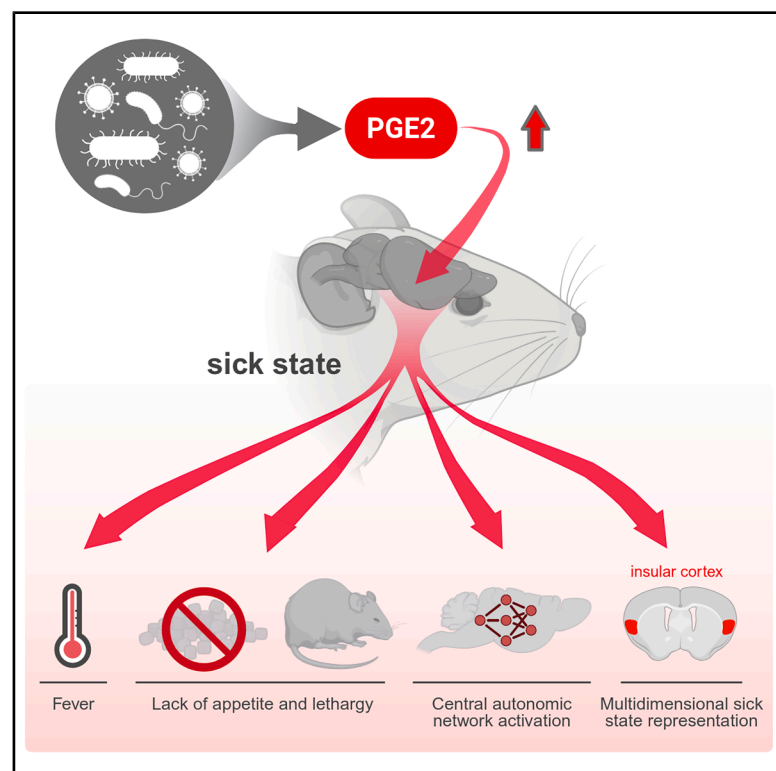


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Central infusion of prostaglandin E2 reveals a unified representation of sickness in the mouse insular cortex

Graphical abstract



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In brief

Kamm et al. demonstrate that central prostaglandin E2 administration serves as a robust mouse model for sickness, showing it shares many characteristics of an internal state. Their findings reveal that the insular cortex preferentially encodes sickness as a unified, multidimensional representation, expanding the framework for studying immune-brain interactions.

Highlights

- Central PGE2 induces a comprehensive sickness response in mice
- Central PGE2 broadly activates the central autonomic network
- Primary somatosensory and insular cortices encode multiple sickness-related traits
- The insular cortex preferentially decodes a multidimensional sick state



Article

Central infusion of prostaglandin E2 reveals a unified representation of sickness in the mouse insular cortex

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SUMMARY

During infections, vertebrates develop stereotypic symptoms such as elevated body temperature, reduced appetite, and lethargy. These changes, collectively known as sickness syndrome, are orchestrated by the brain in response to immune mediators released during systemic inflammation. While the roles of subcortical regions, including the hypothalamus and brainstem, in regulating sickness symptoms are well established, the contribution of the neocortex to the encoding and modulation of sickness remains less well understood. We examined the neuronal correlates of sickness in the neocortex of awake mice following a single intracerebroventricular (i.c.v.) injection of prostaglandin E2 (PGE2), a well-characterized sickness mediator. Behavioral analysis revealed that PGE2 elicits a rapid and robust sickness response, characterized by fever, slower locomotion, quiescence, anorexia, and eye squinting. Whole-brain Fos mapping showed that PGE2 generates a distinct neural activation pattern encompassing much of the interoceptive network. Electrophysiological recordings in awake mice revealed that neuronal population dynamics in the antero-mid insular cortex (IC) and the primary somatosensory cortex (SSp), two regions involved in body-state representation, encode sickness-related information, such as body temperature, walking velocity, grooming, and eye squinting. However, unlike SSp, ongoing neuronal activity in IC exhibited better decoding performance for a multidimensional measure of sickness than for individual sickness traits. Together, these results suggest that PGE2 induces a coordinated physiological and behavioral response akin to a sick state, which is preferentially encoded in the IC, providing a conceptual framework for studying interactions between the immune system and the brain.

INTRODUCTION

Infections often induce profound changes in both behavior and physiology in vertebrates. These manifest both as behavioral symptoms, including decreased locomotion and reduced social and feeding behaviors, and as autonomic and endocrine adaptations, like elevated body temperature and corticosterone/cortisol release.^{1–6} The brain coordinates this, collectively known as sickness syndrome, to prepare the organism to fight infections, thereby improving survival chances.^{7–9}

Sickness responses begin when the immune system detects pathogens and releases inflammatory mediators.⁹ Studies in

humans^{10,11} and mice^{1–3,12–14} show that multiple brain regions respond to peripheral inflammation, including interoceptive areas, such as the IC and primary somatosensory cortex (SSp).¹⁵ Furthermore, in humans, sickness is perceived as a discrete mental state with negative valence, distinct from other affective states.¹⁶ Consequently, we hypothesize that peripheral inflammation induces a distinct internal state,^{10,17,18} which we term a “sick state.”

While neuronal correlates of physiological states like hunger or thirst are extensively studied,^{17–20} how the brain encodes the sick state remains poorly understood. One limitation to further progress is that the standard method for triggering sickness, a



single injection of bacterial lipopolysaccharide (LPS),²¹ produces a slow-developing response lasting several hours.^{1–3} The lengthy, polyphasic nature of LPS-triggered sickness²² makes it challenging to implement during neurophysiological recordings in non-anesthetized animals.

An alternative approach relies on intracerebroventricular (i.c.v.) injections of fast-acting immune mediators. Among the best-studied ones is prostaglandin E2 (PGE2),²³ a short-lived lipid derived from arachidonic acid.^{24,25} During immune challenges, brain PGE2 production increases steeply, and PGE2 concentration in cerebrospinal fluid correlates closely with sickness progression.^{26–28} Neurons can detect PGE2 directly through four specific G protein-coupled receptors²⁹ expressed across multiple brain areas.^{30–33} Brain PGE2 can trigger many sickness-associated symptoms such as fever,³³ increased pain sensitivity,^{34,35} warmth-seeking,³⁶ and anorexia.^{37,38}

Combining behavioral analysis, whole-brain activity mapping, and electrophysiology in mice, we found that a single i.c.v. PGE2 dose induced a suite of features characteristic of internal state.³⁹ First, PGE2 triggered pleiotropic changes, including fever, slower locomotion, quiescence, anorexia, and eye squinting. Second, it activated diverse interoceptive brain regions. Third, antero-medial IC neuronal population activity represented sickness significantly better than SSp activity, suggesting a diversification of body-state representation across the interoceptive neuraxis. Together, our findings establish central PGE2 administration as a robust model for investigating sickness in awake mice, providing a conceptual framework for understanding conditions in which maladaptive immune-brain interactions may drive chronic, sickness-like states, including depression and post-acute infection syndromes.^{40,41}

RESULTS

A central injection of PGE2 generates a robust sickness response in mice

We benchmarked brain injections of PGE2 to induce a short-lived sickness response in mice, using the standard intraperitoneal (i.p.) LPS model for qualitative comparison (Figures 1A and S1A). This profiling included diverse body temperature plots: temporal averages (Figures 1B and S1B), population histograms quantifying distribution across conditions (Figures 1C and S1E), and maximum temperatures capturing physiological strain near the 42°C survival threshold⁴² (Figures 1D and S1C). Finally, raw individual traces highlight inter-individual variability and asynchronous febrile responses (Figures 1E and S1D).

In rodents, mild LPS doses (0.001–0.1 mg kg^{−1}) cause low-to-moderate fever, whereas high doses (> 1 mg kg^{−1}) induce severe hypothermia mimicking septic shock,^{43,44} with a median lethal dose of ~25 mg kg^{−1}.^{45,46} Thus, we selected a mild dose (0.02 mg kg^{−1} LPS i.p. in saline) that reliably induces fever in mice.⁴⁷ LPS generated a moderate fever response developing over 4 h and rarely exceeding 39°C (Figures S1B–S1E). Given this prolonged effect, we evaluated behaviors over this 4-h window.

We found that LPS-injected mice moved significantly less and walked more slowly than saline-injected controls (Figures S1F and S1G). They also exhibited an increase in stasis, defined as locomotion-free intervals exceeding 10 s (Figures S1H and

S1I). Additionally, total time interacting with food, a proxy for food consumption or interest, was reduced (Figures S1J and S1K). These effects on body temperature and behavior were consistent with previous studies^{1,2,33} and validate our automated approach for tracking sickness-related behaviors in home-caged mice, even during partial occlusions such as eating under the food tray (Figures S1L and S1M).

Fever is the most studied effect of central PGE2.⁸ Unlike peripheral LPS, large i.c.v. PGE2 doses near 5 nmol produce a thermal ceiling effect, and no clear lethal dose is established in rodents, as doses exceeding 100 nmol do not cause death.^{48–50} We selected a high dose (4 nmol in vehicle, 1:1 saline:DMSO, i.c.v.), known to elicit a prominent febrile response in mice.^{33,50–52}

PGE2 i.c.v. produced a pronounced, transient febrile response exceeding 39°C that returned to control levels (vehicle-injected mice) within 90 min (Figures 1B–1E). Consequently, we used 90 min as the cutoff point for evaluating the behavioral effects (Figure S2A). PGE2-injected mice exhibited significantly increased stasis and reduced walking speeds, though total displacement remained unaffected (Figures 1F and 1H–1J). Furthermore, PGE2 significantly reduced food interacting time (Figures 1G and 1K).

