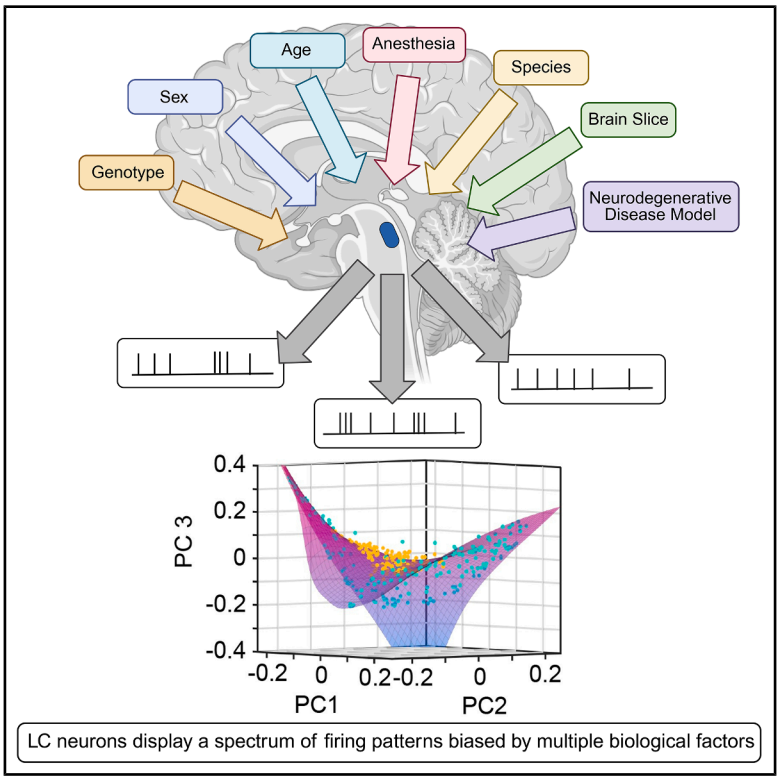
Cell Reports

Resource

Locus coeruleus noradrenergic neurons have diverse activity patterns across species and biological context

Graphical abstract



LC neurons display a spectrum of firing patterns biased by multiple biological factors

Highlights

- Locus coeruleus firing rates vary by species, sex, age, and brain state
- The classic tonic-phasic model fails to describe spontaneous locus coeruleus firing
- Firing patterns form a continuous low-dimensional manifold, not two discrete modes
- Species, age, genotype, and disease models associate with distinct firing-pattern types

Authors

Michael A. Kelberman, Ellen Rodberg, Brandon R. Munn, ..., David Weinshenker, Elena Vazey, Nelson K. Totah

Correspondence

nelson.totah@helsinki.fi

In brief

Kelberman, Rodberg et al. show that the locus coeruleus, though ubiquitous across vertebrates, is not physiologically uniform across species, challenging the tonic-phasic framework applied throughout biology, psychology, and AI. These findings reshape our understanding of neuromodulation's role in cognition and in metabolic, immune, and cardiorespiratory function.

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Locus coeruleus noradrenergic neurons have diverse activity patterns across species and biological context

Michael A. Kelberman,[1,2,51] Ellen Rodberg,[3,4,5,51] Brandon R. Munn,[6] Ehsan Arabzadeh,[7] Chloe J. Bair-Marshall,[8] Craig W. Berridge,[9] Esther Berrocoso,[10,11] Vincent Breton-Provencher,[12] Daniel J. Chandler,[13] Alicia Che,[14,15] Oscar Davy,[16] David M. Devilbiss,[13] Anthony M. Downs,[17,18] Gabrielle Drummond,[19] Roman Dvorkin,[20] Zeinab Fazlali,[21,22,23] Robert C. Froemke,[8,24] Erin Glennon,[8,25] Joshua I. Gold,[26] Hiroki Ito,[16,27] Xiaolong Jiang,[28,29,50] Joshua P. Johansen,[30] Alfred P. Kaye,[14,15,31] Jenny R. Kim,[32] Chao-Cheng Kuo,[32,37] Rong-Jian Liu,[14] Yang Liu,[33] Meritxell Llorca-Torralba,[10,11] Jordan G. McCall,[32] Zoe A. McElligott,[17,18,34] Andrew M. McKinney,[28] Cristina Miguelez,[35,36] Ming-Yuan Min,[37] Alexandra C. Nowlan,[17] Mohsen Omrani,[38]

(Author list continued on next page)

[1]Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO, USA
[2]Department of Human Genetics, Emory University, Atlanta, GA, USA
[3]Department of Biology, University of Massachusetts Amherst, Amherst, MA, USA
[4]Neuroscience and Behavior Program, University of Massachusetts Amherst, Amherst, MA, USA
[5]Department of Psychology, Rhode Island College, Providence, RI, USA
[6]School of Medical Sciences and Brain and Mind Center, The University of Sydney, Sydney, NSW, Australia
[7]Eccles Institute of Neuroscience, John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia
[8]Euroscience Institute, NYU Langone Medical Center, New York University, New York, NY, USA
[9]Department of Psychology, University of Wisconsin-Madison, Madison, WI, USA
[10]Neuropsychopharmacology and Psychobiology Research Group, Department of Neuroscience, School of Medicine, Biomedical Research and Innovation Institute of Cádiz (INiBICA), University of Cadiz, Cadiz, Spain
[11]Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain
[12]Department of Psychiatry and Neuroscience, Université Laval, Québec, QC, Canada
[13]Department of Neuroscience, Virtua Health College of Medicine and Life Sciences, Stratford, NJ, USA
[14]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA
[15]Wu Tsai Institute, Yale University, New Haven, CT, USA
[16]School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK
[17]Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[18]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[19]Department of Brain and Cognitive Sciences, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA
[20]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York, NY 11724, USA

(Affiliations continued on next page)

SUMMARY

The locus coeruleus (LC) brainstem cell group is evolutionarily conserved across vertebrates and influences both mind (cognition) and body (cardiorespiratory, metabolic, and immune functions) via two—putatively distinct—spontaneous activity patterns. Yet, the degree to which spontaneous LC firing rates and patterns are homogeneous across species has never been assessed due to inherently limited sample sizes. To remedy this, we pooled cross-species data from 20 laboratories to show that firing rates differ across species and are modulated by sex, age, and type of *in vitro* or *in vivo* preparation. Contrary to the prevailing dual-mode firing pattern schema, we observed patterns spread across a low-dimensional manifold, with subregions enriched for specific biological factors and neurodegenerative disease models. Our findings show considerable diversity in an ancestral vertebrate neuromodulatory system.

INTRODUCTION

The central nervous system-wide projections of the brainstem noradrenergic cell group, locus coeruleus (LC), have been retained across the vertebrate phylogenetic tree.[1] A core function of the LC—mobilization of cardio-respiratory, metabolic, and sensory-motor-cognitive resources in response to environmental change—has also been conserved across vertebrates.[2–7] This function is thought to be implemented by two modes of activity (tonic or phasic). While phasic activity is

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Gina R. Poe,[39] Anthony Edward Pickering,[16] Yadollah Ranjbar-Slamloo,[21] Jone Razquin,[35,36] Charles Rodenkirch,[33] Anna C. Sales,[16] Rath Satyasambit,[30,40] Stephen D. Shea,[20] Mriganka Sur,[19] John Arthur Tkaczynski,[13] Sonia Torres-Sanchez,[10,11] Akira Uematsu,[41] Chayla R. Vazquez,[32] Amelien Vreven,[42,43,44] Qi Wang,[33] Barry D. Waterhouse,[13] Hsiu-Wen Yang,[45] Jen-Hau Yang,[14,46] Liping Zhao,[47] Ioannis S. Zouridis,[48,49] James M. Shine,[6] David Weinshenker,[2] Elena Vazey,[3,4] and Nelson K. Totah[42,43,44,49,52,*]

[21]School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
[22]Department of Psychiatry, Columbia University, New York, NY, USA
[23]New York State Psychiatric Institute, New York, NY, USA
[24]Department of Otolaryngology, NYU Grossman School of Medicine, New York, NY, USA
[25]Department of Neurology, Weill Cornell Medicine, New York, NY, USA
[26]Department of Neuroscience, University of Pennsylvania, Philadelphia, PA, USA
[27]Department of Urology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[28]Department of Neuroscience, Baylor College of Medicine Neurological Research Institute at Texas Children's Hospital, Houston, TX 1250, USA
[29]Department of Ophthalmology, Baylor College of Medicine Neurological Research Institute at Texas Children's Hospital, Houston, TX 1250, USA
[30]RIKEN Center for Brain Science, Wako-shi, Saitama, Japan
[31]Clinical Neurosciences Division, VA National Center for PTSD, West Haven, CT, USA
[32]Department of Anesthesiology, Washington University in St. Louis, St. Louis, MO, USA
[33]Department of Biomedical Engineering, Columbia University, New York, NY, USA
[34]Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[35]Department of Pharmacology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain
[36]Neurodegenerative Diseases Group, Biobizkaia Health Research Institute, Barakaldo, Spain
[37]Department of Life Science, College of Life Science, National Taiwan University, Taipei, Taiwan
[38]Department of Psychiatry, Queen's University, Kingston, ON, Canada
[39]Integrative Biology and Physiology, UCLA, Los Angeles, CA, USA
[40]Department of Computer Science, Tokyo Institute of Technology, Midori, Yokohama, Japan
[41]Human Informatics and Information Research Institute, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan
[42]Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland
[43]Neuroscience Center, University of Helsinki, Helsinki, Finland
[44]Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[45]Department of Biomedical Sciences, Chung-Shan Medical University, Taichung, Taiwan
[46]Doctoral Program of Clinical and Experimental Medicine, National Sun Yat-sen University, Kaohsiung, Taiwan
[47]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, USA
[48]Graduate Training Centre of Neuroscience, International Max Planck Research School (IMPRS), University of Tübingen, Tübingen, Germany
[49]Department of Physiology of Cognitive Processes, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
[50]Department of Anatomy and Neurobiology, School of Medicine, University of California Irvine, Irvine, CA, USA
[51]These authors contributed equally
[52]Lead contact
*Correspondence: nelson.totah@helsinki.fi
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most commonly attributed to stimulus-evoked firing, spontaneously occurring phasic bursts occur and have a similar neuromodulatory effect on cortical state as stimulus-evoked phasic activity,[8–14] and spontaneous phasic activity occurs during the naturalistic, maternal behavior of pup retrieval that is not attributable to sensory stimuli, motor responses, or action effort.[15] However, spontaneous phasic bursts have not been described in detail. Because the current definition of spontaneous LC phasic bursting is derived from Grace & Bunney's 1984 work on dopaminergic neurons,[16] the field lacks a data-driven definition of spontaneous LC phasic bursts. Despite this absence of information, the tonic-phasic dual-mode terminology has been widely applied to human neuroimaging, cognitive psychology studies, and computational models of cognition and learning.[17–19] Although the application of the two-mode theory of LC function has been primarily toward sensory-guided behavior in these fields, and the theory itself was derived from sensory stimulus-evoked LC activity, it is nevertheless crucial to define spontaneous LC bursts as a core feature of noradrenergic neuromodulation of the central nervous system.

Based on the evolutionary conservation of LC structure and function, we hypothesized that the firing modes of LC neurons have been conserved across vertebrates to fulfill a fundamental functional role. Consider the analogy of breathing, which is fundamentally the same function across mammals. Breathing in mammals, whether the individuals are male or female—young or old—asleep or awake—is driven by homologous brainstem activity patterns in the pre-Bötzinger nucleus[20] regardless of species or these biological factors. The same may be the case for the LC and its resource mobilization function.

