REPORT

Presynaptic dopamine deficit in minimally conscious state patients following traumatic brain injury

Esteban A. Fridman,¹ Joseph R. Osborne,² Paul D. Mozley,³ Jonathan D. Victor¹ and Nicholas D. Schiff¹

Dopaminergic stimulation has been proposed as a treatment strategy for post-traumatic brain injured patients in minimally conscious state based on a clinical trial using amantadine, a weak dopamine transporter blocker. However, a specific contribution of dopaminergic neuromodulation in minimally conscious state is undemonstrated. In a phase 0 clinical trial, we evaluated 13 normal volunteers and seven post-traumatic minimally conscious state patients using ¹¹C-raclopride PET to estimate dopamine 2-like receptors occupancy in the striatum and central thalamus before and after dopamine transporter blockade with dextroamphetamine. If a presynaptic deficit was observed, a third and a fourth ¹¹C-raclopride PET were acquired to evaluate changes in dopamine release induced by L-DOPA and L-DOPA+dextroamphetamine. Permutation analysis showed a significant reduction of dopamine release in patients, demonstrating a presynaptic deficit in the striatum and central thalamus that could not be reversed by blocking the dopamine transporter. However, administration of the dopamine precursor L-DOPA reversed the presynaptic deficit by restoring the biosynthesis of dopamine from both ventral tegmentum and substantia nigra. The advantages of alternative pharmacodynamic approaches in post-traumatic minimally conscious state patients should be tested in clinical trials, as patients currently refractory to amantadine might benefit from them.

1 Feil Family Brain and Mind Research Institute, Weill Cornell Medical College New York, NY, USA

2 Radiology Department, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

3 Radiology Department, Weill Cornell Medical College New York, NY, USA

Correspondence to: Esteban A. Fridman, MD, PhD

LC-802A, New York, NY 10065, USA

E-mail: esf2005@med.cornell.edu

Correspondence may also be addressed to: Nicholas D. Schiff

E-mail: nds2001@med.cornell.edu

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Abbreviations: AST = associative striatum; D2LR = D2-like receptors; MCS = minimally conscious state; SMST = sensorimotor striatum; VST = ventral striatum

Introduction

Patients in a minimally conscious state (MCS) express limited behavioural evidence of consciousness (Giacino *et al.*, 2002), which can remit in some instances with

pharmacological therapies (for review see Fridman and Schiff, 2014). Only one class I study using amantadine, however, has shown a benefit for the treatment of post-traumatic brain injury (TBI) MCS to accelerate the speed and rate of recovery after injury (Giacino *et al.*, 2012).

Although an effect of enhanced dopaminergic neurotransmission was posited to underlie the better outcome of these patients, precise characterization of an underlying dopaminergic dysfunction in TBI MCS has not been demonstrated. Dopaminergic neuromodulation by amantadine relies only on a weak blockade of the dopamine reuptake transporter (Sommerauer *et al.*, 2012), since the drug neither increases dopamine synthesis by itself (Arai *et al.*, 2003) nor binds to postsynaptic receptors (Volonté *et al.*, 2001). Thus, partial TBI damage of presynaptic dopaminergic terminals of the substantia nigra and ventral tegmentum (Matsuda *et al.*, 2003) affecting dopamine biosynthesis would not be expected to improve by blocking the dopamine transporter alone with amantadine, and other dopaminergic drugs that increase dopamine synthesis or bind to postsynaptic receptors might have an important complementary therapeutic role.

Molecular neuroimaging in MCS patients allows *in vivo* exploration of new target biomarkers (Jenkins *et al.*, 2016) for future drug development. ^{11}C -raclopride-PET evaluates dopamine receptors type 2 and 3 occupancy (D2-like receptors, D2LR) within striatal and extrastriatal structures of the brain with a good reproducibility (Alakurtti *et al.*, 2011), because of its selective antagonism of those receptors, allowing quantification of the status of the dopaminergic system at the postsynaptic level. When ^{11}C -raclopride-PET is combined with a single dose of amphetamine, a strong blocker of dopamine transporter reuptake, there is an increase in dopamine levels at the synaptic cleft that reduces D2LR occupancy by the radioligand (Breier *et al.*, 1997; Volkow *et al.*, 1997). Lack of this physiological reduction in ^{11}C -raclopride D2LR occupancy following amphetamine is a marker of a presynaptic deficit. Recent studies of MCS patients point to broad neuronal dysfunction across a mesocircuit involving cortical areas in the frontal lobe (Thengone *et al.*, 2016), posterior parietal cortices (Stender *et al.*, 2015), the central thalamus, and the basal ganglia (Fridman *et al.*, 2014). The importance of dopamine neuromodulation to sustain physiological interactions among these structures is well established (Grillner *et al.*, 2005). For example, tonic and phasic dopamine release are known to be essential in controlling striatal medium spiny neurons firing rates (Gerfen and Surmeier, 2011) and thalamocortical outflow (Haider and McCormick, 2009).

