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1	Antiparkinsonian effects of a metabotropic glutamate receptor 4 agonist in
2	MPTP-treated marmosets
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16	Running title: Antiparkinsonian effects of an mGlu4 agonist
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25	Background: Increased firing across glutamatergic synapses may contribute to both the motor
26	dysfunction and L-DOPA-induced dyskinesia seen in Parkinson's disease. Given their ability to
27	reduce glutamate release, activation of group III metabotropic glutamate receptors such as
28	metabotropic glutamate receptor 4 may prove effective against both motor dysfunction and
29	dyskinesia in Parkinson's disease.
30	
31	Objectives: We hypothesised that activation of metabotropic glutamate receptor 4 by an orthosteric
32	agonist ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
33	nitrophenyl)methyl)phosphoryl)butanoic acid, LSP1-2111) would produce antiparkinsonian activity
34	and reduce expression of dyskinesia in a 1-methyl-4-phenyl,1,2,3,6-tetrahydropyridine (MPTP)-
35	treated marmoset model of Parkinson's disease.
36	
37	Methods: Common marmosets were previously treated with MPTP and pre-primed with L-DOPA for
38	u to 20 down to oversees dualization (CD1 2111 (1, 2 or C reg/lapped)) or ushield (0.00/ colling c.o.) u
50	up to 28 days to express dyskinesia. LSP1-2111 (1, 3 or 6 mg/kg s.c.) or vehicle (0.9% saline s.c.) were
39	administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10%
39 40	administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10% sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored
39 40 41	administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10% sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored for dyskinesia and disability.
 39 40 41 42 	administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10% sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored for dyskinesia and disability.
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 39 40 41 42 43 44 	administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10% sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored for dyskinesia and disability. Results : As expected, L-DOPA reversed motor disability and induced moderate dyskinesia. By contrast, LSP1-2111 alone significantly reduced the motor disability without any accompanying
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 39 40 41 42 43 44 45 46 	up to 28 days to express dyskinesia. LSP1-2111 (1, 3 of 6 mg/kg s.c.) of vehicle (0.9% saline s.c.) were administered immediately prior to L-DOPA (8 mg/kg + benserazide (10 mg/kg) p.o.) or vehicle (10% sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored for dyskinesia and disability. Results : As expected, L-DOPA reversed motor disability and induced moderate dyskinesia. By contrast, LSP1-2111 alone significantly reduced the motor disability without any accompanying expression of dyskinesia. When administered in combination with L-DOPA, LSP1-2111 did not significantly reduce the severity of L-DOPA-induced dyskinesia.
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49 dyskinesia in MPTP-treated marmosets, supporting a role for metabotropic glutamate receptor 4

- 50 orthosteric agonists as promising monotherapy for PD. Conversely, this study found no evidence to
- 51 support their use as antidyskinetic agents within the dose range tested.
- 52

53 Key words: dyskinesia; levodopa; motor disability; Parkinson's disease

54

55 Introduction

56 Parkinson's disease (PD) is a progressive neurodegenerative disorder that presents with motor (e.g.

57 bradykinesia, tremor and postural instability) and non-motor (e.g. pain, anxiety and REM-sleep

58 behaviour disorder) symptoms. The current gold standard treatment for PD is L-3,4-

59 dihydroxyphenylalanine (L-DOPA), which provides relief from motor symptoms. However, within 4-6

60 years after the initiation of L-DOPA treatment, 40% of PD patients experience unwanted involuntary

61 movements in the form of L-DOPA-induced dyskinesia (LID) of a choreic or dystonic nature[1].

62

63 Increased glutamatergic transmission has been implicated in the pathophysiology of both 64 parkinsonian motor symptoms and L-DOPA-induced dyskinesia[2,3]. Increased transmission across 65 the glutamatergic subthalamonigral pathway is believed to contribute towards manifestation of the 66 motor symptoms[4,5] while plasticity of the glutamatergic corticostriatal pathway is implicated in 67 the development of LID[6–9]. In support of the glutamatergic involvement in LID, the weak Nmethyl-D-aspartate (NMDA) receptor antagonist amantadine is one of very few drugs shown to have 68 69 any efficacy against LID[10–13]. However, amantadine has a poor side-effect profile involving 70 psychiatric problems such as hallucination, confusion and depression[14] which reduces its 71 therapeutic utility. An alternative route to the glutamatergic modulation of signalling for potential 72 therapeutic benefit against both the parkinsonian motor symptoms and LID is to target 73 metabotropic glutamate receptors, specifically, the group III metabotropic glutamate receptors 74 (mGluRs) which have shown promise in a range of PD and LID indications[2,3,15].

