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1 **Dynamic Resting State fMRI Analysis in Mice Reveals a**  
2 **Set of Quasi-Periodic Patterns and Illustrates their**  
3 **Relationship with the Global Signal**

4

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## 25 **Abstract**

26 Time-resolved ‘dynamic’ over whole-period ‘static’ analysis of low frequency (LF) blood-oxygen  
27 level dependent (BOLD) fluctuations provides many additional insights into the macroscale  
28 organization and dynamics of neural activity. Although there has been considerable  
29 advancement in the development of mouse resting state fMRI (rsfMRI), very little remains  
30 known about its dynamic repertoire. Here, we report for the first time the detection of a set of  
31 recurring spatiotemporal Quasi-Periodic Patterns (QPPs) in mice, which show spatial similarity  
32 with known resting state networks. Furthermore, we establish a close relationship between  
33 several of these patterns and the global signal. We acquired high temporal rsfMRI scans under  
34 conditions of low (LA) and high (HA) medetomidine-isoflurane anesthesia. We then employed  
35 the algorithm developed by Majeed et al. (2011), previously applied in rats and humans, which  
36 detects and averages recurring spatiotemporal patterns in the LF BOLD signal. One type of  
37 observed patterns in mice was highly similar to those originally observed in rats, displaying  
38 propagation from lateral to medial cortical regions, which suggestively pertain to a mouse Task-  
39 Positive like network (TPN) and Default Mode like network (DMN). Other QPPs showed more  
40 widespread or striatal involvement and were no longer detected after global signal regression  
41 (GSR). This was further supported by diminished detection of subcortical dynamics after GSR,  
42 with cortical dynamics predominating. Observed QPPs were both qualitatively and  
43 quantitatively determined to be consistent across both anesthesia conditions, with GSR  
44 producing the same outcome. Under LA, QPPs were consistently detected at both group and  
45 single subject level. Under HA, consistency and pattern occurrence rate decreased, whilst  
46 cortical contribution to the patterns diminished. These findings confirm the robustness of QPPs  
47 across species and demonstrate a new approach to study mouse LF BOLD spatiotemporal  
48 dynamics and mechanisms underlying functional connectivity. The observed impact of GSR on  
49 QPPs might help better comprehend its controversial role in conventional resting state studies.  
50 Finally, consistent detection of QPPs at single subject level under LA promises a step forward  
51 towards more reliable mouse rsfMRI and further confirms the importance of selecting an  
52 optimal anesthesia regime.

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58 **Keywords**

59 Mouse; Dynamic rsfMRI; Quasi-Periodic Pattern (QPP); Default mode network; Global signal  
60 regression; Medetomidine/isoflurane anesthesia

61 **Abbreviations**

62	<b>CC</b>	cross-correlation
63	<b>GSR</b>	global signal regression
64	<b>HA</b>	High anesthesia
65	<b>LA</b>	Low anesthesia
66	<b>LF</b>	Low frequency
67	<b>PAT</b>	Pattern
68	<b>QPP</b>	Quasi-Periodic Pattern
69	<b>RSN</b>	Resting State Network
70	<b>STC</b>	Sliding Template Correlation
71	<b>CAP</b>	Co-Activation Pattern

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## 84 **1. Introduction**

85 Resting state functional magnetic resonance imaging (rsfMRI) can be used to investigate  
86 functional connectivity (FC) between different brain regions by calculating temporal coherence  
87 between their spontaneous low frequency (LF) blood-oxygen level dependent (BOLD)  
88 fluctuations (Biswal et al. 1995; van den Heuvel & Hulshoff 2010; Damoiseaux et al. 2006). In  
89 humans, this allows identification of several resting state networks (RSNs) (Biswal et al., 1995;  
90 Cordes et al., 2000; Fox et al., 2006; Zhang et al., 2008), including two wide-scale anti-correlated  
91 RSNs, termed the 'default mode network' (DMN), containing regions active during rest, and the  
92 'task-positive network' (TPN), containing regions that become activated during task  
93 performance (Fox et al., 2005; Greicius et al., 2003). By investigating FC changes in these RSNs,  
94 rsfMRI enables the clinical investigation of multiple neurological disorders (Greicius, 2008; Lee  
95 et al., 2013; Zhang and Raichle, 2010).

96 Recently, rsfMRI has been performed in mice, allowing reliable detection of RSNs similar to  
97 those found in humans and primates (Liska et al. 2015; Gozzi & Schwarz 2015; Grandjean et al.  
98 2014; Zerbi et al. 2015; Sforazzini et al. 2014; Jonckers et al. 2011; Nasrallah et al. 2014). Initial  
99 applications in disease models demonstrate its usefulness to track down and disentangle  
100 underlying disease mechanisms (Shah et al. 2013; Shah et al. 2016; Liska & Gozzi 2016;  
101 Sforazzini et al. 2016; Stafford et al. 2014). With strict control over genetic and environmental  
102 conditions available in mice, mouse rsfMRI shows great promise as a pre-clinical tool to study FC  
103 changes in neurological disorders and enable fundamental research into mechanisms underlying  
104 LF BOLD (Keilholz et al., 2016).

105 RsfMRI studies are generally performed with an experimental and methodological paradigm  
106 that either assumes or imposes static FC, meaning that statistical interdependence of LF BOLD  
107 signals between different brain regions stays the same over the length of the entire scan (Biswal  
108 et al., 1995). During the last decade, studies in several species have demonstrated that this is not  
109 the case and that dynamic analysis of rsfMRI FC provides many additional insights into the  
110 macroscale organization and dynamics of neural activity (Calhoun et al., 2014; Deco et al., 2011;  
111 Hansen et al., 2015; Hutchison et al., 2013; Keilholz, 2014). Only just recently, Grandjean et al.  
112 (2017) showed for the first time that dynamic FC could be investigated in mice and allowed  
113 identification of several highly reproducible dynamic functional states. These states display  
114 complex inter- and intra-modular organization and shed new light on information processing in  
115 the mouse brain. Dynamic rsfMRI (drsfMRI) can also be applied to investigate pathology, better  
116 explaining observed FC differences in RSNs and improving distinction between disease and  
117 control groups (Sakoglu et al. 2010; Jones et al. 2012; Damaraju et al. 2014; Rashid et al. 2014;  
118 Grandjean et al. 2017).

119 Most commonly, drsfMRI is based on the sliding-window analysis (SWA) approach, where a FC  
120 metric of interest is investigated within short time windows that are incrementally shifted along  
121 the image series of the scan (Chang and Glover, 2010; Hutchison et al., 2013; Keilholz et al.,  
122 2013). For all windows, region-to-region FC matrices are obtained, which can be clustered to  
123 identify stable neural 'states' (Allen et al., 2014; Damaraju et al., 2014; Gonzalez-Castillo et al.,  
124 2015, 2014). SWA has shown great potential, even just recently in mice (Grandjean et al. 2017),  
125 yet there is a lot of controversy regarding its use. The approach further suffers from the  
126 dependence on user-defined window lengths and limitations of signal-to-noise ratio that can  
127 spuriously induce FC changes (Hindriks et al., 2015; Hutchison et al., 2013; Preti et al., 2016;  
128 Shakil et al., 2016). Given these limitations and the fact that FC is inherently an indirect readout  
129 of spontaneous LF BOLD coherences, recent approaches attempted to track down instantaneous  
130 single volume BOLD configurations that underlie the observed FC (Liu and Duyn, 2013; Preti et  
131 al., 2016; Tagliazucchi et al., 2012; Wu et al., 2013). This spurred the discovery of co-activation  
132 patterns (CAPs), which resemble known RSNs and help to better comprehend dynamic changes  
133 in SWA FC (Karahanoğlu and Van De Ville, 2015).

134 An interesting alternative to the CAP approach is the detection of recurring consecutive  
135 sequences of 'instantaneous' BOLD volumes, or so-called spatiotemporal patterns, which can  
136 better capture the temporal evolution of RSNs. Such patterns were first observed in the  
137 anesthetized rat by Majeed et al. (2009), using a high temporal resting state scan, and consist of  
138 bilateral intensity waves propagating from lateral somatosensory to medial cortical areas.  
139 Majeed et al. further developed an automated algorithm to track down these spatiotemporal  
140 patterns, reproducing their results in rats and discovering similar patterns in humans, where  
141 they alternately involve brain regions which are part of the DMN and TPN (Majeed et al.,  
142 2011). Due to their repeated occurrence and cyclical behavior, they were termed Quasi-Periodic  
143 Patterns (QPPs) (Garth John Thompson et al., 2014). A prominent finding is that QPPs can be  
144 observed across species and are consistently detected at the single subject level with high  
145 occurrences, making them promising candidates to contribute to both static and dynamic FC.  
146 Preliminary data in humans with major depressive disorder supports this hypothesis (Wang et  
147 al., 2016). In rats, QPPs were also detected using cerebral blood volume (CBV)-weighted resting  
148 state scans (Magnuson et al., 2010), confirming their contribution to LF BOLD haemodynamics.  
149 Furthermore, they seem to have a neural precedent through correlation with infraslow local  
150 field potentials (Thompson et al. 2014; Thompson et al. 2015; Pan et al. 2013; Thompson et al.  
151 2014). Altogether, QPPs open up a new perspective on studying FC and dynamics of LF BOLD.

152 In the current study, we thus investigated whether QPPs can be detected in mouse rsfMRI. If  
153 similar patterns could be observed, this would further validate the relevance of QPPs as a

154 mechanism contributing to spontaneous BOLD coherences, and at the same time it would help to  
155 validate mouse rsfMRI as a pre-clinical tool by confirming interspecies preservation of resting  
156 state dynamics. Single subject detection of QPPs would constitute a step forward in more  
157 reliable mouse rsfMRI analysis, provide new perspectives on studying mechanisms underlying  
158 FC, and mark the development of a potential new biomarker for neurological disorders. Given  
159 the controversy on the impact of anesthesia on FC readouts in mice, we compare a low  
160 anesthesia regime of medetomidine and isoflurane, illustrated to allow optimal FC preservation  
161 (Grandjean et al., 2014), with an analogous higher anesthesia regime. Finally, due to the  
162 hypothesized large-scale nature of QPPs, we investigate how their behavior and detection is  
163 affected by global signal regression.

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## 182 **2. Materials and Methods**

### 183 **2.1 Animals, preparation and anesthesia**

184 All procedures were performed in strict accordance with the European Directive 2010/63/EU  
185 on the protection of animals used for scientific purposes. The protocols were approved by the  
186 Committee on Animal Care and Use at the University of Antwerp, Belgium (permit number 2014-  
187 04), and all efforts were made to minimize animal suffering.

188 Eleven male C57BL/6J mice (Charles River) between 22 and 24 weeks old were studied. Animals  
189 were initially anesthetized with 3.5% isoflurane and maintained at 2% during handling. The  
190 animals' heads were positioned with incisors secured over a bite bar and fixed with ear bars.  
191 Ophthalmic ointment was applied to the eyes and a rectal temperature probe was used to  
192 monitor animal temperature, which was kept stable at 37°C via a hot air supply (MR-compatible  
193 Small Animal Heating System, SA Instruments, Inc.). Physiological parameters were measured  
194 via a pressure sensitive pad, to assess breathing rate, and a fiber-optic pulse oximeter placed  
195 over the tail, to assess heart rate and O<sub>2</sub> saturation (MR-compatible Small Animal Monitoring  
196 and Gating system, SA Instruments, Inc.). The respective signals were sampled at 15.895Hz for  
197 the low anesthesia animal group (Signal breakout module, SA Instruments, Inc.). Using Short-  
198 Time Fourier Transform (window size 19.994s; intersperse 0.503s), followed by DC-component  
199 filtering, respiration and cardiac rate were determined as the frequencies corresponding to max  
200 power intensities for each time point.

201 Following preparation, animals received a bolus injection of medetomidine (Domitor, Pfizer,  
202 Karlsruhe, Germany), after which isoflurane was gradually lowered to 0.4% over the course of  
203 15min and maintained at this level for the remainder of the imaging procedures. A subcutaneous  
204 catheter was inserted to allow continuous infusion of medetomidine starting at 15min post-  
205 bolus. Animals were scanned under a high anesthesia regime (HA; bolus 0.3mg/kg & infusion  
206 0.6mg/kg/h; n=11) and two weeks later under a low anesthesia regime (LA; bolus 0.05mg/kg &  
207 infusion 0.1mg/kg/h; n=11), to assess the impact of anesthesia on spatiotemporal dynamics in  
208 LF BOLD. Two animals from the HA group were excluded from the presented analysis, due to  
209 acquisition with offset imaging parameters (flip angle 90° instead of 55°). High temporal  
210 resolution functional resting state scans under HA and LA were acquired respectively 30min  
211 post-bolus, lasting 20min, and 40min post-bolus, lasting 10min. Conventional lower temporal  
212 resolution functional resting state scans were acquired in the LA group 30min post-bolus, lasting  
213 5min. These scans were acquired to compare conventional 'static' rsfMRI analysis across both  
214 scan types in the same session, so that QPPs could be related to whole-brain dynamics (cfr. 2.2-  
215 2.4). Both conventional and spatiotemporal analysis did not show any significant differences in  
216 the first or last 10min of the HA group (data not shown). Great care was taken to keep



217 procedures and conditions identical across animals, with preparatory handling never exceeding  
218 10min.