Although LPS i.p. and PGE2 i.c.v. differ markedly in their modes of action and temporal dynamics, precluding direct potency comparisons, both treatments induced qualitatively similar physiological and behavioral changes. These included altered body temperature, slower locomotion, lethargy, and decreased food interaction. All represent well-characterized symptoms of natural infections commonly observed across established models of sickness and sepsis.^{4,5,53,54}

Fever does not constrain sickness behaviors

Brain temperature can modulate neuronal activity and serve as a reliable predictor of brain states.^{55–58} Given the strong effect of PGE2 i.c.v. on both brain⁵⁹ and body temperature (Figures 1B–1E), we speculated that temperature itself may act as a primary cue for the initiation or maintenance of sickness. If so, sickness-associated behaviors should be constrained by the febrile response and persist as long as body temperature remains elevated.

To test this, we aligned all behavioral responses to the peak of the PGE2-triggered fever (Figure 1L), which was necessary because thermal responses were asynchronous (Figure 1E). Interestingly, all behaviors examined here, namely stasis, food interaction, and walking speed, recovered well before mice reached the peak of their febrile response (Figures 1M–1O). This uncoupling suggests that fever alone does not drive them. Additionally, behavioral changes were confined to the rising phase of the febrile response, indicating this specific phase provided a useful temporal boundary for the sickness state.

Sex minimally influences PGE2- and LPS-induced sickness responses in mice

Infection responses often differ by sex. Females typically mount stronger immune responses but exhibit milder physical and behavioral sickness symptoms than males.^{60–63} Because our study utilized mixed-sex animal cohorts, we evaluated potential

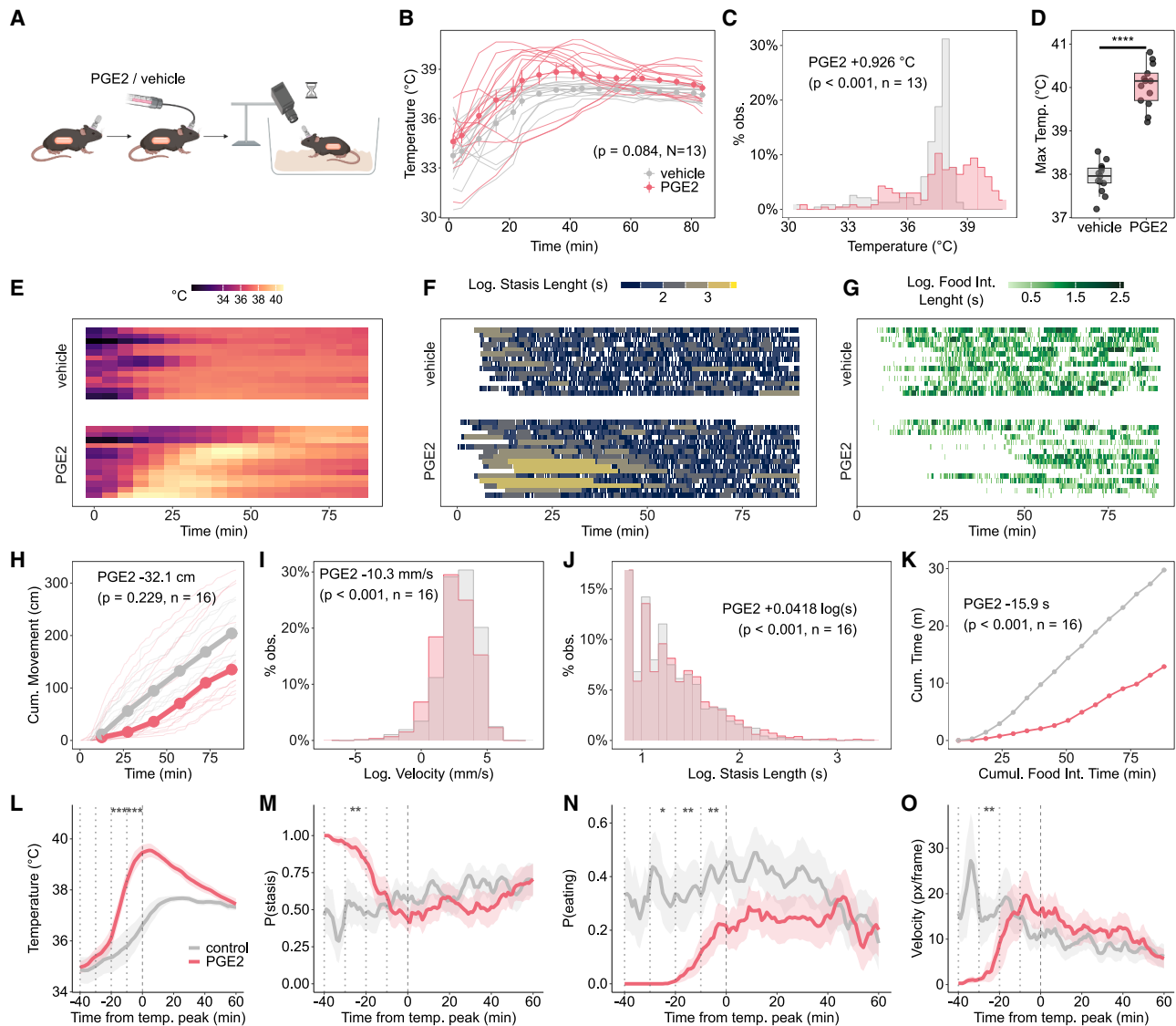


Figure 1. A central injection of PGE2 generates a robust sickness response in mice

(A) Scheme of the experimental approach. Mice were food-deprived for 24 h before injections to promote food consumption. They then received sequentially (in different weeks) an i.c.v. injection of vehicle and PGE2.

(B) Mean body temperature (bold line) and individual temperature profiles (thin lines) over 90 min for mice injected with PGE2 and vehicle.

(C) Histogram of body temperature distributions for PGE2 and vehicle measurements over 90 min, with LME treatment effect annotated (coefficient in $^{\circ}\text{C}$).

(D) Maximum body temperature comparison between PGE2-treated and vehicle-treated mice (paired t test).

(E) Temperature heatmap of individual mice under PGE2 and vehicle conditions over 90 min, ordered by temperature-response magnitude.

(F and G) Stasis (F) and food interaction (G) episodes for PGE2-treated and vehicle control mice over 90 min, where each horizontal segment represents an episode with color indicating the logarithm of episode duration.

(H) Cumulative displacement over 90 min for individual PGE2-treated and vehicle-treated mice (thin lines), with group medians overlaid (LME endpoint comparison, coefficient in cm).

(I) Velocity distribution histogram (log-transformed) for PGE2 and vehicle groups over 90 min (LME, coefficient in mm/s).

(J) Distribution of stasis episode durations (log-transformed) for PGE2 and vehicle groups over 90 min (LME, coefficient in log[s]).

(K) Mean cumulative time spent in food interaction over 90 min for PGE2-treated and vehicle-treated mice (LME endpoint comparison, coefficient in s).

(L) Mean body temperature ($\pm$ SEM) aligned to the onset of peak temperature, comparing PGE2 injection day with paired vehicle control day in the same animals.

(M) Fraction of animals in stasis over time ($\pm$ SEM), aligned to peak temperature onset.

(N) Fraction of animals engaged in food interaction over time ($\pm$ SEM), aligned to peak temperature onset.

(O) Mean velocity over time ($\pm$ SEM), aligned to peak temperature onset.

For (L)–(O), paired Wilcoxon's signed-rank tests. See Table S1 for further details on statistics. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Scheme in (A) created with BioRender.com.

See also Figures S1 and S2 and Table S1.

sex differences in response to i.c.v. PGE2 or i.p. LPS using linear mixed-effects models (LMEs).

We found that sex did not influence the effect of central PGE2 on fever, total movement, stasis, or food interaction (Figures S2B–S2D and S2G). The sole exception was walking velocity, which PGE2 reduced more in females than in males (Figures S2E and S2F). Similarly, sex did not influence LPS-induced fever in time-binned data, total walking, stasis, or food interaction (Figures S2H, S2J, S2L, and S2M). However, peripheral LPS had a stronger effect on pooled body temperature in males and on walking velocity in females (Figures S2I and S2K). Finally, consistent with previous reports,^{64,65} females exhibited higher basal body temperatures in both cohorts during the light and dark phases (Figure S2N).

Overall, our data suggest that the sickness responses studied here did not differ fundamentally by sex, except for walking velocity, which was consistently more affected in females. These findings support the inclusion of both sexes of mice when studying sickness.

PGE2 activates the CAN

The activity of small neuronal populations can induce changes in internal states. However, following this initial activation, multiple brain regions are subsequently engaged, sustaining the pleiotropic effects typically associated with internal states.^{17,39,66}

Interestingly, sickness models in both humans and rodents activate many brain regions, particularly within the central autonomic network (CAN).^{1–3,10,13} This network comprises regions that translate visceral sensory input into conscious interoceptive perception and regulate vital functions, including the insular cortex (IC), amygdala, hypothalamus, periaqueductal gray matter (PAG), parabrachial nucleus (PB), nucleus of the tractus solitarius (NTS), and ventrolateral medulla.^{14,67–69} Therefore, if PGE2 induces a specific sick state, it should engage these critical CAN regions extensively.

To test this hypothesis, we quantified the expression of the early neuronal activation marker Fos⁷⁰ at 1.5 h after delivery of PGE2 (4 nmol in vehicle, 1:1 saline: DMSO, i.c.v.) using iDISCO+^{71,72} (Figures 2A and S3A). We selected this time point based on the rapid onset of response to PGE2 i.c.v. (Figure 1) and the Fos protein expression time course, which typically peaks within 1.5 h post-stimulation.⁷³ Resulting whole-mount Fos expression patterns were registered to the CCFv3 Allen Mouse Brain Atlas, and Fos densities were compared with vehicle-injected controls (Video S1; Table S2).