75

76 Group III mGluRs are G_{i/o}-coupled, presynaptic receptors which reduce exocytosis of

neurotransmitter in response to activation by endogenous glutamate[16–18]. One member of this
family, mGluR4, has received attention as a potential therapeutic target in PD due to its expression
at relevant synapses throughout the basal ganglia[15,19–21]. Indeed, both agonists and positive
allosteric modulators (PAMs) of mGluR4 have been shown to provide antiparkinsonian effects in
acute models of PD in rodents[22–25], while PAMs offer antiparkinsonian relief in a 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaque model of PD[26].

83

84 Studies have also shown the antidyskinetic potential of targeting mGluR4. Thus, the mGluR4 PAM, 85 (1S, 2R)-N1-(3,4-dichlorophenyl)-cyclohexane-1,2-dicarboxamide (Lu AF219234), reduced the 86 development of L-DOPA-induced abnormal involuntary movements (AIMs) in rodent models of 87 LID[24]. Similarly, the mGluR4 PAM, Foliglurax (PXT002331), reduced the expression of well-88 established LID in the MPTP-treated macaques[26]. A systemically-active agonist of mGluR4, (2S)-2-89 amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-nitrophenyl)methyl)phosphoryl)butanoic acid 90 (LSP1-2111) has also shown efficacy against the development of L-DOPA-induced AIMs in 91 rodents[27]. However, whether an mGluR4 agonist will offer beneficial effects in a primate model of 92 PD remains to be examined. This study therefore set out to establish whether the mGluR4 agonist 93 LSP1-2111 provides antiparkinsonian relief in an MPTP-treated marmoset model of PD and whether 94 it also reduces the expression of established LID in this model.

95

96 Materials & Methods

97 Animals

98 Common marmosets (Callithrix jacchus, Harlan, Loughborough, LE12 9TE, UK and Manchester

99 University, UK) aged 7–14 years were housed in female/male (vasectomised) or female/female pairs

at a temperature of 23 ± 2 ⁰C with 50% relative humidity and a 12 hour light/dark cycle[28,29]. They

101 had unlimited access to water and marmoset pellets and received one meal of mashed cereal and 102 one meal of fresh fruit daily. All experiments were performed according to the Animals (Scientific 103 Procedures Act) 1986 under Project Licence No 70/8541, with local approval of the Animal Welfare 104 and Ethical Review Board of King's College London and were compliant with the minimum standards 105 as defined by the European Communities Council Directive (10/63/EU). All animals involved in this 106 study had previously been included in studies assessing the therapeutic value of compounds in PD 107 and LID. Following previous studies, all animals underwent a drug-free 'washout' period of at least 4 108 weeks before the start of this study.

109

110 MPTP-Treatment

Five to seven years prior to this study, marmosets underwent administration of MPTP (Sigma, UK) at
2.0 mg/kg daily for 5 days to induce stable motor deficits [30,31]. This resulted in the animals
exhibiting reduced basal locomotor activity, bradykinesia, rigidity, poor coordination of movement

and reduced alertness/awareness. All animals were primed to express dyskinesia on exposure to L-

115 DOPA through repeated (up to 28 days) oral administration of L-DOPA (8-12.5 mg/kg, Sigma, UK)

116 plus benserazide (10mg/kg, Sigma, UK) in a 10% sucrose solution.

117

118 Drug Treatment

119 Animals (n=8) were selected for the study from a pool (n=10) of MPTP-treated marmosets based on

120 their response to L-DOPA treatment. For this selection process L-DOPA (4, 6 and 8 mg/kg) plus

benserazide (10 mg/kg) was administered p.o. and locomotor activity recorded (as detailed below).

122 L-DOPA (8 mg/kg) was selected as the dose (providing approximately 70% of a maximal response) for

use in the main part of the study. Two animals were removed from the study prior to completion for

124 welfare reasons unrelated to the study, leaving a final group size of n=6 (3 male and 3 female).

125

A modified Latin square design was used to randomise treatments whilst ensuring dosing with LSP12111 (Lundbeck, Denmark) occurred in a dose-escalating manner, to identify any side-effects before
higher doses were given. In this fashion, each animal received all drug combinations once (with an
interval of ≥48 hours between doses). LSP1-2111 was administered subcutaneously in 0.9% sterile
saline (Baxter healthcare) at 0 (vehicle) 1, 3 or 6 mg/kg in a volume of 1 ml/kg. The lowest dose for
LSP1-2111 (1 mg / kg) was selected based on previous data showing emerging significant effects in a
range of behavioural tests in rodents with this dose [25,32].

