



King's Research Portal

Document Version
Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Mann, L., Jackson, M., Lincoln, L., Fisher, R., Salvage, S., & Duty, S. (Accepted/In press). Antiparkinsonian effects of a metabotropic glutamate receptor 4 agonist in MPTP-treated marmosets. *Journal of parkinsons disease*.

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

1 Antiparkinsonian effects of a metabotropic glutamate receptor 4 agonist in
2 MPTP-treated marmosets

3 Elizabeth Mann PhD^a, Michael Jackson BSc^b, Louise Lincoln^b, Ria Fisher^b, Sarah Rose PhD^b, Susan Duty
4 PhD^{a*}

5 King's College London, ^aWolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology
6 and Neuroscience and ^bNeurodegenerative Diseases Research Group, Faculty of Life Science and
7 Medicine, London, SE1 1UL, UK.

8

9 *Corresponding Author

10 Dr Susan Duty, Wolfson Centre for Age-Related Diseases, King's College London, Guy's Campus,
11 London SE1 1UL, U.K.

12 +44207 848 6013

13 susan.duty@kcl.ac.uk

14

15

16 Running title: Antiparkinsonian effects of an mGlu4 agonist

17

18

19

20

21

22

23

24 Abstract

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 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%
40 sucrose p.o.). Locomotor activity was measured in automated test cages and animals were scored
41 for dyskinesia and disability.

42

43 **Results:** As expected, L-DOPA reversed motor disability and induced moderate dyskinesia. By
44 contrast, LSP1-2111 alone significantly reduced the motor disability without any accompanying
45 expression of dyskinesia. When administered in combination with L-DOPA, LSP1-2111 did not
46 significantly reduce the severity of L-DOPA-induced dyskinesia.

47

48 **Conclusions:** Systemic administration of LSP1-2111 reduces motor disability without causing
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 N-
68 methyl-D-aspartate (NMDA) receptor antagonist amantadine is one of very few drugs shown to have
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
77 neurotransmitter in response to activation by endogenous glutamate[16–18]. One member of this
78 family, mGluR4, has received attention as a potential therapeutic target in PD due to its expression
79 at relevant synapses throughout the basal ganglia[15,19–21]. Indeed, both agonists and positive
80 allosteric modulators (PAMs) of mGluR4 have been shown to provide antiparkinsonian effects in
81 acute models of PD in rodents[22–25], while PAMs offer antiparkinsonian relief in a 1-methyl-4-
82 phenyl-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
100 at a temperature of 23 ± 2 °C 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

111 Five to seven years prior to this study, marmosets underwent administration of MPTP (Sigma, UK) at
112 2.0 mg/kg daily for 5 days to induce stable motor deficits [30,31]. This resulted in the animals
113 exhibiting reduced basal locomotor activity, bradykinesia, rigidity, poor coordination of movement
114 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
121 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
123 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

126 A modified Latin square design was used to randomise treatments whilst ensuring dosing with LSP1-
127 2111 (Lundbeck, Denmark) occurred in a dose-escalating manner, to identify any side-effects before
128 higher doses were given. In this fashion, each animal received all drug combinations once (with an
129 interval of ≥ 48 hours between doses). LSP1-2111 was administered subcutaneously in 0.9% sterile
130 saline (Baxter healthcare) at 0 (vehicle) 1, 3 or 6 mg/kg in a volume of 1 ml/kg. The lowest dose for
131 LSP1-2111 (1 mg / kg) was selected based on previous data showing emerging significant effects in a
132 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
135 disability and dyskinesia) was assessed for 60 min as described below. Following the 60 min baseline
136 assessment, LSP1-2111 and L-DOPA (or respective vehicles) were administered according to the
137 randomisation protocol. LSP1-2111 (1, 3 or 6 mg/kg s.c.) or vehicle (1 ml/kg s.c.) was administered
138 immediately followed by L-DOPA (8 mg/kg plus benserazide (10 mg/kg)) or vehicle (10% sucrose plus
139 benserazide (10 mg/kg)) in a combined p.o. administration of 2 ml/kg.