Based on observations of vegetative state and MCS patients refractory to dopamine transporter blockers who later improved with the dopamine precursor L-DOPA or the dopamine agonist apomorphine (Supplementary material) we hypothesized that a disruption in the presynaptic biosynthesis of dopamine is present in MCS patients that would prevent increased dopamine release when only blocking the dopamine transporter. Further, we hypothesized that if such a disruption in the presynaptic biosynthesis of dopamine is present, it could be reversed by administering the dopamine precursor L-DOPA.

Materials and methods

Participants

Twenty participants, 13 normal volunteers (mean age = 34 ± 9 years, seven male) and seven patients with chronic TBI MCS (mean age = 30 ± 10 years, seven male) defined by the Coma Recovery Scale-Revised (CRS-R) (Giacino *et al.*, 2004) (Supplementary material) were recruited as part of an exploratory investigation sponsored by the NINDS-NIH. There was one dropout from the group of normal volunteers (Subject NV3 experienced a panic attack during MRI acquisition). Patients' data are reported in Table 1. The study was approved by the IRB of Weill Cornell Medicine. Informed consent to participate in the study was obtained from the subjects themselves in the case of normal volunteers, and from the legal authorized representative for the MCS patients (see inclusion and exclusion criteria in the Supplementary material).

Image acquisition and analysis

All ^{11}C -raclopride-PET 3D emission acquisitions were obtained in list mode using the same Siemens Biograph mCT PET/CT 64-slice spiral scanner with an axial field of view (21.8 cm), matrix size = $400 \times 400 \times 81$, and 2.025 mm slice thickness. Awake subjects received two ^{11}C -raclopride-PET scans: first, a baseline placebo scan; and second, 3 h after receiving 30 mg of dextroamphetamine via oral or percutaneous endoscopic gastrostomy (amphetamine challenge to evaluate pharmacologically-induced phasic dopamine response), which coincided with the period of peak plasma of the drug (Buckholtz *et al.*, 2010). If a presynaptic deficit was demonstrated, a third ^{11}C -raclopride-PET was acquired 1 h after a single dose of 375 mg of dispersible L-DOPA (levodopa test) (Hughes *et al.*, 1991) to evaluate changes in tonic dopamine release; and, a fourth ^{11}C -raclopride-PET combining L-DOPA and amphetamine (L-DOPA + amphetamine). All normal volunteers or MCS patients received their baseline and amphetamine scans during the same day. Similarly, all MCS patients received their L-DOPA and L-DOPA + amphetamine scans during the same day. Two post-amphetamine studies were discarded: Subject NV5 could not remain awake during the second scanner acquisition, and Patient MCS6 developed agitation following amphetamine. Five of seven MCS patients were able to perform subsequent L-DOPA scans (of the excluded, Patient MCS2 was deceased at time of study and Patient MCS6 because of agitation, as noted earlier). For the fourth scan, two of those five MCS patients experienced intolerance when they received L-DOPA + amphetamine (Patients MCS1 developed hypertension and tachycardia 30 min before the scan and Patient MCS5 vomited 15 min before the scan) disallowing last scan acquisition (see participants flow chart in the Supplementary material).