## 219 **2.2 RsfMRI acquisition**

220 MRI procedures were performed using a Bruker Biospec 9.4T system (Bruker Biospin MRI,  
221 Ettlingen, Germany), with a four-element receive-only phase array coil (RAPID MR international,  
222 Ohio, USA) and a volume resonator for transmission. Anatomical images were acquired in the  
223 sagittal, coronal and axial plane to allow exact and reproducible positioning of axial slices  
224 (0.4mm thickness, 0.1mm inter-slice). Slices were positioned to allow optimal targeting of  
225 cingulate and somatosensory areas, centered 0.1mm caudally of bregma according to a  
226 stereotaxic mouse brain atlas (Paxinos and Franklin, 2007). The anatomical reference scan was  
227 acquired with a spin echo Turbo-RARE sequence: field of view (FOV) (20x20)mm<sup>2</sup>, matrix  
228 dimensions (MD) [256x256], repetition time (TR) 3000ms, effective echo time (TE) 33ms, and  
229 RARE factor 8. B0 field maps were acquired to allow shimming in the target area of interest.  
230 High temporal resolution rsfMRI scans were positioned according to the anatomical reference  
231 scans and were acquired using a gradient-echo echo-planar imaging (EPI) sequence: FOV  
232 (20x20)mm<sup>2</sup>, MD [128x64], **slices 3**, flip angle 55°, bandwidth 400kHz, **TR 500ms**, and TE  
233 16ms. The shorter TR enables an imaging sampling frequency of 2Hz, necessary to investigate  
234 spatiotemporal dynamics at short time scales. Conventional temporal resolution rsfMRI scans  
235 with matching slice positions were also acquired for the LA group, using a gradient-echo EPI  
236 sequence: FOV (20x20)mm<sup>2</sup>, MD [128x64], **slices 16**, flip angle 90°, bandwidth 400kHz, **TR**  
237 **2000ms**, and TE 16ms.

## 238 **2.3 Preprocessing**

239 All analyses were performed using MATLAB2015a (Mathworks, Natick, MA). Motion parameters  
240 for rsfMRI images were computed using 3 rigid body parameters for the high temporal  
241 resolution low slice count datasets, which retains all 3 slices for single subject analysis, and 6  
242 rigid body parameters for the conventional high slice count dataset. RsfMRI images were then  
243 realigned, normalized to a user-defined reference subject, smoothed ( $\sigma = 2$  pixels), and motion  
244 vectors were regressed out of the image series. These preprocessing steps were performed  
245 using Statistical Parametric Mapping (SPM12) software (Wellcome Department of Cognitive  
246 Neurology, London, UK). Afterwards, image series were band-pass filtered with a FIR filter  
247 between 0.01-0.2Hz, quadratic detrended and normalized to unit variance. Before and after  
248 filtering, transient time points at the start and end of the image series were removed.  
249 Consecutive group-level analysis of the high temporal resolution low slice count datasets was  
250 performed solely on the center slice, given that the first and last slices were lost during the  
251 normalization process. For detection of spatiotemporal patterns, images were investigated with

252 and without global signal regression, and a brain mask was employed that excludes the  
253 ventricles to avoid their contribution to spatiotemporal pattern detection.

#### 254 **2.4 Conventional resting state fMRI analysis**

255 Group independent component analysis (ICA) was performed using the GIFT toolbox (v4.0a)  
256 (Calhoun et al., 2004) on data that was not motion regressed. For the high temporal resolution  
257 data, where only the single center slice was investigated, we tested several different numbers of  
258 independent components (IC). When more than six ICs were used, this caused the observation of  
259 unilateral components, while when six ICs were used, this preserved the integrity of bilateral  
260 BOLD signals matching known neuroanatomical regions. Using these criteria, 6 ICs were  
261 empirically determined appropriate for single-slice analysis. For the conventional 16-slice lower  
262 temporal resolution dataset, we employed 15 ICs, based on preceding literature (Shah et al.  
263 2015; Shah et al. 2016; Sforazzini et al. 2014). All ICA analysis was run on variance-normalized  
264 data, filtered between 0.01-0.2Hz, and using the Infomax algorithm with no auto-filling of data  
265 reduction values. A brain mask was used to remove signals exterior to the brain. Stability  
266 analysis was performed using the ICASSO algorithm, rerunning the ICA 50 times with a minimal  
267 cluster size of 30 and maximal of 50. All other default parameters of GIFT were left unaltered.

268 For conventional 'static' functional connectivity (FC) analysis, regions of interest (ROI),  
269 measuring 6 voxels, were selected matching both a stereotaxic mouse brain atlas (Paxinos and  
270 Franklin, 2007) and overlapping with maximal intensities in ICs determined with ICA. ROIs were  
271 subsequently used to construct ROI-based FC matrices and seed-based FC maps. FC values were  
272 Fisher Z-transformed. For within group statistical analysis of ICs and seed-based FC maps, one  
273 sample T-tests (two-tailed,  $p < 0.05$ ) were performed with false discovered rate (FDR)  
274 correction. For between group statistical analysis of the ROI-based FC matrix, a paired T-test  
275 (two-tailed,  $p < 0.05$ , FDR-correction) was used. All statistical analyses were performed using  
276 SPM12 software.

#### 277 **2.5 Spatiotemporal pattern-finding algorithm and k-means clustering**

278 To track down putative recurring spatiotemporal patterns, we employed the algorithm from the  
279 group of Shella Keilholz that was previously used to identify Quasi-Periodic Patterns (QPP) in  
280 humans and rats (Majeed et al., 2011) (The respective MATLAB code is available upon request  
281 via contact with the corresponding author). The algorithm uses a data-driven correlation  
282 approach that identifies spatiotemporally similar subsections in the functional (BOLD) image  
283 series. It essentially increases the signal-to-noise ratio of repetitive spatiotemporal blocks,  
284 allowing averaging and preservation of temporal information. In brief, the algorithm works by  
285 first isolating a random seed section, consisting of a series of consecutive images at a random  
286 starting time point. The length of the spatiotemporal template (i.e. the window size or number of

287 images) is defined by the user. The template is then incrementally shifted (a single TR) along the  
288 image series and a Pearson correlation value is calculated at every time point (**movie 1**). A  
289 Sliding Template Correlation (STC) time series is derived which identifies when the template is  
290 similar to the image series. Peak correlation values, exceeding an arbitrary threshold, are used to  
291 select and average the associated image series at related time points into a new updated  
292 template, which can then be used to extract correlations in the same way. This process is  
293 iteratively repeated until the template no longer changes, i.e. the cross-correlation (cc) of  
294 templates of two consecutive iteration steps  $> 0.9999$ . At this point, the QPP is established and  
295 correlation peaks in the STC exceeding the threshold reflect moments of pattern occurrences. A  
296 more detailed description can be found in the original article (Majeed et al. 2011). In the current  
297 study, we employed the same correlation thresholds.

298 Because the starting time point of the initial random seed template can affect the final outcome,  
299 the entire process described above is repeated several times to derive a set of QPPs for a  
300 respective window size under investigation. To identify a single representative QPP from this  
301 set, each of the obtained QPPs is transformed into a its vector form, which measures the total  
302 amount of voxels comprised in the image mask multiplied by the amount of image frames within  
303 the QPP (i.e. vectors from each masked image frame are concatenated). These vectors are then  
304 clustered via k-means clustering, using correlation as a distance metric. The optimal QPP is  
305 determined by tracking down the QPP, which presents the maximal silhouette value within the  
306 cluster with the highest average silhouette values. A silhouette value indicates how similar an  
307 object is to its own cluster and how much it differs from other clusters. K-means clustering has  
308 been employed previously in rsfMRI literature to cluster functional images (Anderson et al.,  
309 2010; Liu and Duyn, 2013). The way k-means is employed in this study, without prior phasing of  
310 QPPs, essentially tracks down the QPP that was most robustly detected while being at a specific  
311 phase.

312 The procedures described above were performed for all investigated template window sizes.  
313 Data presented in this work was either analyzed at group level, through concatenation of  
314 normalized image series, which allowed pattern identification in a single center slice, or at  
315 individual subject level, which allowed pattern identification in all three slices. We employed  
316 respectively 500 (group) and 200 (subject) random starting time points.

## 317 **2.6 Spatial and temporal cross-correlation**

318 QPPs were compared with each other via two basic ways. The STCs of individual QPPs describe  
319 their similarity and timing with respect to the image series. By performing cc between STCs, one  
320 can establish a measure of QPP similarity, and identify their temporal offsets from one another.  
321 The latter information is used to display QPPs at their appropriate timing (e.g. **Fig.3A**) and to

322 phase-align them (e.g. **Fig.2**). Another option to identify QPP similarity is via circular cc of their  
323 spatiotemporal images. Each single QPP is transformed into its vector form, which measures the  
324 total amount of voxels comprised in the image mask multiplied by the amount of image frames  
325 within the QPP. The resultant vector can be circularly shifted (using the MATLAB 'circshift'  
326 function), with increments measuring the length of indices in the image mask (i.e. a single  
327 frame), to calculate the cc.

## 328 **2.7 Data-driven identification of pattern optimal window size**

329 The ideal window size of putative mouse rsfMRI QPPs is unknown. Previous strategies used  
330 visual inspection and pattern speed as a reference (Majeed et al. 2011; Majeed et al. 2009;  
331 Thompson et al. 2014). Here, we developed an automated processing tool to determine optimal  
332 window sizes in a data-driven fashion, termed fractional average correlation (FA).

333 In a set of QPPs of the same pattern type, where each QPP is determined at a different window  
334 size and at the same phase, each individual pattern is subdivided into all possible consecutive  
335 fractions of a fixed length specified by the smallest window size investigated. To illustrate, in the  
336 presented analysis the smallest window size is set at 6TRs (3s), meaning that a pattern of e.g.  
337 24TRs long will be divided into 19 fractions of 6 consecutive images, each shifted by 1 TR  
338 (**Fig.S1A**). Each individual fraction from a QPP at a specific window size is then treated as a  
339 reference, and the maximal cc value is calculated with respect to the complete 'target' QPP at  
340 another window size (**Fig.S1A-B**). The average of the resultant cc values represents a measure of  
341 how many fractions the reference and target QPP have in common, i.e. the FA. The FA value is  
342 calculated for all possible combinations of window sizes, constructing an  $n \times n$  correlation  
343 matrix, where  $n$  represents the number of different window-sized QPPs and each column  
344 represents the FA values for a specific reference QPP with each respective QPP in the set  
345 (**Fig.S1C**). By averaging the FA matrix across its columns, the set-wise FA value for each QPP is  
346 determined.

347 The power of this approach lies in the notion that target QPPs at smaller window sizes than the  
348 reference QPP under investigation have less fractions in common, given that they only represent  
349 a subpart. Comparing larger reference QPPs with non-matching subparts in short target QPPs  
350 decreases the FA value. Long target QPPs, however, are likely to contain the full pattern and will  
351 therefore show a high FA. The tipping point of increasing FA, before a plateau is reached, reflects  
352 the optimal window size.

## 353 **2.8 Data-driven pattern classification using hierarchical clustering**

354 Within a set of patterns, determined at a given window size for the group wide analysis, multiple  
355 types of spatiotemporal patterns could be visually distinguished. To validate visual classification,

356 set-wise  $n \times n$  cc matrices are constructed via either spatial or STC cc (cfr. 2.6), where  $n$  is the  
357 number of QPPs compared ( $n = 500$ ). The columns of these symmetrical matrices are used to  
358 perform hierarchical clustering, using correlation as a distance metric. Cc scopes QPP similarity  
359 with one another and consecutive clustering of set-wise cc values for each QPP further  
360 accentuates their overall relationship. Opposed to the employed strategy for k-means clustering  
361 (cfr. 2.5), cc inherently aligns QPP phase during the clustering process. Hierarchical clustering is  
362 an unsupervised approach, thus removing potential bias from cluster number pre-selection.  
363 Visual observation of sorted block designs and inspection of their content then serves to validate  
364 pattern subtype separation.

## 365 **2.9 Phase sorting of spatiotemporal patterns**

366 After global signal regression (GSR), opposite phase detections of QPPs dominated hierarchical  
367 clustering off cc matrices. To illustrate that only a single QPP was detected at opposite phases,  
368 QPPs can first be sorted based on their phase, prior to performing clustering. The Cingulate (Cg)  
369 component was present in both LA and HA groups, as determined with ICA and observed in all  
370 established QPPs. Masks were therefore constructed from the Cg independent component  
371 thresholded T-maps (cfr. 2.4), which were subsequently used to calculate the average Cg  
372 intensity across each image frame of the QPPs. This establishes a time series of the Cg region  
373 that can be used to phase sort QPPs based on either displaying first high or low Cg activity.

## 374 **2.10 Global co-activation patterns**

375 To more closely investigate the shape of the global signal, an analysis methodology is employed  
376 that was inspired by the CAP approach (Liu and Duyn, 2013). Briefly, in the latter, supra-  
377 threshold crossings of signal in chosen neuroanatomical seed locations are used to extract fMRI  
378 frames to be averaged or further processed by clustering. This allows the detection of  
379 instantaneous fMRI volumes that contribute to FC and known RSNs. The same strategy is  
380 adapted for the current study, but using the global signal as a seed region, and increasing the  
381 extent of averaged fMRI frames to a window centered on peak intensity time points. Essentially,  
382 all peaks in the global signal of the concatenated group image series are identified and out of  
383 these, the subset highest peaks are chosen. The latter is determined by matching the amount of  
384 chosen peaks to the average occurrences of QPPs (at their ideal window length) that we  
385 hypothesize to be related to the global signal (pattern 2 & 3 for LA; pattern 2 & 4 for HA; cfr.  
386 Results). Given the resultant set of peak time points  $T = \{T_1, T_2, \dots, T_P\}$ , with  $P$  being the number  
387 of peaks, a 3D matrix  $Y = [X_{T_i-WL/2}, X_{T_i-WL/2+1}, \dots, X_{T_i+WL/2}]$  is constructed, with  $X_{T_i}$  being the image  
388 frame at time  $T_i$  and  $WL$  the chosen window length for frames to be averaged. The matrix  $Y$  is  
389 averaged across the third dimension to produce the global CAP.

