1 **Anatomical and physiological foundations of cerebello-hippocampal interactions**

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31   **Conflict of Interest**: The authors declare no competing financial interests.

32

33   **Abstract**

34   Multiple lines of evidence suggest that functionally intact cerebello-hippocampal

35   interactions are required for appropriate spatial processing. However, how the cerebellum

36   anatomically and physiologically engages with the hippocampus to sustain such interactions

37   remains unknown. Using rabies virus as retrograde transneuronal tracer, we reveal that the

38   dorsal hippocampus receives input from topographically restricted and disparate regions of

39   the cerebellum. By simultaneously recording local field potential from both the dorsal

40   hippocampus and anatomically connected cerebellar regions, we additionally demonstrate

41   that the two structures interact, in a behaviorally dynamic manner, through subregion-

42   specific synchronization of neuronal oscillations in the 6-12Hz frequency range. Together,

43   these results reveal a novel neural network macro-architecture through which we can

44   understand how a brain region classically associated with motor control, the cerebellum,

45   may influence hippocampal neuronal activity and related functions, such as spatial

46   navigation.

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Introduction

The cerebellum is classically associated with motor control. However, accumulating evidence suggests its functions may extend to cognitive processes including navigation [1–6]. Indeed, anatomical and functional connectivity has been described between cerebellum and cortical areas that are engaged in cognitive tasks [7–12]. Furthermore, the cerebellum has recently been found to form functional networks with subcortical structures associated with higher-order functions, such as the basal ganglia [13], ventral tegmental area [14] and hippocampus [15–18].

In the hippocampus, spontaneous local field potential (LFP) activity [19–22] and place cell properties [23], are profoundly modulated following cerebellar manipulation [24 for review]. A recent study has also described, at the single cell and blood-oxygen-level-dependent signal level, sustained activation in the dorsal hippocampus during optogenetic enhancement of cerebellar nuclei output in head-fixed mice [25]. These data point towards the existence of an anatomical projection from the cerebellum to the hippocampus. The suggestion of a direct connection between these two structures has been further supported by a recent tractography study in humans [26] and the presence of short-latency evoked field potentials (2-4 ms) in cat and rat hippocampi after electrical stimulation of the cerebellar vermal and paravermal regions [27–31]. However, secondary hippocampal field responses have also been described, at a latency of 12-15ms following cerebellar stimulation, suggesting the existence of an indirect pathway [28].

Taken together, these studies provide compelling physiological evidence of cerebellar influences on the hippocampus. Yet, they do not provide direct evidence of neuroanatomical connectivity between the two regions. Given the known complex, modular functional and anatomical organization of the cerebellum [32] this represents a major gap in

73   our understanding of the network architecture linking the two structures. What's more,

74   these studies provide no direct measure of physiological interactions between the regions,

75   which are thought to be essential for maintaining distributed network functions [e.g. 33].

76   Therefore, this study addresses two fundamental, unanswered questions: which regions of

77   the cerebellum are anatomically connected to the hippocampus and what are the spatio-

78   temporal dynamics of cerebello-hippocampal interactions during behavior? To address

79   these unresolved questions, we used rabies virus as a retrograde transneuronal tracer to

80   determine the extent and topographic organization of cerebellar input to the hippocampus.

81   Based upon the anatomical tracing results, we then studied interactions between the two

82   structures by simultaneously recording LFP from both the cerebellum and the dorsal

83   hippocampus in freely-moving mice. We reveal that specific cerebellar modules are

84   anatomically connected to the hippocampus and that these inter-connected regions

85   dynamically interact during behavior.

86

87   **Results**

88   To study the topographical organization of ascending, cerebello-hippocampal projections,

89   we unilaterally injected rabies virus (RABV), together with cholera toxin β-subunit (CTb),

90   into the left hippocampal dentate gyrus (DG). The use of CTb allowed us to identify and

91   measure the extent of the injection sites (Figure S1).

92   <u>A precise topography of the cerebellum regions projecting to the hippocampus</u>

93   We characterized the presence of retrograde transneuronally RABV-infected neurons after

94   survival times of 30, 48, 58 and 66 h [34,35]. Importantly, we did not find any RABV or CTb

95   labeling in the cerebellum at 30h post infection (p.i.), ruling out the existence of a direct

96   cerebello-hippocampal DG pathway in mice. Rather, RABV/CTb-labeled neurons were found

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97   in two well described subcortical pathways leading to the DG of the hippocampus (first cycle

98   of infection). One labeled pathway included the diagonal band of Broca and the septum. The

99   other labeled hippocampal input pathway included the lateral entorhinal and perirhinal

100   cortices (Figure S2)[36–38]. At 48 h p.i., a few weakly RABV+/CTb- neurons were found in

101   contralateral deep cerebellar and vestibular nuclei (Fig. 1A, inset), likely reflecting the onset

102   of a second infection cycle. In agreement with this hypothesis, after 58 h p.i., i.e. inside the

103   12 h time window required for completion of a viral replication cycle [39] we found robust

104   RABV labeling bilaterally in fastigial, dentate and vestibular nuclei, and a small number of

105   weakly labeled neurons in the posterior nucleus interpositus (Fig. 1B-F). At 58 h p.i. we also

106   observed few labeled cells in the cerebellar cortex (Fig. 1A, inset), suggesting the beginning

107   of an overlapping, third infection cycle. Within the two most labeled cerebellar nuclei,

108   fastigial and dentate, RABV-labeled cells were found to be topographically restricted to

109   caudal and central regions, respectively (Fig. 1G).

110   --FIGURE 1 NEAR HERE--

111   Following 66 h of incubation, the number of strongly labeled cells increased in the DCN and

112   vestibular nuclei (Fig. 1 A); however, the topographical distribution remained unchanged

113   (Fig. S3). At the level of the cerebellar cortex, longitudinal clusters of RABV+ Purkinje cells

114   (PCs) were found in a bilateral manner across highly restricted central and flocculo-nodular

115   regions (Fig. 1L). The bilateral cerebellar patterning of RV labeled cells observed after

116   unilateral hippocampal injections likely reflects the existence of commissural connections

117   within the pathway rather than the existence of bilateral projections arising from the

118   cerebellum. The presence of labeling in the contralateral hippocampus at the earliest

119   survival time (30h; Table 1) and the appearance at 48h of RV labeled neurons exclusively in

120   the contralateral cerebellar output nuclei are consistent with this hypothesis. In the central

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121   cerebellum, clusters were particularly concentrated in lobule VI and Crus I (Fig. 1 H, I, M). In

122   the flocculo-nodular cerebellum, RABV-labeled cells were found in the dorsal and ventral

123   paraflocculus (Fig. 1 J and M and Fig. 2B). Within the vermis we identified a single cluster of

124   RABV+ Purkinje cells that extended across both lobule VIa and lobule VIb-c (Fig. 2). In

125   contrast, within Crus I, RABV-labeled Purkinje cells were arranged in two spatially isolated

126   clusters, one located rostro-laterally and the other caudo-medially (Fig. 2).

127   The topographical arrangement of RABV-labeled PCs in longitudinal clusters is in keeping

128   with the well-described modular organization of the cerebellum [e.g. 32]. Mapping of

129   molecular marker expression patterns, such as zebrin II banding, provides a reliable basis

130   from which modules can be defined and recognized in the cerebellar cortex of rodents.

131   Thus, to further assign the observed PC clusters to previously described cerebellar zones, we

132   used a double immunohistochemical approach to stain for both RABV and aldolase C (zebrin

133   II) in one animal (case S18) 66 h after infection (Fig. 2B) [40,41]. Lobule VI, Crus I and

134   paraflocculus are mostly zebrin positive regions [42] and we found that RABV-labeled

135   Purkinje cells co-localized with zebrin II in all the observed clusters (Fig. 2B). In the vermis,

136   lobule VIa RABV-labeled PCs were mostly located in the a+ band. The few RABV-labeled cells

137   found in lobule VII were confined to the 2+ band. Thus, together, these labeled cells belong

138   to the a+//2+ pair that constitutes part of the cerebellar A module (Fig. 2C) [43]. In Crus I,

139   the rostrolateral cluster of RABV-labeled PCs was aligned with the anterior 6+ zebrin band

140   corresponding to module D2. The caudomedial cluster was in continuation with the

141   posterior 5+ zebrin band suggesting that it is part of the paravermal module C2 (Fig. 2C). In

142   the paraflocculus, the assignment of the RABV-labeled cells to specific modules was not

143   addressed given the complex morphology of this region. However, the presence of RABV-

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144   labeled cells both in the dorsal and ventral paraflocculus suggests the involvement of more

145   than one module (Fig. 2B-C) [44].

146   Cerebellar modules are also defined by their outputs through the deep cerebellar and

147   vestibular nuclei [32,45]. The presence of RABV-labeled cells in the fastigial nucleus is

148   consistent with the involvement of module A. Similarly, the D2 module is routed through

149   the dentate nuclei in which we find robust RABV labeling. We also found RABV+ cells in the

150   nucleus interpositus posterior, which provides the output of module C2. Finally, RABV

151   labeling was observed in the vestibular nuclei, which may represent the output of RABV+

152   Purkinje cells clusters observed in the ventral paraflocculus. Together, our neuroanatomical

153   tracing data indicate that cerebellar projections to the hippocampus emanate from three

154   distinct cerebellar modules subserving diverse functions.

155   --FIGURE 2 NEAR HERE---

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157   <u>Cerebello-hippocampal physiological interactions in a familiar home-cage environment</u>

158   In order to question the potential functional relevance of cerebello-hippocampal anatomical

159   connectivity, we implanted mice (n = 21) with arrays of bipolar LFP recording electrodes in

160   bilateral dorsal hippocampus (HPC) and unilaterally in two highly RABV-labeled regions of

161   the central cerebellum, lobule VI (midline) and Crus I (left hemisphere). For comparison, we

162   also simultaneously recorded LFP from cerebellar regions with minimal RABV labeling

163   (lobule II or lobule III; Fig. 1M; Fig. 3A and B). Data were excluded from further analysis in

164   cases where postmortem histological inspection revealed that electrode positions were off-

165   target Fig. S4).

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166   The spectral profile of cerebellar and hippocampal LFP activity was first assessed during

167   active movement in a familiar home-cage environment (see methods; mean speed, 2.7 ± 0.3

168   cm/s). Within the HPC, a dominant 6-12Hz theta oscillation was similarly observed in both

169   hemispheres (Fig. S5B; left HPC: peak spectral frequency = 7.81 ± 0.13 Hz, mean 6-12Hz z-

170   score power = 1.54 ± 0.07, N = 17 mice; right HPC: peak spectral frequency = 7.72 ± 0.12 Hz,

171   mean 6-12Hz z-score power = 1.54 ± 0.07, N = 19 mice; unpaired t test, t_{34}= 0.007, p = 0.99).

172   Fig. 3C shows combined spectra from both left and right HPC peak spectral frequency = 7.76

173   ± 0.09 Hz, mean 6-12Hz z-score power = 1.55 ± 0.05).

174   Although a clear peak in the 6-12 Hz band was not detected in cerebellar recordings,

175   transient 6-12 Hz oscillations were recorded (Fig. 3C and S6). The mean 6-12 Hz z-score

176   power did not differ between the different cerebellar recording sites (Fig. 3C; Crus I: 0.80 ±

177   0.30, N = 13 mice; lobule II/III: 0.84 ± 0.03, N = 11 mice; lobule VI: 0.79 ± 0.02, N = 19 mice;

178   one-way ANOVA, F $_{(2, 40)}$ = 0.85, p = 0.43).