140

141 Behavioural measurements

142 On test days, animals were acclimatised for 60 min to individual automated test units (50 cm by 60
143 cm by 90 cm). The automated test units were fitted with 2 horizontal wooden perches and a water
144 supply and a clear Perspex door to allow visual observation. Food was not provided during the test
145 period and animals received their normal meal at the end of the test period on return to home
146 caging. Locomotor activity, motor disability and dyskinesia were assessed for up to 6 hours as
147 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

152 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
170 and rare dyskinetic postures and movements; 2 = moderate: more prominent abnormal movements,
171 but not significantly affecting normal behaviour; 3 = marked, frequent and at times continuous
172 dyskinesia affecting the normal pattern of activity; 4 = severe, virtually continuous dyskinetic
173 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, www.graphpad.com). 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 $y = \sqrt{y}$ in order to
185 normalise distribution[33]. This transformation allowed the application of parametric tests to scored
186 data. Time course data was analysed 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 induce
195 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

201 administration (Fig 2a) with scores of 2. Dyskinesia peaked between 90 and 120 min with moderate
202 to marked dyskinetic movements (median scores of 2-3). This effect of L-DOPA lasted approximately
203 3 h.

204

205 LSP1-2111 alone (6 mg/kg s.c.) had no effect on locomotor activity (Fig 1a,b) but significantly
206 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

221 This study set out to examine whether the mGluR4 agonist, LSP1-2111, provided antiparkinsonian
222 relief or reduced the expression of established LID in the MPTP-treated marmoset. LSP1-2111 alone
223 was shown to significantly reduce motor disability in parkinsonian animals without causing
224 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

247 In contrast to the antiparkinsonian effect of LSP1-2111 noted here, treatment with the mGluR4
248 PAM, PXT002331, did not elicit a robust antiparkinsonian effect when given alone to MPTP-treated
249 macaques modelling either early or late stage PD[26]. While this may reflect differences between
250 the macaque and marmoset models of PD, a more likely explanation is that mGluR4 agonists provide
251 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

272 A second aim of this study was to examine the potential of LSP1-2111 to reduce LID in MPTP-treated
273 marmosets. The present data clearly show that LSP1-2111 has no antidyskinetic effect. At all doses
274 tested, co-administration of LSP1-2111 failed to reduce the extent of LID compared to that evoked
275 by administration of L-DOPA alone. Rather, LSP1-2111 tended to increase the expression of LID from
276 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
277 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

