# Alterations in time estimation in multiple system atrophy

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A B S T R A C T

Precise spatiotemporal performance is required by many common tasks and represents a basic aspect of cognition. Time estimation in the second-to-minutes range – known as interval timing – involves the interaction of the basal ganglia and the prefrontal cortex via dopaminergic–glutamatergic pathways. Neurodegenerative disorders such as Parkinson's disease (PD) and multiple system atrophy (MSA) are characterized by basal ganglia dysfunction due to dopamine loss. Although interval timing in PD has been studied, little is known about temporal processing in MSA. In the present work, control, PD and MSA subjects ($n = 8$ for each group) were tested for interval timing in short ($<5$ s), medium (5–15 s) and long ($>15$ s) duration stimuli. MSA differed significantly from controls and PD patients in terms of decreased accuracy in the timing task. Differences between PD and MSA patients (as well as between MSA and controls) were lost after levodopa treatment. We show that time estimation for time bins between 5 and 20 s is affected in subjects with MSA, who had a significant tendency to underestimate time intervals as compared to controls or PD patients. Recordings of cognitive performance related to timing could be considered useful measurements of the progression of movement disorder-related pathologies.

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## Introduction

Timing is crucial to all aspects of our lives. Indeed, biological timing includes diverse time-related mechanisms that encompass several orders of magnitude [1]. More specifically, short perception in the seconds-to minutes range, known as interval timing, is crucial to learning, memory, decision making and other cognitive tasks. Time perception is often thought to depend on cognitive processing of events, as well as on an internal pacemaker–counter mechanism. Recent findings argue for the involvement of cortico-striatal circuits, controlled by dopaminergic modulation of oscillatory activity and lateral connectivity. Indeed, striatal medium spiny neurons are able to detect the coincident activity of specific beat patterns of cortical oscillations [2]. A consistent approach used for studying interval timing in human and animals is a reproduction procedure, in which the subject is presented with a given criterion duration and then required to reproduce this duration. Typically, the participant's responses follow a normal distribution around the criterion duration, and the width of this response distribution is proportional to the target length [3]. Interval timing is altered in several disorders associated with pathological dopaminergic function, including schizophrenia, Parkinson's disease (PD), Huntington's disease and attention deficit disorder [4–6].

PD patients experience specific problems with timing of motor responses, such as increased reaction time, movement time, and speech production time, as well as deficits in programming and synchronizing motor responses. These motor deficits may add a constant variance in timing performance, particularly in tasks with substantial motor requirements such as repetitive tapping [7]. Other cognitive deficits have also been recognized in PD, involving attention, memory, temporal discrimination, frontal lobe function, conceptual ability and visuospatial function [8].

Multiple system atrophy (MSA) is a clinically and pathologically distinctive neurodegenerative disease. MSA is characterized

clinically by symptoms that can be subdivided into pyramidal, extrapyramidal, cerebellar and autonomic categories. Extrapyramidal motor abnormalities such as bradykinesia, rigidity and postural instability are classed as either parkinsonian-type (MSA-P) or cerebellar (MSA-C) and reflect damage to the basal ganglia or cerebellum, respectively [9]. In patients with MSA, the Unified Parkinson's Disease Rating Scale (UPDRS) motor scores correlate with the amount of nigrostriatal dopaminergic neuronal loss and damage to the striatal neurons [10]. Moreover, a more pronounced decline in dopamine transporter (DAT) signal was observed in the caudate and anterior putamen of patients with MSA-P as compared to that in patients with PD [11]. In PD, dopaminergic degeneration is presynaptic, while in MSA it is not only presynaptic but also postsynaptic, showing dopamine 2 (D2) receptor downregulation [12]. In addition, cognitive impairments have been reported in MSA patients, in some cases with higher deficits in MSA-P compared to PD [13,14].

In PD patients there are time estimation deficits compatible with the alteration of an internal clock and partially reversed by levodopa [7]. Although the efficacy of levodopa has been documented in several cases of MSA [15], there is almost no information about time estimation impediments and its treatment in MSA patients.

The objective of this work is to assess putative time estimation deficits in MSA patients, compared to PD patients and a control group. Because dopaminergic degeneration is more severe in MSA compared to PD patients, with these sets of experiments we are testing the hypothesis of more serious deficits in time estimation in MSA patients, which could be partially reversed by levodopa treatment.