390

### 391 **3. Results**

392 We acquired high temporal resolution rsfMRI scans in a set of 11 C57BL/6J mice. Subjects were  
393 scanned under a high anesthesia regime (**HA**, 0.3mg/kg bolus & 0.6mg/kg/h infusion of  
394 medetomidine) and were rescanned two weeks later under a low anesthesia regime (**LA**,  
395 0.05mg/kg bolus & 0.1mg/kg/h medetomidine). Two subjects of the HA group were not  
396 included in the presented analysis due to acquisition with offset parameters. We focus on the  
397 outcome of the LA group, while results under HA are more briefly addressed at the end of each  
398 section and related figures are presented in the supplementary data. A direct comparison is  
399 made in sections 3.1 and 3.7.

#### 400 **3.1. Spectral information and resting state network functional connectivity**

401 Conventional rsfMRI analysis in rodents typically employs a TR between 1-2s and investigates  
402 LF BOLD fluctuations filtered in ranges between 0.01-0.1Hz or 0.01-0.3Hz (Gozzi and Schwarz,  
403 2015; Grandjean et al., 2014; Jonckers et al., 2015; Liska et al., 2015). To enable detection of  
404 propagating spatiotemporal patterns, we acquired scans with a TR of 500ms, providing a  
405 spectrum with a wider range (up to 1Hz) and higher temporal resolution. Visual inspection of  
406 group-average power spectra revealed that under LA the highest spectral information content  
407 was confined to the range below 0.2Hz, while power in the HA group was in general lower  
408 (**Fig.1A**). The spectra of both groups displayed a high peak below  $\sim 0.015$ Hz, consistent with  
409 literature suggesting band-pass filtering above 0.01Hz to remove baseline drift (Bianciardi et al.,  
410 2009; Yan et al., 2009).

411 Group-level analysis was restricted to a single slice, due to image normalization (cfr. 2.3). After  
412 0.01-0.2Hz band-pass filtering, group ICA of the LA data revealed the presence of six meaningful  
413 bilateral RSNs, overlapping with neuroanatomical locations (**Fig.1B**): Ventral Pallidum (VP),  
414 ventro-lateral Caudate Putamen (Cpu vl), dorsal Caudate Putamen (Cpu d), Somatosensory area  
415 1 (S1) forelimb and hindlimb (HL/FL), Somatosensory area 2 (S2), and Cingulate cortex (Cg).  
416 These six RSNs appeared to match known mouse RSNs (Zerbi et al. 2015; Grandjean et al. 2017;  
417 Liska et al. 2015; Sforazzini et al. 2014). To validate if single-slice RSNs in short TR (0.5s) data  
418 match with those in conventional whole-brain lower TR (2s) data, 16-slice rsfMRI scans were  
419 acquired during the same LA sessions. ICA of this data indeed revealed the same RSNs (**Fig.1E**).  
420 However, in this data we observed only a single Cpu component and two somatosensory  
421 components, of which the S1 HL/FL component partially overlapped with S1 barrel field (BF).

422 When LA RSNs of the short and long TR data were compared, it became apparent that S2 and Cg  
423 components, determined in the short TR data, overlap respectively with the large-scale lateral  
424 cortical network and the Default Mode like network (DMN) in the long TR data. The lateral  
425 cortical network has been suggested to represent a potential mouse Task-Positive like network

426 (TPN) (Gozzi and Schwarz, 2015; Liska et al., 2015). These findings suggested that short TR  
427 single-slice RSNs pertain to whole-brain networks. This supported the conceptual paradigm that  
428 a well-positioned single-slice investigation allows a view into whole-brain dynamics. Under HA,  
429 ICA of the short TR data revealed similar single-slice RSNs as under LA, but with a less  
430 pronounced bilateral extent (**Fig.1B**), suggesting compromised FC.

431 Finally, we also directly investigated FC. Left and right ROIs, matching with both the RSN peak  
432 intensities and a mouse stereotaxic brain atlas (Paxinos and Franklin, 2007), were used to  
433 construct a FC matrix in which HA and LA groups were compared (**Fig.1C**). S2, Cg, and Cpu d  
434 showed significantly decreased bilateral FC in the HA group (two-sample T-test, two-tailed,  
435  $p < 0.05$  FDR-corrected). Cpu d – Cg, VP – Cg, S1FL – S2, and Cpu vl – S2 also showed significantly  
436 lowered FC under HA. Seed-based FC analysis of the short TR LA data resulted in FC maps that  
437 were highly similar to the respective RSNs (**Fig.1B&D**). For the HA short TR data, no FC was  
438 apparent after significance thresholding. Seed-based FC analysis of the long TR data similarly  
439 reproduced whole brain RSNs and additionally allowed the detection of both Cpu d and Cpu vl  
440 components (**Fig.1F**).

441 Lastly, it should be noted that, while all observed RSNs matched generally with known mouse  
442 RSNs, some inconsistencies in homotopic representation and functional coupling were apparent  
443 (cfr. 4.6). Therefore, to provide full transparency into our findings, we additionally present the  
444 remaining ICA-derived RSNs that were observed in the LA whole-brain 2s-TR data (**Fig.2**). These  
445 RSNs displayed bilateral coupling similar to what has been observed in other mouse rsfMRI  
446 studies (Grandjean et al., 2014; Liska et al., 2015).

### 447 **3.2. Exploring group wide spatiotemporal dynamics**

448 Without a clear *a priori* knowledge of the time span of putative QPPs, we investigated a series of  
449 different window sizes. This was achieved by running the spatiotemporal pattern finding  
450 algorithm on the center slice image series of all subjects (cfr. 2.5).

451 We observed consistent and well-defined bilateral spatiotemporal patterns for all investigated  
452 window sizes ranging from 3 to 18s (**Fig. 3C**). To interpret the findings across different window  
453 sizes, three major factors need to be considered: (1) the algorithm is to some extent insensitive  
454 to phase, meaning that patterns can be detected at different start times; (2) small window sizes  
455 inherently only scope part of a larger pattern; (3) different window sizes can skew detection  
456 towards different patterns. In a first approach, we addressed this by making use of each  
457 observed pattern's correlation time series, determined via sliding window correlation with the  
458 image series (cfr. 2.5 & 2.6), which we refer to as the Sliding Template Correlation (STC) (**movie**  
459 **1 & Fig.3A**). By calculating the cc of different patterns' STCs at different window sizes, we can

460 establish their temporal overlap and adjust their phase so that the patterns align (**Fig.3B**). Using  
461 visual inspection, this initial exploration of the data revealed a non-redundant full-size QPP at  
462 12s. Phasing to the STC of this QPP allowed a meaningful alignment of all observed QPPs  
463 (**Fig.3C**), facilitating comparison and interpretation.

464 Starting from a window size of 12s and upward, we observed high and consistent STC cc and  
465 QPP spatial cc, with average values of respectively  $(0.96\pm 0.03)$  and  $(0.94\pm 0.01)$ . STC cc from  
466 shorter window sizes with the STC at 12s were however considerably lower and less consistent  
467  $(0.66\pm 0.10)$ , while QPP cc was less diminished  $(0.81\pm 0.04)$ . This discrepancy in STC cc at shorter  
468 and longer window sizes suggested the detection of different spatiotemporal patterns, which  
469 was also hinted by visual inspection of Fig.2C (cfr. 3.3).

470 The same analysis was performed for the HA group (**Fig.S2**), where an optimal window size was  
471 visually determined at 7.5s. STC cc  $(0.84\pm 0.09)$  and QPP cc  $(0.89\pm 0.05)$  were high across all  
472 window sizes. This initially suggested the observation of only one single spatiotemporal pattern.

### 473 **3.3. Multiple Quasi-Periodic Patterns**

474 The group wide QPPs displayed in Fig.2C were derived from iteratively running the algorithm  
475 500 times per window size and selecting the optimal one via k-means clustering and silhouette  
476 classification (cfr. 2.5). We repeated this analysis at each window size with 100 iterations, but  
477 now visually inspected all individual QPPs to determine the full repertoire. We consistently  
478 observed a set of 3 different QPPs (**Fig.4A-B & movie 2**), which could be detected at almost all  
479 window sizes (**Fig. 4D**). Pattern identification was supported by their high spatial similarity with  
480 known RSNs in mice (**Fig.1B&D**, cfr. 4.2). For the purpose of consistent classification, we  
481 employed a set of selection criteria that describes their behavior (**Fig.4B**). Pattern 1 (PAT1) first  
482 displays high intensity in lateral cortices centered on S2, and subsequently also involves S1  
483 areas, Cpu vl, and to some extent the enthorhinal (En) and insular (I) cortices. The pattern  
484 further spreads with lower intensity along Cpu d, towards medial cortical areas centered on Cg.  
485 Regional contrasting high and low intensities are marked by a complementing positive and  
486 negative cycle, followed by a prolonged more global negative intensity. PAT2 displays  
487 simultaneous high intensity in the Cpu d and Cg. PAT3 starts similar to PAT1 with lateral high  
488 intensity, which now becomes more widespread involving a larger area of Cpu, S1, En and Cg.  
489 Both PAT2 and PAT3 display a negative wave to complete the cycle.

490 Classification of QPPs allowed us to estimate the detection rate across window sizes (**Fig.4D**).  
491 PAT2 and PAT3 displayed a bell-shaped curve, with detection rates exceeding that of PAT1 at  
492 window sizes between 7.5-10.5s. PAT1 displayed a U-shaped curve, with higher detection rates  
493 at the smallest and largest window sizes. Especially interesting is the take-over at 12s and



494 upward, exceeding PAT2 and PAT3 detection rates. The observed distributions of detection rates  
495 were strongly in line with QPPs determined in the general analysis using k-means clustering and  
496 silhouette detection (**Fig.3C**). To validate if QPPs in the latter were representative, the k-means  
497 algorithm was iterated 10 times and the outcomes were visually inspected. Below 9s, pattern  
498 detection was highly consistent, always finding the same QPPs, at 9s a mixture of PAT2 and  
499 PAT3 was found, at 10.5s a mixture of PAT1 and PAT3, and above 12s a mixture of PAT1 and  
500 PAT3 with PAT1 dominating (74%). These findings were in line with presented results and  
501 illustrate how k-means clustering becomes less reliable towards longer window sizes.

502 To further investigate this skewed detection, we isolated QPPs and their associated STCs for  
503 each type of pattern at every window size. STC cc was high and consistent at window sizes of  
504 10.5-18s for PAT1 ( $0.93\pm 0.02$ ) and 6-13.5s for PAT2 ( $0.94\pm 0.03$ ), while PAT3 seemed less  
505 confined to a specific range (6-16.5s;  $0.88\pm 0.06$ ) (**Fig.4C**). QPP cc was high across all window  
506 sizes: PAT1 ( $0.94\pm 0.04$ ), PAT2 ( $0.87\pm 0.10$ ) & PAT3 ( $0.92\pm 0.06$ ). PAT2 QPP cc was higher at  
507 window sizes of 6-13.5s ( $0.94\pm 0.02$ ). Further, PAT2 and PAT3 displayed higher occurrence rates  
508 (i.e. the amount of correlation peak threshold crossings in the STC) at window sizes below 10.5s  
509 (**Fig.4F**), but became equal with PAT1 afterwards.

510 After establishing the presence and behavior of 3 individual patterns, we used visual inspection,  
511 QPP detection rate (**Fig.4D**), and FA (cfr. 2.7) (**Fig.4E**) to determine optimal window sizes for  
512 each: **PAT1 12s, PAT2 9s and PAT3 9s**. These optimal sizes seem in line with the skewed  
513 detection rates across window sizes. All three patterns were observed throughout the different  
514 subjects, with PAT1 and PAT3 displaying higher variability (**Table1**, upper panel). An overlay of  
515 each pattern's STC, determined for its respective ideal length, illustrated their overall coincident  
516 behavior (**Fig.4G**). Although there was variation in timings and temporal correlation strength,  
517 patterns appeared to often be co-active, but could nonetheless be separated by the  
518 spatiotemporal pattern-finding algorithm. Specifically, PAT2 & PAT3 appeared to lag behind  
519 PAT1, causing their high intensity phase to fall in between PAT1's S2 - Cg switch (**Fig.4A**).

520 Half cycle times, defined as the time to change from maximal to minimal intensity of a brain  
521 region within the QPP, were similar across all three patterns and different window sizes,  
522 averaging to approximately 4.6s (**Table2**, upper panel). Propagation time, defined as the time  
523 delay of maximal intensity occurring in one brain region within the QPP after maximal intensity  
524 detection in another region, from lateral (S2 or Cpu) to medial regions were different between  
525 the patterns, being shorter for PAT2 and PAT3.

526 Classification analysis was also performed on the HA group, which originally displayed only one  
527 type of pattern (**Fig.S2A**) that appeared highly similar to PAT2. Further inspection revealed

528 detections of patterns similar to PAT1 and a fourth type (**Fig.S3A & movie 3**). The latter  
529 appeared similar to PAT2, but with more widespread and ventral involvement of Cpu, and less  
530 contribution of the medial cortex. QPPs further tended to display more unilateral behavior or  
531 bilateral delays.