179   As an indicator of cross-structure interaction [33], we next calculated coherence between

180   LFP recorded from the different cerebellar subregions and left or right HPC. We found no

181   statistically significant influence of hippocampal laterality on cerebello-hippocampal

182   coherence (Fig. S5C-E hemisphere x combination two-way ANOVA, hemisphere effect F $_{(1, 69)}$

183   = 0.23, p = 0.64, interaction effect, $F_{(2, 69)}$ = 0.06, p = 0.94). Therefore, for further analysis, we

184   grouped these coherence values.

185   A single peak in coherence was observed for all cerebello-hippocampal combinations in the

186   theta frequency range (6-12 Hz, Fig. 3D; Crus I-HPC peak coherence = 7.99 ± 0.13 Hz, lobule

187   II/III-HPC peak coherence = 8.75 ± 0.16 Hz, lobule VI-HPC peak coherence = 8.55 ± 0.11 Hz).

188   However, significant differences across combinations were observed within this bandwidth

189   and LFP oscillations were significantly more synchronised between HPC and lobule VI than

8

190   with lobule II/III (Fig. 3D and E; mean lobule VI-HPC coherence, 0.245 ± 0.006; mean lobule

191   II/III-HPC coherence, 0.223 ± 0.004; Kruskal-Wallis with FDR correction, q = 0.045; lobule VI,

192   n = 33 values/20 mice; lobule II/III, n = 19 values/11 mice). Within lobule VI, coherence was

193   significantly correlated to the mediolateral position of the recording electrode, which was

194   consistent with the mediolateral location of greatest RABV-labeled PCs (Fig. 3F; linear

195   regression, R^2 = 0.35, F $_{(1, 27)}$ = 14.45, p = 0.0007). Mean coherence between HPC and Crus I

196   (0.24 ± 0.01; n = 23 values/13 mice) was not significantly higher than with lobule II/III (Fig.

197   3E; Kruskal-Wallis with FDR correction, q > 0.05).

198   -----------------------------------FIGURE 3 NEAR HERE--

199   Cerebello-hippocampal interactions during the learning of a goal-directed behavior

200   To further characterize the dynamics of cerebello-hippocampal interactions, we quantified

201   cerebello-hippocampal theta coherence during a goal-directed task. A subset of mice (n=8)

202   were trained to traverse a linear track to get a reward (medial forebrain bundle stimulation,

203   see methods) at a fixed position (Fig. 4A).

204   Across training, mice improved their performance as shown by the optimisation of their

205   path (Fig. 4A), significant increase in the number of rewards obtained per trial (Fig. 4B;

206   mean number of rewards obtained on 1st trial = 16 ± 3, mean number of rewards obtained

207   on 20th trial = 81 ± 15; one-way repeated measures ANOVA, F$_{(2.882, 20.18)}$ = 8.93, p < 0.001)

208   and the significant increase in their mean speed (Fig. 4B; mean speed on 1st trial = 6.61 ±

209   0.30 cm/s, mean speed on 20th trial = 13.86 ± 1.56 cm/s; one-way repeated measures

210   ANOVA, F$_{(2.45, 17.15)}$ = 5.631, p = 0.0098). Thus, we next explored the dynamics of LFP power

211   and cerebello-hippocampal 6-12Hz coherence across this learning period.

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212   In the hippocampus, theta oscillations remained dominant in the recorded LFP throughout

213   training. A significant increase in both the mean theta power (Fig. 4C, S7A) and the peak

214   frequency (Fig. S7B) was observed in parallel with the performance and this was

215   independent of the hippocampal hemisphere (theta power: trial effect $F_{(19, 209)}$ = 3.11, p <

216   0.0001, hemisphere effect $F_{(1, 11)}$ = 0.30, p = 0.60; interaction effect, $F_{(19,209)}$ = 1.14, p = 0.31;

217   peak frequency: trial effect, $F_{(19, 209)}$ = 8.84, p < 0.0001, hemisphere effect $F_{(1, 11)}$ = 0.73, p =

218   0.41, interaction effect $F_{(19,209)}$ = 0.16, p > 0.99). In accordance with this finding, post-hoc

219   analysis revealed that mean theta power and peak frequency were significantly different

220   between first and last trials (Fig. 4E-F; mean z-score HPC theta power; mean on 1st trial =

221   1.64 ± 0.05, mean on 20th trial = 1.72 ± 0.04, q = 0.0180; peak frequency, mean trial 1 = 8.02

222   ± 0.15 Hz, mean trial 20 = 8.26 ± 0.13 Hz, q = 0.0205).

223   In the cerebellum, a global variation in the mean theta power was observed across trials but

224   no difference was found between Crus I, lobule VI and lobule II/III and no significant

225   variation was found between the last and first trials (Fig. 4C; Crus I: mean trial 1 theta power

226   = 0.73 ± 0.05 , mean trial 20 theta power = 0.71 ± 0.03, N = 5 mice; lobule II/III: mean trial 1

227   LFP power = 0.86 ± 0.03, mean trial 20 theta power = 0.87 ± 0.07, N = 6 mice; lobule VI:

228   mean trial 1 theta power = 0.78 ± 0.03, mean trial 20 theta power = 0.77 ± 0.06, N = 7 mice;

229   cerebellar region x trial two-way repeated measures ANOVA with FDR correction, cerebellar

230   region effect, $F_{(2, 15)}$ = 2.88, p = 0.09, trial effect $F_{(19, 285)}$ = 3.08, p < 0.0001, interaction effect

231    $F_{(38, 285)}$ = 0.72, p = 0.89, no trial was different from trial 1).

232   However, as learning progressed, cerebello-hippocampal theta coherence evolved in a non-

233   uniform manner (Fig. 4D; trial x combination two-way repeated measures ANOVA, trial

234   effect $F_{(19, 494)}$ = 2.42, p < 0.001, combination effect $F_{(2, 26)}$ = 4.09, p = 0.028, interaction

235   effect $F_{(38, 494)}$ = 3.43, p < 0.0001). Post-hoc analysis revealed that only Crus I-HPC coherence

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236   increased significantly across trials compared to initial values (multiple comparisons against

237   trial 1 with FDR correction; $q < 0.05$ for trials 5 and 7-20; Fig. 4D) and this was independent

238   of hippocampal hemisphere (Fig. S7C). Furthermore, this increase resulted in changes in the

239   differences in coherence observed between cerebello-hippocampal recording combinations.

240   Indeed, while during initial trials no significant inter-regional differences were observed, in

241   later trials, Crus I became significantly more coherent with HPC than lobule II/III and lobule

242   VI (Fig. 4D, multiple comparisons between combinations with FDR correction; Crus I vs

243   lobule II/III $q < 0.05$ for trials 5 and 8-20; Crus I vs lobule VI $q < 0.05$ for trials 8-12 and 14-

244   20). Unlike the observed shift in peak frequency of HPC theta power across trials, the peak

245   frequency of theta coherence remained constant for all the cerebello-hippocampal

246   combinations (Fig. S7D; Crus I-HPC: mean peak frequency = 8.41 ± 0.09 Hz, hemisphere x

247   trial two-way ANOVA, hemisphere effect, $F_{(1, 6)} = 0.98$, $p = 0.36$, trial effect $F_{(19, 114)} = 1.17$, p

248   = 0.30, interaction effect $F_{(19, 114)} = 0.48$, $p = 0.97$; lobule II/III-HPC: 9.08 ± 0.09 Hz,

249   hemisphere x trial two-way ANOVA, hemisphere effect, $F_{(1, 7)} = 1.59$, $p = 0.25$, trial effect,

250    $F_{(19, 133)} = 1.33$, $p = 0.18$, interaction effect, $F_{(19, 133)} = 0.97$, $p = 0.50$; lobule VI-HPC: mean

251   peak frequency = 8.78 ± 0.05 Hz, hemisphere x trial two-way ANOVA, hemisphere effect, $F_{(1,}$

252    $_{10)} = 0.15$ $p = 0.70$, trial effect, $F_{(19, 190)} = 1.21$, $p = 0.25$, interaction effect, $F_{(19, 190)} = 0.53$, p =

253   0.94). Further examination of the power and coherence spectra across a wider frequency

254   range (1 to 45 Hz) in trials 1 and 20 confirmed that the observed changes across training

255   were restricted to the theta band (Fig. 4 E-H).

256   -------------------------------------FIGURE 4 NEAR HERE -------------------------------------

257   To examine whether the observed changes in coherence across learning of the linear track

258   were specifically related to performance of the goal-directed task itself, we next conducted

259   pairwise analysis of cerebello-hippocampal theta coherence levels across the following

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260   conditions: home-cage prior to any linear track training (HC pre LT), first and last trials in the

261   linear track (early and late LT), and home-cage following the end of training in the linear

262   track task (HC post LT).

263   From the three cerebello-hippocampal recording configurations, only Crus I-HPC 6-12Hz

264   coherence varied significantly across task conditions (Fig. 5, Crus I-HPC: Friedman test with

265   FDR correction, Friedman statistic = 15.45, p = 0.0015). At the outset of linear track learning,

266   HPC-Crus I coherence values did not significantly differ from home-cage (HC pre LT = 0.25 ±

267   0.02; early LT = 0.26 ± 0.02; HC pre LT vs early LT q = 0.44). However, during late stage linear

268   track learning, the level of coherence was significantly higher than in home-cage recordings

269   and early stages of learning (late LT = 0.29 ± 0.03; HC pre LT vs late LT q = 0.0015; early LT vs

270   late LT q = 0.0016). When mice were returned to the home-cage environment following

271   completion of linear track training (HC post LT) the level of HPC-Crus I coherence dropped

272   significantly, back to pre-training levels (HC post LT = 0.26 ± 0.02; late LT vs HC post LT q =

273   0.012). We further analyzed changes in running speed and 6-12Hz power across conditions

274   and found that their pattern of modulation was markedly different from the observed Crus

275   I-HPC theta coherence dynamics (Fig. S8 and Fig. 5). While speed significantly varied

276   between all conditions, 6-12 Hz power remained stable in both in the HPC and cerebellar

277   recordings (Fig. S8). Together this suggests that the observed coherence dynamics appear to

278   be at least partially independent of changes in speed or 6-12Hz oscillation power.

279   --FIGURE 5 NEAR HERE--

280

281   <u>Cerebello-hippocampal interactions during locomotion in a virtual environment</u>

282   Our data indicate the presence of dynamic coherence between distinct cerebellar lobules

283   and the dorsal hippocampus during goal-directed behavior. To investigate if this interaction

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284   requires the presence of specific sensory inputs, we further analyzed cerebello-hippocampal

285   6-12Hz coherence under conditions in which such inputs are not relevant for the behavioral

286   task.

