenicline were prepared weekly by dissolving the drug in PBS, and adjusting the pH to 7.4 with 1.0 N NaOH; aliquots were prepared, protected from light and stored at 4°C until use.

2.5. Gait assessment and statistics

As indicated, gait was reassessed for 2 minutes at each speed for all animals at 1, 2, and 3 weeks after baseline testing, and animals in the non-lesioned group were evaluated in parallel with lesioned animals (Fig. 1). Gait measures across assessment times were compared using analysis of variance (ANOVA) with speed and time as repeated measures, and a subsequent repeated measures ANOVA at each speed was used to isolate simple effects; planned comparisons to detect differences between assessments were conducted using Fisher's least significant difference (LSD) test. Data were analyzed for violations of sphericity, and in those instances indicating significant violations, a Geisser-Greenhouse correction was utilized to adjust criteria for significance.

For lesioned animals, behavior was always assessed prior to the daily administration of varenicline to ensure that the acute locomotor effects of the drug did not influence performance on the treadmill. Because 4 of the 45 lesioned animals were unable to complete an uninterrupted 6 stride sequence at 25 cm/s, gait parameters for these animals, irrespective of treatment group, were generated from a minimum 3 stride sequence. This approach was validated by our prior study demonstrating that gait parameters generated from the truncated sequence did not differ significantly from those generated from a longer sequence [25].

A second PCA (termed iPCA) was performed using data generated 1 week post 3-AP to identify the principal components of impaired gait in the lesioned population. This analysis

was used to calculate the iCGI, an index reflecting gait deviations within the impaired population, and used to ascertain whether varenicline affected prototypical impaired gait. The iCGI values were calculated at 1 week following the administration of 3-AP, and following the administration of saline or varenicline. To control for differences in the degree of impairment in each animal, values from saline- or varenicline-injected animals were expressed relative to their 1 week values. Data were compared using a 2×4 repeated-measures ANOVA (assessment x treatment condition) with subsequent planned comparisons using Fisher's LSD test to determine if varenicline administration altered the gait of lesioned animals. A p -value < 0.05 was accepted as criterion for statistical significance for all analyses.

3. Results

3.1. Gait in non-lesioned animals

Non-lesioned animals completed 11 ± 2.1 and 18 ± 2.9 sequences consisting of 6–11 strides during the 25 and 35 cm/s baseline assessments, respectively, and the number of sequences did not change across weeks. Analysis of gait indicated that several temporal and spatial measures differed significantly ($p < 0.05$) from baseline at a treadmill speed of 25 cm/s (Fig. 2). Stride, stance, propulsion and swing durations increased 12–17% and stride length increased 12–14% over time, with a plateau apparent at 2 weeks [for stride duration, $F(3,28) = 9.84$; for stance duration, $F(3,28) = 7.70$; for propulsion duration, $F(3,28) = 9.11$; for swing duration, $F(3,28) = 7.69$; for stride length, $F(3,28) = 9.33$]. Stance width and step angle decreased over time, with significant ($p < 0.05$) effects only at 3 weeks [for stance width, $F(3,28) = 3.68$; for step angle, $F(3,28) = 4.12$]; no other gait parameters exhibited time-dependent alterations. At a treadmill speed of 35 cm/s, all gait parameters exhibited changes similar to, but less than those observed at 25 cm/s (data not shown).

A PCA was conducted using baseline gait measures from all animals at both treadmill speeds to reduce the dimensions of the dataset. This analysis identified four principal components that accounted for 82% of the variance in the data (Table 1). The first factor or Rhythm [eigenvalue (e) = 4.9] included the temporal parameters stride duration, stride frequency, stance duration, and propulsion duration as primary measures (absolute loading value > 0.5), while the second factor or Thrust ($e = 2.1$) included braking duration, stride length, stance width, and step angle as primary measures. Although the absolute loading value for paw angle was > 0.5 for the Thrust component, the magnitude of this measure was greater for the third factor or Contact ($e = 1.14$), which also included paw area, identical to prior observations [25]. However, unlike prior observations, the PCA identified a fourth component, termed Gain ($e = 1.1$), that contained the single measure of swing duration; although step angle had a loading value > 0.5 for Gain, the magnitude of this measure was greater for Thrust. Additional analyses performed using the 1, 2 and 3 week gait measures from non-lesioned animals indicated that the factors identified at baseline were unaffected by repeated treadmill exposure. Therefore, these factors were used to calculate a CGI for non-lesioned animals at each assessment time, reflecting alterations at both treadmill speeds. In agreement with a prior study [25], the CGI did not differ significantly between baseline and assessment week 1 (Fig. 2). However, at 2 and 3 weeks, the CGI increased significantly

