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メタデータ	言語: English 出版者: 公開日: 2020-03-17 キーワード (Ja): キーワード (En): 作成者: Norimoto, Hiroaki, Fenk, Lorenz A., Li, Hsing-Hsi, Tosches, Maria Antonietta, Gallego-Flores, Tatiana, Hain, David, Reiter, Sam, Kobayashi, Riho, Macias, Angeles, Arends, Anja, Klinkmann, Michaela, Laurent, Gilles メールアドレス: 所属:
URL	https://oist.repo.nii.ac.jp/records/1297

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A claustrum in reptiles and its role in slow-wave sleep

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The mammalian claustrum, owing to its widespread connectivity with other forebrain structures, has been hypothesized to mediate functions ranging from decision making to consciousness¹. We report here that a homolog of the claustrum, identified by single-cell transcriptomics and viral tracing of connectivity, exists also in a reptile, the Australian dragon *Pogona vitticeps*. In *Pogona*, the claustrum underlies the generation of sharp-waves during slow-wave sleep. The sharp-waves, together with superimposed high-frequency ripples², propagate to the entire neighboring pallial dorsal ventricular ridge (DVR). Uni- or bilateral lesions of the claustrum suppress sharp-wave ripple production during slow-wave sleep uni- or bilaterally, respectively, but do not affect the regular and rapidly alternating sleep rhythm characteristic of *Pogona* sleep³. The claustrum is thus not involved in sleep-rhythm generation itself. Tract-tracing revealed that the claustrum projects widely to a variety of forebrain areas, including the cortex, and that it receives converging input, among others, from mid- and hind-brain areas known to be involved in wake/sleep control in mammals⁴⁻⁶. An imposed periodic modulation of serotonin concentration in claustrum, for example, caused a matching modulation of sharp-wave production there and in neighboring DVR. Using transcriptomic approaches, a claustrum was identified also in turtles, a distant reptilian relative of lizards. The claustrum is therefore an ancient structure, likely present already in the brain of the common vertebrate ancestor of reptiles and mammals. It may play an important role in the control of brain states due to ascending input from the mid- and hindbrain, to its widespread projections to the forebrain and to its role in sharp-wave generation during slow-wave sleep.

65 Slow-wave sleep (SWS) and rapid-eye-movement sleep (REMS) are the two main macroscopic
 66 components of electrophysiological sleep in mammals and birds⁴⁻⁶, though some mammals
 67 may lack REM⁷. The recent finding of alternating SWS and REMS in a reptile, the Australian
 68 dragon *Pogona vitticeps*³, suggests that these two sleep modes may predate the diversification
 69 of amniotes 320M years ago. Sleep in *Pogona* is particularly interesting because its cycle is
 70 very short (≤ 3 minutes at room temperature) and divided equally into SWS and REMS³.

71

72 The dominant electrophysiological feature of *Pogona* SWS is energy in the δ band (~0-4 Hz)
 73 caused by the reliable occurrence of sharp-waves. Sharp-waves typically contain a high-
 74 frequency ripple, forming a sharp-wave ripple complex (SWR²). SWRs were recorded from
 75 the dorsal ventricular ridge (DVR)⁸, the dominant non-cortical pallial domain of sauropsid
 76 brains⁸⁻¹⁰. REMS, by contrast, is characterized by broad-band energy, measured in the β band
 77 (10-40 Hz) in cortex and DVR³.

78

79 ***Origin of sharp-waves during SWS***

80 SWRs occur reliably in DVR during SWS and SWS alternates regularly with REMS (Fig. 1a-
 81 c; Ext. Data Fig. 1), as reported³. High-frequency ripples (~70-150 Hz) rode on each sharp-
 82 wave and contained action potentials. Local field potentials (LFPs) were highly correlated
 83 across DVR recording sites (peak corr.=.74 over 18h of SWS, mean over 2 animals), but sharp-
 84 waves recorded in the anterior medial pole of DVR (amDVR) preceded their counterparts in
 85 more posterior or lateral regions by up to 200ms, depending on recording-sites spacing (Fig.
 86 1d-e and Ext. Data Fig. 1g-h), suggesting SWR propagation.

87

88 We next recorded from thick anterior transverse, horizontal and para-sagittal slices of DVR in
 89 ACSF (Methods, Ext. Data Fig. 2a-f). All configurations produced spontaneous SWRs,
 90 matching those produced in sleep: biphasic waveform (119 \pm 40 ms) with ripple (~70-150 Hz)
 91 on the trough. SWRs in slices were less frequent than during SWS, (12.4 \pm 1.8min⁻¹, 12 slices,
 92 10 animals; vs. 16.45 \pm 0.98min⁻¹ during SWS; 5 SWS epochs from 2 sleeping animals) though
 93 not significantly so ($p=0.18$, Student's t -test). SWR production in slices was not rhythmically
 94 interrupted by REMS-like activity, as it is during sleep. We patched 12 DVR neurons (Ext.
 95 Data Fig. 2g-j); consistent with sleep data, they typically fired 0-3 action potentials during
 96 SWRs and were silent in between. Under voltage-clamp ($n=2$), neurons displayed coincident
 97 excitatory and inhibitory input during sharp-waves (excitation dominating in current-clamp).

98

99 We also used multielectrode arrays on DVR slices ($n=3$ brains; Methods). As observed *in vivo*,
100 SWRs propagated from anteromedial to lateroposterior poles (Fig. 2a-c). The apparent linear
101 velocity of the wave in the slice plane was $39\text{mm}\cdot\text{s}^{-1}$, although propagation contained local
102 angular components. We further divided DVR slices into “mini-slices” ($n=13$, Fig. 2d). Only
103 those from the anteromedial pole produced SWRs, at rates of $11.9\pm 1.7\text{min}^{-1}$ (Fig. 2e-f), not
104 different from control.

105

106 *scRNA-seq indicates a claustrum homolog*

107 Using a single-cell transcriptomic strategy, we recently mapped the main neuron types of the
108 reptilian pallium¹¹ and described heterogeneity among glutamatergic cell types in the *Pogona*
109 DVR. To characterize amDVR, we generated a deeper and more extensive sampling of *Pogona*
110 single cells (Methods). Using unsupervised graph-based Louvain clustering on transcripts from
111 20,257 cells, we identified 4,054 pallial glutamatergic neurons forming 29 glutamatergic
112 clusters (Fig. 3a and Ext. Data Fig. 3).

113

114 We located these clusters in the *Pogona* telencephalon using the expression of cluster-specific
115 markers detected by *in situ* hybridization (ISH) and/or immunohistochemistry (IHC)¹¹. Two
116 clusters (19 and 20, Fig. 3a) mapped to amDVR, as shown by the expression of the calcium-
117 binding protein *hippocalcin* (*HPCA*) and the RNA-editing enzyme *ADARB2* among others
118 (Fig. 3b-d). Clusters 19 and 20 corresponded to lateral and medial amDVR subdivisions,
119 respectively labeled by expression of the copine 4 (*CPNE4*) and nuclear hormone receptor
120 *RORB* genes (Fig. 3e-f). We repeated mini-slice SWR recordings and labeled those slices *post*
121 *hoc* with a hippocalcin antibody: only hippocalcin-positive mini-slices from the amDVR pole
122 generated SWRs (Ext. Data Fig. 4).

123

124 Some amDVR markers (e.g., *GNG2*, *SYNPR* and *RGS12*, Fig. 3b) are known markers of the
125 mammalian claustrum¹². To explore these molecular similarities further, we used Seurat v3 to
126 project *Pogona* single-cell transcriptomes on mouse cell-type transcriptomes¹³ on the basis of
127 a joint dimensionality reduction analysis (14, Methods). About 63 and 75 percent of amDVR
128 cells (clusters 19 and 20, respectively) projected onto the mouse claustrum transcriptomic
129 cluster (Fig. 3g), suggesting that *Pogona* amDVR and mammalian claustrum are homologous,
130 consistent also with developmental observations^{10,15}.

131

132 To link our transcriptomic and physiological observations, we analyzed ion-channel and
 133 neurotransmitter-receptor gene expression in pallial glutamatergic clusters (143 genes detected
 134 in $\geq 20\%$ of cells of at least one cluster, Methods). These genes were sufficient to distinguish
 135 amDVR from other glutamatergic clusters (Ext. Data Figs. 3,5) and contained clusters of
 136 correlated genes (modules). One module with enriched expression in amDVR (Fig. 3h)
 137 included receptors for noradrenaline (NA), acetylcholine (ACh), dopamine (DA) and serotonin
 138 (5HT). In mammals, these neuromodulators influence sleep rhythms and are released by brain
 139 nuclei from the hypothalamus to the medulla^{4,5,16-18}. Glutamatergic neurons in amDVR were
 140 among the few co-expressing receptors for all four modulators (Ext. Data Fig. 5). Hence,
 141 amDVR expresses receptor types consistent with a sensitivity to input from brain-state
 142 controlling circuits.

143

144 *amDVR is extensively connected*

145 We next mapped the connectivity of amDVR with putative wake/sleep-control areas—as
 146 suggested by the above data—and asked whether amDVR connects widely with the rest of the
 147 pallium, as claustrum does in mammals^{1,12,19-21}. We identified, where possible, the *Pogona*
 148 homologs of mammalian nuclei implicated in sleep⁴⁻⁶. Relying on anatomical studies in related
 149 species (Methods) we used IHC and fluorescent ISH (FISH) to identify and map these nuclei
 150 in the *Pogona* diencephalon, midbrain and brainstem (Fig. 3i, Ext. Data Fig. 6), together with
 151 telencephalic areas mapped by scRNA-seq (blue)¹¹.

152

153 We mapped amDVR connectivity by local tracer injections²² using rAAV2-retro²³ carrying a
 154 fluorescent protein under the CAG or hSyn promoter for (mostly) retrograde labeling
 155 (Methods). rAAV2-retro was sometimes co-injected with (mostly) anterograde AAV2/9-CB7-
 156 mcherry-WPRE for injection-site identification. Because they do not cross synapses²³⁻²⁵ these
 157 tracers revealed direct targets (AAV2/9-CB7) and sources (rAAV2-retro) of the injection site.
 158 The results are summarized in Fig. 3j. On the left are all telencephalic structures whose input
 159 and output connectivity with amDVR (“claustrum”) could be tested. On the right are deeper
 160 structures in which local injection could not be done, for anatomical reasons. For these
 161 structures, connectivity to claustrum was established only by retrograde labeling from amDVR.
 162 Whether claustrum projects to those areas awaits direct demonstration.