A fast bolus injection of $10 \pm 5 \text{ mCi}$ ^{11}C -raclopride produced in-house was administered without any significant difference between the dose used in either group and condition [baseline in normal volunteers = 10.3 ± 0.5 , and baseline in patients with MCS = 11.8 ± 0.5 ($P = 0.095$); and post-amphetamine in normal volunteers = 10.7 ± 0.8 , and post-amphetamine in patients with MCS 9.5 ± 1.0 ($P = 0.33$)]. Serial scans were reconstructed in 15 dynamic frames (t : 0.17–0.5,

Table 1 Demographic and clinical data

	Patient						
	MCS1	MCS2	MCS3	MCS4	MCS5	MCS6	MCS7
Clinical features							
Sex (age, years)	Male (45)	Male (23)	Male (28)	Male (22)	Male (24)	Male (26)	Male (21)
Aetiology	TBI	TBI	TBI	TBI	TBI	TBI	TBI
Time since injury in years	28.5	4.3	6.4	0.9	8.2	3.7	6.7
Diagnosis	MCS	MCS	MCS	MCS	MCS	MCS	MCS
Diagnosis based on Bruno <i>et al.</i> (2011)	MCS (–)	MCS (–)	MCS (+)	MCS (+)	MCS (–)	MCS (+)	MCS (–)
CRS-R subscale scores							
Auditory function	1	2	4	3	1	2	2
Visual function	3	3	2	3	2	3	3
Motor function	5	1	5	3	3	3	3
Oromotor/verbal	1	1	1	2	0	1	1
Communication	0	0	0	1	0	0	0
Arousal	2	2	2	2	2	2	1
Total score	12	9	14	14	8	11	10
PET behavioural test							
CF Baseline	No	No	Yes	Yes	No	No	No
CF AMPH	No	No	Yes	Yes	No	Agitation	No
CF L-DOPA	Yes ^a	^b	Yes	Yes	No	NR	No
CF L-DOPA + AMPH	Cancelled ^c	^b	Yes	Yes	Cancelled ^d	NR	No

– = negative; + = positive; AMPH = amphetamine; CF = command-following; NR = not registered; TBI = traumatic brain injury.

^aSee Supplementary Video 1.

^bDeceased.

^cAdverse event (hypertension and tachycardia).

^dAdverse event (vomiting).

0.5–0.83, 0.83–1.17, 1.5–2.5, 3.5–4.5, 5–7, 7–9, 9–11, 12.5–17.5, 17.5–22.5, 25–35, 35–45, 45–55, and 55–65) and attenuation corrected.

A structural anatomical MRI was acquired on every subject using a Siemens Allegra 3T scanner, T₁–3D MPRAGE sequence (matrix size: 256 × 256 × 176, voxel size: 1 × 1 × 1 mm³), except Patients MCS1 and MCS5, where a 3T GE scanner, T₁–3D FSPGR sequence (matrix size: 256 × 256 × 120, voxel size: 0.93 × 0.93 × 1.2 mm) was used.

Binding potential maps

Each PET scan was reoriented, corrected for motion, denoised using a wavelet algorithm and co-registered to structural T₁–3D (Fridman *et al.*, 2014) using PMOD v.3.403. Anatomical regions of interest were individually drawn on the MRI dividing the striatum into three functional substructures: ventral, associative and sensorimotor striatum (VST, AST and SMST, respectively). The central thalamus was defined using a 0.2 threshold of the thalamus intensity in the mean PET image from frames 1 to 10, consistent with our previous segmentation method (Fridman *et al.*, 2014) (Fig. 1A). The cerebellar atlas (Schmahmann *et al.*, 1999) was used as a single region of interest.

Binding potential non-displaceable values (BP_{nd}) were extracted from regions of interest using the simplified reference tissue model 2 with the cerebellum as a reference tissue (Wu and Carson, 2002) because of its lack of D₂LR receptors (Hall *et al.*, 1994). Delta percentage changes in BP_{nd} induced by amphetamine (%ΔBP_{nd}-amphetamine) were obtained using the formula:

$$\% \Delta BP_{nd} \text{-amphetamine} = 100 \times (BP_{nd} \text{ of basal} - BP_{nd} \text{ of post-amphetamine}) / BP_{nd} \text{ of basal} \quad (1)$$

Since the presence of very low BP_{nd} values on the basal scan can lead to overestimation of values obtained using %ΔBP_{nd}, we did not perform the transformation if basal BP_{nd} was <0.15 (3 of 152 mean region of interest values were <0.15). Changes induced by L-DOPA were obtained using the formula:

$$\% \Delta BP_{nd} \text{-L-DOPA} = 100 \times (BP_{nd} \text{ of basal} - BP_{nd} \text{ of L-DOPA}) / BP_{nd} \text{ of basal} \quad (2)$$