[$F(3, 28) = 4.91$, $p < 0.05$] by 74% and 61%, respectively, indicating an increased deviation from baseline within the non-lesioned population. Further, analysis of each principal component indicated that this divergence within the population could be attributed to a greater than 2-fold increase in Rhythm [$F(3,28) = 4.77$, $p < 0.05$]. Although Thrust and Contact increased by 35–57%, and contributed to the overall increase in the CGI, these increases were not significant; Gain did not change over time.

3.2. Gait in lesioned animals

The effects of olivocerebellar lesions on gait parameters were assessed in rats at weekly intervals at 1 week after the administration of 3-AP and following injections of saline for 2 weeks. One lesioned animal failed to run on the treadmill at 2 and 3 weeks, and thus, was removed from study. The number of 6–11 stride sequences decreased at 1 week following 3-AP administration and remained stable thereafter. At 25 cm/s, the number of sequences at baseline, 1, 2, and 3 weeks after 3-AP administration was 12 ± 1.1 , 7.0 ± 0.94 , 6.3 ± 0.8 , and 7.0 ± 1.1 , respectively. At 35 cm/s, the number of sequences at baseline, 1, 2, and 3 weeks after 3-AP administration was 18 ± 1.4 , 8.8 ± 1.1 , 11 ± 1.5 , and 11 ± 2.1 , respectively.

Gait parameters that differed significantly ($p < 0.05$) over time at a treadmill speed of 25 cm/s are shown in Fig. 3. At 1 week after the administration of 3-AP, stride, stance and propulsion durations decreased significantly ($p < 0.05$) by 10%, 14%, and 19%, respectively [for stride duration, $F(3,27) = 5.39$; for stance duration, $F(3,27) = 8.41$; for propulsion duration, $F(3,27) = 7.40$]. These temporal parameters exhibited recovery over time with stride duration at 3 weeks not significantly different from baseline; stance and propulsion durations at this time were still significantly less ($p < 0.05$) than baseline. Several spatial parameters were also significantly ($p < 0.05$) affected at 1 week following the administration of 3-AP with a 10% shorter stride length, a 42% larger stance width, a 34% smaller paw area, and a 3.6-fold increase in paw angle [for stride length, $F(3,27) = 5.03$; for stance width, $F(3,27) = 9.15$; for paw area, $F(3,27) = 20.2$; for paw angle, $F(3,27) = 33.3$]. By 3 weeks, stride length recovered and values were not significantly different from baseline. Although paw area exhibited some recovery, values at 2 and 3 weeks were significantly ($p < 0.05$) different from those at both baseline and 1 week following the administration of 3-AP. In contrast, neither stance width nor paw angle exhibited recovery, with values at 2 and 3 weeks that remained significantly ($p < 0.05$) different from baseline. At a treadmill speed of 35 cm/s, all gait parameters exhibited changes similar to those at 25 cm/s, albeit the magnitude was less, similar to observations from non-lesioned animals (data not shown).

Lesion-induced alterations in gait parameters at both treadmill speeds are reflected in the CGI, shown in the lower right corner of Fig. 3. The lesion resulted in a significant ($p < 0.05$) 2.9-fold increase in the CGI at 1 week [$F(3,27) = 21.1$], and this increase persisted for the following 2 weeks. Analysis of each principal component (Fig. 4) indicated that this increase was attributed to significant ($p < 0.05$) increases in both Thrust [$F(3,27) = 16.3$] and Contact [$F(3,27) = 23.7$]. Also depicted in the stacked histogram is a comparison of principal components in lesioned versus non-lesioned groups, further illustrating that the increased CGI following the lesion differed from that in non-lesioned rats, which was attributed to increased Rhythm.