## Materials and methods

### Subjects

Eight patients with MSA (parkinsonian-type) and eight matching (age, sex and progression of disease) patients with PD were examined. A matched control group ($n = 8$) was also included. Patients with cognitive impairment (mini-mental state by Folstein below 27) were excluded [16]. Matching criteria were gender, age ($\pm 5$ years), and the Unified Parkinson's Disease Rating Scale (UPDRS) motor score [17] $\pm 15\%$. Patients were examined (a) after an overnight medication fast and (b) after intake of their usual levodopa (L-DOPA) dose. NMDA antagonists, monoamine oxidase-B inhibitors, sedatives, selective serotonin reuptake inhibitors, anxiolytics, and other CNS medications were permitted if dose was stable for the 28 days prior to baseline and likely to remain so for the duration of the experiment. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All subjects (or their families) provided informed consent before study participation.

### Protocol

Patients were seated comfortably in a chair with the computer screen before them. Wheelchair-bound MSA patients were examined in their homes. An examination session consisted of 2 parts: before medication intake after an overnight fast, and 1 h after the usual levodopa dose. At each examination, the time estimation program and a UPDRS motor part was performed, in order to analyze the relationship between changes in time estimation and changes in motor performance. Control subjects were examined only once.

Since most patients cannot operate the computer program properly by pressing the keyboard keys in response to the temporal signals due to their motor impairments – which would produce quite variable, and therefore unreliable, results – in this protocol both MSA and PD patients as well as the control group communicated the examiner via hand grip when to press the computer key. Although the examiners were not blind to patient group, they were randomly assigned to each patient. This procedure was validated in an informal pre-protocol and has been used previously in the literature [18].

Time estimation assessment was performed with a special software that we have developed specifically for this purpose. The procedure was compiled with visual basic and run on a portable PC. The methodology is similar to other time production protocols [19] in which the subjects had to match a variable duration tone to the exact duration of the previous tone that had been presented. This program offered a set of different durations of auditory stimuli (600 Hz, 50 dB tone), which the participant had to estimate and then reproduce. Twenty stimuli were presented per trial, so that an equal proportion of short ($<5$ s), medium ($5$–$15$ s) and long ($>15$ s) stimuli were presented in each trial. The target duration was presented with an uninterrupted tone that lasted between 1 and 20 s. Session duration was of approximately 30 min. No feedback was given to the participants. The computer stored the real and estimated time values, and further analysis characterized the different ratios between these two sets of data, including stratification in short-, medium- and long-duration stimuli.

Mini-mental tests were done before study entry and the Montgomery–Asberg Depression Scale [20] was performed to control for depression-induced impairment of time estimation.

### Data analysis

For each subject, a subjective/real time ratio was calculated. One-way analysis of variance (ANOVA) was applied, followed by a post hoc least significance difference test. All data sets passed the Kolmogorov–Smirnov (KS) normality test.

## Results

Table 1 shows the clinical motor and neuropsychological profiles of both samples of patients. MSA and PD subjects were comparable in all of the variables considered. The mini-mental state examination (MMSE) scores confirmed that patients were free of symptoms of dementia. Most of the patients were undergoing treatment with additional medication other than levodopa.

Fig. 1 summarizes the resulting behavioral performance during the timing task. When all target durations were analyzed together, MSA subjects had a significant tendency to underestimate real time as compared to controls and PD patients ($F_{4,35} = 6.78$, $p = 0.0004$, one-way ANOVA followed by Tukey's test), while PD

**Table 1**
Clinical motor and neuropsychological profiles of PD and MSA patients[a].

| Patients | Age[b] | Mini-mental | Montgomery | UPDRS before | UPDRS after[c] |
|---|---|---|---|---|---|
| PD | $60.62 \pm 7.34$ | $28.11 \pm 2.08$ | $8.0 \pm 5.09$ | $36.88 \pm 11.75$ | $21.11 \pm 11.12$ |
| MSA | $60.50 \pm 7.38$ | $28.0 \pm 1.15$ | $10.57 \pm 7.20$ | $32.71 \pm 13.51$ | $31.86 \pm 13.13$ |