Changes induced by L-DOPA + amphetamine were obtained using the formula:

$$\% \Delta BP_{nd} \text{-L-DOPA + amphetamine} = 100 \times (BP_{nd} \text{ of basal} - BP_{nd} \text{ of L-DOPA + amphetamine}) / BP_{nd} \text{ of basal} \quad (3)$$

The statistical significance of the differences between groups of the mean region of interest value of BP_{nd} from basal ¹¹C-raclopride-PET and, %ΔBP_{nd}-amphetamine were determined by a permutation test, in which group labels (MCS versus normal volunteer) were shuffled, but data from the two hemispheres within each individual remained linked. The distribution of the test statistic under the null hypothesis was obtained by calculating all possible values of the test statistic under rearrangements of the subject labels, yielding estimates of significance that took into account any interdependence of measurements within a subject's two hemispheres.

Figure 1 Tested model of dopamine neurotransmission. (A) ^{11}C -raclopride-PET-MRI fusion of Patient MCS1 displaying VST, AST and SMST and central thalamus. (B) Monosynaptic nigrostriatal pathway from substantia nigra (SN) and mesolimbic and mesothalamic pathways from ventral tegmentum (VT) to target nuclei, respectively. (C) The dopaminergic terminal at rest in target structures from B shows background tonic dopamine (DA) leaving unoccupied D2LR. In normal volunteers (NV), ^{11}C -raclopride binds at rest to the unoccupied D2LR (shown in the first blue square in the top row). Dopamine transporter reuptake (DTA) blockade with amphetamine in normal volunteer initiates a pharmacologically-induced phasic responses that increases dopamine levels at the synaptic cleft reducing D2LR occupancy by ^{11}C -raclopride (second blue square in the top row). Lack of this physiological reduction following amphetamine is a marker of a presynaptic deficit as predicted to occur in MCS (bottom row: first and second red squares for rest and post-amphetamine conditions, respectively). In this setting reversal of the presynaptic dopaminergic deficit is expected with administration of a single high-dose of the dopamine precursor L-DOPA (bottom: green square).

Results were considered significant at $P < 0.05$ using a hypothesis driven one-tailed test since the direction of the critical difference was anticipated based on observations of patients refractory to dopamine transporter blockers (Supplementary material): we hypothesized that $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ in MCS patients would be higher than in normal volunteers because of a disruption in the presynaptic biosynthesis of dopamine preventing an increased dopamine release when only the dopamine transporter is blocked. Error bars representing 95% confidence intervals (CIs) were measured and displayed using the formulae: lower = $-t_a$ SE and upper = $+t_a$ SE, where t_a is the (two-tailed) critical value of the t distribution at level a . For this calculation, data were first averaged across hemispheres within each subject, before estimating mean and standard error (SE), and the number of degrees of freedom is one less than the number of subjects.

To test tonic changes in dopamine release in MCS patients elicited by L-DOPA, our second driven hypothesis, we contrasted mean $\% \Delta \text{BP}_{\text{nd}}\text{-L-DOPA}$ with mean $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ obtained in MCS and normal volunteers. Data were integrated into two groups separating target regions of the nigrostriatal pathway (Gerfen and Surmeier, 2011) originating in substantia nigra (including AST and SMST); and target regions of the mesolimbic (Wise, 2004) and mesothalamic pathways (Beckstead *et al.*, 1979) originating in the ventral tegmental (including VST and central thalamus, respectively) (Fig. 1B). The mean $\% \Delta \text{BP}_{\text{nd}}$ value was compared using the same shuffle approach as described above (Condition: normal volunteer amphetamine, MCS amphetamine, and MCS L-DOPA) for both projection: substantia nigra versus ventral tegmentum. Results were considered significant at $P < 0.05$ following a hypothesis driven one-tailed test, since the direction of the critical difference was anticipated based on observations of patients refractory to dopamine transporter blockers that revert their measured dopamine deficits with the use of L-DOPA (Supplementary material): we hypothesized in MCS patients that $\% \Delta \text{BP}_{\text{nd}}\text{-L-DOPA}$ will be lower than $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ because of the restoration of presynaptic biosynthesis of dopamine by exogenous L-DOPA.

