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Joanes Grandjean, Gabriel Desrosiers-Gregoire, Cynthia Anckaerts, Diego Angeles-Valdez, Fadi Ayad, David A Barrière, Ines Blockx, Aleksandra Bortel, Margaret Broadwater, Beatriz M Cardoso, et al.

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A consensus protocol for functional connectivity analysis in the rat brain

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A list of authors and their affiliations appears at the end of the paper

Task-free functional connectivity in animal models provides an experimental framework to examine connectivity phenomena under controlled conditions and allows for comparisons with data modalities collected under invasive or terminal procedures. Currently, animal acquisitions are performed with varying protocols and analyses that hamper result comparison and integration. Here we introduce StandardRat, a consensus rat functional magnetic resonance imaging acquisition protocol tested across 20 centers. To develop this protocol with optimized acquisition and processing parameters, we initially aggregated 65 functional imaging datasets acquired from rats across 46 centers. We developed a reproducible pipeline for analyzing rat data acquired with diverse protocols and determined experimental and processing parameters associated with the robust detection of functional connectivity across centers. We show that the standardized protocol enhances biologically plausible functional connectivity patterns relative to previous acquisitions. The protocol and processing pipeline described here is openly shared with the neuroimaging community to promote interoperability and cooperation toward tackling the most important challenges in neuroscience.

Understanding the brain requires a multi-level approach across spatial and temporal scales. Distinct brain network features, as revealed by task-free functional magnetic resonance imaging (fMRI), play a central role in the comprehension of healthy brain function and disorder mechanisms. This hemodynamic readout method relies on spontaneous fluctuations in the blood oxygenation level-dependent contrast signal to infer functional connectivity (FC) across the brain. Human neuroimaging has made great strides in understanding of the brain through open data-sharing initiatives focused on task-free fMRI data^{1–5}. Nonetheless, animal models, particularly small rodents, continue to play an important role in neuroscience discovery, partly owing to the feasibility of performing invasive and terminal manipulations on genetically controlled animals⁶. For example, rats are commonly used in pharmacological studies, owing to similarities in drug metabolism, as well as in behavioral neuroscience, owing to their high proficiency in learning complex tasks. By leveraging the same neuroimaging methods and metrics obtained in humans, task-free neuroimaging in rodents may provide a translational bridge from the invasive methods possible only in animal models.

Human neuroimaging sharing initiatives have led to a standardization of fMRI acquisition protocols that aid in the dissemination, aggregation and reuse of data^{4,7,8}. In contrast, preclinical neuroimaging essentially remains without harmonizing guidelines⁹. Animal data acquisitions are performed under diverse protocols that span different strains, restraint and anesthesia conditions, radiofrequency coil designs and magnetic field strengths. These impact the generalization of the results and conclusions. Efforts to propose acquisition and/or pre-processing protocols rarely extend beyond the confines of single laboratories, thus limiting interoperability and widespread adoption^{9,10}. Because of the potential of fMRI in rodents to study the biological basis for connectivity phenomena across the whole brain longitudinally¹¹, an optimized consensus protocol could potentiate future scientific discoveries.

In this pre-registered study, we set out to aggregate and make publicly available representative datasets with various fMRI acquisition protocols in the rat and identify experimental parameters associated with robust and reliable FC detection. We curated the MultiRat_rest collection (646 rats from 65 datasets) representing protocols used

✉ e-mail: joanes.grandjean@radboudumc.nl

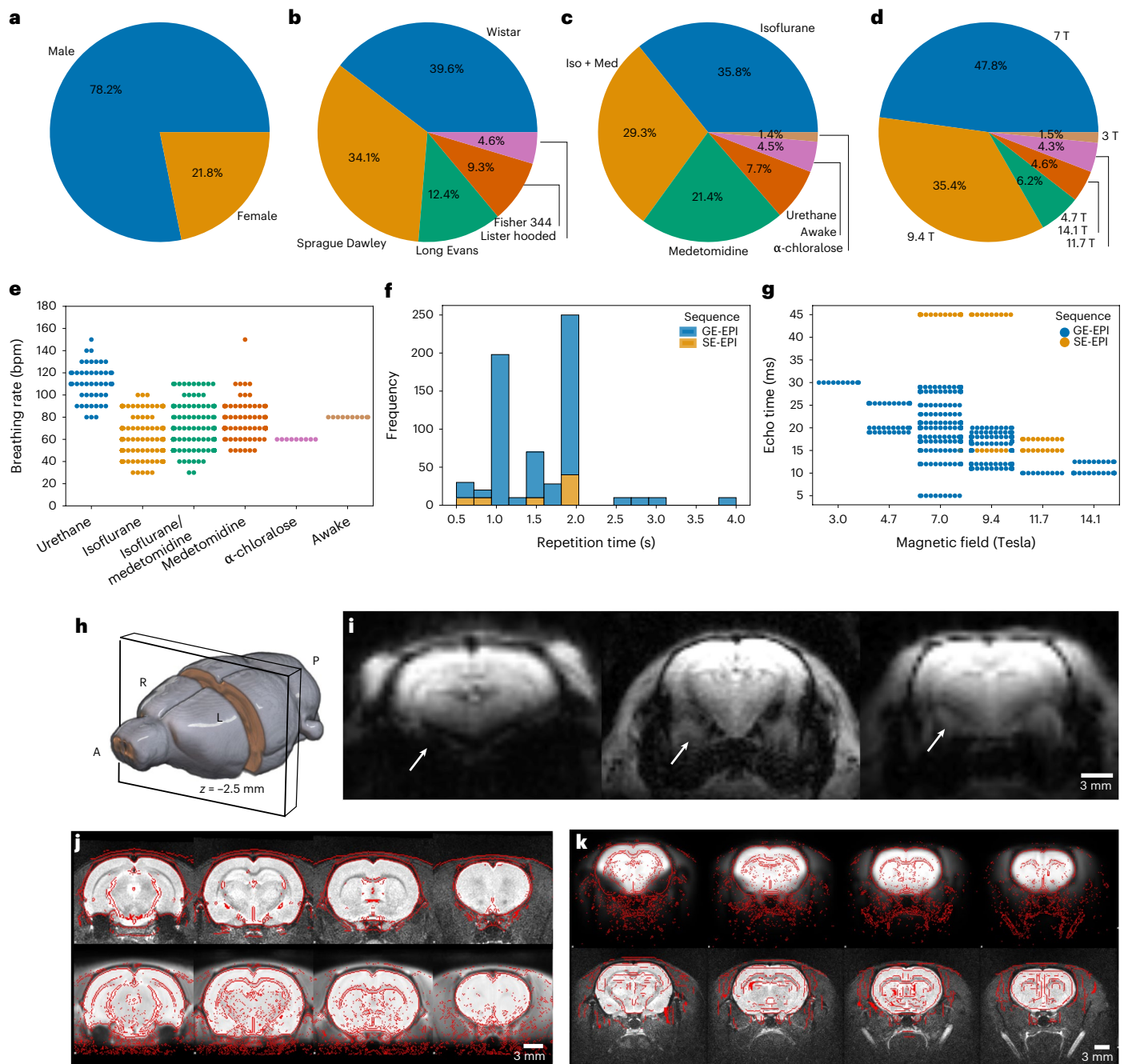


Fig. 1 | MultiRat_rest dataset description. **a**, Sex. **b**, Strain. **c**, Anesthesia. **d**, Magnetic field strength. **e**, Breathing rate as a function of anesthesia. **f**, Repetition time. **g**, Echo time as a function of magnetic field strength. **h**, Slice position for the examples. **i**, Example of representative raw functional images. Arrows indicate different susceptibility artifact-related geometric distortions

in the amygdala. **j**, Successful anatomical (top) to standard (bottom) space registration. Red lines indicate the outlines of the standard image (top) and the anatomical (bottom). **k**, Successful functional (top) to anatomical (bottom) registration. Red lines indicate the outlines of the anatomical image (top) and the functional (bottom).

at 46 institutions. Based on the analysis of the MultiRat_rest collection, we devised a new consensus protocol and used it to aggregate the StandardRat collection (209 rats from 21 datasets). Pre-processing and confound correction were tailored to rodent data of different characteristics using a rodent-adapted fMRI pre-processing and analysis tool. Our primary outcome was the detection of plausible FC patterns corresponding to the biologically expected models. Collating data from 50 centers and 855 rats, we show that standardized acquisition and the associated pre-processing pipeline optimize the detection of distributed fMRI networks in rats. In line with large-scale

studies from other species^{12,13}, we have freely released all data and the generated code.

Results

We aggregated the MultiRat_rest collection of unstandardized fMRI datasets representative of local site acquisition procedures ($n = 65$ datasets and $n = 646$ rats). As expected, we found high heterogeneity in all experimental factors recorded, including rat characteristics (sex and strain, Fig. 1a,b, and age and weight, Extended Data Fig. 1), in-scan physiology (anesthesia/awake and breathing rates; Fig. 1c,e) and