287   Head fixed mice were trained to locate rewards (medial forebrain bundle stimulation) at

288   fixed positions on a virtual-reality based linear track (VR; Fig. 6A and B; see Methods). In this

289   paradigm, vestibular, olfactory and whisker information cannot be reliably used to learn the

290   task and thus it is likely that behavioral performance is linked mainly to visuo-motor

291   information processing. Mice rapidly reached a stable performance level as illustrated by a

292   stable running speed and number of rewards obtained (Fig. 6C) (mean speed: trial 4 = 7.61 ±

293   1.50 cm/s, trial 21 = 8.76 ± 2.78 cm/s, one-way repeated measures ANOVA, $F_{(1.789, 8.945)}$ =

294   0.82, p = 0.82; mean number of rewards: trial 4 = 25 ± 5, trial 21 = 36 ± 16, one-way

295   repeated measures ANOVA, $F_{(1.519, 7.596)}$ = 0.93, p = 0.41; N = 6 mice). Although HPC theta

296   peak frequency was variable across trials with stable behavioral performance (trials 4 to 21,

297   Fig. S9A), mean 6-12Hz power and coherence values were similar (Fig. S9B-C) and therefore

298   collapsed across these trials for further analysis (Fig. 6D-F)

299   In keeping with results obtained in home-cage and real world (RW) linear track experiments,

300   coherence spectra calculated between either Crus I, lobule VI or lobule II/III and HPC

301   contained a single peak in the 6-12Hz theta frequency range (Fig. 6E). In this condition, 6-

302   12Hz coherence levels between HPC and both Crus I and lobule VI were again significantly

303   higher in comparison to lobule II/III (Fig. 6F; Kruskal-Wallis with FDR correction, H = 10.93,

304   Crus I-HPC vs lobule II/III-HPC q = 0.0021, lobule VI vs lobule II/III q = 0.0077, Crus I vs lobule

305   VI q = 0.2998; Crus I n = 5, lobule VI n = 9, lobule II/III n = 8).

306   --------------------------------------FIGURE 6 NEAR HERE--

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307

308   In an effort to isolate the impact of changing sensory information on observed cerebello-

309 hippocampal interactions from the influence of ongoing motor behavior, we next made

310 pairwise comparisons of theta coherence values from recordings made during RW and VR

311 linear track tasks in specific trials in which the number of rewards obtained and mean

312 running speed was similar in both conditions (Fig. 7A; number of rewards: LT = 37 ± 4, VR =

313 36 ± 4, paired t test, t_5 = 0.54, *p* = 0.46; speed: LT = 8.36 ± 0.47 cm/s, VR = 8.50 ± 0.56 cm/s,

314 paired t test t_5 = 0.68, *p* = 0.67; N = 6 mice). Patterns of coherence in the selected RW trials

315 resembled those observed during late training in the LT (Fig. 7B; differences in mean 6-12 Hz

316 coherence: one way ANOVA with FDR correction $F_{(2, 19)}$ = 4.55, Crus I-HPC vs lobule II/III *q* =

317 0.015, Crus I-HPC vs lobule VI-HPC *q* = 0.048, lobule VI-HPC vs lobule II/III-HPC *q* = 0.21;

318 compare with Fig. 4H) and the selected VR trials closely mirrored the overall, pooled data

319 (Fig. 7C; Kruskal-Wallis with FDR correction, H = 9.02, Crus I-HPC vs lobule II/III *q* = 0.009,

320 Crus I-HPC vs lobule VI-HPC *q* = 0.33, lobule VI-HPC vs lobule II/III-HPC *q* = 0.007; compare

321 with Fig. 6E), respectively. In these epochs of comparable motor state, Crus I-HPC coherence

322 was reduced significantly in the VR condition compared to RW (Fig. 7D; RW = 0.2974 ±

323 0.030, VR = 0.2721 ± 0.022; paired t test T_4 = 2.82, *p* = 0.047; n = 5). In contrast, lobule VI

324 and lobule II/III – hippocampal coherence was similar across conditions (Fig. 7D; lobule VI:

325 RW = 0.250 ± 0.011, VR = 0.260 ± 0.014, paired t test T_7 =1.63, *p* = 0.39, n = 9; lobule II/III:

326 RW = 0.239 ± 0.008, VR = 0.216 ± 0.002, paired t test, T_8=0.86 *p* = 0.18, n = 8).

327 ---FIGURE 7 NEAR HERE---

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329 **Discussion**

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331 Taken together, our findings reveal previously undescribed cerebellar inputs to the

332 hippocampus and offer novel physiological insights into a long-range neural network linking

333 disparate brain regions initially assumed to support divergent behavioral functions, namely

334 spatial navigation (hippocampus) and motor control (cerebellum). Topographically

335 restricted regions of cerebellar cortex discretely route through restricted parts of their

336 associated nuclei en-route to the hippocampus. Congruently, our physiological data reveal

337 that these connected cerebellar regions dynamically interact with the hippocampus during

338 behavior, via 6-12 Hz LFP coherence. Our findings thus offer an anatomical and physiological

339 framework for cerebello-hippocampal interactions that could support cerebellar

340 contributions to hippocampal processes [3], including spatial map maintenance [17,23,24].

341 Whilst previous studies provide compelling physiological evidence of cerebellar influences

342 on the hippocampus [19–22,25,46], they do not provide the spatial resolution afforded by

343 neuroanatomical tracing. Indeed, to the best of our knowledge, anatomical tracing studies

344 have failed to report a mono-synaptic ascending cerebello-hippocampal projection. This is

345 consistent with our rabies virus tracing study, in which incubation periods of 48-58 h were

346 required before cell labeling was seen in the cerebellar nuclei. Such a timescale is indicative

347 of a multi-synaptic pathway [35,47–49] potentially involving indirect connectivity through

348 the forebrain navigation circuits.

349 Our anatomical results highlight three main inputs to the hippocampus emanating from the

350 cerebellum. The first input we reveal originates from the vestibulo-cerebellum, specifically

351 from the dorsal and ventral paraflocculus, which is likely routed via the vestibular and

352 dentate nuclei [44]. This anatomical connection between the vestibulo-cerebellum and the

15

353 hippocampus reinforces the already well described influence of the vestibular system on

354 hippocampal dependent functions [50–52].

355 In addition to the classically described vestibular pathway, our data reveal that the central

356 cerebellum also provides inputs to the hippocampus from vermal lobule VI, routed through

357 caudal fastigial nucleus, and from Crus I, routed through the dentate. Using a combination

358 of RABV expression and zebrin II staining we identified three specific cerebellar modules

359 involved in these inputs: (1) the A module in lobule VI, (2) the hemispheric Crus I D2 module

360 and (3) the Crus I paravermal C2 module. Of the latter two modules, C2 is likely less

361 prominently anatomically connected with the hippocampus since the number of RABV+ cells

362 in the nucleus interpositus posterior, its output nucleus [32], was minor compared with the

363 other cerebellar nuclei. The convergence of inputs from disparate cerebellar zones (flocculo-

364 nodular and central zones) and modules from vermal (A), paravermal (C2) and hemispheric

365 (D2) regions in to the hippocampus suggest that its optimal function requires the integration

366 of multiple aspects of sensory-motor processing carried out at these distinct cerebellar

367 locations.

368 The A module in lobule VI is part of the so-called oculomotor vermis. It receives climbing

369 fibers from the caudal medial accessory olive, and sends mainly ascending projections

370 through the caudal portion of fastigial nucleus [53]. The oculomotor vermis receives

371 multiple sensory inputs which include visual, proprioceptive, vibrissae, vestibular and

372 auditory inputs conveyed by both climbing and mossy fibers [44]. It projects to, amongst

373 others, the superior colliculus and other visual structures of the midbrain, the vestibular

374 nuclei, the periaqueductal grey and the ventro-medial nucleus of the thalamus [54].

375 Notably, all of these regions contained RABV+ cells at 48h p.i., and thus they cannot be

376 excluded as potential routes towards the hippocampus. The D2 module receives its climbing

16

377 fiber input from the dorsal cap of the principal olive and projects out of the cerebellum

378 through the rostromedial dentate nucleus [55]. It receives mossy fiber inputs carrying

379 somatosensory, motor [56], and visual [57] information; along with inputs from the

380 prefrontal cortex [7]. Climbing fiber inputs to this module relay information from the

381 parvocellular red nucleus, which receives projections from premotor, motor, supplementary

382 motor and posterior parietal areas. The majority of these cortical areas also receive

383 projections from the D2 module after a thalamic relay in the ventro-lateral nucleus [7,58].

384 Complementary to these anatomical results, our electrophysiological findings reveal

385 coherent activity between the hippocampus and those cerebellar lobules that are

386 anatomically connected with it (lobule 6 and Crus I). This interaction was restricted to the 6-

387 12 Hz range in the awake, behaving animal and showed non-uniform, dynamic profiles that

388 were lobule dependent. Oscillations can align neuronal activity within and across brain

389 regions, facilitating cross-structure interactions [e.g 33,59]. Cerebellar circuits support

390 oscillations across a range of frequencies [for review see 60,61]. Of particular relevance to

391 the current study are reports of oscillations within the theta frequency (~4-12 Hz), which

392 have been described in the cerebellar input layers at the Golgi [62] and granule cell [e.g.

393 63,64] level, and also in the cerebellar output nuclei [9,65]. Indeed, despite of the absence

394 of prominent sustained theta band activity in our overall cerebellar recordings, we could

395 record transient 6-12 Hz cerebellar oscillations (Fig S6).

396 Neuronal coherence has been described across the cerebro-cerebellar system at a variety of

397 low frequencies [9,66–71] and oscillations within the theta range are thought to support

398 inter-region communication across a wide variety of brain regions [72]. Our finding that

399 cerebello-hippocampal coherence is limited to the 6-12 Hz bandwidth is in keeping with

400 previous studies on cerebro-cerebellar communication in which neuronal synchronization

17

401 has been observed between the cerebellum and prefrontal cortex [9,67], primary motor

402 cortex [66,70], supplementary motor area [66] and sensory cortex [66]. Furthermore, local

403 field potentials recorded in the hippocampus and cerebellar cortex are synchronized within

404 the theta bandwidth during trace eye-blink conditioning in rabbits [73]. Human brain

405 imaging studies have also described co-activation of blood oxygen level dependent signals in

406 both cerebellar and hippocampal regions during navigation [15] and spatio-temporal

407 prediction tasks [74], thus highlighting neuronal putative interactions between the two

408 structures. Regarding studies in mice, a recent study has demonstrated the existence of

409 statistically significant co-activation of the dorsal hippocampus and cerebellar lobules IV-V,

410 lobule VI and Crus I after the acquisition of a sequence-based navigation task [16].

411 Multiple lines of evidence suggest that the phase-locking described here is unlikely to have

412 resulted from volume conduction: 1) Rather than using a common reference electrode, our

413 recordings were bipolar, with each recording electrode being locally and independently

414 referenced [75]. 2) If volume conduction of theta oscillations was emanating from a

415 hippocampal source then it could be assumed that cerebellar regions in closer proximity to

416 the hippocampus would show higher levels of coherence (Fig. S5A). However, we found that

417 coherence values were not related to the relative distance between the hippocampus and

418 cerebellar recording site. 3) Hippocampal theta power increased over training in our linear

419 track paradigm, whereas 6-12 Hz power remained stable across all cerebellar recording

420 sites. However, significant coherence was only observed between hippocampus and Crus I.

421 Thus, the observed coherence was unlikely to have resulted from co-variation in theta

422 power between the two areas.

423 Importantly, we have shown for the first time that theta rhythms in the hippocampus

424 preferentially phase lock with those in discrete regions of the cerebellum and that degree of

18

425 this coupling changes depending upon the behavioral context. Lobule VI-hippocampus

426 coherence was dominant during active movement in the home-cage and remained stable

427 during learning of the real world linear track task. On the other hand, Crus I-HPC coherence

428 was highly dynamic, showing a significant increase over the learning of the real world linear

429 track task and becoming dominant after the acquisition of a goal-directed behavior.

430 Interestingly, although multiple streams of sensory input, including those of a vestibular,

431 whisker and olfactory nature, become irrelevant and even confounding in the head-

432 restricted virtual environment task, Crus I-HPC and lobule VI-HPC coherence remained high

433 in this condition. Paired-comparisons of trial epochs containing similar behavioural

434 performances in the real-world and virtual environment revealed that Crus I-HPC coherence

435 is significantly reduced in the latter while no change was observed for lobule VI-HPC.

436 We next consider our results within the modular understanding of cerebellar function.

437 Within lobule VI, the A module receives multi-modal sensory information, mainly arising

438 from collicular and vestibular centres [44]. The superior colliculus plays a role in visual

439 processing and generation of orienting behaviors [76], which might be relevant for the

440 establishment and maintenance of the hippocampal spatial map, and thus may be required

441 constantly during active movement, independent of the specific behavioral task. The

442 persistent and similar levels of lobule VI-HPC coherence during active movement in the

443 homecage and linear track task, in both real world and virtual reality environment tasks is in

444 agreement with such a hypothesis.