To date, this hypothesis has never been tested due to the small sample sizes of individual studies. Typically, only a single neuron is recorded in a brain slice and, in anesthetized or awake animals, only one or two LC neurons are accessible in extracellular recordings,[21–24] which can in principle record activity of many neurons ("single units") simultaneously but have an extremely limited yield in the brainstem due to physical instability of the electrode-tissue interface caused by cardiac, respiratory, and spinal movement.[25]

The LC, via its ascending brain-wide projections, is implicated in cognitive deficits observed in attention deficit and hyperactivity disorder, schizophrenia, and neurodegenerative diseases[26,27] as well as psychological and physical symptoms of post-traumatic stress disorder, anxiety disorder, and symptoms of opioid withdrawal.[28–30] Moreover, via descending projections to the autonomic nervous system and the spinal cord, the LC influences peripheral analgesia, cardiovascular disease, immune dysfunction, metabolic disorders, and obesity.[31–36] The relevance of LC to such a broad range of human health and disease conditions underscores the importance of confirming or refuting whether LC activity varies across species or across other factors such as sex, age, and experimental preparation.

RESULTS

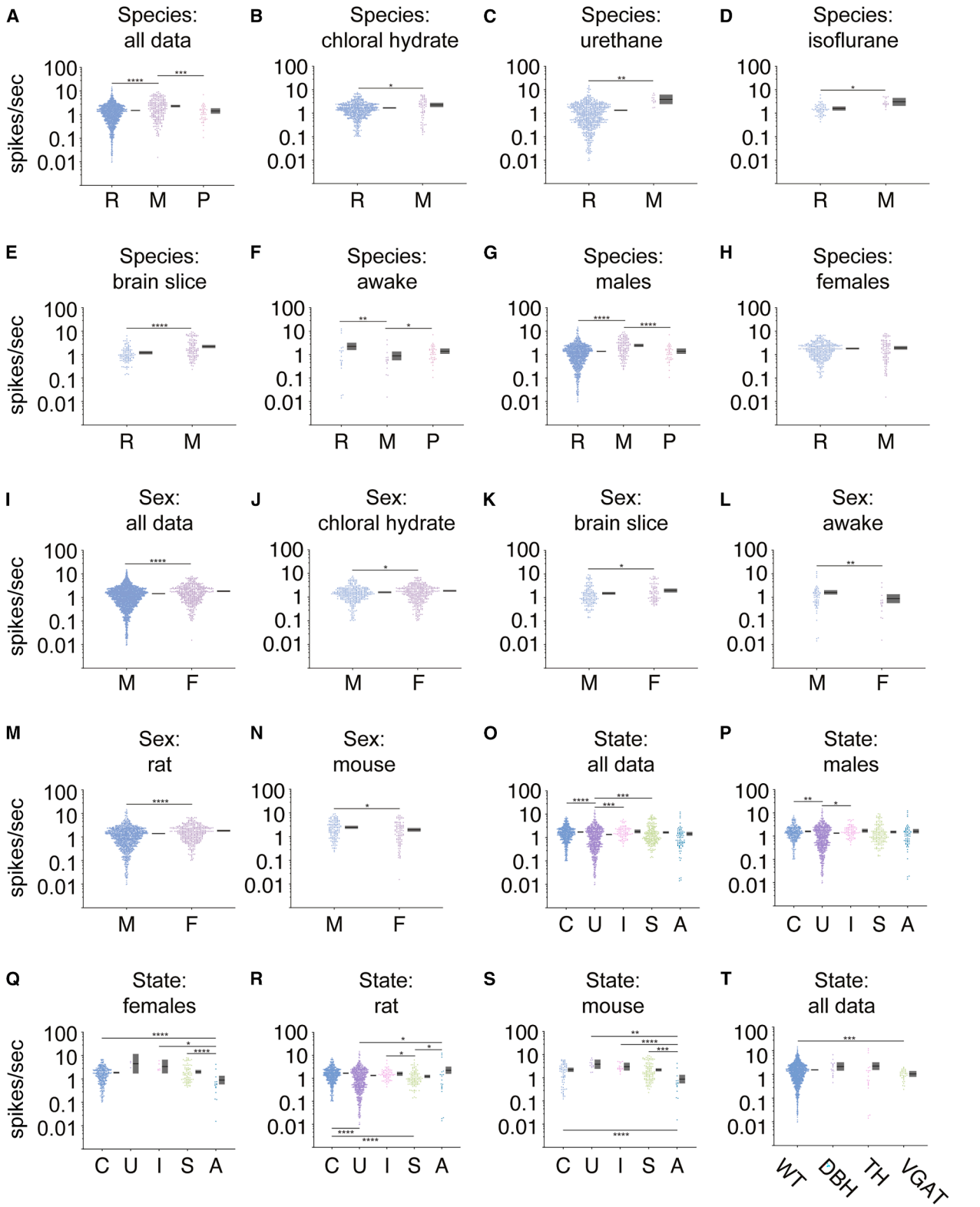
To remedy this issue, we compiled spontaneously occurring spike trains of LC neurons collected across 20 laboratories (Table S1). These data included mice and rats of both sexes, as well as male *Macaca mulatta*, a nonhuman primate (NHP). Animals spanned from relatively young (adolescent) to adult and late age. Spike discharges were recorded either in tissue slices (intracellular), intact animals under anesthesia of various types, or during wakefulness. Additionally, some LC neurons or animals were genetically modified for optogenetic manipulation and, while recordings during optogenetic stimulation were not included in the dataset, the genetic modification was considered as a factor that could influence firing rate. Finally, a subset of mice was pharmacologically manipulated or bred to express disease-specific genotypes. Laboratories providing extracellular recordings were asked to include only single units (henceforth referred to as ''neurons''). Data contributions were also required to have at least one type of verification that the neurons recorded were noradrenergic LC cells, consistent with standard guidelines for verification.[1] Laboratories could use one (or any combination of these STAR Methods): histology, biphasic response to sensory stimuli, clonidine-evoked inhibition of firing, and/or optotagging. Table S2 shows the number of neurons with each verification method and the intersection of those STAR Methods within data from each contributing laboratory. Laboratories contributing slice recordings are verified by visualizing LC neurons in the brain slice. Furthermore, in the interest of applying similar criteria to define single units across laboratories, we performed a quality control sweep of the 2,944 neurons, which yielded 1,855 and 1,708 neurons for firing rate and pattern analysis, respectively (see Supplementary Materials, *Quality control of single unit recordings across laboratories*).

Our goals were to compare neuronal activity levels (firing rate) and to describe tonic and phasic firing modes across species and across a range of intrinsic and extrinsic factors. To analyze neuronal activity levels, we analyzed time-averaged firing rate using negative binomial regression (a marginal model) to explain the individual and combined effects of species, sex, age, genotype, and brain state or experimental preparation type (e.g., awake, anesthesia of various types, or *ex vivo* slice recordings). There was a lack of substantial variation in firing rates between laboratories (Figure S1). We confirmed this by calculating an intraclass correlation value, which ascertains whether firing rates from the same laboratory were more similar than those obtained by other laboratories. The intraclass correlation value was 0.48, which suggests a low to moderate dependence of firing rate on laboratory identity. Including laboratory identity as a factor affecting firing rate (e.g., in a hierarchical model) was not possible because the low variability across laboratories prevented a hierarchical model from fitting the data. Given the limitations of the hierarchical model, we chose to use the marginal model. We examined the main effects of individual factors and considered interactions between these factors (Table S3). Some factors, such as animal strain, were not included in the model (see Supplementary Materials, *Factors excluded from the model of firing rates*). Effects, when significant, are reported as effect sizes in firing rate (spikes/s) with 95% confidence intervals (CIs) to provide an intuitive quantification of how much firing rate differs between factors.[37–39] Importantly, small effect sizes of even 0.5 spikes/s may carry considerable relevance to brain function. First, consider that the baseline discharge of LC neurons is already low (typically ranging from 0.5 to 5 spikes/s) and that a relative change of 0.5 spikes/s is a large difference in this range of activity. Second, our rudimentary understanding of how LC firing rate alters nervous system function largely precludes making a judgment regarding what effect size may (or may not) have consequences.

LC firing rate is not conserved across model species and is influenced by brain state and sex

We start from the premise that LC firing rate is similar across model species due to the conservation of LC function across phylogeny. Our analysis showed a significant main effect of species ($p < 0.0001$; Figure 1A; Table S3). Neurons recorded from mice had higher firing rates compared to rats ($p < 0.0001$) and NHPs ($p = 0.0001$), but rats and NHPs did not significantly differ from each other (see post-hoc comparisons in Table S4). The effect of species on LC activity interacted with the type of experimental preparation defining the state of the animal ($p = 0.001$, Figures 1B–1F; Table S4). The mouse LC is more active relative to the rat LC under all tested anesthetics and during *ex vivo* (brain slice) recordings (Figures 1B–1E; Table S4). However, in the awake state, mouse LC neurons were less active compared to rats ($p = 0.0036$) and NHPs (but to a lesser extent, given the effect size 95% CIs overlap with no difference between means and $p = 0.0491$, see Figure S2F) (Figures 1F; Table S4).

The effect of species also interacted with sex ($p = 0.0103$; Figures 1G and 1H; Table S3). The higher firing rate in mice relative to rats and NHPs was observed specifically in males ($p < 0.0001$ vs rats; $p < 0.0001$ vs NHPs) (Figure 1G; Table S4). The effect sizes between male mice versus male rats and male NHPs were 1.09 and 1.07 spikes/sec, respectively (see Figure S2G). On the other hand, in females, there were no species-specific differences between mice and rats (Figures 1H; Table S4). We did not have data from female NHPs for comparison. It has long been assumed that LC firing rate is conserved across model species, yet our findings show species-specific



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LC firing rates. Intriguingly, these differences depended on sex and animal state, which led us to directly examine sex and state differences in LC firing rate.

Sex impacts LC firing rates in opposing directions in rats and mice

Biological sex is associated with differences in autonomic control, the stress response, sleep, and cognition, as well as symptoms of various clinical disorders.[40–45] These sex differences could be associated with sex-specific LC firing rates. Our analysis revealed a main effect of sex on firing rate ($p < 0.0001$; Figure 1I; Table S3). We found that the female LC was more active than the male LC, with an effect size of 0.4 spikes/s (see Figure S2I). However, there was also an independent interaction between sex and brain state ($p = 0.0044$; Figures 1J–1L; Table S3). Specifically, the female LC was only more active when recorded under chloral hydrate ($p = 0.031$) or from brain slice recordings ($p = 0.024$). On the other hand, in awake animals, the female LC was less active compared to the male LC ($p = 0.004$; Figures 1J–1L; Table S4). The effect of sex also depended on species ($p = 0.0103$; Figures 1M and 1N; Table S3). In rats, the female LC is more active than the male LC ($p < 0.0001$; Figure 1M; Table S4), whereas in mice the opposite was observed ($p = 0.034$; Figure 3N; Table S4). Thus, LC firing rate varies by sex, but species and brain state modify the effect of sex.

