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Jeffrey P. Kaipust
University of Nebraska at Omaha, jkaipust@unomaha.edu

Denise McGrath
University of Nebraska at Omaha

Mukul Mukherjee
University of Nebraska at Omaha, mmukherjee@unomaha.edu

Nicholas Stergiou
University of Nebraska at Omaha, nstergiou@unomaha.edu

Title Page

Gait variability is altered in older adults when listening to auditory stimuli with differing temporal structures

Jeffrey P Kaipust[1], Denise McGrath[1], Mukul Mukherjee[1], Nicholas Stergiou[1,2]

[1] Nebraska Biomechanics Core Facility

Department of Health, Physical Education, and Recreation

University of Nebraska at Omaha

6001 Dodge Street

[2] College of Public Health

Department of Environmental, Agricultural & Occupational Health

University of Nebraska Medical Center

984355 Nebraska Medical Center

Omaha, NE 68198

Corresponding Author:

Nicholas Stergiou, PhD

University of Nebraska at Omaha

6001 Dodge Street, HPER 207

Omaha, NE 68134

Phone: 402-554-3247

Fax: 402-554-3693

nstergiou@unomaha.edu

Abstract and key terms

Gait variability in the context of a deterministic dynamical system may be quantified using nonlinear time series analyses that characterize the complexity of the system. Pathological gait exhibits altered gait variability. It can be either too periodic and predictable, or too random and disordered, as it is the case with aging. While gait therapies often focus on restoration of linear measures such as gait speed or stride length, we propose that the goal of gait therapy should be to restore optimal gait variability, which exhibits chaotic fluctuations and is the balance between predictability and complexity. In this context, our purpose was to investigate how listening to different auditory stimuli affects gait variability. Twenty-seven young and 27 elderly subjects walked on a treadmill for 5 minutes while listening to white noise, a chaotic rhythm, a metronome, and with no auditory stimulus. Stride length, step width, and stride intervals were calculated for all conditions. Detrended Fluctuation Analysis was then performed on these time series. A quadratic trend analysis determined that an idealized inverted-U shape described the relationship between gait variability and the structure of the auditory stimuli for the elderly group, but not for the young group. This proof-of-concept study shows that the gait of older adults may be manipulated using auditory stimuli. Future work will investigate which structures of auditory stimuli lead to improvements in functional status in older adults.

Key terms: Detrended Fluctuation Analysis, chaos, fractal scaling, metronome, walking, locomotion, complexity

Introduction

Human movement variability is now recognized as an important construct in contemporary literature, having previously been dismissed as unwanted noise in the movement signal. It can be described as the normal variations that occur in motor performance across multiple repetitions of a task (33). Gait variability refers to the natural stride-to-stride fluctuations that are present during locomotion. These fluctuations have been described in terms of fractal and chaotic dynamics. The temporal structure of gait variability in healthy people exhibits deterministic processes where each step is correlated with an earlier and a later step, producing long-range correlations over large time series (fractal dynamics) (11). As well as being deterministic, healthy gait variability can also be described as complex (30). Our gait is not bound by determinism; rather it is capable of reorganizing quickly and seamlessly in unpredictable ways in response to changes in our environment (deterministic chaos). Gait variability in the context of a deterministic dynamical system may be quantified using nonlinear time series analyses that characterize the complexity of the system. The complexity of human physiology has been characterized using fractal measures and their dynamics. Fractals are considered as the natural outcome of complex dynamical systems behaving at the frontier of chaos (21).

This optimal combination of predictability and complexity of gait enables us to navigate our environment in a stable but flexible manner. The study of complexity in gait has shown that pathological gait, on the other hand, can be either too periodic and predictable, or too random and disordered (22,23). Hausdorff and colleagues (9) have shown that complexity of stride dynamics matures as a child develops. At the other end of the age spectrum they have shown a degradation of fractal scaling in the gait of older adults. Karmaker *et al.* (18) have shown that older adults at risk of falls exhibit reduced complexity of foot-ground clearance while walking compared to healthy elderly. Thus, there appears to be an optimal level of complexity that is associated with healthy and proficient locomotion. This is the theory of Optimal Movement Variability (32,33), which is based on observations from other biological systems such as the cardio-respiratory systems (8,26,27), and adapted to human movement. Optimal complexity of a physiological system is thought to represent an underlying capability that enables effective cooperation between the participating subsystems which enhances the system's ability to adapt to changing task demands (28,32). Thus, while gait therapies often focus on the restoration of linear measures such as standard deviations of gait speed and spatio-temporal parameters to normative levels, we propose that the goal of gait therapy should be to restore optimal gait variability i.e. the balance between predictability and complexity (32,33) in those populations who exhibit suboptimal patterns of gait variability.