This finding, in concert with results indicating that gait changes in lesioned animals persisted over time, led to a second analysis to determine the principal components of gait in impaired animals, termed the iPCA. This analysis identified factors that accounted for 86% of the total variance in the data (Table 2) and indicated that for lesioned animals, iRhythm ($e = 4.9$) included all the temporal parameters identified in non-lesioned animals with the addition of swing duration; although paw area had a loading value >0.5 for iRhythm, the magnitude of this measure was greater for iContact. iThrust ($e = 1.9$) included those measures identified in non-lesioned animals with the exception of stance width; the loading value for step angle was larger for iContact. iContact ($e = 1.56$) was composed of paw angle and area, which were identified in the non-lesioned population as part of this factor, but also included both stance width and step angle. Although iGain ($e = 1.04$) reflected stride length, which was not identified as part of this factor in the non-lesioned population, the loading value for this measure was greater for iThrust. Thus, the principal components of gait in the lesioned population reflected slightly different relationships and loading factors for gait measures than in the non-lesioned population. The iPCA was used to calculate the iCGI to evaluate the effects of varenicline on gait in lesioned animals. Two animals had iCGI values that were greater than 2 standard deviations from the mean and thus were excluded, resulting in a mean iCGI for all lesioned animals at 1 week following 3-AP administration of 6.91 ± 0.40 ($n = 43$).

3.3. Effects of varenicline on gait in lesioned animals

The effects of varenicline on the iCGI, expressed relative to values determined 1 week after the administration of 3-AP, are shown in Fig. 5 and illustrate that the daily administration of 3.0 mg/kg varenicline increased the iCGI, an effect that was significant [$F(3,37) = 2.98$, $p < 0.05$] relative to all other groups following 2 weeks of drug administration (3 week assessment). Further, at 2 and 3 week assessments, the proportion of animals with increased iCGIs, denoting a greater deviation from the impaired state, was greater for all varenicline-injected groups than for the saline group. For the saline group, 50% of the animals (5/10) exhibited a (+) and 50% exhibited a (−) iCGI change at 2 and 3 weeks following the lesion, whereas in all varenicline-injected groups, greater than 50% (60–90%) of the animals exhibited a (+) change indicating a tendency to change from impaired performance.

Analysis of the factors comprising the iCGI indicated that the increased deviation from impaired gait following varenicline (3.0 mg free base/kg) treatment (Table 3) was attributed to a significant ($p < 0.05$) 3-fold increase in iRhythm [$F(3,37) = 3.61$] and an almost 2-fold increase in iThrust relative to saline-injected group values. Although the overall ANOVA for the latter was not significant [$F(3,37) = 1.62$], a planned comparison of saline versus varenicline (3.0 mg free base/kg) indicated a significant ($p < 0.05$) difference between groups. As a consequence of these findings, individual gait parameters from non-lesioned and lesioned animals following the administration of saline or varenicline (3.0 mg free base/kg) for 2 weeks were compared at 3 weeks. Results are shown in Fig. 6. The administration of varenicline normalized swing duration, i.e., swing duration for varenicline-injected animals was significantly ($p < 0.05$) different than for saline-injected animals, and not significantly different from the non-lesioned group [$F(2,29) = 6.50$]. In addition, saline-injected animals exhibited significant ($p < 0.05$) alterations in stride duration, stride length, and step angle

relative to non-lesioned animals, whereas these gait parameters did not differ between varenicline-injected and non-lesioned animals [for stride duration, $F(2,29) = 5.79$; for stride length, $F(2,29) = 5.44$; for step angle, $F(2,29) = 5.45$]. Thus, varenicline ‘tended to normalize’ stride duration, stride length, and step angle.

4. Discussion

The goal of this study was to determine the dose-response effects of varenicline on 3-AP-induced gait impairments in laboratory rats following olivocerebellar lesions using a PCA and consequent calculation of a single index to characterize gait deviations. Results demonstrate that the gait of ataxic animals differs from that of non-lesioned animals, and that the administration of 3.0 mg free base varenicline for 2 weeks leads to gait adaptations of several parameters. Importantly, these adaptations attenuate 3-AP-induced gait impairments at a walking speed of 25 cm/s, suggesting that these animals compensate for their impairment by altering several components of gait.

The present study extends prior findings [25] and demonstrates that weekly experience on a treadmill for up to 3 weeks, albeit limited to 4 minutes per time, leads animals to acclimate or habituate to the apparatus, resulting in a significant increased deviation of gait relative to baseline determinations. Analysis of the factors that comprised the CGI revealed that the maximal 74% increased deviation over time could be attributed primarily to alterations in Rhythm, which reflected changes in the temporal measures of stride, stance, and propulsion durations. Thus, increased treadmill experience leads to alterations in gait cadence in normal animals over time.