314 Summary

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

321 Acknowledgments

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 Conflicts of Interest

332 The authors report no conflicts of interest in relation to the content of this manuscript.

333

334 References

335

- 336 [1] Hely MA, Morris JGL, Reid WGJ, Trafficante R (2005) Sydney multicenter study of Parkinson's disease:
337 Non-L-dopa-responsive problems dominate at 15 years. *Mov. Disord.* **20**, 190–199.
- 338 [2] Duty S (2012) Targeting Glutamate Receptors to Tackle the Pathogenesis, Clinical Symptoms and
339 Levodopa-Induced Dyskinesia Associated with Parkinson's Disease. *CNS Drugs* **26**, 1017–1032.
- 340 [3] Finlay C, Duty S (2014) Therapeutic potential of targeting glutamate receptors in Parkinson's disease. *J*
341 *Neural Transm* **121**, 861–880.
- 342 [4] Hirsch EC, Périer C, Orioux G, François C, Féger J, Yelnik J, Vila M, Levy R, Tolosa ES, Marin C, Trinidad
343 Herrero M, Obeso JA, Agid Y (2000) Metabolic effects of nigrostriatal denervation in basal ganglia.
344 *Trends Neurosci.* **23**, S78-85.
- 345 [5] Obeso JA, Rodríguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, Rodriguez M (2008)
346 Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. *Mov.*
347 *Disord.* **23**, S548–S559.
- 348 [6] Cenci MA, Lundblad M (2006) Post- versus presynaptic plasticity in L-DOPA-induced dyskinesia. *J.*
349 *Neurochem.* **99**, 381–392.
- 350 [7] Iravani MM, McCreary AC, Jenner P (2012) Striatal plasticity in Parkinson's disease and L-dopa induced
351 dyskinesia. *Parkinsonism Relat. Disord.* **18 Suppl 1**, S123-5.
- 352 [8] Sgambato-Faure V, Cenci MA (2012) Glutamatergic mechanisms in the dyskinesias induced by
353 pharmacological dopamine replacement and deep brain stimulation for the treatment of Parkinson's
354 disease. *Prog. Neurobiol.* **96**, 69–86.
- 355 [9] Zhang Y, Meredith GE, Mendoza-Elias N, Rademacher DJ, Tseng KY, Steece-Collier K (2013) Aberrant
356 Restoration of Spines and their Synapses in L-DOPA-Induced Dyskinesia: Involvement of Corticostriatal
357 but Not Thalamostriatal Synapses. *J. Neurosci.* **33**, 11655–11667.
- 358 [10] Crosby NJ, Deane K, Clarke CE (2003) Amantadine for dyskinesia in Parkinson's disease. *Cochrane*
359 *Database Syst. Rev.*
- 360 [11] Verhagen Metman L, Del Dotto P, van den Munckhof P, Fang J, Mouradian MM, Chase TN (1998)
361 Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology* **50**,
362 1323–6.
- 363 [12] Snow BJ, Macdonald L, Mcauley D, Wallis W (2000) The effect of amantadine on levodopa-induced