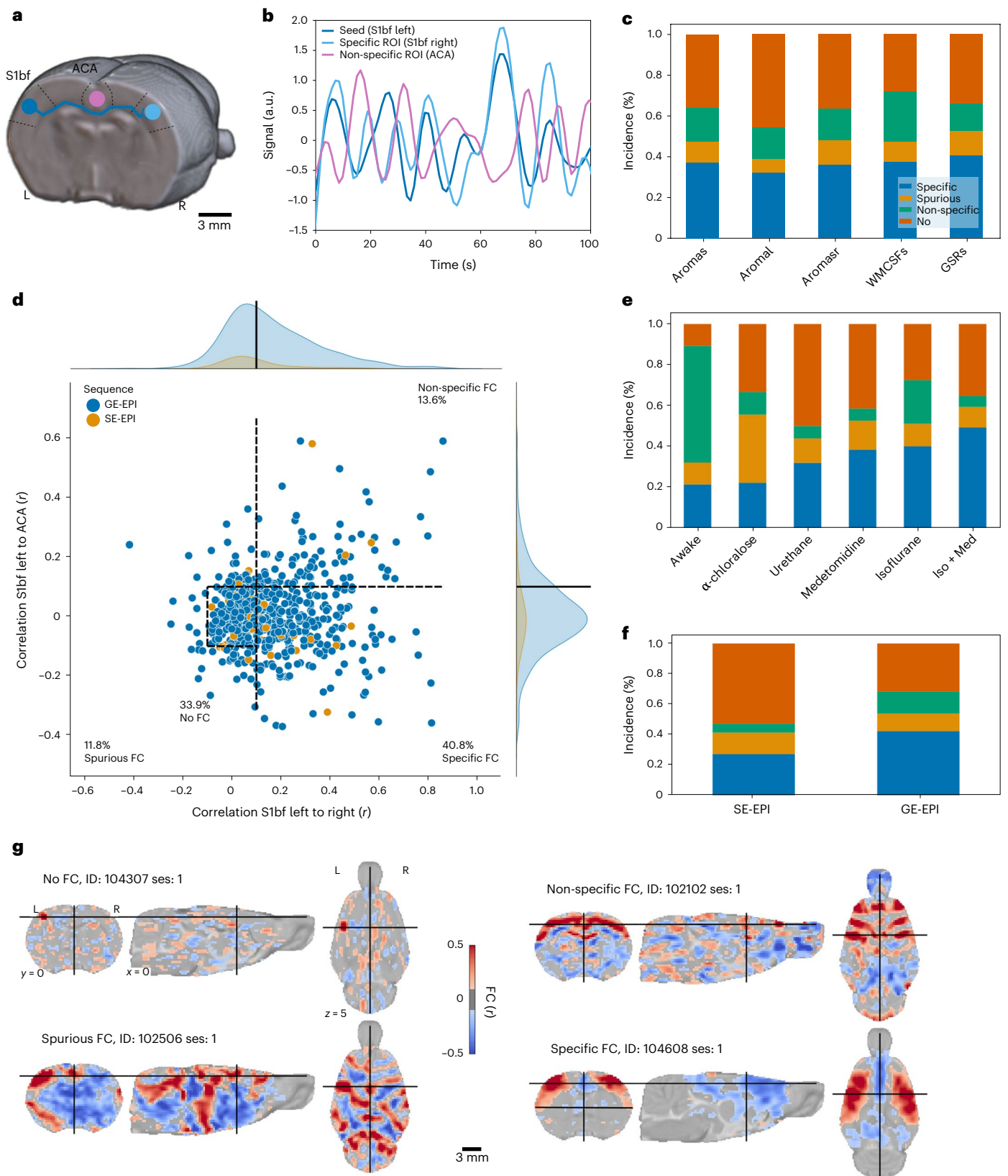


Fig. 2 | FC specificity. **a**, Diagram illustrating the logic behind FC specificity. The sensory (barrel field, S1bf) area (blue) chiefly projects to the contralateral homotopic area (light blue) but not to the ACA area (purple). **b**, Example of temporal dynamics in the resting-state signal. Correlated signal between the ipsilateral and contralateral S1bf and anti-correlated signal from the ACA. **c**, Distribution of FC categories as a function of confound correction models. **d**, FC in left S1bf relative to specific (right S1bf) and non-specific (ACA) regions of interest using the global regression correction model. Dots represent

scans ($n = 638$ rats); dotted lines indicate the thresholds used to delineate the categories. **e**, Distribution of connectivity categories as a function of anesthesia. Example of individual seed-based analysis maps for each connectivity category. **f**, Distribution of connectivity categories as a function of imaging sequence (EPI, echo-planar imaging; GE, gradient echo; SE, spin echo). Group-level FC incidence map ($n = 65$ datasets). **g**, Example of individual seed-based analysis maps for each connectivity category. a.u., arbitrary units; ROI, region of interest; WMCSFs, white-matter + cerebrospinal fluid.

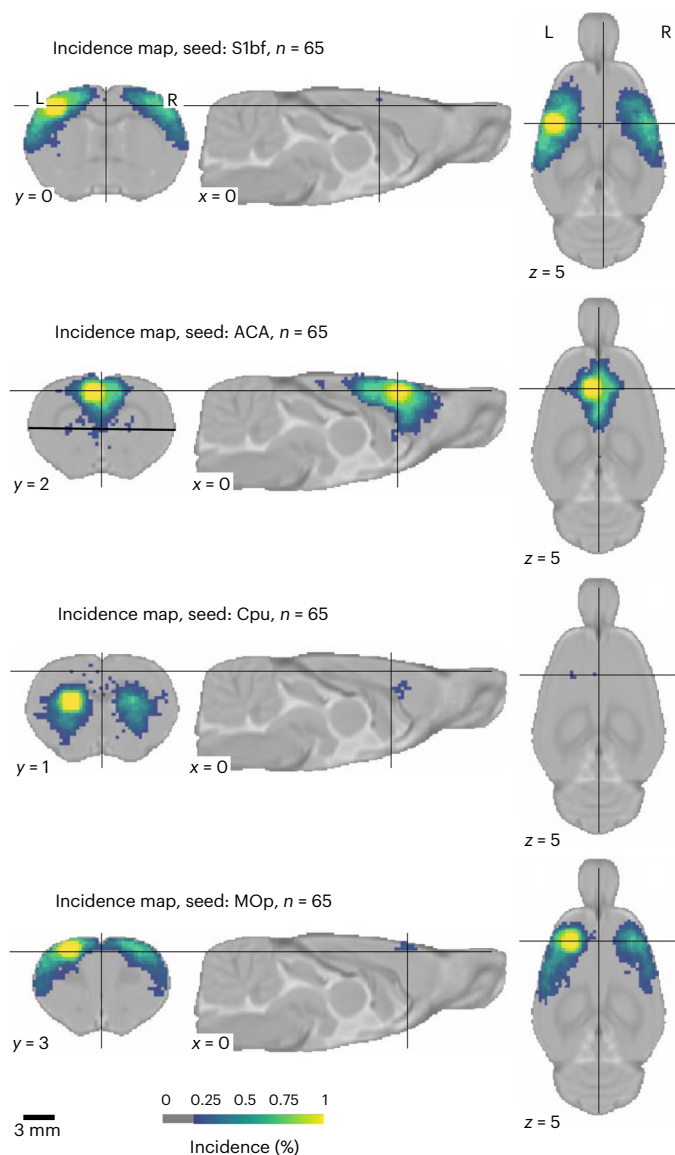


Fig. 3 | Incidence of FC at the group level ($n = 65$ datasets of $n - 10$ subjects per dataset) for four seeds. Cpu, caudate-putamen; MOp, primary motor area. MOp and Cpu seeds were generally observed in 50–75% of the datasets.

image acquisitions (magnetic field strength, sequence and sequence parameters; Fig. 1d,f,g). Notably, there was a large sex bias in favor of males (Fig. 1a). Similarly, the distribution of anesthesia protocols was also in line with current trends in the field (Fig. 1c)⁹. Despite the heterogeneous distribution of the acquisition parameters and ensuing image quality (Fig. 1g,h,i), 638 of 646 of the scans passed pre-processing quality control (one scan with excessive motion, one empty scan and six scans with failed image registrations; Fig. 1j,k and Extended Data Fig. 2). As further quality controls, we described temporal signal-to-noise (Extended Data Fig. 3) and motion parameters (Extended Data Fig. 4). Overall, we found that the aggregated datasets represent current rodent fMRI acquisition trends⁹. Moreover, given the low exclusion rate due to mis-registration, we concluded that the RABIES toolbox can be effectively employed to pre-process rat datasets despite widely varying acquisition parameters¹⁴. This paves the way for reproducible and interoperable data processing across sites.

We focused on examining FC in the sensory cortex, as sensory networks are robust to assess anesthesia effects in the anesthesia depth range typically used in fMRI¹⁵. More specifically, we evaluated

the specificity of the connectivity of the S1 barrel field area (S1bf) using two complementary criteria as indexes of accurate FC identification (Fig. 2a,b)¹². The first criterion was the strong connectivity between inter-hemispheric sensory cortices (barrel field, S1bf). Indeed, in both humans and animals, dating back to the original description of FC¹⁶, most networks, including sensory–motor networks, have a bilateral homotopic organization. The second criterion was a weak or anti-correlation between S1bf and the anterior cingulate area (ACA). The ACA is a major node in the task-negative rodent default mode network. Task-positive (as the S1bf-associated sensory network^{17,18}) and task-negative networks are generally non-correlated or anti-correlated¹⁹.

FC was evaluated for each animal and divided into four categories based on these two criteria referred above: specific, non-specific, spurious and no connectivity. Five confound correction models were tested (Supplementary Table 2 and Fig. 2c). The global signal regression (GSR) nuisance model was the one that performed the best for specific connectivity detection (40.8% of the animals with specific connectivity, 11.8% as non-specific, 13.6% as spurious and 33.9% as containing no detectable FC; Fig. 2c,d,g). To test the generalizability of the specificity metric to other networks, we implemented the same quality control metric to the cingulate cortex, a node of the rodent default mode network, which uncovered lower connectivity specificity (22.1%; Extended Data Fig. 5). Because network inference is often assessed at the group level rather than at the individual level, we performed a one-sample *t*-test per dataset to estimate the incidence of contralateral connectivity detected within groups, relative to the S1bf seed as well as three other seeds (Fig. 3). Up to 70% of the 65 datasets presented limited evidence of contralateral connectivity relative to the seeds, and 50% of the datasets captured the features of a larger sensory network at the group level. We conclude that rat datasets do not capture FC equally, similar to what we reported in the mouse¹². We also found that GSR enhances the incidence of specific connectivity of S1bf seeds, so we decided to use this confound model for the remainder of our analysis.

Our observations underline the need for an improved acquisition protocol to maximize individual-level inferences to potentiate discovery in experimental network neuroscience. To address this, we evaluated parameters associated with increased specificity incidence in the MultiRat_rest collection. Because our analysis relied on categorical data, we tested for differences against the expected frequency distribution of the four connectivity categories as a function of anesthesia and sequence categories using the χ^2 test. Medetomidine/isoflurane anesthesia combination condition was enriched in scans categorized as specific (Fig. 2e; 92/187 scans, χ^2 test *connectivity category - anesthesia*: $\phi = 0.27$, degrees of freedom (dof) = 15, $g = 92.38$, $P = 3.5 \times 10^{-13}$). The use of gradient echo imaging sequence was also associated with higher specificity incidence (Fig. 2f; 241/568 scans, χ^2 test *connectivity category - sequence*: $\phi = 0.11$, dof = 3, $g = 16.00$, $P = 0.001$). Based on these observations, we devised an anesthesia protocol derived from dataset ds01031 (9/10 specific scans) and an imaging sequence based on ds01028 (8/10 specific scans), acquired on a 4.7T mid-field system. The sequence selection is justified as it allows for the detection of specific connectivity on one of the lower-field systems used in this collection and should also perform well for higher-field systems. We optimized relevant sequence parameters (echo time, flip angle and bandwidth) as a function of field strength (Supplementary Table 1). We hypothesized that this protocol would enhance functional specificity while being compatible with lower-field systems that continue to represent a relevant share of the systems used (Fig. 1d)⁹.

