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Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease

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Although patients with Parkinson's disease show impairments in cognitive performance even at the early stage of the disease, the synaptic mechanisms underlying cognitive impairment in this pathology are unknown. Hippocampal long-term potentiation represents the major experimental model for the synaptic changes underlying learning and memory and is controlled by endogenous dopamine. We found that hippocampal long-term potentiation is altered in both a neurotoxic and transgenic model of Parkinson's disease and this plastic alteration is associated with an impaired dopaminergic transmission and a decrease of NR2A/NR2B subunit ratio in synaptic *N*-methyl-D-aspartic acid receptors. Deficits in hippocampal-dependent learning were also found in hemiparkinsonian and mutant animals. Interestingly, the dopamine precursor L-DOPA was able to restore hippocampal synaptic potentiation via D1/D5 receptors and to ameliorate the cognitive deficit in parkinsonian animals suggesting that dopamine-dependent impairment of hippocampal long-term potentiation may contribute to cognitive deficits in patients with Parkinson's disease.

Keywords: α-synuclein; CA1 area; dementia; dopamine; glutamate; synaptic plasticity

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Abbreviations: L-DOPA = L-3,4-dihydroxyphenylalanine; DOPAC = 3,4-dihydroxyphenylacetic acid; EPSP = excitatory postsynaptic potential; 6-OHDA = 6-hydroxydopamine; LTP = long-term potentiation; NMDA = *N*-methyl-D-aspartic acid

Introduction

Parkinson's disease causes impairments in cognitive performance resembling those seen in frontal lobe patients (Robbins and Arnsten, 2009), and progression of these deficits can lead to dementia (Cools *et al.*, 2001; Goetz *et al.*, 2008). While the motor abnormalities in Parkinson's disease result from nigrostriatal dopamine depletion, memory dysfunctions are induced by degeneration of the dopaminergic mesocorticolimbic projections originating from the ventral tegmental area (Calabresi *et al.*, 2006).

The hippocampus is implicated in memory deficits observed in Parkinson's disease (Shohamy *et al.*, 2009) since both structural and functional abnormalities of this structure have been observed in patients suffering from sporadic (Summerfield *et al.*, 2005; Joelving *et al.*, 2006) and genetic forms of the disease (Helton *et al.*, 2008). In addition, hippocampal abnormalities positively correlate with memory deficits (Bruck *et al.*, 2004; Nagano-Saito *et al.*, 2005; Bouchard *et al.*, 2008; Ibarretxe-Bilbao *et al.*, 2008; Jokinen *et al.*, 2009) and behavioural alterations (Ibarretxe-Bilbao *et al.*, 2008).

The hippocampus is essential for encoding spatial and episodic memories (Ryan *et al.*, 2010), and for novelty detection (Jenkins *et al.*, 2004). Novelty detection involves the comparison of an existing memory with new sensory information. Indeed, CA1 pyramidal cells also receive direct sensory inputs from the cortex (Lisman and Otmakhova, 2001). The dopaminergic system is a strong candidate for modulating novelty acquisition and synaptic plasticity in the hippocampal CA1 area. The ventral tegmental area, the major brain source of dopaminergic inputs to the limbic system and hippocampus, is part of a functional loop detecting novelty (Lisman and Grace, 2005).

Hippocampal long-term potentiation (LTP) gates behaviourally relevant information into long-term memory (Lisman and Grace, 2005). Dopamine is critically involved in this process since novel stimuli trigger burst firing in ventral tegmental area cells (Horvitz *et al.*, 1997) whose projections reach the hippocampus (Gasbarri *et al.*, 1997) and make synapses with D1/D5 receptor-expressing CA1 pyramidal cells (Ciliax *et al.*, 2000).

Pharmacological modulation of D1/D5 receptors modifies LTP recorded in the CA1 area (Frey *et al.*, 1991; Huang and Kandel, 1995; Otmakhova and Lisman, 1996). This modulation is correlated with memory tasks (Lemon and Manahan-Vaughan, 2006) suggesting that loss of hippocampal dopamine innervation would cause LTP impairment contributing to memory deficits in Parkinson's disease.

Intracellular accumulation of α-synuclein is the pathological feature of Parkinson's disease and monogenic forms of this disease have been associated with mutations in the gene encoding for this protein (Dawson *et al.*, 2010). Although abnormalities in hippocampal plasticity have been observed in α-synuclein-related models (Steidl *et al.*, 2003; Gureviciene *et al.*, 2009), the mechanisms underlying the alteration of hippocampal LTP and hippocampal-related memory have never been analysed and compared in a toxic and genetic model of Parkinson's disease. To address this issue, we investigated hippocampal dopamine transmission and LTP in 6-hydroxydopamine (6-OHDA) hemilesioned rats and α-synuclein transgenic mice, expressing a truncated form of human α-synuclein (1–120; Tofaris *et al.*, 2006).

Materials and methods

All the detailed methods and any associated references are available in the online Supplementary material.

Rats with 6-hydroxydopamine-induced lesion

Three-month-old 6-OHDA-lesioned rats ($n = 81$) were obtained as previously reported (Picconi *et al.*, 2003, 2008). Briefly, a group of deeply anaesthetized (chloral hydrate, 400 mg/ml/kg) rats were injected unilaterally with 6-OHDA (12 µg/4 µl of saline containing 0.1% ascorbic acid) into the medial forebrain bundle at a rate of 0.38 µl/min (anterioposterior = −4.4, lateral = +1.2, ventrodorsal = −7.8). A group of rats were injected only with vehicle at the same coordinates (sham-operated rats; $n = 43$). Fifteen days later, rats were tested with 0.05 mg/kg subcutaneous apomorphine, and contralateral turns to the lesion were counted for 40 min. The rats that showed more than 200 contralateral turns were enrolled in the study. Sham-operated animals did not show any turning behaviour. The severity of the lesion was also quantified afterward by nigral tyrosine hydroxylase immunohistochemistry. Experiments were performed 4–6 weeks after lesion.

Procedure for L-DOPA treatments

Subchronic treatment with L-3,4-dihydroxyphenylalanine (L-DOPA) was performed by administration of 10 mg/kg L-DOPA plus 6.5 mg/kg benserazide (intraperitoneal) twice a day for four consecutive days. Mice were injected with 20 mg/kg L-DOPA plus 7.5 mg/kg benserazide (intraperitoneal) once a day for four consecutive days. Both L-DOPA and benserazide were dissolved in saline. Seven sham-operated rats and 21 6-OHDA-lesioned rats were implanted with indwelling cannulae six weeks after operation (Rossato *et al.*, 2009). Four groups of seven male Wistar rats were used: (i) sham-operated, intraperitoneal saline-treated, plus saline injected in the dorsal hippocampus (intrahippocampal); (ii) 6-OHDA-lesioned, intraperitoneal saline-treated, plus intrahippocampal injected saline; (iii) 6-OHDA-lesioned, subchronically treated with intraperitoneal L-DOPA, plus intrahippocampal injected saline; and (iv) 6-OHDA-lesioned, subchronically treated with intraperitoneal L-DOPA, plus intrahippocampal injected SCH23390.

Mice transgenic for truncated human α-synuclein (1–120)

Male mice (3–4 months old) transgenic for truncated human α-synuclein (1–120; $n = 23$), produced on a C57BL/6S background (Harlan; Tofaris *et al.*, 2006), and control aged-matched C57BL/6S male wild-type mice which is a strain of C57Bl/6 that lacks endogenous α-synuclein (Harlan; $n = 18$), were used in electrophysiological experiments, dopamine tissue quantification, molecular analysis and for behavioural testing. In these transgenic mice (α-syn120), the expression of truncated human α-synuclein (1–120), driven by the tyrosine hydroxylase promoter on a mouse α-synuclein null background, leads to the formation of pathological inclusions in the substantia nigra and olfactory bulb and to the reduction in striatal dopamine levels. At the behavioural level, the transgenic mice show a progressive reduction in spontaneous locomotion.

Immunohistological procedure

In 6-OHDA rats, the severity of the lesion was confirmed afterward by tyrosine hydroxylase immunohistochemistry. For each rat, tyrosine hydroxylase-positive cells were counted at three different rostrocaudal levels of the substantia nigra compacta at the level of the exiting of the third nerve, 200 μm rostral and 200 μm caudal to this level. Cell number was expressed as the mean number/section and the loss of tyrosine hydroxylase-positive cells was analysed by two-way ANOVA, followed by Tukey's *post hoc* test.

Behavioural procedure

The protocol used for the hole-board test is a modified version of that described by Kemp and Manahan-Vaughan (2008). Horizontal and vertical movements were recorded in an automated apparatus (Imetronic). The hole-board test was performed in two different experimental sections: (i) 6-OHDA-lesioned and sham-operated rats, α-synuclein and wild-type mice and (ii) hemiparkinsonian rats, α-synuclein mice and their relative controls, subchronically treated with L-DOPA. ANOVA was used to analyse statistical differences between groups, and Tukey's Honestly Significant Difference test for *post hoc* comparisons.

Synaptosome preparation and [^{3}H]-dopamine analysis

Synaptosomes were prepared as previously described (Marti *et al.*, 2003). Briefly, hippocampus was homogenized in ice-cold 0.32M sucrose buffer (pH 7.4), then centrifuged for 10 min at $2500g_{max}$ (4°C). The supernatant was centrifuged for 20 min at $9500g_{max}$ (4°C), and the synaptosomal pellet resuspended in oxygenated Krebs solution. Synaptosomes were incubated with 50 nM [^{3}H]-dopamine for 25 min, after which 1 ml aliquots of the suspension (~0.35 mg protein) were injected into nylon syringe filters maintained at 36.5°C and superfused (0.4 ml/min) with preoxygenated Krebs. Under these superfusion conditions, spontaneous [^{3}H]-dopamine efflux was essentially unaffected by reuptake. Sample collection (every 3 min) was initiated after a 20 min period of filter washout. Radioactivity in the samples and in the filter (at the end of experiment) was measured by liquid scintillation spectrophotometry.