Auditory cueing has recently emerged as a promising tool for rehabilitation of gait disorders (29,36). Typically, the patient is instructed to walk in time to a metronome, which emits a periodic, invariant beat. Whilst improvements in stride length, cadence and gait asymmetry have been observed in these experimental conditions, we submit that this approach is not ideal. Training patients to walk to a metronomic beat with no variability runs contrary to the natural stride-to-stride fluctuations that are known to exist in human gait (1). These fluctuations exhibit a chaotic structure where a rich repertoire of locomotor patterns is available when required (20). Elimination of these fluctuations may have implications for reduced ability to interact adaptively and safely to a continuously changing environment, where often our gait must be adjusted in a matter of milliseconds.

The purpose of this proof-of-concept study was therefore to investigate how listening to different auditory stimuli affects gait variability. Each auditory stimulus had a different structure:

white noise, an auditory stimulus based on a chaotic rhythm, and a metronome. The ability of humans to entrain movement to an external rhythm has been demonstrated even when there is a high degree of rhythmic complexity and ambiguity (37). Our experimental design is based upon a theoretical model that describes an inverted-U relationship between complexity and predictability of gait, with optimal gait variability residing at the apex of the inverted U (figure 1). We sought to investigate the underlying trend between auditory stimuli with varying temporal structures that spanned the predictability continuum, and the resulting complexity of spatio-temporal gait parameters. We hypothesized that this would change depending on the structure of the stimulus, in accordance with our theoretical model (figure 1). Previous research by the authors has shown that optimal gait variability degrades with ageing, compared to healthy young adults (1). Changes in gait variability have been associated with an increased risk of falling in older adults (13). We therefore sought to investigate if gait variability can be manipulated using auditory stimuli in a sample of older adults, as well as healthy young subjects. In addition to the three auditory stimuli, we also included a condition where the subjects walked with no auditory stimulation ("No stimulus"). We expected the young and elderly groups to exhibit slightly different trends with respect to the "no stimulus" condition in terms of where it would lie on the inverted-U shaped curve. While we hypothesized that both groups would demonstrate a curvilinear trend similar to figure 1, we expected older adults' gait variability in the "no stimulus" condition to exhibit a trend towards lower levels of complexity than when listening to the chaotic auditory stimulus, due to the changes in gait variability associated with ageing. We did not expect to see this trend in the young group.

Materials and Methods

Participants

Twenty-seven young subjects and twenty-seven elderly subjects participated in this study (Table 1). All subjects were free of any pathological condition that directly affected the musculoskeletal system such as rheumatoid arthritis, arterial disease, neuropathy or myopathy, vertigo, scoliosis, joint replacement, diabetes, stroke, pulmonary diseases, asthma, recent surgery, acute illness, or a history of pulmonary, cardiac, or locomotor disorders. All subjects were required to fill out a medical history questionnaire. Before participating in the study all subjects signed an informed consent approved by the institutional review board of the University's Medical Center.

Experimental Procedures

A Northern Digital 3D Investigator (Northern Digital Inc. Waterloo, Ontario, Canada) six camera system was used to capture spatiotemporal data while subjects walked on a treadmill (Bertec Corporation, Columbus, OH). This active motion capture system was used to collect kinematic data at 100 Hz using the NDI First Principles software. Lightweight, wireless smart markers were tracked by the system, and recorded in 3D space. The markers were arranged in groups of three creating a rigid body. Three such rigid bodies were placed on the lateral aspect of the legs and foot: above the knee on the upper thigh, above the ankle on the lower shank, on the midfoot of the shoe and on the sacrum. The 3D unfiltered position data from individual markers were exported and processed with custom made software developed in MatLab (Mathworks Inc., MA). The position data were used to calculate stride length, step width, and stride interval.

Prior to data collection, subjects were asked to walk on a treadmill for a maximum of 8 minutes to accommodate to treadmill walking. During the eight minute warm-up a self-selected speed was determined for each subject. This speed represented the most comfortable and natural walking speed for the subject. After the warm-up period, a total of four walking conditions were implemented while walking on the treadmill at the self-selected speed: walking while listening to white noise, to a chaotic rhythm, and to a metronome, and walking without listening to any stimulus. Each condition lasted for six minutes. At the beginning of each condition, each subject walked for a minute with the specified auditory stimulus for familiarization. After this one minute of familiarization, a five-minute recording period began. The order of the conditions was randomized.