- 364 dyskinesias in Parkinson's disease: a double-blind, placebo-controlled study. *Clin. Neuropharmacol.* **23**,
365 82–5.
- 366 [13] Luginer E, Wenning GK, Bösch S, Poewe W (2000) Beneficial effects of amantadine on L-dopa-induced
367 dyskinesias in Parkinson's disease. *Mov. Disord.* **15**, 873–8.
- 368 [14] Schwab RS, England AC, Poskanzer DC, Young RR (1969) Amantadine in the Treatment of Parkinson's
369 Disease. *JAMA J. Am. Med. Assoc.* **208**, 1168.
- 370 [15] Duty S (2010) Therapeutic potential of targeting group III metabotropic glutamate receptors in the
371 treatment of Parkinson's disease. *Br J Pharmacol* **161**, 271–287.
- 372 [16] Thomsen C, Kristensen P, Mulvihill E, Haldeman B, Suzdak PD (1992) L-2-amino-4-phosphonobutyrate
373 (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to
374 adenylate cyclase. *Eur. J. Pharmacol.* **227**, 361–2.
- 375 [17] Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S,
376 Mizuno N (1997) Differential presynaptic localization of metabotropic glutamate receptor subtypes in
377 the rat hippocampus. *J. Neurosci.* **17**, 7503–22.
- 378 [18] Conn PJ, Battaglia G, Marino MJ, Nicoletti F (2005) Metabotropic glutamate receptors in the basal
379 ganglia motor circuit. *Nat Rev Neurosci* **6**, 787–798.
- 380 [19] Testa CM, Standaert DG, Young AB, Penney Jr. JB (1994) Metabotropic glutamate receptor mRNA
381 expression in the basal ganglia of the rat. *J Neurosci* **14**, 3005–3018.
- 382 [20] Messenger MJ, Dawson LG, Duty S (2002) Changes in metabotropic glutamate receptor 1-8 gene
383 expression in the rodent basal ganglia motor loop following lesion of the nigrostriatal tract.
384 *Neuropharmacology* **43**, 261–71.
- 385 [21] Bogenpohl J, Galvan A, Hu X, Wichmann T, Smith Y (2013) Metabotropic glutamate receptor 4 in the
386 basal ganglia of parkinsonian monkeys: ultrastructural localization and electrophysiological effects of
387 activation in the striatopallidal complex. *Neuropharmacology* **66**, 242–52.
- 388 [22] Broadstock M, Austin PJ, Betts MJ, Duty S (2012) Antiparkinsonian potential of targeting group III
389 metabotropic glutamate receptor subtypes in the rodent substantia nigra pars reticulata. *Br J*
390 *Pharmacol* **165**, 1034–1045.
- 391 [23] Jones CK, Bubser M, Thompson AD, Dickerson JW, Turle-Lorenzo N, Amalric M, Blobaum AL, Bridges
392 TM, Morrison RD, Jadhav S, Engers DW, Italiano K, Bode J, Daniels JS, Lindsley CW, Hopkins CR, Conn
393 PJ, Niswender CM (2012) The metabotropic glutamate receptor 4-positive allosteric modulator
394 VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in
395 preclinical rodent models of Parkinson's disease. *J Pharmacol Exp Ther* **340**, 404–421.
- 396 [24] Bennouar KE, Uberti MA, Melon C, Bacolod MD, Jimenez HN, Cajina M, Goff LKL, Doller D, Gubellini P
397 (2013) Synergy between L-DOPA and a novel positive allosteric modulator of metabotropic glutamate
398 receptor 4: Implications for Parkinson's disease treatment and dyskinesia. *Neuropharmacology* **66**,
399 158–169.
- 400 [25] Iderberg H, Maslava N, Thompson AD, Bubser M, Niswender CM, Hopkins CR, Lindsley CW, Conn PJ,
401 Jones CK, Cenci MA (2015) Pharmacological stimulation of metabotropic glutamate receptor type 4 in a
402 rat model of Parkinson's disease and L-DOPA-induced dyskinesia: Comparison between a positive
403 allosteric modulator and an orthosteric agonist. *Neuropharmacology* **95**, 121–129.
- 404 [26] Charvin D, Di Paolo T, Bezard E, Gregoire L, Takano A, Duvey G, Pioli E, Halldin C, Medori R, Conquet F
405 (2018) An mGlu4-Positive Allosteric Modulator Alleviates Parkinsonism in Primates. *Mov. Disord.*
- 406 [27] Lopez S, Bonito-Oliva A, Pallottino S, Acher F, Fisone G (2011) Activation of metabotropic glutamate 4
407 receptors decreases L-DOPA-induced dyskinesia in a mouse model of Parkinson's disease. *J.*
408 *Parkinsons. Dis.* **1**, 339–46.
- 409 [28] Pearce RKB, Jackson M, Smith L, Jenner P, Marsden CD (1995) Chronic L-DOPA administration induces

410 dyskinesias in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (*Callithrix*
411 *jacchus*). *Mov. Disord.* **10**, 731–740.

412 [29] Smith LA, Jackson MG, Bonhomme C, Chezaubernard C, Pearce RK, Jenner P (2000) Transdermal
413 administration of piribedil reverses MPTP-induced motor deficits in the common marmoset. *Clin.*
414 *Neuropharmacol.* **23**, 133–42.

415 [30] Smith LA, Jackson MJ, Al-Barghouthy G, Jenner P (2002) The actions of a D-1 agonist in MPTP treated
416 primates show dependence on both D-1 and D-2 receptor function and tolerance on repeated
417 administration. *J. Neural Transm.* **109**, 123–140.

418 [31] Smith LA, Jackson MJ, Hansard MJ, Maratos E, Jenner P (2003) Effect of pulsatile administration of
419 levodopa on dyskinesia induction in drug-naïve MPTP-treated common marmosets: Effect of dose,
420 frequency of administration, and brain exposure. *Mov. Disord.* **18**, 487–495.

421 [32] Wierońska JM, Stachowicz K, Acher F, Lech T, Pilc A (2012) Opposing efficacy of group III mGlu receptor
422 activators, LSP1-2111 and AMN082, in animal models of positive symptoms of schizophrenia.
423 *Psychopharmacology (Berl)*. **220**, 481–494.

