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Presynaptic mechanisms of motor fluctuations in Parkinson's disease: a probabilistic model

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Summary

Levodopa-treated Parkinson's disease is often complicated by the occurrence of motor fluctuations, which can be predictable ('wearing-off') or unpredictable ('on–off'). In contrast, untreated dopa-responsive dystonia (DRD) is usually characterized by predictable diurnal fluctuation. The pathogenesis of motor fluctuations in treated Parkinson's disease and diurnal fluctuation in untreated DRD is poorly understood. We have developed a mathematical model indicating that all these fluctuations in motor function can be explained by presynaptic mechanisms. The model is predicated upon the release of dopamine being subject to probabilistic variations in the quantity of dopamine released by exocytosis of vesicles. Specifically, we propose that the concentration of intravesicular dopamine undergoes dynamic changes according to a log-normal distribution that is associated with different probabilities of release failure. Changes in two parameters, (i) the proportion of vesicles that undergo exocytosis per unit of time and (ii) the proportion of dopamine subject to re-uptake from the synapse, allowed us to model different curves of levodopa response, for the same degree of nigrostriatal damage in Parkinson's disease. The model predicts the following periods of levodopa clinical benefit: 4 h for stable responders, 3 h for wearing-off fluctuators, and 1.5 h for on–off fluctuators. The model also predicts that diurnal fluctuation in untreated DRD should occur some 8 h after getting up in the morning. All these results fit well with clinical observations. Additionally, we calculated the probability of obtaining a second ON period after a single dose of levodopa in Parkinson's disease (the 'yo-yoing' phenomenon). The model shows that the yo-yoing phenomenon depends on how fast the curve crosses the threshold that separates ON and OFF states, which explains why this phenomenon is virtually exclusive to patients with on–off fluctuations. The model supports the idea that presynaptic mechanisms play a key role in both short-duration and long-duration responses encountered in Parkinson's disease. Dyskinesias may also be explained by the same mechanisms.

Keywords: dopamine release; vesicle; probability; motor fluctuations; Parkinson's disease

Abbreviations: c = vesicular level of dopamine below which the patient turns OFF; DA = dopamine; DAT = dopamine transporter; DRD = dopa-responsive dystonia; HVA = homovanillic acid; NMDA = *N*-methyl-D-aspartate; VMAT2 = vesicular monoamine transporter type 2; α = vesicular release rate; β = dopamine re-uptake parameter; λ = shortfall coefficient

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Introduction

Patients with Parkinson's disease usually enjoy a stable response to levodopa during the first years of their illness. Unfortunately, however, a substantial proportion (some 50%) develop motor complications (motor fluctuations and dyskinesias) after 3 years of chronic levodopa treatment (Marsden and Parkes, 1976; Marsden *et al*., 1982). Initially, they begin to notice simply a progressive shortening in the response to each dose of levodopa ('wearing-off' fluctuations) (Marsden and Parkes, 1976; Fahn, 1982; Marsden *et al*., 1982; Fabbrini *et al*., 1988; Kumar *et al*., 2003). In some patients, such predictable fluctuating response precedes the emergence of abrupt unpredictable shifts between ON and OFF states,

unrelated to the timing of levodopa administration ('on–off' fluctuations) (Marsden and Parkes, 1976; Fahn, 1982; Marsden *et al*., 1982; Fabbrini *et al*., 1988; Kumar *et al*., 2003). In such on–off fluctuations, patients who are ON after oral administration of a dose of levodopa suddenly switch OFF for minutes and then ON again (the 'yo-yoing' phenomenon) (Fahn, 1974, 1982). This second ON period is often shorter than the previous one.

Theories on the pathogenesis of motor fluctuations

The nature of motor fluctuations, which have been the subject of a great number of studies, remains unclear (Nutt, 1987; Nutt and Halford, 1996; Kumar *et al*., 2003). Neither pharmacokinetic nor pharmacodynamic mechanisms associated with long-term levodopa treatment satisfactorily explain these complications (Sweet and McDowell, 1974; Shoulson *et al*., 1975; Tolosa *et al*., 1975; Hardie *et al*., 1984; Nutt, 1987; Nutt and Halford, 1996). The prevailing view points to postsynaptic mechanisms as a major cause of on–off fluctuations (Chase *et al*., 1993; Mouradian and Chase, 1994; Chase *et al*., 2001). However, little is known about the nature of such presumed postsynaptic mechanisms. Theories ranging from changes in dopamine receptor affinity to changes occurring in the basal ganglia downstream from the dopaminergic system have been put forward. It was initially suggested that postsynaptic dopamine receptor desensitization could explain the occurrence of a sudden OFF period after a single dose of levodopa (Fahn, 1974; Direnfeld *et al*., 1978). The reversal of this mechanism (i.e. the sudden loss of desensitization) could potentially explain a subsequent ON period (yo-yoing). However, there is evidence that these OFF periods respond to dopaminergic stimuli (Nutt, 1987), which clearly argues against postsynaptic dopamine receptor desensitization as a major mechanism for on–off fluctuations. More recently, it has been suggested that the on–off phenomenon could be related to the changes in the phosphorylation state of *N*-methyl-D-aspartate (NMDA) receptors in relation to daily increases and decreases in levodopa brain levels associated with intermittent levodopa treatment (Chase *et al*., 2001). This theory has received some experimental support. Thus, NMDA antagonists are able to decrease motor fluctuations and dyskinesias (Chase *et al*., 2001). Again, it is difficult to explain the occurrence of the second ON period (i.e. the ON–OFF–ON pattern) by reversal of NMDA receptor sensitization.

Dopamine release: the pathological scenario in Parkinson's disease and dopa-responsive dystonia

Endogenous dopamine is synthesized from L-tyrosine through two consecutive steps. The first is catalysed by the rate limiting enzyme tyrosine hydroxylase and converts L-tyrosine into L-dopa (levodopa) (Cooper *et al*., 1996). In a second step, dopamine is obtained from levodopa by the action of the enzyme dopa-decarboxylase (Cooper *et al*., 1996). The genetic defect in dopa-responsive dystonia (DRD) compromises the synthesis of tetrahydrobiopterin, an essential cofactor for tyrosine hydroxylase, which leads to dopamine deficiency (Ichinose *et al*., 1994). Once synthesized in the cytoplasm, most presynaptic dopamine is packaged in the vesicles present in nigrostriatal terminals by the vesicular monoamine transporter type 2 (VMAT2). This storage process maintains cytoplasmic dopamine at very low levels (0.1–1 μM) (Liu and Edwards, 1997), in contrast to the intravesicular compartment, where dopamine can reach concentrations at least 1000- to 10 000-fold greater (Kelly, 1993; Schuldiner, 1994). In response to an action potential, a small proportion of these vesicles release their contents into the synaptic cleft through exocytosis. After interacting with dopamine receptors, most of the dopamine thus released is taken back up through the plasma membrane dopamine transporter (DAT) (Cooper *et al*., 1996). Some synaptic dopamine is, however, metabolized and lost. Once the vesicles have fused to the plasma membrane and released their content, they are recycled through a complex multistep mechanism (Sudhof, 1995) and refilled with dopamine. It has been estimated that the whole vesicular cycle is complete in 1 min. Dopamine derived from exogenous levodopa is also packed in synaptic vesicles and subject to the same process (Garnett *et al*., 1983).