532 Under HA, PAT2 detection rate seemed to be dominant across all window sizes (**Fig.S3C**), in line  
533 with the QPPs observed in the general analysis. Repeating the k-means algorithm for the HA  
534 group similarly revealed that mostly PAT2 was detected. STC cc revealed that PAT1 ( $0.73\pm 0.09$ )  
535 could now only be consistently detected up to a window size of 7.5s, while PAT2 ( $0.84\pm 0.09$ ) and  
536 PAT4 ( $0.73\pm 0.10$ ) were similar across all window sizes (**Fig.S3B**). Overall cc values were lower  
537 for HA than for LA, illustrating increased difficulty of consistent observations. Occurrence rate  
538 was similar across all three patterns, averaging  $\pm 0.8$  occurrences/min (approx. half that of under  
539 LA:  $\pm 1.5$  occurrences/min), and they were detected in all subjects (**TableS1**, upper panel). Ideal  
540 window sizes for all types of HA patterns were observed at 7.5s (**Fig.S3D**), while half cycle times  
541 averaged to  $\pm 3.7$ s. Consistent with the respective spatiotemporal shape, and as was observed in  
542 the LA group, propagation time from lateral to medial was shorter in PAT2 and PAT4 than in  
543 PAT1 (**TableS2**).

#### 544 **3.4 Data-driven validation confirms multiple Quasi-Periodic Patterns**

545 Visual classification might suffer from user bias, leading to a potentially wrongful identification  
546 of three separate patterns under LA. To validate our findings, we employed a novel approach to  
547 cluster spatiotemporal patterns, utilizing hierarchical sorting of pattern cc matrices (cfr. 2.8).  
548 Individual patterns at respective window sizes (7.5-13.5s) were clustered using either their  
549 spatial structure (**Fig.5A**) or their STC (**Fig.5B**). In both cases clustering was most successful at  
550 shorter window sizes, clearly indicating the presence of three separate clusters. Visual  
551 inspection of their content revealed that clusters predominantly contained a single type of  
552 pattern, confirming the existence of three patterns types. Similarly, the sorted average cc of all  
553 individual patterns with every other pattern revealed step-wise transitions, confirming clear  
554 pattern distinction.

555 With increasing window size clustering became more difficult and pattern separation less clear.  
556 This was similar to the observations made for k-means clustering and was to be expected given  
557 the increasing dimensionality of data to be clustered. Towards longer window sizes PAT1  
558 detection rate increased, which matched preceding results (**Fig.4**). PAT2 and PAT3 ideal window  
559 sizes had been determined at 9s, thus increasing the window size under investigation forces the  
560 detection algorithm to find longer patterns that contain more noise or overlap with other  
561 patterns, potentially contributing to less efficient clustering at higher window sizes. The

562 proposed clustering method at this point seemed not to be sufficiently reliable to replace visual  
563 classification, but did serve valuable to illustrate the existence of three different patterns.

564 Under HA, hierarchical clustering similarly allowed separation of PAT1, PAT2 and PAT4 at short  
565 window sizes (**Fig.S4**). However PAT2 and PAT4 were less clearly separable, suggesting some  
566 potential overlap. At longer window sizes pattern cc was very low and only minor clustering  
567 could be observed.

### 568 **3.5. Global signal regression accentuates pattern 1 while removing spatiotemporal** 569 **dynamics closely related to the global signal.**

570 After GSR, using the k-means algorithm and silhouette scoring, only a single QPP could be  
571 observed across all window sizes (**Fig.S5A**). Repeating k-means clustering 10 times consistently  
572 reproduced this finding. GSR QPPs were highly consistent across window sizes (STC cc  
573  $0.96\pm 0.02$  & QPP cc  $0.96\pm 0.04$ ) (**Fig.S5B**), with their ideal length judged at 9s by taking into  
574 account FA. GSR QPPs were highly similar to PAT1 (QPP cc 0.88), displaying the same lateral to  
575 medial cortical propagation (**Fig.6A & movie 4**). The spatiotemporal profile after GSR was  
576 marked by a sharper contrast between positive and negative intensities and a loss of the  
577 prolonged negative intensity observed in PAT1.

578 Visual inspection of all GSR QPPs indicated that PAT2 and PAT3 detection was abolished.  
579 Hierarchical clustering appeared to produce separate clusters, which would suggest the  
580 detection of different patterns (**Fig.6B**). However, inspection of these clusters revealed they  
581 were composed of a single type of QPP, with some patterns displaying either high (GSR P1) or  
582 low (GSR P2) intensity in the medial Cg component (and vice versa the lateral S2 component)  
583 (**Fig.6A**). This indicated that the phase at which a pattern was detected, became a dominant  
584 factor in the clustering after GSR. Phase sorting of QPPs prior to hierarchical clustering, based on  
585 their intensity time series in the Cg (cfr. 2.9), confirmed the detection of a single GSR QPP  
586 (**Fig.6B**). An overlay of STCs of the QPPs after GSR further confirmed their detection at opposite  
587 phases (**Fig.6C**).

588 An STC overlay of PAT1-3 and the GSR PAT clearly illustrated matching of PAT1 with the GSR  
589 PAT (**Fig.6C**). This was further confirmed by STC cc at each window size, which showed strongly  
590 decreased cc of the GSR PAT with PAT2-3 and high cc with PAT1 (**Fig.6D**, left panel). Direct cc of  
591 PAT1-3 with the global signal further demonstrated its close relationship with PAT2-3, but less  
592 so with PAT1 (**Fig.6D**, right panel). Using the CAP approach (cfr. 2.10), we identified the  
593 spatiotemporal shape associated with global signal and displayed it with its respective timing to  
594 the GSR PAT (**Fig.6A**, lower panel). This illustrated that the global signal falls on the Cg-S2

595 intensity switch. PAT2-3 displayed a similar timing and spatiotemporal shape (**Fig.4A**),  
596 suggesting at least partial overlap with the global signal.

597 Under HA, GSR had very similar effects (**Fig.S6**). PAT2 and PAT4 were no longer observable,  
598 while the GSR pattern displayed similarity with PAT1 (STC cc 0.83 and QPP cc 0.71) (**Fig.S3A &**  
599 **movie 3**). STC cc across window sizes was however ( $0.70\pm 0.13$ ) slightly diminished, not  
600 displaying the high consistency as observed under LA (**Fig.S3B**). PAT2 and PAT4 displayed a  
601 close relationship with the global signal (**Fig.S6D**, right panel). The global CAP displayed a  
602 similar timing as described under LA and its spatiotemporal shape shows consistency with PAT2  
603 and PAT4 (**Fig.S6A**).

### 604 **3.6. Cortex and Caudate Putamen differentially contribute to Quasi-Periodic patterns.** 605 **Global signal regression diminishes subcortical dynamics.**

606 Under LA, PAT2 and PAT3 showed a higher detection rate at window sizes of 6-10.5s. Both  
607 displayed a spatiotemporal pattern that more strongly involves Cpu, while PAT1 was marked  
608 most by lateral to medial cortical propagation and window sizes above 10.5s. To further  
609 investigate this 'skewed' detection of patterns involving different brain regions, we performed  
610 the same general analysis as presented in Fig.2, now with masks comprising either only cortical  
611 or Cpu regions. This allowed, for the cortical mask, observation of a pattern highly similar to  
612 PAT1 (QPP cc 0.92), and for the Cpu mask, observation of a pattern similar to PAT2 (QPP cc  
613 0.70), consisting of bilateral alternating high and low intensities in the full Cpu and Cg (**Fig.7A &**  
614 **movie 4**). Interestingly, when the Cpu mask was employed, the algorithm didn't use information  
615 of the cortex to select whole brain images to be averaged, yet a pattern including the Cg was still  
616 determined, indicating partially preserved coupling with the cortex. A similar outcome was also  
617 observed in rats (Majeed et al., 2011). Timing of the Cpu pattern indicated it as falling in  
618 between the PAT1 S2 - Cg switch, similar to PAT2-3 (**Fig.4**).

619 STC cc with whole brain QPPs obtained via k-means clustering (**Fig.3**) confirmed a high overlap  
620 with Cpu spatiotemporal dynamics at shorter window sizes ( $0.76\pm 0.02$ ), while at high window  
621 sizes QPPs were highly consistent with cortical only dynamics ( $0.94\pm 0.03$ ) (**Fig.7C**). Visual  
622 inspection of the STCs displayed how the Cpu QPP both synchronizes and falls out of phase with  
623 the cortical QPP and whole brain PAT1 (**Fig.7B**). This illustrated a potential common  
624 relationship between the two, which visually disappeared due to differential averaging (e.g. with  
625 a cortical mask in- and out-of phase Cpu occurrences could average to zero, or vice versa).

626 Ideal window sizes for Cpu and cortical QPPs were determined at 9s (visual inspection + FA,  
627 **Fig.7D**). The cortical-mask QPP still displayed the prolonged negative intensity, but less clearly.  
628 Therefore the ideal window size was determined by taking into account FA. Half cycle times for

629 Cpu QPPs appeared shorter in the Cpu ( $\pm 0.6s$ ) compared to PAT2, while propagation time in  
630 cortical QPPs appeared faster than in PAT1 ( $\pm 0.7s$ ) (**Table2**). These differences served to  
631 illustrate different temporal dynamics across brain regions, which likely contributed to the  
632 observation of different patterns. Both Cpu and cortical QPPs were observed across all subjects  
633 and occurrence rates were higher across all window sizes versus whole brain QPPs (**Fig.7E &**  
634 **Table1**, lower panel).

635 We showed earlier that GSR abolished PAT2-3 detection, leaving only a pattern highly similar to  
636 PAT1 (cfr.3.5). Cortical QPP spatiotemporal dynamics were found to be highly similar to GSR  
637 QPPs (QPP cc 0.88) (**Fig.7A**). They further produced a similar STC cc profile as described for the  
638 cortical QPPs, displaying respectively diminished and preserved cc with whole brain QPPs at  
639 lower and higher window sizes (**Fig.7C**). These results indicated that GSR diminished the  
640 detection of subcortical dynamics and the related PAT2/PAT3.

641 Under HA, similar outcomes were observed. The Cpu mask led to the detection of a pattern  
642 highly similar to PAT4 (QPP cc 0.89), while with the cortical mask a pattern similar to PAT1 was  
643 found, which displayed diminished intensities in the lateral cortical S2 component (**Fig.S7**). The  
644 Cpu QPP displayed a similar timing with respect to PAT1 and PAT GSR, as described for LA.  
645 Detection rates are shown in **Table S1**.

### 646 **3.7. Quantitative comparison of Quasi-Periodic patterns between high and low** 647 **anesthesia, before and after global signal regression.**

648 After patterns were determined at their ideal window length, based off the image series of a  
649 specific group (LA – no GSR, LA – GSR, HA – no GSR, HA GSR), they could be compared to those of  
650 other groups via sliding template correlation. This allowed patterns that were hypothesized to  
651 be the same across groups to be compared in terms of how similar they correlate with the  
652 respective time series: e.g. PAT1 determined under LA was used to derive the STC with the HA  
653 image series (STC PAT1 LA->HA), to then be compared with the original STC of PAT1  
654 determined under HA. STC LA->HA cc was determined to be 0.87 for PAT1, 0.89 for PAT2, and  
655 0.89 for PAT GSR. These high cc values suggested that visually classified common patterns  
656 displayed a highly similar interaction with the respective image series and thus pertained to the  
657 same spatiotemporal dynamics across anesthesia groups.

658 In a similar way as described above, reference->target STCs were used to compare detection  
659 rates of patterns before and after GSR in the respective anesthesia groups (**Fig.8**). This showed a  
660 clear suppression of PAT2-4 and Cpu QPP detections after GSR in both groups, with only the Cpu  
661 QPP showing some preservation in the LA group. On the other hand, PAT1, PAT GSR and the  
662 cortical QPP showed consistent detection rates before and after GSR, whilst cortical QPP

663 detections under HA increased after GSR. PAT3 and PAT4 were not clearly visually discerned in  
664 respectively the HA and LA group, but could be compared for their potential presence via this  
665 strategy. Both displayed the lowest occurrence rate in the alternate anesthesia group.

### 666 **3.8. Single subject Quasi-Periodic Pattern detection and consistency with group-level** 667 **analysis**

668 Once we established the repertoire and behavior of QPPs at the group level, we further  
669 investigated whether patterns could be detected at the single subject level. The algorithm was  
670 run on each subject for window sizes of 3 to 15s, with and without GSR. We investigated cc  
671 between the STC of the subject individually and the STC of the same subject, derived from the  
672 group-level analysis. For single-subject data there was no need for image normalization,  
673 allowing all three slices to be included in the analysis. Pattern similarity was therefore visually  
674 confirmed.

675 We present results from three example subjects, displaying QPPs with and without GSR (**Fig.9**).  
676 Subjects were chosen to illustrate, respectively, high PAT1 contribution in subject 11 (**Fig.9A**),  
677 high PAT1 and PAT2 contribution in subject 8 (**Fig.9B**), high PAT2 contribution in subject 4  
678 (**Fig.9C**). The two patterns could clearly be visually observed throughout all three slices, with a  
679 high similarity and timing. Below each illustration, a 200s excerpt is shown from the STCs at  
680 single subject and group level after phasing via cc. Subject 11 and 8 presented a very high degree  
681 of overlap for their respective patterns, while subject 4 showed partial overlap and sporadic  
682 aphasic behavior. These graphs illustrate the high consistency between group and single subject  
683 analysis.

684 After GSR, subject 11's QPP stayed highly similar, while in subject 8 the Cpu contribution seemed  
685 slightly reduced. In subject 4, where no lateral cortical contribution could be observed earlier, a  
686 lateral to medial cortical wave similar to PAT1 could afterwards be appreciated. STCs for group  
687 and subject data with GSR are shown below the figures on the right. In all three subjects a high  
688 overlap could be observed, indicating that GSR allowed reproducible QPP detection.

689 In the lowest middle graph below each subject, an overlay is shown between single subject STC  
690 with GSR and without GSR. Subject 11 and 8 respectively showed high overlap, while subject 4  
691 showed very little overlap. When however for subject 4 the group level STC of PAT1 was  
692 additionally plotted, a high similarity could be observed. This supported the notion that GSR  
693 removed contribution of the Cpu and PAT2, which involved more pronounced subcortical  
694 dynamics. A similar illustration for PAT3 can be appreciated in subject 9 (**movie 5&6**).  
695 Speculatively, in subjects 11 and 8 the cortical contribution, consistent with PAT1, was already  
696 high to start with so the STCs after GSR stayed similar.