[a] Data are expressed as mean $\pm$ S.D., $n = 8$/group.
[b] Expressed in years.
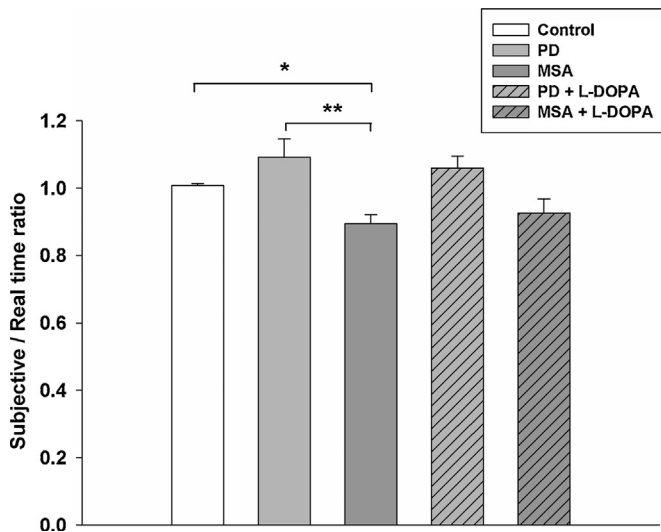[c] Corresponding to 1 h after treatment.

**Fig. 1.** Average time estimation in controls and patients. Subjective/real time ratio from all the intervals tested in control subjects, PD and MSA patients, without and with levodopa (L-DOPA) treatment. **$p < 0.01$, *$p < 0.05$, one-way ANOVA followed by Tukey's test, $n = 8$/group.

patients were not different from control subjects. Although no significant effects were found for levodopa treatment on subjective time estimation in PD or MSA patients, this treatment eliminated the significant difference for MSA patients from both controls and PD groups.

When assessing the relative importance of different time intervals (short, medium or long) for the overall time estimation procedure, it became clear that MSA patients had a greater difficulty for the estimation of medium and long (i.e., >5 s) time

bins, exhibiting a greater deficit in time estimation than PD patients, and, when considering time intervals longer than 15 s, MSA patients underestimated duration when compared to controls (Fig. 2, $F_{4,35} = 3.19$, $p = 0.02$ for short, $F_{4,35} = 11.58$, $p < 0.0001$ for medium, $F_{4,35} = 4.87$, $p = 0.004$ for long time intervals, one-way ANOVA followed by Tukey's test).

When time intervals were <5 s, PD patients overestimated duration compared to controls, while levodopa treatment eliminated this significant difference. MSA patients did not differ significantly from controls for short time intervals.

For medium and long time intervals (5–15 s and <15 s respectively), MSA patients underestimated duration as compared to controls or PD patients. Levodopa did not improve timing performance in MSA patients for medium and long time intervals.

## Discussion

In this work we show that time estimation for short time bins between 1 and 20 s is affected in subjects with multiple system atrophy (MSA). Thus, MSA patients had a significant tendency to underestimate medium and long time intervals as compared to PD patients and a control group. These results indicate that, when all target durations are analyzed together, MSA patients present more serious deficits in time estimation as compared to Parkinson's disease. These data are in agreement with previous findings related to cognitive impairments in MSA patients. Moreover, the temporal discrimination threshold (TDT) – which is the shortest time interval that allows two temporally separated successive stimuli to be perceived as two stimuli – has been reported to be abnormally increased and to correlate with the UPDRS Motor subscores, representing bradykinesia in patients with MSA [21]. On the other hand, PD patients performed better duration discrimination compared to controls for medium and long time intervals (>5 s), consistent with previous reports [22].
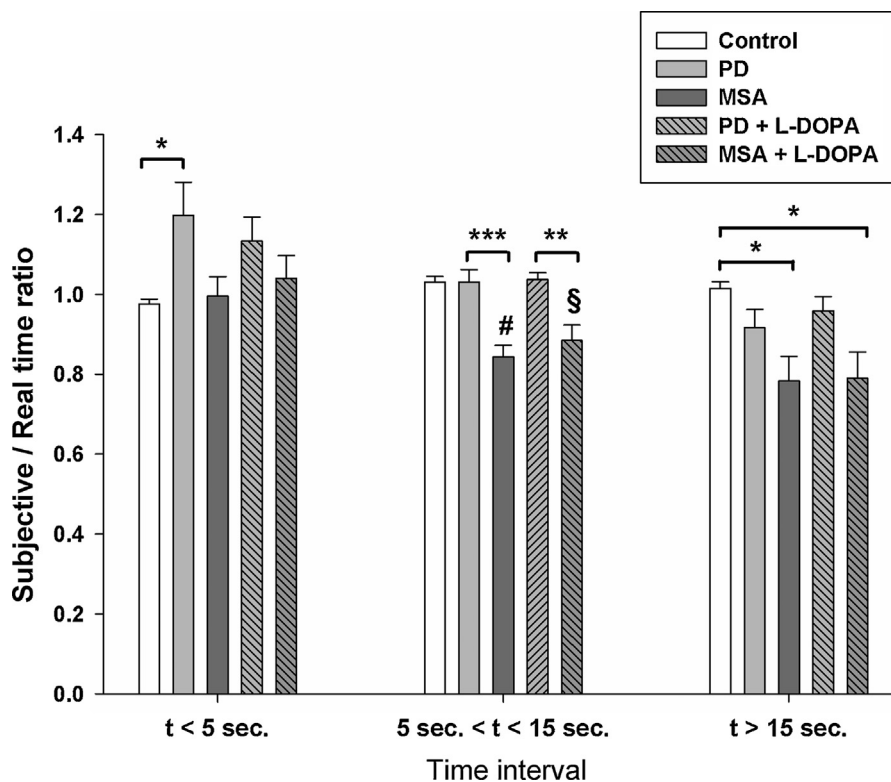