Firing rate in the awake state is increased in rats but decreased in mice

Given the technical challenges of targeting the LC with recording electrodes and sustaining recording signal quality, a substantial basis of our understanding of LC activity has been informed by recordings in anesthetized animals or in brain slices. The pharmacological manipulations (for anesthesia) or afferentation of the LC (for brain slice recordings) could entail a distinct brain state relative to awake animals. LC firing rate may therefore differ in the context of different brain states associated with brain slice recordings, anesthesia, or wakefulness. Indeed, the LC firing rate is suppressed during slow-wave sleep and rapid eye movement (REM) sleep relative to the awake state.[46] However, there is no reason to assume that the LC firing rate differs between the anesthetized and awake states since sleep and anesthesia are not the same, mechanistically or phenomenologically.[47,48] Using the pooled dataset, we observed a main effect of experimental preparation ($p < 0.0001$; Figure 1O; Table S3) in rats and mice (the two species for which we had both *ex vivo* and *in vivo* recordings). Pairwise comparisons showed that firing rates recorded under urethane were around 0.3 to 0.47 spikes/sec lower than those recorded under chloral hydrate ($p < 0.0001$), isoflurane ($p = 0.006$), and in slice ($p = 0.003$) (Figure 1O; Table S4). Although firing rate differs between urethane and the other anesthetics, the firing rates of neurons in the awake state were similar to those recorded under anesthesia and in the deafferented brain slice. However, considering species as a factor modified the relationship between firing rate and brain state. The statistical model indicated an independent interaction between experimental preparation and species ($p = 0.001$; Figures 1P and 1Q; Table S3). In rats, LC neurons from awake animals were more active than those recorded under urethane ($p = 0.034$) or from brain slices ($p = 0.019$). Surprisingly, mice showed a largely opposite pattern relative to what was observed in rats. Specifically, firing rate in awake mice was lower than LC activity under chloral hydrate ($p < 0.0001$), isoflurane ($p = 0.0005$), or urethane ($p = 0.001$), or slice recordings ($p < 0.0001$) (Figure 1Q; Table S4). The opposition between

Figure 1. Dependence of LC firing rate based on interactions with intrinsic and extrinsic biological factors

All panels show the firing rate (antilog) on a logarithmically scaled *y* axis on the left. The individual points are neurons. The sample size (*N*) can be found in Table S1. The box next to the points shows the 95% CI of the firing rate. Asterisks indicate significant differences in firing rate according to the negative binomial regression model (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

(A) There was a main effect of species on LC firing rates, with mouse LC neurons displaying higher activity compared to both rats and nonhuman primates.

(B–F) The effect of species also depended on the state of the animal. Abbreviations: R – rat, M – mouse. Mouse LC neurons were more active when recorded under chloral hydrate (B), urethane (C), isoflurane (D), and in slice (E). In awake animals, this effect was reversed, with rats and NPHs displaying higher firing rates compared to mice (F).

(G and H) There was also a separate species and sex interaction. (G) Male rats and NPHs had lower firing rates compared to mice. (H) There was no difference between rat and mouse LC firing rates when comparing females, while we had no NHP data for comparison.

(I) Sex had a main effect on firing rate. LC neurons recorded from female animals had a higher firing rate compared to males.

(J–L) Sex and brain state (experimental preparation) interacted. Female animals recorded under chloral hydrate (J) and in slice (K) had a higher firing rate than males. (L) Contrastingly, in male animals, LC firing rates were higher than in females in the awake state.

(M and N) Sex and species interacted to affect firing rate. In rats, female LC firing rates were higher than in males. (M) This effect was reversed in mice, where male LC neurons were more active than female LC neurons.

(O) We observed a main effect of brain state on LC firing rates, which revealed that firing rates recorded under urethane anesthesia were lower than those recorded under chloral hydrate, isoflurane, and in slice.

(P and Q) Brain state interacted with sex. (P) LC firing rates under urethane anesthesia were lower than under chloral hydrate anesthesia and isoflurane anesthesia in male animals. (Q) In female animals, firing rate was lower in awake animals compared to chloral hydrate or isoflurane anesthesia and to slice.

(R–S) Brain state interacted with species (only rodents could be tested due to the lack of NHP anesthetized recordings). (R) In rats, LC firing rates in the awake state were higher compared to those recorded under urethane anesthesia or in slice preparations. In addition, firing rates under chloral hydrate anesthesia were higher than in slice and urethane. Finally, firing rates under isoflurane anesthesia were higher than those recorded in slice preparations. (S) In mice, LC firing rates recorded in the awake state were lower than in all other states.

(T) There was also a main effect of genetic modification (Cre expression) on LC firing rates, with VGAT-Cre animals demonstrating a lower firing rate when compared to WT animals. Abbreviations: R – rat, M – mouse, P – NHP, M – male, F – female, C – chloral hydrate, U – urethane, I – isoflurane, S – slice, A – awake, WT – wild-type, DBH – dopamine beta-hydroxylase, TH – tyrosine hydroxylase, VGAT – vesicular GABA transporter. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$, ****$p < 0.0001$.

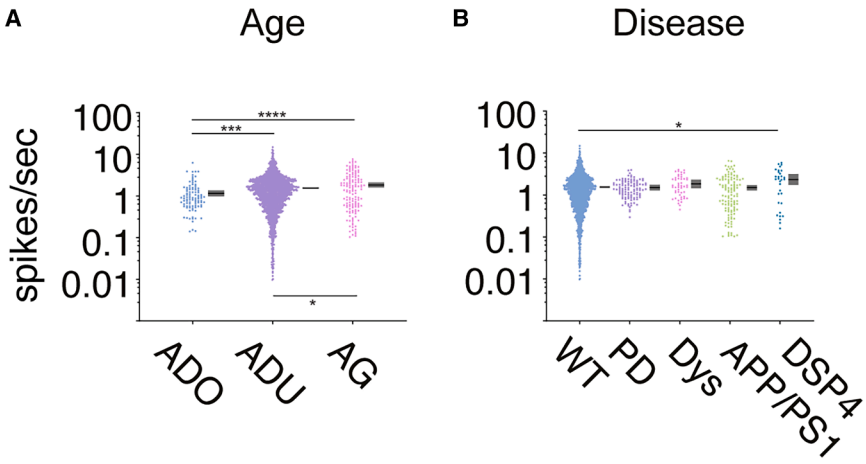


Figure 2. LC firing rate increases with age but does not change after genetic or pharmacological manipulations associated with neurodegenerative disease

All panels are formatted as in Figure 1. Data points are individual neurons. All data are from rats and mice.

(A) There was also a main effect of age on LC firing rates, with firing rate gradually increasing over the course of aging.

(B) There was a main effect of disease model on LC firing rates, and post-hoc analysis revealed that DSP-4 lesions increased LC activity. Abbreviations: ADO – adolescent, ADU – adult, AG – aged, WT – wild-type, PD – parkinsonian, Dys – dyskinetic. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

rats and mice is apparent in the effect size plots (Figures S2P and S2Q), which show positive effect sizes for rats and negative effect sizes for mice.

The female LC has a suppressed firing rate in the awake state

Sex also modified the effect of brain state on LC firing rate. Our analysis revealed an independent interaction between experimental preparation and sex ($p = 0.004$; Figures 1R and 1S; Table S3). In males, activity was similar across anesthetized, sliced, and awake animals except for small differences in firing rate between different types of anesthesia (Figure 1R, see Table S4 for pairwise p-values). In contrast, firing rate was reduced in awake females compared to slice recordings and most anesthetics tested (Figure 1S; see Table S4 for pairwise p-values). The effect sizes compared to the awake state varied from 0.96 to 2.49 spikes/sec (see Figure S2S). Thus, in awake females, LC firing rate is suppressed by a large magnitude (~1 spikes/sec or greater) in comparison to LC neurons recorded in deafferented brain slices or under anesthesia.

Genetic modifications used in optogenetic and chemogenetic experiments may not strongly influence LC firing rates

In addition to the variety of experimental preparations used to study LC neuronal activity, genetic modifications enabling optogenetics and chemogenetics are used in neuroscience research. These genetic modifications typically involve expressing Cre recombinase throughout the brain (e.g., in all catecholaminergic neurons or in all GABA neurons). We compared recordings from (i) dopamine beta-hydroxylase (DBH)-Cre mice, which express Cre in noradrenaline-producing neurons[23]; (ii) vesicular GABA transporter (VGAT)-Cre mice, which express Cre in GABAergic interneurons[49]; and (iii) tyrosine hydroxylase (TH)-Cre rats, which express Cre in all catecholaminergic (dopaminergic and noradrenergic) neurons.[50] There was a significant effect of the type of genetic manipulation on firing rates (p <0.001, Figure 1T; Table S3). Post-hoc testing demonstrated that wild-type animals had higher firing rates compared to VGAT-Cre animals ($p = 0.0007$) but were not different from DBH-Cre or TH-Cre animals (Figures 1T; Table S4). The effect of these genetic manipulations was not dependent on age or sex. Note that we could not assess any interaction effects with state or with species because VGAT-Cre and DBH-Cre data were from mice, while TH-Cre data were from rats (Table S3). This result may indicate that a genetic modification, which is used to enable optogenetic or chemogenetic manipulation of GABAergic interneurons throughout the brain, could reduce LC activity on the order of 0.51 spikes/sec compared to wild-type animals (see Figure S2T). However, VGAT-Cre mice contributed a small number of neurons from a single laboratory. Further study of LC firing rate differences is necessary through the contribution of VGAT-Cre and wild-type mice and rats from a variety of laboratories.

The LC becomes gradually more active over the course of aging

LC dysfunction is a universal feature of Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders beginning with the early aggregation of disease-specific proteins followed by late-stage degeneration.[28] The largest risk for developing neurodegenerative disorders is age.[51] Moreover, age heavily impacts LC structure and function, such as preclinical studies that have reported LC cell body and axon loss over the course of aging.[52–56] We investigated whether there were any changes in LC firing rates during the process of normal aging. Rodents were designated as adolescent (<2 months), adult (2–12 months), or aged (>12 months), while all NHP data came

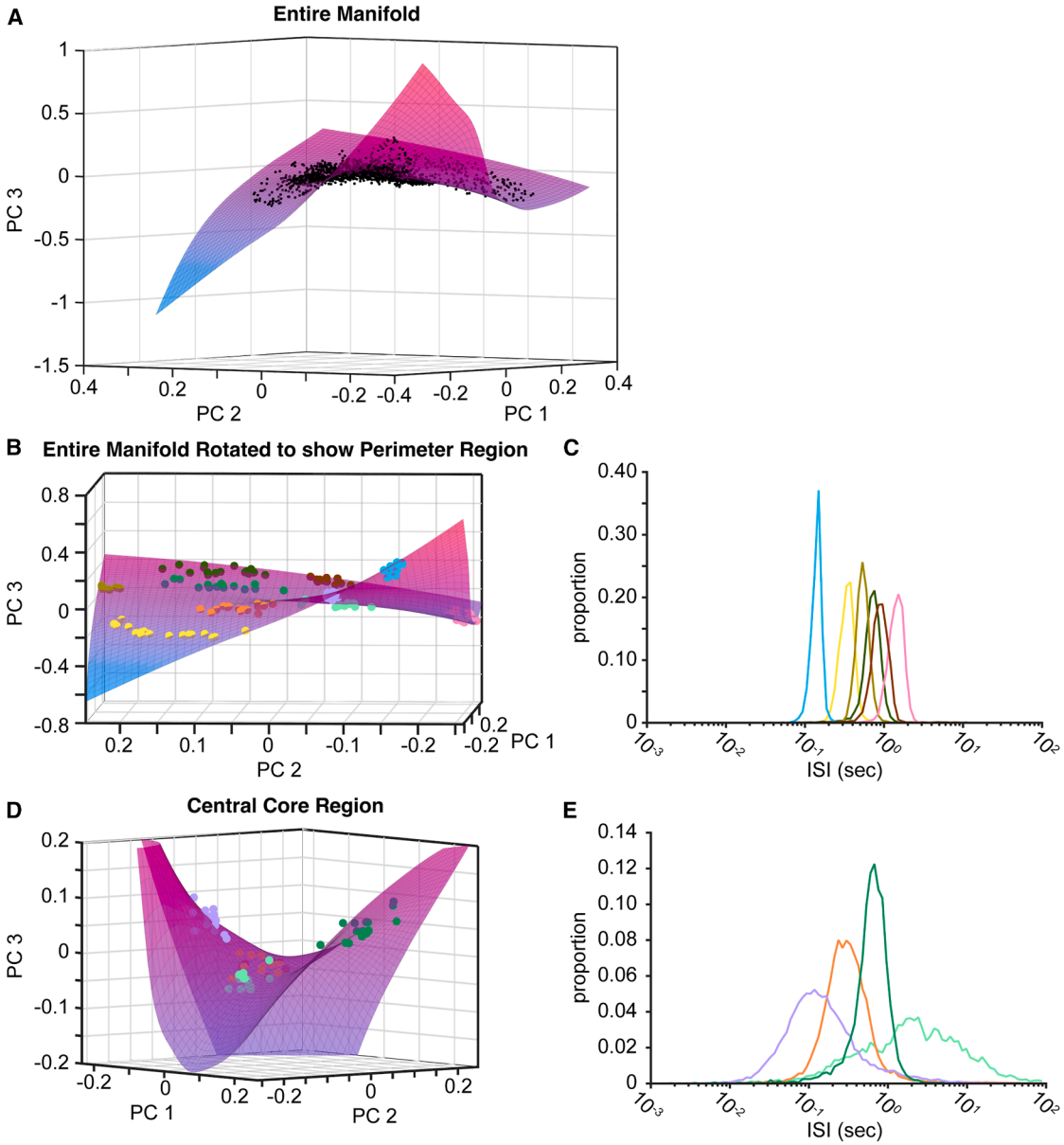


Figure 3. Neuronal firing patterns are distributed along a low-dimensional manifold characterized by diverse firing patterns

(A) The 3D scatterplot shows the principal components (PC) of each neuron on the PC1, PC2, and PC3 axis (N = 1,708 neurons). PCA was performed on the logarithmically binned ISI distribution of each neuron. The pseudocolor surface shows the manifold fit to the points, which is colored according to the PC3 axis. The manifold consists of a dense, central core of neurons and additional neurons spanning the perimeter.