Using this consensus protocol, we curated the StandardRat collection of 21 datasets obtained across 20 centers. This consisted of $n = 209$ rats (93/116 female/male), mainly Wistar (189/209), aged ~2 months (Extended Data Fig. 6). Dataset acquisitions were performed at magnetic field strengths ranging from 4.7T to 17.2T. Pre-processing

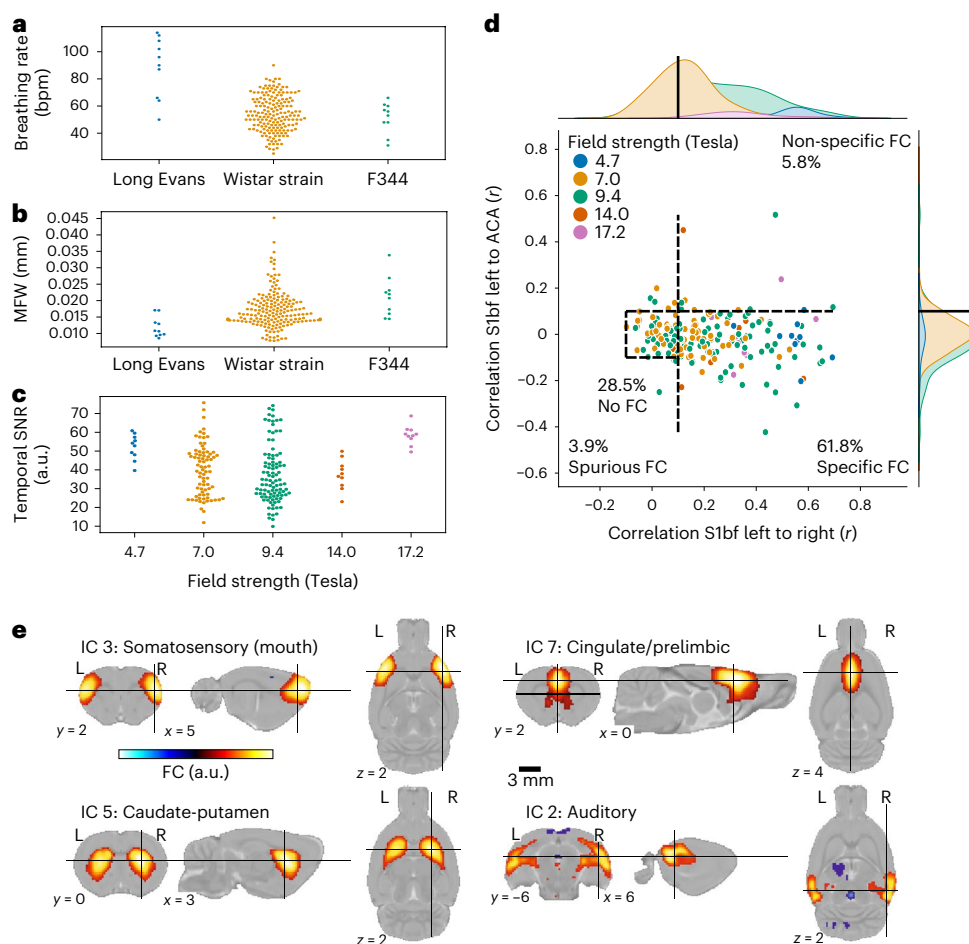


Fig. 4 | StandardRat dataset description. **a**, Breathing rate (bpm) as a function of strain. **b**, MFW as a function of strain. **c**, Temporal signal-to-noise ratio in the sensory cortex as a function of field strength. **d**, FC in left S1bf relative to specific (right S1bf) and non-specific (ACA) regions of interest using the global regression

correction model. Dots represent scans ($n = 207$ rats); dotted lines indicate the thresholds used to delineate the categories. **e**, Representative independent components. a.u., arbitrary units.

was performed similarly to the unstandardized dataset. In total, 207 of 209 scans passed quality assurance (two discards due to image mis-registration). Interestingly, despite the same anesthesia protocol being used, the respiratory rates reported at the start of fMRI acquisition differed as a function of rat strain (Fig. 4a,b; analysis of variance *breathingrate - rat strain*, $\eta^2 = 0.24$, $F_{195,2} = 31.17$, $P = 1.8 \times 10^{-12}$). Finally, there was only a negligible effect on the temporal signal-to-noise ratio as a function of magnetic field strength (Fig. 4c; linear regression *temporal signal-to-noise ratio - field strength*, coefficient = 0.53 [−0.23, 1.30], $r^2 = 0.01$, dof = 201, $T = 1.37$, $P = 0.17$).

The objective of the StandardRat study was to improve the detection of specific connectivity in the individual datasets. We found that 61.8% of the scans were categorized as exhibiting specific connectivity (Fig. 4d) against 40.8% in the MultiRat_rest dataset with unstandardized acquisitions (Extended Data Fig. 8a) when using GSR (χ^2 test *connectivity category - dataset collection*: $\phi = 0.13$, dof = 3, $g = 33.01$, $P = 3.2 \times 10^{-7}$). The difference remained when we compared datasets from centers that contributed to both collections exclusively (χ^2 test *connectivity category - dataset collection*: $\phi = 0.17$, dof = 3, $g = 28.37$, $P = 3.0 \times 10^{-6}$). This is going against the notion that the StandardRat collection outperforms due to characteristics of the contributing laboratories (for example, magnet type or strength and greater experience in data collection). Intriguingly, we could not establish a field strength effect on connectivity specificity (χ^2 test *connectivity category - field strength*: $\phi = 0.19$, dof = 12, $g = 14.89$, $P = 0.25$), suggesting

that acquisition systems are not the limiting factor in this protocol. We conclude that the newly standardized protocol outperforms, on average, previously used protocols within the community for the detection of biologically plausible connectivity patterns. Finally, to explore whole-brain connectivity patterns, we examined connectivity incidence at the dataset level for four selected seeds (Extended Data Fig. 7) and group independent component analysis (Fig. 4e and Extended Data Fig. 10). We found improvement in distal connectivity detection at the dataset level compared to the MultiRat_rest dataset as well as evidence for previously described rodent networks spanning across the whole brain²⁰.

Intriguingly, there remained differences in the connectivity patterns between datasets from the StandardRat collection. Indeed, five of 21 datasets achieved 90% specificity or higher. This underlines the potential of our protocol but also the need to understand what factors hamper other datasets. We next sought to identify the variables associated with greater incidences of specific connectivity patterns. Notably, we could not establish strain, sex or magnetic field strength effects, suggesting that the protocol is applicable for a large range of conditions (Supplementary Table 3). Next, we examined breathing rate and temporal signal-to-noise ratio as indicators of acquisition variability (Extended Data Fig. 9). Overall, scans with breathing rates ranging from 84 to 114 breaths per minute (bpm) and cortical temporal signal-to-noise ratio >53 achieved higher incidences of connectivity specificity among the StandardRat collection. These provide the first

line of evidence to refine the StandardRat protocol by identifying practices that can further enhance connectivity outcomes. The importance of these observations will require confirmations in new datasets, preferably across multiple centers, before the StandardRat protocol can be updated with new guidelines.

Discussion

In summary, we curated two dataset collections (MultiRat_rest and StandardRat), analyzed them and made them an open-access resource. To our knowledge, these are the largest rodent fMRI datasets currently available. We developed and deployed a pre-processing and confound correction strategy generalizable to most scans and every dataset. Using information from the MultiRat_rest collection, we provide useful population parameter estimates to enhance the comparison of rat fMRI datasets. We proposed and evaluated a new standardized protocol and found that this consensus acquisition and pre-processing pipeline outperformed the previous acquisitions for connectivity specificity. To allow replication and to inspire new analyses, we release all raw and processed data to the broader community.

On average, the standardized protocol yielded improvements over previous acquisitions gathered in the MultiRat_rest collection. However, individual-level inferences remain limited to 61.8% of the scans acquired. For comparison, we estimated 55% specificity in the 7T Human Connectome Project dataset (based on 184 scans) using the same quality assurance metrics⁵. This underlines the importance of implementing sound quality assurance metrics based on assumptions of biologically plausible FC. Improving output quality, through understanding the factors leading to successful acquisitions, enhanced protocols, pre-processing or nuisance regression models, would lead to tangible outcomes capable of further potentiating future data acquisitions and reducing animal use by reducing discards.

Notably, our new protocol relies on light sedation to restrain the animals. Although optimized for fMRI, this protocol may not generalize to other procedures, such as electrophysiology. We also found that existing awake restraining protocols, on average, lead to a lower incidence of specific connectivity patterns (Extended Data Fig. 8b). A previous report indicated similar values in a dataset in awake rats²¹, which we confirmed with pre-processing using the RABIES pipeline (Extended Data Fig. 8b). Due to the impact of anesthesia on networks^{9,10}, it remains central to develop awake imaging as an alternative. However, these protocols should be examined through the lens of quality control metrics to ensure that plausible connectivity patterns are achieved consistently. Furthermore, physiological factors, such as heart rate, were scarcely reported. This limited our ability to examine the possible contribution of these factors on connectivity outcomes. We take this opportunity to encourage the community to acquire these data and report them in ensuing publications. Finally, the acquisition sequence in StandardRat is designed to run on many systems. The effectiveness of new sequences should be examined against the current protocol—for example, isotropic resolution²² or multiband acquisition²³.

Our project's methodological and conceptual advancements are the first step toward large multi-site rat neuroimaging acquisitions. Coordinated open-science projects in neuroimaging and other disciplines are transforming the scientific landscape²⁴. Through the concerted efforts of our centers and potentiated by a substantially improved protocol, rat functional brain imaging is set to tackle urgent questions in neuroscience and mental health research.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41593-023-01286-8>.