445 In monkeys and humans, Crus I is anatomically and functionally associated with prefrontal

446 cortex [7,15]. In rat Crus I, the D2 module receives convergent sensory and motor

447 information [77]. Furthermore, this module has been found to contain internal models, a

448 neural representation of one's body and the external world based on memory of previous

19

449   experiences, that are used for visuo-motor coordination [78]. Similarly, the C2 module has

450   been found to also participate in visuo-motor processing related to limb coordination during

451   goal-directed reaching [79]. Both modules might be particularly important during the

452   acquisition of a goal-directed behavior such as our real-world linear track task in which

453   animals needed to reach non-cued reward zones. Our finding that Crus I-HPC coherence

454   increases during task learning fits with this hypothesis. Furthermore, the observed reduction

455   of Crus I-HPC coherence levels in the head-restricted, virtual environment task may reflect

456   the reduced recruitment of cerebellar modules that are involved in processing of non-

457   relevant sensory modalities, since only visuo-motor information can be reliably used to

458   learn the task.

459   In summary, our results suggest the existence of both, an anatomically discrete

460   hippocampal-cerebellar network interactions and a topographical dynamic weighting of

461   these interactions potentially tailored to the prevailing sensory context and behavioral

462   demands.

463

464

20

465 **Methods**

466 Anatomical tracing studies were performed under protocol N°00895.01, in agreement with

467 the Ministère de l'Enseignement Supérieur et de la Recherche. RABV injections were

468 performed by vaccinated personnel in a biosafety containment level 2 laboratory.

469 All behavioral experiments were performed in accordance with the official European

470 guidelines for the care and use of laboratory animals (86/609/EEC) and in accordance with

471 the Policies of the French Committee of Ethics (Decrees n° 87–848 and n° 2001–464). The

472 animal housing facility of the laboratory where experiments were made is fully accredited

473 by the French Direction of Veterinary Services (B-75-05-24, 18 May 2010). Surgeries and

474 experiments were authorized by the French Direction of Veterinary Services (authorization

475 number: 75-752).

476 A total of 39 adult, male mice were used for this study. 18 adult male C57BL6-J mice were

477 used for the anatomical tracing study, (Charles River, France) and 21 for the

478 electrophysiology study (Janvier, France). 3 adult male CD-L7ChR2 mice were used for the

479 dual hippocampal LFP and cerebellar unit-recording study (in-house colony derived from

480 Jackson labs stock, USA).

481 Mice received food and water *ad libitum*, were housed individually (08: 00–20: 00 light

482 cycle) following surgery and given a minimum of 5 days post-surgery recovery before

483 experiments commenced.

484 **1. Anatomy**

485 **Rabies virus injections**

21

486   All the RABV (the French subtype of Challenge Virus Standard; CVS-N2C) inoculations were

487   performed in the Plasticity and Physio-Pathology of Rhythmic Motor Networks (P3M)

488   laboratory, Timone Neuroscience Institute, Marseille, France. Mice (n= 18) were injected

489   intraperitoneally with an anesthetic mixture of ketamine (65 mg/kg; Imalgene, France) and

490   xylazine (12 mg/kg; Rompun, Bayer) to achieve surgical levels of anesthesia, as evidenced by

491   the absence of limb withdrawal and corneal reflexes and lack of whisking and were then

492   placed in a stereotaxic frame (David Kopf Instruments, USA). The scalp was then incised, the

493   skull exposed and a craniotomy drilled above the hippocampus.

494   Mice were injected with 200 nL of a mixture of one part 1% CTb Alexa Fluor® 488 Conjugate

495   (Invitrogen, distributed by Life Technologies, Saint Aubain, France) and four parts RABV in

496   the left hippocampus (AP -2.0, ML +2.0, DV 1.97; Fig. 1, Fig. S1). Injections (200 nL/min)

497   were performed using a pipette connected to a 10 μL Hamilton syringe mounted on a

498   microdrive pump. Following infusion, the pipette was left in place for 5 min. The incision

499   was then sutured and the animals allowed to recover in their individual home cage for

500   either 30h (n= 5); 48 h (n= 3), 58h (n= 5) or 66 h (n=5). All animals were carefully monitored

501   during the survival period and, in line with previous studies using these survival times, were

502   found to be asymptomatic [48].

503   **Tissue preparation**

504   At the end of the survival time, mice were deeply anesthetized with sodium pentobarbitone

505   (100mg/kg, intraperitoneal) then transcardially perfused with 0.9 % saline solution

506   (15mL/min) followed by 75 mL of 0.1M phosphate buffer (PB) containing 4 %

507   paraformaldehyde (PFA; pH = 7.4). The brain was then removed, post fixed for 2-3 days in 4

508   % PFA and then stored at 4°C in 0.1 M PB with 0.02% sodium azide. Extracted brains were

509   then embedded in 3 % agarose before being coronally sectioned (40 μm) on a vibratome.

22

510   Serial sections were collected and divided in 4 vials containing 0.1 M PB so consecutive

511   slices in each vial were spaced 160 µm.

512   **Injection site visualization**

513   Sections from vial 1 were used to visualize the injection site by the presence of CTb. In most

514   of the cases, the injected CTb was fluorescent and sections were directly mounted with Dapi

515   Fluoromount G (SouthernBiotech®, Alabama, USA). In the other cases (S4-5, S11-13 and

516   S17-18), the sections were first rinsed with PB 0.1M and then permeated with PB 0.1 M and

517   0.3 % Triton X-100. They were then incubated overnight in a choleragenoid antibody raised

518   in goat (goat anti-CTb, lot no. 703, List Biological Laboratories, USA) diluted 1: 2000 in a

519   blocking solution (PB 0.1 M, 5 % BSA). Subsequently, the sections were rinsed in PB 0.1M

520   and incubated 4 h at room temperature with donkey anti-goat secondary antibody (1: 1000

521   in the blocking solution; Alexa Fluor® 555, Invitrogen, distributed by ThermoFisher Scientific,

522   Massachusetts, USA). Finally, they were also mounted with Dapi Fluoromount G.

523   The injection site was then visualized using a fluorescence microscope equipped with a

524   fluorescein isothiocyanate filter (Axio Zoom V16, Carl Zeiss, France).

525

526   **Rabies virus labeled cell quantification**

527   Sections from vial 2 were used for quantification and 3 D reconstruction of the RABV labeled

528   cells. Sections mounted on gelatin-coated SuperFrost ®Plus slides (Menzel-Glaser,

529   Braunschweig, Germany) were first rinsed with PB 0.1 M and pre-treated with 3 % H_2O_2

530   during 30 minutes for blocking reaction against endogenous peroxidase. Following

531   pretreatment, the sections were incubated overnight at room temperature with an anti-

532   rabies phosphoprotein mouse monoclonal antibody [80] diluted at 1: 10000 in a blocking

23

533   solution (PB 0.1 M, 0.1 % BSA, goat serum 2 % and 0.2 % Triton X-100). The day after, the

534   sections were rinsed in PB 0.1M and incubated 2 hours with a biotinylated affinity-purified

535   goat anti-mouse IgG (1: 2000 in blocking solution; Santa-Cruz, Heidelberg, Germany). Then,

536   they were also incubated using an avidin-biotin complex method (Vectastain Elite ABC-

537   Peroxidase kit R.T.U. Universal, Vector Laboratories, Burlingame, CA, USA) to enhance

538   sensitivity. For visualization, the sections were incubated in a 3,3'-diaminobenzidine-

539   tetrahydrochloride (DAB) solution (0.05 % DAB and 0.015 % H_2O_2 in PB 0.1 M). Finally, they

540   were counterstained with Cresyl and cover-slipped.

541   Quantitative analyses of rabies-positive nuclei were performed using a computerized image

542   processing system (Mercator, Exploranova, France) coupled to an optical microscope. The

543   quantification of rabies-positive nuclei was carried out at 10x magnification. Structures were

544   defined according to a standard atlas [81]. Immunoreactive neurons were counted

545   bilaterally. Representative images were obtained using an Axio Zoom V16 microscope (Carl

546   Zeiss, France).

547   **3-D reconstruction**

548   A Nikon Eclipse E800 microscope equipped with a digital color camera (Optronics, USA) was

549   used to visualize mounted cerebellar sections under brightfield illumination. The contour of

550   every 4 th section was then manually drawn using Microfire software (Neurolucida, MBF

551   Bioscience, USA) and cell counts were performed. The sections were then aligned and

552   stacked (160 µm spacing).

553   **Rabies virus-zebrin II double immuno-staining**

554   For case S18, sections from vial 3 were mounted on gelatin-coated SuperFrost ®Plus slides

555   (Menzel-Glaser, Braunschweig, Germany), rinsed with PB 0.1M and then permeated and

24

556   blocked in a solution of PB 0.1 M, 0.2 % Triton X-100 and bovine serum 2.5% for 30 minutes.

557   Then they were incubated during 48h at 4°C in a mix of rabbit polyclonal anti-Aldolase C

558   primary antibody (a kind gift from Izumi Sugihara [41]; No. 69075; 1:500000) and the mouse

559   anti-rabies antibody used for the single RABV staining (1:5000) in a blocking solution (PB 0.1

560   M, 0.1 % Triton X-100 and bovine serum 1 %). Subsequently, the sections were first rinsed

561   with PB 0.1 % and then incubated in a mix of RRX-Goat anti-rabbit IgG (1: 5000; ref 111-295-

562   144, Jackson Immuno Research) and donkey anti-mouse secondary antibody (1: 5000; Alexa

563   Fluor® 647, Invitrogen distributed by ThermoFisher Scientific, Massachusetts, USA) in

564   blocking solution. Finally, they were also mounted with Dapi Fluoromount G.

565   Images were obtained using an Axiozoom v16 microscope (Carl Zeiss, France) and contour

566   and labeled neurons in the cerebellum were manually draw for reconstruction of the zebrin

567   bands and cerebellar modules and location of the RABV+ cells.

568   **2. Electrophysiology procedures**

569   **Subjects and surgical protocols**

570   Bipolar LFP recording electrodes (interpolar distance of ~0.5mm; 140μm diameter Teflon

571   coated stainless-steel, A-M system, USA) were stereotaxically targeted to hippocampus (AP -

572   2.2, ML +2.0, DV 1.0), lobule 6 (AP -6.72, ML 0.0, DV 0.1), lobule 2 (AP -5.52, ML 0.0, DV 1.8)

573   and Crus I (AP -6.24, ML 2.5, DV 0.1) of 21 C57BL6-J mice. Pairs of flexible stainless-steel

574   wires were used to also record neck EMG (Cooner wire, USA)

575   In 15 C57BL6-J mice, bipolar stimulation electrodes (140-μm-diameter stainless steel; A-M

576   system, USA) were also implanted at the left medial forebrain bundle [MFB; to serve as a

577   reward signal; AP -1.4, ML +1.2, DV +4.8 [Cf. 82,83]. All electrode assemblies were fixed to

578   the skull using a combination of UV activated cement (SpeedCem, Henry Shein, UK),

25

579   SuperBond (SunMedical, Japan) and dental cement (Simplex Rapid, Kemdent, UK). Four

580   miniature screws (Antrin, USA) were also attached to the skull for additional support and to

581   serve as recording ground.

582   In 6 mice, a lightweight metal head fixation device (0.1g) was also implanted. The total

583   implant weight did not exceed 2.5g (including head fixation post and cement).

Recording

585   Signals from all electrodes were attached to an electronic interface board (EIB 18,

586   Neuralynx, USA) either during surgery. Differential recordings were made via a unity-gain

587   headstage preamplifier (HS-18; Neuralynx, USA) and Digital Lynx SX acquisition system

588   (Neuralynx, USA). LFP and EMG Signals were bandpass-filtered between 0.1 and 600 Hz and

589   sampled at 1 kHz. Mouse position was tracked at 30Hz using video tracker software and

590   infra-red LEDs attached to the headstage (Neuralynx, USA).