163

164 The cortical sources of input to amDVR were anterior and posterior dorsal cortices (Fig. 3j,
 165 Ext. Data Fig 6c). Retrograde and anterograde tracers revealed no direct projections from
 166 hippocampus (x, Fig. 3j; DMC = CA fields; MC = Dentate Gyrus) to amDVR, even though
 167 amDVR projects to both. In subcortical pallium, aDVR and pDVR showed strong projections
 168 to amDVR. amDVR also received input from dorsal thalamic nuclei (DMT, DLT, DLPT), from
 169 prethalamus, hypothalamus, VTA, substantia nigra and the periaqueductal grey in the
 170 midbrain, and from locus coeruleus, subcoeruleus and the raphe nucleus in the brainstem (Ext.
 171 Data Fig. 6).

172
 173 amDVR projected to hippocampus (MC and DMC), posterior DC (potential subiculum
 174 homolog) and to aDC, the neocortex homolog¹¹. In subcortical pallium, projections to anterior
 175 DVR (aDVR) were dense and extensive, consistent with sharp-wave propagation (Figs 1,2).
 176 Projections between amDVR and some of its targets appeared ordered: the more lateral
 177 amDVR projected to rostral aDVR; central amDVR projected to caudal aDVR. Conversely,
 178 input to amDVR from cortex (aDC and pDC) was strongest laterally, weakest medially (absent
 179 from DMC and MC—hippocampus).

180
 181 Hence, amDVR is connected with the pallial forebrain and receives input from areas implicated
 182 in wake/sleep control, consistent with the widespread expression of many receptor genes
 183 specific to these areas. Based on these transcriptomic and anatomical data, we conclude that
 184 amDVR is the reptilian homolog of the mammalian claustrum.

185

186 ***The claustrum homolog in turtles***

187 Having applied similar transcriptomic approaches in *Pogona* and in the turtle *Trachemys*
 188 *scripta*¹¹, two species on distinct branches of the reptilian tree, we looked for a claustrum in
 189 *Trachemys*. Comparison of transcriptomic data (Methods) yielded four potential turtle clusters
 190 (Ext. Data Fig. 7). Cells in these clusters lay in a region known as the pallial thickening
 191 (PT)^{11,26,27}. Turtle PT and lizard amDVR are both in the anterior pallium, consistent with their
 192 similar developmental origin in antero-lateral pallium¹⁰; but turtle PT is lateral to aDVR and
 193 close to olfactory cortex, rather than fused to the rest of DVR, as is claustrum in *Pogona*.
 194 Architectonics also differed: *Pogona* claustrum is nuclear and composed of isotropically
 195 distributed multipolar neurons; turtle PT forms a curved sheet extending anterior-dorsal cortex,
 196 traversed from below by LGN axons en route to visual cortex²⁷. Indeed, principal neurons in

197 turtle PT (rAAV2-retro injection in DMC) are pyramid-like, with apical and basal dendrites
198 (Ext. Data Fig. 7d). Despite these differences, slices of turtle PT produced SWRs, leading those
199 in DVR, like in *Pogona*. Thus, PT appears to be the turtle claustrum and a claustrum homolog
200 likely existed already in the common ancestor of amniotes.

201

202 ***Manipulating claustrum activity***

203 We developed a reduced *ex vivo* *Pogona* forebrain preparation, enabling direct access to the
204 non-cortical pallium after cortex removal (Methods). This preparation generated spontaneous
205 SWRs in claustrum and DVR, similar to those recorded *in vivo* during sleep and in
206 DVR/claustrum slices (Ext. Data Fig. 8). SWRs occurred continuously but more frequently
207 ($21.6 \pm 5.4 \text{ min}^{-1}$, 4 brains) than in slices ($12.4 \pm 1.8 \text{ min}^{-1}$, $n=13$). Claustrum led DVR (Ext. Data
208 Fig. 8f) with delays similar to those observed during sleep or in slices of claustrum+DVR (11-
209 141ms, peak mean corr.=.57; 4 brains). To test the causal role of claustrum in SWR generation,
210 we injected TTX selectively in claustrum *ex vivo* ($n=4$, 3 animals), causing a prolonged
211 silencing of claustrum and the concomitant cessation of SWRs in ipsilateral DVR (Ext. Data
212 Fig. 8b-d).

213

214 We next lesioned one or both claustra *in vivo* using ibotenic acid (Methods; 3 animals).
215 Bilateral recordings from DVR in sleeping lesioned animals revealed that the rhythmic
216 modulation of β activity (REM) was unaffected, but that SWRs, characteristic of slow-wave
217 sleep, were eliminated on the claustrum-lesioned side(s) (Fig 4a-d, Ext. Data Fig. 9). Hence
218 the claustrum is required for DVR SWR production during slow-wave sleep; its action is
219 unilateral; and it is not involved in the alternating SW/REM sleep rhythm.

220

221 Because claustrum receives direct input from areas implicated in sleep-wake production in
222 mammals and expresses receptors for their transmitters (Fig. 3), we tested the sensitivity of
223 SWR production to those transmitters^{4-6,16}. DA significantly increased SWR production rate;
224 ACh and 5HT decreased it (Fig 4e). We selected 5HT for further experiments. Consistent with
225 tracing data indicating serotonergic input from the Raphe, claustrum contained 5HT-positive
226 fibers (Ext. Data Fig. 10a). 5HT at concentrations $\geq 1\mu\text{M}$ suppressed SWRs ($n=9$
227 claustrum+DVR slices, 9 animals, Ext. Data Fig. 10b). This effect was best mimicked by the
228 HTR1D agonist L703,664 (Fig. 4f), consistent with scRNA-seq results (Ext. Data Fig. 5). We

229 then superfused slices with caged-5HT (Methods): SWRs were suppressed within seconds of
230 illumination onset and resumed when illumination ceased (Fig. 4g,h).

231

232 The mammalian claustrum is hypothesized to play a role in higher cognition^{1,28,29} because of
233 its hub-like connectivity^{12,30-32}. Direct experimental tests, however, are difficult due to
234 claustrum anatomy^{12,33}. Using single-cell RNAseq and tract-tracing techniques, we identified
235 a claustrum in two distant reptiles, suggesting an origin predating the common ancestor of
236 amniotes. The claustrum probably derives from the lateral pallium and may correspond to parts
237 of the mesopallium in birds^{34,35}. Thus, if the claustrum plays a role in higher cognition in
238 mammals, this role may be derived from other functions in a common amniote ancestor.
239 Claustrum assumes different architectonics, reflected in neuronal morphology, in two distant
240 reptiles. (Differences exist also between marsupial and eutherian mammals³⁶.) Because
241 claustrum produces SWRs in both reptiles, architectonics likely play little role in SWR
242 generation.

243

244 Claustrum participates in the generation and relaying of SWRs, characteristic of slow-wave
245 sleep in *Pogona*. Given the claustrum's widespread connectivity and its input from sleep/wake
246 controlling areas, it may be implicated in coordinating forebrain states during sleep. Early
247 experiments in cats³⁷ describe sleep-like behavior after (though not during) low-frequency
248 claustrum stimulation. These results remain uncertain because selective stimulation of the
249 mammalian claustrum is difficult. Recent results in rodents using markers of synaptic activity³⁸
250 suggest claustrum activity during REM sleep. Other^{39,40} suggest that claustrum acts to shut
251 down cortex via dominant projections onto cortical interneurons. This action would cause a
252 general cortical downstate, as possibly seen during certain phases of SWS⁴⁰. These results
253 collectively suggest tentative links between claustrum and sleep in mammals.

254

255 During sleep in *Pogona*, SWRs originate in claustrum and propagate to the rest of the non-
256 cortical pallium, the mammalian amygdaloid complex homolog¹¹. By virtue of ascending input
257 from areas controlling wake/sleep, the claustrum is ideally positioned to act as a relay for
258 wake/sleep-related states in the forebrain. During sleep the claustrum alternates between SWR
259 production and REM, presumably driven by alternating ascending inputs, themselves
260 independent of claustrum integrity. Claustrum projections suggest a distributed action on
261 cortex, hippocampus, amygdala and other forebrain areas. SWRs in sleeping *Pogona in vivo*

262 are each correlated with a short phasic inhibition of cortex [consistent with stimulation
263 experiments (Ext. Data Fig. 8) and with results in rodents^{39,40}], followed by cortical excitation³
264 (consistent with CA1-mPFC coordination in rodents⁴¹). The mechanisms underlying this
265 coordination must now be characterized, as does the nature of sleep-related inputs to claustrum.

266

267 **Acknowledgments**

268 We thank K. Steele and A. Schwartzlose for help with 5HT immunocytochemistry and neural
269 tract tracing, Drs. E. Northrup and G. Wexel for veterinary care, T. Klappich for reptile care,
270 S. Junek, F. Vollrath and C. Polisseni for help with imaging and microscopy, G. Tushev for
271 help with genome reannotation, E. Desfilis (University of Lleida, Spain) for help with lizard
272 neuroanatomy and the Laurent lab for help and comments. Funded by the Max Planck
273 Society, the European Research Council under the European Union's Seventh Framework
274 Programme (FP7/2007-2013) (ERC grant agreement n° 322705), the European Research
275 Council under the European Union's Horizon 2020 research and innovation programme
276 (ERC grant agreement n° 834446) and the DFG (CRC1080) (G.L.), postdoctoral fellowships
277 from the JSPS (SPD and for Research Abroad) and from the Kanae Foundation for the
278 promotion of medical science (H.N.), and an EMBO Long-Term Fellowship (ALTF 421-
279 2017) (L.A.F.).