In contrast to the role of temporal measures (Rhythm) underlying the increased CGI in non-lesioned animals over time, analysis of the factors contributing to the increased CGI as a consequence of the lesion indicated a role for Thrust and Contact, reflecting primarily stride length, stance width, step angle, and paw area and angle. Thus, the primary deficit in lesioned animals may be attributed to spatial alterations, and are likely responsible for the inability of these animals to maintain the temporal parameters involved in normal rhythm. Due to the major differences in gait between lesioned and non-lesioned animals, a second PCA was performed on lesioned animals to identify components of the principal factors in the impaired population, and thus serve as a reference for evaluating the effects of varenicline.

Results from the iPCA determined from gait measures in lesioned animals were similar, but not identical to those from the PCA determined from baseline gait measures from non-lesioned animals. The major difference between these analyses was a change in the association of swing duration from Gain for the PCA to iRhythm for the iPCA. In addition, stance width and step angle, which were associated only with Thrust for the PCA, were associated with both iThrust and iContact for the iPCA, with the loadings for iContact greater than those for iThrust. Thus, following 3-AP administration, the timing of the gait cycle (iRhythm) is limited by the duration of the swing phase, and the contribution of stance width and step angle to Thrust is reduced. Importantly, the large increase in association of stance width with iContact likely indicates a postural change to increase the base of support

of the animal. Because step angle is directly influenced by stance width, the increased association of step angle with iContact is most likely due to this change in stance width. Thus, the iPCA identified a new basis that best represents the dynamics of gait in the lesioned population relative to the non-lesioned population, and as such, represents a valid reference for evaluating the effects of varenicline. When the iCGI was calculated from the iPCA and the dose-response effects of varenicline determined, results indicated that the highest dose of drug (3.0 mg free base/kg) administered for 2 weeks led to a nearly 2-fold increase in the iCGI, indicating that gait following the administration of this dose of varenicline was unlike that of the impaired population. Further, this increase was attributed to increases in iRhythm and iThrust. Analysis of individual gait parameters indicated that the increase in iRhythm reflected a drug-induced increase in swing duration and consequent increased stride duration to values that did not differ from the non-lesioned population. Similarly, the increase in iThrust reflected an increase in stride length and consequent decrease in step angle to values that were not significantly different from those in the non-lesioned population. Thus, varenicline enabled animals to compensate for their impairment by normalizing swing duration and stride length, thereby rectifying the timing of the gait cycle.

Although several studies have used a treadmill system to investigate alterations in both temporal and spatial gait parameters in animal models of disease [26–30], it can be difficult to conceptualize how alterations in the parameters generated affect overall gait, especially because of differences in magnitude and direction. Using a PCA to identify the key components of gait enables calculation of an index representing gait deviations from a defined population. Although this method has been used clinically to characterize gait deficits in several clinical conditions [21–24], it has not been adopted for use in preclinical studies. This approach has tremendous power in that it provides a single value from a large and often redundant dataset that is sensitive to small changes in individual gait measures, and should be utilized because of its translational potential to provide a succinct and reliable indicator of gait deviation.

Results from this preclinical study, as well as clinical evidence [17], clearly indicate that varenicline is efficacious for alleviating both balance and gait deficits in ataxia. It remains to be determined whether these actions: 1) are mediated by the varenicline-induced activation or desensitization of neuronal nicotinic receptors, and which specific receptor subtypes are involved; and 2) occur as a consequence of a direct effect on the cerebellum or other anatomic regions. Indeed, varenicline has been shown to have full and/or partial agonist activity at $\alpha 4\beta 2$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 6$ -containing, and homomeric $\alpha 7$ nicotinic receptors [31–34], all of which are expressed in the cerebellum [35–37]. Alternatively, it is possible that the direct effects of varenicline are mediated by other neuroanatomical regions. This idea is supported by studies indicating that the loss of balance resulting from cortical impact injury leads to compensatory changes in brain regions remote to the site of injury, and that administration of the cholinesterase inhibitor physostigmine, which alleviated the impairment, increased the activity of these remote pathways [38].