MFB Stimulation

592   Intracranial rewarding stimulation consisted of a 140Hz stimulation train lasting 100ms

593   delivered through the headstage to the implanted electrodes (SD9k, Grass Technologies,

594   USA). Optimal voltage for intracranial MFB was determined for each mouse with a nose-

595   poke task prior to training (range, 1-6V [Cf. 82]).

Histology

597   After completion of all the experiments, mice were deeply anesthetized with

598   ketamine/xylazine solution (150mg/kg) and electrolytic lesions created by passing a positive

599   current through the electrodes (30μA, 10sec). With the electrodes left *in situ*, the animals

600   were perfused transcardially with saline followed by paraformaldehyde (4%).

601   Brains were extracted and post-fixed in paraformaldehyde (4%; 24h) then embedded in

602   agarose (24h). A freezing vibratome was used to cut 50μm thick sagittal cerebellar and

26

603   coronal hippocampal sections. The sections were mounted on gelatinised slides and stained

604   with cresyl violet. Recording locations were identified by localised lesions in the cerebellum

605   and hippocampus and plotted on standard maps with reference to a stereotaxic atlas [81].

27

606 **3. Behavioral procedures**

607 **Familiar environment**

608 All recordings were made in the animal's home-cage (30 cm x 10 cm x 10 cm plastic box),

609 with the lid removed and lasted a maximum of 4 hours. Recordings were made during the

610 day between the hours of 10 am to 18:00 pm.

611 **Linear track – real world**

612 The linear track was made in-house from 100 cm x 4 cm x 0.5 cm of black plastic positioned

613 20cm above the surface of the experimental table. The behavioral assembly was located in a

614 separate room from the experimenter and was surrounded on four sides by black curtains.

615 Three salient visual cues were placed at fixed locations along the edge of the track (10 cm

616 from the edge). Mice were trained to run in a sequential manner from one end of the track

617 to the other in order to receive a reward, which consisted of an electrical stimulation of the

618 MFB. The reward stimulation was delivered automatically when the mice reached a 5 cm

619 wide goal-zone, which was located 10 cm from the end of the track. Timing of the reward

620 signal was logged on the electrophysiological recordings via TTL signals. Sessions lasted 12

621 mins and were repeated 3 times per day with an inter-session time of 5 mins over 7 days.

622 Between sessions, the track was cleaned with 20 percent ethanol.

623 **Linear track – virtual reality environment**

624 A commercially available virtual-reality environment was used (Jet Ball, Phenosys,

625 Germany), utilising an air cushioned Styrofoam ball (200 mm), which served as a spherical

626 treadmill for head restrained mice [Cf. 84] (Supplementary Fig. 12). The floating ball

627 assembly was positioned 20 cm from a series of six octagonally arranged TFT surround

628 monitors (19 inch) such that the head restrained mice had an unobstructed view of the

28

629   visual scene. Rotation of the Styrofoam ball was detected by an optical sensor (sampling

630   frequency 5700 dots per inch at 1 kHz). The vertical axis signals were interpreted by the VR

631   software as the forward and backward movement of the virtual position of the animal.

632   Position within the VR was then translated to a voltage signal (zero to five volts, with 5 volts

633   indicating the end of the track), and sent to the Digital Lynx SX (Neuralynx, USA)

634   electrophysiology system via a DACQ interface (DACQ 6501, National Instruments, USA). The

635   start of the VR display was logged on the electrophysiology recordings via a TTL signal. To

636   provide a reward signal, when the mice reached a given location within the VR (10 cm from

637   the end of the track) a TTL marker was sent to both the electrophysiological recording

638   system (to provide a timestamp-marker of the event) and an electrical stimulus generator

639   linked to the HS-18 headstage (in the same manner as for RW linear track experiments).

640   The virtual scene consisted of a 1 m long track with grey walls and included 3 salient visual

641   cues. After 3 x 12 mins sessions of habituation to the head fixation on the floating-ball

642   assembly, mice were trained to run on the linear track in 12 min sessions, 3 times per day

643   with an inter-session interval of approximately 5 mins during 7 days. The number of rewards

644   received by the animal was logged in the electrophysiology software (Cheetah 5.6.3,

645   Neuralynx, USA).

Behavioral and electrophysiological analysis

All data were processed in Matlab (Mathsworks, USA), Spike 2 (Cambridge Electronic Design, UK) and Prism (Graphpad, USA).

1. Behavior

In the home-cage environment, behavioral data were selected using a custom-made Matlab script. Interactive cursors were used to define periods of active movement, based upon speed (derived from video tracking data), EMG and LFP signals. For the purpose of further analysis, we focused on periods of active movement (indicated by high EMG amplitude, speed and hippocampal 6-12 Hz oscillations). The overall mean speed of each mouse was then calculated across all the selected data epochs.

For RW linear track experiments, in each 12 minute trial (3 trials per day) the number of rewards (indicated by TTL markers), distance traveled, speed and an efficiency score (distance traveled per reward) were calculated using a custom Matlab script. These parameters were calculated on a trial-by-trial basis (from trial 2 onwards).

In virtual reality-based experiments, for each 12min trial (3 trials per day), the number of rewards (timestamped by TTL markers) and virtual speed was calculated using a custom Matlab script. Virtual speed was calculated using the virtual environment X and Y coordinate values (recorded as voltage signals in Neuralynx Cheetah software).

30

2. Electrophysiology

Multi-taper Fourier analyses (Chronux toolbox [85]) were used to calculate power and coherence of the LFP data. We used a 10s sliding window (9s overlap) and 19 tapers for all analysis, except for the example presented in Fig. S6 in which we used a 2s sliding window (1.5s overlap) and 3 tapers. Statistical comparison was restricted to the 6-12 Hz frequency band unless otherwise stated.

For recordings made in the home-cage environment, LFP data were manually selected, as described above for behavioral data. Analysis was focused on LFP gathered during periods of active movement. Selected data were then filtered to remove any large-amplitude, low frequency artifacts using a stationary wavelet transform [86]. Spectral power and coherence were then calculated. The spectral power between 0.1 and 45 Hz was z-scored due to inter-animal variations in LFP magnitude. Mean power and coherence were calculated in the 6-12Hz frequency range for all cerebello-hippocampal combinations. Data duration in the home-cage environment varied across mice (range, 12 to 132 min). Therefore, to reduce the impact of data length on subsequent analyses and also to match with subsequent linear track experiments (duration of 12 min), for each mouse we concatenated the LFP in to 12 min blocks. When multiple 12 min blocks were available (number of data blocks ranged from 1 to 11) we calculated the average coherence across all blocks. The number of 12 minute blocks used was found to have no correlation with the overall level of calculated coherence (See Fig. S5F).

For RW and virtual linear track recordings, general electrophysiological analysis methods were the same as described for home-cage recordings including artifact removal procedures (in this case MFB stimulation artifacts were also removed using the methodology described in [86]). In addition to pooled calculations (in which analysis was conducted across all trials

31

688   of the task), power and coherence was also calculated on a trial-by-trial basis across

689   learning.

690   **Statistical analysis**

691   Statistical analyses were conducted using Matlab Statistical Toolbox and Prism (Graphpad,

692   USA). Normality was assessed using a Shapiro-Wilk test. Parametric and non-parametric

693   tests were then used accordingly.

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695   **Acknowledgements**

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937   **Figure Legends**

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939   **Figure 1. Topographically restricted regions of cerebellar cortex are connected to the**

940   **hippocampus.**

941   **A**, Left, mean number of labeled cells in the deep cerebellar nuclei (DCN), vestibular nuclei

942   (VN) and cerebellar cortex at different survival times following rabies injection in left

943   hippocampal dentate gyrus. Box shows a magnification of the labeling at 48 and 58 h (n = 5

944   mice for all data points except 48 h, n = 3). Middle, schematic representation of rabies

945   injection site and survival times required to reach the cerebellar and vestibular nuclei (58 h,

946   dashed blue line, polysynaptic pathway), and cerebellar cortex (66 h, orange line). Upper

947   right, schematic view of the posterior cerebellar cortex indicating regions of highest labeling

948   following rabies virus injection (red, vermis lobule VI; green, Crus I; grey, paraflocculus). **B-E**,

949   Representative photomicrographs showing labeling in the contralateral cerebellar and

950   vestibular nuclei 58 h post infection. Left panels show low magnification view, right panels

951   show magnified view of area indicated by dashed box. Solid arrow heads indicate the

952   presence of the very few labeled cells in the IntP. **F**, Pooled, normalized counts of rabies

953   labeled cells in the ipsi- and contralateral cerebellar and vestibular nuclei 58 h post infection

954   (n = 5 mice). No significant differences were found between ipsi- and contralateral nuclei

955   (nuclei x hemisphere two-way ANOVA, hemisphere effect $F_{(1, 4)} = 1.31\times10^{-5}$, p = 0.99,

956   interaction effect $F_{(3, 12)} = 2.79$, p = 0.09, nuclei effect $F_{(3, 12)} = 9.38$, p = 0.002). **G**,

957   Normalised cell counts in the fastigial nucleus (left) and dentate nucleus (right), according to

958   their rostro-caudal position relative to bregma. Open circles, contralateral count; filled

959   circles, ipsilateral count (n= 5 mice). **H-K**, Representative photomicrographs of the resultant

960   labeling in lobule VI, Crus I, paraflocculus and lobule II at 66 h post infection. **L**, Normalised

961   count of rabies labeled cells in anterior (black bar; lobule II to lobule IV/V); central (dark

962   grey bar; lobule VI to Crus II); posterior (clear bar; lobule VIII and lobule IX) and

963   flocculonodular (Floc. Nod., light grey bar; lobule X, flocculus and paraflocculus) cerebellum

964   66 h post infection (n= 5 mice; one-way ANOVA with FDR correction, $F_{(3, 16)} = 19.11$, p <

965   0.0001). **M**, Normalised cell count of rabies labelled cells in all assessed lobules 66 h post

966   infection. Colour coding of bars indicate assignment of lobules to either anterior, central,

967   posterior or vestibular cerebellum as indicated in **L**. Abbreviations: Dent., Dentate nucleus;

968   Fast., fastigial nucleus; Fast. DL, dorsolateral fastigial nucleus; Floc. Nod., flocculonodular

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969   lobe; Interp., nucleus interpositus; IntA, nucleus interpositus anterior; IntDL, dorsolateral

970   nucleus interpositus; IntP, nucleus interpositus posterior; i-Sim, ipsilateral simplex lobule; c-

971   Sim, contralateral simplex lobule; i-Crus I, ipsilateral Crus I; c-Crus I, contralateral Crus I; i-

972   Crus II, ipsilateral Crus II; c-Crus II, contralateral Crus II; i-Par, ipsilateral paramedian lobule;

973   c-Par, contralateral paramedian lobule; i-CP, ipsilateral copula; c-CP, contralateral copula; i-

974   Floc, ipsilateral flocculus; c-Floc, contralateral flocculus; i-PF, ipsilateral paraflocculus; c-PF,

975   contralateral paraflocculus; Vestib., vestibular nuclei.   ** $q < 0.01$, *** $q < 0.001$.