Finally, due to the adverse events observed when combining large single doses of L-DOPA + amphetamine we present only descriptive results in the Supplementary material.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Results

Figure 1C outlines the conceptual model underlying our experiments. First, we assessed D2LR occupancy by measuring resting BP_{nd} , within normal volunteer and MCS patients groups (Fig. 2A and Supplementary Fig. 1). One-way between groups permutation analysis showed an effect of mean BP_{nd} when contrasting groups. Specifically, there was a significant reduction of BP_{nd} in the VST, AST and central thalamus for MCS patients, but BP_{nd} of SMST did not differ (Fig. 2A and Supplementary Table 1). The observed pattern in MCS is consistent with a partial postsynaptic denervation of VST, AST and central thalamus neurons.

To examine the pharmacologically-induced phasic dopaminergic response we measured $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ (Fig. 2B). There was a significant effect of mean $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ between normal volunteer and MCS groups (Supplementary Table 2). One-way permutation analysis demonstrated the following differences: $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ of the VST, SMST and central thalamus were significantly altered in MCS but $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ of AST did not differ (Fig. 2B). Whereas $\% \Delta \text{BP}_{\text{nd}}\text{-amphetamine}$ of normal volunteers showed the typical physiological response in the striatum and central thalamus, dopamine release in VST, SMST and central

Figure 2 Background and pharmacologically-induced phasic dopamine responses measured using ^{11}C -raclopride. (A) Box plot for background D2LR occupancy in 12 normal volunteers (NV) and seven MCS patients: reduction in BP_{nd} of VST, AST, and central thalamus in MCS (one-sided permutation test); no difference in SMST. (B) Box plot shows $\%\Delta\text{BP}_{\text{nd}}$ -amphetamine for phasic responses in 11 normal volunteers and six MCS patients: lower reduction in $\Delta\text{BP}_{\text{nd}}$ of VST, SMST, and central thalamus in MCS (one-sided permutation test) indicating a presynaptic deficit of the three main pathways; no difference in AST.

thalamus of MCS patients was significantly reduced with preservation of response in AST. The low responses observed in MCS patients are consistent with a presynaptic deficit affecting phasic responses of neurons in both ventral tegmentum and substantia nigra.

To evaluate whether the presynaptic dopaminergic deficits in MCS patients could be reversed further, we tested five patients after administering L-DOPA. There was a significant change of the mean $\%\Delta\text{BP}_{\text{nd}}$ for MCS patients (Supplementary Table 3). Four of five patients showed a large increment of dopamine release in target regions from both ventral tegmentum and substantia nigra (Supplementary Table 3) following L-DOPA (one-way between groups shuffle permutation analysis), reaching the physiological levels observed with amphetamine in normal volunteers (Fig. 3A). Two single subject observations are, in addition, noted. First, Patient MCS1, who remained for 28 years without showing reproducible upper-limb command-following, displayed reliable appendicular command-following 90 min after L-DOPA (Fig. 3B and Supplementary Video 1); and second, the only case that did not increase dopamine release with L-DOPA (Patient MCS5) showed bilateral structural damage within the substantia nigra and ventral tegmentum suggesting a broad loss of presynaptic dopaminergic neurons (Fig. 3C).

Finally, the combination of L-DOPA + amphetamine increased the phasic response in target structures of both dopaminergic pathways in all three patient subjects (see Supplementary Fig. 2 for descriptive unanalysed data).

Discussion

Here we demonstrate first that MCS patients present a dopaminergic deficit affecting mainly presynaptic neurons projecting from ventral tegmentum and substantia nigra to the striatum and central thalamus. These neurons can no longer increase dopamine availability at the synaptic cleft by only blocking the dopamine transporter reuptake