697 We observed similar trends over all subjects, with individual subjects displaying differences in  
698 which patterns seemed to be dominantly present (**movie 5&6**). To illustrate this, we present a  
699 visual overview of STC cc of each subject, per window size, with the group-level patterns before  
700 and after GSR (**Fig.S8**). Some subjects showed lower pattern detection (e.g. subject 7) and QPPs  
701 could not be detected for all window sizes. This was especially the case after GSR.

702 Under HA, single subject detection of QPPs was much more challenging and visual assessment of  
703 pattern type was often not possible (**Fig.S9 & movie 7**). After GSR, this slightly improved, but  
704 patterns remained challenging to discern and tended to display lateralization (**movie 8**). STC  
705 overlap of group and single subject data further illustrated substantial difficulty to find reliable  
706 matching.

## 707 **4. Discussion**

### 708 **4.1 Overview**

709 Often, the assumption is made that BOLD FC is stationary, but recent studies indicate that  
710 dynamic analysis of FC better captures the interaction between different brain regions and  
711 resting state networks (RSNs), providing additional insights into the macroscale organization  
712 and dynamics of neural activity (Calhoun et al., 2014; Deco et al., 2011; Hutchison et al., 2013;  
713 Keilholz, 2014). Only just recently, Grandjean et al. (2017) applied sliding window analysis  
714 (SWA) and dictionary learning to identify for the first time several highly reproducible dynamic  
715 functional states in mice. Other dynamic rsfMRI techniques focus directly on the LF BOLD  
716 fluctuations, tracking down instantaneous single volume BOLD configurations that underlie  
717 observed FC and RSNs, e.g. the CAP approach (Liu and Duyn, 2013; Preti et al., 2016), and in an  
718 alternative extension their recurring spatiotemporal evolution (Majeed et al., 2011). It has been  
719 speculated that SWA and spatiotemporal dynamics both scope different aspects of the neural  
720 basis underlying dynamic rsfMRI (Keilholz, 2014). Being able to apply and compare both  
721 techniques in mice would thus represent an important step forward.

722 We investigated such spatiotemporal dynamics by acquiring high temporal rsfMRI scans in mice  
723 under an analogous HA and LA condition. Using the pattern detection algorithm developed by  
724 Majeed et al. (2011), we report the detection of a set of group-level QPPs, which appear to  
725 capture the spatiotemporal occurrence of BOLD configurations resembling known RSNs. We  
726 present an initial framework for the interpretation of observed QPPs, illustrating the influence of  
727 analysis window size on skewing detection towards either more cortical (PAT1) or widespread  
728 and subcortical (PAT2-4) spatiotemporal dynamics. PAT1-2 and the pattern after GSR were both  
729 visually and quantitatively determined to be the same across both anesthesia conditions, where  
730 they display different occurrence rates and lower lateral cortical intensities under HA. PAT3 and

731 PAT4 were identified separately under respectively LA and HA, and both displayed a similar  
732 spatiotemporal shape to the global CAP. We went on to illustrate the relationship between  
733 observed patterns and the global signal, showing how GSR removed detection of PAT2-4 and  
734 diminished the detection of subcortical spatiotemporal dynamics. This resulted in the dominant  
735 detection of PAT1. To aid interpretation, we developed a novel data-driven approach to guide  
736 identification of optimal window sizes, we proposed a clustering approach to confirm different  
737 pattern subtypes, we added an extension of the CAP approach to investigate the global signal  
738 spatiotemporal pattern, and provided a means of quantitatively comparing patterns across  
739 groups. Interestingly, our findings suggest that QPPs and their interaction with the global signal  
740 were consistent across anesthesia conditions, but that their detection rates were diminished  
741 under higher anesthesia levels.

742 PAT1 is highly similar to the QPPs detected in preceding rat studies, displaying a propagating  
743 intensity from lateral S2 towards medial Cg cortical areas, with almost the same propagation  
744 time and half cycle length (Magnuson et al., 2010; Majeed et al., 2011, 2009) (**movie 2**). This  
745 interspecies consistency supports that QPPs are a robust phenomenon and further validates  
746 mouse rsfMRI as a pre-clinical tool. In the current study, QPPs could only be investigated in a  
747 single slice. By utilizing conventional resting state analysis on both low and high temporal  
748 resolution datasets, acquired in the same LA session, we illustrate how single slice investigations  
749 allow a view into brain-wide BOLD dynamics. We suggest that the S2 and Cg components of  
750 PAT1 pertain to anti-correlated interaction between the mouse DMN-like and lateral cortical  
751 networks. We further speculate that the lateral cortical network might represent a mouse TPN-  
752 like network. These networks have been conjectured to be present in mice (Liska et al., 2015),  
753 and match a similar DMN-TPN anti-correlation (Fox et al., 2005) and quasi-periodicity in  
754 humans (Majeed et al., 2011; Yousefi et al., 2017). Although the exact subcortical patterns shown  
755 in the current study were not reported in rats, Majeed et al. (2011) did indicate the presence of a  
756 pattern including Cpu. The latter similarly locked in-and-out of phase with the rat whole-brain  
757 pattern and displayed shorter cycle lengths, consistent with our findings.

758 Comparison of group-level QPPs with single subject multi-slice QPPs, by means of STC cc,  
759 allowed us to investigate detection reliability at the subject-level. The latter seemed consistent  
760 under conditions of LA and was improved by GSR. At group-level, subjects displayed occurrences  
761 of all patterns, but contributions were skewed towards one or multiple subtypes. Visual  
762 inspection and STC cc of single-subject with group-wide QPPs confirmed this observation. The  
763 variability in QPP contribution and occurrence across subjects might be related to the commonly  
764 observed inter-subject variability in rodent rsfMRI, which knows numerous origins (Keilholz et  
765 al., 2016). It is interesting to speculate that different contributions of QPPs might contribute to



766 inter-subject differences in FC readouts. In humans it was already indicated that QPPs contribute  
767 to FC (Wang et al., 2016). Single subject investigation of QPPs promises a step forward towards  
768 more reliable resting state fMRI.

#### 769 **4.2 Anesthesia and resting state network resemblance**

770 Anesthesia type and dosage are known to alter neurovascular coupling, haemodynamics and  
771 BOLD FC patterns (Grandjean et al., 2014; Jonckers et al., 2014; Keilholz et al., 2016; Masamoto  
772 and Kanno, 2012; Schlegel et al., 2015; Schroeter et al., 2014; Williams et al., 2010). Several  
773 rodent rsfMRI studies point at a combination of low dosage medetomidine and isoflurane  
774 (MedIso) as a potential optimal anesthesia regime that preserves vascular reactivity, preserves  
775 FC within and between cortical and subcortical structures, and allows high retention of local  
776 activity measured via regional homogeneity (Bukhari et al., 2017; Fukuda et al., 2013; Grandjean  
777 et al., 2014; Wu et al., 2017). We therefore scanned animals with a similar regime (**LA** – low  
778 anesthesia) and also under a higher dosage (**HA**) for comparison. Our results confirm the  
779 importance of choosing optimal anesthesia and are in line with the outcomes of several studies.

780 BOLD configurations of the observed QPPs match well with several RSNs described in  
781 (Grandjean et al., 2014) and those in a follow-up study of the same lab (Zerbi et al., 2015). The  
782 lateral cortical components of PAT1 and PAT3 match the bilateral sensory cortical map obtained  
783 with a seed-based analysis in (Grandjean et al., 2014), which displays involvement of  
784 somatosensory areas (S1 & S2), a ventral part of the Cpu, and partially extends to enthorinal and  
785 insular cortices. After GSR, this FC map displays anti-correlation between S1BF/S2 and Cg,  
786 similar to the contrast observed in PAT1. A seed in the dorsal Cpu further indicates a bilateral  
787 striatal network that we observe throughout all patterns. Zerbi et al. (2015) used ICA to identify  
788 bilateral RSNs, which also match with QPPs. The configuration with co-active dorsal Cpu and Cg  
789 was not shown, yet a high correlation was determined between their time series. Furthermore,  
790 in a recent study employing MedIso anesthesia, this configuration could be observed and it was  
791 even correlated to underlying monosynaptic structural connectivity (Grandjean et al. 2017). It  
792 was also observed as a part of the DMN module and with CBV-weighted rsfMRI, when halothane  
793 was used as an optimal anesthesia regime (Liska et al., 2015; Sforazzini et al., 2014). In these  
794 two studies, similar RSN topologies as described above were identified.

795 Under HA, we observe diminished cortical contribution to the QPPs, while spatiotemporal  
796 dynamics displaying bilateral striatal co-activation predominate. This is in line with diminished  
797 cortico-cortical and preserved striatal connectivity observed at higher dosages of medetomidine  
798 (Grandjean et al. 2014; Nasrallah et al. 2014). Medetomidine is a potent vasoconstrictor (Ganjoo  
799 et al., 1998), exerting its effect via interaction with  $\alpha$ 2-adrenoreceptors (Lakhlani et al., 1997;

800 Lukasik and Gillies, 2003), which have different expression densities throughout the brain  
801 (Nasrallah et al., 2012). Cortical expression is higher than in striatum, leading to local  
802 diminished vascular reactivity, which supports observations in our study and that of (Grandjean  
803 et al., 2014).

#### 804 **4.3 Haemodynamics**

805 QPP half cycle times across different pattern subtypes under LA were consistent, averaging to  
806 4.6s across relevant window sizes. Interestingly, for QPPs derived with a cortical and subcortical  
807 mask, these values average respectively to 4.4s and 3.8s. Although the temporal resolution in the  
808 current experimental setup is limited to 0.5s, this difference was determined to be significant (T-  
809 test,  $p < 0.01$ ) across window sizes, supporting our hypothesis that subcortical and cortical  
810 spatiotemporal dynamics differ. The latter might skew detection of pattern subtypes, depending  
811 on the window size under investigation. As described above, differences in haemodynamics due  
812 to regional expression variation in anesthetic-binding receptors could contribute to this  
813 phenomenon. Regional differences in haemodynamics were indicated before in rats (Devonshire  
814 et al., 2012; Sloan et al., 2010), and more recently also in mice (Schlegel et al., 2015; Schroeter et  
815 al., 2014). Visual inspection of the mouse S1 haemodynamic response function (HRF),  
816 determined under medetomidine in (Schlegel et al., 2015), suggests a similar cycle time (9-10s)  
817 as we observe for mouse QPPs. On the other hand, for a subcortical structure (thalamus) the  
818 HRF was determined to be shorter, which is in line with our observed shorter subcortical  
819 dynamics. The authors suggested that this could be attributed to regional differences in vessel  
820 structure and blood supply.

821 It thus becomes interesting to speculate that QPPs reflect spontaneous haemodynamic events. It  
822 has already been suggested that haemodynamics in the mouse somatosensory cortex, due to  
823 spontaneous neural activity, resemble stimulus-evoked haemodynamics (Bruyns-Haylett et al.,  
824 2013). Further support comes from other multimodal imaging modalities. Particularly, two  
825 studies in mice employed wide-field optical imaging to visualize calcium and intrinsic optical  
826 signals, to investigate and relate respectively neuronal activity with haemodynamics (Ma et al.,  
827 2016; Matsui et al., 2016). It was shown how in the resting state, spontaneous symmetrical  
828 events in cortical synchronized neural activity translate into similar patterns of haemodynamics,  
829 which may reflect the basis of RSNs as detected by rsfMRI. (Matsui et al., 2016) went on to show  
830 global waves of neural activity propagating across the cortex, with functionally connected  
831 cortical regions co-activating at different time points along the wave. These events could be  
832 translated into spatially similar haemodynamic co-activations. In both studies, haemodynamics  
833 under anesthesia were on the order of  $\sim 10$ s and illustrated the existence of transiently co-  
834 activating large-scale patterns. Although speculative, there seems to be a consistency with the

835 currently detected QPPs, which were also on the order of  $\sim 10$ s. Future studies applying the  
836 pattern detection algorithm of (Majeed et al., 2011) on these types of data, or multimodal  
837 experiments combining rsfMRI with neuronal recordings in mice, might answer this hypothesis.

#### 838 **4.4 Impact of physiology, motion, spectral range and processing**

839 A critique to the above statement is that QPPs might be confounded by contributions of  
840 physiological noise or could arise from imaging/processing artifacts. However, phase  
841 randomization of the respective BOLD data and analysis of data acquired in a dead rat does not  
842 allow detection of QPPs, addressing the latter concern (Majeed et al., 2011) (**Fig.S10**). In the  
843 original study in rats (Majeed et al., 2009), in which a highly similar QPP was detected to the one  
844 we observe in mice (PAT1 & GSR), rsfMRI was acquired with a TR of 100ms to prevent aliasing  
845 of cardiac and respiratory noise into the lower frequencies under investigation. With short TRs,  
846 the BOLD signal becomes more weighted to cerebral blood flow (CBF), which then might  
847 predominantly underlie QPPs, but a subsequent study with CBV-weighted BOLD imaging also  
848 allowed detection of similar QPPs (Magnuson et al., 2010). The consistent regional FC and  
849 observation of QPPs between 'CBF-' and CBV-weighted rsfMRI in this study further confirms a  
850 relationship with neurovascular coupling and suggests that both readouts are primarily  
851 reflective of vascular fluctuations.