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978   **Figure 2. Different cerebellar modules project to the hippocampus.**

979   **A**, 3-D reconstruction showing the location of RABV+ Purkinje cells in the most labeled

980   cerebellar lobules at 66 h post-infection. Red, blue and green dots represent RABV+ Purkinje

981   cells in lobule VI, Crus I and paraflocculus, respectively. **B**, Photomicrographs from case S18

982   showing double staining against zebrin II (green, left column), RABV (red, central column)

983   and merge (right column) in lobule VI (i), Crus I (ii and iii) and paraflocculus (iv). RABV+

984   Purkinje cells were also zebrin positive and were organized in clusters of strongly labeled

985   RABV+ cells (filled arrow-heads) surrounded by weakly labeled RABV+ Purkinje cells (unfilled

986   arrow-heads). **C**, Assignment of the RABV+ clusters to specific cerebellar modules for case

987   S18 in the anterior (AZ; left), central (CZ; central column) and posterior (PZ; right column)

988   zones. First row shows stacked sections with zebrin positive Purkinje cells (white dots) and

989   RABV+ Purkinje cells, which were also zebrin positive (purple dots, strong and weakly

990   labeled cells included); central row shows reconstructed principal zebrin bands (delineated

991   by yellow dashed lines and named from 1+ to 7+; nomenclature from [42] and cerebellar

992   modules (capital letters; defined as in [42]); and bottom row shows the location of the

993   RABV+/zebrin Purkinje cells (purple dots) in relation to reconstructed zebrin bands and

994   modules. Abbreviations, I, lobule I; III, lobule III; IV/V, lobule IV/V; VIa and VI b-c, lobule VIa

995   and VI b-c, respectively; IX, lobule IX; X, lobule X; Sim, lobule simplex; Cr I, Crus I; Cr II, Crus

996   II; Par, paramedian lobule; CP, copula, PFL, paraflocculus, FL, flocculus.; dPFC and vPFC,

997   dorsal and ventral paraflocculus, respectively.

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Figure 3. Assessment of cerebello-hippocampal interactions during active movement in the home-cage.

A, Schematic representation of recording and stimulation electrode implant positions. **B**, Representative simultaneous LFP and EMG recording made during active movement in the homecage condition. Colored lines: raw LFP (filtered from 0.1 to 600 Hz). Overlaid grey lines: LFP filtered from 6-12 Hz. Note prominent 6-12 Hz oscillations in left hippocampal recording and deflections on neck EMG trace, reflecting active movement (EMG rectified and smoothed to 2.5 ms). **C**, Pooled z-score of the power spectra of hippocampal LFP recorded from the left (n = 16 mice) and right (n= 18 mice) hemispheres during homecage exploration and from cerebellar Crus I (n = 12), lobule II/III (n = 10) and lobule VI (n = 18). **D**, Pooled coherence between cerebellar cortical regions (colour coded) and hippocampus (left, Crus I, n = 11; lobule II/III, n = 8; lobule VI, n = 15; right, Crus I, n = 11; lobule II/III, n = 9; lobule VI, n = 16) during homecage exploration. **E**, Mean cerebello-hippocampal coherence in the 6-12 Hz range (Crus I, n = 21 values/12mice; lobule II/III, n = 17 values/10 mice; lobule VI, n = 31 values/18 mice). Lobule VI-hippocampus coherence level was significantly higher than that observed with lobule II/III (*, q < 0.05; Kruskall-Wallis with FDR correction). **F**, Mean 6-12 Hz coherence between lobule VI and hippocampus plotted against estimated medio-lateral recording electrode position in lobule VI (red dots; n = 16 mice, coherence with right and left hippocampus pooled; linear regression, R^2 = 0.348, p < 0.001). In grey, number of RABV+ cells counted across lobule VI, 66 h after injection in the left hippocampus as a function of medio-lateral position (0.2 mm bins; n = 5 mice). Shading indicates S.E.M. Abbreviations, LFP, local field potential; HPC, dorsal hippocampus; lob II/III, lobule II/III; lob VI, lobule VI; EMG, electromyogram.

Figure 4. Cerebello-hippocampal interactions during goal-directed behavior

A, Mice learned to traverse a 1 m linear track to receive a medial forebrain bundle stimulation upon reaching invisible goal zones (lightening symbols indicate MFB stimulation; n = 8 mice). Representative trajectories from early (trial 1) and late training (trial 20) show the transition from exploratory to goal-directed behavior. **B**, Mice improved their performance in the task across trials as shown by increases in the mean number of rewards obtained (purple line; plotted against left Y axis; one-way repeated measures ANOVA, time

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1032   effect p < 0.0001) and the mean speed (orange line; plotted against the right Y axis; one-way

1033   repeated measures ANOVA, time effect p = 0.0098).**C**, Mean z-score 6-12 Hz power of the

1034   recorded LFP signals (colour coded; Crus I, n = 5; lobule II/III, n = 6; lobule VI, n = 7; HPC left

1035   , n = 6; HPC right, n = 7; left and right HPC values are pooled as no difference was observed

1036   across hemispheres, hemisphere x trial two-way ANOVA with FDR correction, hemisphere

1037   effect *p* = 0.5974, interaction effect *p* = 0.3132, trial effect *p* < 0.0001) across trials. Solid

1038   blue line indicate trials where hippocampus values were significantly higher than those in

1039   trial 1 (q < 0.05).**D**, Mean coherence in the 6-12 Hz range between cerebellar regions (colour

1040   coded) and hippocampus (pooled bilaterally) during learning of the linear track task (Crus I,

1041   n = 8 values/5 mice; lobule II/III, n = 9 values/6 mice; lobule VI, n= 12 values/7 mice).

1042   Significant changes in coherence were observed over trials (combination x trial two-way

1043   ANOVA with FDR correction, trial effect p < 0.001, combination effect p = 0.0285,

1044   interaction effect p < 0.0001). Solid green rectangles indicates trials where Crus I-

1045   hippocampus coherence was higher than those in trial 1 (q < 0.05). This increase in Crus I-

1046   hippocampus coherence was also reflected in significant changes relative to other lobules:

1047   grey bordered rectangle corresponds to trials significantly higher than lobule II/III-

1048   hippocampus coherence (q < 0.05) while red bordered rectangles indicates those

1049   significantly higher than lobule VI-hippocampus coherence (q < 0.05). **E-F**, Pooled power

1050   spectra from hippocampal and cerebellar LFPs (z-score normalized, colour coded) from

1051   representative trials of early (**E**; trial 1) and late (**F**; trial 20) stages of training. **G-H**, Pooled

1052   coherence between cerebellar cortical regions and bilateral hippocampus from

1053   representative trials of early (**G**; trial 1) and late (**H**; trial 20) stages of training. Shading

1054   indicates S.E.M. Abbreviations, HPC, dorsal hippocampus; lob II/III, lobule II/III; lob VI, lobule

1055   VI.

1056

1057

1058   **Figure 5. Cerebello-hippocampal interaction dynamics**

1059   Mean 6-12 Hz coherence for each cerebello-hippocampal recording combination (Crus I, n =

1060   8 values/5 mice; lob II/III, n = 9 values/6 mice; lob VI, n = 12 values/7 mice) obtained during

1061   homecage recordings before training in the linear track (HC pre LT), during a representative

1062   trial of early training in the linear track (trial 1; early LT), during a representative trial of late

1063   training in the linear track (trial 20; late LT) and during homecage recording following

1064 completion of linear track training (HC post LT). A significant increase in Crus I–hippocampus

1065 coherence was observed during late LT compared to both HC pre LT and early LT, which

1066 then returned to pre-training levels during HC post LT (paired Friedman test with FDR

1067 correction, p = 0.0037; * q < 0.05, ** q < 0.01.). Lobule VI - hippocampus and lobule II/III -

1068 hippocampus coherence levels did not change significantly across conditions (paired

1069 Friedman test, FDR corrected p-value = 0.1718 and 0.5319, respectively). Abbreviations, lob

1070 II/III, lobule II/III; lob VI, lobule VI.

1071

1072 **Figure 6. Cerebello-hippocampal coherence patterns during goal-directed behavior in**

1073 **virtual reality.**

1074 **A**, Schematic of the virtual reality system and recording setup. Head-fixed mice were trained

1075 to move an air-cushioned Styrofoam ball in order to navigate through a virtual environment

1076 displayed on six TFT monitors surrounding the animal. **B**, Example recording of the virtual

1077 position as a mouse traversed a virtual linear track to receive rewards (MFB stimulation

1078 indicated by a lightning symbol, n = 7). **C**, Behavioural performance remained stable across

1079 trials as illustrated by the mean number of rewards (purple line; plotted against left Y axis;

1080 one-way repeated measures ANOVA, p = 0.4070) and the mean speed (orange line; plotted

1081 against the right Y axis; one-way repeated measures ANOVA, p = 0.4583). **D**, Pooled, z-

1082 scored normalized power spectra of hippocampal LFP recorded from the left (n = 16 mice)

1083 and right (n = 18 mice) hemispheres during homecage exploration and from cerebellar Crus I

1084 (n = 12), lobule II/III (n = 10) and lobule VI (n = 18). **E**, Averaged coherence between

1085 cerebellar cortical regions (colour coded) and bilateral hippocampus, pooled across all trials

1086 (Crus I-HPC, n = 5 values/3 mice; lobule II/III-HPC, n = 8 values/5 mice; lobule VI-HPC, n = 9

1087 values/5 mice). **F**, Mean cerebello-hippocampal coherence in the 6-12 Hz frequency range.

1088 Both lobule VI and Crus I showed significantly higher coherence with hippocampus than

1089 lobule II/III (Kruskal Wallace with FDR correction, p = 0.0137; ** q < 0.01). Shading indicates

1090 S.E.M.

1091

1092 **Figure 7. Comparison of cerebello-hippocampal interactions during epochs of similar**

1093 **behavioural performances in real-world and virtual-reality conditions.**

47

1094   **A**, For each mouse (n = 6) we selected one trial from the real world linear track (LT) and one

1095   from the virtual reality (VR) condition in which behavioural performance was similar, as

1096   defined by non-significant changes in the number of rewards obtained (purple circles;

1097   plotted against left Y axis; paired t test, p = 0.5279) or the mean speed (orange circles;

1098   plotted against right Y axis; paired t test, p = 0.6119). **B**, Overall coherence between

1099   cerebellar cortical regions (colour coded) and bilateral hippocampus (Crus I, n = 5 values/3

1100   mice; lobule II, n = 8 values/5 mice; lobule VI, n= 9 values/5 mice) in the selected trials from

1101   the linear track. **C**, Same as in **B** but for the selected trials from the virtual reality. **D**, For

1102   each recording combination, we compared 6-12Hz coherence values obtained from the

1103   selected linear track and virtual reality epochs. Crus I levels were significantly reduced in the

1104   virtual reality trials compared with the linear track (paired t test, *, p = 0.0480) while lobule

1105   VI or lobule II/III levels were similar in both conditions (Paired t tests, p = 0.1465 and 0.4165,

1106   respectively).

1107

1108   **Figure S1. Location of rabies virus injection for the 4 experimental groups.**

1109   RABV was co-injected with fluorescent CTB to visualize the injection spread. The location of

1110   the injection is indicated by the red area on a standard coronal outline of the left

1111   hippocampus adapted from [81]. Rostro-caudal position relative to bregma is indicated on

1112   the left (mm). Experimental ID for each case is shown above the sections. **A**, Injection sites

1113   of the 5 mice from the 30h survival time group **B**, Injection sites of the 3 mice from the 48h

1114   survival time group. **C**, Injection sites of the 5 mice from the 58h survival time group. **D**,

1115   Injection sites of the 5 mice from the 66h survival time group.

1116

1117   **Figure S2. Rabies virus main labelled structures 30h after hippocampal rabies injection.**

1118   **A**, Cumulative sum of labelled cells per structure after 30h post hippocampal rabies

1119   injection (colour coded for each case, n = 5 mice). **B-C,** Localisation and representative

1120   photomicrographs of RABV most labelled regions at 30h, lateral entorhinal cortex (**B**) and

1121   nucleus of the diagonal band (**C**). The position is indicated by a blue insert on a standard

1122   coronal brain section adapted from [81], and rostro-caudal position according to Bregma is

1123   indicated in the top-right corner**.** Abbreviations, ADN, antero-dorsal nucleus of the

1124   thalamus; LDN, latero-dorsal nucleus of the thalamus; Lat entorhinal cortex, lateral

1125   entorhinal cortex; NDB, nucleus of the diagonal band; TRN, thalamic reticular nucleus.