In sum, results indicate that varenicline improves gait in laboratory animals with olivocerebellar lesions, a finding similar to that reported for patients with SCA3 [17]; the

cellular mechanisms involved remain to be elucidated. Results also underscore the benefit of using a PCA to consolidate specific gait measures into a limited number of factors to characterize gait. This analysis can then be used to provide a meaningful index that is sensitive to subtle changes in gait function. This approach represents a powerful tool that has been underutilized in both preclinical and clinical studies.

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Abbreviations

3-AP	3-acetylpyridine
CGI	Cumulative Gait Index
PCA	Principal Component Analysis

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Highlights

- Principal component analyses identified key components of gait for rats
- A cumulative gait index represented gait deviations from population norms
- Olivocerebellar lesions altered principal gait components and increased deviations
- Varenicline (3 mg free base/kg/day for 2 weeks) improved impaired gait

Fig. 1.

Experimental paradigm. Rats were trained to walk on the DigiGait™ system for 5 consecutive days and gait assessments were conducted at baseline and weekly thereafter for up to 3 weeks. Non-lesioned (control) animals were assessed in parallel with lesioned animals, the latter receiving injections of 3-AP following baseline assessment. At 1 week after the lesion and reassessment, rats received daily injections of either saline or varenicline (0.3, 1.0, or 3 mg free base/kg); gait was reassessed following 1 or 2 weeks. A PCA was conducted using gait data generated from all animals at baseline, and results used to calculate a CGI for non-lesioned and lesioned/saline-injected rats. Similarly, an analysis was also performed with gait data from all lesioned animals (iPCA), and results used to calculate the iCGI for lesioned/saline-injected and lesioned/varenicline-injected rats. The CGI or iCGI were calculated using z-transformations of each gait measure and the loadings from the PCA or iPCA, respectively.

Fig. 2. Gait parameters and the CGI in control rats. Temporal and spatial gait parameters were determined in rats for 2 minutes at treadmill speeds of 25 and 35 cm/s at weekly intervals; data shown is from 25 cm/s. Each point is the mean $\pm$ s.e.m. of determinations from 11 animals. Data were analyzed by a one-way ANOVA and Fisher's LSD test. The asterisks denote significant ($p < 0.05$) differences from baseline (Base).

Fig. 3.

Gait parameters and the CGI in lesioned rats. Temporal and spatial gait parameters were determined in rats for 2 minutes at treadmill speeds of 25 and 35 cm/s at baseline (Base) and weekly intervals following the administration of 3-AP; data shown is from 25 cm/s. Each point is the mean $\pm$ s.e.m. of determinations from 10 animals. Data were analyzed by a one-way ANOVA and Fisher's LSD test. The single asterisks denote significant ($p < 0.05$) differences from baseline; the double asterisks denote significant ($p < 0.05$) differences from 1 week determinations.

Fig. 4.

Effects of 3-AP on CGI principal component deviations. Z-transformations of each gait measure and the loadings from the PCA were used to calculate the deviations attributed to each factor. Values for each factor from non-lesioned (NL) and lesioned (Les) animals were calculated following weekly assessments and compared using a 2×4 repeated-measures ANOVA (treatment $\times$ assessment) and Fisher's LSD test. Data represent mean $\pm$ s.e.m. of determinations from 11 (NL) or 10 (Les) animals per group. The asterisks denote significant ($p < 0.05$) differences from corresponding group baseline values.

Fig. 5.

Effects of varenicline on the iCGI. Temporal and spatial gait parameters were determined in rats for 2 minutes at treadmill speeds of 25 and 35 cm/s following the administration of 3-AP, and a PCA was conducted using gait data generated from all lesioned animals (iPCA), with results used to calculate the iCGI. Following the 1 week gait assessment, animals received daily injections of saline or varenicline (0.3, 1.0 or 3.0 mg free base/kg), and gait reassessed weekly for the following 2 weeks. The mean iCGI of all lesioned animals at 1 week following the administration of 3-AP was 6.91 ± 0.40 ($n=43$) and did not differ significantly by group assignment. The iCGI values at 2 and 3 weeks after 3-AP were: 7.2 ± 0.47 and 7.7 ± 1.3 , respectively, for the saline group; 8.5 ± 1.4 and 8.0 ± 0.8 , respectively, for the 0.3 mg/kg varenicline group; 8.2 ± 0.77 and 7.9 ± 1.1 , respectively, for the 1.0 mg/kg varenicline group; and 8.8 ± 1.2 and 10.5 ± 1.2 , respectively, for the 3.0 mg/kg varenicline group. Data are expressed relative to pretreatment (1 week assessment) values to control of individual differences in impairments. The bars represent group mean values $\pm$ s.e.m., and the ratio in each bar is the number of animals exhibiting a (+) change relative to initial lesioned values relative to the total number of animals (n) in each group. Data were analyzed by a 2×4 ANOVA (assessment $\times$ treatment) and Fisher's LSD test. The asterisk denotes significant ($p < 0.05$) differences from saline group values.