Data, means ± SEM of 6–8 determinations per group, were calculated as absolute content (pmol/mg protein), fractional release (i.e. tritium efflux expressed as percentage of the tritium content in the filter at the onset of the corresponding collection period) or net fractional release, i.e. K^+-evoked tritium overflow as percentage of the tritium content in the filter at the onset of the corresponding collection period. Statistical analysis was performed (Prism software) by one-way ANOVA followed by the Newman–Keuls test for multiple comparisons. When only two groups were compared, the Student *t*-test was used. *P*-values < 0.05 were considered to be statistically significant.

Microdialysis

Microdialysis experiments were carried out in awake, freely moving animals. Rats were anaesthetized, mounted in a stereotaxic frame (David Kopf Instruments) and implanted with a guide cannula (stainless steel, shaft outer diameter of 0.38 mm, length 4 mm; Metalant AB), in the hippocampus ipsilateral to the 6-OHDA-lesioned side (anterioposterior = −3.6; lateral = +1.84). Experimental procedures were performed as previously reported (Pascucci *et al.*, 2007). Following the onset of perfusion, rats were left undisturbed for 2 h and then dialysates were collected at 20-min intervals for 3 h. Dialysate samples were transferred to high-performance liquid chromatography systems for biogenic amine detection. Both catecholamines were simultaneously measured at the following conditions: the conditioning cell was set at +400 mV, electrode 1 at +200 mV and electrode 2 at −250 mV; the mobile phase was described previously (Westerink *et al.*, 1998). For 5-hydroxytryptamine detection, the conditioning cell was set at +350 mV, electrode 1 at −150 mV and electrode 2 at +200 mV; the mobile phase was described previously (Gartside *et al.*, 2003). A Nova-Pack C18 column (3.9 × 150 mm; Waters) equipped with a Sentry Guard Nova-Pack C18 pre-column (3.9 × 20 mm) maintained at 32°C was used. The limit of sensitivity of the assay was 0.1 pg. The flow rate was 1.2 ml/min.

Tissue analysis

Tissue analysis was carried out as previously reported (Puglisi-Allegra *et al.*, 2000). Briefly, following decapitation, the brain was dissected and put on an aluminium surface at 0°C. The punches of hippocampus were kept frozen and stored at −80°C. On the day of analysis, punches were weighed and homogenized in 0.05 M $HClO_4$. Tissue levels of dopamine, norepinephrine, homovanillic acid and 3,4-dihydroxyphenylacetic acid (DOPAC) were assessed simultaneously by a high-performance liquid chromatography system.

Subcellular fractionation and western blot analysis

Purification of triton-insoluble postsynaptic fraction and western blot analysis were performed as previously reported (Gardoni *et al.*, 2006). The following antibodies were used: polyclonal antibody anti-NR2B and monoclonal antibody anti-NR2A from Zymed Laboratories, monoclonal antibody anti-α-Tubulin from Sigma-Aldrich.

Electrophysiological recordings

Mice and rats were anaesthetized with halothane before decapitation. Under visual control, a stimulating electrode was inserted into the Schaffer collateral fibres, and a recording electrode was inserted into the CA1 region of the hippocampal slice (Sgobio *et al.*, 2010). Field excitatory postsynaptic potentials (fEPSPs) were filtered at 3 KHz, digitized at 10 KHz and stored on a PC. For all of the experiments, data are presented as mean ± SEM (n is the number of slices). Off-line analysis was performed using Clampfit (Molecular Devices) and GraphPad Prism 3 (GraphPad Software) software. Two-way ANOVA

was used for statistical analysis. The significance level was established at $P < 0.05$.

For patch-clamp recordings, neurons of the CA1 region were visualized using differential interference contrast (Nomarski) and infrared microscopy (Olympus). Whole-cell voltage-clamp (holding potential, −60 mV) recordings were performed with borosilicate glass pipettes. Postsynaptic currents (PSCs) of half-maximal amplitude were evoked every 10 s; LTP was induced by a high-frequency stimulation protocol consisting of three trains stimulating at same postsynaptic current strength. Details are given in the Supplementary material.

Results

Features of dopamine denervation: substantia nigra pars compacta versus ventral tegmental area

6-OHDA injected into the rat medial forebrain bundle caused loss of dopamine neurons located in both the substantia nigra pars compacta and the ventral tegmental area (Fig. 1A). This procedure was accompanied by loss of the efferent nigral projections to the striatum and of the dopaminergic projections from the ventral tegmental area ($P < 0.001$). However, as shown in Fig. 1A and B, the dopaminergic loss was more evident in the substantia nigra pars compacta than the ventral tegmental area ($P < 0.001$) mimicking the pattern observed in Parkinson's disease (Damier *et al.*, 1999). In fact, while some dopamine neurons were spared in the ventral tegmental area, the dopamine denervation was virtually complete in the substantia nigra pars compacta.

Both spontaneous and stimulated dopamine release is reduced in the hippocampus of hemiparkinsonian rats

In order to assess whether loss of mesencephalic dopamine neurons was associated with changes of dopamine release in the hippocampus, hippocampal synaptosomes were obtained from either dopamine-depleted or sham-operated rats. [^{3}H]-dopamine accumulation was slightly reduced (~20%) in synaptosomes prepared from the dopamine-depleted hippocampus of hemiparkinsonian rats with respect to the hippocampus of sham-operated rats (0.67 ± 0.022 and 0.84 ± 0.027 pmol mg prot^{-1} min^{-1}, respectively; Fig. 1C). Basal [^{3}H]-dopamine efflux was reduced by ~22% in synaptosomes prepared from the dopamine-depleted hippocampus (4.0 ± 0.2%) compared with controls (6.19 ± 0.2%; Fig. 1D). Spontaneous tritium efflux was unaffected by omission of Ca^{2+} from the perfusion medium both in 6-OHDA-treated and sham-operated rats. A 2 min K^+ pulse evoked a tritium overflow of 5.28 ± 0.23%, which was largely (~85%) Ca^{2+}-dependent and therefore exocytotic in nature. K^+-evoked tritium overflow was dramatically reduced (~70%) in the dopamine-depleted hippocampus. Also, in these conditions, K^+-evoked tritium overflow was largely Ca^{2+}-dependent (Fig. 1E).

Biogenic amine outflow was evaluated by intracerebral microdialysis in the hippocampus of dopamine-depleted ($n = 8$) or sham-operated ($n = 8$) rats under basal conditions. The two groups did not differ for either norepinephrine (sham-operated: 0.989 ± 0.248 pg/20 µl; dopamine-depleted: 1.221 ± 0.286 pg/20 µl) or 5-hydroxytryptamine (sham-operated: 3.920 ± 0.492 pg/20 µl; dopamine-depleted: 4.721 ± 1.066 pg/20 µl) levels. Conversely, basal dopamine levels were significantly reduced in dopamine-depleted (0.711 ± 0.201 pg/20 µl) compared with sham-operated (2.758 ± 0.773 pg/20 µl; $^*P < 0.05$) rats (Fig. 1F–H).

Hippocampal-dependent learning is impaired in experimental parkinsonism: reversal by L-DOPA

In order to explore whether endogenous hippocampal dopamine is implicated in cognitive deficits observed in Parkinson's disease, we measured the ability of both 6-OHDA-depleted and sham-operated rats ($n = 10$ for both groups) to recognize environmental spatial novelty by utilizing an open-field hole-board (Fig. 2A–C). This test has been demonstrated to involve the dorsal hippocampus and to be dopamine-dependent (Lemon and Manahan-Vaughan, 2006). In Session 1 (Fig. 2A and B), no significant differences in locomotor (horizontal and vertical) activities were observed between groups ($P > 0.05$). In the exposure of hole-board sessions (Fig. 2C), two-way ANOVA revealed a significant interaction between Group × Session main factors ($P < 0.001$). *Post hoc* analysis showed a significant reduction of hole explorations in sham-operated rats ($P < 0.001$) but not 6-OHDA-lesioned animals, indicating that parkinsonian rats have a recognition deficit of novel context feature (hole-board). Subchronic L-DOPA treatment administered (twice a day for four consecutive days) 4 h before test restored normal performance in lesioned rats ($^{**}P < 0.01$; Fig. 2C).

To demonstrate that the behavioural effect induced by systemic L-DOPA was really dependent on the activation of hippocampal dopamine receptors, we injected the D1 receptor antagonist SCH23390 (1.5 µg/µl saline) in the hippocampus of dopamine-depleted ($n = 21$) and sham-operated rats ($n = 7$). SCH23390 or saline were injected (20 min before systemic L-DOPA administration) once a day for four consecutive days.

Intrahippocampal application of saline in sham-operated animals did not produce significant alterations in recognition ability of new context. Also, in hemiparkinsonian lesioned rats, cannula implantation and handling procedure for injection did not alter the deficit in recognition novelty and the capability of L-DOPA to restore habituation process. Interestingly, intrahippocampal delivery of SCH23390 fully prevented the L-DOPA-induced therapeutic effect in parkinsonian animals (Fig. 2C). This observation supports the critical involvement of hippocampal D1/D5 receptors in the observed behavioural effects induced by L-DOPA. Moreover, between-group *post hoc* comparisons revealed that sham-operated rats explored the hole-board significantly more than the other groups during first exposure (Fig. 2C). These results revealed an effect of 6-OHDA on basal arousal activity of the animals. L-DOPA treatment did not restore normal exploration activity