Our Fos iDISCO+ map showed that parts of the IC (particularly the left medial IC [mIC]) had significant activation after PGE2 injection (Figures 2B and S3B–S3D). We also detected a significant reduction of Fos signal in the thalamus, including the ventral medial nucleus (VM), the mediodorsal nucleus (MD), and the magnocellular part of the subparafascicular nucleus (SPFm) (Figures 2C, S3E, and S3F). Notably, PGE2 prominently activated several hypothalamic nuclei that regulate body temperature, stress responses, and energy homeostasis,^{8,33,74,75} including PGE2 receptor-enriched regions,^{33,76} such as nuclei within the preoptic area (POA), the paraventricular (PVH), and the arcuate nucleus (ARH) of the hypothalamus (Figures 2D and S3G–S3I).

In the midbrain, we observed a significant increase in Fos+ cell density in the lateral PAG (Figures 2E and S3J). Additionally,

regions involved in processing ascending spinal and visceral information^{77,78} and that express ER receptors or bind PGE2,^{32,79} such as the PB, NTS, and neighboring areas, showed likewise activation (Figures 2F and S3K–S3M).

Overall, we found that PGE2 i.c.v. activated multiple brain regions, including numerous subnuclei across the CAN, further supporting the hypothesis that sickness reflects a specific internal state.

Convergent brain activation patterns across peripheral and central sickness models

Multiple independent studies evaluated Fos gene expression throughout the mouse brain following i.p. administration of LPS at pyrogenic-to-hypothermic doses.^{1–3,13,14} Despite methodological and dosage differences, these studies consistently demonstrate a robust pattern of brain activation^{67,68,80} (Figure S3N).

Systemic LPS-induced inflammation reaches the brain via peripheral pathways and bloodstream-released immune mediators, subsequently stimulating central PGE2 production.^{9,12,27} Conversely, PGE2 i.c.v. bypasses peripheral mechanisms, acting directly on central EP receptors with minimal spillover outside the blood-brain barrier.^{8,81} Given these distinct pathways, we further characterized the i.c.v. model by assessing the extent to which centrally infused PGE2 recapitulates the map of neuronal activation induced by peripheral LPS.

We compared our PGE2 dataset with a whole-brain Fos map from Ilanges et al. following LPS administration (3 h after 0.5 mg kg^{−1} i.p.).¹ Both studies used similar tissue-clearing technologies and induced comparable sickness behaviors. However, the Ilanges model generated transient fever followed by mild hypothermia (35°C). Crucially, pyrogenic (0.1 mg kg^{−1}) to hypothermic (1 mg kg^{−1}) systemic LPS doses significantly elevate central PGE2 levels in mice.^{82,83} This means that their observed thermal phenotypes were likely accompanied by elevated central PGE2 levels. Therefore, comparing these datasets provides an ideal opportunity to cleanly disentangle central PGE2's specific contribution to brain activation from additional humoral and peripheral effectors present during systemic inflammation.

We observed that more than 70% of the regions activated by PGE2 i.c.v. were also significantly activated by LPS i.p., with 90 regions shared between the two conditions and 34 regions uniquely activated by PGE2 (Figure 2G; Table S3).

Across shared areas, we identified multiple CAN regions. In the neocortex, these included interoceptive cortices,⁶⁸ such as parts of the agranular insular (AI) and gustatory (GU) cortices. Subcortical structures involved in motivation, salience, and behavioral control,⁸⁴ including the CEA and the bed nuclei of the stria terminalis (BST), were also activated by both models. In the hypothalamus, overlapping activation was observed in key regions involved in thermoregulation, stress response, and energy homeostasis,⁸ including the median, medial, and lateral POAs (MEPO, MPO, and LPO), as well as PVH and ARH. Additionally, areas involved in ascending and descending relay of visceral, humoral, and motor information,⁶⁷ including the area postrema (AP), PB, dorsal motor nucleus of the vagus (DMX), raphe pallidus (RPA), and aspects of the medullary reticular formation, were activated by both sickness models (Figure 2G; Table S3).

In addition, we found that both PGE2 i.c.v. and LPS i.p. reduced Fos expression across diverse thalamic nuclei. These

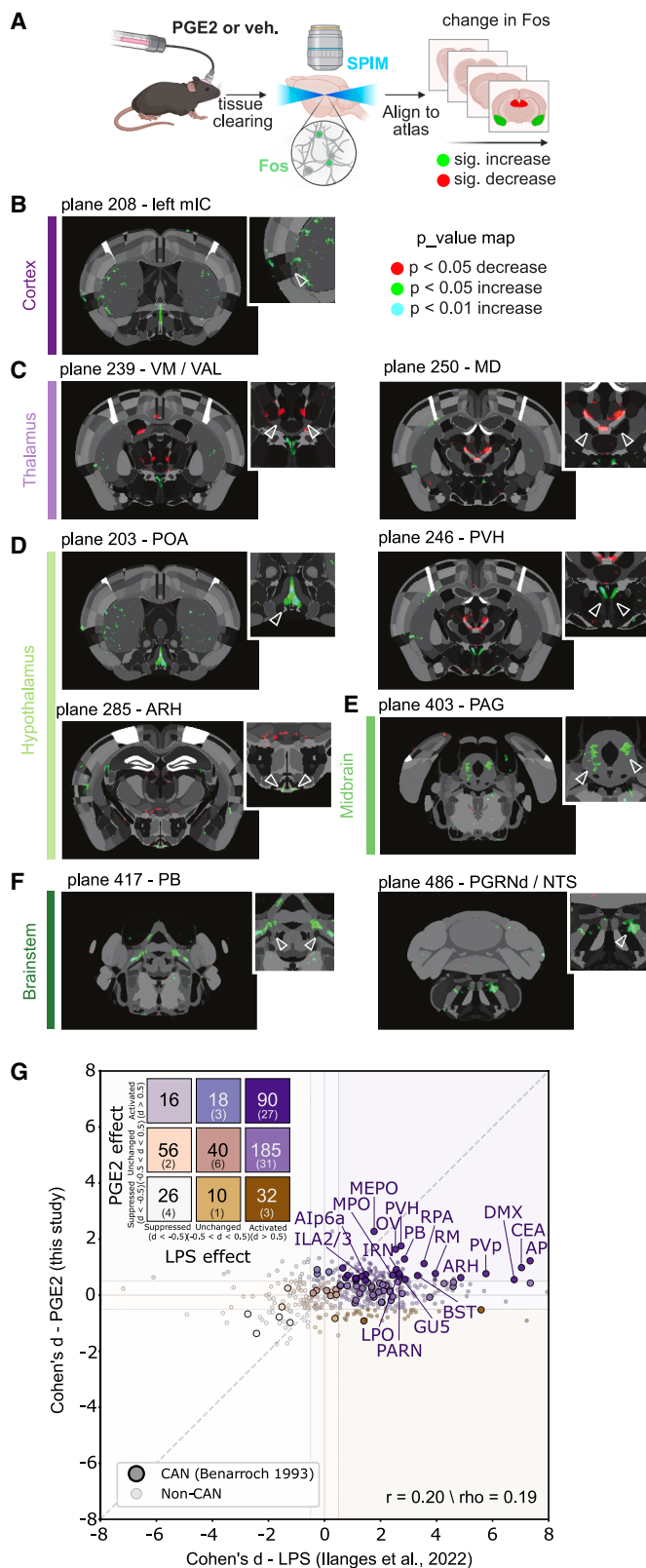


Figure 2. PGE2 activates the CAN

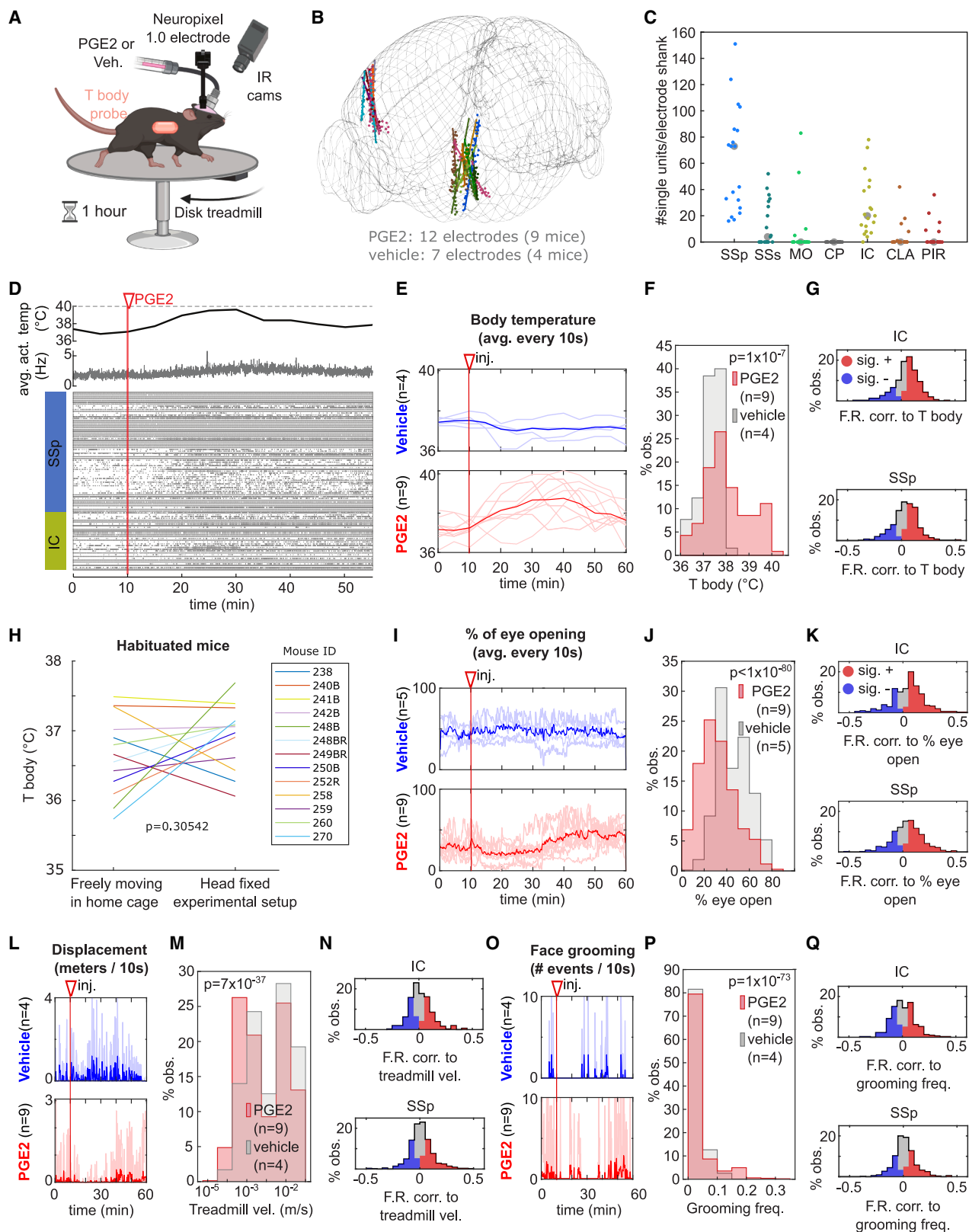
(A) Schematic representation of the experimental approach.