424 [33] Fisher R, Lincoln L, Jackson MJ, Abbate V, Jenner P, Hider R, Lees A, Rose S (2018) The effect of
425 *Banisteriopsis caapi* (*B. caapi*) on the motor deficits in the MPTP-treated common marmoset model of
426 Parkinson’s disease. *Phyther. Res.* **32**, 678–687.

427 [34] Smith LA, Jackson MJ, Al-Barghouthy G, Rose S, Kuoppamaki M, Olanow W, Jenner P (2005) Multiple
428 small doses of levodopa plus entacapone produce continuous dopaminergic stimulation and reduce
429 dyskinesia induction in MPTP-treated drug-naïve primates. *Mov. Disord.* **20**, 306–314.

430 [35] Marino MJ, Williams DL, O’Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Hess
431 JF, Kinney GG, Conn PJ (2003) Allosteric modulation of group III metabotropic glutamate receptor 4: A
432 potential approach to Parkinson’s disease treatment. *Proc. Natl. Acad. Sci.* **100**, 13668–13673.

433 [36] Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG, Conn PJ (2003) Group III metabotropic
434 glutamate receptor-mediated modulation of the striatopallidal synapse. *J. Neurosci.* **23**, 7218–26.

435 [37] Gubellini P, Melon C, Dale E, Doller D, Kerkerian-Le Goff L (2014) Distinct effects of mGlu4 receptor
436 positive allosteric modulators at corticostriatal vs. striatopallidal synapses may differentially contribute
437 to their antiparkinsonian action. *Neuropharmacology* **85**, 166–177.

438 [38] Valenti O, Mannaioni G, Seabrook GR, Conn PJ, Marino MJ (2005) Group III metabotropic glutamate-
439 receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta
440 dopamine neurons. *J Pharmacol Exp Ther* **313**, 1296–1304.

441 [39] Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends*
442 *Neurosci.* **12**, 366–375.

443 [40] Wichmann T (2019) Changing views of the pathophysiology of Parkinsonism. *Mov. Disord.* **34**, 1130–
444 1143.

445 [41] Cuomo D, Martella G, Barabino E, Platania P, Vita D, Madeo G, Selvam C, Goudet C, Oueslati N, Pin J-P,
446 Acher F, Pisani A, Beurrier C, Melon C, Kerkerian-Le Goff L, Gubellini P (2009) Metabotropic glutamate
447 receptor subtype 4 selectively modulates both glutamate and GABA transmission in the striatum:
448 implications for Parkinson’s disease treatment. *J. Neurochem.* **109**, 1096–1105.

449 [42] Mathiesen JM, Ramirez MT (2006) The metabotropic glutamate receptor 4 is internalized and
450 desensitized upon protein kinase C activation. *Br J Pharmacol* **148**, 279–290.