The auditory stimuli were delivered through headphones with adjustable volume for the subjects' comfort (Turtle Beach, Voyetra Turtle Beach Inc., Elmsford, NY). The chaotic music was created from the WolframTones website (http://tones.wolfram.com) using Rule 30 as it is a class III rule that generates chaos which is found in nature (39). It is a complex system based on simple rules that is robust to small perturbations, but produces very different behavior when larger perturbations are introduced, typical of a chaotic system. White noise was downloaded from whitenoisemp3.com. The metronome beat was delivered via a portable metronome (Boss Dr. Beat DB-30 metronome, Roland Corporation, Los Angeles, CA). To determine the frequency of the metronome, the cadence of the self-selected pace was calculated. This cadence was calculated from the last minute of the eight minute warm-up period and the metronome was set to this frequency. The headphones were plugged directly into the metronome to produce the sound for the subject. Lastly, for the 'no stimulus' condition, subjects were still asked to wear the headphones but nothing was played through the headphones in this condition. The subjects were not explicitly instructed to walk to the beat of the rhythm.

Data Analysis

Treadmill data from the three-dimensional marker trajectories were exported and processed in custom software using MATLAB (MathWorks Inc., Natick, MA). This software was used to calculate the stride length, step width, and stride interval time series. Stride length was defined as the distance between two consecutive heel contacts of the same leg. To calculate step width, first the midpoint between the heel and the toe for each foot was determined during the walking trial. Step width was then defined as the distance between the midpoints of one foot during its stance phase and the subsequent midpoint of the opposing foot stance phase. Stride interval was defined as the time between two consecutive heel contacts of the same leg. Stride length, step width, and stride intervals were calculated from 151 continuous strides for all conditions. Detrended Fluctuation Analysis (DFA) was then performed on these time series. Previous research has similarly used five minutes of walking to investigate the fractal scaling of stride interval time series (17), however Damouras et al. (3) suggest that upwards of 600 strides are required to estimate the DFA scaling exponent α with an accuracy of ±0.1. Naturally, longer data series are more representative of the variable under investigation. However, since this investigation is not concerned with the absolute α-value, but rather the trend exhibited by the data as the subjects are exposed to different auditory conditions, we deemed this data length to be appropriate.

Many dynamic systems generate outputs with fluctuations characterized by 1/f-like scaling of the power spectra, S(f) where f is the frequency (16). The 1/f spectrum in the fluctuations is thought to result from the presence of many components interacting over a wide range of time or space scales (7). Fluctuations exhibiting 1/f-like behavior are often termed “complex,” since they obey a scaling law indicating a hierarchical fractal organization of their frequency time scale components rather than being dominated by a single frequency (16). DFA has been proposed as a method to quantify the complexity of a physiological signal. It evaluates the presence of long-range, power-law correlations as part of multifractal cascades that exist over a wide range of time scales. Many biological signals are noisy, heterogeneous and exhibit nonstationarities which can affect the correlation properties of the signal. One of the main advantages of using this method is that it allows for the detection of long-range power-law correlations in noisy signals with embedded polynomial trends that can match the true correlation in the fluctuation of the signal (2).

The DFA algorithm was implemented in MATLAB according to the methods used by Peng et al. (26). This method first forms an accumulated sum of the time series, sectioning it into windows, and then the log of the average size of fluctuation for a given window size is plotted against the log of the window size. In brief, if $B(i)$ is the ith interval and B_{ave} is the average interval then:

$$y(k) = \sum_{i=1}^{k}[B(i) - B_{ave}] \quad \text{Equation 1}$$

Thus, the time series is divided into boxes of equal length, n. In each box of length n, a least-squares line is fit to the data. The y coordinate of the straight-line segments is denoted by $y_n(k)$. The time series is detrended, $y(k)$, by subtracting the local trend, $y_n(k)$, in each box and then the root mean square fluctuation of this integrated and detrended time series is calculated by equation 2. This calculation is repeated across the entire times series to provide a relationship between $F(n)$, the average fluctuation as a function of box size, and the box size n. A linear relationship on a double log graph indicates the presence of scaling. The fluctuations can be characterized by the scaling exponent α, the slope of the line relating log $F(n)$ to log n (26).