(B–F) p value prior to false discovery rate (FDR) correction map of the difference between Fos staining signal density from vehicle-injected brains versus PGE2-injected brains across selected brain planes from the Allen Brain Atlas.

(G) Bivariate comparison of LPS and PGE2 Fos activation across brain regions. Scatterplot of Cohen's d effect sizes for LPS (x axis¹) versus PGE2 (y axis; this study). Large points denote regions belonging to the central autonomic network (CAN; Benarroch⁶⁹); small points denote non-CAN regions. Dashed lines indicate the $d = \pm 0.5$ classification thresholds. Pearson's r and Spearman's ρ correlation coefficients are shown. The top-left panel displays the 3×3 color legend with region counts per category (CAN region counts in parentheses).

Abbreviations: see Figure S3. Scheme in (A) was created with BioRender.com.

See also Figure S3, Tables S2 and S3, and Video S1.



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regions include key relay stations for ascending visceral sensory inputs to the IC^{85,86} (Table S3). Moreover, LPS i.p. produced broader activation than PGE2 i.c.v., activating 307 nuclei compared with 124 (Figure 2G; Table S3). This pattern was particularly evident in the neocortex, where LPS elicited extensive activation spanning most cortical layers, whereas PGE2 preferentially targeted deeper layers (Figure S3O; Table S3).

Overall, both PGE2 i.c.v. and LPS i.p. activated most critical CAN nodes, with LPS engaging a wider range of neocortical layers than PGE2. These findings support the hypothesis that sickness triggered peripherally or centrally activates similar core neuronal networks, suggesting a shared perceived/predicted internal state across sickness models.

Single-unit analysis of the neuronal correlates of sickness in head-fixed mice

Based on current models of interoceptive state representation, sickness can be understood as a predicted internal state that the brain infers from immune signals and evolutionary programs. Mechanistically, this framework proposes that visceromotor cortices, such as the anterior IC, generate highly integrated interoceptive predictions and regulate emotional salience. Conversely, exteroceptive cortices, including the SSp, receive these interoceptive predictions but primarily maintain representations of incoming somatic inputs.^{67,87–90} Consequently, we hypothesize that the IC encodes sickness as a highly multivariable state, whereas the SSp preferentially tracks individual somatosensory signals associated with it.

To explore this, we obtained high-density neuronal recordings targeting primarily the antero-mid subdivisions⁹¹ of the IC and the SSp using Neuropixel electrodes⁹² (Figures 3A–3D; STAR Methods). We included both agranular and GU areas in our insular recordings (Table S4), as these regions can represent current and predicted physiological states in mice.⁶⁶

We first used the body temperature fluctuations triggered by PGE2 as a proxy for the sick state. PGE2 i.c.v. triggered a rapid fever response in head-fixed mice with temperatures reaching 40°C (Figures 3E and 3F). Our single-unit analysis revealed that neither PGE2 nor vehicle infusion produced overall changes in firing rates across the neuronal populations in IC

or SSp (Figures 3D and S4A–S4F). However, we noted a tendency for a slight increase in the proportion of units that decreased their average firing frequency (FF) compared with vehicle infusion (Figures S4C and S4D), which was present across left and right IC and SSp as well (Figures S4E–S4H). Nevertheless, the mean change in population firing rate was very close to 0 Hz and did not change significantly (Figure S4B). Additionally, we observed that IC and SSp had comparable proportions of neurons showing significantly correlated activity (positively and negatively) with body temperature (Figure 3G; Table S1). Importantly, head-fixed, habituated mice exhibited baseline body temperatures comparable to their home-cage conditions at the start of Neuropixels recordings (Figure 3H). Altogether, this indicates that changes in brain activity following i.c.v. injections were driven neither by head-fixation stress nor by the positive Q10 temperature sensitivity of synaptic transmission.⁹³

Next, we examined sickness behaviors. PGE2-injected mice displayed eye squinting (Figures 3I and 3J), a facial expression linked to physical pain,^{85,86} significantly slower walking (Figures 3L and 3M), and increased facial grooming (Figures 3O and 3P). Both the IC and SSp contained comparable proportions of neurons whose activity correlated positively and negatively with these three behaviors (Figures 3K, 3N, and 3Q; Table S1).

Altogether, PGE2 i.c.v. induced a comprehensive sickness response in head-fixed mice, mirroring freely moving conditions. Neuropixels recordings, however, revealed heterogeneous correlations with temperature and behavior in both the IC and SSp, without substantial single-cell differences between these regions.

A sickness-associated state across neocortical regions

There is a growing consensus that low-dimensional patterns of population neuronal activity may play a crucial role in complex brain functions, such as encoding sensory stimuli, motor tasks, and internal states.^{94–98} To test whether sickness-related variables could be identified in low-dimensional representations of our neuronal recordings, we used CEBRA.⁹⁹ Using the multi-session CEBRA-behavior algorithm, we mapped neuronal

Figure 3. Single-unit analysis of the neuronal correlates of sickness in head-fixed mice

- (A) Schematic representation of the experimental design.
- (B) Reconstruction of the electrode tracks from recorded mice aligned to the brain atlas.
- (C) Number of single units recorded per brain area. Each point represents data from one probe insertion. SSp, principal somatosensory cortex; SSs, secondary somatosensory cortex; MO, primary motor cortex; CP, caudate putamen; IC, insular cortex; CLA, claustrum; PIR, piriform cortex.
- (D) Representative dataset. Top: body temperature trace. Middle: average population firing rate (F.R.). Bottom: Raster plot of simultaneously recorded single units across SSp (blue) and IC (yellow) with one probe. Red line: time of injection of PGE2.
- (E) Body temperature recorded across the experiment for vehicle (top) and PGE2-injected mice (bottom). Light colors: individual mice; solid colors: average across mice. Vertical line: injection time.
- (F) Histograms of pooled body temperatures recorded in response to the injections of PGE2 (9; red) and vehicle (4; gray). Two-sample Kolmogorov-Smirnov test.
- (G) Histograms of the correlation coefficients between recorded single units' F.R. dynamics and body temperature across the experiment for IC (top) and SSp (bottom) from PGE2-injected mice. Red and blue areas represent significantly positive and negative correlations, respectively (Pearson's correlation coefficient $p < 0.05$).
- (H) Pairwise comparison of average core body temperature in head-fixed mice (30 min pre-i.c.v. infusion) versus their home-cage baseline (48 h pre-recording, post-habitation). Statistics: Wilcoxon's test.
- (I–K) Same as (E)–(G), but for % of eye opening.
- (L–N) Same as (E)–(G), but for treadmill velocity.
- (O–Q) Same as (E)–(G), but for grooming frequency.

Scheme in (A) created with BioRender.com. See Table S1 for further details on statistics.

See also Figure S4 and Tables S1 and S4.