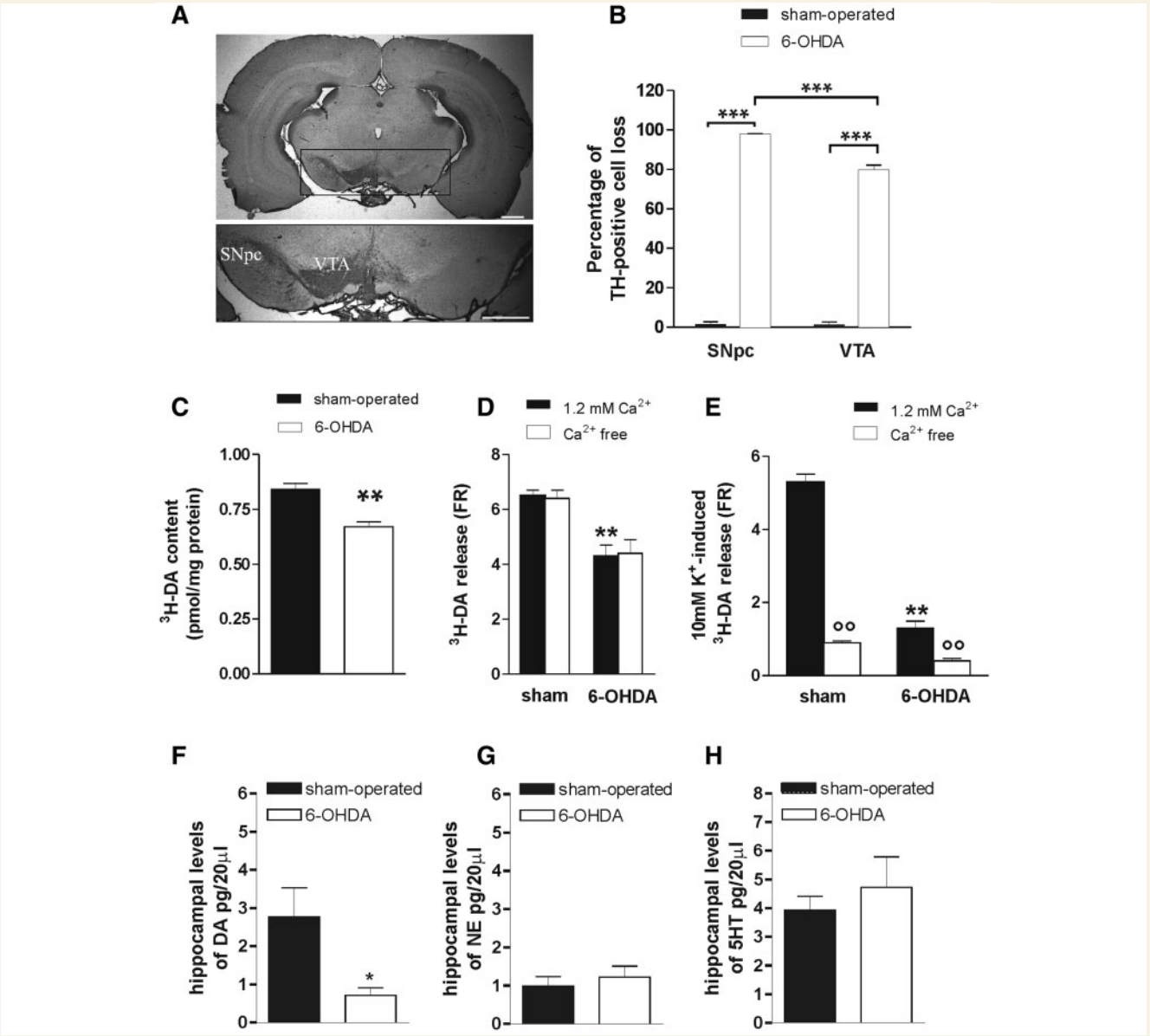

Figure 1 Tyrosine hydroxylase-immunostaining and dopamine release in the hippocampus of sham-operated and hemiparkinsonian rats. (**A**) Tyrosine hydroxylase (TH)-immunostaining in coronal section of midbrain of 6-OHDA-lesioned rat (scale bar = 1 mm). (**B**) 6-OHDA treatment produces a selective cell death of dopamine (DA) neurons in both substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) regions. Significant interaction Group × Region: $F(1,18) = 37.6$; $^{***}P < 0.001$. However, the dopaminergic denervation was more evident in the substantia nigra than in the ventral tegmental area ($^{***}P < 0.001$). (**C–E**) A synaptosomal preparation was obtained from the dopamine-depleted hippocampus of hemiparkinsonian and sham-operated rats. [^{3}H]-dopamine accumulation (**C**), efflux (**D**) and K^+-evoked overflow (**E**) were measured. K^+ was applied for 2 min. Spontaneous [^{3}H]-dopamine efflux and K^+-evoked overflow were also measured in Ca^{2+}-free conditions (**D** and **E**). Perfusion with Ca^{2+}-free Krebs solution started 9 min before the K^+ pulse and was maintained until the end of experiments. Data, means ± SEM of six determinations per group, are expressed in absolute values (**C**), fractional release (FR) (**D**) or net fractional release (**E**: refer to 'Materials and methods' section for calculation details). ($^{**}P < 0.01$ different from sham-operated rats, $^{\circ\circ}P < 0.01$ different from synaptosomes in 1.2 mM Ca^{2+} Krebs.) (**F–H**) Biogenic amine outflow measured in hippocampus of sham-operated and 6-OHDA rats by *in vivo* microdialysis. (**F**) Hippocampal release of DA is reduced in 6-OHDA-lesioned in comparison with sham-operated rats [dopamine-depleted 0.711 ± 0.201 pg/20 µl versus sham-operated 2.758 ± 0.773 pg/20 µl; $F(1,14) = 6.571$, $^*P < 0.05$], while the two groups did not differ for either norepinephrine (NE) (**G**) or 5-hydroxytryptamine (5HT; **H**) hippocampal levels.

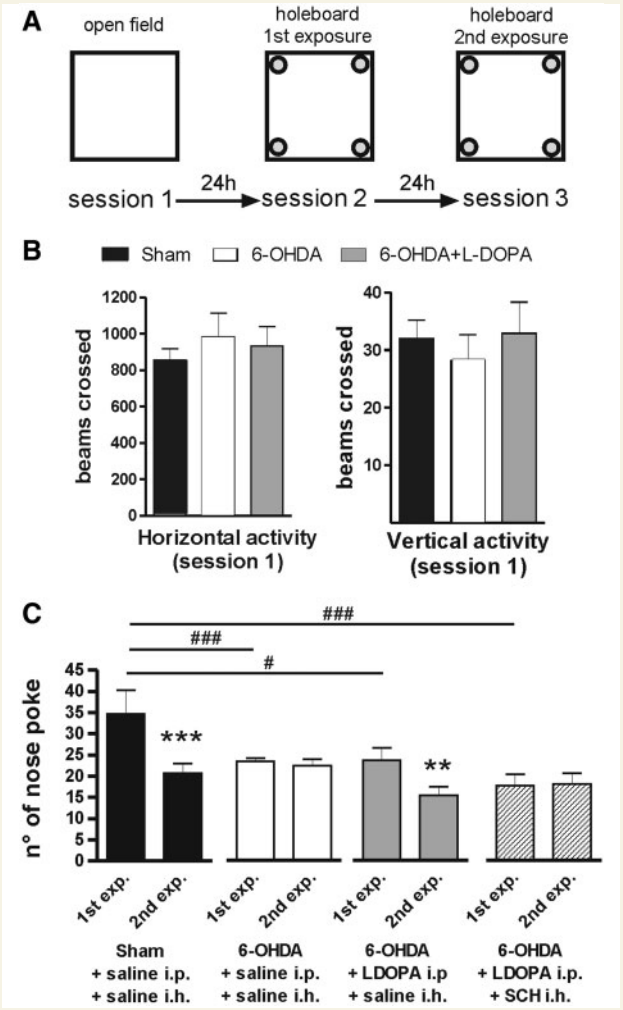

Figure 2 Hippocampal-related memory is altered in a dopamine-dependent manner in hemiparkinsonian rats. (**A**) Representative scheduling and session features of the open-field hole-board test. (**B**) Horizontal (*left*) and vertical (*right*) activity revealed no differences in locomotion between groups. (**C**) A significant reduction of hole explorations in sham-operated group but not in parkinsonian group indicated a recognition deficit of novel context in 6-OHDA-lesioned animals whereas subchronic L-DOPA treatment restored normal performance in lesioned rats. Two-way ANOVA revealed a significant Group × Session interaction [$F(2,15) = 12.8$; $P < 0.001$; Bonferroni *post hoc*: Sham, first experiment versus second experiment $^{***}P < 0.001$; 6-OHDA + L-DOPA, first experiment versus second experiment $^{**}P < 0.01$]. Intrahippocampal (i.h.) application of saline did not alter habituation observed in sham-operated rats ($^{***}P < 0.001$). Saline (intrahippocampal) also failed to affect the deficit in habituation observed in 6-OHDA-denervated animals ($P > 0.05$) and the restorative effect of L-DOPA on habituation ($^{**}P < 0.01$). Conversely, intrahippocampal administration of the D1 receptor antagonist SCH23390 fully prevented the restorative effect of systemic L-DOPA in parkinsonian animals ($P > 0.05$, 1st exp versus 2nd exp). For between-group comparisons, Sham-operated rats explore hole significantly more than the other group during first exposure. Bonferroni *post hoc*: Sham versus 6-OHDA $^{###}P < 0.001$; Sham versus 6-OHDA + L-DOPA, $^{#}P < 0.05$; Sham versus 6-OHDA + L-DOPA + SCH, $^{###}P < 0.001$. i.p. = intraperitoneal.

suggesting that L-DOPA might fail to correct all the deficits caused by dopamine denervation and that non-dopaminergic systems might contribute to this deficit. Nonetheless, increasing brain dopamine with L-DOPA allows the animals to habituate to the hole-board in relation to their own level of arousal.

Hippocampal long-term potentiation is reduced in hemiparkinsonian rats

CA1 pyramidal neurons were patch-clamped in slices from hemiparkinsonian ($n = 9$) and sham-operated ($n = 6$) rats. The current–voltage relationship revealed no differences in basal membrane properties between neurons recorded in slices from sham-operated and 6-OHDA-lesioned rats (Fig. 3A; $P > 0.05$). Postsynaptic currents (PSCs) and extracellular field potential (field EPSP) recordings were subsequently obtained from hippocampal slices taken from hemiparkinsonian and sham-operated rats ($n = 20$ and $n = 8$ for field EPSP recordings, respectively). At the beginning of each experiment, an input–output curve was obtained by stimulating the collateral Schaffer fibres and recording from the CA1 region of the slice. The comparison of the curves obtained from 6-OHDA and sham-operated slices revealed no significant difference between groups for both the postsynaptic current (Fig. 3B and C; $n = 4$ for each group, $P > 0.05$) and for the field EPSP ($n = 8$ slices for each group).