1126

1127 **Figure S3. The topographical distribution of DCN labelling at 66h.**

1128 **A-D**, representative photomicrographs showing labeling in the ipsilateral cerebellar and

1129 vestibular nuclei 66h after hippocampal rabies injection. Left panels show low magnification

1130 view, right panels show high magnification view of area indicated by dashed box. **E**, Pooled,

1131 normalized counts of rabies labeled cells in the ipsi- and contralateral cerebellar and

1132 vestibular nuclei (n = 5 mice). No significant differences were found between ipsi- and

1133 contralateral nuclei (nuclei x hemisphere two-way ANOVA, hemisphere effect $F_{(1, 4)}$ = 1.14, p

1134 = 0.35, interaction effect $F_{(3, 12)}$ = 0.21, p = 0.89, nuclei effect $F_{(3, 12)}$ = 7.88, p = 0.004). **F**,

1135 Normalized cell counts in the fastigial nucleus (left) and dentate nucleus (right) according to

1136 their rostro-caudal position relative to Bregma. Open circles, contralateral count; filled

1137 circles, ipsilateral count (n = 5 mice). Abbreviations: Dent., Dentate nucleus; Fast., fastigial

1138 nucleus; Fast. DL, dorsolateral fastigial nucleus; Interp., nucleus interpositus; IntA, nucleus

1139 interpositus anterior; IntDL, dorsolateral nucleus interpositus; IntP, nucleus interpositus

1140 posterior; Vestib., vestibular nuclei.

1141

1142 **Figure S4. Reconstructed location of the implanted electrodes.**

1143 The position of the implanted electrodes are represented by black dots on a standard

1144 sagittal outline of the cerebellum (**A**) or coronal outline of the hippocampus (**B**) [adapted

1145 from 81]. The medio-lateral (in **A**) or rostro-caudal (in **B**) positions relative to midline or

1146 bregma, respectively, are indicated next to the outlines. Abbreviations, II, lobule II; III, lobule

1147 III; IV/V, lobule IV/V; VI, lobule VI; VII, lobule VII; VIII, lobule VIII; IX, lobule IX; X, lobule X;

1148 Sim, lobule simplex; PM, paramedian lobule.

1149

1150 **Figure S5. Cerebello-hippocampal coherence patterns are similar across hemispheres**

1151 **during active movement in homecage.**

1152 **A**, Schematic indicating approximate distances between recording sites in the cerebellum

1153 and left/right dorsal hippocampus (HPC). **B**, Overall power spectra from right (n = 18) and

1154 left (n = 16) HPC and cerebellar recordings made from Crus I (n = 12), lobule II/III (n = 10)

1155 and lobule VI (n = 18) during active movement in the homecage environment. **C**, Overall

1156 coherence between cerebellar cortical regions (Crus I, n = 11; lobule II, n = 8; lobule VI, n =

1157 15; colour coded) and left HPC during active homecage movement. **D**, Overall coherence

49

1158   between cerebellar cortical regions (Crus I, n = 10; lobule II, n = 9; lobule VI, n = 16; colour

1159   coded) and right HPC. **E**, Mean 6-12 Hz coherence between cerebellar recordings and left or

1160   right HPC. No differences were observed between hemispheres (hemisphere x combination

1161   one-way ANOVA with FDR correction, hemisphere effect p = 0.6355). **F**, The number of

1162   12min analysis blocks was not correlated to the mean 6-12 Hz level of coherence obtained.

1163   Coherence values shown for all cerebello-hippocampal recording combinations (n= 57

1164   values/23 mice; solid line indicates linear regression; dashed lines indicate 95% confidence

1165   intervals).   Shading   indicates   S.E.M.

1166

1167   **Figure S6. Transient 6-12 Hz oscillations are present in the cerebellar cortex.**

1168   **A**, Upper trace, 200s epoch of LFP (0.1 to 600Hz) recorded from lobule VI during active

1169   movement in the homecage environment. Lower panel, spectrogram of the LFP. Periods of

1170   intense spectral power in the 6-12Hz band demarked by red boxes. **B**, 20s period of intense

1171   oscillation (from area marked by dashed white lines in **A**) is shown on a larger timescale. In

1172   red, same epoch filtered in the 6-12 Hz frequency band. **C**, Power spectra calculated from

1173   the whole period (black line), from epochs of high intensity in the 6-12Hz frequency band

1174   (epochs demarked by red boxes in A; red line) and from epochs without high intensity in the

1175   6-12Hz frequency band (blue line). Arrow indicates peak in spectra within the 6-12Hz range,

1176   apparent in the selected high intensity periods.

1177

1178   **Figure S7. Cerebello-hippocampal coherence patterns are conserved across hemispheres**

1179   **during running on the linear track. A**, Mean z-score 6-12 Hz power of the recorded LFPs

1180   from left (n = 6) and right (n = 5) hippocampus (HPC) and from the cerebellum (colour

1181   coded; Crus I, n = 5; lobule II/III, n = 6; lobule VI, n = 7) across trials. No laterality effect was

1182   found in the HPC (hemisphere x trial two-way ANOVA with FDR correction, hemisphere

1183   effect *p* = 0.5974, interaction effect *p* = 0.3132, trial effect *p* < 0.0001). Solid blue line

1184   indicate trials where HPC values were significantly higher than those in trial 1 (q < 0.05). **B**,

1185   Over trials, there was a significant increase in the peak frequency of the power spectra in

1186   both left and right HPC recordings; however, no differences were found between

1187   hemispheres (hemisphere x trial two-way ANOVA with FDR correction, trial effect p <

1188   0.0001, hemisphere effect p = 0.4124, interaction effect p > 0.9999; solid blue line shows

1189   trials significantly different from trial 1, q < 0.05). **C**, No differences were observed in the

50

1190   mean 6-12 Hz coherence between left or right HPC and Crus I at early (trial 1) or late (trial

1191   20) stages of training (Crus I-HPC left, n = 4; Crus I-HPC right, n = 4; hemisphere x trial two-

1192   way ANOVA, hemisphere effect p = 0.9026, interaction effect p = 0.7272, trial effect p =

1193   0.0183). **D**, Peak frequency of cerebello-hippocampal coherence was not affected by the

1194   hippocampal hemisphere and did not change across trials for any combination (colour

1195   coded; Crus I-HPC left, n = 4, Crus I-HPC right, n = 4, hemisphere x trial two-way ANOVA,

1196   hemisphere effect p = 0.3601, interaction effect p = 0.9652, trial effect p = 0.2954; lobule

1197   II/III-HPC left, n = 4, lobule II/III-HPC right, n = 5, hemisphere x trial two-way ANOVA,

1198   hemisphere effect p = 0.2485, interaction effect p = 0.5048, trial effect p = 0.1767; lobule VI-

1199   HPC left, n = 6, lobule VI-HPC right, n = 6, hemisphere x trial two-way ANOVA, hemisphere

1200   effect p = 0.7025, interaction effect p = 0.9446, trial effect p = 0.2543). Shading indicates

1201   S.E.M.

1202

1203

1204   **Figure S8. Speed and power spectrum dynamics across homecage and real world linear**

1205   **track conditions.**

1206   **A,** Significant changes in speed were found across all conditions (HC pre LT, early and late LT

1207   and HC post LT; one-way repeated measures ANOVA with FDR correction, n = 8 mice). **B**, No

1208   significant differences were observed in hippocampal 6-12Hz z-score power (pooled values

1209   from both hemispheres) across conditions (repeated measures Friedman test, p = 0.1764, n

1210   = 13). **C**, No significant differences were observed across conditions in 6-12 Hz z-score

1211   power of Crus I (repeated measures Friedman test, p = 0.0755, n = 5), lobule VI (repeated

1212   measures Friedman test, p = 0.4188, n = 7) or lobule II/III (repeated measures Friedman test,

1213   p = 0.3751, n = 6). * q < 0.05, ** q < 0.01, *** q < 0.001. Abbreviations as for Fig. 5.

1214

1215

1216   **Figure S9. Cerebello-hippocampal coherence patterns are similar across hemispheres**

1217   **during goal-directed behavior in virtual reality.**

1218   **A**, Peak frequency of the power spectra from left (n = 4) and right (n = 6) hippocampus

1219   (HPC) across trials. There was no difference between hemispheres but there was a

1220   significant effect of the trial (hemisphere x trial two-way ANOVA with FDR correction, trial

1221   effect p = 0.004, hemisphere effect p = 0.8795, interaction effect p = 0.9998). **B**, Mean z-

1222   score 6-12 Hz LFP power (colour coded; Crus I, n = 3; lobule II/III, n = 5; lobule VI, n = 5)

1223   across trials. No laterality effect or trial effect was found in the HPC (hemisphere x trial two-

1224   way ANOVA with FDR correction, hemisphere effect *p* = 0.7357, interaction effect *p* =

1225   0.9865, trial effect *p* = 0.4180). Similarly, no differences were observed between cerebellar

1226   regions and no changes across trials (cerebellar region x trial two-way ANOVA with FDR

1227   correction, cerebellar region effect p = 0.8290, interaction effect p = 0.9993, trial effect p =

1228   0.1781). **C**, Pooled, mean 6-12 Hz cerebello-hippocampal coherence (colour coded; Crus I-

1229   HPC = 5 values/3mice, lobule II/III = 8 values/5 mice, lobule VI-HPC = 9 values/5mice) across

1230   trials in the virtual reality condition. No differences across trials were observed at any

1231   cerebello-hippocampal combination (cerebellar region x trial two-way ANOVA with FDR

1232   correction, trial effect p = 0.0724, interaction effect p = 0.1265). **D**, Overall z-score power

1233   spectrum from cerebellar cortical regions (colour coded) and bilateral HPC averaged across

1234   all trials. **E**, Overall coherence between cerebellar cortical regions (Crus I, n = 2; lobule II/III,

1235   n = 3; lobule VI, n = 4; colour coded) and left HPC averaged across all VR trials. **F**, Overall

1236   coherence between cerebellar cortical regions (Crus I, n = 3; Lobule II/III, n = 5; Lobule VI, n

1237   = 5; colour coded) and right HPC. **G**, Mean 6-12 Hz coherence between cerebellar recordings

1238   and left or right HPC. No differences were observed between hemispheres (hemisphere x

1239   combination one-way ANOVA with FDR correction, hemisphere effect p = 0.6355). Shading

1240   indicates S.E.M.

1241

1242   **Table 1**

1243   Overview of RABV labeling intensity in different brain regions for all animals in the four

1244   experimental groups (30h, 48h, 58h and 66h post RABV injection). (-) denotes no labeling,

1245   (+/-) few cells, (+) minor labeling, (++) fair labeling, (+++) strong labelling. Question mark

1246   indicates that the area was not available for analysis. When the labeling was different

1247   between ipsilateral (i.) and contralateral (c.) hemispheres, the regions are split in two

1248   columns.

1249

A — DCN/VN, Cerebellar Ctx. Number of labeled cells vs Survival time (h): 30, 48, 58, 66. Inset shows 48 and 58.

66 H, 58 H. Lob VI, Crus I, PF.

Caudal Medial + Lateral Rostral

58h:DCN/VN

B — Fast.DL, Fast., IntP, Fast.

C — Fast., IntP, IntDL, IntP, IntDL

D — IntA, Fast., Dent., Dent.

E — IntA, Dent., Vestib. 500 μm, Vestib. 100 μm

66h:Cerebellar Cortex

H — Lob VI

I — Crus I

J — PF

K — Lob II, 100 μm

F — Normalised cell count (% total labeled cells per animal). Ipsi., Contra. Dent., Interp., Fast., Vestib.