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Joanes Grandjean ^{1,2}✉, Gabriel Desrosiers-Gregoire^{3,4}, Cynthia Anckaerts^{5,6}, Diego Angeles-Valdez⁷, Fadi Ayad^{8,9,10}, David A. Barrière ¹¹, Ines Blockx^{5,6}, Aleksandra Bortel^{9,10,12}, Margaret Broadwater^{13,14,15}, Beatriz M. Cardoso¹⁶, Marina Célestine¹⁷, Jorge E. Chavez-Negrete¹⁸, Sangcheon Choi ^{19,20}, Emma Christiaen²¹, Perrin Clavijo²², Luis Colon-Perez ²³, Samuel Cramer²⁴, Tolomeo Daniele²⁵, Elaine Dempsey ^{26,27}, Yujian Diao^{28,29}, Arno Doelemeyer³⁰, David Dopfel²⁴, Lenka Dvořáková³¹, Claudia Falfán-Melgoza³², Francisca F. Fernandes ¹⁶, Caitlin F. Fowler^{3,8}, Antonio Fuentes-Ibañez ¹⁸, Clément Garin ¹⁷, Eveline Gelderman ¹, Carla E. M. Golden ³³, Chao C. G. Guo ¹, Marloes J. A. G. Henckens ^{1,34}, Lauren A. Hennessy ^{35,36}, Peter Herman ^{37,38}, Nita Hofwijks¹, Corey Horien ³⁷, Tudor M. Ionescu³⁹, Jolyon Jones⁴⁰, Johannes Kaesser⁴¹, Eugene Kim ⁴², Henriette Lambers ⁴³, Alberto Lazari⁴⁴, Sung-Ho Lee ^{13,14,15}, Amanda Lillywhite ^{45,46}, Yikang Liu²⁴, Yanyan Y. Liu⁴⁷, Alejandra López -Castro ⁷, Xavier López-Gil ⁴⁸, Zilu Ma²⁴, Eilidh MacNicol ⁴², Dan Madularu^{8,49}, Francesca Mandino ³⁷, Sabina Marciano³⁹, Matthew J. McAuslan²⁶, Patrick McCunn⁵⁰, Alison McIntosh^{26,27}, Xianzong Meng¹, Lisa Meyer-Baese²², Stephan Missault^{5,6}, Federico Moro ⁵¹, Daphne M. P. Naessens ⁵², Laura J. Nava-Gomez ^{53,54}, Hiroi Nonaka⁵⁵, Juan J. Ortiz¹⁸, Jaakko Paasonen³¹, Lore M. Peeters^{5,6}, Mickaël Pereira ⁵⁶, Pablo D. Perez²⁴, Marjory Pompilus⁵⁷, Malcolm Prior⁵⁸, Rustam Rakhmatullin⁵⁹, Henning M. Reimann⁶⁰, Jonathan Reinwald⁶¹, Rodrigo Triana Del Rio⁶², Alejandro Rivera-Olvera¹, Daniel Ruiz-Pérez⁵⁹, Gabriele Russo⁶³, Tobias J. Rutten¹, Rie Ryoke⁵⁵, Markus Sack³², Piergiorgio Salvan ⁴⁴, Basavaraju G. Sanganahalli ^{37,38}, Aileen Schroeter⁶⁴, Bhedita J. Seewoo ^{35,36,65}, Erwan Selingue⁶⁶, Aline Seuwen⁶⁴, Bowen Shi ⁶⁷, Nikoloz Sirmipilatzé ^{68,69,70}, Joanna A. B. Smith^{71,72,73}, Corrie Smith²², Filip Sobczak ^{19,20}, Petteri J. Stenroos⁷⁴, Milou Straathof⁷⁵, Sandra Strobel⁴¹, Akira Sumiyoshi^{55,76}, Kengo Takahashi^{19,20}, Maria E. Torres-García¹⁸, Raul Tudela⁷⁷, Monica van den Berg ^{5,6}, Kajo van der Marel⁷⁵, Aran T. B. van Hout¹, Roberta Vertullo¹, Benjamin Vidal ⁵⁶, Roël M. Vrooman ¹, Victora X. Wang⁷⁸, Isabel Wank⁴¹, David J. G. Watson⁴⁵, Ting Yin⁷⁹, Yongzhi Zhang⁸⁰, Stefan Zurbrugg⁸¹, Sophie Achard ⁸², Sarael Alcauter¹⁸, Dorothee P. Auer^{58,83}, Emmanuel L. Barbier ⁷⁴, Jürgen Baudewig⁶⁸, Christian F. Beckmann^{1,44}, Nicolau Beckmann ³⁰, Guillaume J. P. C. Becq ⁸⁴, Erwin L. A. Blezer⁷⁵, Radu Bolbos⁸⁵, Susann Boretius ^{68,69,70}, Sandrine Bouvard⁵⁶, Eike Budinger ^{86,87}, Joseph D. Buxbaum ³³, Diana Cash ⁴², Victoria Chapman^{45,46,83}, Kai-Hsiang Chuang ⁸⁸, Luisa Ciobanu⁶⁶, Bram F. Coolen⁵², Jeffrey W. Dalley⁴⁰, Marc Dhenain ¹⁷, Rick M. Dijkhuizen⁷⁵, Oscar Esteban ⁸⁹, Cornelius Faber ⁴³, Marcelo Febo⁵⁷, Kirk W. Feindel⁶⁵, Gianluigi Forloni ⁹⁰, Jérémie Fouquet³, Eduardo A. Garza-Villarreal⁷, Natalia Gass³², Jeffrey C. Glennon⁹¹, Alessandro Gozzi ⁹², Olli Gröhn³¹, Andrew Harkin^{26,27}, Arend Heerschap ², Xavier Helluy^{63,93}, Kristina Herfert ³⁹, Arnd Heuser ⁹⁴, Judith R. Homberg ¹, Danielle J. Houwing¹, Fahmeed Hyder ^{37,38}, Giovanna Diletta Ielacqua⁹⁴, Ileana O. Jelescu ²⁸, Heidi Johansen-Berg⁴⁴, Gen Kaneko ⁹⁵, Ryuta Kawashima⁵⁵, Shella D. Keilholz ²², Georgios A. Keliris ^{5,6}, Clare Kelly ^{27,96,97}, Christian Kerskens^{27,98}, Jibrán Y. Khokhar⁵⁰, Peter C. Kind^{71,72,73,99}, Jean-Baptiste Langlois⁸⁵, Jason P. Lerch^{44,100}, Monica A. López-Hidalgo⁵⁴, Denise Manahan-Vaughan⁶³, Fabien Marchand¹⁰¹, Rogier B. Mars^{1,44}, Gerardo Marsella¹⁰², Edoardo Micotti⁹⁰, Emma Muñoz-Moreno ⁴⁸, Jamie Near ^{3,103}, Thoralf Niendorf^{60,104}, Willem M. Otte^{75,105}, Patricia Pais-Roldán ^{19,106}, Wen-Ju Pan²², Roberto A. Prado-Alcalá¹⁸, Gina L. Quirarte ¹⁸, Jennifer Rodger ^{35,36}, Tim Rosenow ¹⁰⁷, Cassandra Sampaio-Baptista ^{44,108}, Alexander Sartorius ⁶¹, Stephen J. Sawiak ¹⁰⁹, Tom W. J. Scheenen ^{2,110}, Noam Shemesh ¹⁶, Yen-Yu Ian Shih ^{13,14,15,111}, Amir Shmuel ^{8,9,10,12,112}, Guadalupe Soria ¹¹³, Ron Stoop⁶², Garth J. Thompson ⁶⁷, Sally M. Till^{71,72,73}, Nick Todd⁸⁰, Annemie Van Der Linden^{5,6}, Annette van der Toorn ⁷⁵, Geralda A. F. van Tilborg ⁷⁵, Christian Vanhove ²¹, Andor Veltien ², Marleen Verhoye ^{5,6}, Lydia Wachsmuth⁴³, Wolfgang Weber-Fahr³², Patricia Wenk⁸⁶, Xin Yu^{19,114}, Valerio Zerbi ^{115,116}, Nanyin Zhang ²⁴, Baogui B. Zhang⁴⁷, Luc Zimmer ^{56,85,117}, Gabriel A. Devenyi ^{3,118}, M. Mallar Chakravarty^{3,8,118} & Andreas Hess ⁴¹

¹ Donders Institute for Brain, Behaviour, and Cognition, Radboud University, Nijmegen, The Netherlands. ² Department for Medical Imaging, Radboud University Medical Center, Nijmegen, The Netherlands. ³ Cerebral Imaging Centre, Douglas Mental Health University Institute, Verdun, QC, Canada.

⁴ Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada. ⁵ Bio-imaging Lab, University of Antwerp, Antwerp, Belgium.

⁶μNEURO Research Centre of Excellence, University of Antwerp, Antwerp, Belgium. ⁷Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, Mexico. ⁸Biological and Biomedical Engineering, McGill University, Montreal, QC, Canada. ⁹McConnell Brain Imaging Centre, McGill University, Montreal, QC, Canada. ¹⁰Montreal Neurological Institute, McGill University, Montreal, QC, Canada. ¹¹UMR INRAE/CNRS 7247 Physiologie des Comportements et de la Reproduction, Physiologie de la reproduction et des comportements, Centre de recherche INRAE de Nouzilly, Tours, France. ¹²Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada. ¹³Center for Animal MRI, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ¹⁴Department of Neurology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ¹⁵Biomedical Research Imaging Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ¹⁶Preclinical MRI, Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon, Portugal. ¹⁷Laboratoire des Maladies Neurodégénératives, Molecular Imaging Research Center (MIRCent), Université Paris-Saclay, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA), CNRS, Fontenay-aux-Roses, France. ¹⁸Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, México. ¹⁹Translational Neuroimaging and Neural Control Group, High-Field Magnetic Resonance, Max Planck Institute for Biological Cybernetics, Tuebingen, Germany. ²⁰Graduate Training Centre of Neuroscience, International Max Planck Research School, University of Tuebingen, Tuebingen, Germany. ²¹Institute Biomedical Technology (IBiTech), Electronics and Information Systems (ELIS), Ghent University, Ghent, Belgium. ²²Department of Biomedical Engineering, Emory University/Georgia Institute of Technology, Atlanta, GA, USA. ²³Department of Pharmacology & Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, USA. ²⁴Translational Neuroimaging and Systems Neuroscience Lab, Biomedical Engineering, Pennsylvania State University, University Park, PA, USA. ²⁵Centre for Advanced Biomedical Imaging, University College London, London, UK. ²⁶Neuropsychopharmacology Research Group, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland. ²⁷Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland. ²⁸CIBM Center for Biomedical Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. ²⁹Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. ³⁰Musculoskeletal Diseases Department, Novartis Institutes for BioMedical Research, Basel, Switzerland. ³¹Biomedical Imaging Unit, A.I.V. Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland. ³²Translational Imaging, Department of Neuroimaging, Central Institute of Mental Health, Medical Faculty Mannheim, Mannheim, Germany. ³³Seaver Autism Center for Research & Treatment, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York City, NY, USA. ³⁴Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands. ³⁵Experimental and Regenerative Neurosciences, School of Biological Sciences, University of Western Australia, Crawley, WA, Australia. ³⁶Brain Plasticity Group, Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia. ³⁷Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, CT, USA. ³⁸Quantitative Neuroscience with Magnetic Resonance (QNMR) Core Center, Yale University School of Medicine, New Haven, CT, USA. ³⁹Werner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, University of Tuebingen, Tuebingen, Germany. ⁴⁰Department of Psychology, University of Cambridge, Cambridge, UK. ⁴¹Institute of Experimental and Clinical Pharmacology and Toxicology, FAU Erlangen-Nürnberg, Erlangen, Germany. ⁴²Biomarker Research And Imaging in Neuroscience (BRAIN) Centre, Department of Neuroimaging King's College London, London, UK. ⁴³Experimental Magnetic Resonance Group, Clinic of Radiology, University of Münster, Münster, Germany. ⁴⁴Nuffield Department of Clinical Neurosciences, Wellcome Centre for Integrative Neuroimaging, FMRIB, University of Oxford, John Radcliffe Hospital, Headington, Oxford, UK. ⁴⁵School of Life Sciences, University of Nottingham, Nottingham, UK. ⁴⁶Pain Centre Versus Arthritis, University of Nottingham, Nottingham, UK. ⁴⁷Brainnetome Center, Institute of Automation, Chinese Academy of Sciences, Beijing, China. ⁴⁸Magnetic Imaging Resonance Core Facility, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. ⁴⁹Center for Translational Neuroimaging, Northeastern University, Boston, MA, USA. ⁵⁰Khokhar Lab, Department of Anatomy and Cell Biology, Western University, London, ON, Canada. ⁵¹Laboratory of Acute Brain Injury and Therapeutic Strategies, Department of Neuroscienze Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Milan, Italy. ⁵²Biomedical Engineering and Physics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands. ⁵³Facultad de Medicina, Universidad Autónoma de Querétaro, Querétaro, México. ⁵⁴Escuela Nacional de Estudios Superiores, Juriquilla, Universidad Nacional Autónoma de México, Querétaro, México. ⁵⁵Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan. ⁵⁶Lyon Neuroscience Research Center, Université Claude Bernard Lyon 1, INSERM, CNRS, Lyon, France. ⁵⁷Febo Laboratory, Department of Psychiatry, University of Florida, Gainesville, FL, USA. ⁵⁸School of Medicine, University of Nottingham, Nottingham, UK. ⁵⁹Comparative Medicine Unit, Trinity College Dublin, Dublin, Ireland. ⁶⁰Berlin Ultrahigh Field Facility (B.U.F.F.), Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany. ⁶¹Translational Imaging, Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany. ⁶²Psychiatric neurosciences, Center for Psychiatric Neuroscience, Lausanne University and University Hospital Center, Unicentre, Lausanne, Switzerland. ⁶³Department of Neurophysiology, Medical Faculty, Ruhr University Bochum, Bochum, Germany. ⁶⁴Institute for Biomedical Engineering, University and ETH Zurich, Zurich, Switzerland. ⁶⁵Centre for Microscopy, Characterisation & Analysis, Research Infrastructure Centres, University of Western Australia, Nedlands, WA, Australia. ⁶⁶NeuroSpin, CEA Saclay, Paris, France. ⁶⁷iHuman Institute, ShanghaiTech University, Shanghai, China. ⁶⁸Functional Imaging Laboratory, German Primate Center - Leibniz Institute for Primate Research, Göttingen, Germany. ⁶⁹Faculty of Biology and Psychology, Georg-August University of Göttingen, Göttingen, Germany. ⁷⁰DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany. ⁷¹Simons Initiative for the Developing Brain, University of Edinburgh, Edinburgh, UK. ⁷²Patrick Wild Centre, University of Edinburgh, Edinburgh, UK. ⁷³Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, UK. ⁷⁴Univ. Grenoble Alpes, Inserm, U1216, Grenoble Institut Neurosciences, Grenoble, France. ⁷⁵Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, University Medical Center Utrecht & Utrecht University, Utrecht, The Netherlands. ⁷⁶National Institutes for Quantum Science and Technology, Chiba, Japan. ⁷⁷Group of Biomedical Imaging, Consorcio Centro de Investigación Biomédica en Red (CIBER) de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), University of Barcelona, Barcelona, Spain. ⁷⁸BioMedical Engineering and Imaging Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA. ⁷⁹Animal Imaging and Technology Section, Center for Biomedical Imaging, École polytechnique fédérale de Lausanne, Lausanne, Switzerland. ⁸⁰Focused Ultrasound Laboratory, Department of Radiology Brigham and Women's Hospital, Boston, MA, USA. ⁸¹Neurosciences Department, Novartis Institutes for BioMedical Research, Basel, Switzerland. ⁸²Inria, University Grenoble Alpes, CNRS, Grenoble, France. ⁸³NIHR Biomedical Research Centre, University of Nottingham, Nottingham, UK. ⁸⁴Gipsa-lab, University Grenoble Alpes, CNRS, Grenoble, France. ⁸⁵CERMEP - Imagerie du vivant, Lyon, France. ⁸⁶Combinatorial NeuroImaging Core Facility, Leibniz Institute for Neurobiology, Magdeburg, Germany. ⁸⁷Center for Behavioral Brain Sciences, Magdeburg, Germany. ⁸⁸Queensland Brain Institute and Centre for Advanced Imaging, University of Queensland, St. Lucia, QLD, Australia. ⁸⁹Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland. ⁹⁰Biology of Neurodegenerative Disorders, Department of Neuroscience Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Milan, Italy. ⁹¹Conway Institute of Biomedical and Biomolecular Sciences, School of Medicine, University College Dublin, Dublin, Ireland. ⁹²Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto, Italy. ⁹³Department of Biopsychology, Institute of Cognitive Neuroscience, Ruhr University Bochum, Bochum, Germany. ⁹⁴Max-Delbrück Center

