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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Abstract

Background: Increased firing across glutamatergic synapses may contribute to both the motor

dysfunction and L-DOPA-induced dyskinesia seen in Parkinson's disease. Given their ability to

reduce glutamate release, activation of group III metabotropic glutamate receptors such as

metabotropic glutamate receptor 4 may prove effective against both motor dysfunction and

dyskinesia in Parkinson's disease.

Objectives: We hypothesised that activation of metabotropic glutamate receptor 4 by an orthosteric

agonist ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-

nitrophenyl)methyl)phosphoryl)butanoic acid, LSP1-2111) would produce antiparkinsonian activity

and reduce expression of dyskinesia in a 1-methyl-4-phenyl,1,2,3,6-tetrahydropyridine (MPTP)-

treated marmoset model of Parkinson's disease.

Methods: Common marmosets were previously treated with MPTP and pre-primed with L-DOPA for

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

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.

Conclusions: Systemic administration of LSP1-2111 reduces motor disability without causing

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

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

3

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 $23 \pm 2\,^0C$ 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

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 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 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

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

Acknowledgments

325

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

Conflicts of Interest

331

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

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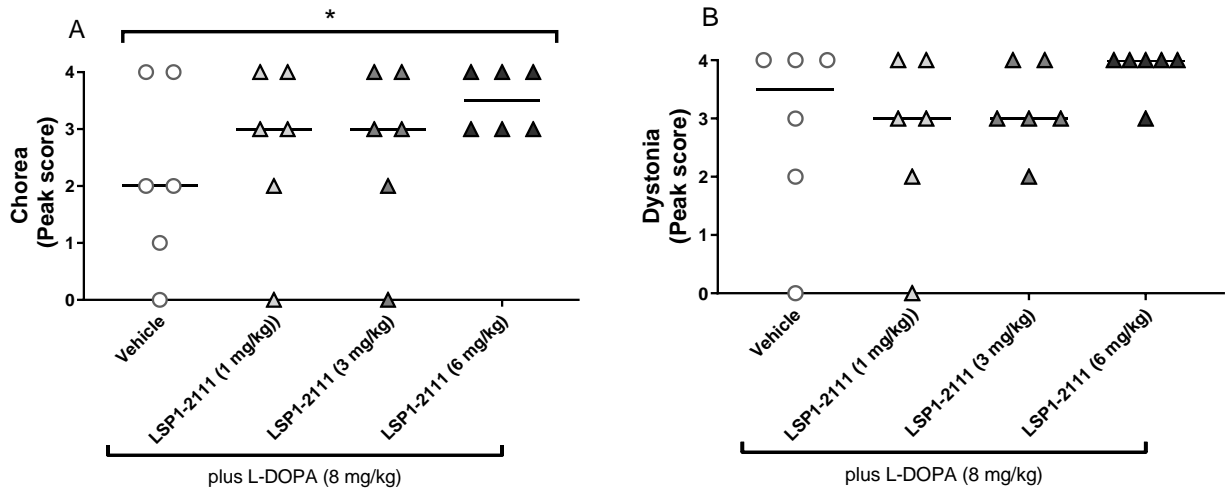
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Supplementary Figure 1.

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

455 not dystonia.

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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).

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Figure Legends

Figure 1. 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 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 (♦) (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).