(amphetamine challenge), or even maintain the resting tone as shown following L-DOPA. Further, we demonstrate in a small sample of MCS patients that administration of L-DOPA generally restores the pattern of tonic background dopaminergic (Figs 2 and 3). Since in our MCS patients administration of L-DOPA reversed the presynaptic dopamine deficit by restoring the substrate for the biosynthesis of dopamine (i.e. L-DOPA itself), we suggest that a deficiency of the enzyme tyrosine hydroxylase is present and alters the first step in biosynthesis of dopamine: from L-tyrosine to endogenous L-DOPA, considered as the main limiting factor of the biosynthesis of dopamine (Supplementary Fig. 3) (Tabrez *et al.*, 2012). We speculate that the enzyme tyrosine hydroxylase deficiency in our MCS patients results from acquired post-traumatic cellular dysfunction (e.g. secondary inflammatory injury) (Fridman and Schiff, 2014; Liu *et al.*, 2016). It should be noted that these findings are not proposed to underlie the full spectrum of the disorder of consciousness present with MCS level function in these patients; rather, the findings identify a mechanism of dopamine dysfunction that at least in these few subjects, in which its partial reversibility is demonstrated, can provide an avenue for improving brain function.

Nonetheless, this loss of dopamine is likely an important factor underlying reduced behavioural expression in these MCS subjects. Sharp changes in frontostriatal network activity depends on dopamine release (Grillner *et al.*, 2005). Dopamine reduction would be expected to affect excitatory glutamate neurotransmission (Fan *et al.*, 2014) and, subsequently, firing patterns of neurons that likely underpin large shifts in the conscious state of MCS patients (Schiff, 2010; Liu *et al.*, 2015; Baker *et al.*, 2016). Furthermore, as the central thalamus has high levels of dopamine exceeding those observed in the substantia nigra (Sano *et al.*, 1959) and a preponderant distribution of D2LR (Rieck *et al.*, 2004), our findings suggest that specific interruption of brainstem dopaminergic projections to the central thalamus is likely an important

Figure 3 Elicitation of dopamine response with L-DOPA administration. (A) Box plot shows %ΔBP_{nd} in 11 normal volunteers (NV) using amphetamine (AMPH), six MCS patients using amphetamine, and four MCS using L-DOPA. A clear reversibility of the presynaptic deficit from ventral tegmentum (VT) and substantia nigra (SN) follows a single dose of L-DOPA, reaching physiological values obtained from normal volunteers. (B) ¹¹C-raclopride-PET-MRI fusion displaying a restoration of the physiological reduction in binding potentials (BP) following administration of L-DOPA in Patient MCS1. (C) Mesencephalon (midbrain) axial anatomic high-resolution MRI in a representative normal volunteer and the five MCS patients that performed the ¹¹C-raclopride-PET after a single dose of 375 mg of dispersible L-DOPA. All patients (Patients MCS1, MCS3, MCS4 and MCS7) demonstrated higher dopamine release following L-DOPA administration show relative preservation of the midbrain structures, compared with anatomical image from Patient MCS5, who did not respond to a single dose of L-DOPA, which reveals extensive bilateral damage within the substantia nigra and ventral tegmentum.

contributor in the pathophysiology of MCS. However, the primary loss of long-range glutamergic projecting neurons across cortico-cortical and cortico-thalamic networks likely accounts for the MCS level of global dysfunction in our subjects (Schiff, 2010).

Finally, noting that in a large randomized clinical trial amantadine only modestly improved the primary outcome measure for MCS patients (i.e. 1.4-point difference in a 30-point scale) and that almost 60% of MCS patients in this study lacked consistent command-following at trial end (Giacino *et al.*, 2012), our findings raise the possibility that compounds with different dopaminergic pharmacodynamic profiles may provide additional support to MCS patients. Improving dopamine function is likely an essential aspect of optimal neuropharmacological management of disorders of consciousness due to TBI. Thus, the findings here have implications for future clinical research; the possible general advantages of targeting the presynaptic deficit or stimulating postsynaptic receptors in MCS should be tested in clinical trials. Our findings further suggest that dopamine modulation alone or in conjunction with amantadine might have significant benefit, as the former drug is an NMDA antagonist and likely primarily drives fronto-striatal system as bulk activator of glutamatergic neurons (Fridman and Schiff, 2014) similarly to the effects of ketamine (Brown *et al.*, 2010; Esterlis *et al.*, 2018). In MCS patients a role for release of anterior forebrain activation via a combination of dopaminergic and glutamatergic tone has been proposed (Schiff, 2010) and supported by studies of resting glucose metabolism (Fridman *et al.*, 2014). From clinical perspective, MCS patients currently refractory to amantadine or holding a clinical plateau using this drug might find benefit from compounds with different dopaminergic pharmacodynamic profiles.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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