**Fig. 2.** Stratified time estimation in controls and patients. Subjective/real time ratio from stratified time intervals, short (<5 s), medium (5–15 s) or long (>15 s) intervals. $p < 0.0001$ for groups (controls, PD and MSA) and $p < 0.0001$ for different time intervals, two-way ANOVA. ***$p < 0.001$, *$p < 0.05$, #$p < 0.001$ vs. control, §$p < 0.01$ vs. control, one-way ANOVA followed by Tukey's test, $n = 8$/group.

Since the most affected brain areas in MSA-P are the striatonigral system and the basal ganglia with its cortical targets of striatal projections, poor performance in the timing task could be associated with these particular regions. Indeed, several evidences indicate that interval timing requires attentional mechanisms and involves basal ganglia and related cortical structures [2]. Although dopamine supplementation did not improve time estimation in MSA patients, it tended to decrease the differences from both control and PD groups.

More importantly, our data are consistent with the "migration effect", in which shorter durations are overestimated and longer durations underestimated [23,24]. In a study of Parkinson's disease effects on interval timing, Malapani et al. [23] reported that temporal reproductions by off-medication patients were too long for relatively short intervals (8-s) and too short for relatively long intervals (21-s) when these were tested together in the same session. In contrast, on-medication patients accurately estimated both short and long intervals. The migration effect was hence dependent on learning two intervals. When patients are required by the task to remember two different intervals, memory for an earlier learned interval appears to affect production of a later learned interval by causing a migration of criterion times toward each other. These findings were later replicated by Koch et al. [24] in tasks with shorter durations, mixing both sub-second short (about 500 ms) and supra-second long (about 2000 ms) target durations. The pattern shown by off-medication patients follows the so-called Vierordt's Law [25] and was also reported in working memory capacity and temporal reproduction [26]. Our results indicate that MSA patients underestimated longer (>15-s) and medium (between 5-s and 15-s) durations, and that L-DOPA medication did not revert this situation. PD patients, however, overestimated shorter (<5-s) durations but this effect was reverted by L-DOPA treatment. Moreover, PD patients had a tendency to underestimate longer intervals (>15-s) although this difference was not significant (Fig. 2).

Recent research has used a combination of pharmacological, neurophysiological and behavioral manipulations to gain understanding of how temporal information is processed. Current pharmacological research suggests that different stages of temporal processing may involve separate brain regions and can be modified by different neurotransmitter systems. In particular, the internal clock used to time durations in the seconds-to-minutes range appears linked to dopamine function in the basal ganglia, while temporal memory and attentional mechanisms – related to longer time durations – appear linked to acetylcholine function in the frontal cortex [1]. These two systems are connected by frontal–striatal loops, thus allowing for the completion of the timing sequences involved in duration discrimination.

Timing is a primary aspect of movement since most acts in real life demand the production of an appropriate force–time pattern. Deficits in timing are evident in most motor abnormalities reported in PD: increased reaction time and movement time, as well as prolonged inter-onset latencies when performing simultaneous and sequential movements are well documented [27]. Rhythm generation has been regarded as a possible structural basis for timing, and PD patients in particular have a certain inability to maintain a specific rhythm in tapping, a fact consistent with the suggestion of a deficit in the perception and/or reproduction of the timing component in PD [28]. Indeed, the progression of MSA disease correlates with a higher deficit in temporal discrimination [21].

## Conclusions

In summary, the results of this study indicate that MSA patients present abnormalities in short-time estimation, and suggest that, although more research is needed in the field, recordings of cognitive performance related to timing could be considered useful measurements of the progression of movement disorder-related pathologies.

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