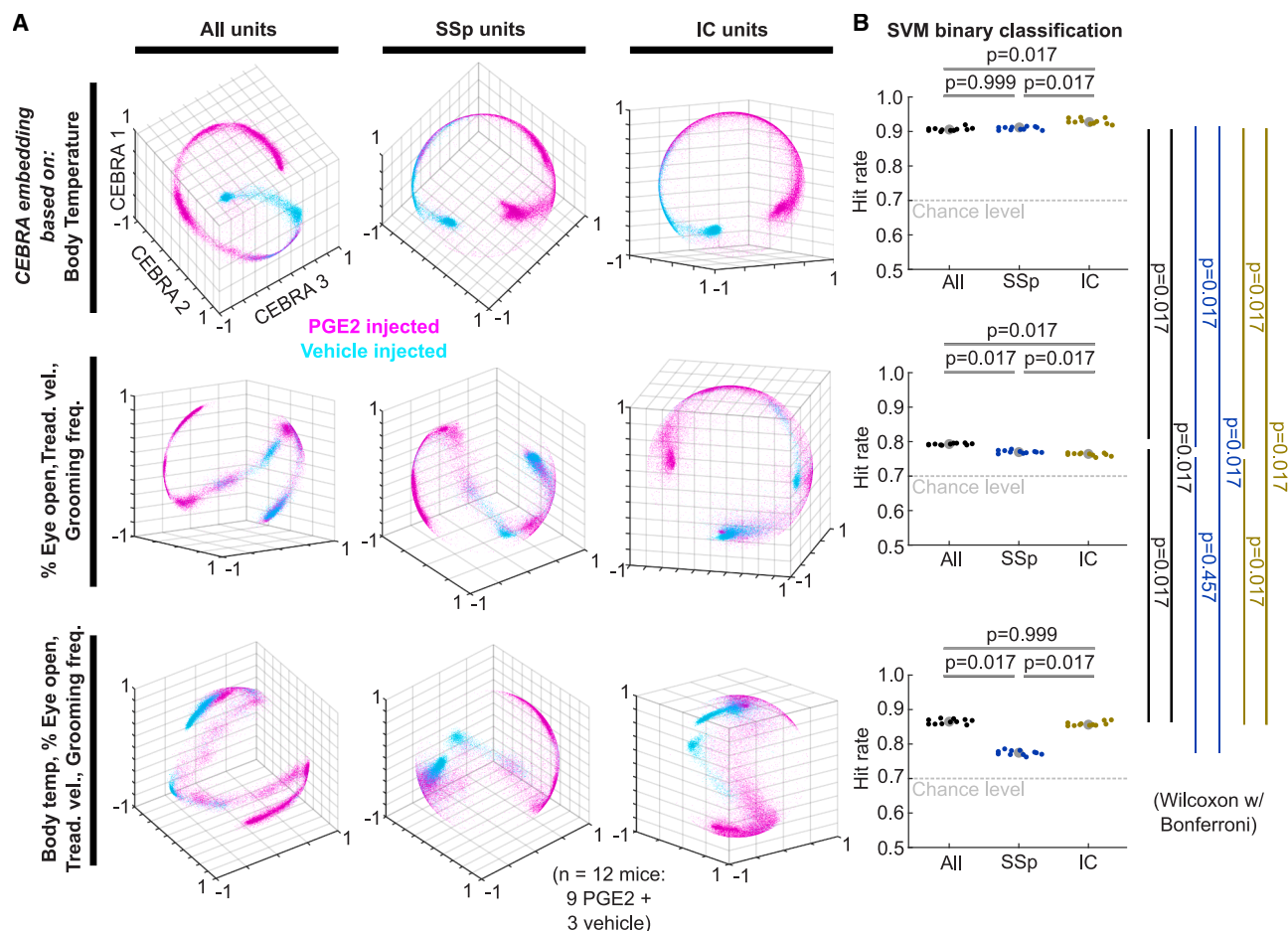


Figure 4. A sickness-associated state across neocortical regions

(A) 3D embeddings of the F.R.s recorded in 1 s time bins (plotted points) across 48 min post-injection of either vehicle (cyan, $n = 3$ mice, 5 probes) or PGE2 (magenta, $n = 9$ mice, 12 probes) obtained through multi-session CEBRA-behavior supervised dimensionality reduction (STAR Methods). Embeddings for data from all units simultaneously recorded by each probe across brain regions (left), or specifically from SSp (center) or IC (right), are plotted. Multi-session CEBRA-behavior embeddings were obtained using either body temperature (top), the three behavioral readouts studied (middle), or all 4 readouts together (bottom) as accessory variables.

(B) Hit rates for predicting the correct treatment labels (vehicle or PGE2) obtained through cross-validated binary classification of the corresponding embedded points in (A) using a support vector machine (SVM) classifier. The chance-level hit rate (Chl) obtained by scrambling the treatment labels is plotted as gray dotted lines. Statistics: Wilcoxon's signed-rank tests with Bonferroni correction.

See also Figure S5 and Table S1.

activity from all recorded regions (All), the SSp, or the IC to CEBRA embeddings generated based on body temperature, sickness behaviors (eye-opening, walking speed, and grooming), or a combination of all factors. We then trained a support vector machine (SVM) classifier to determine whether the embedded neuronal activity could distinguish PGE2 from vehicle conditions.

3D CEBRA generated well-defined embeddings across all three categories, both in the full dataset and in subsets containing only units from the IC and SSp (Figures 4A, S5A, and S5B). The SVM classifiers decoded the injection type (PGE2 or vehicle) significantly above chance (scrambled labels on embedded data) for all comparisons, with fractions of correctly classified time bins (hit rate) ranging from 0.76 to 0.92 (Figure 4B; Table S1). Interestingly, decoders trained on IC data outperformed those trained on SSp data, particularly when body

temperature or all factors were used as auxiliary variables (Figure 4B). These results suggest that central PGE2 induced discernible changes in neuronal population dynamics across all recorded regions, a characteristic associated with bodily states.¹⁰⁰

Multiple studies have reported lateralization of engaged neocortical regions, including the IC, during systemic inflammation.^{10,101–103} Interestingly, decoders trained on left-hemisphere data outperformed those trained on right-hemisphere data when using body temperature as an auxiliary variable. A similar pattern was observed in IC when body temperature was combined with sickness behaviors. Conversely, sickness behaviors alone were better decoded from embeddings obtained from the right hemisphere in both SSp and IC (Figures S5C and S5D). These results suggest a complex pattern of sickness-representation lateralization in the IC and SSp.

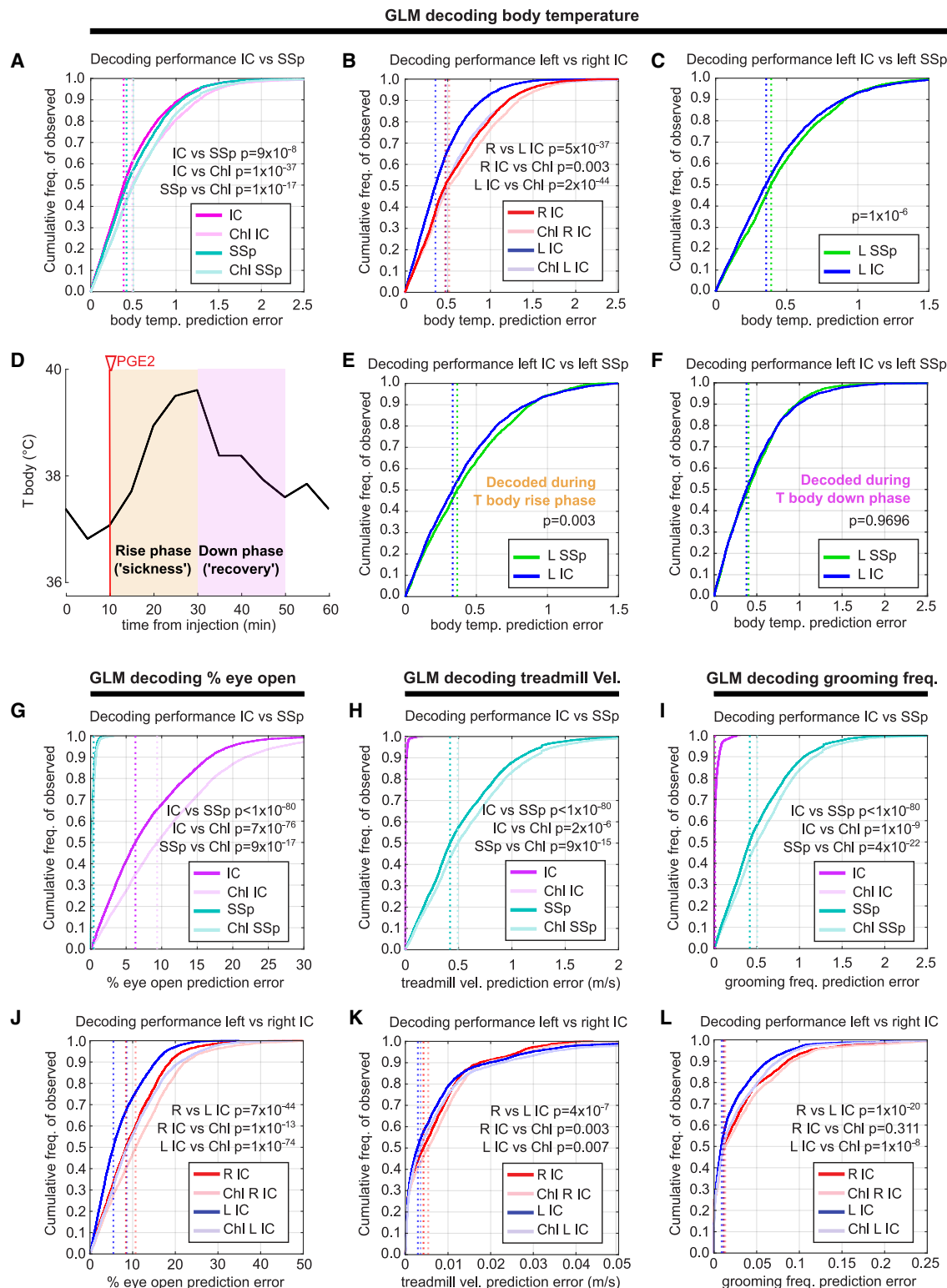


Figure 5. A divergent representation of sickness behaviors between the IC and SSp

(A) Cumulative distribution plots of the decoding errors ($^{\circ}$ C, T body – predicted) obtained using GLMs on the simultaneously recorded IC populations (magenta) and corresponding SSp populations of equal size (cyan) across the datasets from 9 PGE2-injected mice. The chance-level distributions (Chl) obtained by scrambling the temperature traces are plotted in light magenta and light cyan, respectively. Vertical dotted lines correspond to each distribution's median. Statistics: two-sample Kolmogorov-Smirnov tests with Bonferroni correction.

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A divergent representation of sickness behaviors between IC and SSp

CEBRA enables the integration of neuronal recordings from different individuals. However, to rule out potential bias in the consistency of CEBRA-generated embeddings (Figure S6A), we performed a decoding analysis using generalized linear models (GLMs) on direct measurements of neuronal activity during sickness.