for Molecular Medicine in the Helmholtz Association, Berlin, Germany. ⁹⁵School of Arts & Sciences, University of Houston-Victoria, Victoria, TX, USA. ⁹⁶School of Psychology, Trinity College Dublin, Dublin, Ireland. ⁹⁷Department of Psychiatry, School of Medicine, Trinity College Dublin, Dublin, Ireland. ⁹⁸Trinity Centre for Biomedical Engineering, School of Medicine, Trinity College Dublin, Dublin, Ireland. ⁹⁹Centre for Brain Development and Repair, Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India. ¹⁰⁰Department of Medical Biophysics, University of Toronto, Toronto, QC, Canada. ¹⁰¹Université Clermont Auvergne, Inserm U1107 Neuro-Dol, Pharmacologie Fondamentale et Clinique de la Douleur, Clermont-Ferrand, France. ¹⁰²Animal Care Unit, Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Milan, Italy. ¹⁰³Physical Sciences Platform, Sunnybrook Research Institute, Toronto, QC, Canada. ¹⁰⁴Experimental and Clinical Research Center, A Joint Cooperation Between the Charité Medical Faculty and the Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany. ¹⁰⁵Department of Pediatric Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht & Utrecht University, Utrecht, The Netherlands. ¹⁰⁶Medical Imaging Physics (INM-4), Institute of Neuroscience and Medicine, Forschungszentrum Juelich, Juelich, Germany. ¹⁰⁷Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Crawley, WA, Australia. ¹⁰⁸School of Psychology and Neuroscience, University of Glasgow, Glasgow, UK. ¹⁰⁹Translational Neuroimaging Laboratory, Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK. ¹¹⁰Erwin L. Hahn Institute for MR Imaging, University of Duisburg-Essen, Essen, Germany. ¹¹¹Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ¹¹²Department of Physiology, McGill University, Montreal, QC, Canada. ¹¹³Laboratory of Surgical Neuroanatomy, Institute of Neuroscience, University of Barcelona, Barcelona, Spain. ¹¹⁴Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA. ¹¹⁵Neuro-X Institute, School of Engineering (STI), EPFL, Lausanne, Switzerland. ¹¹⁶Centre for Biomedical Imaging (CIBM), Lausanne, Switzerland. ¹¹⁷Hospices Civils de Lyon, Lyon, France. ¹¹⁸Department of Psychiatry, McGill University, Montreal, QC, Canada. ✉ e-mail: joanes.grandjean@radboudumc.nl

Methods

Pre-registration

This study was pre-registered (<https://doi.org/10.17605/OSF.IO/EMQ4B>). The following are notable deviations. We used the SIGMA rat template²⁵ instead of the Papp et al. template²⁶, owing to fewer artifacts, additional relevant assets and improved in vivo contrast. Some datasets had field of view cropped to ease image registration. Some datasets had time series cropped to ease the computational load. We used temporal signal-to-noise instead of signal-to-noise ratio, as these were shown to be correlated¹². Detailed deviations are listed here: <https://github.com/grandjeanlab/MultiRat>.

Animals

All acquisitions were performed after approval from the respective local and national ethical authorities. Participating laboratories were instructed to provide $n = 10$ rat imaging acquisitions consisting of one anatomical and one resting-state functional run. Exclusion criteria were unsuitability for RABIES pre-processing (for example, dedicated image reconstruction needs and restricted field of view). The MultiRat_rest collection consists of $n = 65$ datasets from 46 research centers and $n = 646$ rats (141/505 female/male). StandardRat consists of $n = 21$ datasets and $n = 209$ rats (93/116 female/male) from 20 research centers. For comparison, 224 scans ($n = 39$ rats) from a single-center awake rat dataset were included²¹. Exclusions were based on the following criteria: image misregistration (Extended Data Fig. 2), excessive motion and missing or corrupted data.

Standardized fMRI acquisition protocol

The standardized protocol was determined based on the outcomes of the analysis of MultiRat_rest and used to acquire the StandardRat dataset. Acquisitions were performed chiefly in ~2-month-old free-breathing Wistar rats, mixed sex, and anesthetized using 4% isoflurane and 0.05 mg kg⁻¹ medetomidine subcutaneous (s.c.) bolus for induction and 0.4% isoflurane and 0.1 mg/kg/h medetomidine s.c. for maintenance. Imaging with a gradient-echo echo-planar imaging technique was conducted 40 minutes after anesthesia induction, with repetition time = 1,000 ms, echo time/flip angle/bandwidth defined as a function of field strength (Supplementary Table 1), repetitions = 1,000, matrix size [64 × 64], field of view 25.6 × 25.6 mm² and 18 interleaved axial slices of 1 mm with 0.1-mm gap. The full protocol is available here: <https://github.com/grandjeanlab/StandardRat>.

Data pre-processing and confound correction

Scans were organized according to the BIDS format²⁷. Pre-processing was performed on each scan session separately using a reproducible containerized software environment for RABIES 0.3.5 (Singularity 3.7.3–1.el7, Sylabs)¹⁴. The pre-processing was performed using auto-box²⁸, N4 inhomogeneity correction²⁹, motion correction²⁹, a rigid registration between functional and anatomical scans²⁹, non-linear registration between anatomical scan and template and a common space resampling to 0.3 × 0.3 × 0.3 mm³. A volumetric image registration and brain masking workflow was developed to address discrepancies in brain size, image contrast and susceptibility distortions that are found in rodent images. Visual inspection was performed on pre-processing outputs for all scans for quality control. Five confound correction models were tested, using three approaches based on ICA-AROMA³⁰, white matter and ventricle signal or GSR (Supplementary Table 2). These were done together with motion regression, spatial smoothing to 0.5 mm³, a high-pass filter of 0.01 Hz and a low-pass filter of either 0.1 Hz or 0.2 Hz. The ICA-AROMA method was adapted from humans to rats by using dedicated rat cerebrospinal fluid and brain edge masks and by training the classifier parameters based on a set of rodent images. A visual inspection of the components and their classifications indicated that less than 5% of the plausible signal components were erroneously labeled as noise.

Data analysis

To determine FC in individual rats, seed-based analysis was performed with RABIES in template space using spherical seeds of 0.9-mm diameter located on the S1bf and the ACA, caudate-putamen and primary motor area. Functional connectivity was calculated as the Pearson's correlation coefficient between regional time courses. Functional connectivity specificity was defined relative to the left S1bf seed, using the contralateral right S1bf region of interest as the specific region of interest and the ACA as the non-specific region of interest¹². FC was evaluated for each animal and divided into four categories: specific ($r_{\text{S1bf left to right}} > 0.1$ AND $r_{\text{S1bf left to ACA}} < 0.1$); non-specific ($r_{\text{S1bf left to right}} > 0.1$ AND $r_{\text{S1bf left to ACA}} > 0.1$); no ($r_{\text{S1bf left to right}} [-0.1, 0.1]$ AND $r_{\text{S1bf left to ACA}} [-0.1, 0.1]$); and spurious connectivity (remaining cases). To assess connectivity specificity in the default mode network, the same approach was implemented with a seed in the ACA. The specific region of interest was located in the ACA (3.3 mm posterior to the seed), and the non-specific region of interest was located in the S1bf. To assess whole-brain connectivity, group independent component analysis was performed with $n = 20$ components using Nilearn (https://github.com/grandjeanlab/MultiRat/blob/master/assets/nifti/canica_resting_state_clean.nii.gz). Components' biological plausibility was assessed using criteria defined in Zerbi et al.²⁰. For comparison against humans, the same quality control metric was implemented on the 7T Human Connectome Project dataset⁵. The FIX-denoised scans ($n = 184$ participants) were bandpass corrected (0.01–0.1 Hz) and smoothed (2.5 mm²) using 3dTproject. Regions of interest were positioned in the sensory cortex and ACA.

Statistics and reproducibility

Sample size was determined on the basis of the available datasets forming this collection. No randomization was applied. Data collection and analysis were not performed blinded to the conditions of the experiments. One-sample t -test voxel-wise maps and group independent component analysis were estimated using Nilearn 0.7.1 (ref. ³¹). Comparisons between FC specificity and categorical variables (for example, magnetic field strength, strain and sex) were determined using χ^2 tests, as implemented in SciPy 1.6.2 (ref. ³²). Continuous variables (for example, mean framewise displacement (MFW)) were transformed into six categorical bins to allow comparison with χ^2 tests. Linear regression and analysis of variance were performed using Pingouin 0.5 (ref. ³³). Individual seed-based maps are represented as color-coded overlays thresholded at $r > 0.1$. Given the emphasis on detection of FC and the factors that affect it, we mitigated against false negatives by applying a liberal threshold of $P_{\text{uncorrected}} < 0.05$ to the one-sample t -test maps, following pre-registration specifications. This thresholding is justified because we did not want to exclude any datasets with weak potential traces of FC. Slice positions are indicated in millimeters relative to the anterior commissure.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw datasets are available here: unstandardized resting-state fMRI (MultiRat_rest) (<https://doi.org/10.18112/openneuro.ds004114.v1.0.0>); standardized resting-state fMRI (StandardRat) (<https://doi.org/10.18112/openneuro.ds004116.v1.0.0>). The pre-processed volumes, time series and quality control files are available here: <https://doi.org/10.34973/1gp6-gg97>. Image pre-processing, confound correction and connectivity analysis were performed using RABIES 0.3.5 (<https://github.com/CoBrALab/RABIES> (ref. ¹⁴)).