The average intravesicular concentration of dopamine is critical to the normal function of the nigrostriatal pathway, as it affects the quantal release size of dopamine. It has been shown experimentally that, under normal conditions, each vesicle contains a number of neurotransmitter molecules corresponding to a quantum (Stevens, 1993; Geppert *et al*., 1994; Sudhof, 1995). Hence, any decrease in the intravesicular concentration of dopamine will increase the likelihood of response failure.

Both Parkinson's disease and DRD are associated with striatal dopamine depletion. Several mechanisms are set in motion to compensate for such a dopamine deficiency (Calne and Zigmond, 1991); among them, increased dopamine turnover probably occurs even at very early stages of disease (de la Fuente-Fernández *et al*., 2001*b*; Sossi *et al*., 2002). While Parkinson's disease is associated with the progressive loss of nigrostriatal dopamine terminals (Bernheimer *et al*., 1973; Kish *et al*., 1988, 1992), with consequent reduced ability to synthesize and store enough dopamine, DRD is a pure biochemical model of dopamine deficiency with no structural damage to the nigrostriatal system (Rajput *et al*., 1994). Based on these observations, we hypothesized that DRD should be characterized by low intravesicular levels of dopamine. A recent study using PET with (±)-α-[^{11}C]dihydrotetrabenazine has given support to this notion (de la Fuente-Fernández *et al*., 2003*a*). In Parkinson's disease, on the other hand, it seems reasonable to predict that the loss of dopamine resulting from increased turnover may well exceed the rate of synthesis of endogenous

dopamine. Consequently, the average intravesicular concentration of dopamine could also be reduced in surviving Parkinson's disease terminals.

Presynaptic model of motor fluctuations

We develop here a simple mathematical model that shows how motor fluctuations in Parkinson's disease can be explained by alterations in presynaptic mechanisms of dopamine release. Specifically, the model predicts that on–off fluctuations obey probabilistically determined oscillations in vesicular dopamine release. We present support for this model based on recent observations derived from PET studies, as well as from experimental data on both the quantal release of dopamine and dopamine re-uptake.

Methods

We modelled the kinetics of intravesicular dopamine levels in the nigrostriatal system in different disease stages of Parkinson's disease (stable response, wearing-off fluctuations, and on–off fluctuations). We also included DRD in the analysis because this disorder is characterized by (i) a combination of dystonia and parkinsonism that typically worsens late during the day (diurnal fluctuation) (Segawa *et al*., 1976); and (ii) an excellent response to levodopa treatment (i.e. stable response pattern) (Segawa *et al*., 1976; Hwang *et al*., 2001; Nutt and Nygaard, 2001). We will begin by describing the relationship between the requirements for dopamine in the nigrostriatal system and the amount of dopamine provided by the doses of levodopa usually employed in clinical practice.

Loading vesicles with dopamine: the effect of levodopa treatment

The human nigrostriatal dopaminergic pathway consists of about 1 million pigmented neurons (counting both sides of the substantia nigra) (McGeer *et al*., 1977; Pakkenberg *et al*., 1991). Each nigral dopaminergic cell has between half a million and one million release sites (boutons or varicosities) along its highly branched axon in the striatum (Anden *et al*., 1966; Doucet *et al*., 1986; Grace, 1991; Nicholls, 1994). The number of synaptic vesicles per release site is 200–500 (Harris and Stevens, 1988, 1989) and the average number of dopamine molecules per vesicle is probably somewhere between 2000 (Ryan *et al*., 1993) and 5000 (Bruns and Jahn, 1995). Hence, it can be estimated that the normal nigrostriatal system contains some 10^{18} molecules of dopamine. In Parkinson's disease, this number is reduced by at least 50%.

A small proportion (10%) of the levodopa administered orally with a peripheral dopa-decarboxylase inhibitor reaches the brain (Nutt *et al*., 1994), but only about 1% of the original dose may be available for dopamine synthesis within the nigrostriatal pathway. Now the question is: how does this amount of levodopa translate into the synthesis of dopamine molecules? If, for example, a tablet of 100 mg of levodopa (with carbidopa) is taken, is it enough to refill the nigrostriatal system if it were structurally intact but empty of dopamine? In this example, we can anticipate that only 1 mg of levodopa will be converted into dopamine in nigrostriatal terminals. Since the molecular weight of levodopa is 197.2 (Mathews and van Holde, 1990), and the number of molecules in a mole is 6.02×10^{23} (Avogadro's number), we can estimate that a tablet containing 100 mg of levodopa can provide the nigrostriatal system with some 3×10^{18} molecules of levodopa. One can also predict that, because of the high activity of the enzyme dopa-decarboxylase (Cooper *et al*., 1996), this large number of molecules of levodopa can in fact be converted into dopamine in the nigrostriatal system. In addition, given the molecular dopamine turnover by VMAT2 as 150–300 molecules of dopamine per min (Scherman and Boschi, 1988; Henry and Scherman, 1989; Peter *et al*., 1994), and taking into account the fact that each synaptic vesicle contains one to three VMAT2 sites (Scherman and Boschi, 1988; Henry and Scherman, 1989), a (hypothetical) structurally intact nigrostriatal pathway containing empty vesicles could store in its vesicles 10^{18} dopamine molecules in some 10–15 min. Hence, 100 mg of levodopa taken orally would provide a sufficient number of dopamine molecules to fully replenish an intact but 'empty' nigrostriatal system.

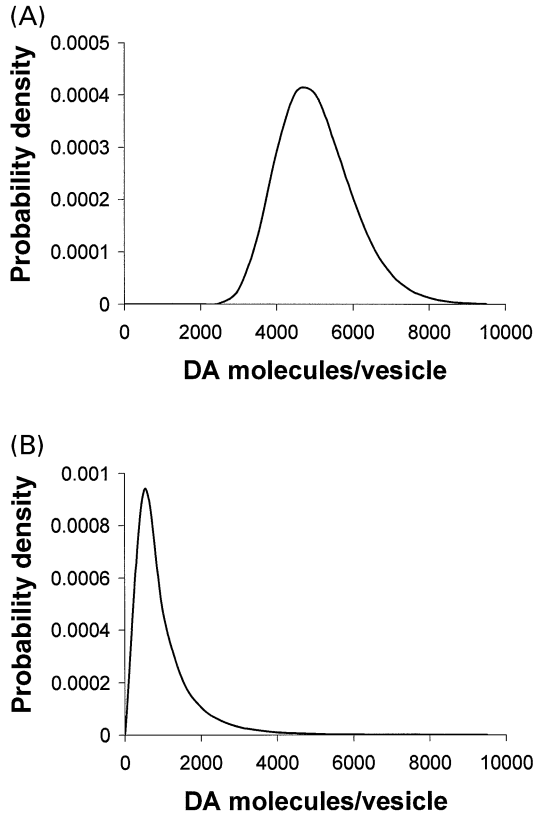

Fig. 1 The log-normal model. We assume that the intravesicular concentration of dopamine follows a log-normal distribution, which is approximately symmetrical (**A**) at time 0 (i.e. once the system has been replenished with dopamine after levodopa administration; mean, 5000 molecules/vesicle; SD, constant over time, 1000 molecules/vesicle). Note that the distribution becomes highly skewed to the right (**B**) as the mean decreases with time. DA = dopamine.