280

281 **Authors contributions**

282 H.N. and L.A.F. contributed equally and have equal right to list themselves first in
283 bibliographic documents; project conception: H.N, L.A.F and G.L.; animal surgery: M.K.,
284 H.N. and L.A.F.; electrophysiology: H.N., L.A.F. and S.R.; pharmacology: H.N., R.K., and
285 L.A.F.; single-cell RNAseq: M.T.G.F., D.H., A.M. and M.A.T.; bioinformatics: M.A.T. and
286 D.H.; tracing, anatomy and histology: L.A.F., H-H.L., R.K., M.T.G.F., A.A. and M.K.;
287 experimental design, data interpretation and analysis: H.N., L.A.F., H-H.L., M.A.T., M.T.G.F.,
288 D.H., S.R., and G.L.; project management and supervision: G.L.; manuscript writing: G.L.,
289 with input from all.

290

291 **Competing interests**

292 The authors declare no competing interest.

293

294 **Materials & Correspondence**

295 Sequences, code and links can be found at <https://brain.mpg.de/research/laurent->
 296 [department/software-techniques.html](https://brain.mpg.de/research/laurent-department/software-techniques.html). Data are available upon request. Correspondence:
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Figure Legends

Fig. 1 | SWRs originate in the antero-medial DVR in sleeping *Pogona*.

a, Simultaneous recordings from two sites in DVR (subcortical). See Ext. Data Fig.1. **b**, Auto- and cross-correlations of δ/β from sites in **a** calculated over 8h of sleep. Colored strips: δ/β over one single 1000-s stretch of sleep. **c**, Short segment of data analyzed in **b** (same colors). (i) Zoom-in on short segment of SW sleep, illustrating SWR coordination and antero-posterior delay. (ii) Detail of a SWR (bottom) and high-pass components (middle and top). **d**, Cross-correlation between broadband LFP waveforms (**c**) during 3.42h of SW sleep. Reference (0) is anterior recording site. **e**, Delay distribution of SWs in anterior (A) (or posterior, P) DVR triggered on simultaneously recorded P (or A) DVR (See Methods and Ext. Data Fig. 1).

452 **Fig. 2 | SWRs occur spontaneously in DVR slices and originate at the antero-medial pole.**
453 **a-c** CMOS-MEA-recordings of SWR propagating across horizontal DVR slice (outlined) (see
454 Ext. Data Fig 2). **a**, Instantaneous voltage samples at 20-60ms interval. Squares 1-5: recordings
455 sites for **b**. Note initiation at anterior pole. *z*: z-score. **b**, SWR from sites 1-5 in **a**. Note
456 amplitude and onset time differences across sites. **c**, Signal latency relative to earliest channel
457 over slice plane (mean of 12 SWRs, same slice as in **a**). **d-f**, SWRs in mini slices; 252-site
458 MEA, 200 μm pitch. **d**, Thick horizontal DVR slices were sub-divided. **e**, Simultaneous LFPs
459 recorded from colored sites in **d**. **f**, Mean SWR frequency in intact slices (ctrl): $n = 12$ slices;
460 amDVR: $n = 13$ minislides; plDVR: $n = 9$ minislides. Ctrl vs. amDVR: $p=1$, $t_{23}=0.04$; Ctrl vs.
461 plDVR: $p=7.2 \times 10^{-6}$; $t_{19}=6.3$; amDVR vs. plDVR: $p=4.6 \times 10^{-6}$, $t_{19}=6.3$, two-sided Bonferroni
462 test. Data are mean \pm s.e.m. A: anterior; D: dorsal; L: lateral; M: medial; P: posterior; V:
463 ventral.
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467 **Fig. 3 | sc-RNAseq and viral tract tracing show that amDVR is a reptilian claustrum**
468 **a**, UMAP (Uniform Manifold Approximation and Projection⁴⁵) representation of single-cell
469 transcriptomes of 4,054 *Pogona* glutamatergic pallial neurons; cells color-coded by cluster (1-
470 29). aDVR, amDVR, pDVR: anterior, anterior-medial, posterior DVR; CoA: cortical
471 amygdala; DLA: dorsal lateral amygdala. See Ext. Data Fig. 3,5. **b**, Expression across clusters
472 of markers with high, specific expression in amDVR (clusters 19, 20). These include markers
473 of mammalian claustrum. Dot size: fraction of cells in which the gene is detected; color:
474 expression level. **c-e** Anterior transverse sections of *Pogona* telencephalon with
475 immunostaining for hippocalcin (*HPCA*) (**c**); *in situ* hybridization (ISH) for *ADARB2* (**d**);
476 double ISH with *RORB* and *CPNE4* probes (**e**). Scale bars: 500 μ m. **f**, Diagram of amDVR
477 subdivisions. **g**, Transcriptomic similarity between lizard and mouse clusters, measured as
478 fraction of single-cell transcriptomes mapping from *Pogona* to mouse clusters (Methods)
479 (mouse data from *I3*). **h**, Average expression in the *Pogona* clusters of 143 ion channel and
480 receptor genes (Ext. Data Fig. 5). Genes with enriched expression in amDVR listed at right. **i**,
481 Schematic of *Pogona* brain. Forebrain areas (blue) identified by sc-RNAseq + *in situs* (*11*, this
482 paper). Diencephalic (green), mesencephalic (orange) and rhombencephalic (pink) areas
483 identified by IHC and FISH (details in Ext. Data Fig. 6). **j**, Summary of claustrum (amDVR)
484 connectivity with areas in **a**, determined by viral tracing. Line arrows: connections. x: absence
485 of connection (absence of anterograde and retrograde labeling). Stippled arrows: tentative (due
486 to inconsistent labeling) connections. Claustral projections to pDVR/DLA not conclusively
487 tested, due to failure to inject rAAV2-retro specifically into those small areas.
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498 **Fig. 4 | Dependency of SWR production in DVR on claustrum integrity and modulation.**
 499 **a-d:** Ibotenic acid lesions of claustrum and SWRs in sleeping lizards (Ext. Data Fig. 9). **a**, Short
 500 sleep segment showing LFP (< 150 Hz) from left and right DVRs after unilateral CLA lesion.
 501 Sham-lesioned hemisphere (blue, CLA⁺); lesioned (red, CLA⁻). Arrowheads: sharp-waves in
 502 DVR. Sleep rhythm is intact but SW sharp-waves are nearly absent on CLA⁻ side. **b**, Same as
 503 **a**, in animal with bilateral CLA lesions. **c**, Cross-correlation of β -band (REM) power across
 504 hemispheres in lesioned animals. **d**, Number of sharp-waves per SWS cycle in sham and CLA-
 505 lesioned hemispheres. ***: $p < 1.73 \times 10^{-60}$, $W = 64252$, Wilcoxon signed-rank test (data from two
 506 animals, four nights, 375 cycles). Box conventions: see Methods/statistics. **e-h:** CLA-DVR
 507 slice experiments. **e**, Effects of superfused NA ($n = 7$, 25 μ M), DA agonist SKF38393 ($n = 7$,
 508 10 μ M), ACh agonist carbachol ($n = 5$, 50 μ M) and 5HT ($n = 4$, 10 μ M) on spontaneous SWR
 509 frequency. **f**, Action of 5HT-R agonists on spontaneous SWR rate in isolated CLA slices. $n = 3$
 510 experiments (5HTR-1A); $n = 4$ (1B); $n = 5$ (1D); $n = 5$ (2C); $n = 4$ (7). *** $p = 8.0 \times 10^{-3}$, $T = 15$, two-
 511 sided Wilcoxon rank-sum test, * $p = 0.04$, $t_4 = -2.9$, # $p = 0.049$, $t_8 = -2.3$, paired t -test. Means \pm SEM
 512 (**e,f**). **g**, Light-uncaging of 5HT suppresses spontaneous SWRs in CLA-DVR slices. **h**,
 513 summary of 8 experiments as in **g**. Bins: 10s. $n = 8$ slices. Circles: mean \pm s.e.m. Control: light
 514 pulses on ACSF-superfused slices. *** $p = 1.5 \times 10^{-4}$, $T = 36$, two-sided Mann-Whitney rank-sum
 515 test.

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527 Methods

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529 ANIMALS

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531 Lizards: Animals (*Pogona vitticeps* known as “Australian dragon”) of either sex, weighing
532 100-400 grams, were obtained from our institute colony, selected for sex, size, weight, health
533 status, and wild-type coloring.

534

535 Turtles: Wild-type turtles (*Trachemys scripta elegans* or *Chrysemys picta*) of either sex,
536 weighing 200 to 400 g, were obtained from an open-air breeding colony (NASCO Biology,
537 Wisconsin, USA). The animals were housed in our state-of-the-art animal facility.

538

539 All experimental procedures were performed in accordance with German animal welfare
540 guidelines: permit #V54- 19c 20/15- F126/1005 delivered by the Regierungspraesidium
541 Darmstadt, Darmstadt, Germany (Dr. E. Simon).

542

543

544 RECORDINGS

545

546 Lizard surgery for chronic recordings

547 Twenty-four hours before surgery, the lizard was administered analgesics (Butorphanol: 0.5
548 mg/kg s.c., Meloxicam: 0.2 mg/kg s.c.) and antibiotics (marbofloxacin, Marbocyl, 2 mg/kg).
549 On the day of surgery, anesthesia was initiated with isoflurane, and maintained with isoflurane
550 (1-4 Vol. %) after intubation. The lizard was placed in a stereotactic apparatus after ensuring
551 deep anesthesia (absence of corneal reflex). Body temperature during surgery was maintained
552 at 32°C using a heating pad and esophageal temperature probe. Heart rate was monitored using
553 a Doppler flow detector. The skin covering the skull was disinfected using 10% Povidone-
554 iodine solution before removal with a scalpel. A small (~3x2 mm) craniotomy was then drilled
555 postero-lateral to the parietal eye along the midline. The dura and arachnoid layers covering
556 the forebrain were removed with fine forceps, and the pia was removed gently over the area of
557 electrode insertion (dorsal/dorsomedial cortex). The exposed skull was covered with a layer of
558 UV-hardening glue, and the bare ends of two insulated stainless steel wires were secured in
559 place subdurally with UV-hardening glue to serve as reference and ground.

560

561 Insertion of silicon probes: probes were mounted on a Nanodrive (Cambridge Neurotech) and
562 secured to a stereotactic adaptor. On the day after the surgery, probes were slowly lowered into
563 the tissue (~ 0.9-1.2mm). The brain was covered with Duragel followed by vaseline. After
564 connecting grounds, the skull, craniotomy, and probes were secured with dental cement.
565 Following surgery, lizards were released from the stereotax and left on a heating pad set to
566 32°C until full recovery from anesthesia.