Paired-pulse ratios of field EPSP slope, obtained at increasing stimuli intervals (50–300 ms), revealed no differences between 6-OHDA and sham-operated slices (Fig. 3D; $n = 7$ slices for each group, $P > 0.05$). For each experiment, after the recording of a stable field EPSP for 20 min, a high-frequency stimulation protocol was delivered to the slice. As presented in Fig. 3E, 60 min after high-frequency stimulation protocol, a LTP occurred in the two groups of animals. Interestingly, LTP recorded in 6-OHDA-lesioned rats ($n = 9$ slices) was significantly reduced with respect to that in sham-operated animals ($n = 9$ slices, $P < 0.001$). These data were confirmed by voltage-clamp patch-clamp recordings in 6-OHDA-lesioned and sham-operated rats (Fig. 3F). The analysis of the amplitude of postsynaptic current measured pre- and post-the high-frequency stimulation showed a significant decrease of LTP amplitude in 6-OHDA-lesioned rats compared with sham-operated animals (Fig. 3F; $n = 5$ neurons for both groups; $P < 0.001$). In sham-operated rats, LTP was significantly reduced by bath application of the D1/D5 receptor antagonist SCH23390 (Supplementary Fig. 1A; sham in standard solution versus SCH23390-treated rats, $n = 9$ and $n = 11$ slices, respectively; $P < 0.001$). LTP in the CA1 contralateral to the lesioned side was also significantly reduced by SCH23390 (Supplementary Fig. 1B; $n = 4$ slices for both groups; $P < 0.001$).

L-DOPA administration restores long-term potentiation in the hippocampus of hemiparkinsonian rats via D1/D5 dopamine receptors

We investigated the possibility to restore hippocampal LTP with L-DOPA. Bath application of 30 μM L-DOPA was able to rescue LTP, as shown by recovery of field EPSP potentiation (Fig. 4A;

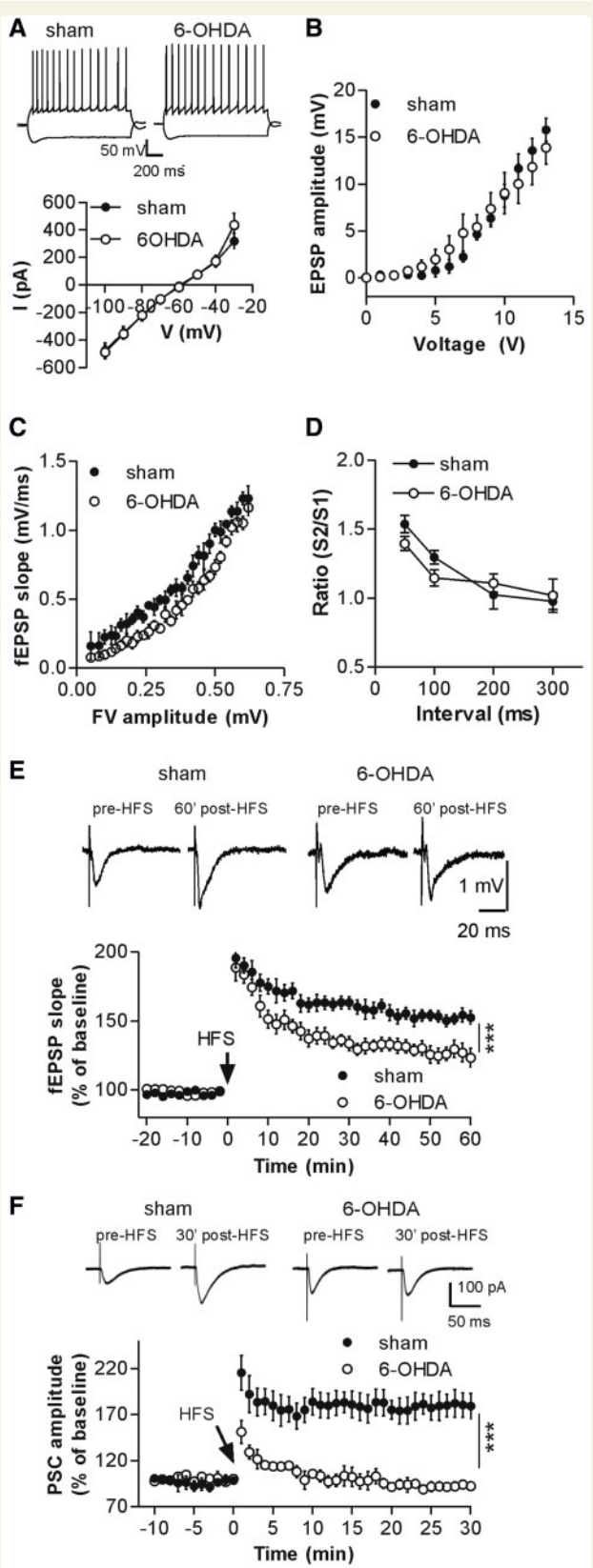

Figure 3 Hippocampal-related LTP is altered in hemiparkinsonian rats. (**A**) Representative traces showing voltage membrane changes to hyperpolarized and depolarized steps of current injected into CA1 pyramidal neurons patched from slices of sham-operated (*left*) and 6-OHDA lesioned rats (*right*). The current–voltage relationship shows no difference in membrane properties between neurons recorded from sham-operated and 6-OHDA lesioned rats. (**B** and **C**) Input–output plots obtained in hippocampal slices from sham-operated and 6-OHDA-lesioned rats by stimulating Schaffer fibres and recording EPSPs in neurons by patch clamp measurements (**B**) or field EPSPs (fEPSP) by extracellular field potential measurements (**C**) from the CA1 region. (**D**) Paired-pulse ratios (S2/S1) at different interstimulus interval (50–300 ms) from slices cut from sham-operated and 6-OHDA rats. (**E**) Representative traces and time-course of field EPSP slopes in slices from sham-operated and 6-OHDA rats. Arrow indicates the time at which the high-frequency stimulation (HFS) protocol is delivered. Note the reduced LTP in 6-OHDA group respect to the sham-operated group; [in 6-OHDA rats 123.2 ± 7.0%, versus sham-operated animals 150.7 ± 3.5%, $n = 9$ field EPSP for both, $F(40,640) = 5.19$; $^{***}P < 0.001$]. (**F**) Representative traces and time-course of postsynaptic currents (PSCs) amplitude in slices from sham-operated and 6-OHDA-lesioned rats. 6-OHDA rats show a reduced LTP compared with the sham-operated group [Sham 179.1 ± 14.0% versus 6-OHDA 92.3 ± 4.5%, $n = 5$ neurons for both groups, $F(40,320) = 13.47$; $^{***}P < 0.001$]. FV = fibre volley.

$n = 9$ slices for each group; $P < 0.001$). Interestingly, L-DOPA did not affect LTP amplitude in the hippocampus of sham-operated rats (Supplementary Fig. 1A) or in the contralateral hippocampus of 6-OHDA-lesioned rats (Supplementary Fig. 1B).

To test the hypothesis that L-DOPA effects were dependent on its conversion to dopamine, we performed experiments in the presence of the DOPA-decarboxylase inhibitor carbidopa. L-DOPA (30 μM) plus 100 μM carbidopa were bath applied 20 min prior to the induction of LTP and throughout the experiment. In these conditions, LTP was not different from that observed in untreated 6-OHDA slices (Fig. 4A; $n = 8$ slices, $P > 0.05$).

To confirm the dopamine-dependence of hippocampal LTP, we analysed LTP in slices obtained from 6-OHDA-lesioned animals subchronically treated with L-DOPA. Similar to the acute effect of L-DOPA, LTP was restored in L-DOPA treated animals (Fig. 4B; $n = 6$ for L-DOPA-treated rats and $n = 9$ for 6-OHDA rats; $P < 0.001$).

To investigate the dopamine receptor subtype involved in L-DOPA action, we applied 30 μM L-DOPA in the presence of the D1/D5 receptor blocker SCH23390 (10 μM). In this condition, L-DOPA failed to restore LTP, suggesting that the action of L-DOPA was due to its conversion to dopamine acting on D1/D5 receptors (Fig. 4C; L-DOPA plus SCH23390, $n = 5$ versus control in the standard solution, $n = 9$ slices, $P > 0.05$). In slices obtained from dopamine-denervated animals, SCH23390 failed to decrease the LTP amplitude further (Supplementary Fig. 1C).

To confirm further the involvement of D1/D5 receptors, bath application of the D1 dopamine receptor agonist SKF38393 mimicked the action of L-DOPA. In fact, bath application of SKF38393 (10 μM) in 6-OHDA slices restored an LTP (Fig. 4D; $n = 6$ slices; $P < 0.001$) similar to that measured in sham-operated animals. Conversely, the D2 receptor agonist quinpirole (10 μM) was ineffective (Fig. 4D; $n = 6$ slices; $P > 0.05$).