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[y(k) - y_{n(k)}]^2} \quad \text{Equation 2}$$

The α-value resulting from DFA is described in the time series framework as follows: $\alpha > 0.5$ indicates that an increasing trend in the past is likely to be followed by an increasing trend in the future e.g. a long stride length is correlated with a long stride length. The series is said to be persistent. Conversely, $\alpha<0.5$ signifies that an increasing trend in the past is likely to be followed by a decreasing trend in the future e.g. a long stride length is followed by a short stride length. The series is then said to be anti-persistent (5); α=0.5 suggests a random, or uncorrelated, time series i.e. white noise.

Statistical Analysis

The mean α-values for each condition were calculated for stride length, step width, and stride interval time series. Linear and quadratic trend analyses (19) were performed for the young and elderly group. This is a theoretically driven analysis that evaluates the trend components that describe the data. The independent variable represents different amounts of a single common variable i.e. predictability. The variables are ordered conceptually along the x-axis in terms of the levels of predictability that they represent. This approach provides information about the form of the relationship between the independent and dependent variables, and thus enables us to test our inverted-U hypothesis. The analysis begins with an assessment of the linear component to see if the simplest mathematical function will describe the data. We do not expect this to be the case, and will therefore proceed to test for the more complex quadratic trend component. A quadratic trend is one that displays concavity e.g. an inverted u-shape. Trend analysis enables us to determine whether an idealized inverted-U shape is present in our data. Young and elderly groups were examined separately as slightly different trends with respect to the "no stimulus" condition were expected. The independent variable was auditory stimulus with four conditions – white noise, chaotic auditory stimulus, no stimulus, and metronome – ordered along the predictability dimension illustrated in figure 1. The trend analysis enabled us to examine the form of the relationship between the auditory stimuli and the α-value, as a measure of complexity. Our hypothesis stated that this relationship should take the form of an inverted-U (Figure 1), indicating that gait variability may be manipulated using auditory stimuli in accordance with the theory of

Optimal movement variability. We therefore expected the test for a linear trend to be non-significant, and the test for a quadratic trend to be significant for all gait parameters. Significance was set at an alpha value of 0.05. Coefficients for the linear trend were (-3, -1, 1, 3) and coefficients of quadratic trend analysis were (1, -1, -1, 1). Analysis of variance was not implemented as it is based on linear modeling, whereas our hypothesis was of a curvilinear nature.

Results

In the elderly group, the linear trend analysis did not reveal any significant trends for the three gait parameters. The quadratic trend analysis on the other hand, revealed significant trends for stride length ($F(3,104)$:2.79; $p=0.044$), step width ($F(3,104)$:4.88; $p=0.003$), and stride interval ($F(3,104)$:9.54; $p=0.0001$). These results suggest that an inverted-U shaped relationship exists between the temporal structure of auditory stimuli ordered along the predictability continuum, and the complex structure of gait variability (figure 2). As expected, walking with the chaotic auditory stimulus resulted in a trend towards more complex gait than when walking with no stimulus in this group. In contrast, no significant linear or quadratic trends were observed in our young group of subjects for any of the three gait parameters (Figure 3). Quadratic trend results were as follows: stride length (F:1.42; df:3,104; $p=0.242$), step width (F:4.88; df:3,104; $p=0.154$), and stride interval (F:0.48; df:3,104; $p=0.69$). This suggests that the younger subjects were not as sensitive to the effect of the auditory stimuli as their older counterparts.

Discussion

This was a proof-of-concept study that aimed to investigate the effect of different auditory stimuli on gait variability. The study was based on the theory of Optimal Movement Variability that proposes an inverted-U shaped relationship between predictability and complexity of gait. We sought to determine if such an inverted-U shaped relationship exists when gait is manipulated using auditory stimuli with differing temporal structures. Our results show significant quadratic trends for all three gait parameters in the elderly group, indicating that the structure of gait variability can be manipulated using auditory stimulation. Furthermore, the auditory condition that produced optimal gait variability for all parameters (i.e. at the apex of the inverted-U shaped curve) was the stimulus with the chaotic structure. Thus, we accept our hypothesis concerning the elderly group. In contrast, we reject our hypothesis that relates to the young group, as significant quadratic trends were not observed in any gait parameter. As expected, no consistent trend was observed in terms of the chaotic stimulus and no stimulus conditions, as outlined in the introduction. Unlike the elderly group, who consistently exhibited a trend towards less complex gait in the no stimulus condition compared to the chaotic condition, no such trend was observed in the young. We propose a possible explanation for these findings.