133

134 Following a 60 min acclimatisation period, baseline motor function (locomotor activity, motor

disability and dyskinesia) was assessed for 60 min as described below. Following the 60 min baseline

assessment, LSP1-2111 and L-DOPA (or respective vehicles) were administered according to the

randomisation protocol. LSP1-2111 (1, 3 or 6 mg/kg s.c.) or vehicle (1 ml/kg s.c.) was administered

immediately followed by L-DOPA (8 mg/kg plus benserazide (10 mg/kg)) or vehicle (10% sucrose plus

benserazide (10 mg/kg)) in a combined p.o. administration of 2 ml/kg.

140

141 Behavioural measurements

On test days, animals were acclimatised for 60 min to individual automated test units (50 cm by 60 cm by 90 cm). The automated test units were fitted with 2 horizontal wooden perches and a water supply and a clear Perspex door to allow visual observation. Food was not provided during the test period and animals received their normal meal at the end of the test period on return to home caging. Locomotor activity, motor disability and dyskinesia were assessed for up to 6 hours as described below.

148

149 Locomotor activity

150 Each behavioural test unit was fitted with 8 photoelectric emitters/detectors (light beams) arranged

151 horizontally to permit optimal assessment of locomotor activity. Interruption of a light beam was

automatically recorded as a single locomotor count which were accumulated in 30 min time

153 segments for 1 hour before and 5 hours following drug treatment.

154

155 Motor disability

156	Motor disability was assessed simultaneously with locomotor activity, by observation via a one-way
157	mirror, by experienced observers blinded to the treatment. Basal disability was assessed once every
158	30 minutes, for 30 minutes before and 5 hours after drug treatment using an established motor
159	disability rating scale; alertness (normal = 0, reduced = 1, sleepy = 2); checking (present = 0, reduced
160	= 1, absent = 2); posture (normal = 0, abnormal trunk +1, abnormal tail + 1, abnormal limbs + 1,
161	flexed = 4); balance (normal = 0, impaired = 1, unstable = 2, spontaneous falls = 3); reaction to
162	stimuli (normal = 0, reduced = 1, slow = 2, absent = 3); vocalisation (normal = 0, reduced = 1, absent
163	= 2); motility (normal = 0, bradykinesia = 1, akinesia = 2). These values were summed, a maximum
164	score of 18 indicating severe motor disability, a minimum score of 0 indicating maximum reversal of
165	motor disability.

166

167 Dyskinesia

168 Dyskinesia was assessed simultaneously with motor disability by experienced observers blinded to

169 treatment. The following established dyskinesia rating scale was used; 0 = absent; 1= mild, fleeting

- and rare dyskinetic postures and movements; 2 = moderate: more prominent abnormal movements,
- but not significantly affecting normal behaviour; 3 = marked, frequent and at times continuous
- dyskinesia affecting the normal pattern of activity; 4 = severe, virtually continuous dyskinetic

activity, disabling to the animal and replacing normal behaviour.

174

175 Data handling and statistical analysis

176	The area under the curve (AUC) for locomotor activity, motor disability and dyskinesia was
177	determined from the time course data over 5 hours following drug administration (GraphPad Prism
178	version 8.0.0 for Windows, GraphPad Software, San Diego California USA, <u>www.graphpad.com</u>). The
179	AUC for locomotor activity and dyskinesia was calculated from values greater than baseline and for
180	reversal of motor disability values lower than baseline. For AUC figures therefore, increased
181	locomotor activity, reversal of motor disability and increased severity of dyskinesia are all
182	represented by rising values.
183	
184	Prior to analysis, motor disability and dyskinesia data were transformed by $y = vy$ in order to
185	normalise distribution[33]. This transformation allowed the application of parametric tests to scored
186	data. Time course data was analyses by 2-way ANOVA. If the effect of treatment was significant,
187	individual differences at each time point were analysed by Dunnett's test. Repeated measures 1-
188	way ANOVA with Sidak's multiple comparisons test was applied to area under the curve (AUC) data,
189	comparing each group to its respective vehicle condition (L-DOPA alone and LSP1-2111 alone
190	compared to the vehicle/vehicle condition and L-DOPA with 1, 3 or 6mg/kg LSP1-2111 compared to
191	L-DOPA alone).
192	

193 Results

194 Vehicle treatment had no effect on either locomotor activity or motor disability and did not induce195 dyskinesia expression (Fig 1-3).