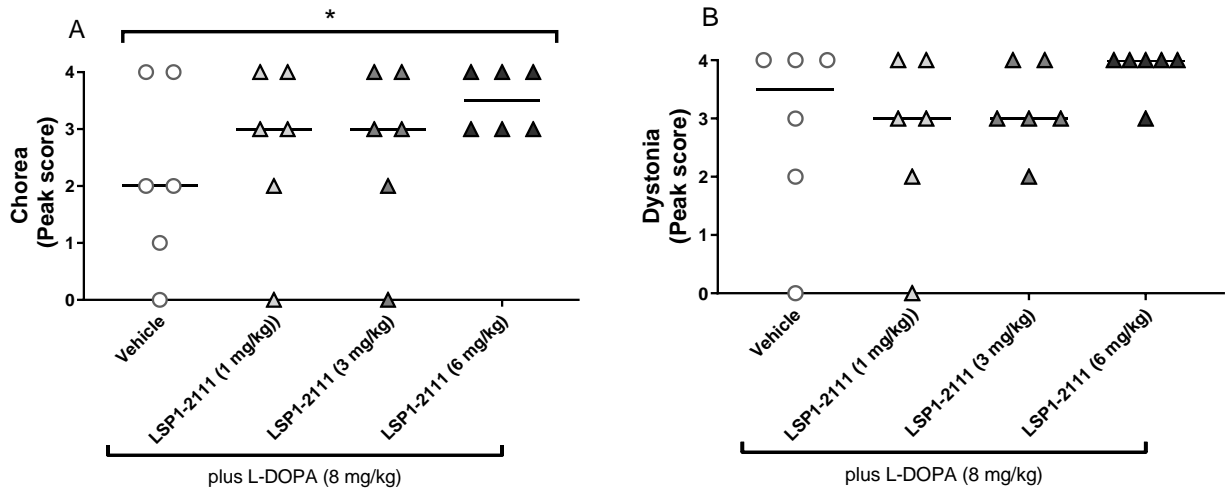
451

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



457

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

465

466 Figure Legends

467

468 Figure 1. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
469 nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
470 presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on locomotor activity. A)
471 The time course of effect with treatment administered at time T=0. Data are presented as mean
472 locomotor counts per 30 minutes (n=6). * p < 0.05 versus vehicle alone (two-way ANOVA plus Holm-
473 Sidak's multiple comparison test on transformed data). B) Total locomotor activity counts (AUC).
474 Data are presented as mean (line) and individual counts. * p<0.05 versus vehicle alone (◆) (one-way
475 ANOVA plus Holm-Sidak's multiple comparison test on transformed data).

476

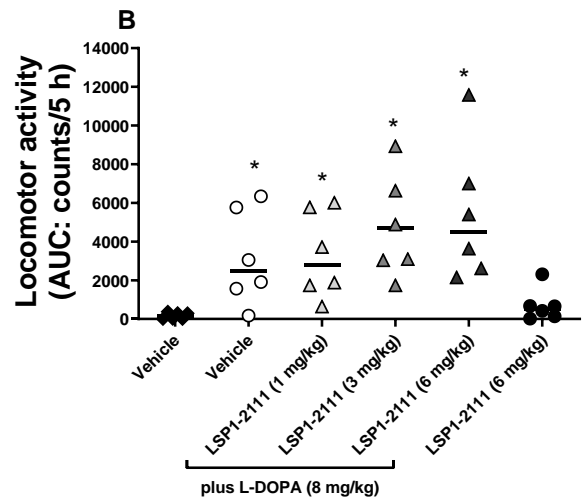
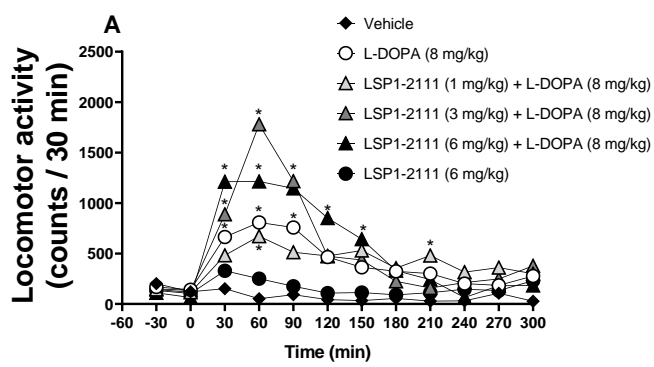
477 Figure 2. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
478 nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
479 presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on motor disability. A)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

486

487 Figure 3. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-
488 nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the
489 presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on dyskinesia expression.
490 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
497