Code availability

Jupyter notebooks demonstrating the analysis code are available under the terms of the Apache-2.0 license (<https://github.com/grandjeanlab/MultiRat>; <https://doi.org/10.5281/zenodo.7614670>).

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Author contributions

J.G. designed, planned and executed the study and wrote the manuscript. G.D.G., G.A.D. and M.M.C. provided the software and hardware environment for the analysis. All authors contributed experimental data and edited the manuscript. A.H. initiated the study.

Competing interests

A.S. is an employee of Bruker, the manufacturer of preclinical MRI systems used for the acquisition of most of the datasets in this collection. E.L.B. is a consultant for Bruker. B.V. is an employee of TheraNexus. S.Z., A.D. and N.B. are employees of Novartis Pharma AG. T.N. is founder and CEO of MRI.TOOLS GmbH. The other authors declare no competing interests.

Additional information

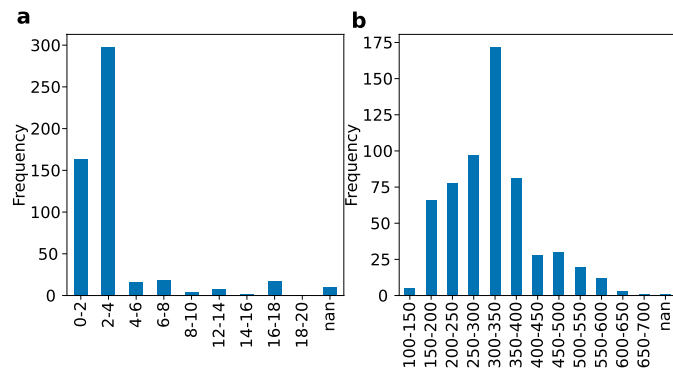
Extended data is available for this paper at <https://doi.org/10.1038/s41593-023-01286-8>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41593-023-01286-8>.

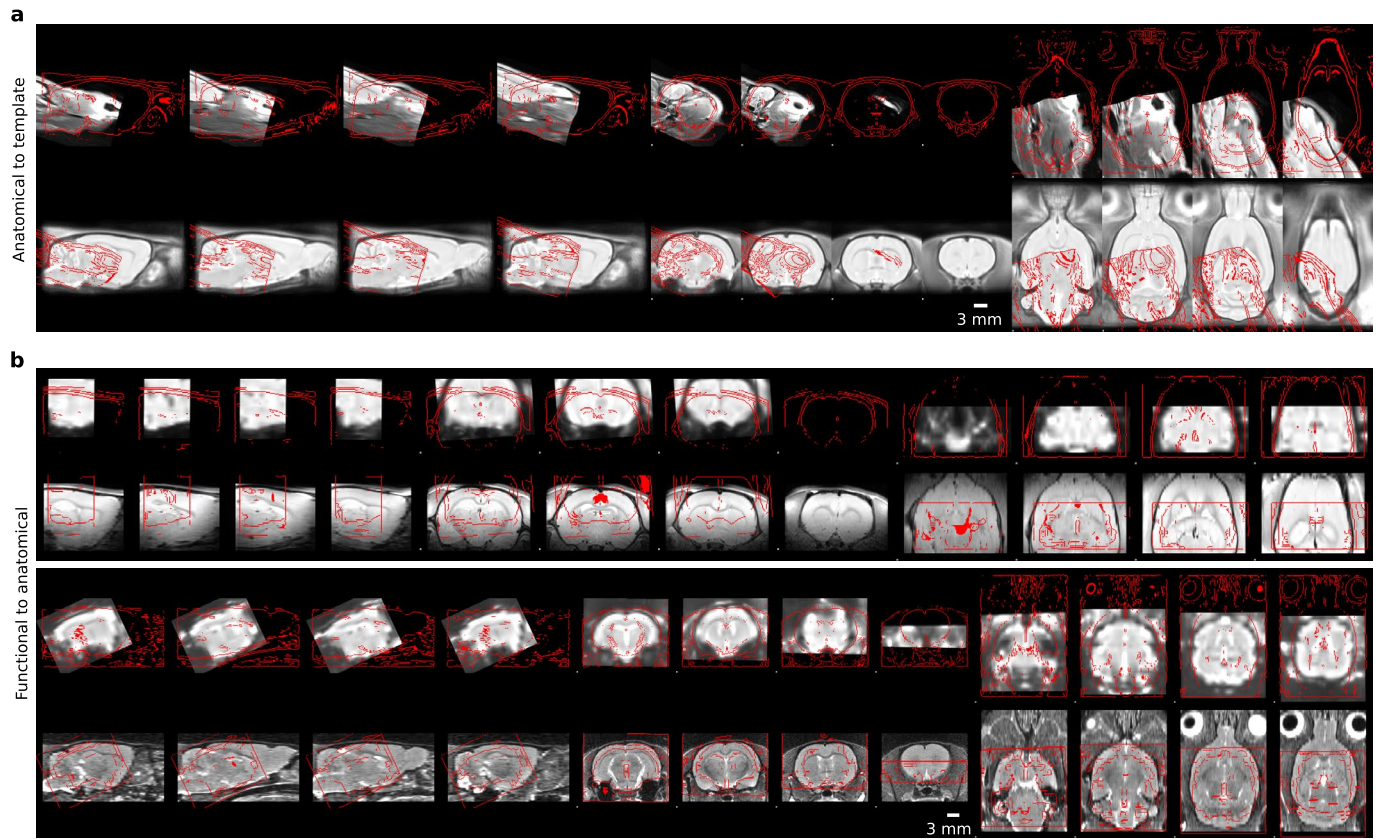
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Peer review information *Nature Neuroscience* thanks Peter Klink, Afonso Silva and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

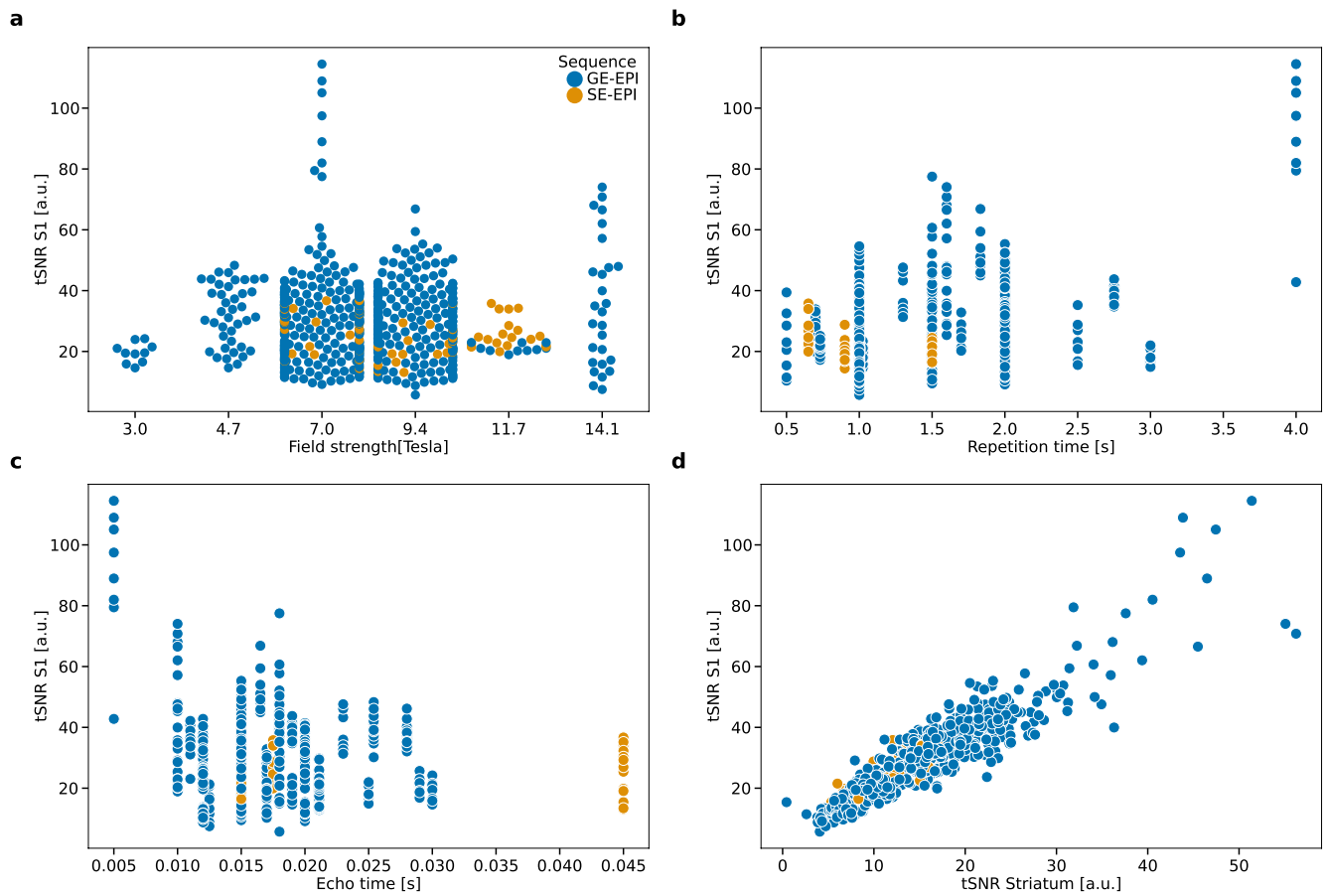
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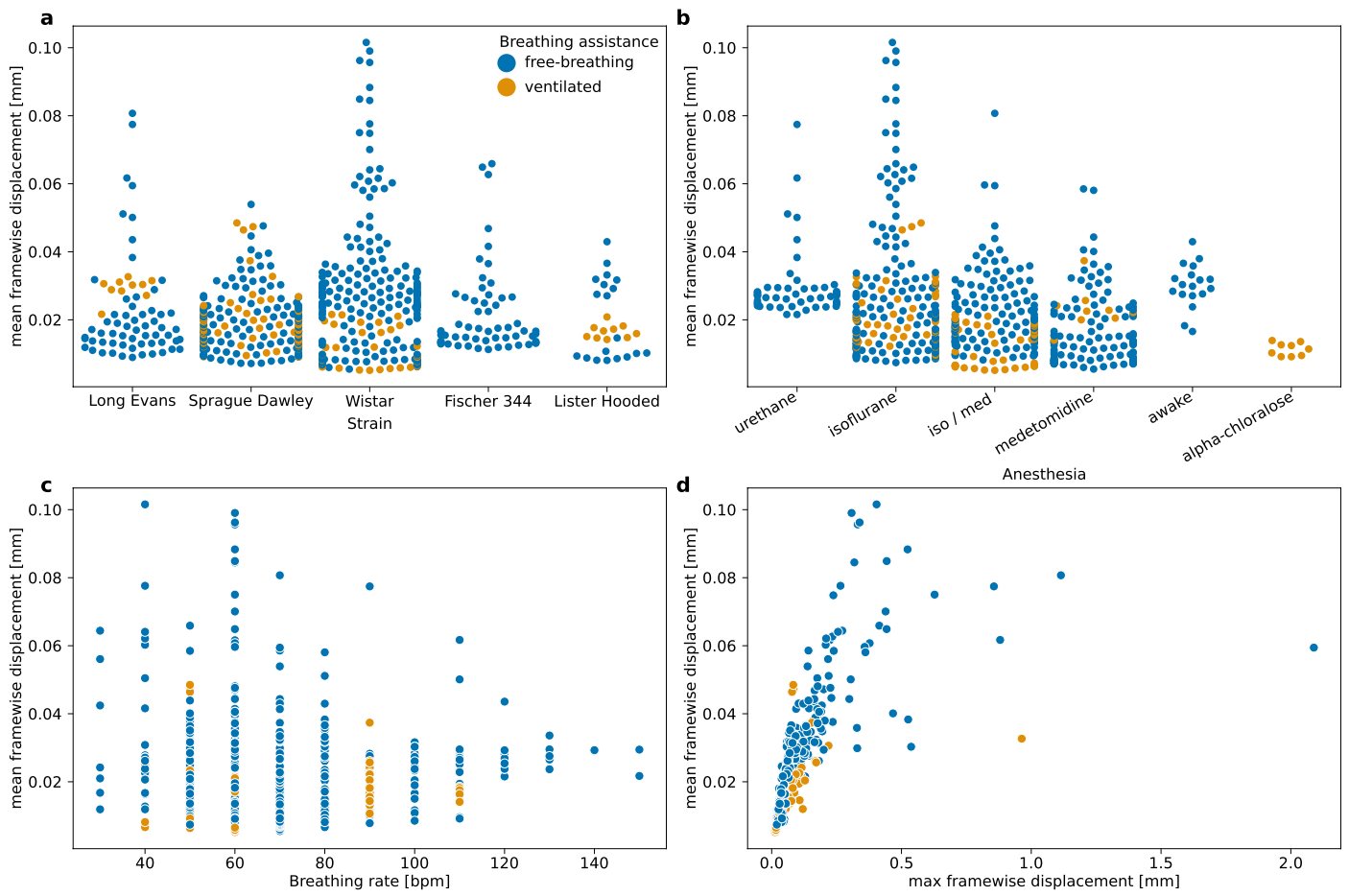
Extended Data Fig. 1 | Age and weight distributions. Age (a) and weight (b) distribution for the rats in the MultiRat_rest collection.