Fig. 6.

Effects of saline or varenicline on absolute gait measures. Temporal and spatial gait parameters were determined in lesioned rats following the daily administration of saline (Sal) or varenicline (Var, 3.0 mg free base/kg) for 2 weeks, and in corresponding non-lesioned (NL) rats. Bars represent the mean $\pm$ s.e.m. of determinations from 10 (Sal or Var) or 11 (NL) animals/group. Data were analyzed by ANOVA and Fisher's LSD test. The single asterisk denotes significant ($p < 0.05$) differences from NL group values and the double asterisk denotes significant ($p < 0.05$) differences from Sal group values.

Table 1

Principal component analysis at baseline.

Gait Measure	Rhythm (factor 1)	Thrust (factor 2)	Contact (factor 3)	Gain (factor 4)
Stride Duration	.979	-.146	.120	.012
Stride Frequency	-.979	-.110	-.120	-.004
Stance Duration	.977	-.044	.034	-.126
Propulsion Duration	.910	-.300	-.021	-.032
Braking Duration	.467	.642	.169	-.327
Swing Duration	.338	-.440	.390	.612
Stride Length	-.458	-.548	.285	.262
Stance Width	.187	.663	-.129	.460
Step Angle	.208	.570	-.376	.524
Paw Angle	.032	.527	.697	.022
Paw Area	.365	-.260	-.683	.023
% Total Variance	40.5	19.5	12.8	9.6

A principal component analysis was conducted using gait measures from baseline assessments of all animals (n=59). For each measure, the largest absolute loading value across the 4 factors is bolded, representing primary components of each factor.

Table 2

Principal component analysis in impaired animals (iPCA).

Gait Measure	iRhythm (factor 1)	iThrust (factor 2)	iContact (factor 3)	iGain (factor 4)
Stride Duration	.983	.150	-.071	-.033
Stride Frequency	-.969	-.157	.113	-.006
Stance Duration	.952	.194	.014	-.193
Propulsion Duration	.936	.013	.045	-.213
Braking Duration	.330	.662	-.104	-.001
Swing Duration	.738	-.015	-.321	.475
Stride Length	.302	-.652	-.065	.624
Stance Width	.034	.450	.635	.493
Step Angle	-.111	.589	.606	.104
Paw Angle	-.343	.621	-.537	.161
Paw Area	.505	-.481	.548	-.156
% Total Variance	43.8	19.2	13.6	9.1

A principal component analysis was conducted using gait parameters generated from animals (n = 45) at 1 week after the administration of 3-AP, termed the iPCA. For each variable, the largest absolute loading value across the 4 factors is bolded, representing primary components of each factor.

Table 3

Effects of varenicline on iCGI principal component deviations.

Group	iRhythm (factor 1)	iThrust (factor 2)	iContact (factor 3)	iGain (factor 4)
Saline	1.17 ± 0.24	1.27 ± 0.33	2.14 ± 0.85	2.23 ± 0.88
Varenicline (mg free base/kg)				
0.3	1.40 ± 0.29	1.79 ± 0.31	1.43 ± 0.33	2.05 ± 0.94
1.0	1.59 ± 0.44	1.71 ± 0.35	1.07 ± 0.23	1.35 ± 0.37
3.0	3.51 ± 0.99*	2.39 ± 0.44*	1.53 ± 0.55	3.52 ± 1.85

Animals received 3-AP followed 1 week later by daily injections of saline or varenicline for 2 weeks. The z-transformations of each gait measure and the loadings from the iPCA were used to calculate the deviations attributed to each factor. Values for each factor were calculated and compared using ANOVA, and Fisher's LSD test was used for planned comparisons of saline versus varenicline. Data represent mean ± s.e.m. of determinations from 10 (saline and varenicline, 1.0 and 3.0 mg/kg) or 11 varenicline, 0.3 mg/kg) animals per group. The asterisks denote significant ($p < 0.05$) differences from corresponding saline group values.