(B) Subsets of 15 neurons were selected (at random) from different areas of the core and perimeter of the manifold. Neurons from different areas are plotted in different colors.

(C) The average ISI distribution of the randomly selected neurons from the perimeter of the manifold (N = 15 neurons per average distribution). Note that the x axis is logarithmically scaled.

(D) The PC3 axis of the manifold has been limited (±0.2) to focus on the central core region of the manifold. The different colored points are 15 randomly selected neurons in 4 different groups. Two groups (light green and orange) have negative PC3 values but are separated in the PC1 and PC2 axes. Two groups (purple and dark green) have positive PC3 values and are separated in the PC1 and PC2 axes. These points are also all visible in the central area of the manifold in panel B.

(E) The average ISI distributions for these central core groups (N = 15 neurons per average distribution). Note that the x axis is logarithmically scaled.

from adult animals (10–16 years).[57,58] There was a main effect of age on firing rates (p = 0.006; Figure 2A; Table S3). Adolescent firing rates were lower than both adult (p = 0.0002) and aged animals (p < 0.0001), while LC activity in adults was lower than in aged animals (p = 0.035) (Figure 2A; Table S4). The effect sizes for the change in firing rate with age are shown in Figure S3A. Note that the interaction of age with species and state was not estimable (Table S3). There were no interactions between age

and any other factor, suggesting that the gradual increase in LC activity during aging is not genotype- or sex-dependent. Thus, LC firing rates gradually increase with age, which has implications for understanding the contributions of LC activity to behavioral changes during aging and disease prevalence during aging.

LC firing rate is unaffected in models of neurodegenerative disease unless the degeneration specifically targets LC neurons

Given our finding that LC firing rate increases with age, which is a risk factor for developing neurodegenerative disorders, we wondered whether LC activity might be disrupted in genetic and pharmacological rodent models of neurodegenerative diseases. We analyzed the firing rate of neurons recorded from TgF344-AD rats that carry transgenes expressing autosomal dominant, early-onset Alzheimer's disease-causing APP/PS1 mutations.[59] Importantly, these rats develop hyperphosphorylated tau in the LC prior to amyloid or tau pathology in forebrain regions,[52] similar to the temporal pattern of tau deposition observed in humans.[60] The dataset also included three pharmacological manipulations to induce neurodegenerative disease-like states. The first manipulation was the selective adrenergic neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), which induces LC noradrenergic axonal degeneration.[61] The second manipulation was that rats were unilaterally injected with 6-OHDA into the medial forebrain bundle to induce dopaminergic system neurodegeneration while sparing the noradrenergic system using pretreatment with desipramine.[9] This second manipulation produces a hemiparkinsonian disease model. The third manipulation combined 6-OHDA injections into the medial forebrain bundle with 3 weeks of L-DOPA treatment, which produces a dyskinetic disease model.[62] We asked whether these manipulations led to changes in LC firing rates that could be relevant for the presentation of neurodegenerative disorders. There was a main effect of disease model type on firing rates ($p < 0.0001$; Figure 2B; Table S3). Post-hoc analysis revealed that animals treated with DSP-4 had higher firing rates than wild-type animals ($p = 0.0249$; Figure 2B; Table S4). The effect size for the change in firing rate for DSP-4 versus wild-type is shown in Figure S3B. Overall, we were surprised to find that LC activity did not differ from control animals in any of the other disease models.

Due to the relationship between neurodegenerative disorders and age,[51] the age of the animal might interact with the preclinical models to produce a change in LC activity. However, we found that the effect of these disease models on firing rate was not dependent on sex or age (Table S3). Note that interactions with species or brain state were not estimable. Overall, our findings are unexpected given that these disease models presumably relate to symptoms through altered noradrenergic neuromodulation by the LC but resulted in no change in baseline LC activity except for the DSP-4 pharmacological lesion model.

LC activity spans a spectrum of diverse firing patterns that fall upon—but are constrained to—a low-dimensional manifold

We have shown that the time-averaged firing rates of LC neurons vary across species and multiple intrinsic and extrinsic biological factors. It is plausible that the patterns of LC activity may also vary. Traditionally, these patterns have been labeled as either a "tonic mode" or "phasic mode" of firing.[63–65] Tonic spiking has been described as irregular discharging of spikes with inter-spike intervals (ISIs) lasting several hundred milliseconds (resulting in a firing rate below ~6 spikes/s), while the phasic mode consists of 2–3 spikes within a 50–100 msec window[65] and correspondingly short (<80 ms) ISIs.[9,14] This two-mode dichotomy is used not only in studies of single-unit LC activity in animals but also in human cognitive neuroscience studies where single LC neuronal activity is inaccessible, as well as in computational models of cognition and learning.[17–19]

We detected the predominant firing patterns across neurons by conducting a principal component analysis (PCA) of the logarithmically binned ISI distributions (ranging from 1 ms to 100 s) across 1,708 neurons (see exclusion criteria from Figure 1). These 1,708 distributions characterized the firing patterns from the entire recording of each neuron. The first three principal components (PC) explained 59.56% of the variance in the data, with a drop to 7.89% at PC4 and <5% for all subsequent PCs. Thus, we plotted the first three PCs of the ISI distributions. We expected two discrete clusters of points that would correspond to neurons with irregular, infrequent spike times that are, or are not, mixed with phasic bursts of spiking (< 80 ms ISIs). Instead, the firing patterns of individual neurons spread along a low-dimensional manifold with a dense central "core" area along the third PC zero axis and a "perimeter" area characterized by a dispersal of points in the positive and negative third PC axis (Figure 3A; Video S1). Figure 3B shows the manifold from panel A rotated leftward (partly collapsing the PC1 axis and spreading the PC2 axis). From this vantage point, we sampled units from various areas around the perimeter of the manifold. The units in each area are noted by distinct colors. Figure 3C shows the average ISI distributions of the units for each area of the manifold. These ISI distributions have different means, mostly ranging from 0.3 to 1.0 s ISI (firing rate of 3.3 spikes/sec down to 1.0 spikes/sec), with the cyan-colored distribution having a mean ISI of 0.15 s (firing rate of 6.6 spikes/sec) and the pink distribution having a mean ISI of 1.05 s (firing rate of 0.95 spikes/s). All distributions are narrow distributions and therefore qualitatively rhythmic. By contrast, zooming in on the central core region of the manifold (Figure 3D) and selecting units from different areas of the core shows groups of neurons with relatively more variability in the ISI (Figure 3E). In this central core region, the mean ISIs (0.1 s–2.0 s) were approximately similar to those in the perimeter of the manifold (0.15 s–1.05 s). However, comparing the span of the distributions in Figures 3D and 3E (which have the same *x* axis) shows that units in the core have wider distributions and are therefore relatively less rhythmic in firing. In summary, looking across the manifold, a diversity in firing patterns is apparent. PCA effectively identified and clustered similar firing patterns, with the spread of these points showing the diversity in LC firing patterns. This stands in contrast to the notion that a single ISI cutoff divides LC activity into two firing patterns. This continuum of firing patterns among 1,708 LC neurons is evidence against tonic and phasic spiking modes that are characterized by rigid descriptors in spontaneous spiking. However, we note that stimulus-evoked firing may produce two distinct firing patterns.

Specific LC activity patterns were not on the manifold, such as a highly positive PC2 and highly positive PC3. This suggests that some activity dynamics may not be permissible (or not expressed in this dataset). Therefore, despite the diversity in the patterns of LC spiking, there are patterns which are not observed and may not occur naturally. Intriguingly, deafferented brain slices, which made up one component of this dataset, are not natural. This begs the question of whether brain slice spiking patterns might map onto a specific area of the low-dimensional space. Therefore, we next assessed whether any factors (sex, species, age, and brain state) were biased toward specific areas of the manifold.

Firing patterns can occupy specific areas of the manifold when brain state, species, age, and genotype are considered

Brain slice firing patterns fell along the perimeter of the manifold, while firing patterns in awake organisms were concentrated in the core (Figures 4A and 4B). The firing patterns under anesthesia were spread across the entire manifold but avoided the perimeter occupied by slice recordings. The difference in firing patterns between slice and awake recordings manifested as more rhythmic firing with a lower limit of approximately 100 ms, while LC firing patterns during wakefulness exhibited comparably shorter (down to approximately 10 ms) as well as longer (up to approximately 10 s) ISIs (Figure 4C). We used the two-sample Kolmogorov-Smirnov test to compare shapes of the ISI distributions. This test does not compare summary statistics (e.g., the mean ISI); rather, it directly compares the distribution shapes by comparing the cumulative distribution functions. The two-sample Kolmogorov-Smirnov test does not compare the collapsed mean ISI. We used this test to compare the dynamic shape of the ISI distributions. The test calculates empirical cumulative distribution functions (CDFs), finds the maximal distance between the two CDFs, and compares it to a critical threshold. Applied in this manner, the two-sample Kolmogorov-Smirnov test detects differences in distribution shape that can include variance, skew, and bi- (or multi-) modality.

We found that the ISI distribution shapes significantly differed between awake recordings and slice recordings (KS = 0.374, $p < 0.001$), as well as from anesthetized recordings (KS = 0.353, $p < 0.001$). Awake and anesthetized recordings had similar ISI distributions (KS = 0.111, $p = 0.549$). Overall, LC neurons recorded from brain slices exhibit clearly distinct firing patterns from awake and anesthetized recordings.

Species-specific and age-specific firing patterns were also observed. NHPs, mice, and rats all occupied large portions of the manifold (Figure 4D), with NHPs occupying the core of the manifold (Figure 4E). The ISI distributions for each species were thus similar (Figure 4F), although the firing patterns for mice and NHPs may differ (KS = 0.202, $p = 0.030$). However, this may be due to NHPs being recorded only during wakefulness, which we found was associated with the core area of the manifold (Figure 4B). The perimeter of the manifold contained both rat and mouse neurons, which corresponds with the contribution of slice recordings from both species. We also assessed whether rodent species might separate males and females on the manifold and found no evidence that sex was associated with different patterns of firing (Figure S2). While neurons recorded from adolescent and adult animals were spread across the manifold (Figure 4G), those recorded in aged animals were localized to one side of the core (Figure 4H; Video S4). Given that a large number of adult neurons were also concentrated in the core, it is unsurprising that the ISI histograms for adults and aged animals did not significantly differ (Figure 4I, KS = 0.131, $p = 0.338$). Although adolescent neurons appear to be isolated around the perimeter of the manifold and have a significantly different ISI distribution from both adult (KS = 0.394, $p < 0.001$) and aged (KS = 0.343, $p < 0.001$) animals, this is explained by adolescent neurons being recorded exclusively in slice recordings. *In vitro* recordings are typically done in brain slices from young animals. These results highlight the need for single laboratories to record LC single-neuron activity in a variety of states (e.g., anesthetized and awake NHPs) and across a variety of ages (e.g., brain slices from young, adult, and aged animals). Despite the need for more data in some contexts, it is nevertheless apparent that aged neurons occupy a specific area of the manifold in this dataset.