498 Figure 1.

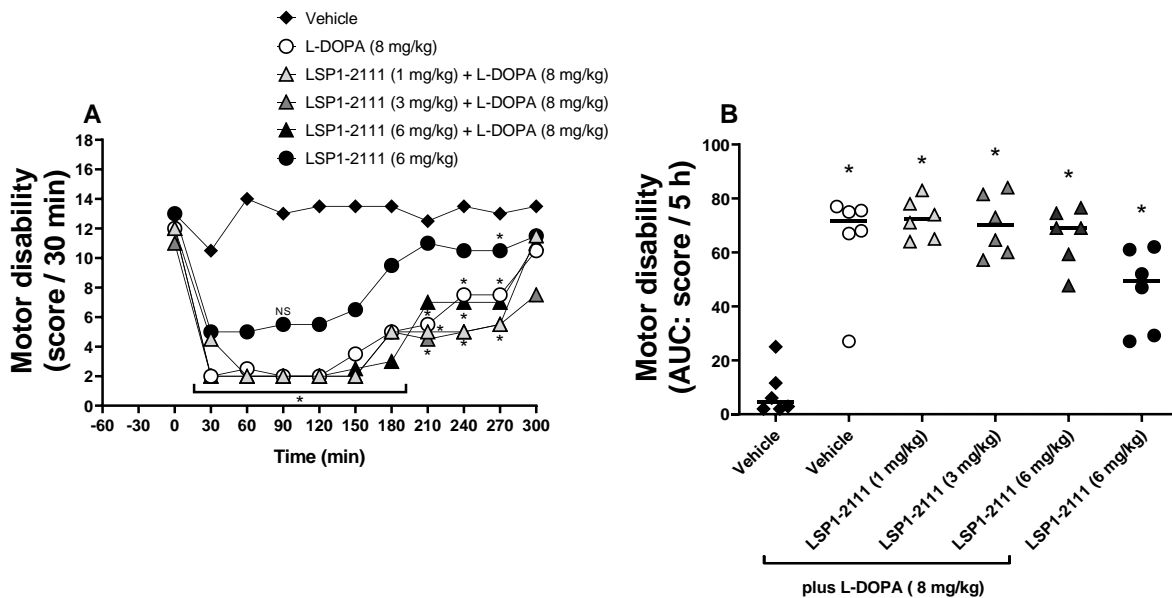


499

500

501 Figure 2.

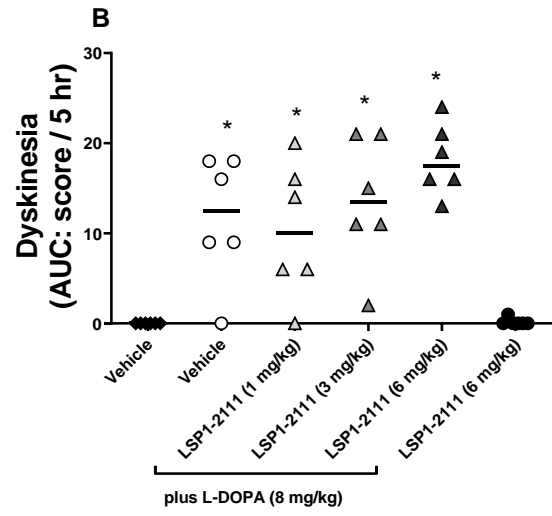
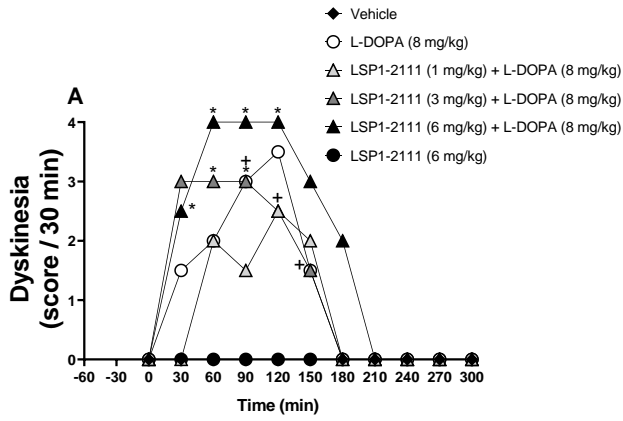
502



503

504

505 Figure 3.



506

507

508