We found that models trained on IC data performed significantly better at predicting body temperature than those trained on SSp data (Figure 5A). Furthermore, decoders trained on left-hemisphere data performed better at predicting body temperature than those trained on right-hemisphere data (Figures 5B and S6B). In the left hemisphere, population dynamics of the left IC yielded better decoding performance than those trained on data from the left SSp (Figure 5C). In line with the CEBRA findings, the GLM analysis suggested that information from ventrally and left-located brain areas yielded better body temperature predictions. Specifically, the left IC produced a more accurate representation of body temperature following PGE2 injection, regardless of which lateral ventricle had been injected (Figures S7A–S7C; Table S1).

Next, we separately analyzed the population dynamics during the upward and the downward phases of the fever response, as they likely represent different body states: sickness and recovery, respectively (Figures 1L–1O and 5D). Interestingly, the better decoding performance for body temperature information from the left IC than from the left SSp was observed only during the upward phase of fever (Figures 5E and 5F). This suggests that the information carried by the left IC during the sickness phase was the primary factor driving the better decoding performance of body temperature fluctuations observed in the left IC compared with the left SSp. On the other hand, the difference in decoding performance between right- and left-located brain areas was preserved in both phases between IC and SSp (Figures S6D–S6I).

We then extended our GLM decoding analysis to predict individual sickness behaviors. Decoders trained on the data from IC and SSp predicted each of the three behaviors better than chance. However, eye squinting was more accurately decoded using SSp data, whereas walking speed and grooming were better predicted by IC data (Figures 5G–5I). Similar to the pattern observed for fever, decoding performance was better from data obtained from the left hemisphere in both IC and SSp (Figures 5J–5L and S6J–S6L).

The IC encodes a unified readout of sickness, outperforming fever alone

So far, our GLM approach examined different behavioral and physiological traits of sickness separately. However, as highlighted by our CEBRA analysis, all physiological and behavioral symptoms contribute to the overall sick state. To evaluate their joint contribution, we defined a unified readout of sickness, named the sickness index (SI) (STAR Methods). The SI generally tracked body temperature profiles, although its temporal dynamics differed, with the SI plateauing earlier on average (Figures 6A and 3E). Moreover, PGE2-treated mice exhibited a significantly higher SI than vehicle-infused controls (Figure 6B).

Individual neuronal analysis indicated that IC and SSp had comparable proportions of neurons with activity positively or negatively correlated with SI (Figure 6C; Table S1). At the population level, we found that information about SI was decoded better than at chance across all studied areas. However, we observed that models trained on IC data performed remarkably better than those trained on SSp data (Figure 6D). Interestingly, the IC's superior SI encoding compared with SSp was evident in both hemispheres, with data from the right and left ICs yielding better SI predictions than their SSp counterparts (Figures 6E and 6F). Furthermore, left-hemisphere data consistently provided better decoding performance of SI than right-hemisphere data for both IC and SSp recordings (Figure S7D and S7E).

Intrigued by the remarkable difference in SI decoding performance between IC and SSp, we next compared the performance of decoders trained on SI versus normalized body temperature alone. We found that neuronal population activity of the IC encoded SI better than temperature alone (Figure 6G), and this was the case for both the left and right IC (Figures 6H and 6I). By contrast, only a modest improvement in decoding performance was achieved with the SSp data (Figure 6J), and this effect was not observed in either the left or right SSp alone (Figures 6K and 6L).

Altogether, aligning with the proposed role in representing integrated physiological states,^{89,90} we found a more prominent encoding of sickness in the IC than the SSp. This difference was most clearly revealed when using a unified measure of the sick state (the SI) rather than individual readouts such as fever.

DISCUSSION

Our study introduces a novel methodology and conceptualization to investigate real-time changes in internal state associated

(B) Same as in (A), but comparing the IC decoding error distributions from probes implanted in the right hemisphere (red, $n = 4$) versus the left hemisphere (blue, $n = 8$). The respective chance levels are shown in light red and blue. Same statistics as (A).

(C) Same as in (A), but for probes implanted in the left hemisphere ($n = 8$), comparing the decoding error distributions obtained from the left IC (blue) and the left SSp (green). Same statistics as (A).

(D) Representative body temperature response to PGE2 injection, demarcating the fever rise phase and down phase.

(E and F) Same as in (C), but decoding IC population dynamics during the temperature rise phase (E) and down phase (F). Same statistics as (A).

(G–I) Cumulative distribution plots of the decoding errors for eye-opening percentage (G), treadmill velocity (H), and grooming frequency (mean #events/s in 5 min, I) obtained using GLMs on the simultaneously recorded IC populations (magenta) and corresponding SSp populations of equal size (cyan) across the datasets from 9 PGE2-injected mice. The chance-level distributions obtained by scrambling each behavioral readout are plotted in light red and light blue, respectively. Vertical dotted lines correspond to each distribution's median. Statistics: two-sample Kolmogorov-Smirnov tests with Bonferroni correction.

(J–L) Same as in (G)–(I), but comparing the IC decoding error distributions from probes implanted in the right hemisphere (red, $n = 4$) versus the left hemisphere (blue, $n = 8$). Respective chance levels are in light red and blue; the same statistics as (G).

See Table S1 for further details on statistics.

See also Figures S6 and S7.

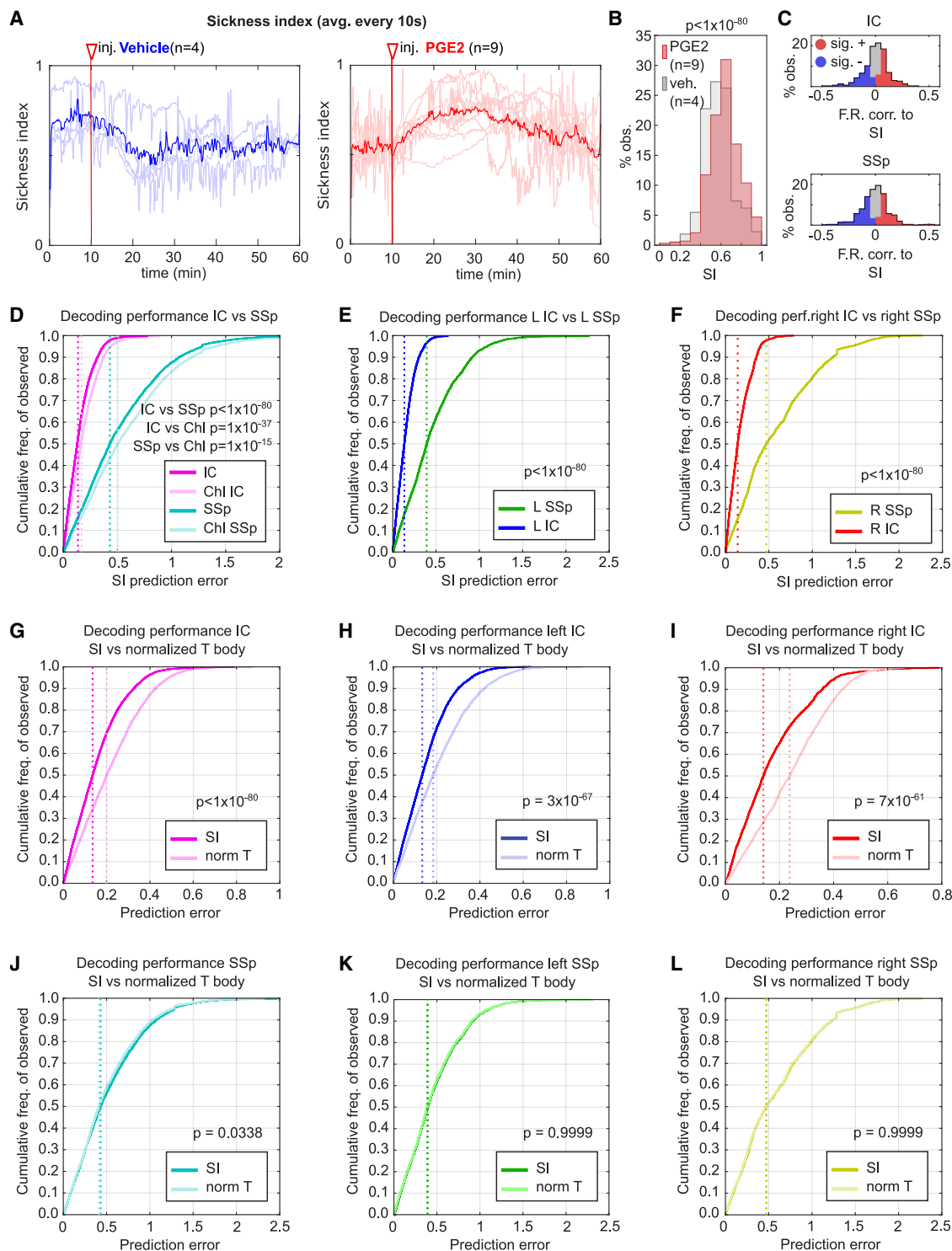


Figure 6. The IC encodes a unified readout of sickness, outperforming fever alone

(A) SI recorded across the experiment for vehicle (left) and PGE2-injected mice (right). The values plotted were averaged across 10 s time bins. Light colors, individual mice; solid colors, average across mice. The highlighted vertical lines: injection time.

(B) Histograms of SI recorded in response to the injections, excluding the 10 min pre-injection and 2 min during injection, from 9 PGE2-injected and 4 vehicle-injected mice (red and gray, respectively). Statistics: two-sample Kolmogorov-Smirnov tests.

(C) Correlation coefficient histograms for single units' F.R. dynamics and SI, excluding the 10 min pre-injection and 2 min during injection, for IC (top) and SSp (bottom). Red areas, significantly positive correlations; blue areas, negative correlations (Pearson's correlation coefficient $p < 0.05$).