The mathematical model

For simplicity, the model, which appears fully developed in the Appendix, begins once the system has been refilled with dopamine

Table 1 *Baseline parameter values for normal subjects and dopa-responsive dystonia (DRD) and Parkinson's disease (PD) patients*

Feature	Normal	DRD	PD (stable)	PD (wearing-off)	PD (on–off)
Vesicles per release site	300	300	300	300	300
Vesicles released per minute	30	60	45	60	120
Proportion of vesicles released per minute (α)	0.10	0.20	0.15	0.20	0.40
Dopamine molecules per vesicle					
Mean [$\zeta(0)$]*	5000	1250	1750	1750	1750
Standard deviation (τ)**	1000	1000	1000	1000	1000
Proportion of dopamine molecules taken back up (β)	0.985	0.985	0.950	0.950	0.950
Shortfall coefficient (λ)	1	0.25	0.35	0.35	0.35
Threshold for OFF state (c)	NA	1500	2300	2300	2300

*Although we assumed that the mean intravesicular concentration of dopamine [$\zeta(0)$] in surviving Parkinson's disease terminals is decreased at baseline and then reaches normal values (i.e. 5000 molecules/vesicle) after levodopa treatment, the model gives the same results if we take the baseline values to be normal and then increased by the same proportion after levodopa (see text). **The standard deviation (τ) was obtained from experimental observations (e.g. Pothos, 2002). In Parkinson's disease, we used the same shortfall coefficient ($\lambda = 0.35$) to show that different patterns of response can be obtained for the same degree of damage to the nigrostriatal dopaminergic system. Naturally, fluctuators will, in general, have more severe parkinsonism (e.g. $\lambda = 0.25$) than stable responders. NA = not applicable; PD = Parkinson's disease.

following levodopa administration. We have seen that 100 mg of levodopa/carbidopa given orally can potentially replenish the system with dopamine. Also for simplicity, we will use the terms 'terminal' and 'vesicular release' instead of 'release site' and 'vesicular exocytosis', respectively.

We assume that the intravesicular concentration of dopamine follows a log-normal distribution (Johnson and Kotz, 1970), which is approximately symmetrical at time 0 (i.e. once the system has been replenished with dopamine) (Fig. 1A), and becomes progressively skewed to the right as the system loses dopamine with time (Fig. 1B). We applied the central limit theorem (Feller, 1950; Loeve, 1963) to derive the distribution of the amount of dopamine released into the synapse at any given time. Finally, we calculated the probability of observing a yo-yoing pattern of response after the administration of a single dose of levodopa (Fig. 2) (Loeve, 1963; Feller, 1966).

Table 1 shows the normal parameter values used in the model. We will take 300 as the number of vesicles per terminal (Sudhof, 1995), each vesicle containing 5000 molecules of dopamine (Bruns and Jahn, 1995). At any given time, there are 20–30 readily releasable vesicles per terminal (Sudhof, 1995). Classical dopamine cells have a firing rate ranging from 0.1 to 8 Hz (Fiorillo *et al*., 2003). An average spontaneous firing frequency of 1 Hz has recently been reported (Liss *et al*., 2001); others give 3–4 Hz as the mean value (Grace, 1991). However, the probability of vesicular release at central synapses is very low [at most only one vesicle (one quantum) is released every two to three stimuli (Hessler *et al*., 1993; Goda and Stevens, 1994), but this probability can be as low as 0.10 (one vesicle released every 10 action potentials)] (Sudhof, 1995). We can then estimate that 10% of the vesicles (i.e. 30 out of 300) will be released in each terminal each minute. Hence, taking 1 min as the time unit, $\alpha = 0.10$ is the proportion of vesicles released per terminal under normal conditions (note that 1 min is a convenient time unit because, as we mentioned earlier, the vesicle cycle is complete also in 1 min). As to the dopamine re-uptake parameter (β), there is evidence that most (>95%) dopamine released into the synapse is taken up again by DAT (Ross, 1991; Onn *et al*., 2000); we will use $\beta = 0.985$ as the normal value. The 'shortfall coefficient', λ (see Appendix), will determine the baseline levels of dopamine in DRD and Parkinson's disease.

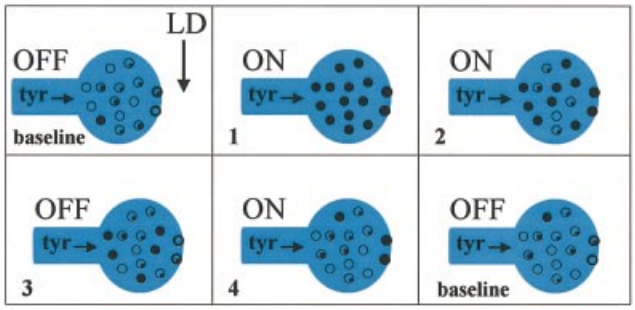

Fig. 2 The on–off phenomenon: a probabilistic model based on the dynamics of vesicular dopamine release. In Parkinson's disease, levodopa (LD) administration increases the intravesicular concentration of dopamine (solid symbols) from baseline values, which leads to an ON state (here represented by the release of two full vesicles). Since releasable vesicles (thick circles) are randomly selected at any given time, it is possible to switch OFF while there are still available vesicles full of dopamine, and then switch ON again, before reaching baseline levels (the yo-yoing phenomenon). As shown in the figure, the intravesicular baseline levels of dopamine may be reduced in surviving Parkinson's disease terminals, whenever the synthesis of endogenous dopamine (from tyrosine, tyr) is insufficient to compensate for the loss of dopamine (this is related, among other factors, to increased dopamine turnover). The model, however, gives identical results assuming normal baseline levels (see text).