Finally, we assessed how manipulation of genotype could affect where LC neuronal activity patterns fall upon this manifold. These genetic modifications expressed Cre recombinase throughout the brain with specificity for distinct neuron types. DBH-Cre targeted noradrenaline-producing neurons, TH-Cre targeted all catecholaminergic (dopaminergic and noradrenergic) neurons, and VGAT-Cre targeted GABAergic interneurons. VGAT-Cre neurons skewed toward one end of the manifold (Figures 4J and Video S5) and exhibited particularly rhythmic ISIs (Figure 4K). The ISI distribution for VGAT-Cre significantly differed from the distributions for TH-Cre (KS = 0.263, $p = 0.002$), DBH-Cre (KS = 0.323, $p < 0.001$), and Gad2-Ires-Cre (KS = 0.242, $p = 0.005$). DBH-Cre also appeared to be more diffusely spread across the manifold relative to TH-Cre, and the ISI distributions for those groups also significantly differed (KS = 0.273, $p = 0.001$). These results suggest that merely expressing Cre in a variety of neurons—brain wide—may alter the spontaneous firing patterns of LC neurons.

The temporal dynamics of LC spike patterns exhibit a variety of attractor dynamics that are predominantly expressed according to species, age, sex, and genetic modification

Spike train patterns are dynamic over time. The PCA of ISI distributions (Figures 3 and 4) reflected dynamic state transitions within the recordings. This motivated our next analysis aimed at honing in on spontaneous state transitions by examining return maps (also known as Poincaré maps) to characterize how preceding ISIs influence the subsequent ISI. Return maps are visualizations of the joint relationship between successive inter-spike intervals. The return map can show temporal patterns including stable states (attractors) of neuronal firing.[66,67] Figure 5A illustrates this concept using synthetic data for two neurons. The return maps show the relationship between pairs of ISIs, with one ISI on the *x* axis plotted against the following ISI on the *y* axis. How often a specific pair of ISIs occurs is shown as a density plot, with blue indicating few ISI pairs and yellow

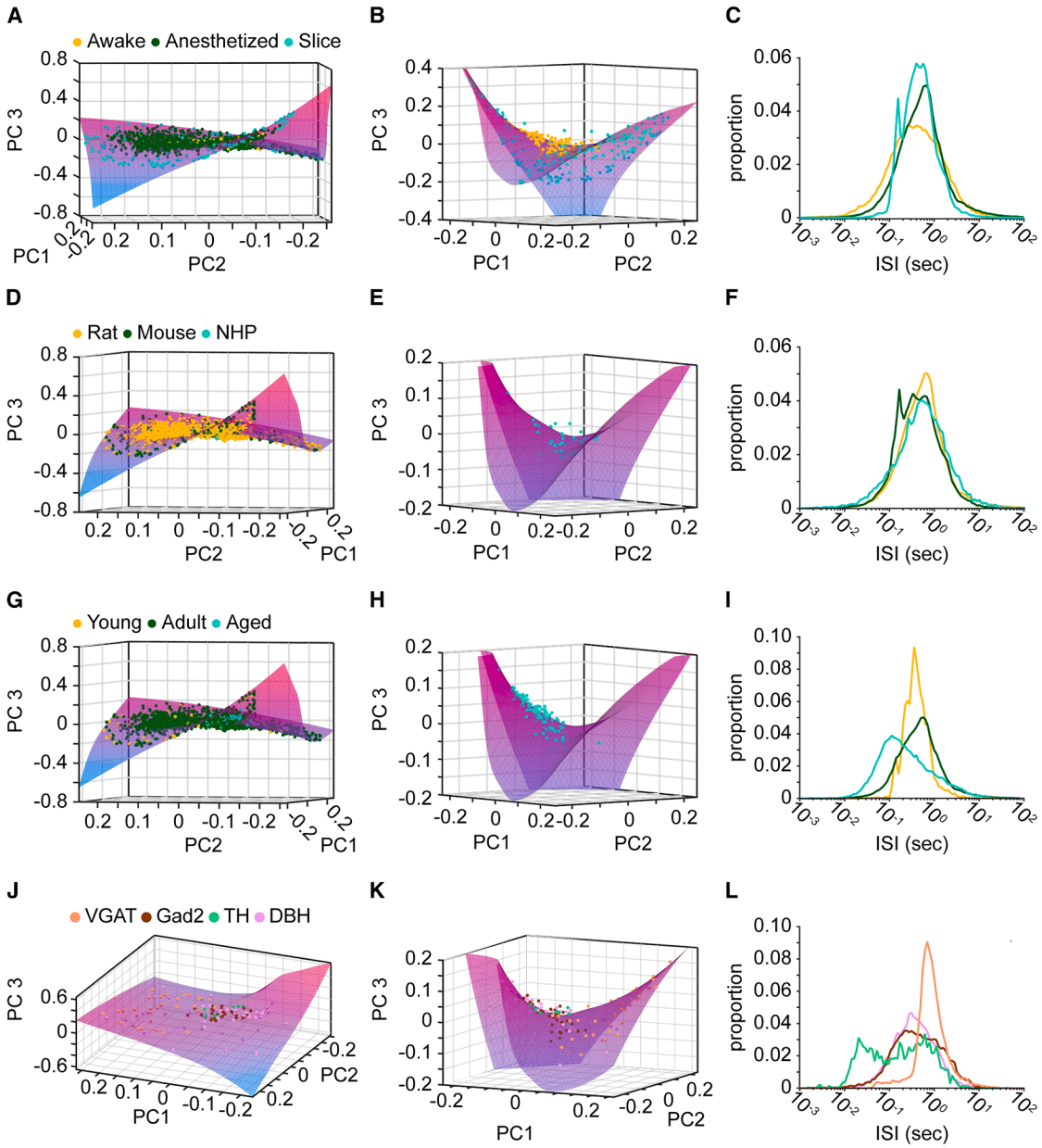


Figure 4. Intrinsic and extrinsic factors were biased toward particular areas of the manifold.

(A) The PC scores of neurons recorded in the awake, anesthetized, or brain slice states are plotted on a 3D scatterplot of PC1, PC2, and PC3. *N* = 136 awake neurons; 1,376 anesthetized neurons; and 196 brain slice neurons.

(B) The PC3 axis has been limited (±0.4) to show the central core area of the manifold. The neurons recorded in the awake state and brain slice state are shown.

(C) The ISI distribution of all awake neurons (orange line, average of *N* = 136 neurons), all brain slice neurons (cyan line, average of *N* = 196 neurons), and all anesthetized neurons (green line, average of *N* = 1,376 neurons). Note that the *x* axis is logarithmically scaled.

(D–F) The plots are the same format as panels A through C. The PC scores are shown for different species. *N* = 1,375 rat neurons; 292 mouse neurons; and 41 NHP neurons. In panel (E), the PC3 axis of the manifold has been limited (±0.2) to show the central core area. The NHP neurons were constrained to this central area. Note that the *x* axis is logarithmically scaled. Differences between the distributions appear smaller on a log scale.

(G–I) The plots are the same format as panels (A–C). The PC scores are shown for different ages. *N* = 41 young animal neurons; 1,552 adult animal neurons; 115 aged animal neurons. In panel (H), the PC3 axis of the manifold has been limited (±0.2) to show the central core area. The aged animal neurons were constrained to the negative PC1 axis and (mostly) positive PC3 axis of the central area.

(J–L) The plots are the same format as panels (A–C). The PC scores are shown for different genotypes. *N* = 57 DBH-Cre animal neurons; 32 Gad2-Cre animal neurons; 9 TH-Cre animal neurons; and 35 VGAT-Cre animal neurons. The VGAT-Cre animal neurons are lateralized on the manifold toward positive PC1 scores.

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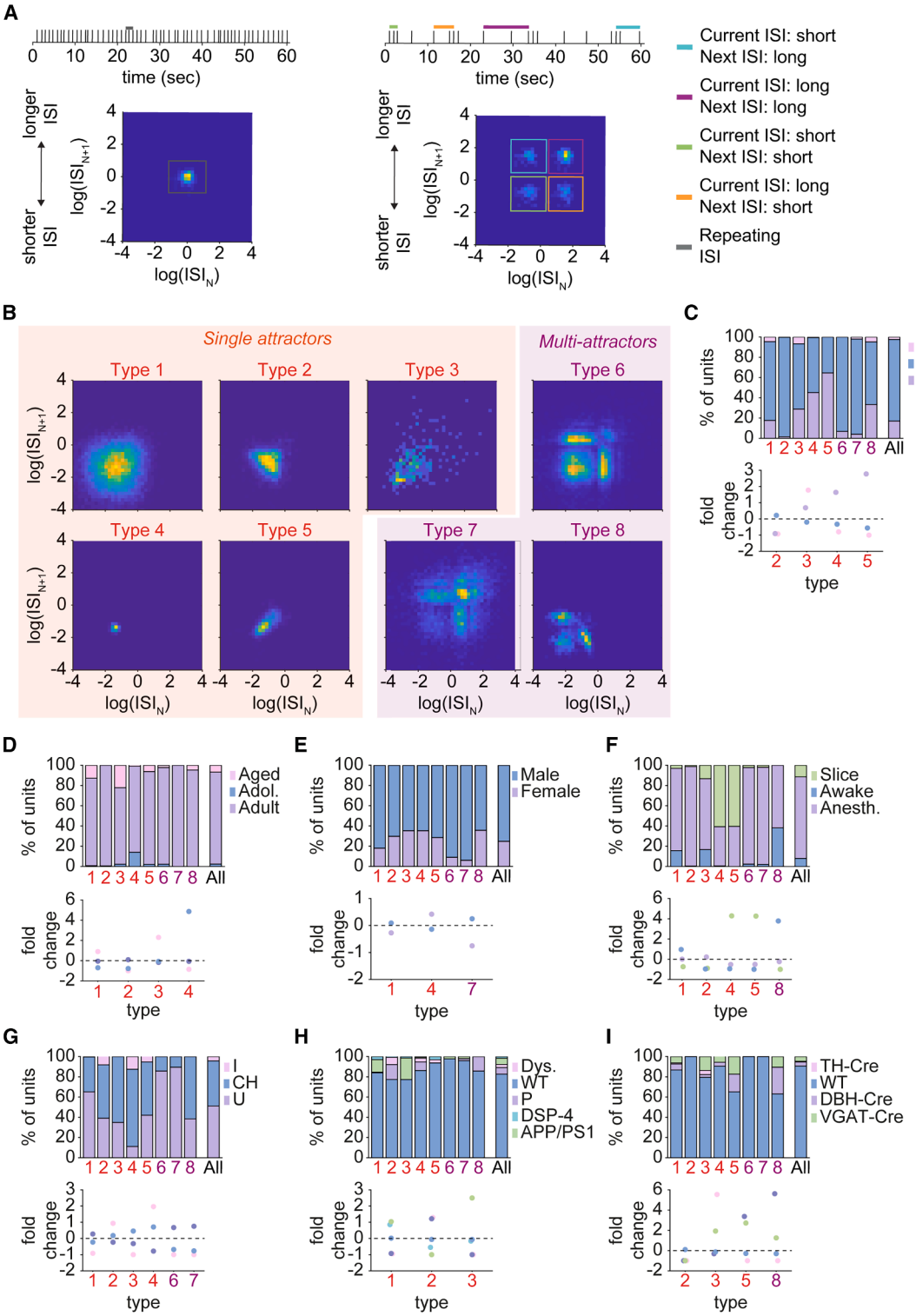


Figure 5. Intrinsic and extrinsic factors are associated with different types of attractor dynamics

(A) Synthetic data showing the spike train characteristics displayed as return map density plots. The return maps show the relationship between pairs of ISIs in a single neuron, namely the ISI of a given neuron on the *x* axis against the following ISI of that same neuron on the *y* axis. The frequency with which those relationships occur for a given unit is represented in the color, where blue is no/infrequent ISI pairings and yellow is a pairing that occurs often. The upper panels show two synthetically generated spike trains (left and right panel). The lower panels show the return maps. The left panel shows the return map associated with a

(legend continued on next page)

showing frequently observed pairs. The left panel shows the return map associated with a regular/tonic firing pattern, which results in a return map with one point (i.e., a single attractor). The right panel shows a tonic-phasic firing pattern. In the spike train, a burst (i.e., a short ISI followed by a short ISI) is highlighted by a green rectangle. Note that the specific ISI is not meant to mimic a spontaneous LC neuron burst and is only to illustrate how a shorter ISI would appear on a return map. A burst initiation in the context of tonic firing (i.e., a long ISI followed by a short ISI) is denoted by an orange rectangle. Tonic firing (which may or may not precede a burst) is shown by a purple rectangle (in this example, the tonic firing precedes the start of a burst). Finally, a burst termination (i.e., a short ISI followed by a long ISI) is indicated with a blue rectangle. These four patterns appear in the return map as 4 separate points. Each of these 4 points can be thought of as an attractor because the return map, which shows the density of the ISI patterns, illustrates that most of the time firing is constrained to these 4 specific ISI patterns. The example synthetic neuron firing pattern is therefore a multi-attractor.