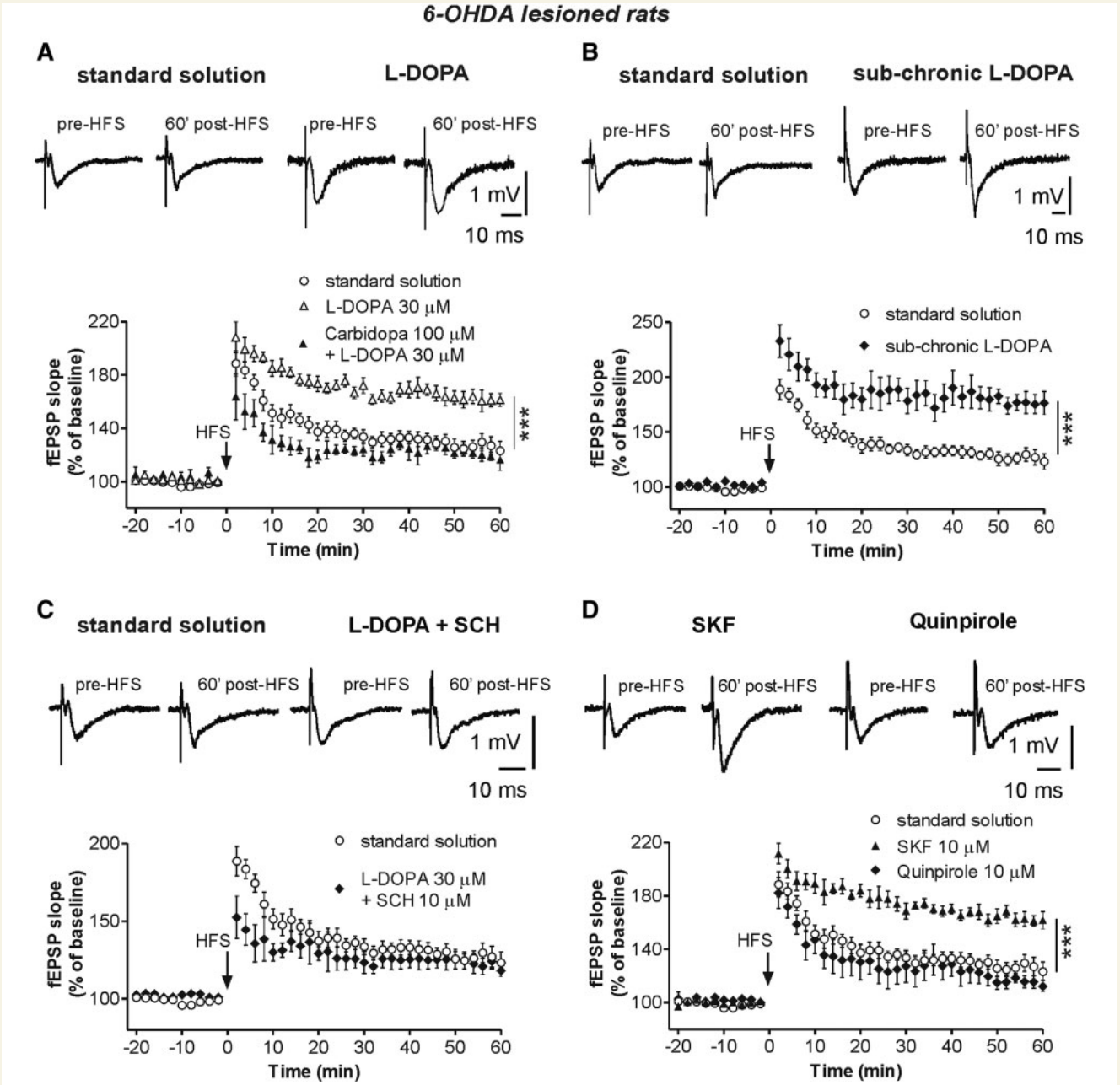

Figure 4 L-DOPA restores long-term potentiation in the hippocampus of 6-OHDA-lesioned rats by its conversion into DA activating D1 receptors. (**A**) Traces and time-course plots of field EPSP (fEPSP) recorded in the standard solution, in the presence of 30 μM L-DOPA and in the presence of 30 μM L-DOPA plus 100 μM carbidopa. Note the enhanced LTP in the presence of L-DOPA but not in the presence of L-DOPA plus carbidopa; [161.3 ± 5.0%, and 123.2 ± 7.0% in the presence and the absence of L-DOPA, respectively, $n = 9$ field EPSPs for each group, $F(80,920) = 1.82$; $^{***}P < 0.001$]. (**B**) Traces and time-courses of field EPSPs from slices of 6-OHDA lesioned rats and of 6-OHDA rats subchronically treated with L-DOPA [intraperitoneal; field EPSPs potentiation after systemic L-DOPA treatment, 166.8 ± 9.5% versus field EPSPs potentiation in untreated hemiparkinsonian animals 123.2 ± 7.0%, respectively; $n = 6$ for L-DOPA-treated rats and $n = 9$ for 6-OHDA rats, $F(40,520) = 5.97$; $^{***}P < 0.001$]. (**C**) The traces and time-course plots show field EPSPs recorded in the standard solution and in the presence of 30 μM L-DOPA plus the D1 DA receptor antagonist SCH23390 (SCH, 10 μM). (**D**) Traces and time-course of field EPSPs recorded in the standard solution, in the presence of the D1 DA receptor agonist SKF38393 (SKF, 10 μM) [155.5 ± 1.9%, $n = 6$ field EPSPs, $F(80,720) = 5.93$; $^{***}P < 0.001$] and in the presence of the D2 DA receptor agonist quinpirole (10 μM; $P > 0.05$). HFS = high-frequency stimulation.

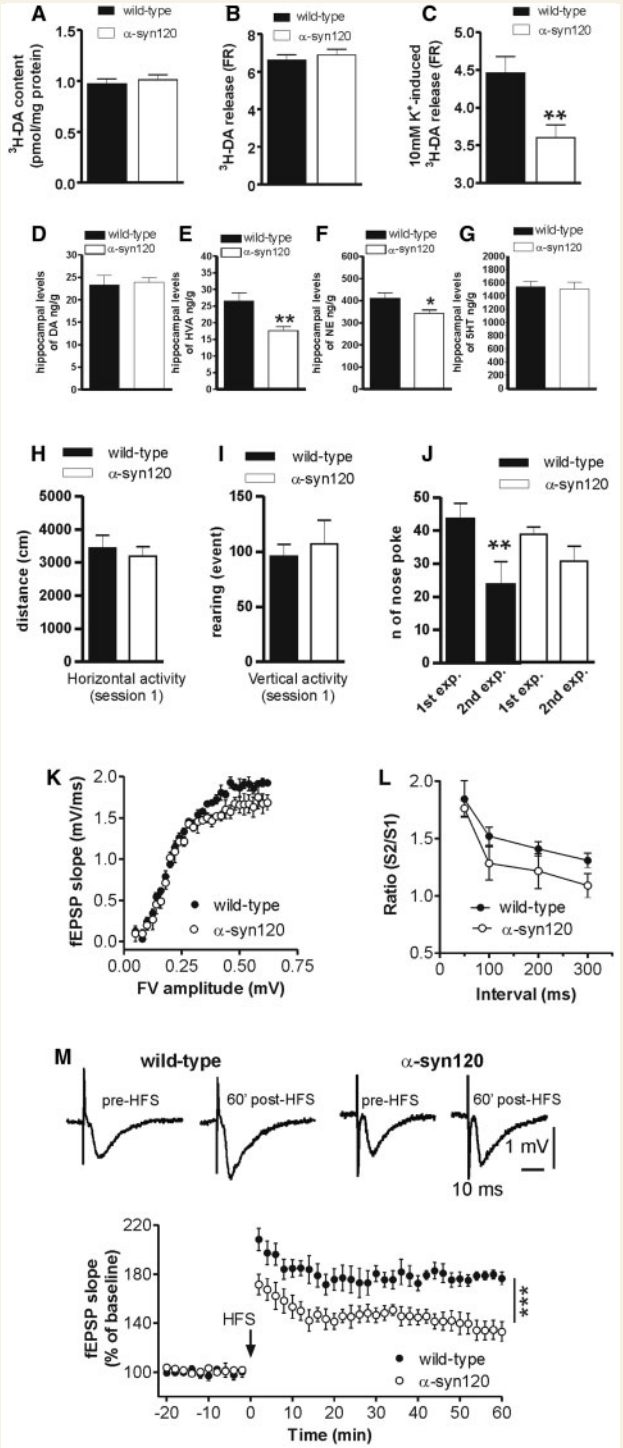

Figure 5 Dopamine release, hippocampal-related memory and long-term potentiation are altered in transgenic α-syn120 mice. A synaptosomal preparation was obtained from the hippocampus of wild-type and transgenic α-syn120 mice. [^{3}H]-dopamine (DA) accumulation (**A**), efflux (**B**) and K^+-evoked overflow (**C**) were measured. K^+ was applied for 2 min. Data, means ± SEM of six determinations per group, are expressed in absolute values (**A**), fractional release (FR) (**B**) or net fractional release (**C**) (**$P < 0.01$). (**D–G**) DA, homovanillic acid, norepinephrine and 5-hydroxytryptamine (5HT) tissue levels (ng/g wet weight) in hippocampus of wild-type and transgenic α-syn120 mice. The two groups did not differ for hippocampal dopamine, DOPAC or 5-hydroxytryptamine levels, while norepinephrine [α-syn120 342.7 ± 14.8 ng/g versus wild-type 409.3 ± 25.6 ng/g, $t(10) = 2.25$; *$P < 0.05$] and homovanillic acid [α-syn120 17.62 ± 1.2 ng/g versus wild-type 26.52 ± 2.4 ng/g, $t(10) = 3.24$; **$P < 0.01$] tissue levels from the hippocampus of α-syn120 mice were significantly reduced. (**H** and **I**) Locomotor activities of transgenic α-syn120 mice were not altered ($P > 0.05$). (**J**) Two-way ANOVA revealed a significant Group × Session interaction in hole-board sessions [$F(1,8) = 6.08$; $P < 0.05$]. Exposure of mice to hole-board sessions showed a significant habituation in wild-type mice (**$P < 0.01$) but not in α-syn120 transgenic animals ($P > 0.05$). (**K**) Input–output plots obtained in hippocampal slices from wild-type mice and α-syn120 mice by stimulating Schaffer fibres and recording in the CA1 region. (**L**) Paired-pulse ratios (S2/S1) at different interstimulus interval (50–300 ms) from slices cut from wild-type and α-syn120 mice. (**M**) Example traces of field EPSPs from a slice of a wild-type mouse (*left*) and a α-syn120 mouse (*right*). Time-course of field EPSP slopes showing a reduced LTP in slices from α-syn120 mice with respect to wild-type mice; [α-syn120 137.2 ± 7.8% versus wild-type 169.2 ± 7.5%, $n = 10$ field EPSPs, $F(40,720) = 7.59$; ***$P < 0.001$].