567

568 ***In vivo* electrophysiology**

569 One week before surgery, animals were habituated to a sleep arena for a minimum of 2 nights.
570 One to two hours before lights off, the lizard was placed in the sleep arena, itself placed in a
571 3x3x3m EM-shielded room. The animal was let to sleep and behave naturally overnight, and
572 returned to its home terrarium 3-4 hours after lights on. The animal then received food and
573 water. Recordings were made from the cortex, anterior DVR (including claustrum) and/or
574 posterior DVR of chronically implanted adult lizards. Electrodes were 32-channel silicon
575 probes (50 µm pitch, 177 µm² surface area for each site; in two rows of 16 contacts).

576

577 Recordings were performed with a Cheetah Digital Lynx SX system and HS-36 headstages of
578 unity gain and high input impedance (~1 TΩ). The headstage was connected with a
579 headstage adapter to a connector on the head, and a lightweight shielded tether cable connected
580 the headstage to the acquisition system. Recordings were grounded and referenced against one
581 of the reference wires. Signals were sampled at 32 kHz, with wide-band 0.1–9,000 Hz.
582 Electrophysiological traces were typically filtered <150Hz with a 2-pole Butterworth filter for
583 display.

584

585 **Ibotenic-acid lesion experiments**

586 In preparation for claustrum-lesion experiments we carefully removed, using fine forceps in
587 anesthetized animals, the pia overlaying dorsal cortex and inserted a beveled quartz
588 micropipette at an angle of 90° to the surface, to a depth of 1050–1150 µm from the surface, at
589 appropriate a-p and m-l coordinates to reach the center of the claustrum. 400–600 nl of ibotenic
590 acid (5 µg/µl in phosphate-buffered saline, PBS, pH 7.2) were injected at a rate of 50–100
591 nl/min (UMP3, World Precision Instruments, USA). The injection pipette was retracted 3 min
592 after the end of injection. Two silicon recording probes were subsequently positioned

593 bilaterally, as described above, for DVR recordings. For sham claustrum lesions, we injected
594 PBS alone (same methods and volumes) on the sham-lesion side. Recordings were carried out
595 each night from one to 6 days after surgery. Effects of the lesions could already be observed
596 24 hrs after surgery. A week after each experiment, the animal was sacrificed, its brain
597 sectioned and stained (Nissl) for histological confirmation.

598

599 **SWR delay calculation**

600 Sharp-waves were detected as described previously (template-based detection³). After
601 independently detecting SWRs on probes in anterior and posterior DVR throughout a dataset,
602 the delay between SWRs across probes was calculated by pairing SWRs on one probe with the
603 SWR closest in time on the second probe. Pairs occurring >500 ms apart were ignored.

604

605 **SWRs at the SWS-REMS transition point**

606 REMS and SWS periods and the timing of their transition, were calculated as described
607 previously³. Average SWR rate and amplitudes were calculated by averaging these values
608 triggered on all SWS-REMS transition points within 100ms bins, and smoothing the resulting
609 histogram with a Gaussian filter (std. 25 ms).

610

611 In ibotenic-acid lesion experiments, sleep cycles were determined using median filtered beta-
612 band power (10-40 Hz, as above), for a 6-hour period beginning 3 hours after recording start
613 time. The time course of beta was filtered above 0.001 Hz with a 2-pole Butterworth filter, and
614 additionally smoothed with a Gaussian filter (std, 20s). Periods of SWS were conservatively
615 defined as ones in which this signal was less than 1 s.d. below the mean. To avoid false SW
616 detections observed in lesioned animals (which demonstrate reduced low-frequency power),
617 SWs were detected through thresholding the voltage trace (1.5-2.5 s.d. below the mean) after
618 low-pass filtering at 4 Hz with a 2-pole Butterworth filter. The threshold was adapted to each
619 lesion experiment and was the same for both hemispheres within each experiment.

620

621 **Sharp-wave shape statistics**

622 For comparison with *ex vivo* and slice sharp-waves, sharp-waves detected *in vivo* were low-
623 pass filtered at 20Hz using a 2-pole Butterworth filter.

624

625 ***Ex vivo* and slice preparation**

626 Adult lizards or turtles were deeply anesthetized with isoflurane, ketamine (60 mg/kg, and
627 midazolam (2mg/kg). After loss of the corneal reflex, the animals were decapitated, and the
628 heads were rapidly transferred into cooled artificial cerebrospinal fluid (ACSF) solution
629 (Lizard: 126 mM NaCl, 3 mM KCl, 1.8 mM CaCl₂, 4 mM MgCl₂, 24 mM NaHCO₃, 0.72 mM
630 NaH₂PO₄, 20 mM glucose, pH 7.4, Turtle: 96.5 mM NaCl, 2.6 mM, KCl, 4 mM CaCl₂, 2 mM
631 MgCl₂, 31.5 mM NaHCO₃, 20 mM glucose, pH 7.4) bubbled with carbogen gas (95% O₂, 5%
632 CO₂).

633

634 Ex vivo intact subcortical preparation: After isolation of the lizard brain, subcortical slabs were
635 prepared with iridectomy scissors.

636

637 Slice preparation: Coronal, horizontal or sagittal subcortical area slices (700 μm thick) were
638 prepared using a vibratome (VT 1200S, Leica) in ice-cold, oxygenated ACSF. The slices were
639 allowed to recover for at least 60 min and then submerged in a chamber filled with oxygenated
640 ACSF (Lizards: 126 mM NaCl, 3 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 24 mM
641 NaHCO₃, 0.72 mM NaH₂PO₄, 20 mM glucose, pH 7.4, Turtle: 96.5 mM NaCl, 2.6 mM, KCl,
642 4 mM CaCl₂, 2 mM MgCl₂, 31.5 mM NaHCO₃, 20 mM glucose, pH 7.4) at 20–22°C.

643

644 Ex vivo/Slice physiology and SWR detection: During recordings, oxygenated ACSF (Lizard:
645 126 mM NaCl, 3 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃, 0.72 mM
646 NaH₂PO₄, 20 mM glucose, pH 7.4, Turtle: 96.5 mM NaCl, 2.6 mM, KCl, 4 mM CaCl₂, 2 mM
647 MgCl₂, 31.5 mM NaHCO₃, 20 mM glucose, pH 7.4) was constantly superfused at 18-20°C (*ex*
648 *vivo*) and 18-21°C (slices) at 4 ml/min. Local field potentials (LFPs) were recorded using
649 micro-electrode arrays (MEAs), silicon probes, or glass pipettes filled with ACSF. The
650 electrodes were carefully placed in the targeted areas with micromanipulators. Signals were
651 low-pass filtered at 2kHz and digitized at 20kHz. For analysis of SWs, the traces were further
652 low-pass filtered at 20 Hz using a 2-pole Butterworth filter. SWRs were detected at a threshold
653 of 3× s.d. of the total signal. The detected events were visually scrutinized and manually
654 rejected if they were erroneously detected. Events lasting less than 30ms were also discarded
655 as they were typically artifacts. For claustrum electrical stimulation experiments, stimulation
656 pulses lasted 50μs and were delivered with bipolar electrodes. Multi-unit extracellular
657 recordings in cortex were carried out with glass micropipettes filled with ACSF. Mini-slices
658 were cut with a sharp razor blade and were 0.61-3.12 mm² in surface area.

659

660 CMOS MEA experiments

661 The slices were placed over a high-density MEA (3Brain AG) of 4,096 electrodes (electrode
662 size, 21×21µm; pitch, 81µm; 64×64 matrix; 5.12×5.12mm area). During recording, ACSF
663 perfusion was interrupted to avoid movements of the slices and noise due to ACSF flux. Signals
664 were sampled at 18kHz with high-pass filter at 1Hz.

665

666 Saturating or damaged channels were detected as channels whose voltage crossed $\pm 500\mu\text{V}$ and
667 were removed from later analysis. Channel data were low-pass filtered at 20Hz, z-scored, and
668 troughs greater than 5(z) below the mean on the channel with the largest signal were taken as
669 SWs. The signal $\pm 400\text{ms}$ from these peak times, on all channels, was taken as a SWR episode.
670 For calculation of SWR latency, SWRs were averaged on each channel and the time that the
671 average signal crossed 1(z) below the mean was taken as the start of the SWR on that
672 channel. Latency was calculated relative to the time of the earliest channel's SWR. Channels
673 that did not cross 1(z) were considered maximum latency. The resulting latency image was
674 filtered with a 3×3 median filter to remove the impact of bad channels, and up-sampled by a
675 factor of 10 for display.

676

677 Whole-cell patch-clamp (WCPC) recordings of DVR and claustrum neurons

678 Long-shank patch pipettes (6-8 MΩ) were pulled from borosilicate glass with a Sutter P1000
679 electrode puller. Pipettes were filled with internal solution (140mM K-gluconate, 4mM NaCl,
680 14mM Phosphocreatine, 10mM HEPES, 4mM Mg-ATP, 0.3mM Na-GTP, 4mg/ml biocytin).
681 Experiments were carried out on an upright Olympus BX61WI microscope with 5x and 40x
682 water-immersion objectives and cells were patched under visual guidance. EPSCs and IPSCs
683 were recorded in the voltage-clamp configuration with the same cell held at either -70 mV or
684 +10 mV. Simultaneous patch-clamp and LFP recordings were carried out with an EPC10
685 Quadro amplifier (HEKA).

686

687 Pharmacology

688 5HT hydrochloride (0.1-30 µM), carbamoylcholine chloride (50 µM), noradrenaline bitartrate
689 (25 µM), SKF38393 hydrobromide (10 µM), (R)-(+)-8-Hydroxy-DPAT hydrobromide (2 µM),
690 L-703,664 succinate (1 µM), CP 809,101 hydrochloride (0.1 µM), LP44 (0.2 µM), and TTX
691 (20 µM) were diluted to their final concentrations in ACSF (126 mM NaCl, 3 mM KCl, 1.8

692 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃, 0.72 mM NaH₂PO₄, 20 mM glucose, pH 7.4).
693 For slice experiments, drugs were continuously bath-applied after a baseline recording period
694 of 5-20 min. For *ex vivo* experiments in Ext. Data Fig. 8, TTX dissolved in ACSF was injected
695 into the claustrum through a glass micropipette using a 10-ml syringe pressurizer (20-30 hPa
696 for 15 min). For 5HT uncaging, RuBi-5HT (Abcam) (10 μM) was bath-applied, and white-
697 light (400-700nm, 0.11 W/cm², TH4-200, Olympus) was turned on and off at chosen intervals
698 (e.g., 80s).