196

197 As expected, the submaximal dose of L-DOPA (8 mg/kg p.o.) produced a small but significant rise in

198 locomotor activity (Fig 1a,b), a significant reversal of motor disability (Fig 2a,b) and significant

199 expression of dyskinesia (Fig 3a,b). Locomotor activity peaked at 60 min (Fig 1a), whilst the

200 improvement in motor disability showed maximum effect between 30 and 90 min after

administration (Fig 2a) with scores of 2. Dyskinesia peaked between 90 and 120 min with moderate
to marked dyskinetic movements (median scores of 2-3). This effect of L-DOPA lasted approximately
3 h.

204

LSP1-2111 alone (6 mg/kg s.c.) had no effect on locomotor activity (Fig 1a,b) but significantly

improved motor disability with a sub-maximal reduction in score between 30 and 60 min (Fig 2a,b).

207 Interestingly, LSP1-2111 (6 mg/kg s.c.) did not induce any dyskinesia (Fig 3a,b).

208

209 When given in combination with L-DOPA (8 mg/kg p.o.), LSP1-2111 (1-6 mg/kg) appeared to increase 210 locomotor activity in a dose-related manner, although this effect was not significant (Fig 1a,b). In 211 spite of the reversal of motor disability by LSP1-2111 (6 mg/kg s.c.) alone, when given in 212 combination, LSP1-2111 (1-6 mg/kg s.c.) did not alter the L-DOPA-induced reversal of motor 213 disability (Fig 2a,b). However, in parallel with the non-significant rise in locomotor activity, LSP1-214 2111 produced a non-significant increase in the expression of L-DOPA-induced dyskinesia at the 215 highest dose tested (Fig 3a,b). This included a dose-related increase in chorea, but not dystonia 216 (Supplementary Figure 1) with fleeting bouts of severe choreic activity at peak effect after the 217 combination of LSP1-2111 (6 mg/kg) and L-DOPA. For this reason, the effects of further increments 218 in dose of LSP1-2111 were not explored.

219

220 Discussion

This study set out to examine whether the mGluR4 agonist, LSP1-2111, provided antiparkinsonian
relief or reduced the expression of established LID in the MPTP-treated marmoset. LSP1-2111 alone
was shown to significantly reduce motor disability in parkinsonian animals without causing
dyskinesia. However, LSP1-2111 did not reduce established LID when co-administered with L-DOPA.

225

226 Regarding the potential antiparkinsonian efficacy of LSP1-2111, the significant reduction in motor 227 disability seen with LSP1-2111 alone compared to vehicle treatment supports an antiparkinsonian 228 effect of this mGluR4 agonist. Although the reduction in motor disability was non-significantly lower 229 than that achieved with L-DOPA treatment, these animals were clearly 'switched on' as defined by a 230 score of 8[34]. Importantly, in contrast to the response with L-DOPA, this beneficial effect of LSP-231 2111 was not accompanied by a significant increase in locomotor activity, indicating less 232 hyperactivity, and more naturalistic antiparkinsonian effect. Furthermore, administration of LSP1-233 2111 alone did not evoke the expression of dyskinesia in L-DOPA-primed animals.

234

235 LSP1-2111 did not have any significant additive effects in reversing motor disability when given 236 alongside the submaximal dose of L-DOPA (8 mg/ kg) used here. This suggests that the LSP1-2111 237 operates via the same downstream mechanism as L-DOPA to achieve this antiparkinsonian response. 238 Existing evidence points towards a mechanism involving modulation of indirect pathway of the basal 239 ganglia to counteract pathological alterations in firing. For example, in vitro slice work has shown 240 that activation of mGluR4 receptors, using either agonists or PAMs, reduces GABAergic transmission 241 across the striatopallidal pathway, reflecting the heteroreceptor role of these receptors[35–37] and 242 glutamatergic transmission across the subthalamonigral[22,38] and corticostriatal[24,37] pathways, 243 reflecting the autoreceptor roles. The outcome of each of these actions is to reduce the overall 244 activity in the indirect pathway, restoring the balance of firing between the direct and indirect 245 pathways which is thought to be disrupted in PD[39,40], thereby restoring motor function.