In DRD, 100% nigrostriatal terminals are present, and the same values of α, β and λ apply to each of them. Therefore, the mean number of dopamine molecules per vesicle at time 0 (i.e. once the nigrostriatal system has been replenished with dopamine following levodopa administration), is probably the same as that of normal controls (i.e. 5000 molecules/vesicle) (Table 1). Because of the enzymatic defect, at baseline (and steady state) this quantity is expected to be some 25% of the normal value (i.e. 1250 molecules/vesicle) (Table 1), and so $\lambda = 0.25$. Naturally, the re-uptake capacity is normal in DRD (Furukawa *et al*., 1999; de la Fuente-Fernández *et al*., 2003*a*). Hence, $\beta = 0.985$. The ratio between homovanillic acid and dopamine (HVA/DA), which reflects dopamine turnover,

Table 2 *Predicted duration of ON state in dopa-responsive dystonia (DRD) and Parkinson's disease*

Condition	Time to OFF (h)
DRD	
Diurnal fluctuation (untreated DRD)	8
Levodopa withdrawal	15
Parkinson's disease	
Stable responders	4
Wearing-off fluctuators	3
On–off fluctuators	1.5

For Parkinson's disease, we modelled different response patterns to levodopa for the same severity of nigrostriatal damage (Table 1).

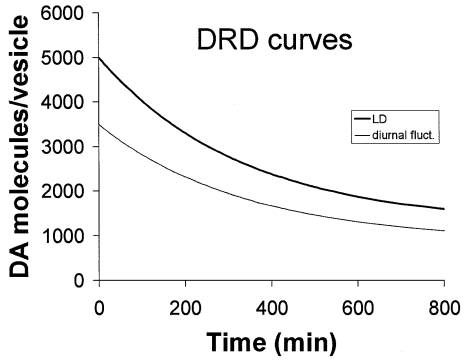

Fig. 3 Dopa-responsive dystonia (DRD) curves for diurnal fluctuation (i.e. motor deterioration in untreated DRD) (thin curve) and time to OFF after levodopa (LD) withdrawal (thick curve). The model predicts that (i) diurnal fluctuation occurs 8 h after getting up in the morning; and (ii) the OFF state is reached 15 h after levodopa withdrawal. The assumptions here are that, while levodopa treatment fully refills the vesicles with dopamine (5000 molecules/vesicle), sleep benefit in untreated DRD leads to incomplete refilling of vesicles (3500 molecules/vesicle), which explains the occurrence of diurnal fluctuation. DA = dopamine. Time is given in minutes (min).

can be used to estimate α. Post-mortem studies have shown that the striatal HVA/DA ratio is up to four times higher in DRD than in controls (Rajput *et al*., 1994; Furukawa *et al*., 1999). This would suggest that $\alpha = 0.40$. However, post-mortem studies tend, by definition, to include mostly patients with advanced disease. Consequently, we used an intermediate value (i.e. $\alpha = 0.20$) in our model. Recent PET studies are also compatible with this notion (de la Fuente-Fernández *et al*., 2003*a*).

In Parkinson's disease, on the other hand, the nigrostriatal system has 50% (or less) surviving terminals. Still, patients turn ON when on levodopa, which implies that the remaining terminals (and presumably their vesicles) transiently increase their dopamine contents after levodopa administration. Although we do not know whether the mean baseline vesicular concentration of dopamine in surviving Parkinson's disease terminals is normal, it must be considerably lower than that obtained after levodopa administration. Indeed, there is experimental evidence that the administration of levodopa increases the dopamine quantal size (Pothos, 2002). Hence, λ is also less than 1 in Parkinson's disease. We will assume that the baseline vesicular levels are decreased in each surviving terminal and normalize after levodopa administration. It should be noted, however, that, for the same value of λ, the model gives identical predicted ON times using either this approach or assuming that the baseline levels are normal and then increase after levodopa. As the model is based on surviving terminals, only relative alterations in re-uptake capacity must be taken into account. Recent PET studies in Parkinson's disease suggest a 3–8% reduction in DAT sites relative to VMAT2 sites (Lee *et al*., 2000). Hence, we used $\beta = 0.95$ and $\beta = 0.90$ (instead of the normal value, $\beta = 0.985$). On the other hand, we know from post-mortem studies that the striatal HVA/DA ratio is approximately four times higher in Parkinson's disease patients than in controls (Hornykiewicz, 1982). Consequently, we estimated that the maximum value for α in Parkinson's disease should be four times its normal value (i.e. $\alpha = 0.40$).

Since the number of terminals is reduced in Parkinson's disease and not in DRD, one can anticipate that, for the same degree of overall striatal dopamine depletion (and the same degree of motor impairment), DRD must have lower baseline levels of intravesicular dopamine per terminal than Parkinson's disease. The same applies to the threshold (*c*; vesicular level of dopamine below which the patient turns OFF)—lower in DRD than in Parkinson's disease. Post-mortem studies have shown that Parkinson's disease symptoms appear when there is some 50% cell loss (see above), which corresponds to 75–80% loss in striatal dopamine levels (Barolin *et al*., 1964; Hornykiewicz, 1982). Therefore, *c* in Parkinson's disease must be between 2000 and 2500 molecules/vesicle. In DRD, on the other hand, *c* is most likely slightly above the baseline value after 75% striatal dopamine depletion (i.e. >1250 molecules/vesicle). As a first approximation, we will take *c* to be 2300 molecules/vesicle in Parkinson's disease and 1500 molecules/vesicle in DRD. We will see that, in addition to λ and *c*, changes in α (vesicular release) and β (dopamine re-uptake) allow one to model different disease states (Table 1). In other words, although the parameter values are approximations only, the model provides testable hypotheses and, as we will see, remarkably accurate predictions.

Results

Dopa-responsive dystonia: diurnal fluctuations

Taking $\alpha = 0.20$ (i.e. twice the normal rate of vesicular release), $\beta = 0.985$ (i.e. preserved re-uptake capacity), $\lambda = 0.25$ (i.e. 75% reduction in baseline levels of dopamine) and a threshold *c* of 1500 molecules/vesicle (instead of the normal value of 5000 molecules/vesicle) (Table 1), DRD patients are expected to turn OFF some 15 h after discontinuing levodopa treatment (Table 2; Fig. 3). However, the OFF state may take 2 days to occur if, for example, $\lambda = 0.30$ (instead of $\lambda = 0.25$). These results are in keeping with clinical observations (Hwang *et al*., 2001; Nutt and Nygaard, 2001). For comparison with Parkinson's disease (see below), if we take 2300 molecules/vesicle as the threshold, DRD patients would be expected to turn OFF some 7 h after levodopa withdrawal.