699 We tested several metabotropic 5HT-R agonists. Of those, 1D agonist L-703,664 best
700 mimicked the effects of 5HT, consistent with the high expression of 5HT1D-R in glutamatergic
701 neurons in claustrum (Extended Data Fig. 5a). 5HTR7 agonist LP44 had no effect (Fig. 4f),
702 also consistent with the low expression of the 5HTR7 in claustrum excitatory neurons.
703 5HTR2C agonist CP 809,101 increased the rate but not the amplitude of SWRs.

704

705

706 SINGLE-CELL TRANSCRIPTOMICS

707

708 **Single-cell RNA sequencing libraries**

709 Adult male lizards (150-400g) were deeply anesthetized with isoflurane, ketamine (50 mg/kg)
710 and midazolam (0.5 mg/kg) and decapitated. The head was immersed in ice-cold, oxygenated
711 ACSF (126 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 4 mM MgCl₂, 24 mM NaHCO₃, 0.72 mM
712 NaH₂PO₄, 20 mM glucose, pH 7.4). The brains were perfused to remove blood from the
713 vasculature. The data shown originate from four libraries constructed from data from one male
714 lizard (160g, 20 months old).

715

716 Thereafter, the brain was removed and immersed in oxygenated, ice-cold ACSF. The brain was
717 embedded in 4% low melting agarose, glued to the base of a vibratome (VT1200S, Leica),
718 immersed in ice-cold oxygenated ACSF and 500μm-thick sections were prepared (speed: 0.08
719 mm/s). The sections were individually inspected under a dissection microscope (Stemi 2000-
720 C, Zeiss) and anatomical regions of interest were dissected (telencephalon, amDVR). These
721 slices were cut with fine scissors (Fine Science Tools) into small cubes of tissue
722 (~500x500x500 μm).

723

724 These were transferred to dissociation buffer (20 U/ml papain, 200 U/ml DNase I, 25 μg/ml

725 liberase TM, 1 μ M tetrodotoxin (TTX), 100 μ M D-APV) and triturated with fire-polished,
726 silanized glass pipettes of decreasing tip diameter (~10 passes per pipette). After every pipette
727 change the supernatant (dissociated cell suspension) was removed and filtered through a 100
728 μ m-mesh-diameter strainer.

729

730 The pooled dissociated cell suspension was diluted to 20 ml (with Hibernate A - CaCl₂),
731 transferred to a 50-ml reaction tube and filtered with a 40- μ m mesh diameter strainer. Then 5
732 ml of 4% bovine serum albumin (BSA) in Hibernate A - CaCl₂ was added to the bottom of the
733 tube with a long-stemmed glass pipette. The solution was spun in a centrifuge at 4°C with 300g
734 (lowest acceleration and brake) for 5 min. The supernatant was removed and the cell pellet
735 resuspended in 20 ml of Hibernate A - CaCl₂. This procedure was repeated for a second
736 gradient clean-up. The pellet was then resuspended in an appropriate amount (50-200 μ l) of
737 Hibernate A - CaCl₂-MgCl₂ and the cell concentration was measured with a Fuchs-Rosenthal
738 cell counting chamber (Brand).

739

740 The cell suspension was then diluted to 466 cells/ μ l and used as input to half a chip (four
741 samples) of the 10x Chromium system (Chemistry v3) with a targeted cell recovery of 7,000
742 cells/sample. The library construction was performed according to the manufacturer's
743 instructions.

744

745 The final four libraries were quantified using Qubit fluorometer (ThermoFisher) and sequenced
746 five times on a DNA sequencer (NextSeq 500, Illumina) with an average depth of 442,806,563
747 reads/library.

748

749 **Analysis of transcriptomics data**

750 Raw sequencing data were processed using Cellranger v3.0 (10X Genomics). Raw reads were
751 demultiplexed and filtered with the cellranger mkfastq function with default settings. To
752 generate digital gene expression matrices, demultiplexed reads were aligned to the *Pogona*
753 genome with the cellranger count function, setting the force-cells parameter to 7000. For reads
754 alignment, we re-annotated the *Pogona* genome (assembly 1.1.0, NCBI accession number
755 GCF_900067755.1, April 10th 2017) using the same 3'-end MACE (Massive Analysis of
756 cDNA Ends) data and the approach described in ref. 11.

757

758 Digital gene expression matrices were analyzed in R, using the Seurat v3.0 package¹⁴. Cells
759 were filtered by number of genes (> 800 genes/cell) and percentage of mitochondrial genes
760 (>5%), yielding a total of 20,257 cells, with a median number of 2,278 transcripts and 1,349
761 genes per cell. Data were normalized by the total number of transcripts detected in each cell,
762 and regressed by the number of genes and of transcripts (by setting `vars.to.regress =`
763 `c("nFeature_RNA","nCount_RNA")` in `ScaleData` function). Variable genes were identified
764 after variance standardization from an estimate of the mean-variance relationship
765 (`FindVariableFeature, method="vst"`), and the top 1,000 highly variable genes were used for
766 principal component analysis. The first 30 principal components were used for Louvain
767 clustering (`FindClusters, resolution=0.2`) and for dimensionality reduction with UMAP⁴⁵
768 (`RunUMAP` with default settings).

769

770 After this first round of analysis, neuronal clusters (characterized by high expression of pan-
771 neuronal markers, such as the synaptic protein *SNAP25*) were reanalyzed using the same
772 procedure as above and the following settings: >800 genes/cell, 2000 highly variable genes, 30
773 principal components, clustering resolution=2. This led to the identification of 33 neuronal
774 clusters. Two clusters of doublets, recognized by the co-expression of glutamatergic and
775 GABAergic markers, were filtered out at this stage, leaving 9,777 neurons in 29 clusters
776 (Extended Data Fig. 3).

777

778 From this neuronal dataset, we identified 4,054 pallial glutamatergic neurons (with >1000
779 genes/cell) co-expressing the vesicular glutamate transporters *SLC17A7* and *SLC17A6*. Further
780 sub-clustering of these cells (analysis settings: 2,000 highly variable genes, 34 principal
781 components, clustering resolution=3) led to the identification of 29 clusters (Fig. 3a and
782 Extended Data Fig. 3). To assign an identity to each of these clusters, we analyzed the
783 expression of marker genes with known tissue expression patterns (data and approach in ref.
784 *11*). This allowed us to define the pallial region to which each cluster belongs (for example,
785 hippocampus for *ZBTB20*-expressing clusters). Further annotation of cluster identities
786 (Extended Data Fig. 3) was based on the expression of selective markers or combination of
787 marker genes, identified from the transcriptomics data.

788

789 **Analysis of ion channels and neurotransmitter receptor genes**

790 We mined the *Pogona* genome for the following gene families: noradrenaline, acetylcholine,
791 serotonin and dopamine receptors, calcium, chloride, sodium and potassium channels, GABA,
792 glutamate, adenosine, cannabinoid, glycine and histamine receptors. This yielded 270 genes in
793 total. Of these, 143 were kept for further analysis, because they were detected in at least 20%
794 of the cells of at least one glutamatergic cluster (Extended Data Fig. 5a).

795

796 To calculate pairwise cluster correlations (Pearson correlations, Extended Data Fig. 5b), we
797 used this set of 143 genes and average cluster expression data (calculated from normalized and
798 log-transformed data with the AverageExpression function in the Seurat package). A distance
799 matrix was calculated from the correlation matrix, and used for hierarchical clustering (R
800 package hclust) with the Ward.D2 linkage method.

801

802 The gene expression matrix from above was transposed to calculate gene-gene correlations
803 (Fig. 3g). The gene dendrogram was also calculated with hierarchical clustering and the
804 Ward.D2 linkage method.

805

806 The heatmap in Fig. 3h was generated from the matrix of 29 glutamatergic clusters (columns)
807 and average expression of the 143 genes (rows). The data matrix was scaled by columns, and
808 the heatmap was plotted with the heatmap.2 function from the R package gplots. The
809 dendrogram of glutamatergic clusters is based on Euclidean distance and Ward.D2 linkage.

810

811 **Mapping of single-cell transcriptomes across species**

812 To map *Pogona* single-cell transcriptomes on mouse single-cell data, we used the dataset from
813 ref. 13, available on the dropviz.org website. In this dataset, pallial glutamatergic neurons were
814 sampled from three regions: “hippocampus”, “frontal cortex” and “posterior cortex”. These
815 dissections encompass several cell types; for example, “frontal cortex” includes claustrum and
816 “hippocampus” includes subiculum and entorhinal cortex. Raw data were processed through
817 the Seurat pipeline (normalization, scaling, variable genes selection) and glutamatergic clusters
818 and subclusters were selected, according to the cluster and subcluster identities provided by
819 Saunders et al. (ref. 13) and dropviz.org. Subclusters were downsampled to a maximum
820 number of 200 cells/subcluster, yielding a total of 17,455 cells.

821

822 *Pogona*-mouse comparative analysis were limited to one-to-one orthologs, according to the
823 orthology annotations provided by Ensembl (*Pogona* assembly pv1.1 and mouse assembly
824 GRCm38.p6, one-to-one orthologs downloaded on May 1st, 2019). Of 13,273 one-to-one
825 orthologs, 10,693 were detected in both the mouse and *Pogona* datasets and used for the
826 comparative analysis.