The subjects were not explicitly instructed to walk to the beat of the rhythm. It is possible that rather than synchronizing with the auditory stimuli, subjects adopted a strategy whereby they ignored the auditory stimuli. Following the protocol, the subjects reported that the white noise in particular was distracting. They may therefore have tried to make a conscious effort not to attend to the stimuli. The increased use of portable electronic devices in the younger population suggests that walking and listening to auditory stimulation may be an accustomed activity for some individuals. If this is the case, then the younger subjects may have been more successful in ignoring the auditory stimuli. A recent study by Neider et al. (24) showed that dual-task costs were largely absent in a younger adult group when asked to cross a busy road while listening to auditory stimuli, compared to an older group. Younger adults may therefore have dedicated less attentional resources to the auditory stimuli in this study, perhaps due to habituation of similar tasks in their daily lives. This speculation cannot be proven at this time however, as we did not record previous history of listening device usage in this study. We have since developed our research paradigm by designing a chaotic auditory stimulus that is individualized according to each person's natural stride cadence. This stimulus is embedded into familiar music with a stronger beat than the stimulus used in the current study. This significant modification appears to enhance the auditory-motor coupling in young adults.

One observation that does appear consistent across gait parameters and groups (with the exception of stride length in the elderly group) is that the metronome condition yielded the lowest α-value compared to the other three auditory conditions. While all of our data fell within the α-values larger than 0.5 range (i.e. persistent correlations) (Table 2), the consistently lower values for the metronome condition suggests a trend towards less complex walking behavior. This trend supports our suggestion that a metronomic beat may not be the optimal temporal structure for a rhythmic auditory stimulus, commonly used in gait rehabilitation. In contrast, for the elderly group, α-values for the chaotic auditory stimulus were located around the apex of the inverted-U shaped curve, above the level of their normal walking in the no stimulus condition. Fractal properties are indicative of intrinsic stability within a complex system that emerges from a subtle cooperation between the many components of that system (4). A degradation of this cooperation within the locomotor system is likely to have negative consequences for the individual such as poor control of gait. Previous research has reported a degradation of long-range correlations in gait parameters when listening to a metronome (12,15,35,38), yet Delignères and Torre (4) have concluded that the intrinsic complexity of the system is still at work in metronomic conditions, but simply expressed differently: in the asynchronies to the metronome. Unfortunately, our experimental design did not facilitate the calculation of asynchronies to the beats, so we were not able to explore this notion in our study. However, previous research has shown elderly adults with low fractal scaling (as was the case in this study when walking to a metronome) are more likely to fall than those with a high fractal scaling, and this index is a better predictor of falling than other indices (14).

While the consistently lower α-values for the metronome condition indicated a less persistent gait pattern compared to the other conditions, all α-values remained above 0.5. This result is at odds with some recent literature that has demonstrated a strong anti-persistent pattern (i.e. $\alpha < 0.5$) in the control variable when walking to an auditory metronome (31,34). A possible explanation for this difference is that the subjects in our study were not instructed to walk in time to the metronome beat. The modality of gait control in this case may have remained in the more automated/unconscious mode resulting in a persistent pattern across strides, compared to when subjects are consciously trying to walk to a beat, which tends to produce an anti-persistent pattern (as proposed by Terrier and Deriaz (34)). Another possible explanation for the discrepancy may relate to the nature of metronomic walking. If walking to a metronome leads to a breakdown of power law behavior, then any measurement of an alpha value is, by definition, spurious and potentially unreliable.

While it is important to determine what precise nonlinear dynamics are at play when walking to a metronome, the broader and arguably more important question is how this relates to motor learning and function. Our study has shown that the fractal scaling of gait follows a trend towards less complexity when listening to a metronomic beat in a group of elderly adults. Future work needs to investigate if using a metronome for gait rehabilitation in populations with declining gait capabilities has better or worse functional outcomes than using a chaotic auditory stimulus. A recent study has applied similar concepts to those described here to the rehabilitation of gait in Parkinson's disease (PD) patients (15). This worked showed that the fractal scaling of PD patients' gait may be temporarily restored with short-term carry-over effects after synchronizing with nonlinear auditory rhythms. Another exciting outcome of this study was that the PD patients reported greater perceived stability when synchronizing to the nonlinear rhythms compared to a stimulus with fixed temporal structure. A subjective feeling of stability such as this would have important implications for older adults with fear of falling, which results in activity curtailment and other psycho-social problems (25).