Levodopa-naive DRD patients with sleep benefit (i.e. patients able to partially replenish their nigrostriatal system with endogenously derived dopamine during their nightly sleep; for example, from 1250 molecules/vesicle to 3500

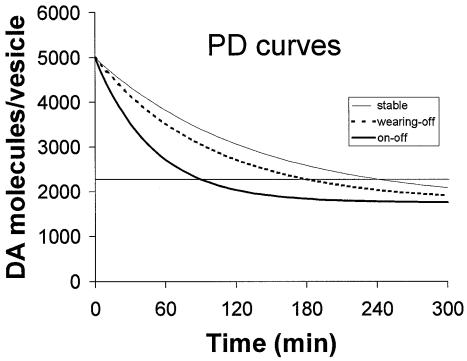

Fig. 4 Parkinson's disease (PD) curves for ON time after a dose of levodopa in stable responders (thin, broken curve; 4 h), wearing-off fluctuators (thick, broken curve; 3 h), and on–off fluctuators (solid curve; 1.5 h). These three curves were obtained from the model assuming between-group differences in vesicular release rate (i.e. differences in dopamine turnover) and the same degree of nigrostriatal damage. The horizontal line represents the threshold that separates ON (above) and OFF (below) states. DA = dopamine. Time is given in minutes (min).

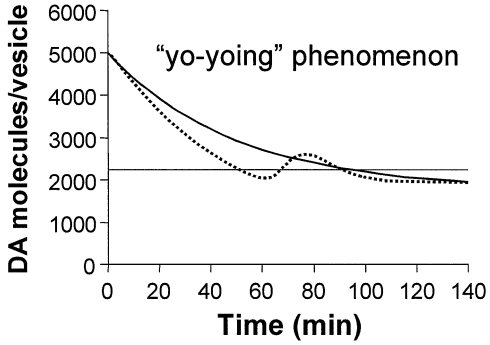

Fig. 5 The yo-yoing phenomenon in Parkinson's disease: mean curves based on $\alpha = 0.20$, $\beta = 0.90$ and $\lambda = 0.35$ (i.e. a parameter set known to give curves of the on–off type; see Results). The model predicts that the probability of obtaining a second ON period after a single dose of levodopa increases as the time to OFF shortens. Thus, the probability of a yo-yoing response with a second ON period of 10–30 min (broken curve) is 0.038 for on–off fluctuators (solid curve), but only 0.0017 for wearing-off fluctuators and virtually zero for stable responders. Again, the horizontal line represents the threshold that separates ON and OFF states. DA = dopamine. Time is given in minutes (min).

molecules/vesicle) are predicted to experience motor deterioration (i.e. diurnal fluctuations) some 8 h after getting up (Table 2; Fig. 3). Again, this result fits clinical observations well (Segawa *et al*., 1976; Hwang *et al*., 2001).

Parkinson's disease: from stable response to the yo-yoing phenomenon

As previously indicated, the threshold c in Parkinson's disease is assumed to be less than half the normal value (i.e. 2300 molecules/vesicle). Also, Parkinson's disease is most likely associated with increased α (i.e. a higher fraction of vesicles is released per unit of time). As to β (the re-uptake capacity of surviving terminals), it is probably reduced either as a regulatory change (i.e. an attempt to increase synaptic dopamine levels) or as a consequence of early damage to the plasma membrane DAT sites (de la Fuente-Fernández *et al*., 2003*a*, *b*). Naturally, λ, which gives the proportion of molecules present at baseline (and steady state) with respect to those present at time 0 (i.e. after refilling surviving terminals with dopamine), must be, as we have seen earlier, less than 1.

Although there may be differences in β and λ between stable responders and patients with wearing-off or on–off fluctuations, we first modelled the impact of vesicular release rate (α) on motor fluctuations. Thus, assuming that these three Parkinson's disease groups have identical $\beta = 0.95$ and $\lambda = 0.35$ (i.e. the same degree of nigrostriatal damage), and taking $\alpha = 0.15$ for the stable group, $\alpha = 0.20$ for the wearing-off group and $\alpha = 0.40$ for the on–off group (Table 1), we obtain three different curves (Table 2; Fig. 4). These curves predict that, on average, stable responders turn OFF at 4 h, wearing-off patients at 3 h and on–off patients at 1.5 h (Table 2; Fig. 4). These results are in keeping with clinical observations (Fabbrini *et al*., 1988). Also, the notion that a change in a single parameter may be sufficient to explain motor fluctuations in Parkinson's disease supports our previous PET findings (de la Fuente-Fernández *et al*., 2001*b*). Both lines of evidence suggest that differences in the severity of nigrostriatal dopamine damage are not strictly necessary to explain the occurrence of motor fluctuations. In other words, patients with a stable response to levodopa treatment may have the same degree of striatal dopamine depletion as fluctuators. Nevertheless, it is obvious that the larger the lesion to the nigrostriatal pathway, the higher the threshold in our model (i.e. higher levels of dopamine in surviving terminals are needed to maintain the patient ON). Such an increase in the threshold would lead to a reduction in the time ON and, consequently, the occurrence of motor fluctuations. Although other factors are implicated (de la Fuente-Fernández *et al*., 2001*a*, *b*), this explains why Parkinson's disease progression is associated with motor fluctuations (Fahn, 1982; Marsden *et al*., 1982).

Interestingly, different changes of parameters can give similar curves. For example, keeping $\lambda = 0.35$ and $c = 2300$ as before, but using $\alpha = 0.20$ and $\beta = 0.90$ (instead of $\alpha = 0.40$ and $\beta = 0.95$), we obtain an identical on–off type curve (i.e. ON time = 1.5 h). This illustrates that, though clinically identical, different patients may have different kinetic parameters.

Importantly, the model allowed us to estimate the probability of obtaining a second ON period after the administration of a single dose of levodopa (the yo-yoing phenomenon) (Fig. 5). Thus, for example, using $\alpha = 0.20$, $\beta = 0.90$ and $\lambda = 0.35$, we obtain an on–off type curve which crosses the threshold line at 1 h (first OFF) and has a probability of 0.038 of crossing back the threshold line (second ON) for 10–30 min some 10–30 min later, before

Table 3 *Sensitivity analysis*

Release (α)	Threshold (c)	Standard deviation (τ)	Shortfall coefficient $\lambda = 0.25$	Shortfall coefficient $\lambda = 0.35$
$\alpha = 0.15$	2200	1000	5.33×10^{-9}	1.01×10^{-11}
	2300	750	6.16×10^{-12}	$<10^{-12}$
		1000	8.35×10^{-8}	3.16×10^{-10}
		1500	1.96×10^{-4}	1.34×10^{-5}
$\alpha = 0.20$	2200	1000	1.39×10^{-5}	6.73×10^{-8}
	2300	750	5.56×10^{-7}	2.52×10^{-10}
		1000	8.35×10^{-5}	8.03×10^{-7}
		1500	5.84×10^{-3}	6.06×10^{-4}
$\alpha = 0.40$	2200	1000	0.10	0.018
	2300	750	0.076	0.011
		1000	0.13	0.038
		1500	0.20	0.12

Cells under $\lambda = 0.25$ and $\lambda = 0.35$ give the probability of yo-yoing response. In all cases, the re-uptake capacity (β) was taken to be 0.95.