827

828 The *Pogona* and mouse data were analyzed jointly following the approach described in ref. 14.
829 Briefly, after normalization and scaling, 1,500 highly variable genes were identified in each
830 dataset. The union of these sets of variable genes was used for a joint canonical correlation
831 analysis (CCA). The first 15 canonical components were then used to identify 2,626 transfer
832 anchors, that is, pairs of cells with matching neighborhoods (“mutual nearest neighbors”) in
833 the two transcriptomics spaces (function FindTransferAnchors from Seurat). These anchors
834 were then used to project *Pogona* cells (“query” dataset) on the mouse dataset (“reference”
835 dataset), using the TransferData function from Seurat. The projection is based on a weighted
836 classifier, that assigns a classification score based on the distance of each cell from the transfer
837 anchors. Fig. 3g represents the result of the classification, showing the fraction of single cells
838 from each *Pogona* cluster mapping on each of the mouse subclusters (mouse subclusters
839 without matching lizard cells are not indicated in the figure).

840

841 The approach described above was also used to project the transcriptomes of turtle pallial
842 glutamatergic cells on the *Pogona* data (Ext. Data Fig. 7a). The turtle data are from ref. 11. The
843 comparison was based on 9,820 one-to-one orthologs detected in both species. For this
844 analysis, the top 2,000 variable genes of each dataset were used for CCA. The first 25 canonical
845 components were used to compute 3,406 transfer anchors.

846

847 ANATOMY

848

849 **Identification of *Pogona* brain areas with potential role in brain-state regulation**

850 Areas known to play a role in controlling brain state have been, over the past decades, identified
851 in a number of mammalian species. Those areas can be identified by their location (*e.g.*, within
852 the hypothalamus, midbrain or brainstem), their axonal projections, and by the neuroactive
853 substances their neurons contain and release (and thus potential marker genes). To our
854 knowledge, no such description exists at present for the brain of the bearded dragon (*Pogona*)

855 but anatomical studies of homologous areas have been performed by other lizard species (refs.
 856 43,44,46-56). These references were used to identify relevant brain areas, including POA,
 857 SUM⁵⁷ and TMN in the hypothalamus, VTA, SN and PAG in the midbrain, and LDT, LoC,
 858 SC, Ra in the brainstem. The location and identity of these areas were established in *Pogona*
 859 by IHC and/or FISH using appropriate neuronal markers, combined with Nissl stains of brain
 860 sections. Tyrosine hydroxylase (TH, marker of catecholaminergic neurons) was used to
 861 identify POA, VTA, SN, PAG and LoC (Extended Data Figs. 6). Choline acetyltransferase
 862 (ChAT) was used to identify LDT (Extended Data Figs. 6a). Histamine (His) was used to
 863 identify TMN (Extended Data Fig. 6a). Serotonin (5HT) was used to identify the raphe
 864 (Extended Data Fig. 6a). SC identification was based on the prior identification of LDT and
 865 LoC and by the expression of *SLC17A6* (vesicular glutamate transporter 2, vGluT2,
 866 glutamatergic neuron marker) by ISH (Extended Data Fig. 6a). The expression of *SLC17A6* by
 867 ISH was used also for the identification of SUM (Extended Data Fig. 6a) (see also ref. 57).

868

869 **Abbreviations**

870 LoC: Locus coeruleus; LDT: lateral dorsal tegmental nucleus; PAG: periaqueductal grey;
 871 POA: preoptic area; Ra: Raphe; SC: subcoeruleus; SN: substantia nigra; SUM:
 872 supramammillary nucleus; TMN: tuberomammillary nucleus; VTA: ventral tegmental area.

873

874 ***Pogona* whole-brain images**

875 *Pogona* brain reconstruction (Fig. 3i) was based on images obtained with a μ CT scanner, and
 876 the *surface* function of the Imaris software (Oxford Instruments). The boundaries of relevant
 877 nuclei were determined from consecutive serial histological sections. The serial images were
 878 aligned and assembled to 3D volumes using the Voloom software, and then imported into
 879 Imaris and aligned with the 3D data. The boundaries of some areas identified by retrograde
 880 tracing were defined from GFP and Nissl staining patterns.

881

882 **Immunohistochemistry and *in situ* hybridization**

883 The lizards were deeply anesthetized with isoflurane, Ketamine (60 mg/kg) and Midazolam (2
 884 mg/kg) until loss of the foot-withdrawal reflex. Pentobarbital (10 mg/kg) was then
 885 administered by intraperitoneal injection. After loss of the corneal reflex, the lizard was
 886 perfused transcardially with cold phosphate-buffered saline (PBS; 1.47×10^{-3} M KH_2PO_4 ,
 887 8.10×10^{-3} M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2.68×10^{-3} M KCl, 1.37×10^{-1} M NaCl) followed by 4%

888 paraformaldehyde (PFA) in PBS. The brain samples were post-fixed with 4% PFA/PBS for
889 16h at 4°C and subsequently immersed in 30% sucrose for 24h at 4°C. The brain area was
890 sectioned coronally (60µm) with a microtome at -24°C. The sections were permeabilized for
891 30 min at room temperature (RT) in blocking solution (PBST: PBS with 0.3% Triton X-100
892 and 10% goat serum) and incubated with primary antibodies (anti-GFP, A10262, Invitrogen,
893 Chicken, 1:1000; Hippocalcin, ab24560, abcam, rabbit, 1:1000; ChAT-choline
894 acetyltransferase, AB144P, Merk, Goat, 1:100; mTH-tyrosine hydroxylase, 22941,
895 Immunostart; mouse, 1:100; rabTH, AB152, Merk, rabbit, 1:200; Histamine,
896 22939, Immunostart, rabbit, 1:100; Serotonin, MAB352, Merk, rat, 1:100) in blocking solution
897 overnight at 4°C. After washing with PBST 3 times, the samples were incubated with
898 secondary antibodies conjugated with appropriate secondary antibodies (1:500, All from
899 Invitrogen) in blocking solution for 4 h at RT. Ensued three washes with PBST. Some slices
900 were counterstained with NeuroTrace 435/455 blue-fluorescent Nissl stain (N21479,
901 Invitrogen, 1:200) in PBS for 2h at RT. After rinsing with PBS, the samples were mounted
902 with Dako Fluorescence Mounting Medium (S3023, Dako) or Roti-Mount FluorCare DAPI
903 (HP20.1, Carl Roth). Images were acquired using a confocal system or fluorescent microscopy
904 at 10x, 20x or 40x. Chromogenic ISHs and dual colorimetric ISHs were performed following
905 the protocols previously described in ref 11.

906

907 **Fluorescent in situ hybridization by RNAscope**

908 The lizards were deeply anesthetized as described above. After loss of corneal reflex, the
909 animals were sacrificed by decapitation. Brains were dissected out immediately, embedded in
910 OCT on dry ice/ethanol bath and stored at -80 °C. Fresh-frozen brains were sectioned at 25µm
911 on a Thermo Fisher Scientific CryoStar NX70 cryostat and placed onto SuperFrost-coated
912 (Thermo Fisher Scientific) slides. Some slides were stored at -80 °C after air drying.
913 RNAscope hybridization was performed according to the manufacturer's instructions. We
914 used the RNAscope Multiplex Fluorescent assay (Advanced Cell Diagnostics) for fresh-frozen
915 sections. Target genes and probe catalogue numbers were Pv-CHAT-C2, 522631-C2; Pv-
916 SLC17A6-C1, 529431-C1. Fluorescent Nissl was used for counterstaining. Slides were
917 mounted with ProLong Gold Diamond Antifade Mountant (P36970, Thermo Fisher Scientific).
918 Images were acquired with a digital slide scanner (Pannoramic MIDI II, 3DHISTECH) at 20x
919 magnification.

920

921 **Tract tracing**

922 The lizards were anesthetized as described for *in vivo* recordings. Extensive preliminary search
923 for useful AAV serotypes for reptilian brains and for appropriate incubation conditions were
924 carried out by Lorenz Pammer²². The tracers (rAAV2-retro-CAG-GFP, 37825-AAVrg;
925 rAAV2-retro-hSyn-EGFP, 50465-AAVrg; AAV9-CB7.Cl.mCherry.WPRE.RBG, 105544-
926 AAV9; all from Addgene, <https://www.addgene.org>) were injected in one or two forebrain
927 locations (e.g., dorso-medial cortex, DVR, amDVR, etc). Four to 6 weeks later, the animals
928 were deeply anesthetized as described above; after loss of corneal reflex, the animals were
929 sacrificed by decapitation. Brains were dissected out, processed for histology, sectioned and
930 imaged. The data presented come from 18 of 30 injected brains. The remaining 12 brains were
931 rejected either because the viral injections failed or because the injections were not sufficiently
932 specific. Targeting specific regions in the brain of *Pogona* and *Trachemys* is difficult because
933 the brain is loosely contained in the cranial cavity and its position relative to the cranium and
934 reliable landmarks is thus variable: the brain floats in CSF, attached by cranial nerves. As a
935 consequence, there exists no reliable stereotactic coordinates based on cranium landmarks. The
936 lateral ventricles are large. The external appearance of the forebrain also lacks reliable
937 landmarks (e.g., blood vessels or sulci). Finally, these animals are not standardized species,
938 bred over generations to reduce variability.

939

940 Note that, because rAAV2-retro does not infect all neuron types equally²³, *negative* retrograde-
941 labeling results should be confirmed with other methods. Conversely, connectivity estimated
942 using the tracers we used is likely underestimated.

943

944

945 **STATISTICS and REPRODUCIBILITY**

946

947 Unless stated otherwise, data are mean \pm s.e.m. For comparisons of two groups we performed
948 a two-tailed unpaired t-test, two-tailed paired t-test, Mann-Whitney rank-sum test or Wilcoxon
949 signed-rank test, as appropriate (all two-sided). For multiple comparisons we performed a
950 Bonferroni test. Significance was determined with the 0.05 alpha level for all statistical tests.
951 Box plot (Fig 4d): margins are 25th and 75th percentiles; red: median; whiskers: boundaries
952 before outliers; outliers (+) are values beyond $1.5 \times$ interquartile range from the box margins.
953 Experiment numbers and repetitions are indicated in the table below.