Stimulated but not spontaneous dopamine release is reduced in the hippocampus of α-synuclein 1–120 transgenic mice

Taking advantage of the human α-synuclein (1–120) genetic model of Parkinson's disease (Tofaris *et al.*, 2006), we first explored dopamine release in the hippocampus and analysed hippocampal synaptic plasticity. [^{3}H]-dopamine accumulation in hippocampal synaptosomes did not differ between α-syn120 and wild-type (1.01 ± 0.049 and 0.97 ± 0.048 pmol mg prot^{-1} min^{-1}, respectively) mice (Fig. 5A). Likewise, basal [^{3}H]-dopamine efflux was similar (fractional release 6.9 ± 0.3% and 6.6 ± 0.3%, respectively; Fig. 5B). Conversely, 10 mM K^+-evoked tritium overflow was 20% lower in synaptosomes prepared from the hippocampus of α-syn120 mice (net fractional release 3.6 ± 0.17%) than wild-type mice (net fractional release 4.4 ± 0.22%; Fig. 5C).

Moreover, we used high-performance liquid chromatography to measure tissue level of endogenous dopamine content in the hippocampus of α-syn120 ($n = 3$) and wild-type ($n = 3$) mice. No difference in dopamine content was found between α-syn120 and control mice (Fig. 5D; $P > 0.05$) whereas a decrease in homovanillic acid (a dopamine metabolite) was detected in α-syn120 mice (Fig. 5E; $P < 0.01$). Conversely, the levels of DOPAC (the metabolite generated by monoamine oxidase) did not change (data not shown). This evidence should be interpreted as a decrease in dopamine turnover associated with a possible catechol-*O*-methyltransferase (COMT) defect in the hippocampus of this genetic Parkinson's disease model. In addition, α-syn120 mice presented a slight reduction in norepinephrine (Fig. 5F;

$P < 0.05$), but no difference in 5-hydroxytryptamine levels (Fig. 5G; $P > 0.05$) compared with control mice, which may point to the role of others catecholaminergic neurotransmitters in cognitive disturbances in these mice.

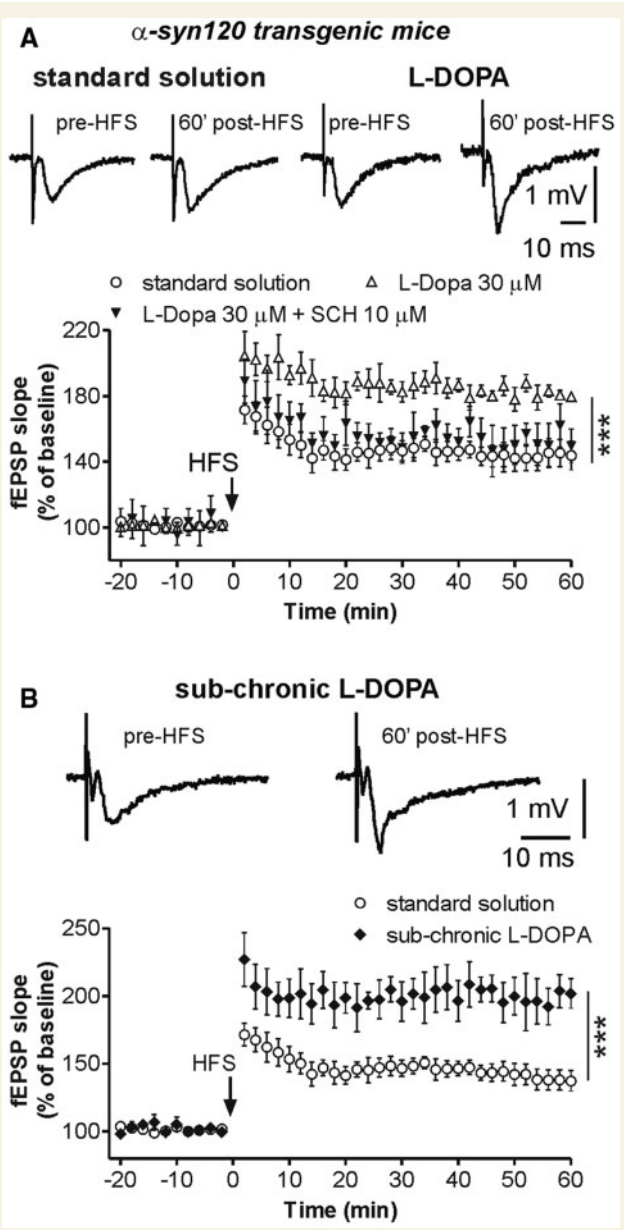

Figure 6 L-DOPA restores long-term potentiation in the hippocampus of α-syn120 transgenic mice. (**A**) Traces and time-course plots of field EPSP (fEPSP) recorded in the standard solution and in the presence of 30 μM L-DOPA; L-DOPA bath application restored LTP [180.4 ± 2.5%, $n = 7$ field EPSPs, $F(40,480) = 4.17$; $^{***}P < 0.001$] whereas it had no effect in restoring LTP when coapplied with 10 μM SCH23390 (149.6 ± 6.0%, $n = 4$). (**B**) Traces and time-courses of field EPSPs recorded from slices of α-syn120 mice subchronically treated with L-DOPA (intraperitoneal); LTP was also restored in transgenic mice that had been systemically treated with L-DOPA for 4 days [204.3 ± 6.7%, $n = 8$ field EPSPs, $F(40,560) = 9.66$; $^{***}P < 0.001$]. HFS = high-frequency stimulation.

Hippocampal-dependent learning and long-term potentiation are impaired in α-synuclein 1–120 transgenic mice

Hole-board task in α-syn120 transgenic mice ($n = 5$; Fig. 5H–J) revealed a deficit in hippocampal-dependent learning similar to that observed in 6-OHDA hemilesioned rats. Locomotor activities of these animals (horizontal and vertical) were not altered in the first session. However, two-way ANOVA revealed a significant Group × Session interaction in hole-board sessions ($P < 0.05$). In particular, *post hoc* comparison showed a significant habituation in hole exploration in wild-type (Fig. 5J; $P < 0.01$) but not α-syn120 transgenic mice. L-DOPA treatment partially restored the hippocampal-dependent learning deficit in α-syn120 transgenic mice (data not shown).

Basic electrophysiological properties as well as short-term and long-term synaptic plasticity were then studied in CA1 hippocampal area. Field EPSPs were recorded in hippocampal slices either from α-syn120 transgenic ($n = 10$) or wild-type ($n = 10$) mice to obtain input–output curves. No significant genotype differences emerged from curves comparison (Fig. 5K; $n = 6$ slices for each group, $P > 0.05$).

Paired-pulse ratios of field EPSP slope (interstimulus interval 50–300 ms) showed no difference between α-syn120 and wild-type slices (Fig. 5L; $n = 6$ slices for each group, $P > 0.05$). After acquiring a stable field EPSP for 20 min, the high-frequency stimulation protocol was delivered. Interestingly, the LTP obtained in slices recorded from α-syn120 mice was significantly reduced (Fig. 5M; $n = 10$ slices) with respect to the LTP in wild-type mice (Fig. 5M; $n = 10$ slices; $P < 0.001$).

L-DOPA restores long-term potentiation in the hippocampus of α-synuclein 1–120 transgenic mice

In order to assess whether the LTP impairment in α-syn120 transgenic mice could be related to an altered dopamine transmission, we tested whether L-DOPA reversed this deficit acting on D1/D5 receptors. Bath application of L-DOPA (30 μM) restored LTP (Fig. 6A; $n = 7$ slices; $P < 0.001$). This effect was reversed by SCH23390 (10 μM, Fig. 6A, $n = 4$). LTP was also restored in transgenic mice systemically treated with L-DOPA for 4 days (Fig. 6B; $n = 8$ slices; $P < 0.001$). SCH23390 failed to decrease the amplitude of LTP recorded from transgenic mice further (Supplementary Fig. 1D).

Altered distribution of NMDA receptor subunits in 6-hydroxydopamine-lesioned rats and in α-synuclein 1–120 transgenic mice

NR2 subunits of *N*-methyl-D-aspartic acid (NMDA) receptor play a key role in hippocampal plasticity and LTP. To explore the possibility that LTP impairment observed in 6-OHDA hemilesioned rats and α-syn120 transgenic mice also share common dysfunction of glutamate synapse, levels of NR2A and NR2B NMDA receptor