We calculated return maps for the 1,708 LC neurons that met requisite conditions for ISI analysis. We manually grouped these return map patterns because automated STAR Methods, such as PCA, histogram of oriented gradients analysis, and k-means clustering, performed poorly. We then assessed how distinct types of second-order firing patterns mapped onto various factors. We identified 8 return map patterns among the 1,708 neurons. Figure 5B shows an example unit from each of these 8 types of return maps. Types 1 through 5 (accounting for 62.5% of the patterns produced and most of the neurons) show a single stereotyped firing pattern (i.e., an attractor) with different shapes due to ISI variability. This pattern is expected from irregular tonic baseline activity. For example, the return density plots of types 1 and 4 are both evenly distributed around a common ISI value, but the variability in type 1 is larger than type 4, which is highly discrete and the result of extremely regular activity similar to the synthetic raster shown in Figure 5A (left panel). Types 6 through 8, on the other hand, have multiple attractors that also differ in their shape and variability. The neurons with multi-attractor firing patterns are generated by tonic firing interspersed with bursting at discrete relative frequencies. This identifies specific relative relationships in spontaneous bursting activity of LC neurons. The number of units in each type varied from 21 to 689. More complex multi-attractor firing patterns were observed in a minority of neurons (N = 115, 6.73%), while most neurons fired in type 1 or type 2 single-attractor patterns (N = 1249, 73.13%). Descriptive values for each type can be found in Table S5. These findings show that LC neurons produce firing patterns that are stereotyped attractors, and some neurons display transitions between multiple attractors.

Different extrinsic and intrinsic factors might bias LC neurons toward firing in particular second-order patterns. We tested this idea by characterizing to what degree different factors were expressed in each return map type by comparing the proportion of neurons with a specific factor to the overall population (Table S5). Our analysis revealed that some firing patterns are almost entirely expressed by a single species (Figure 5C). Type 2 (single attractor) was almost exclusively rat LC neurons. Types 4 and 5 (single attractor) had a greater proportion of neurons from mice and rarely included neurons recorded from NHPs. However, NHP LC neurons were overrepresented in type 3 (single attractor), which also contained a large proportion of mouse LC neurons. Therefore, we found species-specific variations in tonic baseline firing intervals from mouse, rat, and NHP LC.

We also examined the effect of age on LC spike train dynamics (Figure 5D). Types 1 and 3, both single attractors with wide variability, had a higher proportion of aged animals. Adolescent neurons were especially common in type 4, which showed the most consistent ISIs. Type 2 was almost entirely made up of adult neurons with little/no adolescent or aged neurons. Thus,

regular/tonic firing pattern, which results in a return map with one point (i.e., a single attractor). The right panel shows a tonic-phasic firing pattern. In the spike train, a burst (i.e., a short ISI followed by a short ISI) is highlighted by a green rectangle. A burst initiation in the context of tonic firing (i.e., a long ISI followed by a short ISI) is denoted by an orange rectangle. Tonic firing (which may or may not precede a burst) is shown by a purple rectangle (in this example, the tonic firing precedes the start of a burst). Finally, a burst termination (i.e., a short ISI followed by a long ISI) is indicated with a blue rectangle. These four patterns appear in the return map as 4 separate points. Each of these 4 points can be thought of as an attractor because the return map, which shows the density of the ISI patterns, illustrates that most of the time firing is constrained to these 4 specific ISI patterns. The example synthetic neuron firing pattern is therefore a multi-attractor.

(B) Example return maps for the 8 types of attractor patterns that were detected across all neurons. Types 1–5 are single attractors, while Types 6–8 are multi-attractors. The number of neurons for Types 1 to 8 was: N = 689, N = 560, N = 90, N = 206, N = 48, N = 44, N = 50, N = 21) (C-I) The bar plots (upper panels) show the proportion of factors (e.g., species, age, sex, etc.) represented in each of the 8 return map Types (type ID on x axis) and the population as a whole (rightmost bar). The proportion of factors in each cluster is identified in each bar graph and was tested for differences compared to the overall population with a chi-squared test. Fold change in proportional representation (lower panels) is shown for types where there was a significantly different proportion of factors. For each type, the fold change was calculated by first subtracting the factor's proportion in the population from the factor's proportion within the type. This difference was then divided by the proportion in the population.

(C) Four types were comprised of neurons across species in different proportions compared to the population. Type 2 contained almost entirely neurons from rats. Mouse neurons were over-represented in Type 4 and Type 5. NHP neurons were over-represented in Type 3.

(D) Age was associated with specific types. Types 1 through 4 were comprised of significantly different proportions of neurons based on age. There was an increase in adolescent neurons in Type 4, adult neurons in Type 2, and aged neurons in Type 1 and Type 3.

(E) Three of the Types (1,4, and 7) contained different proportions of neurons recorded from males or females.

(F) Brain state varied greatly across the eight types. Type 4 and Type 5 contained more neurons from slice recordings. Type 1 and Type 8 had more neurons from awake animals. Type 2 was almost entirely anesthetized neurons.

(G) Almost all types had different proportions of anesthetics compared to the overall population of neurons.

(H) Neurodegenerative disease-associated manipulations were differentially represented in Type 1, Type 2, and Type 3. There was an over-representation of APP/PS1 and DSP-4 neurons in Type 1 and Type 3, and an over-representation of Parkinsonian and dyskinetic pharmacological manipulation neurons in Type 2.

(I) Type 3, Type 5, and Type 8 contained increased proportions of neurons from genetically manipulated (Cre) animals, while Type 2 was almost entirely wild-type animals. $^{*}p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$, $^{****}p < 0.0001$.

the adult LC may express a specific tonic firing pattern that distinguishes it from tonic activity patterns in adolescence or later life. Of note, variability (spread) between relative ISIs is very low in the type with more adolescent animals (type 4), increasing in the adult animal type (type 2), and the greatest variability is observed in aged animals (types 1 and 3). Thus, distinct timepoints in development and aging are associated with specific LC second-order firing patterns. (Note, however, all LC neurons from adolescent animals were obtained from brain slice recordings. While aged animals had distinct firing patterns, the developmental transition of firing patterns from adolescence to adult animals may instead be due to the experimental preparation.)

We investigated whether firing patterns were expressed to a greater extent in males compared to females and vice versa. However, no return map type was entirely specific to one of the sexes (Figure 5E). Male neurons predominate types 1 and 7, whereas type 4 had a higher proportion of female neurons. These results show that sex has some influence on second-order firing patterns, but all patterns are expressed in both sexes.

We next examined the role of experimental preparation on second-order firing dynamics (Figure 5F). Type 4 and 5 return maps (single attractors without much variability) had a significantly higher proportion of neurons recorded in a slice preparation and contained no neurons recorded in awake animals. Type 2 (single attractor) was almost entirely made up of neurons from anesthetized animals. Finally, type 1 (single attractor) and 8 (multiple attractors) were comprised of a higher proportion of neurons recorded in the awake state with a lower proportion of neurons recorded in the slice preparation. These results suggest that the preparation in which LC neurons are recorded is associated with specific second-order spike train statistics.

Given that experimental preparation is associated with altered LC spike train dynamics, we assessed whether the dynamics were influenced by the type of anesthetic agent utilized in anesthetized recordings (Figure 5G). Neurons recorded under urethane anesthesia were the majority in types 1, 6, and 7. Moreover, these types lacked neurons recorded under isoflurane. Similarly, there were no isoflurane neurons in type 3, but this type contained a higher proportion of chloral hydrate neurons. Finally, types 2 and 4 contained neurons recorded under isoflurane, as well as those recorded under chloral hydrate, in greater proportions. Thus, we found 75% of the second-order spike train patterns identified differed in their composition of neurons recorded under urethane, chloral hydrate, and/or isoflurane.

We tested whether models of neurodegenerative disease (i.e., genetic modification or pharmacological manipulations) were over- or under-represented in any of the return map types. We found that type 2 had an increased proportion of neurons from pharmacological animal models of dyskinesia and Parkinson's disease but a decreased proportion of neurons from the APP/PS1 genetic model and DSP-4 pharmacological model of neurodegenerative disease (Figure 5H). In contrast, we observed the opposite patterns in types 1 and 3. These clusters contained more APP/PS1 and DSP-4 neurons, but almost entirely lacked neurons from the pharmacological models of Parkinson's disease and dyskinetic disorders.

Lastly, we assessed whether genetic manipulations for opto/chemo-genetics (Cre-expression) were differentially expressed across the 8 types of return density patterns (Figure 5I). We found type 2 was almost entirely comprised of wild-type animals. Type 3 had a higher proportion of TH-Cre neurons, while types 5 and 8 had a greater proportion of neurons recorded from DBH-Cre animals. Although not a large difference, types 3, 5, and 8 also had an increased proportion of neurons from VGAT-Cre animals. These results show that TH-Cre, DBH-Cre, and VGAT-Cre-expressing neurons may each be biased toward specific relative firing patterns. Importantly, however, one type of firing pattern (type 2) was expressed only in wild-type subjects.

DISCUSSION

The LC is an ancestral brain region whose evolutionarily conserved function is thought to be achieved by two modes of LC firing: slow and irregular tonic activity or fast phasic burst activity. In contrast to this two-mode concept for spontaneous LC activity, we found many more than two modes of firing, which spread along a low-dimensional manifold. The temporal dynamics of these firing patterns can be described as different types of attractors. Neither specific firing patterns nor average activity levels are conserved across species.

We used a negative binomial regression model to make a direct comparison of LC firing rate across species and across other key intrinsic and extrinsic biological factors. The statistical model controlled for the individual and combined effects of various factors. Crucially, however, model-based significance testing was fully supported by assessments of effect size with 95% CIs. The 95% CI is an 83% prediction interval, meaning it has an 83% chance of covering any future experiment's mean effect size.[37,39] Thus, all findings were supported by both a statistical model and an approach not based upon *p*-values. We found that mean firing rate was higher in the mice compared to rats and NHPs. The model revealed that the effect of species upon mean firing rate also interacted with the brain state. The mouse LC was more active under anesthesia and in brain slices compared to the awake state, whereas in rats the opposite pattern was observed. Species and sex also interacted. In rats, neurons recorded from females were more active than those recorded in males, while the opposite pattern was observed in mice. It is known that the LC is a sexually dimorphic structure.[41] Our findings may implicate the increased activity of female LC as a driver of sex-specific vulnerability to stress, anxiety, and substance-use disorder,[41,68] but our results also show that studies on the role of the LC in sex-specific vulnerabilities must be interpreted in a species-specific context. New data are needed to test whether species differences—specifically during wakefulness—could be due to sex of the animal, since neurons recorded during the awake state in mice came from females but, in rats and NHPs, they came from males.