The α-values reported in this study were somewhat different than previous studies. Hausdorff and colleagues reported values between 0.76 and 0.87 for healthy young adults, and 0.68 for elderly for stride interval time series (11,12), compared to 0.60 for healthy adults and 0.84 for elderly in the current study (table 2). These differences may be due to the fact that overground walking was analyzed in other studies as opposed to treadmill walking in this study, where the gait velocity is controlled. Dingwell and Cusumano (6) have investigated the effect of the treadmill on stride-to-stride fluctuations using DFA. They developed a model that shows that individuals constantly adapt their instantaneous speed to the treadmill speed. As a result, if individuals are subjected to auditory stimuli while walking on a treadmill, it is possible that they have to adapt their gait to two different constraints, i.e. the speed constraint and the auditory constraint. Additionally, the short time series used in this study may account for the differences. Continuous data series in excess of 600 data points have been recommended for accurate DFA (3), and while this not consistently observed in the gait literature (10), it may be seen as a limitation in this study. Finally, stride time intervals were determined based on a sampling rate of 100 Hz. The temporal resolution is hence quite low for measures of gait variability since most stride interval values are expected to fall within 0.1 seconds, which suggests a temporal resolution of 10 samples. Our subsequent work has addressed these limitations by performing a similar experiment on overground walking, sampling a stride analyzer at higher sampling frequencies. Our preliminary results are very promising, showing even stronger outcomes than what are reported here.

This study has presented a new perspective in gait variability, where sensory inputs can be manipulated to influence the nonlinear dynamics of the locomotor system. We have reinforced the theory of Optimal Movement Variability by demonstrating a curvilinear relationship between gait variability and the temporal structure of auditory stimuli. This proof-of-concept study shows that the gait of older adults may be manipulated using auditory stimuli. Future work will investigate which structures of auditory stimuli in terms of fractal scaling will lead to improvements in functional status in populations with impaired gait.

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Conflict of Interest Statement

There are no conflicts of interest relating to this manuscript.

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Tables, Figures and Captions

Table Captions

Table 1: Subject demographics

Table 2: DFA α-values, mean(SD), for elderly and young groups across all four conditions, for stride interval, stride length and step width.

Table 1.

	Age (yrs)	Height (cm)	Weight (kg)	Speed m/s
Elderly	71.4 ± 4.4	172.88 ± 11.15	74.64 ± 13.38	0.75 ± 0.22
Young	25.7 ± 3.0	175.96 ± 8.71	72.89 ± 12.38	0.89 ± 0.19

Table 2.

	White	**Chaotic**	**No Stimulus**	**Metronome**
Stride Interval				
Elderly	0.769 (0.21)	0.882 (0.26)	0.846 (0.23)	0.721 (0.32)
Young	0.600 (0.20)	0.589 (0.20)	0.600 (0.20)	0.535 (0.21)
Stride Length				
Elderly	0.691 (0.19)	0.803 (0.22)	0.758 (0.27)	0.741 (0.28)
Young	0.797 (0.23)	0.823 (0.23)	0.775 (0.19)	0.699 (0.24)
Step Width				
Elderly	0.679 (0.20)	0.764 (0.22)	0.740 (0.23)	0.655 (0.23)
Young	0.706 (0.17)	0.737 (0.18)	0.757 (0.23)	0.688 (0.20)

Figure Captions

Figure 1: Schematic illustrating how our hypothesis maps to the theory of Optimal Movement Variability. The white noise stimulus will produce a disordered and unpredictable gait pattern. Movement will lack any organization, therefore complexity is low. The metronomic stimulus will produce a highly predictable, periodic gait pattern that lacks flexibility. The beat is one-dimensional, therefore the complexity is low. The chaotic auditory stimulus will produce an optimally complex and predictable gait pattern that contains a repeated pattern at multiple levels of organization.

Figure 2: Quadratic Trend Analysis results for the elderly group presented within the Optimal Movement Variability framework. The α-value exhibited a significant quadratic trend (i.e. the actual trend matched the predicted quadratic trend) for all gait parameters, confirming the presence of an inverted-U relationship between predictability and complexity, driven by the auditory stimuli.