852 Spurious repeating patterns, captured by the spatiotemporal pattern detection algorithm, might  
853 reflect sporadic or respiratory/cardiac-induced motion. To ensure that motion was not  
854 causative to QPP events, we calculated frame-wise displacement (FD), based on the backwards  
855 looking temporal derivations of the motion time series (Power et al., 2012), and cross-correlated  
856 the resultant FD time series with the STCs of the 3 main patterns found under LA. This was done  
857 for both raw motion time series and motion time series pre-processed in the same way as the  
858 functional data (filtering, detrending and normalization to unit variance). In both cases cross-  
859 correlation was minimal across all subjects, never exceeding 0.11 (**Fig.S11**), suggesting there is  
860 no influence of motion on detected QPPs. Mean FD across subjects was low ( $0.0066 \pm 0.0005$   
861 mm). Illustrative motion time series, FD time series and lack of overlap with STCs can be  
862 appreciated in **Fig.S12A-C** for individual subjects, matching those described in **Fig.9**.

863 Several studies investigating QPPs in rats employed medetomidine as an anesthetic, which  
864 revealed power spectra peaking close towards 0.2Hz and containing most of the spectral  
865 information below this point (Magnuson et al. 2010; Majeed et al. 2011; Magnuson et al. 2014).  
866 Similar power spectra under medetomidine have been observed in mice (Grandjean et al., 2014),  
867 and also in the current study. In rats, QPPs were variably investigated in frequency ranges  
868 higher than those in conventional rsfMRI ( $0.05 > f < 0.3$ Hz; cfr. respective articles), while for the

869 currently presented mice data we chose 0.01-0.2Hz. Majeed et al. (2009, 2011) split these  
870 spectral confines into a lower and higher frequency range, concluding that the range of 0.08-  
871 0.2Hz was most appropriate to investigate rat spatiotemporal dynamics. Although we also  
872 observed some differences between lower and higher frequencies, they were not sufficiently  
873 compelling for us to do the same. The spectral information content in Fig.1A clearly indicated the  
874 relevance of the investigated frequency range.

875 Although these frequency ranges isolate the hypothesized relevant spectral information of the  
876 BOLD signal and were shown to cohere significantly with infraslow LFPs under medetomidine  
877 anesthesia (Pan et al., 2013), inclusion of temporal content above 0.1Hz risks contribution from  
878 slow frequency vascular phenomena such as vasomotion and Mayer waves (Baudrie et al., 2007;  
879 Bumstead et al., 2017; Drew et al., 2011; Julien, 2006; Tsai et al., 2015). Vasomotion is the  
880 intrinsic spontaneous oscillation of blood vessel tone leading to flow motion, while Mayer waves  
881 represent slow frequency changes in arterial blood pressure.

882 Given potential confounds from physiological noise and the fact that Mayer waves relate to  
883 heart rate variability (HRV) (Elghozi and Julien, 2007), we used multiple linear regression  
884 analysis to investigate in LA subjects the relationship between two parameters of the observed  
885 QPPs, namely occurrence rate and power (i.e. the average correlation value for above-threshold  
886 peak detections), with four physiological parameters, namely cardiac rate, cardiac rate STD (i.e.  
887 HRV), respiration rate, respiration rate STD (**Table S3**). No significant interactions could be  
888 observed, only after GSR there was a trend towards correlation between breathing rate and QPP  
889 occurrence rate, and between cardiac rate STD and QPP power. GSR is generally considered to  
890 remove contributions from physiological noise (Chang and Glover, 2009; Murphy et al., 2013),  
891 making these results seem somewhat surprising. It would however be expected that with  
892 stronger CBF-weighting at shorter TRs, systemic parameters that affect CBF would correlate  
893 more to the BOLD signal and derived readouts. The latter might be accentuated by GSR.

894 As an additional control, we performed the QPP analysis at a lower frequency range (0.01-0.1Hz)  
895 that should theoretically exclude contributions from vasomotion and Mayer waves. The same  
896 QPPs could be detected, with or without GSR, although be it with altered detection rates and  
897 slower temporal dynamics, which is to be expected due to temporal filtering (**Fig.S13**). The  
898 current experimental setup does not allow to fully exclude contributions from physiological  
899 signals, which should be investigated more in depth in future experiments together with the role  
900 of spectral range. Nonetheless, there seems to be substantial support for a neuronal component  
901 in the QPPs and their high similarity with known RSNs implicates their contribution to BOLD LF  
902 FC, regardless of their origin.

#### 903 **4.5 Global signal regression impact on spatiotemporal dynamics**

904 GSR remains a controversial tool for rsfMRI processing, with the current consensus being that  
905 data should be compared with and without GSR (Murphy and Fox, 2016). In the investigation of  
906 the spatiotemporal dynamics in mouse BOLD, we observed that GSR removes PAT2-4 detection  
907 and decreases detection of subcortical patterns, leading to the sole detection of the lateral to  
908 medial cortical QPP (PAT1), which was also observed in rats (Majeed et al., 2011). The removal  
909 of PAT2-4 through GSR was further supported by their similar timing and spatiotemporal shape  
910 with the global signal CAP. In QPPs after GSR, subcortical dynamics are still present, indicating  
911 that their removal is targeted to the specific coincident timing with the global signal and  
912 potentially to periods between respective PAT1 occurrences. The removal of QPPs matches with  
913 a recently suggested mechanism for GSR, where it acts as a temporal down-weighting process,  
914 attenuating data from time points with a large global signal contribution and leaving data from  
915 low global signal time points largely unaffected (Nalci et al., 2017). With regard to the current  
916 study, PAT1 would represent the low global signal time points and PAT2-4 the high global signal  
917 time points, which appeared to fall between the first and second part of PAT1.

918 The 'anti-correlated' structure of PAT1 and the QPPs after GSR have also been shown for  
919 measures of RSN FC in several rodent studies and in humans, between analogues DMN (medial  
920 Cg, i.e. mouse DMN-like network) and TPN (lateral S2, i.e. mouse lateral cortical network) (Fox et  
921 al., 2005; Gozzi and Schwarz, 2015; Grandjean et al., 2014). It is very relevant to note here that  
922 the anti-correlated nature of these two networks has been debated to be a potential artifact of  
923 GSR (Fox et al., 2009; Murphy and Fox, 2016), but in the current study PAT1 was both detected  
924 before and after GSR. Similarly, Nalci et al. (2017) suggested that censoring of high global signal  
925 time points in the time series, rather than GSR, still allowed detection of anti-correlated  
926 interactions between DMN and TPN.

927 Although the suggested DMN-TPN interaction might thus be comparable with or without GSR  
928 (PAT1 is present in both cases), it does not address the question if GSR has a positive or negative  
929 role. We however show that without GSR, three separate QPPs could be observed, which  
930 resemble known RSNs and therefore suggest their neuronal relevance. A neural basis for QPPs  
931 was already directly indicated in rats, where a correlation is observed with infraslow LFPs  
932 (Thompson et al. 2014; Thompson et al. 2015; Pan et al. 2013). All QPPs observed in the current  
933 study displayed a high coincidence with each other and the global signal. It was shown that the  
934 global signal itself, as measured with rsfMRI, might also be related to a global neural signal  
935 (Schölvinck et al., 2010). In the latter study, global signal coupling with the neuronal signal was  
936 increased during the eyes-closed condition, which has been correlated to changes in vigilance  
937 and attention (Wong et al., 2016). Similarly the presence and magnitude of the global signal has

938 been related to the level of arousal (Liu et al., 2017; Wong et al., 2013, 2012) and global neuronal  
939 events were found to match with micro-arousal fluctuations (Liu et al., 2015). The relationship  
940 between arousal and the global signal provides a potential interpretation for the results of the  
941 current study, given that QPPs and spontaneous large-scale BOLD fluctuations have been linked  
942 with attention and behavior performance in humans (Abbas et al., 2016; Fox et al., 2007; Monto  
943 et al., 2008) & cfr. (Keilholz, 2014; Keilholz et al., 2016).

944 Based on our results, we suggested that GSR might be removing relevant information from  
945 neuronal origins related to arousal. Investigating and isolating QPPs that are related to the  
946 global signal thus provides a potentially more physiologically relevant alternative to standard  
947 GSR. For this interpretation, it should be stressed that global signal in this study is calculated  
948 from either one or three slices, and might thus not fully match the global signal as described in  
949 other studies. On the other hand, the observed impact of GSR on proposed DMN-like and TPN-  
950 like networks matches with preceding literature.

#### 951 **4.6 Study limitations**

952 In section 4.2, we described the similarity of QPPs with RSNs in preceding literature. In the  
953 current study, QPPs also matched with RSNs determined in the presented data itself. However,  
954 while the overall configuration of RSNs appeared to match existing literature and monosynaptic  
955 connectivity between different brain areas (Grandjean et al., 2017), it is important to state that  
956 these RSNs also displayed some variability in their homotopic representation across the  
957 hemispheres. Furthermore, some RSNs displayed functional coupling that was not as strong and  
958 pronounced as in other studies. These observations were most noticeable for S1HL/FL/BF  
959 components. We also determined differences between short and long TR data, where in long TR  
960 data only a single Cpu component was observed when ICA was used to determine RSNs. These  
961 RSN topology differences and lower functional coupling, compared to preceding mouse rsfMRI  
962 literature, might be attributed to differences in sample size and pre-processing data cleanup  
963 strategies. When interpreting the presented results, these differences should be kept in mind.

964 Another potential limitation of the current study is the timing of HA followed by LA, which might  
965 bias LA results through habituation and interference with neural activity. A prior study however,  
966 which performed rsfMRI scans in young C57BL/6J mice two weeks apart, showed no significant  
967 differences between both time points, addressing concerns about habituation. To address  
968 potential remaining concerns, we performed novel experiments in C57BL/6J mice (n=4) at the  
969 age of 3.5 months, which were prior not exposed to anesthesia. High temporal rsfMRI data was  
970 acquired under the same experimental conditions and same LA regime. Analysis of this data  
971 revealed reproducible QPPs, showing the same timing with respect to each other, similar

972 clustering and interaction with the global signal (**Fig.S14**). This high reproducibility supports  
973 the validity of the presented findings.

974 A last limitation is reflected by this study's restriction to single slice investigation, so that QPPs  
975 could only be investigated in a sub-sample of the brain. This was an active choice, to enable high  
976 spatial resolution EPI-acquisition with a short TR, so that earlier rat work could be reproduced.  
977 However, while normal low temporal rsfMRI could be used to relate QPPs to large-scale brain  
978 networks, QPP propagation across the rostro-caudal axis could not be investigated. In humans,  
979 QPPs propagate across the entire brain, involving mainly DMN and TPN areas (Majeed et al.,  
980 2011; Yousefi et al., 2017). Similarly, different whole-brain CAPs appeared to display some form  
981 of temporal sequence, suggestive of QPP-like behavior (Chen et al., 2015; Liu and Duyn, 2013).  
982 Finally, within the current study, it should be stated that the detection of multiple pattern types,  
983 which highly coincide with each other, might be a consequence of limited slice count and QPP  
984 variability across subjects. It is therefore not un-plausible that all observed patterns relate to a  
985 single QPP that shows a close interaction with the global signal. Future studies with larger brain  
986 coverage will be needed to investigate rostro-caudal and whole-brain properties of mouse QPPs,  
987 and to further elucidate their relationship with the global signal.

#### 988 **4.7 Conclusion & Perspectives**

989 Dynamic rsfMRI has been shown to reveal new insights into the macro-scale organization of  
990 functional networks, stepping closer to the underlying neural activity (Calhoun et al., 2014;  
991 Keilholz, 2014). In this study, we tease at the repertoire of dynamic processes, focusing in  
992 particular on the large-scale and repetitive background BOLD fluctuations that in recent years  
993 have become apparent as propagating spatiotemporal activity waves. We report the detection of  
994 a set of recurring QPPs in mice, which show similarity with known RSNs and represent  
995 promising contributors to BOLD FC. Their shape and properties confirm interspecies  
996 consistency and the importance of anesthesia in rodent rsfMRI research. High consistency of  
997 QPP detection, even at the single subject level, and a suggestive mechanistic role for GSR, marks  
998 advance towards more reliable and comprehensive rsfMRI research. These findings open up a  
999 new approach to study mouse LF BOLD spatiotemporal dynamics and mechanisms underlying  
1000 FC, as was shown recently in humans (Wang et al., 2016).

1001 It has been suggested that within the spectrum of neural activity, QPPs represent the infraslow  
1002 LFP contribution, playing a role in attention and task performance, while SWA captures BOLD  
1003 dynamics related with higher frequency LFPs, scoping state changes in cognitive processing  
1004 (Abbas et al., 2016; Keilholz, 2014; Keilholz et al., 2016, 2013; Thompson et al., 2015). Together  
1005 with the recent application of SWA in mice by Grandjean et al. (2017), the findings in this study

1006 suggest that we can now tackle this dual dynamic repertoire in mice rsfMRI. This promises a  
1007 considerable step forward in the field, encompassing a wide range of new research strategies  
1008 and potential applications for pre-clinical disease models.