turning definitely OFF again (Figs 5 and 6). Since patients with on–off fluctuations usually take six (or more) doses of levodopa per day, this result indicates that such a patient would be expected to experience a yo-yoing phenomenon with these specific characteristics at least once a week. By contrast, using parameters that give a wearing-off type curve ($\alpha = 0.20$, $\beta = 0.95$ and $\lambda = 0.35$), the probability of observing such a second ON period is only 0.0017 (i.e. once every 4 months at the most). This shows that yo-yoing fluctuations probably reflect a presynaptic phenomenon, which is related to how fast the patient turns OFF after levodopa administration. Our model shows that, while rather common in patients with on–off fluctuations, the yo-yoing phenomenon is very rare in wearing-off patients; the probability that stable responders could experience a yo-yoing pattern of response is virtually zero. Again, these results are in keeping with clinical observations (Fahn, 1982; Marsden *et al*., 1982). A sensitivity analysis of the likelihood of obtaining a yo-yoing pattern of response under different parameter values is given in Table 3. Clearly, the probability of yo-yoing generally increases as α increases, and decreases as λ increases (i.e. as λ approaches its normal value). This probability also decreases as β increases, so that, independently of the values of α and λ, it is virtually 0 (i.e. $<10^{-10}$) when β is normal (i.e. when $\beta = 0.985$), as in DRD. As expected, the probability of yo-yoing increases as the threshold (c) and standard deviation (τ) increase.

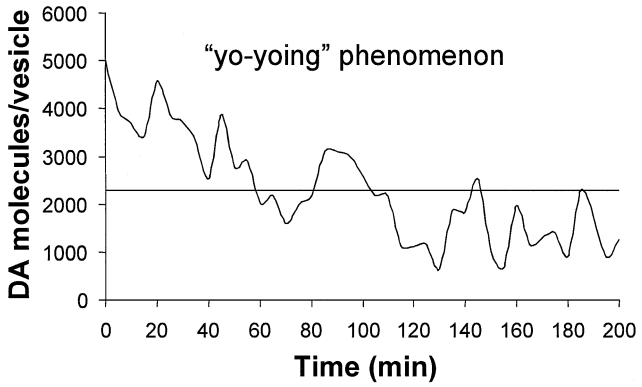

Fig. 6 A randomly simulated trajectory (random path), illustrating typical variations in dopamine levels in a patient who experiences a yo-yoing phenomenon. Again, the trajectory is based on $\alpha = 0.20$, $\beta = 0.90$ and $\lambda = 0.35$, the horizontal line represents the threshold that separates ON and OFF states, time is given in minutes (min) and DA represents dopamine. The first OFF occurs when the path first crosses the threshold downwards (at ~60 min). The second ON occurs when the path recrosses the threshold upwards (at ~80 min). The last OFF then follows when the path once again crosses the threshold downwards (at ~100 min). Compare Fig. 5, which shows the corresponding mean curve for the group of on–off fluctuators.

The long-duration response in Parkinson's disease

In addition to the short-duration response (modelled above), Parkinson's disease patients also show the long-duration response (Nutt and Halford, 1996). This second type of response refers to the observation that chronically treated Parkinson's disease patients often undergo a progressive deterioration in motor function in successive days after levodopa withdrawal. Characteristically, patients experience morning ON periods, which become progressively smaller in magnitude and shorter in duration over consecutive days until a definitive OFF state is reached (Nutt and Halford, 1996). In Table 4 we show that our model can also accommodate this long-duration response. As for DRD (see above), we assume that such daily ON periods are related to sleep benefit, which is equivalent to a morning dose of (endogenous) levodopa. For simplicity, the sleep benefit will be assumed constant (1400 molecules/vesicle). As shown in Table 4, now the key parameter is λ (i.e. Parkinson's disease severity). This is in keeping with clinical observations: the long-duration response to levodopa is inversely related to disease severity; i.e. it decays more rapidly in more severely affected patients (Nutt and Halford, 1996). Other parameters (e.g. α) only determine the daily duration of the ON period (if present). It should be noted that now there are two baseline values to consider: one baseline is reached while on chronic levodopa therapy and the other (lower) after levodopa withdrawal. The

Table 4 *Long-duration response in Parkinson's disease*

	Shortfall coefficient $\lambda = 0.35$			Shortfall coefficient $\lambda = 0.25$		
	DA levels (morning)	ON duration (minutes)	DA levels (night)	DA levels (morning)	ON duration (minutes)	DA levels (night)
Day 0	5000	240	1750	5000	170	1250
Day 1	3150	75	1103	2650	30	663
Day 2	2503	20	876	2063	0	516
Day 3	2276	0	797	1916	0	479
Day 4	2197	0	769	1879	0	470
Day 5	2169	0	759	1870	0	467
Day 6	2159	0	756	1867	0	467
Day 7	2156	0	755	1867	0	467
Day 8	2155	0	754	1867	0	467
Day 9	2154	0	754	1867	0	467
Day 10	2154	0	754	1867	0	467

Two degrees of severity of Parkinson's disease are considered, from less severe ($\lambda = 0.35$) to more severe ($\lambda = 0.25$) parkinsonism; in both cases, $\alpha = 0.15$ and $\beta = 0.95$. Levodopa is stopped after the first morning dose on day 0. A constant sleep benefit is assumed, which would provide 1400 molecules of dopamine (DA) per vesicle each morning. The threshold (c) to define ON periods is taken to be 2300 molecules/vesicle.

smaller the sleep benefit, the shorter the long-duration response.

Discussion

We have modelled mathematically the transition from ON to OFF after levodopa administration in several clinical scenarios: DRD, Parkinson's disease with stable response, Parkinson's disease with wearing-off fluctuations, and Parkinson's disease with on–off fluctuations. We have shown that our model gives predictions for ON times, corresponding to the short-duration response, that fit well with clinical observations. The model also accommodates the long-duration response.

According to the model, DRD patients on optimal treatment with levodopa are expected to turn OFF some 15 h after levodopa withdrawal (or later if the treatment is able to increase the vesicular levels of dopamine above normal values—i.e. $\zeta(0) > 5000$ molecules/vesicle). In addition, the model predicts that untreated DRD patients are likely to experience motor deterioration (i.e. diurnal fluctuations) some 8 h after getting up.