Figure 3: Quadratic Trend Analysis results for the young group presented within the Optimal Movement Variability framework. The α-value did not exhibit a significant quadratic trend (i.e. the actual trend did not match the predicted quadratic trend) in any gait parameter.

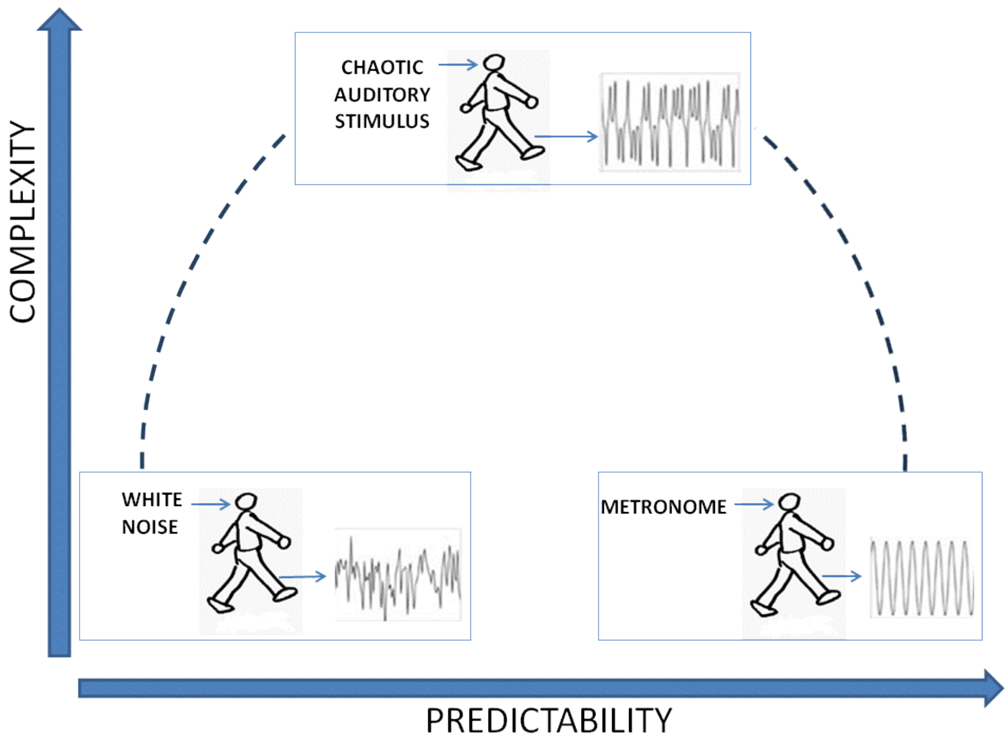

Figure 1 (Kaipust)