246

In contrast to the antiparkinsonian effect of LSP1-2111 noted here, treatment with the mGluR4
PAM, PXT002331, did not elicit a robust antiparkinsonian effect when given alone to MPTP-treated
macaques modelling either early or late stage PD[26]. While this may reflect differences between
the macaque and marmoset models of PD, a more likely explanation is that mGluR4 agonists provide
greater activation of the relevant receptors. To activate mGluR4, an orthosteric agonist like LSP1-

252 2111 does not require additional endogenous glutamate. However, a PAM such as PXT002331
253 requires the presence of endogenous glutamate to stimulate the orthosteric site, before the action
254 of the PAM is manifest. Although sufficient glutamate might be anticipated at the corticostriatal and
255 subthalamonigral synapses to support actions of a PAM, this is unlikely to be so at the GABAergic
256 striatopallidal synapse. Therefore, one possible explanation why the mGluR4 agonist but not PAM is
257 antiparkinsonian when administered alone, is that the additional activity of the agonist at the
258 striatopallidal synapse is key to underpinning the antiparkinsonian efficacy.

259

260 Although not effective when administered alone, the mGluR4 PAM, PTX002331, did enhance the 261 locomotor response to L-DOPA[26] and this L-DOPA sparing action was also not accompanied by the 262 emergence of dyskinesia. In partial agreement with this, in the present study LSP1-2111 tended to 263 enhance the locomotor activity AUC with L-DOPA from 3134±999 counts/5 h (L-DOPA alone) to 264 5395±1440 counts/5 h (L-DOPA plus 6 mg/kg LSP1-2111) although this failed to reach significance. 265 However, an L-DOPA sparing action per se was not examined in this study. This would have required 266 administering LSP1-2111 with a subthreshold dose of L-DOPA. Given our primary aim was to explore 267 the anti-dyskinetic effect of LSP1-2111, it was only given here alongside suprathreshold doses of L-268 DOPA that elicited significant dyskinesia. Nevertheless, our data provide support for mGluR4 269 agonists being more effective than PAMs as a monotherapy, while PAMs may prove more effective 270 as an adjunct to L-DOPA.

271

A second aim of this study was to examine the potential of LSP1-2111 to reduce LID in MPTP-treated marmosets. The present data clearly show that LSP1-2111 has no antidyskinetic effect. At all doses tested, co-administration of LSP1-2111 failed to reduce the extent of LID compared to that evoked by administration of L-DOPA alone. Rather, LSP1-2111 tended to increase the expression of LID from a median AUC score/5 h of 12.5 (range 18; L-DOPA alone) to 17.5 (range 11; L-DOPA plus 6 mg/kg LSP1-2111). Although this failed to reach significance, a dose-related increase in choreic movements

278 was observed, and prevented higher doses being tested. This lack of antidyskinetic effect agrees 279 with previous studies in rodents which also found no beneficial effect of a single administration 280 LSP1-2111 to animals with pre-established dyskinesia[25,27]. Given that plasticity across the 281 corticostriatal synapse is central to the pathophysiology of LID[6–9], the lack of antidyskinetic 282 efficacy with LSP1-2111 suggests that modulation across this synapse using an mGluR4 agonist is not 283 likely to have a functional outcome in vivo. Accordingly, this also points to effects at either the 284 striatopallidal synapse (as previously discussed) or subthalamonigral synapse underlying the above 285 antiparkinsonian actions of LSP1-2111, rather than an action on the corticostriatal synapse.

286

287 In contrast to the lack of antidyskinetic efficacy with LSP1-2111, the single published primate study 288 with an mGluR4 PAM (PTX002331) did reveal an antidyskinetic effect in a macaque model of late 289 stage PD expressing established LID[26]. The reason behind these different outcomes with agonist 290 versus PAM remains to be established. One possibility is that a modulatory action on the relevant 291 mGluR4 receptors -most likely those at the corticostriatal synapse for dyskinesia – is more likely to 292 normalise firing levels compared to outright activation with an agonist which might instead lead to 293 too much inhibition of glutamate release and excessively reduced firing in downstream pathways. 294 An alternative explanation is that the mGluR4 agonist may act at multiple sites in the striatum that 295 counteract each other. For example, a related mGluR4 agonist, LSP1-3081 has been shown to inhibit 296 GABA release in the striatum, as well as glutamate release[41]. If LSP1-2111 acts similarly on 297 heteroreceptors to reduce GABA release in the striatum, this could counter any potential 298 antidyskinetic efficacy of LSP1-2111's action on the corticostriatal pathway. Depending on the 299 location of these heteroreceptors, it is plausible that they are not modulated by an mGluR4 PAM, 300 due to a lack of sufficient glutamate at the orthosteric site, permitting the antidyskinetic effects of 301 the PAM to prevail.