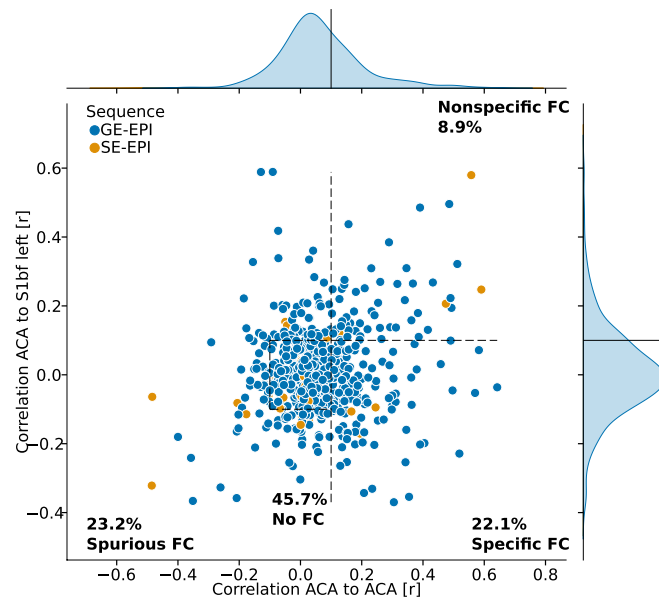
Extended Data Fig. 2 | Quality control examples. Failed quality controls for anatomical to template registrations (a) and functional to anatomical registrations (b). The top rows are the moving objects, bottom rows are the reference objects. The red lines indicate the outlines of the other object. Four slices along the sagittal, axial, and coronal axis are shown for each case.



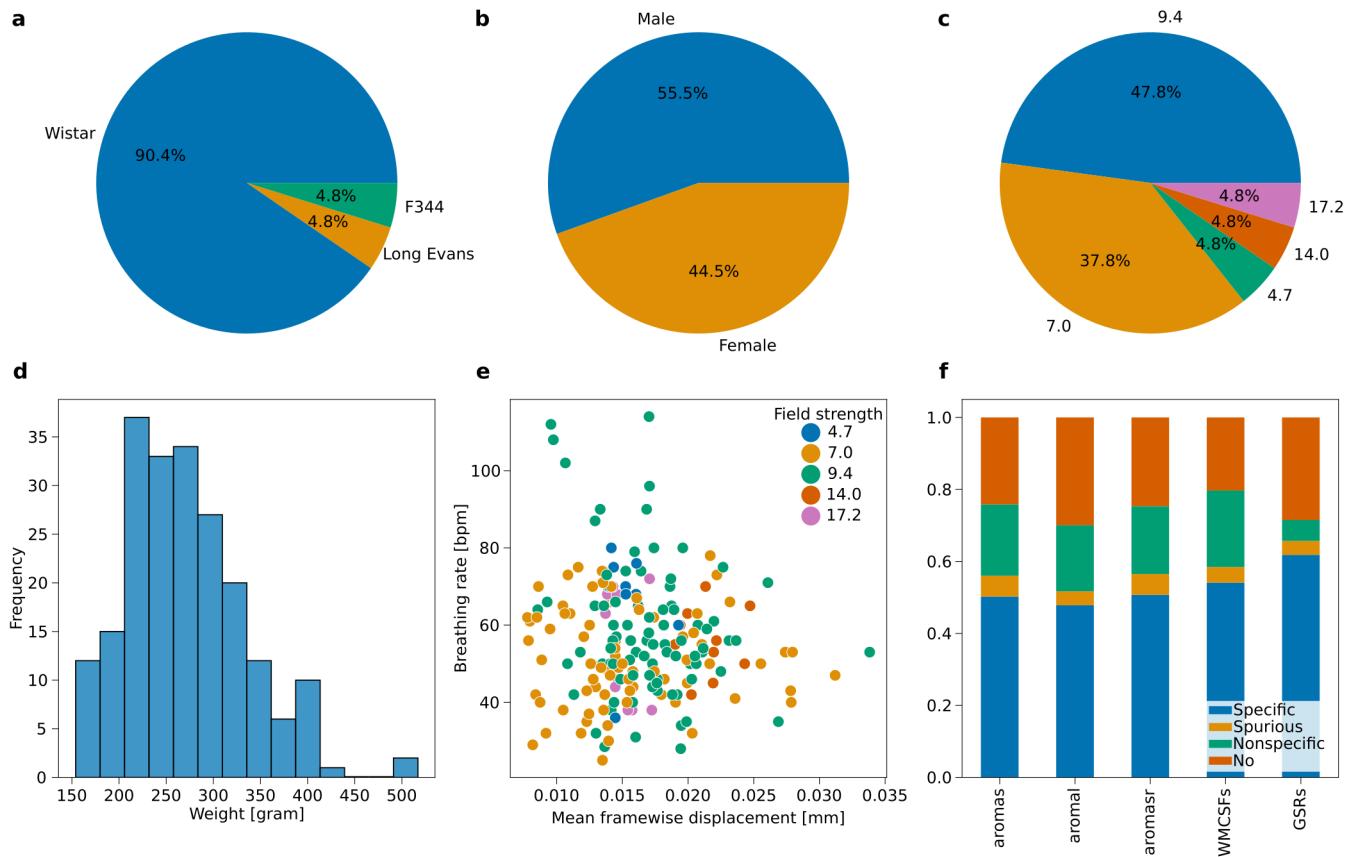
Extended Data Fig. 3 | Temporal signal-to-noise ratio. Temporal signal-to-noise ratio in the sensory cortex (tSNR S1) in the MultiRat_rest dataset collection as a function of (a) magnetic field strength, (b) repetition time, (c) echo time, (d) temporal signal-to-noise ratio in the striatum.



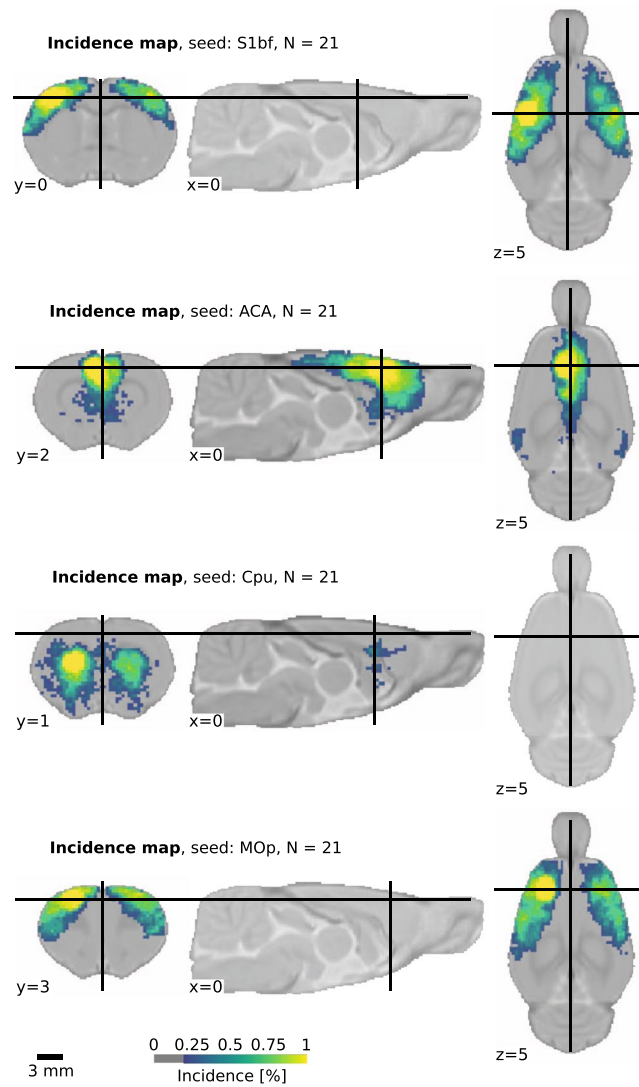
Extended Data Fig. 4 | Framewise displacement. MFW in the MultiRat_rest dataset collection as a function of (a) strain, (b) anesthesia, (c) breathing rate, (d) maximal framewise displacement.