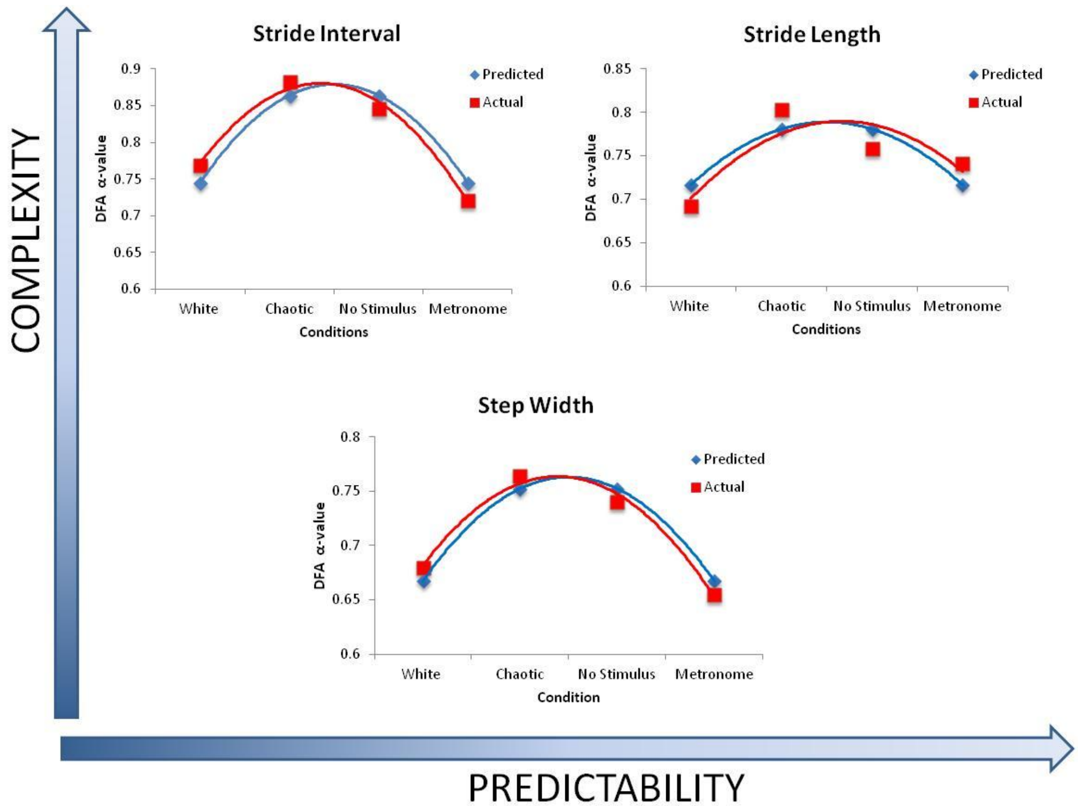

Figure 2 (Kaipust)

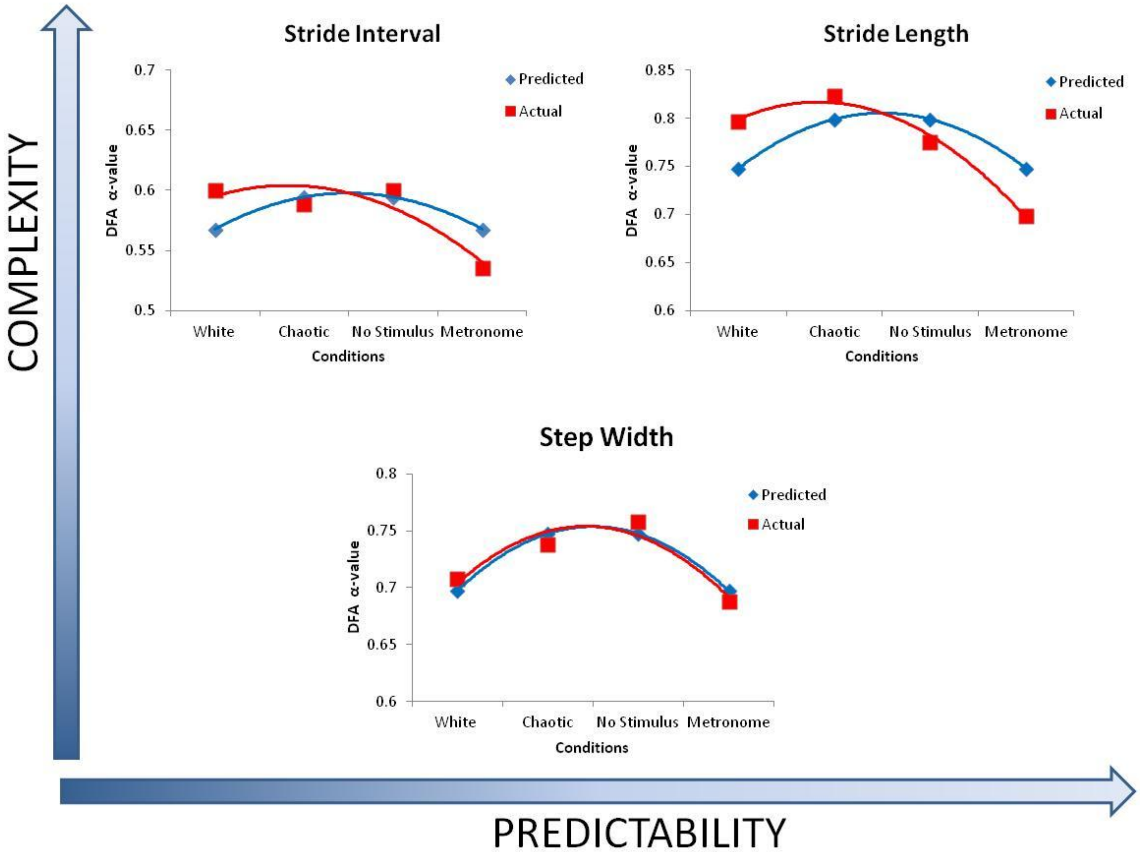

Figure 3 (Kaipust)