The model shows that the occurrence of motor fluctuations in Parkinson's disease, both predictable (wearing-off) and unpredictable (on–off) fluctuations, can be explained through presynaptic mechanisms that regulate vesicular dopamine release. This observation is at variance with conventional views (Bravi *et al*., 1994). In an interesting experiment, Bravi and colleagues (Bravi *et al*., 1994) found that the response to apomorphine (a direct dopamine agonist) is shorter in fluctuators than in stable responders. Based on the assumption that apomorphine has a pure postsynaptic mechanism of action, they argued that up to 75% of such shortening in motor response is due to postsynaptic mechanisms. However, while the clinical response to apomorphine is most likely related to its action on postsynaptic dopamine receptors, this drug also acts on nigrostriatal autoreceptors (Przedborski *et al*., 1995), which leads to a reduction in cell firing, dopamine synthesis and dopamine release (Przedborski *et al*., 1995; Cooper *et al*., 1996). Indeed, it has long been suggested that changes in dopamine autoreceptor function can be relevant to the pathogenesis of motor fluctuations (Carlsson, 1983; Cooper *et al*., 1996). We propose that fluctuators have autoreceptor dysfunction (e.g. autoreceptor desensitization or autoreceptor downregulation), something that may be a homeostatic regulatory mechanism to increase dopamine release. In keeping with this notion, there is preliminary *in vivo* PET evidence suggesting that autoreceptor dysfunction could be at the very heart of the increased dopamine turnover found in fluctuators (de la Fuente-Fernández *et al*., 2001*a*, *b*). We argue that the results of Bravi and colleagues can be explained as follows. Stable responders would tend to accumulate presynaptic dopamine while on apomorphine; the release of this (surplus) dopamine as the action of apomorphine decreases leads to prolonged motor response. By contrast, fluctuators would continue to release dopamine because of the lack of autoreceptor responsiveness to apomorphine and, consequently, the motor response would end as the action of apomorphine finishes.

Whereas we recognize that downstream changes probably contribute to motor complications (both fluctuations and dyskinesias) in Parkinson's disease, several clinical observations suggest that postsynaptic mechanisms are not primarily responsible for these phenomena. Thus, OFF periods are terminated by apomorphine (Poewe *et al*., 1988), which indicates that postsynaptic dopamine receptors (and downstream mechanisms) remain responsive. It is also relevant that motor fluctuations are ameliorated by continuous infusion of dopamine agonists (Obeso *et al*., 1986, 1987) or by therapeutic manoeuvres that steadily refill vesicles with dopamine, such as continuous intravenous (Quinn *et al*., 1982, 1984) or intraduodenal (Sage *et al*., 1988) administra-

tion of levodopa. Even in typical 'unpredictable' (on–off) fluctuations, a relation between levodopa doses and the appearance of OFF periods becomes evident when serial daily charts of motor performance are analysed through several consecutive days (Quinn *et al*., 1982, 1984).

We have shown that a change in a single parameter (vesicular release rate) leads to dramatic differences in motor response (from stable response to on–off fluctuations) for the same degree of nigrostriatal damage. This is in keeping with our previous PET studies (de la Fuente-Fernández *et al*., 2001*b*) and emphasizes the role of increased dopamine turnover in the pathogenesis of motor fluctuations. Perhaps the most striking finding provided by the model is that the yo-yoing phenomenon (i.e. the occurrence of a second ON period after the administration of a single dose of levodopa) can be explained by purely presynaptic mechanisms. Again, dopamine turnover is a key factor: the faster the patient turns OFF, the more likely the occurrence of the second ON. This explains why the yo-yoing phenomenon is virtually exclusive to patients with on–off fluctuations (i.e. those Parkinson's disease patients with the shortest duration of motor response). The possibility exists that some unpredicted ON periods (particularly those of longer duration) may be due to the release of endogenous dopamine. It has been shown that the biochemical basis of the placebo effect in Parkinson's disease is the release of endogenous dopamine in the striatum (de la Fuente-Fernández *et al*., 2001*c*) and this is also likely to be the mechanism responsible for the phenomenon of kinesia paradoxica (de la Fuente-Fernández and Stoessl, 2002). These observations indicate that Parkinson's disease patients still have a significant reserve of endogenous dopamine, which can be brought into play under special circumstances.

Although we did not attempt to model levodopa-related dyskinesias, some conclusions in this respect can be derived from our observations. Again, the vesicular release rate may be a key parameter. Thus, our model predicts larger changes in the synaptic concentration of dopamine (and, consequently, larger changes in dopamine receptor occupancy) in those patients with greater vesicular release rate (usually fluctuators). Indeed, we now have PET evidence supporting this notion (i.e. peak-dose dyskinesias may reflect dramatic changes in synaptic dopamine levels; de la Fuente-Fernández *et al*., unpublished observation). The model also involves a prominent random component, which leads to substantial oscillations in dopamine levels at the end of the ON period (Fig. 6). Such variations in dopamine levels may explain the emergence of diphasic dyskinesias at the end of the clinical benefit induced by levodopa. While not modelled here, the same pattern is expected to occur at the beginning of the ascending part of the curve, which may explain diphasic dyskinesias at the beginning of the ON period.

Finally, our observations have profound clinical implications. The model indicates that levodopa therapy, by itself, is not the cause of motor complications in Parkinson's disease. Instead, these complications reflect the way in which levodopa is handled by the nigrostriatal dopaminergic system. Hence, the potential occurrence of motor complications should not heavily influence clinical decisions aimed at optimizing the initial treatment of Parkinson's disease (i.e. levodopa versus direct dopamine agonist).

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Appendix

The mathematical model

Let X(t) represent the concentration of dopamine within each of the N vesicles of any given terminal at time t. Thus, X(0) measures the initial concentration among the N vesicles (i.e. once the terminals have been replenished with dopamine derived from the exogenous administration of levodopa). We assume that, initially, the distribution of X(0) across the vesicles is approximately symmetrical (i.e. quasinormal), with mean $\zeta(0)$ and variance $\tau^2(0)$ (Fig. 1A). Let A(0) represent the total amount of dopamine initially distributed among the vesicles. Let the unit of time measurement correspond to the (average) time interval between successive releases of vesicles into the synapse. Assume that, at each unit of time, corresponding to each new release, a proportion α of the vesicles is released per terminal, and that, prior to the next release, a proportion β of the dopamine previously released into the synapse is taken up again and is available for redistribution among the vesicles within the terminal. Finally, assume that the endogenous synthesis of dopamine proceeds in the terminal at a constant rate and provides γ molecules of dopamine per unit time.

Now, let A(t) represent the total amount of dopamine available in the terminal just prior to time t, with X(t) measuring the concentration of dopamine within each of the vesicles at that time. Let $\zeta(t)$ and $\tau^2(t)$ represent, respectively, the mean and the variance of X(t). Then we have:

$A(0) = N\zeta(0)$

$A(1) = A(0)[1 - \alpha] + \beta\alpha A(0) + \gamma = A(0)[1 - \alpha + \beta\alpha] + \gamma = A(0)\delta + \gamma$,

where $\delta = 1 - \alpha + \beta\alpha$ ($0 < \alpha \leqslant 1$, and $0 \leqslant \beta < 1$), and

$A(2) = A(1)[1 - \alpha] + \beta\alpha A(1) + \gamma = A(1)\delta + \gamma = A(0)\delta^2 + \gamma[1 + \delta]$.