Figure 1b-e	The experiment was repeated 7 times independently with similar results.
Figure 2a-c	The experiments were repeated 4 times independently with similar results.
Figure 2e	The experiments were repeated amDVR:13 times; plDVR: 9 times independently with similar results.
Figure 3	a-b, d-h: The experiments were repeated 4 times; c:10 times independently with similar results.
Figure 4a-c	The experiments were repeated 3 times independently with similar results.
Extended Figure 1a-d	The experiments were repeated 7 times independently with similar results.
Extended Figure 1h	The experiments were repeated 3 times independently with similar results.
Extended Figure 2b	The experiments were repeated 15 times independently with similar results.
Extended Figure 2g&i	The experiments were repeated 12 times independently with similar results.
Extended Figure 2h&j	The experiments were repeated 2 times independently with similar results.
Extended Figure 3f	The experiments were repeated 3 times independently with similar results.
Extended Figure 4a	The experiments were repeated 3 times independently with similar results.
Extended Figure 4b	The experiments were repeated; amDVR:13 times; plDVR: 9 times independently with similar results.
Extended Figure 6a-c	Except c5-7, the experiments were repeated at least 3 times independently with similar results. C5-7 were reproduced once in 5 experiments (see Fig. 3 legend)
Extended Figure 8a, e-f	The experiments were repeated 4 times independently with similar results.
Extended Figure 8b-d	The experiments were repeated 4 times independently with similar results.
Extended Figure 7b	The experiments were repeated 3 times independently with similar results.
Extended Figure 7c	The experiments were repeated 5 times independently with similar results.
Extended Figure 7d	The experiments were repeated 4 times independently with similar results.
Extended Figure 7e,g	The experiments were repeated 3 times independently with similar results.
Extended Figure 8a, e-f	The experiments were repeated 4 times independently with similar results.
Extended Figure 8b-d	The experiments were repeated 4 times independently with similar results.
Extended Figure 9a-d	The experiments were repeated 2 (a), 2 (b) and 3 (c) times with similar results. Lesion of claustrum (d) was confirmed for all experiments.
Extended Figure 10a-b	The experiments were repeated 2 (a) and 3–4 times (b) independently and with similar results

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959 DATA AVAILABILITY

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961 Sequencing data have been deposited in the NCBI Sequence Read Archive: BioProjects
 962 PRJNA591493 (lizard); PRJNA408230 (turtle); Links to those archives and to analysis code
 963 can be found at: <https://brain.mpg.de/research/laurent-department/software-techniques.html>.

964 Data available upon request to GL (gilles.laurent@brain.mpg.de).

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Extended Data Legends

Extended Data Figure 1 | Further description of SWR statistics and propagation *in vivo*
a, SW amplitude and frequency vary as the animal transitions between SW and REM sleep. Top: illustrative LFP trace (<150 Hz) showing a decrease in sharp-wave amplitude and frequency around the SW-REM transition point. Open circles indicate detected sharp-waves (see Methods and ref. 3). Data in **a-d** are from the same animal and a single night, corresponding to the recording in Fig. 1 (anterior recording site, red). Statistics based on $n = 11,123$ sharp-waves. **b**, Distribution of sharp-wave width (measured at half peak amplitude) and peak amplitude from animal in Fig. 1 and Extended Data Fig. 1a. **c**, Mean sharp-wave ± 1 SD (grey), $n = 11,123$ sharp-waves. **d**, Inter-Event Interval (IEI) for sharp-waves recorded during SWS. y-axis in log scale. **e, f**, Summary of data recorded over 5 nights from 2 animals. Each circle represents the mean of one night; black line shows the median. **e**, Mean inter-event intervals (IEIs) during SW sleep. **f**, Mean sharp-wave width and amplitude ($n = 8,055$ – $13,494$ sharp-waves/night). **g**, Delay distributions of sharp-waves in anterior (or posterior) DVR, triggered on simultaneously recorded posterior (or anterior) DVR. Sharp-waves from 3 nights (animal 1, $n = 24,501$ SWs), and 2 nights (animal 2, $n = 13,070$ SWs). **h**, Locations of simultaneous recording sites in aDVR (circles). Left: schematic of recording configuration. Middle and right: Confocal images highlighting recording sites, as identified by electrolytic lesions and DiI applied to the back of the silicon probes. *Post-hoc* staining with an antibody against hippocalcin was used to determine claustral borders (see Fig 3).

1034 **Extended Data Figure 2 | Comparison of SWR statistics across preparations and**
 1035 **recording conditions**

1036 **a**, Slice preparation (see Methods)) for field-potential recordings. **b**, Spontaneous sharp-waves
 1037 (LFP, <150 Hz) and corresponding ripples (HP, 70-150 Hz) in amDVR. Insets: top left,
 1038 expanded SWR in box; top right: 350 ripples; high-pass signal intensity (HPI, >70 Hz) aligned
 1039 on trough of sharp-wave (overlaid as average). **c**, Distribution of amplitude (x) and width (y ,
 1040 full width at half-maximum) of SWR events in a representative DVR slice. **d**, Distribution of
 1041 SWR amplitude and width (as in **c**) in a representative *ex vivo* preparation. **e**, Ratio of amplitude
 1042 (μV) to width (ms). $n = 5$ sleep epochs from 3 animals (*in vivo*), 4 *ex vivo* brains, and 12 slices.
 1043 Colored lines: means. **f**, Autocorrelation function of sharp-wave times showing that the
 1044 characteristic rhythmic modulation of sharp-wave generation (due to the alternation of SWS
 1045 and REMS with 2-3min period) in sleeping animals (*in vivo*, blue) is absent from both *ex vivo*
 1046 brain (red) and slice (green) preparations. $n = 5$ epochs from 3 animals (*in vivo*), 4 *ex vivo*
 1047 brains, and 12 slices. **g**, Whole-cell patch-clamp (WCPC) recording in current-clamp mode of
 1048 a DVR neuron (V_m) together with LFP recording in neighboring region (LFP) with a glass
 1049 micropipette. Note simultaneous depolarization of the neuron and SWRs, and moderate
 1050 neuronal depolarization giving rise to occasional firing (3 action potentials here). Experiment
 1051 repeated with 12 neurons. **h**, WCPC recording of an amDVR neuron in V-clamp mode, held at
 1052 depolarized (cyan) and hyperpolarized (red) holding potentials (V_h). Note volleys of excitatory
 1053 (red) and inhibitory (cyan) currents at each SWR (LFP), and near absence of synaptic input in
 1054 between. **i**, Spike times of patched amDVR neuron in relation to sharp-wave. Note locking to
 1055 sharp-wave trough ($t=0$), and absence of firing otherwise. $n = 2$ amDVR neurons. **j**, Mean
 1056 excitatory (g_e) and inhibitory (g_i) conductances ($n = 20$ and 21 events, respectively).
 1057 Black/grey: averaged SWs recorded with **i** and **e** conductances. Traces aligned on sharp-wave
 1058 trough.

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1064 **Extended Data Fig. 3 | Additional single-cell transcriptomic characterization**
 1065 **a**, UMAP⁴⁵ representation of 20,257 *Pogona* telencephalic cells, color-coded by cluster. EG:
 1066 ependymoglia cells; ExcNeur: excitatory neurons; InhNeur: inhibitory neurons; MG:
 1067 microglia; Mur: mural cells; NPC: neural progenitor cells; Olig: oligodendrocytes; OPC:
 1068 oligodendrocyte progenitor cells; RBC: red blood cells. **b**, Dotplot showing expression of
 1069 canonical cell markers (along each row) across telencephalic cell clusters (columns). Dot size:
 1070 percentage of cells in a cluster in which the gene has been detected; color: expression level. **c**,
 1071 UMAP representation 9,777 lizard telencephalic neurons, color-coded by cluster. **d-e** UMAP
 1072 representations of glutamatergic (*SLC17A7*) and GABAergic (*SLC32A1*) neurons in the
 1073 telencephalon dataset. **f**, Double colorimetric *in situ* hybridization in a frontal section through
 1074 the anterior *Pogona* forebrain. Scale bar: 1,000 μ m. *SLC32A1* (blue) labels GABAergic
 1075 neurons in the subpallium and scattered GABAergic neurons that have migrated from
 1076 subpallium to pallium. *SLC17A6* (orange) labels glutamatergic neurons in the pallial region. **g**,
 1077 Ordered matrix of pairwise Pearson's correlations between expression of 143 ion channels and
 1078 neurotransmitter receptor genes detected in this glutamatergic pallial *Pogona* dataset (see
 1079 Extended Data Fig. 5). Dendrogram (top) based on correlation coefficients and Ward.D2
 1080 linkage; red indicates a gene module with enriched expression in amDVR. **h**, Average
 1081 expression, in the 29 glutamatergic *Pogona* clusters, of the 143 genes in **g** (and Extended Data
 1082 Fig. 5). Genes with enriched expression in amDVR listed at right. **i**, UMAP representation
 1083 4,054 lizard pallial glutamatergic neurons, color-coded by cluster (same as in Fig. 3). **j**, Dot-
 1084 plot showing expression of specific cluster markers (along the rows) in the 29 pallial
 1085 glutamatergic clusters (along each column). Dot size: percentage of cells in a cluster in which
 1086 a gene is detected; color: expression level.

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1097 **Extended Data Fig. 4 | Minislices of DVR and localization of SWR generation**

1098 **a**, Left, Recording configuration of mini-DVR slices on a planar 252 channel MEA. Dots
1099 represent electrodes. Right, *posthoc* immuno-staining of the slices at left. Red: Nissl, Green;
1100 Hippocalcin (HPCA). **b**, Left, Spatial distribution of SWR waveforms as recorded from mini-
1101 slices in **a**. Right, illustrative LFP traces recorded from the amDVR or claustrum (1) and
1102 posterior lateral DVR (2) (see recording positions on the micro-electrode array at left). In
1103 conclusion, SWRs occur spontaneously in amDVR, and are absent from plDVR once it is
1104 disconnected from amDVR (claustrum).

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1130 **Extended Data Fig. 5 | Ion-channel and neurotransmitter-receptor mRNAs in the**
1131 **glutamatergic cell clusters of the *Pogona* telencephalon.**

1132 **a**, Dotplot showing expression of ion-channel and neurotransmitter-receptor genes (rows) in
1133 *Pogona* glutamatergic clusters (columns 1-29). The plot shows only genes detected in at least
1134 20% of the cells of at least one cluster. Dot size indicates the percentage of cells in a cluster
1135 where the gene was detected; dot colors indicate expression level. Clusters 19 and 20 (box)
1136 correspond to the amDVR or claustrum. They differ by the expression of some ACh- and 5HT-
1137 receptor subtypes (see also Fig. 3h). **b**, Ordered pairwise Pearson's correlation matrix of
1138 cluster transcriptomes, calculated from the expression of the ion-channel and neurotransmitter-
1139 receptor genes in **a**. This gene set is sufficient to distinguish the amDVR clusters (19 and 20)
1140 from all others. The dendrogram is based on Pearson correlations and Ward.D2 linkage.