L — Normalised cell count (% total labeled cells per animal). Anterior, Central, Posterior, Floc. nod. ***, **

G — Normalised cell count (% total labeled cells per animal). Fastigial, Dentate. Ipsi., Contra. Distance from bregma (mm): −5.55, −5.71, −5.87, −6.03, −6.19, −6.35, −6.51, −6.67, −6.83

M — Normalised cell count (% total labeled cells per animal). Lob II, Lob III, Lob IV/V, i-Sim, c-Sim, Lob VI, Lob VII, i-Crus I, c-Crus I, i-Crus II, c-Crus II, Lob VIII, Lob IX, Lob X, i-Par, c-Par, i-CP, c-CP, i-Floc, c-Floc, i-PF, c-PF. Anterior, Central, Posterior, Floc. Nod.

A

S14
S15
S16
S17
S18

Dorsal
Lateral
Rostral

B

i VIb-c — zebrin — RV — merge

ii Crus I (rostral)

iii Crus I (caudal)

iv dPFL — vPFL — 200 µm

C

Sections
Zebrin
RV/Zebrin

AZ

VIa Sim
Cr I
I III
IV-V
FL
PFL

Zebrin

A AX X/B C1
C2
C3
D1 D0
a+ a+
4+
3+
5+ 3+ 2+ 2+ D2
6+ 1+
5+
6+

RV/Zebrin

A AX X/B C1
C2
C3
D1 D0
D2

CZ

VIa
Cr I
Cr II
X
Par
PFL

Zebrin

I A AX
2+ a+ 1+ a+ C2
6+ 5+ 2+ 5+ D1
7+ D2
4+
7+

RV/Zebrin

I A AX
C2
D1
D2

PZ

VIb-c
Cr I
Cr II
IX CP Par

Zebrin

A A2/C1
4+ 3+ 2+ a+ 2+ 3+ 4+ C2
C3
6+ 5+ 1+ 5+ D1
7+ 6+ D0
D2
7+

RV/Zebrin

I A A2/C1
C2
C3
D1
D0
D2

A MFB stim, HPC LFP, lob II/III LFP, Crus I LFP, lob VI LFP

B HPC left, Crus I, lob II/III, lob VI, EMG, 5 mV, 250 msec

C z-score power vs Frequency (Hz); HPC, Crus I, lob II/III, lob VI

D Coherence vs Frequency (Hz); Crus I, lob II/III, lob VI

E Mean Coherence (6-12 Hz); Crus I, Lob II/III, Lob VI; *

F Mean Coherence (6-12 Hz) / Number of labeled cells vs Estimated Distance from Midline (mm); R² = 0.348, *p* < 0.001

A

B **LT**

C **VR**

D

Crus I   Iob VI   Iob II/III

A   **30 h**

S1   S2   S3   S4   S5

-1.46
-1.70
-1.94
-2.18
-2.46
-2.70
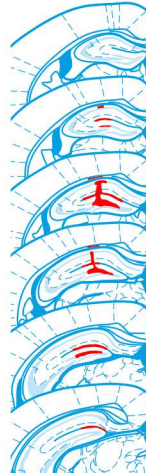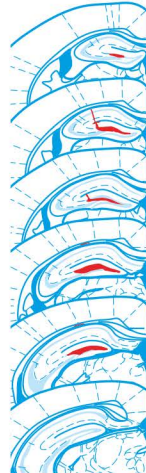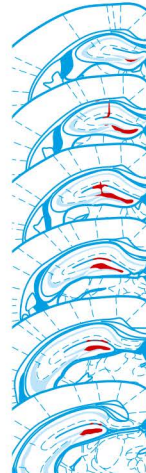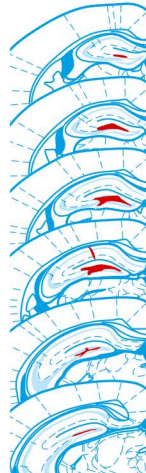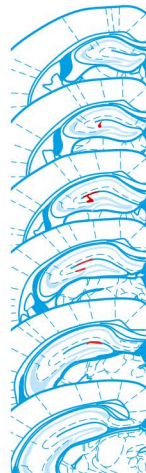
B   **48 h**

S6   S7   S8
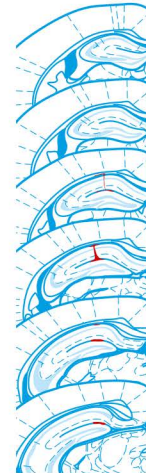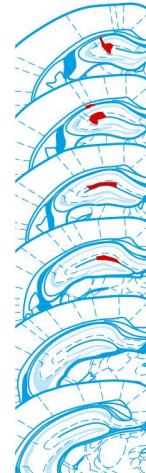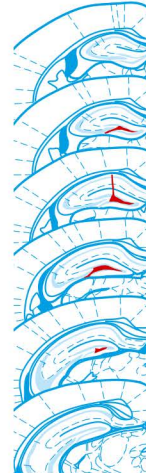
C   **58 h**

S9   S10   S11   S12   S13

-1.46
-1.70
-1.94
-2.18
-2.46
-2.70
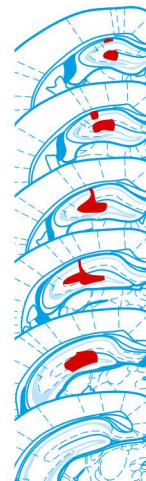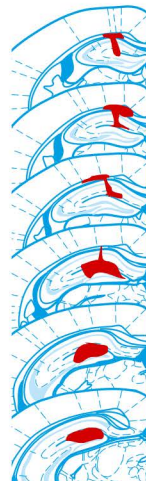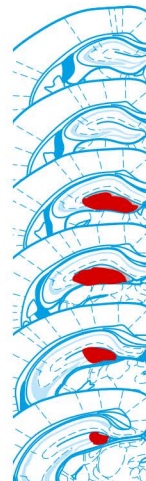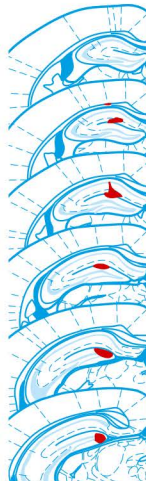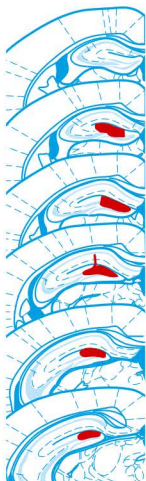
D   **66 h**

S14   S15   S16   S17   S18
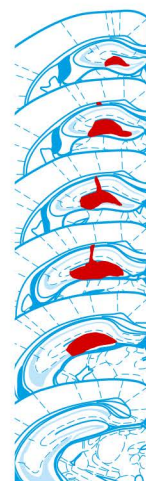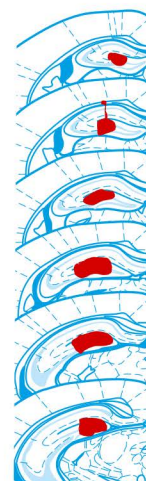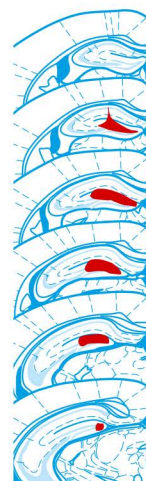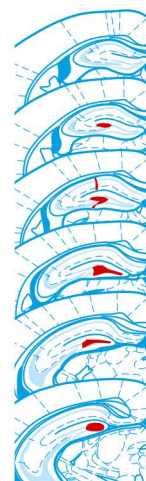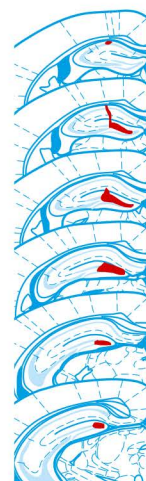
A Sum of labeled cells per structure at 30h post rabies virus injection (n=5)

Legend: S1, S2, S3, S4, S5

Structures: NDB, Medial septum, Lat entorhinal cortex, Perirhinal cortex, Supramammillary bodies, Reuniens, Antherodors thal nu, Laterodors thal nu, Reticular nu of the thal, thalamus, Retrosplenial cortex, Somatosensory cortex, Auditory cortex, Visual cortex, Ectorhinal cortex, Piriform cortex, Cingulate gyrus anterior, Dorsal subiculum, Central nucleus raphe, Hypothalamus, Globus pallidus, Subiculum transition area, Med Amygd nu

Groupings: Thalamus, Cortex, Others

B -3.64 mm
100 µm

C 0.86 mm
100 µm

A

Sim
Crus I
Crus II
PM

2.76 mm   2.64 mm   2.52 mm   2.4 mm

0.84 mm   0.72 mm   0.60 mm   0.48 mm   0.36 mm   0.24 mm

IV/V
VI
III
VII
II
VIII
IX
X
0.12 mm

1 mm

B

-1.96
-2.70

-2.18
-2.80

-2.30
-2.92

-2.46

1mm

A

LFP

strong osc.

Frequency (Hz)

Time (seconds)

B

LFP

LFP
(6-12Hz)

5s

C

LFP power

Frequency (Hz)

		30h p.i.					48h p.i.			58h p.i.					66h p.i.				
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
LEC	i.	++	+	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++-	+++	+++
	c.	+	-	-	+	++	-	++	++	++	++	++	++	++	++	++	++	++	++
NDB/MS	i.	+++	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++
	c.	++	++	++	++	+	+++	+++	?	++	+++	+++	+++	+++	+++	++	+++	+++	+++
SUM	i.	-	+++	++	++	-	+++	+++	+++	++	+++	+++	+++	+++	++	+++	++	+++	+++
	c.	-	++	++	++	-	+++	+++	+++	++	+++	+++	+++	+++	++	+++	++	+++	+++
Thalamus		+/-	+	+	-	-	+	++	+	+	+++	++	+++	+++	+++	+++	+-	++	+++
Hypothalamus		-	+	-	-	+	++	++	++	++	++	+++	+++	+++	+++	+++	++	+++	+++
Raphe nu		-	+	-	+	-	++	++	++	++	++	++	++	++	+++	+++	++	+++	+++
Cortex		-	-	+	-	+	++	++	++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
MEC	i.	-	-	-	-	-	-	+	++	+	-	++	++	++	?	+	++	++	++
	c.	-	-	-	-	-	-	+/-	+	+/-	-	+	+	+	?	+/-	+	+	+
Perirhinal ctx.	i.	-	-	-	-	-	-	+	+	+	+	+	+	+	++	++	+	++	++
	c.	-	-	-	-	-	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MM		-	-	-	-	-	++	++	++	++	++	++	+/-	+	++	++	+	++	++
PAG		-	-	-	-	-	++	++	++	++	++	+++	++	+++	+++	+++	++	+++	+++
Pont. nu.	i.	-	-	-	-	-	+++	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
	c.	-	-	-	-	-	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Amygdala	i.	-	-	-	-	-	+	+	+	+	++	++	+++	+++	++	+++	++	+++	+++
	c.	-	-	-	-	-	+	+	+	+	+++	+++	++	++	+++	+++	++	+++	+++
Vestibular nu.	i.	-	-	-	-	-	-	-	-	+	+	+	+	+	++	++	++	++	++ (+)
	c.	-	-	-	-	-	-	-	+/-	+	+	++	++	+	++	++	++	++	++ (+)
DCN	i.	-	-	-	-	-	-	-	-	+	+	+	++	++	+++	+++	+++	+++	+++
	c.	-	-	-	-	-	+/-	+/-	+/-	+	+	++	++	++	+++	+++	+++	+++	+++
Cerebellar ctx.	i.	-	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	++	++	++	++	++
	c.	-	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	++	++	++	++	++