302

303 When administered to rodents in combination with L-DOPA, LSP1-2111 did reduce LID induction in 304 one[27] but not another[25] study. It will therefore be important in the future to determine whether 305 this mGluR4 agonist can reduce the incidence or severity of LID when given in combination with L-306 DOPA in *de novo* treated marmosets. Such an outcome is also not yet known for mGluR4 PAMs. 307 308 One potential disadvantage of mGluR4 agonists over PAMs that requires consideration is the risk of 309 triggering receptor desensitisation with chronic use. However, given that chronic administration of 310 LSP1-2111 was efficacious in a rodent model of LID[27], it seems that desensitisation may not be of 311 concern with this agonist. Indeed, studies have shown that desensitisation of mGluR4 is independent 312 of agonist activation[42], thus mGluR4 agonists remain serious contenders for use in PD. 313 Summary 314 315 In summary, this study is the first to examine the antiparkinsonian and antidyskinetic efficacy of an 316 mGluR4 agonist in a primate model of PD. Although unable to reduce the severity of established LID, 317 our data reveal that LSP1-2111 produces an anti-parkinsonian effect, without provoking dyskinesia 318 in L-DOPA primed MPTP-treated marmosets, supporting further examination of the potential of 319 mGluR4 agonists in the treatment of PD. 320 Acknowledgments 321 322 We are grateful to Lundbeck for their generous gift of LSP1-2111. This work was funded by an MRC 323 PhD CASE Studentship joint with Eisai Ltd., awarded to EM under the supervision of Dr Peter 324 Atkinson. 325

326 Author contributions

327	SD and EM conceived the study; EM, MJ, SR and SD designed the study; EM, LL and RF executed the		
328	study; EM, MJ, SR and SD reviewed the data, performed statistical analyses and prepared the		
329	manuscript.		
330			
331	Confl	icts of Interest	
332	The au	thors report no conflicts of interest in relation to the content of this manuscript.	
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- 452

453 Supplementary Figure 1.

454 When given in combination with L-DOPA, LSP1-2111 produced a dose -related increase in chorea but

455 not dystonia.

456



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458

459 Figure shown the effect of treatment with L-DOPA (8 mg/kg p.o.) alone (vehicle) and in combination

460 with increasing doses of ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-

461 nitrophenyl)methyl)phosphoryl)butanoic acid) (LSP1-2111; 1, 3 and 6 mg/kg p.o.) on A) peak chorea

462 score and B) Peak dystonia score. Data are presented as median (line) and individual counts. *

463 p<0.05 (Friedman's one-way ANOVA).

464

466 Figure Legends

467

nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on locomotor activity. A)
The time course of effect with treatment administered at time T=0. Data are presented as mean
locomotor counts per 30 minutes (n=6). * p < 0.05 versus vehicle alone (two-way ANOVA plus Holm-
Sidak's multiple comparison test on transformed data). B) Total locomotor activity counts (AUC).
Data are presented as mean (line) and individual counts. * p<0.05 versus vehicle alone (\blacklozenge) (one-way
ANOVA plus Holm-Sidak's multiple comparison test on transformed data).
Figure 2. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on motor disability. A) The
time course of effect with treatment administered at time T=0. Data are presented as median scores
per 30 minutes (n=6). * p < 0.05 all groups versus vehicle alone, NS indicates single point of non-
significance (two-way ANOVA plus Holm-Sidak's multiple comparison test on transformed data).
B) Total reversal of motor disability (AUC). Data are presented as median (line) and individual counts.
* p<0.05 versus vehicle alone (♦) (one-way ANOVA plus Holm-Sidak's multiple comparison test on
transformed data).
Figure 3. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on dyskinesia expression.

A) The time course of effect with treatment administered at time T=0. Data are presented as median

- 491 scores per 30 minutes (n=6). + p < 0.05 vehicle versus L-DOPA alone, * p < 0.05 vehicle versus L-
- 492 DOPA plus LSP1-2111 combinations (two-way ANOVA plus Holm-Sidak's multiple comparison test on
- 493 transformed data). B) Total dyskinesia score (AUC). Data are presented as median (line) and
- 494 individual counts. * p<0.05 versus vehicle alone (♦) (one-way ANOVA plus Holm-Sidak's multiple
- 495 comparison test on transformed data).

496





plus L-DOPA (8 mg/kg)

501 Figure 2.



plus L-DOPA (8 mg/kg)

505 Figure 3.