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## 1320 **Figure captions**

### 1321 **Figure 1. Spectral range and resting state networks**

1322 All ICA and FC maps display thresholded T-values (one-sample T-test,  $p < 0.05$  FDR-corrected). **A-D)**  
1323 **Single-slice high temporal resolution data. A)** Group average multitaper power spectral density of the  
1324 center brain slice for the low (LA, blue) and high (HA, red) anesthesia groups. Patches indicate standard  
1325 deviations. Note the higher power under LA. The frequency range displays the highest spectral content,  
1326 with the full range shown on top. Based on this observation, all data were filtered between 0.01-0.2Hz. **B)**  
1327 LA (top) and HA (bottom) RSNs determined with ICA. Top row text indicates similarity with resting state  
1328 networks, lower row indicates overlap with anatomical parcellations (Paxinos and Franklin, 2007). **C)**  
1329 ROI-based zFC matrix for LA (top right) and HA (bottom left). Significant differences are indicated with 'S'  
1330 (two-sample T-test, FDR  $p < 0.05$ ). ROIs are indicated on a representative EPI image. **D)** LA (top) and HA  
1331 (bottom) seed-based FC maps, using left ROIs (C). Note for HA, the loss of FC, and for LA, the similarity  
1332 with ICA-derived RSNs (B). **E-F) Whole-brain low temporal resolution data.** The matching slice,  
1333 investigated in the high temporal resolution data, is indicated in blue. **E)** LA whole brain RSNs matching  
1334 those shown in (B). Note the speculative mouse TPN and DMN, matching single slice lateral and cingulate  
1335 ctx networks. Only two whole-brain striatal networks were observed, and two S1 networks instead of one.  
1336 **F)** LA Seed-based FC maps illustrate similarity with RSNs (E). A third striatal network is now again  
1337 observed. **Abbreviations.** LA, low anesthesia; HA, high anesthesia; ctx, cortex; Cg, Cingulate; S1,  
1338 somatosensory area 1; FL, forelimb; HL, hindlimb; BF, barrel field; S2, somatosensory area 2; Cpu, caudate  
1339 putamen; d, dorsal; vl, ventro-lateral; VP, ventral pallidum; Pir, piriform ctx; I, insular ctx; En, enthorhinal  
1340 ctx; Tea, temporal association ctx; HC, hippocampus; TH, thalamus; DMN, default mode network; TPN, task  
1341 positive network; RSN, resting state network.

### 1342 **Figure 2. Additional whole-brain resting state networks**

1344 This figure is complimentary to Fig.1E and displays the remaining ICA-derived RSNs, obtained from the  
1345 whole-brain low temporal resolution data. Note the observation of bilateral RSNs that display similarity  
1346 with preceding mouse rsfMRI literature. Maps display thresholded T-values (one-sample T-test,  $p < 0.05$   
1347 FDR-corrected).

### 1348 **Figure 3. Spatiotemporal patterns detected at the group level**

1350 **A)** Illustration of the Sliding Template Correlation (STC) time series associated with QPPs observed at  
1351 different window sizes. Upper panel. Single STC excerpt at a window size of 12s. Red line indicates the  
1352 threshold for pattern detection, with QPP occurrences indicated by black triangles. Lower Panel. Close-up  
1353 of several STCs at different window sizes, illustrating phase offsets between detected patterns. Red  
1354 indicates anti-phasic detections, versus similar phase detections in blue. **B)** Cross-correlation (cc) matrix  
1355 of STCs at different window sizes. Lower triangle indicates max cc values, while upper triangle shows  
1356 phase offsets (seconds) between detected patterns. Note the high cc from window size 12s upwards. **C)**  
1357 Rows present QPPs determined for different window sizes of analysis (vertical axis), while their temporal  
1358 unfolding is shown across the columns (horizontal axis; images interspersed by 1.5s). Images display  
1359 normalized BOLD signals. QPPs are phased using the time delays of their STC cc (left panel). The resultant  
1360 alignment can be visually appreciated. Note that the figure suggests that several types of QPPs could be  
1361 observed (e.g. at 7.5s, 10.5s & 12s). At 12s we observed a full non-redundant pattern, displaying bilateral  
1362 S2 towards medial Cg intensity propagation, followed by a low intensity wave (green square). Red square  
1363 indicates redundancy or repeating parts of the cycle.

### 1364 **Figure 4. Detection of multiple Quasi-Periodic Patterns based on window size and visual inspection**

1367 **A)** Three different types of QPPs could be identified and are displayed at their respective ideal window  
1368 sizes, after phase-alignment (1s intersperse). PAT1 is marked by contributions in cortical regions with  
1369 opposing intensities. PAT2 and PAT3 display stronger involvement of Caudate Putamen, which are co-  
1370 active with medial cortical regions. PAT2 does not display lateral cortical high intensities. Both PAT2 and  
1371 PAT3 high intensities coincide with PAT1's S2-Cg intensity switch. **B)** Schematic illustration of the  
1372 spatiotemporal flow of the three patterns. Circles indicate key regions that were used to visually classify

1373 patterns, while activity propagation is indicated by arrows. Red indicates high and blue low intensities. All  
1374 involved brain regions are indicated in green on the middle illustration. **C)** STC cc matrices across all  
1375 window sizes, for each pattern. PAT1 was more reliably detected at longer window sizes, PAT2 more at  
1376 shorter ones. PAT3 appeared similarly correlated across most window sizes. **D)** Detection rate of each  
1377 pattern, as determined by visual classification of a 100 patterns per window size. Note the bell-shape  
1378 curve of PAT2 and PAT3 at shorter window sizes, and the U-curve for PAT1, which takes over after 12s  
1379 (red circle). These curves illustrate skewed pattern detection dependent on window size. **E)** Fractional  
1380 average correlation per window size (cfr. 2.7). Red circles indicate the start of a plateau, representing the  
1381 ideal window size. **F)** Occurrence rate across window sizes. Note the higher occurrence rates for PAT2 and  
1382 PAT3 at shorter window sizes. **G)** Illustration of the overlap between non-phase-corrected STCs,  
1383 determined for each pattern's ideal window size. Although there is variation in peak timing and temporal  
1384 correlation, individual patterns display coincident behavior with one another.

### 1385 **Figure 5. Hierarchical clustering confirms three Quasi-Periodic Patterns**

1386 All 500 individual QPPs, determined at each displayed window size, were hierarchically clustered using a  
1387 maximal cross-correlation (cc) matrix based on: **A)** QPP spatial similarity. **B)** QPP temporal occurrence  
1388 similarity, i.e. STC cc (cfr. 2.8). Columns indicate the respective window size under investigation. Upper  
1389 row panels show clustered cc matrices of the QPPs. Clusters were visually inspected and their content  
1390 marked above the panels (MIX = mixture of all pattern subtypes). Note the clear presence of three clusters  
1391 at shorter window sizes, especially via STC cc, confirming the prior visual classification. Lower row panels  
1392 show the average sorted cc of each QPP with all other QPPs (black trace, STD indicated by grey patch).  
1393 This serves as an indicator of overall QPP (dis)similarity, supporting the notion of different subtypes. Blue  
1394 curves indicate the 10% fraction of QPPs that displayed the highest cc plateaus. Note the sharp transitions  
1395 at shorter window sizes, indicating clear distinction between different pattern subtypes.

### 1397 **Figure 6. Global signal regression removes detection of PAT2 and PAT3, while preserving** 1398 **only PAT1. PAT2 and PAT3 display high similarity with the global signal.**

1399 **A)** QPPs observed after GSR. The three displayed patterns are the same, but due to differences in phase  
1400 detection, the starts and ends display higher intensities. P1 and P2 respectively refer to high and low  
1401 intensities in the Cg. GSR P1 and P2 are shown phase-aligned to PAT GSR. A global CAP (cfr. 2.10) is shown  
1402 below to illustrate its timing as falling between the S2-Cg switch. **B)** To illustrate the detection of only one  
1403 pattern after GSR, hierarchical clustering was employed, but patterns were first sorted based on their  
1404 temporal intensities in the Cg. Respective average Cg time series are displayed in red and blue, while black  
1405 lines indicate unsorted patterns (center phase). A comparison is shown on the left under conditions of no  
1406 GSR. Clusters were visually inspected and their content marked in red or blue to indicate relationship to  
1407 Cg phase. **C)** Upper panel. Illustration of the overlap between non-phase-corrected STCs for PAT1-3 and  
1408 PAT GSR. Note the high STC overlap and similarity between PAT1 and PAT GSR. Lower panel. All three  
1409 apparent GSR patterns are displayed at the same timing as the above panel. Note their clear anti-phasic  
1410 behavior, indicating they are the same. **D)** Left panel. STC cc between PAT1-3 and PAT GSR. Note the clear  
1411 and low cc of PAT GSR with PAT2-3, suggesting that GSR removes their occurrences. Right panel. STC cc  
1412 with the global signal. Note higher cc values for PAT2-3. **Abbreviations.** GSR, global signal regression; CAP,  
1413 co-activation pattern.

### 1414 **Figure 7. Relationship with cortex, Caudate Putamen and global signal regression**

1415 **A)** QPPs observed without GSR, after GSR, with a cortical mask, and a Cpu mask. Patterns are shown  
1416 phase-aligned with each other. Note the high similarity between GSR and cortical QPPs, lacking a clear Cpu  
1417 contribution. With a Cpu-mask, a bilateral alternating high and low intensity could be observed in Cpu,  
1418 with preserved coupling to the Cg area. Note the timing of the Cpu pattern between the GSR pattern's S2-  
1419 Cg switch. **B)** STCs of the patterns described in (A). Note the overlap between all STCs, except for that of  
1420 the Cpu pattern, which synchronized and dephased through time. This illustrates how subcortical patterns  
1421 could behave independently of cortical patterns, but still couple at specific time points, potentially  
1422 contributing to the observation of patterns like PAT2 and PAT3. **C)** STC cc between patterns illustrated in  
1423 (A - 3 lower panels) and whole brain patterns observed in Fig.3C. Note the high cc with Cpu-masked QPPs



1424 at shorter window sizes and the high cc with cortical-masked QPPs at longer window sizes. GSR strongly  
1425 lowered the cc at shorter window sizes, suggesting it diminished Cpu spatiotemporal dynamics. **D)** FA-  
1426 values indicated the ideal window size for each QPP. Grey patch indicates the range of interest for the  
1427 different patterns. **E)** Occurrence rates at all window sizes.

### 1429 **Figure 8. Pattern occurrence rate before and after global signal regression**

1430 All described QPPs were determined from the image series of 4 groups: LA – no GSR, LA – GSR, HA – no  
1431 GSR, HA GSR. QPPs of one group were compared with the image series of others via sliding template  
1432 correlation, to quantify occurrence rates across conditions. Panels display the occurrence rates of patterns  
1433 before and after GSR, in their respective anesthesia groups. Both clearly indicate that PAT2-4 were no  
1434 longer detected after GSR. Cpu QPP detections were lowered in the LA group and no longer seen in the HA  
1435 group. PAT3 and PAT4, which were not visually identified in respectively the HA and LA group, were  
1436 compared with the other anesthesia group in which they displayed the overall lowest occurrence rates.  
1437 **Abbreviations.** LA, low anesthesia; HA, high anesthesia; GSR, global signal regression.

### 1439 **Figure 9. Single subject detection of Quasi-Periodic Patterns and the relationship with** 1440 **group analysis**

1441 Illustrations of QPPs detected for single subject three-slice images, with (left) and without (right) GSR: **A)**  
1442 subject 11, high PAT1 contribution **B)** subject 8, high PAT1 & PAT2 contribution **C)** subject 4, high PAT2  
1443 contribution. Below each panel an excerpt of the subject's STC and its STC, derived from the group-level  
1444 analysis, are shown. The middle lowest panel shows the overlay of single subject STCs with and without  
1445 GSR. **A-B)** Note the consistent high overlap for subject 11 and 8 across all panels. These subjects displayed  
1446 strong cortical contributions in their QPPs. **C)** Subject 4's QPP, without GSR, was dominated by Cpu  
1447 intensities and showed less STC overlap. After GSR, a cortical component could be observed in the QPP  
1448 and the STCs nicely overlapped. The subject's STC after GSR overlapped with PAT1 at the group level,  
1449 indicating removal of PAT2 and the Cpu contribution.

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### 1451 **Table 1. Pattern occurrence rate per subject**

### 1452 **Table 2. Pattern half cycle time and propagation time from lateral to medial, averaged** 1453 **across relevant window sizes**

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### 1456 **Figure S1. Fractional average correlation**

1457 **A)** Illustration of the comparison between a reference (R) and target (T) QPP. The R QPP is split into all  
1458 possible fractions  $\{Rf_1, Rf_2, \dots, Rf_L\}$ , where  $L = R_{WL} - F_{WL} + 1$ , with  $R_{WL}$  the window length of the R QPP and  
1459  $F_{WL}$  the chosen window length for fractions (e.g. 6TRs). **B)** A cross-correlation (cc) is calculated for each  $Rf$   
1460 with regard to the T QPP. The average of these cc values provides the fractional average correlation (FA).  
1461 **C)** All QPPs in the set under investigation are compared. Each QPP, with increasing window size, is treated  
1462 as an R QPP to be compared with all others. The determined FA values of these comparisons are filled in  
1463 as a column vector in the displayed  $n \times n$  matrix, where  $n$  is the number of QPPs in the current set.  
1464 Illustrative FA values are indicated in the matrix, to indicate that comparisons of longer R QPPs  
1465 comparisons with shorter T QPPs results in low FA values. By averaging across columns, the set-wise FA  
1466 value at each window size can be determined.

### 1467 **Figure S2. Spatiotemporal patterns under high anesthesia**

1468 **A)** QPPs observed for different window sizes of analysis. Images display a normalized BOLD signal. QPPs  
1469 are phased using the calculated time delays of their STC cc. Note that only a single type of QPP can be  
1470 observed. At 7.5s we observe a full non-redundant pattern, displaying bilateral high intensity propagation

1471 from Caudate Putamen to medial cingulate cortex, followed by a low intensity wave (green square). **B)** cc  
1472 matrix of STCs at different window sizes. Lower triangle indicates max cc values, while upper triangle  
1473 shows phase offsets (seconds) between detected patterns. Note the overall high cc indicating 1 pattern  
1474 type.