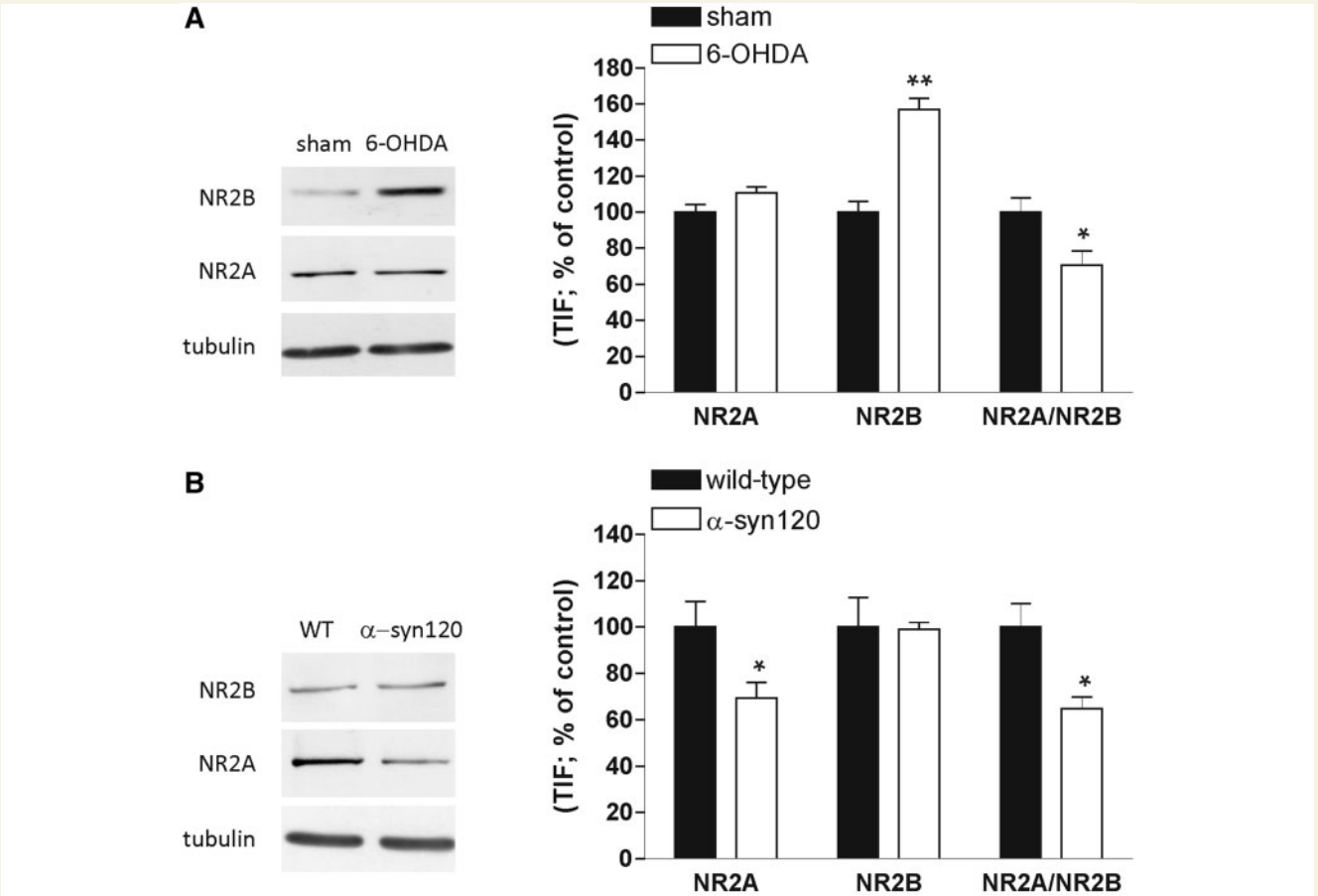

Figure 7 Altered distribution of NMDA receptor subunits in 6-OHDA-lesioned and in α-syn120 transgenic animals. Hippocampal Triton-insoluble fractions (TIF) from parkinsonian and control animals were analysed by western blot analysis with antibodies for NMDA receptor NR2A and NR2B subunits. The same amount of protein was loaded per lane. Histograms show the quantification of western blotting performed in hippocampal TIF from 6-OHDA and sham-operated rats (**A**) and from α-syn120 transgenic and wild-type mice (WT) (**B**); (*$P < 0.05$, **$P < 0.005$).

subunits were analysed in hippocampal purified Triton-insoluble postsynaptic fractions (TIF) from 6-OHDA-lesioned ($n = 5$) and sham-operated rats ($n = 5$; Fig. 7A). In 6-OHDA-lesioned animals, the level of NR2A subunit in Triton-insoluble postsynaptic fractions was normal. However, dopamine-denervated animals were characterized by a significant increase in the NR2B immunostaining ($+57.0 \pm 6.2\%$, $P < 0.005$ compared with sham-operated); accordingly, the NR2A/NR2B ratio was significantly decreased ($-29.4 \pm 7.6\%$, $P < 0.05$ compared with sham-operated) suggesting the presence of a profound rearrangement of the NMDA receptor composition in 6-OHDA-lesioned rats.

We also evaluated whether the LTP impairment in α-syn120 transgenic mice ($n = 5$) was correlated with changes in NMDA receptor composition in the postsynaptic compartment. Interestingly, a decrease in NR2A ($-30.7 \pm 6.7\%$, $P < 0.05$ compared with wild-type, $n = 5$) and a concomitant decrease in the NR2A/NR2B ratio ($-35.2 \pm 4.9\%$, $P < 0.05$ compared with wild-type) were detected in α-syn120 transgenic mice compared with wild-type mice (Fig. 7B). These findings demonstrate that, although toxic and genetic models of Parkinson's disease show distinct neurochemical and molecular patterns, they can share similar alterations in the NR2A/NR2B ratio, possibly leading to a reduced hippocampal LTP.

Influence of NMDA receptor subunits on hippocampal long-term potentiation in sham-operated and 6-hydroxydopamine-lesioned rats

In order to investigate the role of NMDA receptor subunits on CA1 hippocampal LTP recorded in sham-operated and 6-OHDA denervated animals ($n = 5$ for each group), we analysed synaptic plasticity in the presence of ifenprodil, an antagonist of NR2B receptor subunit. Ifenprodil (1 μM) significantly reduced the LTP observed in sham-operated animals (Fig. 8A; $n = 5$, $P < 0.001$), while it did not affect the LTP amplitude in dopamine-denervated rats (Fig. 8B; $n = 4$, $P > 0.05$). Previous studies have shown the capability of cell-permeable TAT peptides fused to the C-terminal domain of NMDA receptor subunits to reach and to disrupt

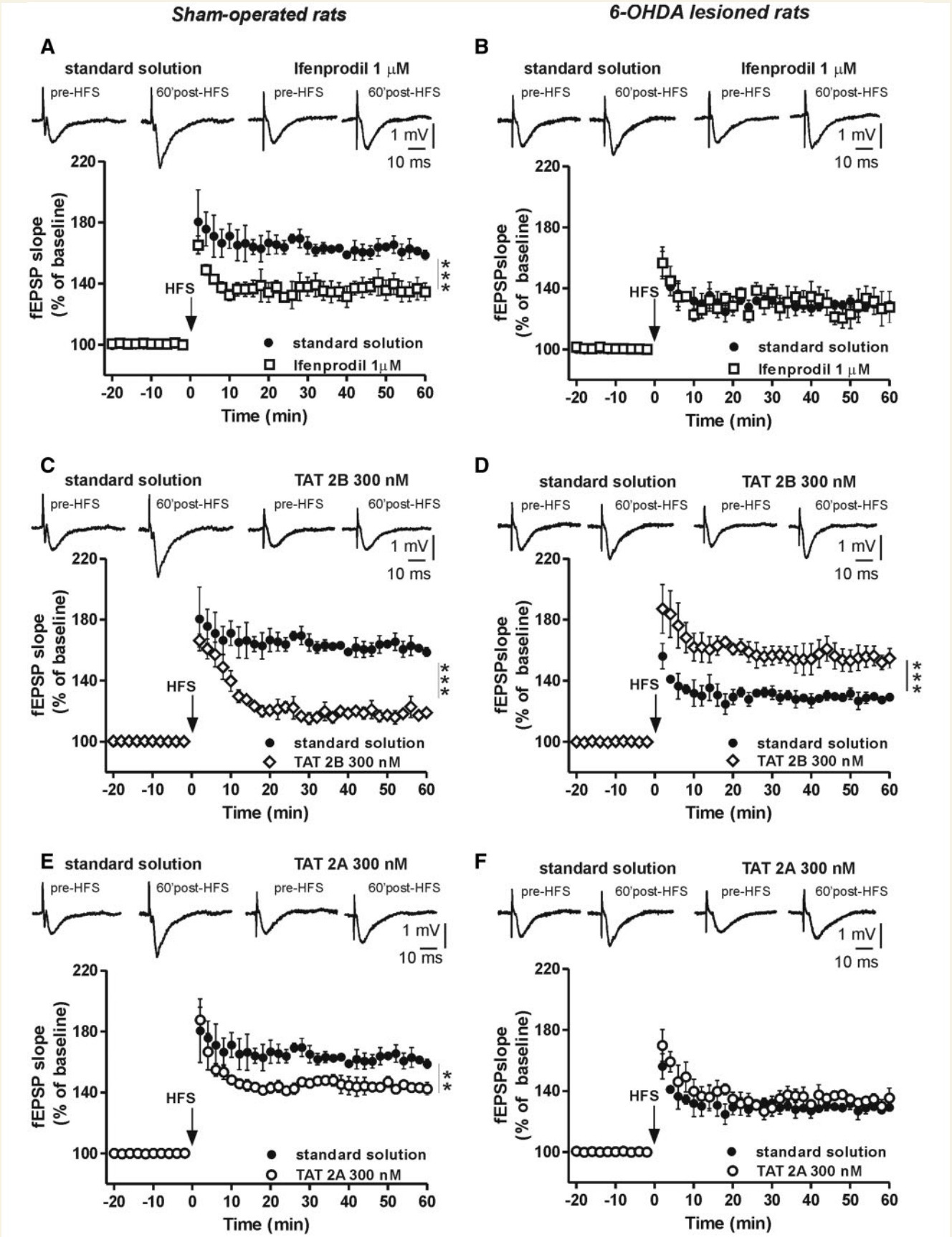

Figure 8 Influence of NMDA receptor subunits on hippocampal LTP in sham-operated and 6-OHDA-lesioned rats. Traces and time-course plots of field EPSP (fEPSP) recorded from sham-operated (**A**, **C** and **E**) and 6-OHDA-lesioned rats (**B**, **D** and **F**). The treatment

(continued)

NMDA/PSD-MAGUKs (PSD-95-like membrane associated guanylate kinases) association both in *in vitro* and *in vivo* studies (Aarts *et al.*, 2002; Gardoni *et al.*, 2006). Thus, we also analysed LTP in the presence of the cell-permeable peptides TAT2A and TAT2B, respectively, and selectively targeted NR2A and NR2B subunits of NMDA receptor in both sham-operated and 6-OHDA denervated rats. In these experiments, slices were incubated with either TAT2B or TAT2A before (at least 2 h) and during the electrophysiological recordings. The application of 300 nM TAT2B peptide significantly decreased LTP in sham-operated animals (Fig. 8C; $n = 3$, $P < 0.001$), as previously reported for CA1 hippocampal LTP in control animals (Gardoni *et al.*, 2009). However, this peptide, in sharp difference with ifenprodil, fully restored LTP to control levels in 6-OHDA denervated rats (Fig. 8D; $n = 5$, $P < 0.001$) suggesting that the correct assembly of the NMDA receptor subunits, rather than their pharmacological blockade, is necessary to restore physiological plasticity. In line with this observation, we also found that the TAT2A peptide significantly decreased CA1 LTP in sham-operated animals (Fig. 8E; $n = 8$, $P < 0.001$) but not in dopamine-denervated animals (Fig. 8F; $n = 4$, $P > 0.05$), confirming the molecular data and further supporting the hypothesis that a correct balance between NR2A and NR2B is critically important for LTP induction.