It is important to consider that comparisons across factors inherently become comparisons across laboratories because the expertise of individual laboratories is usually centered upon one species and one experimental preparation (i.e., only awake, or with one type of anesthesia, or brain slices). We found that inter-lab variability in firing rates was low, and intraclass correlation indicated that firing rate had low-to-moderate dependence on laboratory identity. Factors that often differ across

laboratories, such as habituation, room lighting, and sensory (visual, auditory, and olfactory) cues, are unlikely to affect our results, as 95.9% of our data were collected from brain slices and anesthetized animals, where these environmental factors are irrelevant. Under anesthesia, LC neurons do not respond to sensory cues (except for painful stimuli).[65,69]

Electrode location within the LC could also differ across laboratories or between experiments within a laboratory. Along the dorsal-ventral axis of the LC, neurons have different firing rates and waveform shapes in rats[70] as well as in mice.[71] Waveform shape is associated with differences in membrane excitability and oscillatory firing pattern properties,[70,72] as well as intra-LC functional connectivity and potentially nonsynaptic (gap junction-mediated and noradrenergic lateral inhibition-mediated) connectivity,[70,73] and cell morphologies.[71,73] Therefore, dorsal-ventral location within the LC may be an important factor to consider. However, given the small dorsal-ventral extent of the LC, it is unlikely that different laboratories or factors (sex, age, etc.) were exclusively associated with the dorsal or the ventral LC. We encourage the field to use laminar multi-electrode probes to map the LC based on electrophysiological landmarks and LC anatomy following the specific guidelines suggested by multiple laboratories.[1] Similarly, we urge the field to report whether neurons were recorded in the LC-core (likely the vast majority of the literature), LC-horn, or the sub-Coeruleus rostroventral to the rostral LC horn.[1] Since both firing rate and waveform shape vary with the LC dorsal-ventral axis, single-unit waveform shape should be characterized as in prior work[70] and note that signal filters must also be reported, per LC recording guidelines.[1]

We also considered whether noncontrolled circadian rhythms might influence the results. Prior research indicates that LC activity changes approximately 1.5-fold between the min/max of the circadian cycle[74], suggesting that any circadian influence on our data would be even less given that this prior research was *a priori* designed to detect a maximal circadian effect. However, our work showed some even larger effect sizes, such as the 2.26-fold change for rat versus mouse. Firing rate dependence on a variety of factors could be influenced but not fully explained by circadian rhythms, which were not unified across laboratories.

Attributing inter-laboratory differences in recording STAR Methods and conditions does not fully account for all of our findings. For instance, 14 labs recorded male only, 0 labs recorded exclusively female, and 8 labs recorded both sexes. Two things would need to be true for lab variability to be the primary explanation for differences in sex: First, the 22 laboratories that recorded males would need to record identically, and the 8 laboratories that recorded females would need to be systematically recording in a different way. Second, there are a subset of laboratories recording both conditions (e.g., 8 laboratories recorded both sexes), so individual laboratories would need to record males and females differently within each laboratory. In our view, this is highly unlikely. We assert that our analysis reveals that LC activity is more diverse across species and conditions than previously understood.

Our central claim is that LC activity is less uniform (across species and other factors) than previously thought. Even if inter-laboratory variability influenced the results, our data still show that LC activity is not uniform, and it simply shifts the source of the diversity to differences across laboratories. If true, this underscores a pressing need to address reproducibility issues in LC research, potentially necessitating a reevaluation of 60 years of studies in systems neuroscience. Resolving any inter-laboratory effect on our results will require individual laboratories to record diverse datasets by working across species and multiple experimental preparations and recording from both sexes. It must be recognized that laboratory variability is inherent to systems neuroscience: we compare studies, *post hoc*, across laboratories. The large sample size of nearly 2,000 neurons combined into one common analysis offers robust insight compared to previous studies involving much smaller sample sizes. Our results imply the need for future studies designed to control for inter-laboratory variability using an *a priori* design.

One of the most important observations from this large, pooled dataset is that LC firing patterns are highly diverse but still constrained onto a low-dimensional manifold. In other words, LC firing patterns spread along a spectrum of potential patterns, but some "off-manifold" patterns were not observed in this dataset. The dynamics of these patterns can be described as different types of single attractors and multi-attractors. Studies of LC neuronal activity have defined tonic activity and phasic activity by dividing these firing modes using a threshold of either a firing rate >6 spikes/s,[64,65] or ISI of less than 80 ms.[9,14] Our results suggest that, rather than using a rigid cut-off in firing rate or ISI to define two modes of activity, spontaneous LC neuronal activity patterns must instead be appreciated as heterogeneous and falling across a spectrum. Our results show that spontaneous LC activity, which can similarly affect cortical state and behavior as much as stimulus-evoked activity,[10,13,15] has many firing modes that perhaps defy simple labels of "tonic" and "phasic." LC firing patterns are simply diverse.

While we show that there is no single cut-off or defining feature of an LC neuron burst, clear cut-offs or definitions for bursting might be useful for specific research questions. For instance, if a researcher notes that noradrenaline release changes at a different rate when a specific ISI threshold is exceeded (either in spontaneous spiking or due to stimulation entraining an LC response), then that observation might prompt the researcher to use that ISI cut-off when assessing the context and conditions during which that specific firing pattern is observed. Similar experiments could be conceived for LC-associated changes in cortical state or behavior. The key finding of our work is that there is no singular definition of a burst in spontaneous LC activity that is inherent to and shared by all LC neurons. There are a multitude of ways to define a burst in the LC, and those definitions may change when we consider different circumstances (slice, awake, adolescent, and female). Finally, it is crucial to note that stimulus-evoked activity may have a clearly defined ISI cut-off for phasic bursting. This remains to be determined in future work leveraging a large dataset collected from a variety of laboratories employing the same stimuli in the same set of species across a variety of ages, genotypes, and experimental preparations in both sexes.

The data-driven characterization of spontaneous LC phasic bursts, presented here, has implications not only for understanding LC neuromodulation of cortical state and behavior but also for human neuroimaging studies, cognitive psychology studies, and computational neuroscience studies which invoke the dual-mode conceptual framework.[17–19] The broadening of LC modes of operation also has importance for understanding and interpreting pupillometry data as a correlate of LC activity,[75,76] which is commonly used to noninvasively index ongoing, spontaneous fluctuations in LC activity in human subjects. The heterogeneity of LC firing patterns also has implications for experiments probing LC neuromodulation of cortical state and behavior through optogenetic or electrical neuro-stimulation manipulation of ongoing LC activity. Such studies have driven LC neurons at a specific timing and pattern[77–80] that do not necessarily reflect the underlying spontaneous firing pattern heterogeneity reported here. Diversity in firing patterns is also important for understanding neuromodulatory functions of the LC throughout the central nervous system because when rodent LC neurons emit spikes at a short ISI, their axons can become depolarized so that subsequent spikes in the burst are conducted faster.[81] Axonal acceleration of spikes may affect the magnitude of NE release[82] and the propensity for LC neurons to release co-transmitters.[24,83,84]

We observed that firing patterns differed based on species, sex, age, and brain state. For instance, we observed firing patterns that were specific to rats and others that were specific to mice. Moreover, brain slice recordings were biased toward the perimeter of the manifold, and the attractor dynamics were pacemaker-like (type 4 and type 5 return plots), which may be explained by the severance of afferent connections and the removal of external sensory input relative to *in vivo* anesthetized and awake recordings. While pacemaker-like activity was most prominent in brain slices, such patterns were still observed in anesthetized and awake animals and are therefore not "unnatural" brain activity patterns. While the diversity in LC firing patterns is shared across different experimental preparations, some preparations are particularly appropriate for studying some areas of the manifold. The differential expression of diverse firing patterns across species and biological factors suggests that the neurotransmitter release properties and functional effects of LC neurons could have nuanced and previously unappreciated differences based on species, sex, age, and brain state.

Lastly, our findings have implications for understanding aging and neurodegenerative disorders. LC firing transitioned through different attractor dynamics from semi-rhythmic firing in the adolescent LC that becomes predominantly irregular in adulthood and finally more rhythmic, faster activity in aged animals. Mean firing rate gradually increased with age in multiple species, both sexes, and across *in vitro* recordings, *in vivo* anesthetized recordings, and awake recordings. This is consistent with human neuroimaging[85] and mouse slice recordings[86] but discrepant with a prior anesthetized rat study, which may be due to its smaller sample size.[87] Age-related changes could be a compensatory adaptation to the NE fiber loss that occurs naturally with age in rodents and humans.[52,53]

LC cell body and fiber loss also occurs in neurodegenerative diseases.[28] We compared multiple neurodegenerative disease-related genetic or pharmacological manipulations and found, among those models, a convergence upon single attractor spiking dynamics. The APP/PS1 and DSP-4 animals were overrepresented in type 1 and type 3 single-attractor return plots and underrepresented in type 2 single-attractor return plots, whereas dyskinetic and parkinsonian rat models had opposite patterns. Our results suggest that single-attractor (rather than multi-attractor) dynamics are a shared feature across neurodegenerative disorder-related LC modifications.

Using the shared contributions of data from multiple laboratories, our analysis uncovered diverse neuronal firing patterns, which can be described as a low-dimensional manifold. The dynamics of these firing patterns are expressed as attractors. Both firing patterns and average firing rates depended on intrinsic biological factors, such as species, sex, and age. Although the LC is an ancestral brain region shared by vertebrates, our data show that its activity has not been tightly conserved. The LC likely integrated into vertebrate nervous systems within the variable context of the evolutionary pressures that sculpted the nervous system of each species. Via its broad central nervous system-wide connectivity, this ancestral region may contribute to sex, species, and age-related differences in sensory processing, cognitive functions, motor control and behavior, as well as cardiovascular, immune, and metabolic function.

Limitations of the study

Although inter-laboratory variability in LC firing rate was low, many of the biological factors in this study were inherent properties of the methodology of a single laboratory. In the future, collaborative efforts should aim for each laboratory in the consortium to collect data from multiple species, a range of ages, both sexes, numerous genotypes, and various recording preparations. Factors, such as duration of recording and zeitgeber timing, should be normalized across laboratories.

Our findings provide the first data-driven definition of LC spontaneous burst firing and, by revealing the diversity of spontaneous activity patterns, provide evidence against a dichotomous tonic-phasic LC firing mode framework. However, whether LC firing patterns are similarly diverse when evoked by a sensory stimulus remains an open question to be investigated.

In neither our analysis of firing rate nor of firing patterns did we control for precise electrode location within the LC, which could also differ across laboratories or between experiments within a laboratory. Location along the LC dorsal-ventral axis may be an important factor given that firing rate, firing pattern, neuronal excitability, and intra-LC connectivity all vary on this axis. Subsequent studies should use laminar multi-electrode probes to map the LC based on electrophysiological landmarks and LC anatomy (per recommended guidelines[1]).

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Nelson K. Totah (nelson.totah@helsinki.fi).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data have been deposited at the Etsin repository (part of Finland's Fairdata services provided by the Ministry of Education and Culture and produced by the Center for Scientific Computing) and are publicly available at https://doi.org/10.23729/fd-71c807fc-fcbe-3639-b562-739415b5b749 as of the date of publication.
- All original code has been deposited at the Etsin repository (part of Finland's Fairdata services provided by the Ministry of Education and Culture and produced by the Center for Scientific Computing) and is publicly available at https://doi.org/10.23729/fd-71c807fc-fcbe-3639-b562-739415b5b749 as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

This study reused data from the following papers.[9,23,59,61,62,70,75,88–95] For this study, the scientific contributions of all authors were limited to providing previously collected electrophysiological data, and the authors were credited with investigation for independently collecting these data. None of the authors participated in joint experiments with any other institution. None of the authors participated in joint data analysis with any other participating laboratory, except for M. Kelberman, E. Rodberg, B. Munn, J.M. Shine, E. Vazey, D. Weinshenker, and N.K. Totah, who jointly analyzed the data.