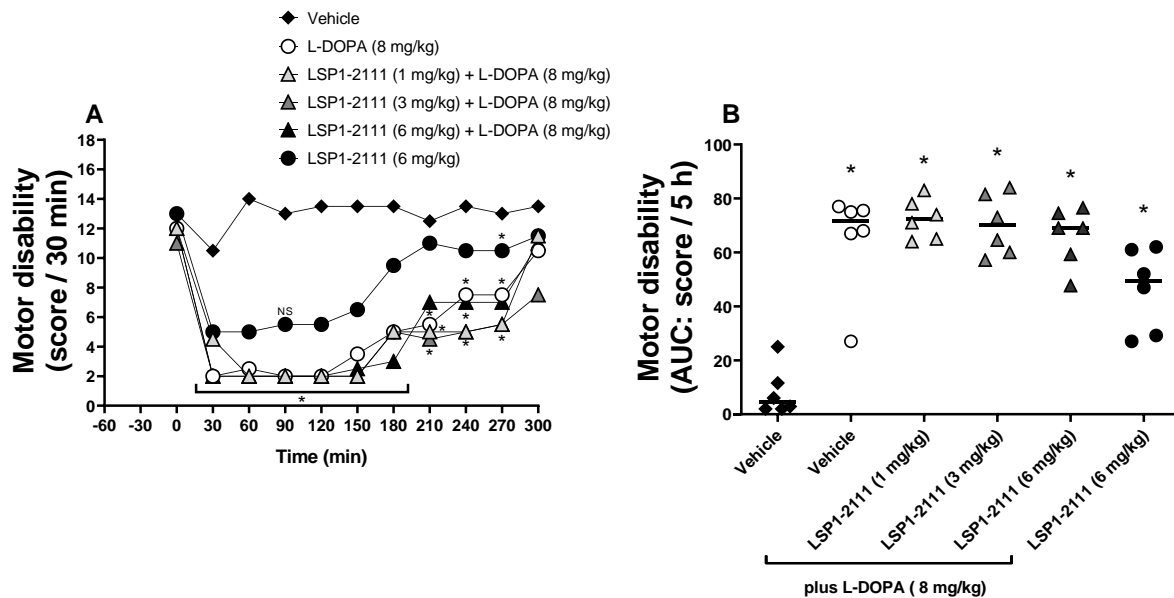Figure 3. The effect of treatment with ((2S)-2-amino-4-(hydroxy(hydroxy(4-hydroxy-3-methoxy-nitrophenyl)methyl)phosphoryl)butanoic acid (LSP1-2111; 6 mg/kg p.o.) alone or with L-DOPA in the presence of increasing doses of LSP1-2111 (vehicle, 1, 3 and 6 mg/kg p.o.) on dyskinesia expression. A) The time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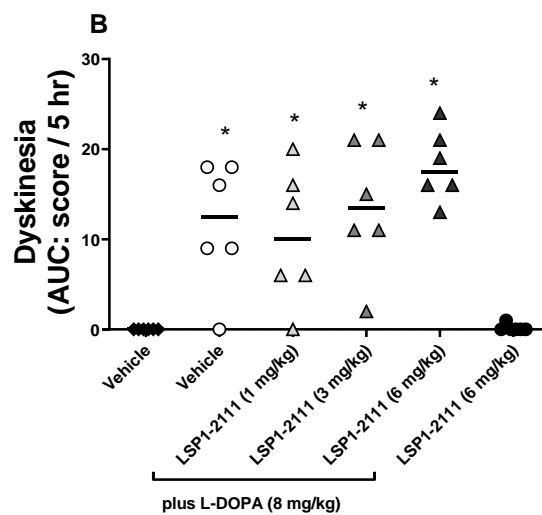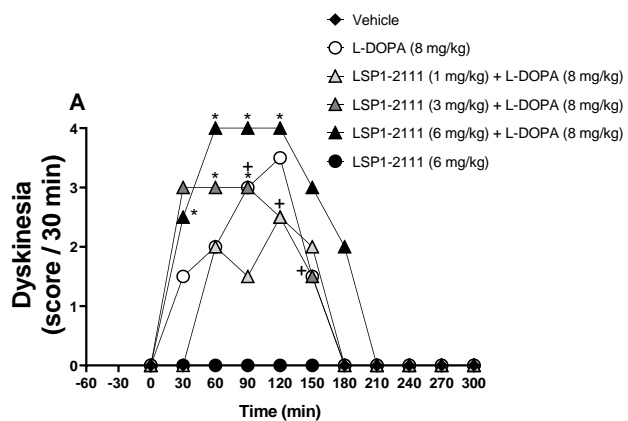
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498   Figure 1.
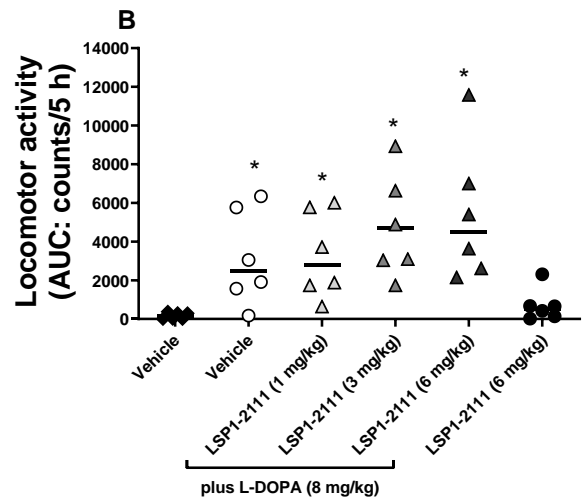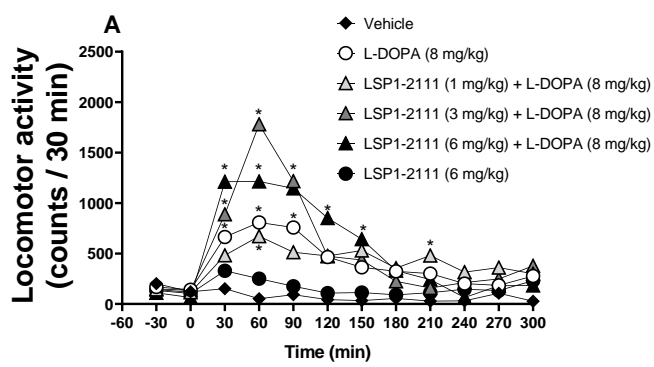
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