Discussion

In the present study, we have shown that CA1 hippocampal LTP is reduced in both a neurotoxic and a genetic model of Parkinson's disease. This plastic alteration is associated with neurochemical changes of dopamine transmission, deficits of hippocampal-related memory tasks and abnormalities in the expression of hippocampal NMDA receptor subunits. We achieved these results by analysing, for the first time, the role of hippocampal synaptic plasticity in both a toxic and a genetic model of Parkinson's disease using combined electrophysiological, behavioural, molecular and neurochemical approaches. The 6-OHDA-induced lesion is still the most widely used model for replicating a Parkinson's disease-like loss of nigral dopaminergic neurons and it is currently adopted for the analysis of striatal synaptic plasticity and its link with motor symptoms. Surprisingly, this toxic model has been less utilized to investigate altered synaptic plasticity in other brain areas such as hippocampal LTP and the correlated memory deficits. On the other hand, the genetic model used in the present study expresses truncated human α-synuclein (1–120), leading to the formation of pathological inclusions in the substantia nigra pars compacta and olfactory bulb and to a reduction in striatal dopamine levels. At the behavioural level, transgenic mice show a progressive reduction in spontaneous locomotion mimicking the pathological and clinical features of Parkinson's disease (Tofaris *et al.*, 2006). However, hippocampal dopamine transmission and plasticity and their possible involvement in memory deficits have never been deeply investigated in genetic models.

Two major findings suggest that the reduction of endogenous hippocampal dopamine plays a major role in the decrease of LTP in experimental parkinsonism. First, in the 6-OHDA denervated hippocampus the release of dopamine during membrane depolarization is significantly reduced in comparison with control animals. Secondly, in both neurotoxic and genetic models of Parkinson's disease, the administration of L-DOPA, a dopamine precursor, restores a physiological LTP. The effect of L-DOPA is blocked by hippocampal administration of a DOPA-decarboxylase inhibitor such as carbidopa, further supporting the idea that L-DOPA does not exert its therapeutic action on hippocampal synaptic plasticity *per se* but only after its conversion to dopamine.

In our 6-OHDA-induced lesioned model of Parkinson's disease, the dopaminergic loss was complete in substantia nigra pars compacta while some dopamine neurons were spared in the ventral tegmental area. This condition partially mimics Parkinson's disease where there is a more severe loss of dopamine cells in substantia nigra pars compacta than in ventral tegmental area. However, a significant cell loss in the ventral tegmental area has been observed in Parkinson's disease brains (Uhl *et al.*, 1985; German *et al.*, 1989).

Interestingly, an impaired hippocampal LTP and an altered hippocampal-dependent memory were also observed in a transgenic mouse model for α-synuclein aggregation obtained by the expression of human α-syn120 under the control of the tyrosine hydroxylase promoter (Tofaris *et al.*, 2006). These findings do not match with a previous observation in which no difference in hippocampal LTP was reported in a transgenic mouse line carrying the α-synuclein A30P mutation (Steidl *et al.*, 2003). These data, however, were obtained in a mouse line showing normal dopamine levels, dopamine receptor number and dopamine function, features that are importantly different from those of the α-syn120 model, where dopamine signalling was affected (Tofaris *et al.*, 2006; Garcia-Reitbock *et al*., 2010).

The absence of motor abnormalities observed in mutant mice in our study is in line with a previous study showing that, in these mice, spontaneous locomotor activity was altered at 18 but not at 6 months of age (Tofaris *et al.*, 2006). Thus, at least in this genetic model, deficits in hippocampal function seem to precede motor symptoms.

Figure 8 Continued
with 1 μM ifenprodil, a drug targeting the NR2B NMDA receptor subunit, reduces the CA1 LTP with respect to control in sham-operated rats [134.7 ± 13%, $n = 5$ field EPSPs, $F(39,273) = 3.12$; ***$P < 0.001$] (**A**) but not in 6-OHDA-lesioned animals (**B**). The treatment with 300 nM TAT2B, targeting NR2B assembly into functional NMDA receptors, reduces the CA1 LTP with respect to control in sham-operated rats [117.7 ± 2%, $n = 3$ field EPSPs, $F(39,195) = 6.43$; ***$P < 0.001$] (**C**) whereas LTP is restored in 6-OHDA animals [154.7 ± 14.4%, $n = 5$ field EPSPs, $F(39,234) = 3.44$; ***$P < 0.001$] (**D**). The treatment with 300 nM TAT2A, targeting NR2A NMDA receptor subunit, slightly reduces the LTP with respect to control in sham-operated rats [142.4 ± 8.2%, $n = 4$ field EPSPs, $F(39,234) = 1.85$; **$P < 0.01$] (**E**) but it does not alter LTP in 6-OHDA animals (**F**). HFS = high-frequency stimulation.

Unlike patients with Parkinson's disease and 6-OHDA rats, α-syn120 mice do not show significant neuronal cell death (Tofaris *et al.*, 2006). Thus, impairment of dopamine transmission in hippocampus (present study) and striatum (Tofaris *et al.*, 2006) cannot be attributed to dopamine cell loss but more likely to functional changes of the release machinery caused by the early accumulation of α-syn120 in presynaptic terminals. Accordingly, alterations in dopamine release associated with redistribution of SNARE proteins have recently been described in the striatum of these mice (Garcia-Reitbock *et al.*, 2010).

The hippocampus represents a key structure for the formation of spatial and episodic memories (Ryan *et al.*, 2010) as well as for the detection of novelty (Jenkins *et al.*, 2004). This structure is critically involved in the storage of information such as memory for places (Muzzio *et al.*, 2009). In line with this view, in the present study we found that habituation to a novel environment is altered in hemiparkinsonian animals bearing impaired hippocampal LTP, and that these behavioural and biochemical deficits, as well as abnormalities in synaptic plasticity, can be reversed by systemic L-DOPA. Thus, our data provide a novel link among spatial memory, hippocampal LTP and neurochemical changes underlying Parkinson's disease.

The ventral tegmental area/hippocampal dopaminergic loop regulates the flux of information into long-term memory and this system is believed to play an important role in information acquisition and synaptic plasticity (Lisman and Grace, 2005). It has been proposed that the CA1 neurons could detect mismatches between predictions from the dentate gyrus–CA3 network and sensory input from the cortex (Lisman and Otmakhova, 2001). The behavioural function exerted by the CA1 hippocampal area could be of critical importance since novelty detection involves the comparison of an existing memory with new sensory information.

D1/D5 dopamine receptors play a major role in hippocampal CA1 LTP and associative learning (Frey *et al.*, 1991; Otmakhova and Lisman, 1996; Sajikumar and Frey, 2004; Granado *et al.*, 2008; Ortiz *et al.*, 2010). D1/D5 receptors are involved in object configuration learning and are required for LTP induced by exploration of empty space (Lemon and Manahan-Vaughan, 2006). In line with these data obtained in physiological conditions, in the present study, we found that the therapeutic action of L-DOPA on hippocampal LTP in parkinsonian animals could be blocked by a D1/D5 receptor antagonist and mimicked by a D1/D5 but not D2/D3 receptor agonist.

Our data allow the establishment of an intriguing association between the roles of D1/D5 receptors in hippocampal synaptic plasticity and cognitive dysfunction in Parkinson's disease. In fact, although both L-DOPA (acting on all the different types of dopamine receptors after its conversion to dopamine) and D2 receptor agonists are widely used in patients with Parkinson's disease to improve motor function, they cause different modulation on neuropsychological functions (Robbins and Arnsten, 2009).

Several studies have suggested that NR2A- and NR2B-containing receptors have different roles in the regulation of the induction of LTP in the hippocampus. Enhanced expression of NR2B seems to facilitate the induction of LTP (Tang *et al.*, 1999; Foster *et al.*, 2010). It has been postulated that the NR2B subunit plays a critical role for LTP, presumably by recruiting relevant molecules important for LTP via its cytoplasmic tail (Foster *et al.*, 2010). In contrast, NR2A does not seem to be critical for the LTP and its cytoplasmic tail seems to carry inhibitory factors for LTP (Foster *et al.*, 2010). Accordingly, hippocampal LTP is severely impaired by experimental approaches selectively targeting NR2B NMDA subunit, such as selective NR2B antagonists (Bartlett *et al.*, 2007), antisense knockdown of NR2B (Clayton *et al.*, 2002) and cell-permeable peptides reducing the NR2B localization to synaptic sites (Gardoni *et al.*, 2009). Conversely, Liu *et al.* (2004) showed that NR2A but not NR2B subunits are required for hippocampal LTP. Differences in the experimental approaches used might possibly account for the obtained conflicting results. However, an emerging concept derived from all these studies is the requirement of a correct balance in the NR2A/NR2B subunit composition at hippocampal synapses as a critical condition for LTP induction (Liu *et al.*, 2004; Bartlett *et al.*, 2007; Bellone and Nicoll, 2007; Morishita *et al.*, 2007; Gardoni *et al.*, 2009). In agreement with these studies, here we show that, although a toxic and a genetic model of Parkinson's disease both show distinct neurochemical and molecular changes, they can induce a significant decrease of NR2A/NR2B subunit ratio in hippocampal synaptic NMDA receptors possibly leading to LTP impairment and memory dysfunctions. These molecular findings are also supported by our electrophysiological experiments showing that, while the TAT2B peptide reduces LTP in sham-operated animals showing a normal NR2A/NR2B ratio, in 6-OHDA denervated rats it restores this form of synaptic plasticity to a control level. These molecular and electrophysiological findings suggest that changes in the NR2A/NR2B ratio at CA1 hippocampal synapses might be a key factor to explain both plastic and cognitive dysfunction in Parkinson's disease.

In conclusion, the concomitant deficits in hippocampal LTP observed in the present study together with the altered dopamine-dependent basal ganglia synaptic plasticity reported in parkinsonian animals (Calabresi *et al.*, 2007) and patients with Parkinson's disease (Calabresi *et al.*, 2009; Prescott *et al.*, 2009) might represent the synaptic mechanisms underlying the complex neuropsychological scenario associated with human Parkinson's disease.

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Supplementary material

Supplementary material is available at *Brain* online.

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