(legend continued on next page)

with inflammation in awake, behaving mice. Our behavioral analysis demonstrated that a single i.c.v. injection of PGE2 generated a robust sickness response, including fever, slower locomotion, quiescence, anorexia, and eye squinting. All of these physiological and behavioral features have been widely observed across vertebrate species during systemic inflammation.^{4,5,7,53,85,86}

Additionally, PGE2 i.c.v. induced a distinct brain-wide Fos activation pattern that overlapped with a subset of regions activated by peripheral LPS. Interestingly, both models engaged nearly every major node of the interoceptive neuraxis, consistent with a coordinated response to a complex internal state.^{17,20,67,104} However, while LPS activates most layers across multiple neocortical areas, central PGE2 preferentially targeted deeper layers, such as layer 6. Future studies must confirm this preference for deeper layers and determine whether it arises from direct binding to central EP receptors, long-range neuromodulatory signals, or specific synaptic inputs. Furthermore, a minor subset of regions showed activation exclusively in response to PGE2 but not LPS. This difference underscores the necessity for further investigation to elucidate the mechanisms underlying this divergence.

Next, our Neuropixels recordings revealed that neither PGE2 nor the vehicle altered the overall firing of IC and SSp neurons. The absence of widespread hyperexcitability during hyperthermia has been previously described in the mouse neocortex and may arise from balancing mechanisms that protect the brain from fever-induced activity changes.¹⁰⁵ Interestingly, using dimensionality reduction with CEBRA and decoding with GLMs, we found that IC consistently yielded a better representation of body temperature than SSp. This difference was likely driven by the warming phase of the fever response. Previous work has shown that the posterior IC (pIC) encodes skin warming better than SSp, while skin cooling is equally represented in both pIC and SSp.¹⁰⁶ Our findings suggest that this preferential representation of warming in IC over SSp may extend to internal temperatures.

Additionally, GLMs trained on IC data outperformed SSp in decoding most sickness behaviors, except for eye squinting, which was better represented in SSp. Because intense eye squinting is linked to visceral pain in rodents,⁸⁵ its better decoding in SSp aligns well with its role in pain representation.¹⁰⁷ Furthermore, combining individual readouts into a single SI revealed the largest separation between regions, with decoding of IC data significantly outperforming SSp and exceeding the performance achieved

using body temperature alone. These findings suggest that ongoing population activity in IC preferentially encodes a multidimensional representation of sickness, a distinction that emerges at the level of population activity patterns rather than integrated single-cell firing rates. Furthermore, GLM decoders trained on left IC data also consistently outperformed those trained on the right IC, a pattern observed similarly in SSp. The relative contributions of the left and right IC to sickness and immune state representation remain debated.^{10,101–103,108,109} Further work will be needed to clarify the extent and functional significance of lateralization in sickness representation and to dissect the contributions of different cytoarchitectonic subdivisions of the IC to sick state representation.

Altogether, our work establishes PGE2 i.c.v. as a powerful approach for studying immune-brain communication in awake mice and identifies the IC as a key region that preferentially represents complex, multivariate interoceptive information. Additionally, it demonstrates that PGE2-triggered sickness exhibits key features of an internal state, including pleiotropic behavioral and physiological effects, widespread neuronal activation, and representation as a unified, multivariate state within an interoceptive neocortex. Finally, this study provides a complementary framework for investigating sickness as an internal state and positions it among the growing list of internal states characterized in animal models.

Limitations of the study

Because PGE2 is centrally infused, this study overlooks humoral mediators and visceral inputs present during natural infections,¹² assuming that effects result primarily from direct activation of brain EP receptors. However, central PGE2 rapidly drives peripheral changes, including ACTH and corticosterone release and splenic nerve activation,^{110–113} which mediate rapid peripheral immunosuppression within an hour.¹¹² Substantial evidence shows that the IC receives information regarding peripheral immune function, stores memory-like traces of past inflammatory events, and coordinates environment-immune associations.^{3,10,11,101–103,108,109} Currently, combining Neuropixels recordings, i.c.v. infusions, and *in vivo* immune imaging, such as lymphocyte mobilization, remains technically challenging. Overcoming these methodological barriers to decoding real-time immune information from insular activity represents an exciting future avenue.

An additional limitation is the divergence in thermal profiles between our model and the LPS dataset from Ilanges et al.¹ While

(D) Cumulative distribution plots of the decoding errors (SI, predicted) obtained using GLMs on the simultaneously recorded IC populations (magenta) and corresponding SSp populations of equal size (cyan) across the datasets from 9 PGE2-injected mice. Light magenta and light cyan: chance-level distributions. Vertical dotted lines: medians. Statistics: two-sample Kolmogorov-Smirnov tests with Bonferroni correction.

(E) Same as in (D), but for probes implanted in the left hemisphere ($n = 8$), comparing the decoding error distributions obtained from the left IC (blue) and the left SSp (green). Same statistics as (D).

(F) Same as in (E), but for probes implanted in the right hemisphere ($n = 4$), comparing the decoding error distributions obtained from the right IC (red) and the right SSp (yellow).

(G) Comparison of the decoding error distributions for SI (magenta) and body temperature normalized to the same scale as SI (0 to 1 range, light magenta), obtained using GLMs on the simultaneously recorded IC populations (magenta) across the datasets from 9 PGE2-injected mice. Vertical dotted lines: medians. Statistics: two-sample Kolmogorov-Smirnov tests with Bonferroni correction.

(H) Same as in (G), but for probes implanted on the left hemisphere ($n = 8$; blue, SI; light blue, normalized body temp.).

(I) Same as in (G), but for probes implanted in the right hemisphere ($n = 4$; red, SI; light red, normalized body temp.).

(J–L) Same as (G)–(I), respectively, but for corresponding SSp populations of equal size to the IC populations studied.

See also Figure S7 and Table S1.

central PGE2 induces high fever, the Ilanges LPS model generates a biphasic response culminating in hypothermia at the time of tissue collection. As both models fundamentally rely on central PGE2,^{8,114} this difference likely stems from mediators present during systemic inflammation but absent in this model. Notably, central PGE2 never induces hypothermia in mice, even at high doses, and body temperature plateaus around 40°C–41°C. Therefore, additional peripherally derived mediators likely drive the hypothermic phase observed by Ilanges.^{50,115,116} Future comparisons with pyrogenic LPS doses or viral and parasitic infection models will be necessary to reveal the extent to which distinct sickness models converge on a common neuronal network.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Robert Prevedel (rprevedel@embl.de).

Materials availability

This work did not generate any new reagents.

Data and code availability

- Data supporting Figures 3, 4, 5, and 6 and their supplements are available at <https://www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD2379>.
- Additional raw data, analysis code, and further information can be requested from and will be fulfilled by the lead contact upon reasonable request.

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AUTHOR CONTRIBUTIONS

G.B.K. conceived, designed, and performed the research. J.C.B. collaborated with Neuropixels recordings and analyzed the electrophysiology data. D.R. and N.R. conducted the Fos stainings and analysis. M.N.H., J.C.B., G.B.K., and A.C. analyzed behavioral data. J.C.B. and M.N.H. performed statistical analysis and prepared the figures. G.B.K. led the project with collaboration from M.Y.A.E.H., M.N.H., M.S., N.R., H.A., and R.P. G.B.K. and R.P. acquired funding and supervised the project. G.B.K. wrote the paper, and all authors commented on and approved the final version of the manuscript.

DECLARATION OF INTERESTS

G.B.K. and J.C.B. are married.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

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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
Antibodies		
Rabbit monoclonal anti-cFos	Synaptic System	cat#226-008; RRID:AB_2891278
Chemicals, peptides, and recombinant proteins		
Atipamezol hydrochloride	Vetoquinol GmbH	N/A
CM-Dil Cell-Labeling Solution	ThermoFisher	cat#V22888
Dimethyl sulfoxide	Sigma Aldrich	cat#D2650-5X5ML
Fentanyl citrate	Piramal Critical Care Deutschland GmbH	PZN#13624067
Flumazenil	CHEPLAPHARM Arzneimittel GmbH	PZN#03488574
Glutaraldehyde solution	Merck Sigma	cat#340855-25ML
Isoflurane	Baxter	N/A
Lidocaine hydrochloride (Xylocaine)	Aspen Germany GmbH	PZN#01138002
Lipopolysaccharides from Escherichia coli O111:B4 (LPS)	Sigma Aldrich	cat#L2630-10MG
Medetomidine hydrochloride	CP-Pharma	cat#798-491
Meloxicam	Boehringer Ingelheim Vetmedica GmbH	cat#796-617
Midazolam	CHEPLAPHARM Arzneimittel GmbH	PZN#03085793
Paraformaldehyde solution 4% in PBS	ThermoScientific	cat#30525-89-4
PGE2	STEMCELL Technologies	cat#72194 and 72192
Saline sterile	Braun	PZN#02737756
Sodium azide 0.05%	Merck Sigma	cat#RTC000068-1L
Tergazyme	Alconox	cat#1304-1
Experimental models: Organisms/strains		
C57BL/6J	EMBL's colony and Charles River Laboratories	Strain #:000664; RRID:IMSR_JAX:000664
Software and algorithms		
Matlab	Mathworks	2024a,b
Bonsai	https://bonsai-rx.org/	N/A
SHARP-Track	https://github.com/cortex-lab/allenCCF/tree/master/SHARP-Track	N/A
Kilosort4	https://github.com/MouseLand/Kilosort	Version 4
FaceMap	https://github.com/MouseLand/facemap	N/A
Boris	https://www.boris.unito.it/	N/A
CEBRA	https://github.com/AdaptiveMotorControlLab/cebra	0.4.0

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

Experimental procedures on living animals followed the European Union Council Directive 2010/63/EU on the protection of animals used in scientific research. EMBL's Institutional Animal Care and Use Committee (IACUC) reviewed and approved under license number 22-005_HD_RP and 25_009_HD_RP all animal experiments in this work. Mice of both genders were obtained from EMBL's colonies and Charles River Laboratories. Animals were housed in groups of 1-5 in Makrolon type 2L cages on ventilated racks at room temperature and 50% humidity with a 12 hr light cycle and ad libitum access to food and water unless otherwise specified.