So,

$A(t) = A(t - 1)[1 - \alpha] + \beta\alpha A(t - 1) + \gamma = A(0)\delta^t + \gamma[1 + \delta + \delta^2 + \ldots + \delta^{t-1}] =$

$= A(0)\delta^t + \gamma(1 - \delta^t)/(1 - \delta)$.

When the endogenous synthesis of dopamine is sufficient to maintain a constant dopamine level in the terminal over time, then $A(0) = A(1) = \ldots = A(t)$. This occurs, clearly, when $\gamma = A(0)(1 - \delta)$ (i.e. when the synthesis of dopamine equals the loss of molecules of dopamine per unit of time). The mean of X(t) at any time t is now given by $\zeta(t) = A(t)/N$.

Clearly, in both DRD and Parkinson's disease, symptoms (motor impairments) reflect an insufficient production of endogenously derived dopamine in the nigrostriatal system. Furthermore, the synthesis of dopamine is also insufficient to maintain the patient ON after levodopa administration. Thus, the vesicular levels of dopamine decrease and eventually reach a threshold (denoted as *c*), below which the patient turns OFF. We define $\lambda = \gamma/[A(0)(1 - \delta)]$ to represent the shortfall coefficient, measuring the deficiency in the rate of the endogenous synthesis of dopamine relative to the equilibrium rate level of $A(0)(1 - \delta)$.

We further assume that X(t) follows a log-normal distribution, with mean $\zeta(t)$ and variance $\tau^2(t)$. The log-normal model allows for right-skewness in the distribution of X(t), and provides the flexibility of increasing the skewness with time, as the mean of X(t) decreases (Fig. 1B). The variance $\tau^2(t)$ of X(t) is assumed to remain constant over time, to allow for a stable range of potential values for X(t), but with diminishing probabilities of assuming the large values in its range, as the skewness of the distribution increases and the mean of X(t) decreases. At time 0, however, we assume that the log-normal distribution of X(0) approximates a symmetrical normal distribution (as mentioned above). This is achieved by ensuring that the variance of $Y(0) = \log X(0)$ is sufficiently small (Johnson and Kotz, 1970).

Under the above assumptions, we can estimate probabilities of various events relating to X(t) at any time t, by calculating the corresponding probabilities for the normally distributed logarithmic transform (Johnson and Kotz, 1970). Thus, for example, to calculate the probability that $a < X(t) < b$, for any non-negative numbers *a* and *b* with $a < b$, we simply calculate the corresponding probability that $\log a < Y(t) < \log b$, where Y(t) follows the normal distribution, with mean $\mu(t)$ and variance $\sigma^2(t)$, related to $\zeta(t)$ and to $\tau^2(t)$, the mean and variance of X(t), by the equations (Johnson and Kotz, 1970)

$\mu(t) = \log [\zeta^2(t)/(\zeta^2(t) + \tau^2(t))^{1/2}]$

and

$\sigma^2(t) = \log [1 + (\tau^2(t)/\zeta^2(t))]$.

Naturally, the assumption of a log-normal distribution for X(t) also allows us to derive the approximate distribution of W(t), the amount of dopamine released into the synapse at time t. Indeed,

W(t) can be regarded as the sum of αN independent variables, each having the same distribution as X(t). For sufficiently large αN, we can apply the central limit theorem to this sum (Feller, 1950; Loeve, 1963). It thus follows that at any time t, W(t) has, approximately, a normal distribution, with mean $\alpha N\zeta(t)$ and variance $\alpha N\tau^2(t)$.

Modelling oscillations

Suppose that at time $t + 1$, $\zeta(t + 1) < c$ for the first time, where c represents the 'threshold' intravesicular level of dopamine that separates ON and OFF states. We wish to estimate the probability that $X(s)$ remains below c for k units of time after that (i.e. for any time s such that $t + 1 \leqslant s \leqslant t + k$), then crosses back to exceed c for another k units of time, and then falls below c once again. We assume that, while the mean of the stochastic process X(t) is time-dependent, as described above, the distributions of $X(t_1)$ and $X(t_2)$ at two distinct time points are independent. This is due to the assumption of the randomness of both the release and the refilling processes. Then, the probability of the event described above becomes $P[X(t) > c, X(t + 1) < c, X(t + 2) < c, \ldots, X(t + k) < c, X(t + k + 1) > c, X(t + k + 2) > c, \ldots, X(t + 2k) > c, X(t + 2k + 1) < c, X(t + 2k + 2) < c, \ldots]$. Applying the logarithmic transformation to X(t), X(t + 1) etc. and using the symbol $\Phi(z)$ to represent the probability that a standard normal variable is $\leqslant z$, the probability becomes (Loeve, 1963; Feller, 1966) $[1 - \Phi(\{\log c - \mu(t)\}/\sigma(t))] \times \Phi(\{\log c - \mu(t+1)\}/\sigma(t+1)) \times \Phi(\{\log c - \mu(t + 2)\}/\sigma(t + 2)) \times \ldots \times [1 - \Phi(\{\log c - \mu(t + k + 1)\}/\sigma(t + k + 1))] \times \ldots \times \Phi(\{\log c - \mu(t + 2k + 1)\}/\sigma(t + 2k + 1)) \times \ldots$. This method was used to calculate the probabilities of observing the sequence ON–OFF–ON (i.e. the yo-yoing phenomenon; Fig. 2) after a single dose of levodopa, for different clinically acceptable values of α, β and λ. For example, a typical configuration of these parameters was taken to be $\alpha = 0.20$, $\beta = 0.90$ and $\lambda = 0.35$, with the threshold set at 2300. The calculations then proceeded by summing the probabilities of all individual trajectories (sample paths) which followed the log-normal distribution described above, but fulfilled special conditions. These consisted of requiring the first ON period to last for ~1 h, guaranteeing that for the last 5 min of that period the trajectories were still above the threshold. In the next 5 min the trajectories were forced to remain below the threshold (OFF). At this stage, to allow for a variable length of 10–30 min in this first OFF period, shorter OFF trajectories were allowed to remain below the threshold for another 5 min, and then forced to cross and remain above the threshold for the following 5 min, entering a second ON period. Longer trajectories were forced to cross into the second ON period after successively longer sojourns in the OFF stage (up to 30 min), by guaranteeing that for the last 5 min of their sojourn they remained below the threshold, and then crossed and stayed above the threshold for 5 min. An analogous procedure then ensured that the trajectories remained in this second ON stage for 10–30 min, and after the last 5 min in their second ON stage would once again cross the threshold and remain below, in a new OFF stage, for at least 5 min. The probabilities of all these trajectories were summed, as described above. The results are shown in the text. A typical random path was plotted and is shown in Fig. 6. A sensitivity analysis was carried out, assessing the effects of varying the values of the parameters on the estimated probability of the yo-yoing phenomenon (Table 3).