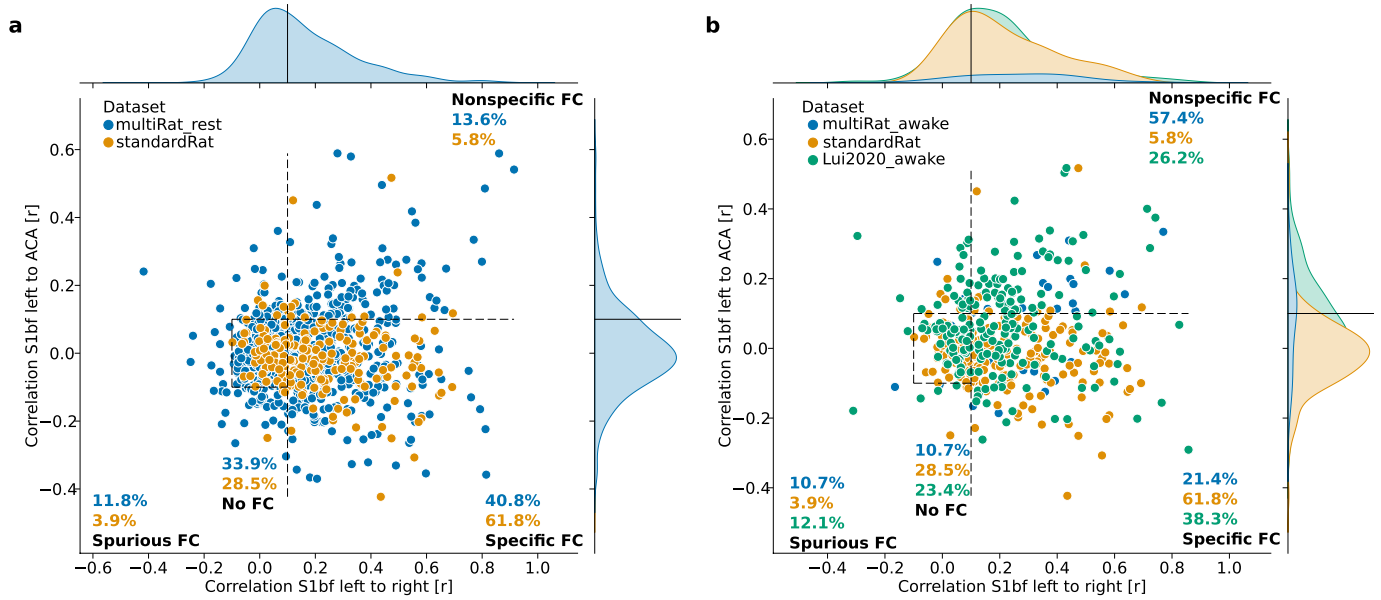
Extended Data Fig. 5 | FC in the default-mode network. The reference seed is positioned in the anterior cingulate cortex (Fig. 2a), the specific region-of-interest is positioned 3.3 mm posterior in the cingulate cortex and the nonspecific region-of-interest is positioned in the S1bf.



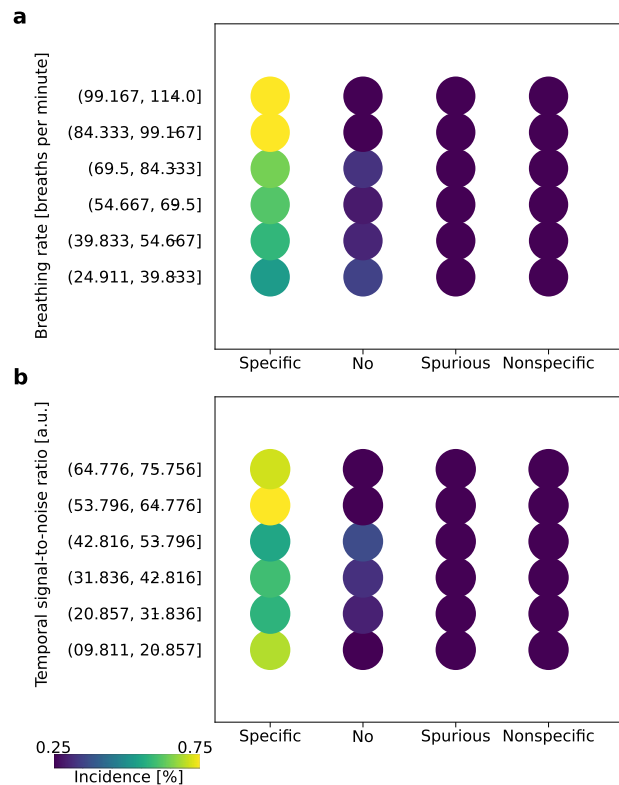
Extended Data Fig. 6 | StandardRat dataset description. a. Strain. b. Sex. c. Field strength. d. Weight. e. Breathing rate as a function of MFW. f. FC specificity as a function of confound correction models.



Extended Data Fig. 7 | FC incidence. Incidence of FC at the group level in the StandardRat collection for four selected seeds ($n = 21$ datasets, $n = 10$ subjects per dataset). Connectivity incidence is improved in the StandardRat collection relative to MultiRat_rest (Fig. 3).

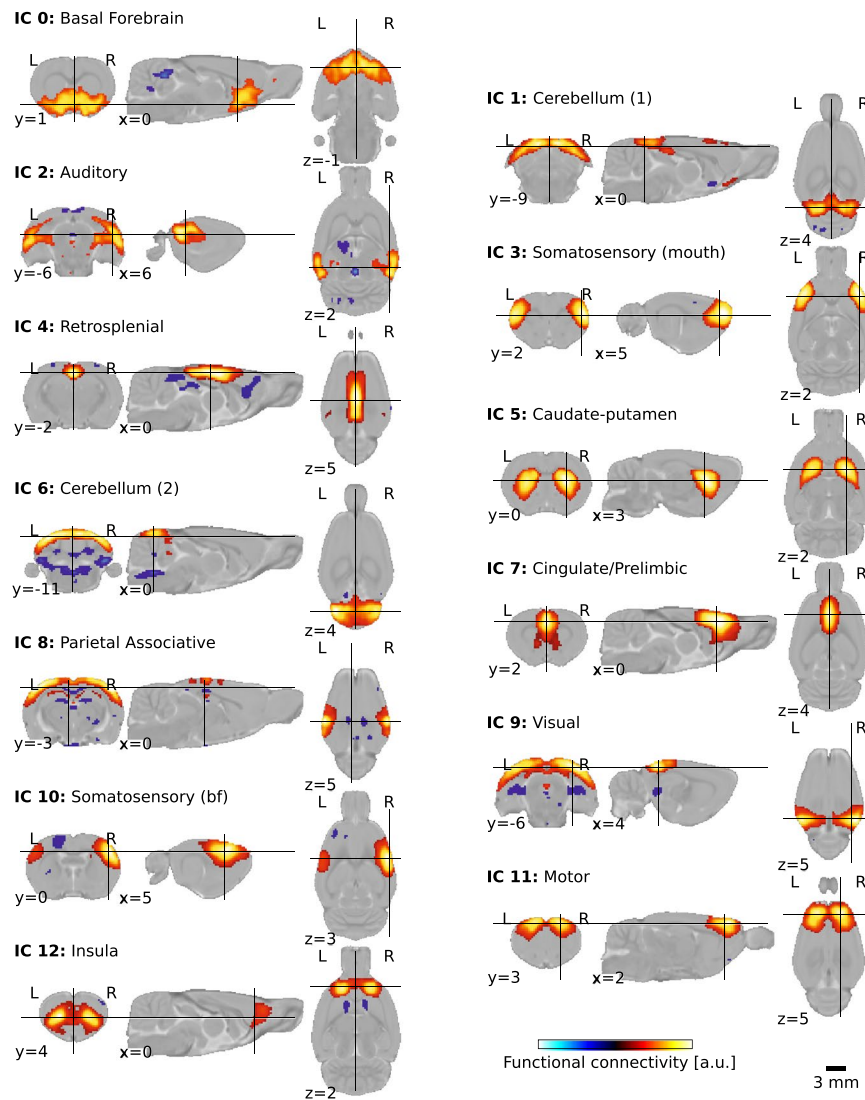


Extended Data Fig. 8 | Between-datasets connectivity comparisons. FC category comparison between MultiRat_rest and StandardRat (**a**) and between the awake datasets of MultiRat_rest and the awake dataset from Lui et al. 2020 (**b**).



Extended Data Fig. 9 | Connectivity specificity as a function of breathing rate and signal-to-noise ratio. FC specificity as a function of binned breathing rate (**a**) AND temporal signal-to-noise ratio (**b**) in the StandardRat collection. The percentage of each condition is size and color-coded. High levels of connectivity specificity were achieved in scans where the breathing rates were in the 84 to 114

bpm range. Similarly, higher connectivity specificity incidences were found when the cortical temporal signal-to-noise ratio was > 53 . These observations support the notion of an optimal breathing rate when applying the StandardRat protocol, along with temporal signal-to-noise ratio and movement targets.



Extended Data Fig. 10 | Group independent components analysis. Plausible independent components overlapping with known rodent networks, obtained after group-level decomposition with $n = 20$ components. Labels are based on the SIGMA anatomical atlas.

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 - Standardized resting-state fMRI (StandardRat) (<https://doi.org/10.18112/openneuro.ds004116.v1.0.0>).
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Wild animals	<input type="text" value="No wild animals were used"/>

Reporting on sex	Sex effect was tested
Field-collected samples	No field collected sampled were used
Ethics oversight	<p>This study was approved by the authorities of:</p> <p>Radboudumc, The Netherlands; Univ. Grenoble Alpes, France; The University of Western Australia, Australia; University of Queensland, Australia; Brigham and Women's Hospital, USA; McGill University, Canada; University Hospital Münster, Germany; University of Florida, USA; University of Eastern Finland, Finland; German Primate Center - Leibniz Institute for Primate Research, Germany; Icahn School of Medicine at Mount Sinai, USA; Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Germany; École polytechnique fédérale de Lausanne, Switzerland; Universidad Nacional Autónoma de México, Mexico; The University of Edinburgh, UK; King's College London, UK; Pennsylvania State University, USA; Tohoku University, Japan; Max Planck Institute for Biological Cybernetics, USA; University and ETH Zurich, Switzerland; Trinity College Dublin, Ireland; University of Antwerp, Belgium; University Medical Center Utrecht, Netherlands; The University of North Carolina at Chapel Hill, USA; University of Barcelona, Spain; Ghent University, Belgium; Yale University School of Medicine, USA; University of Nottingham, UK; Institute of Automation, Chinese Academy of Sciences, China; University of Cambridge, UK; Amsterdam UMC location University of Amsterdam, Netherlands; CEA Saclay, France; FAU Erlangen-Nürnberg, Germany; Champalimaud Centre for the Unknown, Portugal; University of Guelph, Canada; Emory University/Georgia Institute of Technology, USA; Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Italy; Novartis Institutes for BioMedical Research, Switzerland; Ruhr University Bochum, Germany; Northeastern University, USA;</p>

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Magnetic resonance imaging

Experimental design

Design type	resting-state
Design specifications	One run and one session per subject.
Behavioral performance measures	na

Acquisition

Imaging type(s)	functional
Field strength	3 to 17.6T
Sequence & imaging parameters	Gradient and spin echo echo-planar imaging
Area of acquisition	Whole-brain
Diffusion MRI	<input type="checkbox"/> Used <input checked="" type="checkbox"/> Not used

Preprocessing

Preprocessing software	RABIES 0.3.5 default parameters
Normalization	ANTs 2.3.5
Normalization template	SIGMA rat template
Noise and artifact removal	RABIES 0.3.5 default parameters
Volume censoring	na

Statistical modeling & inference

Model type and settings	Chi-square test between categorical data
Effect(s) tested	Differences in the expected distribution of the functional connectivity categories
Specify type of analysis:	<input checked="" type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input type="checkbox"/> Both
Statistic type for inference (See Eklund et al. 2016)	Effect size and confidence intervals
Correction	na

Models & analysis

n/a	Involvement in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Functional and/or effective connectivity
<input checked="" type="checkbox"/>	<input type="checkbox"/> Graph analysis
<input checked="" type="checkbox"/>	<input type="checkbox"/> Multivariate modeling or predictive analysis
Functional and/or effective connectivity	Pearson's correlation