Conceptualization: NKT
Data curation: MAK, EM, LZ
Formal analysis: MAK, BRM, ER, JMS, LZ
Investigation: VBP, CJBM, DJC, CCC, RFC, OD, DMD, AMD, GD, RD, ZF, EG, JCH, HI, MAK, JRK, CCK, YSK, RJL, YL, MLT, AMM, CAN, YRS, JR, ER, CR, ACS, RS, SDS, HCT, JAT, STS, AU, CRV, AV, HWY, JHY, HJY, LZ, ISZ
Methodology: MAK, ER, NKT, LZ
Project administration: NKT, EV, DW
Resources: EA, EB, DMD, RCF, JIG, ZJ, JPJ, APK, JGM, ZAM, CM, MYM, AEP, SDS, MS, NKT, EV, QW, DW
Software: MAK, ER, NKT, LZ
Supervision: NKT, EV, DW
Validation: MAK, ER, NKT, LZ
Visualization: MAK, ER, NKT
Writing – original draft: MAK, ER, NKT
Writing – Review and Editing: EV, DW, GRP, BDW

DECLARATION OF INTERESTS

Qi Wang and Charles Rodenkirch are the founders of Sharper Sense, a company developing methods of enhancing sensory processing with neural interfaces. Alfred P. Kaye receives or has received research funding from Transcend Therapeutics and Freedom Biosciences and has filed a provisional patent for combination psychedelic pharmacotherapies in PTSD. Anthony E. Pickering reports research funding from Eli Lilly and is a member of the advisory board for Lateral Pharma. All other authors report no disclosures or conflicts of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Data	N/A	https://doi.org/10.23729/fd-71c807fc-fcbe-3639-b562-739415b5b749
Software and algorithms		
MATLAB	Mathworks	N/A
SAS	SAS Institute	N/A
DABEST toolbox	https://github.com/ACCLAB/DABEST-matlab	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

We acquired data on species, sex, strain, state (awake, slice, anesthesia and type), age, pharmacology and events (type and timestamp), and genotype. The genetically modified rodents were modified as follows: APP/PS1 (TgF344-AD) rats (transgene modification), TH-cre rats (BAC transgene modification), DBH-cre mice (BAC transgene modification), VGAT-cre mice (targeted knock-in). Data included male and female rats and mice. Non-human primate data was exclusively male. Rodents were designated as adolescent (<2 months), adult (2–12 months), or aged (>12 months), while all NHP data came from adult animals (10–16 years). The sample size was 2,944 neurons (prior to the quality control sweep, see Table S1). The sample size in number of animals is not available because that information was not provided by the laboratories contributing data.

All animal studies in the United States were performed with approval from the Institution Animal Care and Use Committee at Baylor College of Medicine, Cold Spring Harbor, Columbia University, Emory University, Massachusetts Institute of Technology, New York University, Rowan University, University of North Carolina Chapel Hill, University of Pennsylvania, University of Wisconsin-Madison, Washington University School of Medicine, University, and Yale University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval for animal studies outside of the United States were approved by the Animal Care and Experimentation Committee of the Institute for Research in Fundamental Sciences, the Animal Care and Use Committees of the RIKEN Brain Science Institute, the Institutional Animal Care and Use Committee of National Taiwan University, the Institutional Animal Care and Use Committee of Chung-Shan Medical University, the University of Bristol Animal Welfare and Ethical review body according to the UK Animals (Scientific Procedures) Act 1986 (license PPL3003362), the Committee for Animal Experimentation at the University of Cadiz and University of the Basque Country (UPV/EHU), in compliance with the European Community Guidelines for the Care and Use of Laboratory Animals (2010/63/EU) and Spanish Law for the protection of animals used for research experimentation and other scientific purposes (RD 53/2013), the Committee for Animal Experimentation at the University of Cadiz, The Regierungspräsidium Tuebingen & The Regional State Administrative Agency for Southern Finland according to German Law for the Protection of Animals in experimental research (Tierschutzversuchstierverordnung) & the European Community Guidelines for the Care and Use of Laboratory Animals (EU Directive 2010/63/EU).

METHOD DETAILS

Data collection and organization

Data was sourced from various labs and imported into a custom structure in MATLAB (MathWorks; version R2019a) for analysis. LC recordings were imported as timestamps of each individual spike within a given recording. Firing rates were calculated as the number of spikes divided by the timestamp of the last recorded spike.

Quality control of single unit recordings across labs

A total of 2,944 neurons were submitted across 20 labs. We discarded neurons for which more than 2% of the interspike intervals were less than 10 msec per prior work.[70] This excluded 319 neurons from further analysis. Next, we removed spiking occurring during pharmacological manipulation with the exception of anesthesia. We sought to characterize only temporally stable recordings by removing neurons which did not spike more than 30 times throughout the recording (457 neurons were removed). At the request of the contributors of those data we also removed 199 neurons that were recorded from animals subjected to a stressor. Additionally, we removed 113 neurons in which the sex of the animal was unknown; this was necessary because the negative binomial regression model required each neuron to have information about each factor that could affect firing rate. These exclusions left only one neuron recorded under pentobarbital anesthesia, which was removed from subsequent analyses. After submitting data to this quality control

procedure, a total of 1,855 neurons remained for analysis of firing rates. The number of neurons for each factor are reported in Table S1. Finally, since some recordings were provided with stimulus-evoked spiking following a stimulus-free baseline epoch, we determined for each neuron whether there was significant difference in the mean firing rate between the epoch of the recording without stimulation and the subsequent recording epoch containing sensory stimuli. We reasoned that, since phasic LC responses to stimuli are transient biphasic events lasting ~1000 msec, such transient changes would not lead to an overall change in firing rate averaged over a comparably much larger time window (the duration of this subset of recordings ranged from ~5 s to ~7 h, and the median duration was 444 s). We performed a Student's *t*-test comparing multiple samples of firing rate (split into 10 equal time bins) between the recording epochs with, or without, sensory stimuli. If the introduction of sensory stimuli led to a significant ($p < 0.05$) alteration of time-averaged firing rate compared to the preceding stimulus-free epoch, then the spike train of that neuron was analyzed only until the first stimulus and the remaining data were discarded. Out of 1,855 neurons, 560 were exposed to sensory stimuli; of those 560, we discarded the recording epoch with stimuli for 352 neurons. In addition to analyzing firing rate, we also analyzed firing patterns and, for that analysis, only included spike trains of individual neurons prior to any stimuli. Further, we excluded units that did not have at least 100 interspike intervals (ISIs) prior to any stimulus or pharmacology application but included neurons from animals with an unreported sex to increase sample size (1,708 neurons analyzed).

Factors excluded from the model of firing rates

Animal strain was not included in the model because strain could be completely encompassed by another factor, in this instance species (i.e., Fischer animals are only in rats, C57Bl/6 animals are only mice). The contributing laboratories did not provide substrain information for specific strains within a species (e.g., C57B6J versus C57B6N mice). Therefore, genetic background was not included as a factor. For similar reasons, the interactions between age and species (adolescent and aged neurons were only obtained from rats), age and anesthesia (aged animals were all recorded under chloral hydrate anesthesia while all adolescent animals were recorded in slice), species and genotype (specific genotypes came from the same species, such as APP/PS1 animals all being rats), and genotype and anesthesia (neurons from specific genotypes were recorded under the same anesthetic) could not be estimated.

QUANTIFICATION AND STATISTICAL ANALYSIS

Description of statistical analyses, software, and location of the statistical details for each analysis

The statistical analysis of firing rate (Figures 1 and 2; Tables S1, S3, and Table S4) were performed using SAS software. The SAS PROC GENMOD algorithm was used to estimate the independent effects of individual factors and their interactions on firing rates (see section on "statistical analysis of firing rates" for method details). The number of samples (in neurons), N, is stated in Table S1. In Figures 1 and 2, each point is a neuron. The box shows the median (line) and the 95% confidence intervals (box). The effect sizes for differences in firing rate (Figures S2 and S3) were calculated using the DABEST toolbox running in MATLAB. The error bar around the effect size is the 95% confidence interval. The N (number of neurons) used for the effect size plots in Figures S2 and S3 are stated in the figure legends.

The statistical analysis comparing ISI histograms (Figure 4) used a two-sample Kolmogorov-Smirnov test to compare shapes of the ISI distributions. This was performed in MATLAB. The N for each group (i.e., number of neurons) is stated in the legend of Figure 4.

The statistical analysis comparing the proportion of neurons from each return map type (Figure 5) was a chi-squared test with Bonferroni correction for multiple comparisons performed in MATLAB. The N (in number of neurons) is stated in the legend of Figure 5. There are no error bars since proportions were compared.

For all the above statistical analyses, the data met the assumptions of the statistical approach.

Statistical analysis of firing rates

Descriptive statistics were used to describe the characteristics of the sample of firing rates. Given the multitude of variables present in our analyses, we fit a regression to explain the individual and combined effects of factors on firing rates. Initial examination of the data revealed overdispersion. We therefore chose a negative binomial regression model, which is commonly used to model overdispersed data. Negative binomial regression is a generalization of a Poisson regression with less restrictive assumptions of equal mean and variance which was also present in our data. Given that we wanted to leverage cross-lab comparisons, we removed lab as a random effect and fit a marginal model instead. This was further justified by the large size of our dataset and model fit statistics (ratio of the deviance to degrees of freedom and dispersion parameter). A generalized estimating equation algorithm was then used to estimate the independent effects of individual factors and their interactions on firing rates (SAS PROC GENMOD).

Generalized estimating equations (GEE) are one popular algorithm for fitting marginal models when outcome is a discrete variable. Using GEE with offset of measurement time, the independent effect of age, sex, species, genotype and anesthesia/state as well as their effect modification on firing rate were assessed. If a significant effect modification (interaction) was present, estimated firing rate was compared at a different level of the components of the interaction. If the interaction was not significant, estimated firing rate was reported with adjustment of other features. GEE was also applied to the comparison of firing rate between single or various combination of STAR Methods which were used to verify LC neurons. To correct for multiple comparisons, a false-discovery rate of < 20% was applied and Benjamini-Hochberg procedure was used. All analyses were performed in SAS 9.4 (SAS Institute Inc).

All tests performed were two-tailed. Effect size plots showing the difference between the estimated means ± 95% confidence interval of the estimate was generated using the DABEST toolbox in MATLAB.

Principal components analysis of inter-spike interval (ISI) distributions

ISIs were calculated by subtracting the time of each spike from the following spike time ($spike_{x+1}$-$spike_x$). The calculation was performed from the first spike recorded until the last spike of the recording prior to a pharmacological manipulation or external event (e.g., a sensory stimulus), if either was present. ISI histograms (calculated in 100 logarithmically-spaced bins from 1 msec to 100 s) were analyzed using PCA. A smooth surface (i.e., manifold) was fit to the first three PCs using the MATLAB (v2025a) function, loess. The root-mean-square deviation was smaller for loess compared to the lowess function.

Return maps

Second-order ISI patterns were visualized using return maps. These density plots show the ISI of a single neuron on the x axis plotted against the following ISI of that same neuron on the y axis. A two-dimensional histogram was created for each unit (ISI_n x ISIn+1) binned in 40 bins on each axis centered around the maximum value in the two-dimensional histogram (±5) plotted on a logarithmic scale for better visualization. The bin with maximum density was centered in the plot to visualize the shape of the return map. We manually clustered the patterns into 8 types. We compared the proportion of a given factor within each return map type to the overall population of neurons in the dataset. Differences in the frequency of a given factor in each return map type were compared to the frequency of the factors in the total population of neurons. The proportion of neurons was compared using the chi-squared test with Bonferroni correction for multiple comparisons.