1475 **Figure S3. Multiple Quasi-Periodic Patterns under high anesthesia, determined with and**  
1476 **without global signal regression**

1477 **A)** Three different types of QPPs that could be observed, at their respective ideal window sizes. PAT1 and  
1478 PAT2 were similar under low anesthesia, but PAT1 is now less spatially defined. A fourth pattern is also  
1479 observed, displaying more wide-spread and ventral involvement of the Cpu and less contribution of the  
1480 Cg. After GSR, a pattern similar to PAT1 was observed. **B)** STC cc matrices across all window sizes, for each  
1481 type of pattern. PAT1 was now only reliably detected up to 7.5s, while PAT2 and PAT4 showed high cc  
1482 across window sizes. GSR allowed relatively high cc across window sizes, but appeared less reliable than  
1483 PAT2 and PAT4. **C)** Detection rate of each pattern, as determined by visual classification of a 100 patterns  
1484 per window size. Note the overall dominant detection of PAT2 across window sizes. Red rectangle  
1485 indicates the ideal window size for each pattern and the highly dominant detection of PAT2 and PAT4.  
1486 Detection rates are not indicated after window sizes of 12s, due to the high difficulty of visual  
1487 classification. **D)** FA per window size. Red square indicates the ideal window size for each type of QPP. **E)**  
1488 Occurrence rate of the patterns across window sizes.

1489 **Figure S4. Hierarchical clustering under high anesthesia**

1490 All 500 individual QPPs, determined at each displayed window size, were hierarchically clustered using a  
1491 cc matrix based on: **A)** QPP spatial similarity. **B)** QPP temporal occurrence similarity, i.e. STC cc. Columns  
1492 indicate the respective window size under investigation. Upper row panels show clustered cc matrices of  
1493 the individual QPPs. Clusters were visually inspected and their content marked above the panels. Note the  
1494 presence of three clusters at the shorter window size, using STC cc, and the partial clustering using spatial  
1495 cc. PAT2 and PAT4 appeared less clearly separable. At high window sizes, little cc intensity was left and  
1496 QPPs clustered very limitedly. Lower row panels show the average sorted cc of each QPP with all other  
1497 QPPs (black trace, STD grey patch). This serves as an indicator of overall QPP (dis)similarity, supporting  
1498 the notion of different subtypes. Blue curves indicate the 10% fraction of QPPs that displayed the highest  
1499 cc plateaus. Note the sharp transition at the shorter window size for STC cc, indicating distinction between  
1500 at least 2 pattern subtypes.

1501 **Figure S5. Quasi-Periodic Patterns under low anesthesia, determined with k-means**  
1502 **clustering after global signal regression**

1504 **A)** QPPs observed for different window sizes of analysis after performing GSR and using k-means  
1505 clustering. Images display a normalized BOLD signal. QPPs are phased using the calculated time delays of  
1506 their STC cc. Note that only a single type of QPPs could be observed, which was highly consistent with  
1507 PAT1. **B)** cc matrix of STCs at different window sizes. Lower triangle indicates max cc values, while upper  
1508 triangle shows phase offsets (seconds) between detected patterns. Note the overall very high cc.

1509 **Figure S6. Global signal regression under high anesthesia removes detection of PAT2 and**  
1510 **PAT4, preserving only PAT1. PAT2 and PAT4 display similarity with the global signal.**

1511 **A)** QPPs observed after GSR. Displayed patterns are the same, but were detected at different phases, with  
1512 phase defined by the average intensity time series in the Cg component. The latter is indicated at the left  
1513 side of the panel with respective color code and sinusoid. Patterns are shown phased to each other. A  
1514 global CAP is shown below to illustrate its timing as falling between the S2 - Cg switch. **B)** To illustrate the  
1515 detection of only one pattern after GSR, hierarchical clustering was employed after phase sorting QPPs  
1516 based on their temporal intensities in the Cg. QPP sorted Cg time series are displayed in red and blue,  
1517 while black lines indicate unsorted QPPs (center phase). A comparison is shown on the left under  
1518 conditions of no GSR. Clusters were visually inspected and their content marked above the panels in colors  
1519 matching Cg phase. Little cc intensity remained after GSR, but 2 clusters appeared visually with STC cc.  
1520 These showed to be a single cluster after phase sorting. **C)** Upper panel. Illustration of the overlap

1521 between the non-phased STCs, of PAT1,2-4, and PAT GSR. Note the overlap and consistency of PAT1 and  
1522 PAT GSR. Lower panel. Both GSR patterns are displayed at the same timing as the above panel. Note their  
1523 anti-phasic behavior, indicating they are the same. **D)** Left panel. STC cc between the three different types  
1524 of patterns and the pattern after GSR. Note the very clear and low cc with PAT2&4, indicating that GSR is  
1525 removing their contribution and causing the sole detection of PAT1. Right panel. STC cc with the global  
1526 signal. Note higher cc values for PAT2&4.

1527 **Figure S7. Relationship with cortex, Caudate Putamen and global signal regression under**  
1528 **high anesthesia**

1529 **A)** Displayed are PAT2, the pattern achieved with a cortical mask, with a Cpu mask, and the pattern after  
1530 GSR. Patterns are shown phased with each other. With a Cpu-mask, a bilateral alternating high and low  
1531 intensity could be observed in Cpu, which preserved some coupling to the Cg. Note the timing of the Cpu  
1532 pattern as falling between the S2 - Cg switch. This was also partially the case for PAT2. The GSR and  
1533 cortical patterns show similarity, but the lateral cortical intensity was much less pronounced with the  
1534 cortical mask, opposed to what is found under LA (Fig.7A).

1535 **Figure S8. Visual overview of single subject STC cross-correlation under low anesthesia**

1536 Each panel shows the cc, per window size, of individual subject STCs with their STCs derived at the group  
1537 level for PAT1 (left upper panel), PAT2 (right upper panel) and PAT3 (left lower panel). In the lower right  
1538 panel, STC cc is shown after GSR on the single subject and group level. Note the overall higher cc after GSR.

1539 **Figure S9. Single subject Quasi-Periodic Pattern detection under high anesthesia**

1540 Illustrations of QPPs detected for single subject three-slice brain volumes, with (left) and without (right)  
1541 GSR. Below each panel an excerpt of the subject's STC and its STC derived from the group-level analysis  
1542 are shown. The middle lowest panel shows the overlay of single subject STCs with and without GSR. All  
1543 subjects proved difficult to visually attribute a pattern type. Subject 11 shows nice overlap with group-  
1544 level STCs, and displayed a bilateral cortical pattern after GSR (**A**). Subject 8 and 4 however showed very  
1545 poor overlap in STCs and GSR led to detection of lateralized patterns. This illustrates the overall increased  
1546 difficulty of consistently detecting single subject QPPs under HA.

1547 **Figure S10. Patterns and Sliding Template Correlation after phase-randomization**

1548 To investigate the likelihood that QPPs would occur by chance or emerge as an intrinsic property of the  
1549 preprocessed signal, the full group dataset under LA was subjected to the detection algorithm after  
1550 performing phase randomization, while retaining the magnitude spectra. For methodology please refer to  
1551 Majeed et al. (2011). **A)** Normal analysis. Example of a QPP detected at group level and the related Sliding  
1552 Template Correlation (STC), marking the QPP's occurrence over time and across subjects (black triangles).  
1553 **B)** Same analysis after phase randomization. Note the loss of observable spatiotemporal dynamics and  
1554 peak detections.

1555 **Figure S11. Cross-correlation of frame-wise displacement with sliding Template**  
1556 **Correlation**

1557 Frame-wise displacement (FD), for all subject in the LA short TR data set, was calculated at each point by  
1558 taking the sum of absolute backwards looking temporal derivatives for all three motion time series  
1559 (Power et al., 2012). To compute rotational displacement and convert degrees to millimeters, an  
1560 assumption was made where the mouse brain is considered as a sphere with a diameter of 10mm. **A)**  
1561 Resultant FD time series were cross-correlated with the STCs of PAT1-3. **B)** FD time series were also  
1562 constructed after applying filtering, detrending and variance normalization (as was done for the  
1563 functional data) to the motion time series, and were then cross-correlated with the STCs of PAT1-3. Both  
1564 conditions showed minimal cross-correlation.

1565 **Figure S12. Subject-specific motion metrics and overlap with sliding Template Correlation**

1566 Single subject motion metrics, determined via 3 rigid body parameters, for the LA short TR data are  
1567 presented, together with FD, FD based on pre-processed motion time series, and PAT1-3 STCs. Results are

1568 shown for 3 illustrative subjects: **A)** Subject 11, **B)** Subject 8, and **C)** Subject 4. Upper 5 panels represent  
1569 non-pre-processed motion time series and lower 5 panels the pre-processed motion time series.

1570 **Figure S13. Quasi-Periodic Patterns after filtering between 0.01-0.1 Hz under low**  
1571 **anesthesia**

1572 Rows present QPPs observed for different window sizes of analysis (vertical axis). Images display a  
1573 normalized BOLD signal filtered between 0.01-0.1Hz. QPPs are phased using the calculated time delays of  
1574 their STC cc. Green boxes indicate ideal window sizes as determined via visual observation and fractional  
1575 average correlation. **A)** QPPs determined without GSR. Most prominently PAT3 was detected at most  
1576 window sizes, while PAT2 is observed at 6-7.5s. **B)** QPPs after GSR indicated consistent detection of the  
1577 same lateral-medial cortical pattern (PAT1) that was observed in the 0.01-0.2Hz frequency range. The  
1578 temporal dynamics of patterns detected at the lower frequency range were slower, which is expected due  
1579 to temporal smoothing. The 'lower' frequency range 0.01-0.1Hz is more often applied in conventional  
1580 rsfMRI and excludes the frequency range in which vasomotion and Mayer waves contribute to the signal.  
1581 Similar detection of patterns in the lower range thus supports the notion that QPPs reflect a neuronal  
1582 origin.

1583 **Figure S14. Quasi-Periodic Patterns reproducibility under LA conditions**

1584 Novel experiments were performed in 4 C57BL/6J mice at the age of 3.5 months, which were prior not  
1585 exposed to anesthesia. High temporal rsfMRI data was acquired with the same LA conditions and in the  
1586 same way as the primary LA group, starting at 40min post-bolus and lasting for 10min. An illustrative  
1587 analysis at a window size of 12s is shown, after running the detection algorithm 250 times. **A)** Examples of  
1588 PAT1-3, without GSR, and the global CAP. Patterns are phased to display them at their proper timing with  
1589 respect to each other. **B)** Examples of the same 3 apparent types of patterns after GSR (phased to each  
1590 other), as were shown in Fig.6A. **C)** Spatial cc clustering with or without GSR. Pattern clustering appeared  
1591 similar as for the main LA group.

1592 **Table S1. Pattern occurrence rate per subject under high anesthesia**

1593 **Table S2. Pattern half cycle time and propagation time from lateral to medial, averaged**  
1594 **across relevant window sizes, under high anesthesia**

1595 **Table S3. Physiological parameters under low anesthesia and interaction with patterns**  
1596 **determined via multiple linear regression analysis**

1597 Upper panel. Physiological parameters acquired during rsfMRI acquisitions in animals under LA. Note the  
1598 low STDs, indicating stable physiology during the experiments. No parameters could be stored for subject  
1599 2, but visual observation during the experiment confirmed stable respiration and cardiac rate. Lower  
1600 Panel. Multiple linear regression analysis of all four physiological parameters with each pattern's  
1601 occurrence rate and power (i.e. the average correlation value with the image series at peak crossings). No  
1602 significant interactions could be determined. Only after GSR a trend could be observed (orange boxes) and  
1603 the model fit ( $R^2$ ) was improved. Related Pearson correlation values ( $\rho$ ) are indicated below.

1604 **Movie 1.**

1605 Illustration of the Sliding Template Correlation (STC) approach to identify sets of images throughout the  
1606 image series, which are similar to the template. The upper panel shows the temporal evolution of a single  
1607 slice, with below the spatiotemporal template that is correlated at each incremental (1TR) overlap. An  
1608 arbitrary correlation threshold (red line) is employed to determine which images will be averaged into an  
1609 updated template during the iterative procedure, and to determine pattern occurrences once the final  
1610 template is derived.

1611 **Movie 2.**

1612 Visualisation of the three QPPs (PAT1-3), determined at the group level under LA. Images are shown for  
1613 the center slice, after phase alignment, and are displayed per TR (0.5s) for the duration of 12s.

1614 **Movie 3.**

1615 Visualisation of the three QPPs (PAT1,2 & 4) and the pattern detected after GSR, determined at the group  
1616 level under HA. Images are shown for the center slice, after phase alignment, and are displayed per TR  
1617 (0.5s) for the duration of 7.5s.

1618 **Movie 4.**

1619 Visualisation of PAT1 and QPPs after GSR, with a cortical mask, and a subcortical Caudate Putamen (Cp)  
1620 mask, determined at the group level under LA. Images are shown for the center slice, after phase  
1621 alignment, and are displayed per TR (0.5s) for the duration of 12s.

1622 **Movie 5.**

1623 Visualisation of QPPs for each individual subject, determined under LA. Images are shown for the full  
1624 volume of three slices (left to right = posterior to anterior) and are displayed per TR (0.5s) for the  
1625 duration of 9s.

1626 **Movie 6.**

1627 Visualisation of QPPs for each individual subject, determined after GSR and under LA. Images are shown  
1628 for the full volume of three slices (left to right = posterior to anterior) and are displayed per TR (0.5s) for  
1629 the duration of 9s.

1630 **Movie 7.**

1631 Visualisation of QPPs for each individual subject, determined under HA. Images are shown for the full  
1632 volume of three slices (left to right = posterior to anterior) and are displayed per TR (0.5s) for the  
1633 duration of 7.5s.

1634 **Movie 8.**

1635 Visualisation of QPPs for each individual subject, determined after GSR and under HA. Images are shown  
1636 for the full volume of three slices (left to right = posterior to anterior) and are displayed per TR (0.5s) for  
1637 the duration of 7.5s.