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1163 **Extended Data Fig. 6 | Identification of potential regulatory areas of brain states and**
 1164 **distribution of GFP labeled neurons after claustrum injection of rAAV2-retro.**

1165 **a**, Left, Schematic of the *Pogona* brain in sagittal view, showing the regions defined by
 1166 immunohistochemistry, *in situ* hybridization and retrograde tracing. 1-7 indicate levels of
 1167 transverse sections shown at right. Panels 1-7: Micrograph and corresponding schematic
 1168 representation of relevant areas (in red), identified by IHC, ISH (*in situ*) and Nissl stains. Scale
 1169 bars: 500 μ m. To the right in each panel: magnified view of area(s) delineated as box(es) in
 1170 photomicrographs. Scale bars: 100 μ m. **b**, Identification of AAVrg-*hSyn*-eGFP injection sites.
 1171 Scale bar: 500 μ m. (Rightmost panel: red channel not shown.) **c**, Illustrative examples of
 1172 retrograde labeling of claustrum connectivity, in transverse sections. 1-2: Inputs to claustrum
 1173 revealed by rAAV2-retro injection in claustrum. Panel 1: injection site in lateral claustrum;
 1174 claustrum indicated by anti-hippocalcin immunostain (pink); note retro-labeled cells in aDC
 1175 (box, magnified at right). Panel 2: Same brain as in 1, more posterior section; labeled region in
 1176 box is DLA. 3-12: Representative images illustrating the distribution of GFP-labeled neurons
 1177 in DLPT, DLT, DMT, prethalamus, SUM, MN, TMN, VTA, SN, PAG, LoC and SC, with
 1178 projections to claustrum. Catecholaminergic neuron marker tyrosine hydroxylase (TH) used to
 1179 indicate location of VTA, SN and LoC. Scale bar: 500 μ m; Magnified area calibrations: DLPT,
 1180 DLT, DMT, prethalamus, SUM, MN, TMN, VTA, LoC: 50 μ m; SN, PAG, SC: 100 μ m.

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1182 **Abbreviations:** CLA:claustrum; aDC: anterior dorsal cortex; pDC: posterior dorsal cortex;
 1183 DMT: dorso-medial thalamus; DLA: dorso-lateral (basolateral) amygdala; DLT: dorso-lateral
 1184 thalamus; DLPT: dorso-lateral posterior thalamus; DMC: dorso-medial cortex; aDVR:
 1185 anterior dorsal ventricular ridge; pDVR: posterior dorsal ventricular ridge; LC: lateral cortex;
 1186 LoC: Locus coeruleus; LDT: lateral dorsal tegmental nucleus; MC: medial cortex; MN:
 1187 mammillary nucleus; PAG: periaqueductal grey; POA: preoptic area; SC: subcoeruleus; SN
 1188 substantia nigra; SUM: supramammillary nucleus; TMN: tuberomammillary nucleus; VTA:
 1189 ventral tegmental area.

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1195 **Extended Data Fig. 7 | The claustrum of lizard and turtle differ in position and**
1196 **architectonics, but both are autonomous sources of SWRs.**

1197 **a**, Transcriptomic similarity between turtle and lizard clusters, measured as fraction of single
1198 cells mapping from the turtle pallium dataset to *Pogona* clusters (Methods). Note that the turtle
1199 cell clusters e03-06 (pallial thickening, or PT) map on the lizard cluster 19 (amDVR or
1200 claustrum). Turtle data and clusters from ref. 11. **b**, ISH in an anterior transverse section
1201 showing expression of PT marker *CRHBP*. Scale bar: 500 μ m. **c**, Architectonic of lizard
1202 claustrum. Retrograde labeling of claustrum neurons by rAAV2-retro injected in aDVR (right).
1203 Left panel: magnification of boxed area in right panel (in claustrum). Note disordered
1204 distribution of multipolar neurons. Pink: anti-hippocalcin immunostaining. Calibration: 100 μ m
1205 (L); 500 μ m (R). **d**, Architectonic of turtle claustrum. Retrograde labeling of claustrum neurons
1206 by rAAV2-retro injected in DMC (right panel). Left panel: magnification of boxed area in right
1207 panel. Note arrangement of bipolar neurons within PT layer (see also b for layering of PT).
1208 Scale bar: 100 μ m (L); 500 μ m (R). **e**, Spontaneous sharp-waves recorded simultaneously in
1209 claustrum and DVR in turtle slice preparation. Red dots (schematic): recording sites. Note
1210 sharp-wave (LFP) and ripple in high-pass (HP) band. **f**, Bottom: 295 successive spontaneous
1211 ripples; high-pass signal intensity (HPI, >70Hz) aligned on trough of sharp-wave. Average of
1212 295 sharp-waves, aligned on waveform troughs; grey: SD. **g**, Representative cross-correlogram
1213 of LFP traces recorded simultaneously from claustrum and DVR (reference: claustrum),
1214 showing DVR trailing claustrum.

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1216 **Extended Data Fig. 8 | Sharp-wave ripple recordings and stimulation experiments with**
 1217 **lizard *ex vivo* brain preparations.**

1218 **a-f:** Experiments in *ex vivo* brain preparation (**a**, top left) after cortex removal. **a** bottom,
 1219 Spontaneous SWRs recorded in the claustrum (CLA, <150 Hz). HP: 70-150-Hz filtered LFP,
 1220 showing ripples (bottom trace). **b**, Local pressure injection of 20 μ M TTX in claustrum and *post*
 1221 *hoc* assessment of injection with Evans blue (red, transverse section, bottom). **c**, TTX injected
 1222 in claustrum (shading) silences sharp-wave activity in CLA, but also (indirectly) in DVR. **d**,
 1223 Analysis of 4 experiments as in **c**. Filled circles: mean \pm s.e.m. CLA: * $p=0.029$, $T=26$, two-sided
 1224 Mann-Whitney rank-sum test. DVR: * $p=0.029$, $T=26$, two-sided Mann-Whitney rank-sum test.
 1225 **e**, Average trace (top) and standard deviation (shading) from 3,842 sharp-waves recorded from
 1226 claustrum of an *ex vivo* forebrain (alignment on trough). Bottom: high-pass signal intensity
 1227 (HPI, >70 Hz) aligned on sharp-wave trough, showing ripple alignment. **f**, Top: simultaneous
 1228 recordings from ipsilateral CLA and DVR in an *ex vivo* preparation. Bottom: cross-correlation
 1229 between simultaneous recordings in ipsilateral CLA and DVR, showing that CLA precedes
 1230 DVR by ~ 100 ms. **g**, Peri-stimulus time histogram for multiunit activity in cortex, in response
 1231 to ipsilateral CLA activation in an intact *ex vivo* forebrain. Experiment carried out in normal
 1232 ACSF at room temperature in the presence of 30 μ M 5HT to suppress spontaneous SWRs in
 1233 claustrum and 50 μ M CCh to raise cortex excitability. Claustrum stimulus: single 50 μ s
 1234 electrical pulse, delivered with a bipolar electrode. Cortex multi-unit activity recorded with
 1235 glass micropipette. **h**, Change in cortical firing rate (FR) measured in 200-ms-bin-after *vs.*
 1236 200ms-bin-before the CLA stimulus (as in **g**). Control: as in **g**. GBZ: gabazine (5 μ M), CGP;
 1237 CGP52432 (GABA_B antagonist, 2 μ M), $n = 4$ *ex vivo* brains from 3 animals each. The control
 1238 experiment shows that CLA stimulation has an immediate and reliable inhibitory effect on
 1239 cortex (#: significantly different from baseline, $p=0.017$, $t_3=4.8$, two-sided paired t -test). The
 1240 stimulation experiment in GABA blockers shows that CLA stimulation now slightly excites
 1241 cortex (**: significantly different from control, $p=2.0 \times 10^{-3}$, $t_6=-5.22$, two-sided Student's t -
 1242 test), suggesting that claustrum projections both activates and inhibits cortical neurons,
 1243 probably via direct excitatory projections and indirect inhibitory ones through interneurons (see
 1244 ref. 39 for rodent experiments). Short horizontal lines indicate means.

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1246 **Extended Data Fig. 9 | Further analysis of *in vivo* ibotenic-acid lesion experiments in**
1247 **sleeping *Pogona*.**

1248 **a**, Autocorrelation (top) and cross-correlation (bottom) of β -band activity in L and R DVR
1249 during sleep in an animal with bilateral claustrum lesions (see lesions in **d**). Note that the sleep
1250 rhythm (~3-min period) remains after claustrum lesions and therefore does not seem to depend
1251 on claustrum integrity. **b-c**, same as **a**, but with unilateral ibotenic-acid lesion in two animals
1252 (I and II). The non-lesioned (sham) side was injected with the same volume of PBS vehicle but
1253 with no ibotenic acid. Top: stippled line: sham; solid line: lesion. **d**, Nissl stains (1-3) of
1254 transverse sections of the brain of bilateral-CLA-lesion animal in **a** (shown also in Fig. 4b), at
1255 levels indicated in schematic at left. Note the claustral lesions (arrows, 1) visible as cell body
1256 loss, and the recording sites in L (2) and R (3) DVRs (stippled circles).

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1278 **Extended Data Fig. 10 | Further data on 5-HT projections to claustrum and their effects**
1279 **on sharp-wave ripple generation**

1280 **a**, Transverse section of claustrum double-labeled with DAPI (blue, nuclei) and 5HT (axonal
1281 fibers) antibodies. Note dense meshwork of serotonergic fibers. Scale bar: 50 μ m. **b**,
1282 Spontaneous SWR frequency in claustrum mini-slices as a function of superfused 5HT
1283 concentration. Red circles, individual experiments (slices). Black: mean and s.e.m.

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