METHOD DETAILS

Pre-incisional stereotaxic procedures

All surgical procedures were conducted in aseptic conditions. 8–12 weeks-old mice of both genders were anesthetized with a 5 μ l/g intraperitoneal injection of a mixture of Fentanyl (0.07 μ g/g), Midazolam (14 μ g/g), and Medetomidine (1 μ g/g) (aka FMM). Anesthetized mice were transferred to a digital stereotaxic apparatus (cat# 68018; RWD). The head was carefully fixed to the setup using a snout mask, a bite bar, and ear bars. Eyes were protected with hydrating ointment (Bepanthen with 5% dexpanthenol; Bayer), and body temperature was monitored and maintained using a rectal probe and a warm blanket connected to a homeostatic monitoring system (cat#69027; ThermoStar, RWD). Hair from the head was removed with depilatory cream (Veet; Reckitt). Mice received a 5 μ l/g subcutaneous injection of Meloxicam (1 μ g/g) in sterile saline for postoperative analgesia. Before the incision, the head's skin was disinfected using alcohol pads (cat#9160612; Braun), and mice received a 50 μ l subcutaneous injection of Xylocaine 1% (PZN#01138002; Aspen Germany GmbH) under the skin of the scalp. The skin of the scalp was removed using fine scissors (cat#15006-09; Fine Scientific Tools).

Brain cannula and head bar implantations

All surgical procedures were conducted in aseptic conditions. Two small craniotomies of $\sim$ 1mm were drilled using a microdrill (cat#78001; RWD). One craniotomy was drilled over the lateral ventricle (from bregma AP=0.00 mm, ML= $\pm$ 0.80 mm), and the second craniotomy was drilled over the parietal bone. The exposed dura was carefully torn with fine forceps (Dumont#5; Fine Science Tools). A sterilized stainless steel surgical screw (cat# 00-96x1/16, Plastic One) was fixed to the skull over the parietal craniotomy, and a sterilized i.c.v. guide cannula (OD 0.48x ID 0.34x L3 mm; C:2.5MM, cat#62003; RWD) was carefully lowered onto the brain through the craniotomy over the lateral ventricle. When required, a custom-made head bar was additionally placed over the skull. The cannula's pedestal, head bar, surgical screw, exposed skull, and skin on the head were secured with glue (Cyano retarder; Hager Werken) and dental cement (Paladur; Heraeus). Once the glue was set, a sterilized dust cap (O.D. 0.30 mm/M3.5, length 2.5mm; cat#62102; RWD) was crewed on the guide cannula to keep the cannula clean and to prevent dirt from entering the brain.

Logger implantation

All surgical procedures were conducted in aseptic conditions. After stereotaxic procedures, mice were removed from the stereotaxic frame and were carefully placed in a supine position over a warm blanket connected to a homeostatic monitoring system (cat#69027; ThermoStar, RWD). Hair on the belly was removed with depilatory cream (Veet; Reckitt), and the exposed skin was disinfected with alcohol pads (cat#9160612; Braun). A small incision of $\sim$ 1 cm was performed on the skin of the abdomen. A sterile implantable temperature logger (DST nano-T; Star::Oddi) was inserted into the abdominal cavity, and muscle and skin layers were sutured separately with sterile absorbable surgical threads (Marlin 17241041; Catgut, Germany).

Craniotomies for Neuropixels electrodes

Two small craniotomies of $\sim$ 1mm were drilled over the right and left insular cortices (from bregma AP=1.00 mm, ML= $\pm$ 3.25 mm) using a microdrill (cat#78001; RWD), and the exposed dura was carefully torn with fine forceps (Dumont#5; Fine Science Tools). After that, the craniotomies were covered with an absorbable hemostatic gelatin sponge (Equispon; Invotec) embedded in sterile cortex buffer (mM: NaCl 125, KCl 5, Glucose 10, Hepes 10, CaCl₂ 2, MgCl₂ 2, pH 7.4) and sealed with non-toxic silicon glue (Kwik-Cast; World Precision Instruments).

Postsurgical care

FMM anesthesia was antagonized with a 10 μ l/g subcutaneous injection of Flumazenil (0.74 μ g/g) and Atipamezole (2.8 μ g/g). Mice were transferred to their home cage and kept overnight over a heating pad. The following day, mice received a 5 μ l/g subcutaneous injection of Meloxicam (1 μ g/g) in sterile saline for pain management, and wound healing was closely monitored. Mice carrying brain cannulas were single-housed to prevent cage mates from nibbling on cannula pedestals.

Training on the treadmill apparatus

This training aimed to habituate mice to the experimenters and to walk on the treadmill on the Neuropixel setup. The experimenter gently handled mice for $\sim$ 5 to 15 minutes/day. While handling, mice were offered a sweetened soy drink (cat#4337185832758; Take it veggie) at random intervals as a reward using a Pasteur pipette. After handling, the mice were returned to their home cage. This step was repeated between 3 to 7 days, depending on mouse habituation performance.

Next, mice were briefly anesthetized with isoflurane 5% in O₂ and head-fixed to the custom-made treadmill apparatus using the previously implanted head bar. Once in the behavioral setup, mice remained on the treadmill between 15 and 60 minutes/day, depending on their habituation progress. While head-fixed, mice were offered a sweetened soy drink (cat#4337185832758; Take it veggie) at random intervals using a Pasteur pipette. After habituation, mice were returned to their home cage. This step was repeated for 3 to 10 days, depending on habituation progress.

Electrophysiological recordings

24 hours after Neuropixels craniotomy drilling, mice were anesthetized with isoflurane 5% in O₂ and transferred to the stereotaxic apparatus, where 1–2% isoflurane in O₂ was vaporized through a snout mask at a flow rate of 0.4–0.6 LPM during surgical procedures. The silicon sealant was carefully removed, and exposed insular craniotomies were covered in sterile cortex buffer (mM: NaCl 125, KCl 5, Glucose 10, Hepes 10, CaCl₂ 2, MgCl₂ 2, pH 7.4). While still anesthetized, mice were head-fixed to a custom-made treadmill apparatus and transported to the recording setup. The cap covering the guide cannula was unscrewed, and an i.c.v. injection needle (O.D.0.30mm-with M3.5 G1:0.5MM; cat#62203; RWD) was lowered through the guide cannula. Two Neuropixel 1.0 electrodes⁹² with their shanks coated in CM-Dil Cell-Labeling Solution (cat#V22888; ThermoFisher), were lowered through the insular craniotomies at ~10 μm/s using micromanipulators (SMX single-axis; Sensapex piezo-driven micromanipulator system and MPC-385; Sutter motorized micromanipulator system). The electrodes were allowed to settle for ~15 minutes before starting the recordings. Neuropixel recordings were acquired for 60 minutes, as detailed before.¹¹⁷ Synchronized with Neuropixel data acquisition, body posture, face conformation, and pupil diameter were monitored and recorded using two IR-sensitive cameras equipped with a CMOS OV2710 sensor (cat#USBFHD05MT-KL361R; ELP) at 30 fps and 720p resolution under IR illumination (cat#B0BJFG-F2YP; Serlun). Synchronization between IR cameras and neuropixels acquisition was performed using Bonsai (<https://bonsai-rx.org/>) and an Arduino Uno board to capture the cameras' recording start signal through a digital channel of the neuropixels acquisition system through an NI DAQ board (National Instruments, PXIe-6341).

Ten minutes into the recordings, 2 μl of a sterile solution of PGE2 2 nmol/μl (cat#72194 and 72192; STEMCELL Technologies) in DMSO 1:1 (cat#D2650-5X5ML; Sigma Aldrich) or vehicle (sterile saline and DMSO 1:1) were injected in the lateral ventricle at 1 μl/minute using the previously placed i.c.v. injection needle. After i.c.v. perfusion, electrophysiological recordings were allowed to proceed for another 50 minutes. Upon experiment completion, the Neuropixels electrodes and i.c.v. injection needle were removed from the brain. Mice were released from the treadmill apparatus, transported to an euthanasia chamber, and sacrificed by CO₂ inhalation. Euthanized mice were transcardially perfused with PBS followed by PFA 4% in PBS (cat#30525-89-4; ThermoFisher). Brains were collected for electrode track reconstruction.

The injection side was not randomized due to technical constraints of the recording configuration. To target the lateral ventricle, the guide cannula was implanted at ±0.8 mm ML rather than on the midline, which created spatial asymmetry for Neuropixels electrode insertion. The probe ipsilateral to the i.c.v. cannula was inserted at an angle to avoid collision between the Neuropixels base and the cannula pedestal, whereas the contralateral probe was inserted perpendicular to the cortical surface. These configurations required specific positioning of the micromanipulators on the breadboard of the antivibration table, and changing the injection side required a full reconfiguration of the setup.

Initial recordings were therefore performed with the cannula targeting the left lateral ventricle. After preliminary analyses suggested stronger sickness related encoding in the left IC, the setup was reconfigured and additional recordings were performed with PGE2 injected into the right lateral ventricle. The lateralization of sickness encoding to the left IC persisted, indicating that it was unlikely to result from the side of PGE2 injection (see Table S1 for additional information regarding the targeted ventricle in each experimental subject).

Neuronal and behavioral analysis in head-fixed mice was limited to one hour, consisting of 10 minutes before i.c.v. injection and 50 minutes after i.c.v. administration to minimize discomfort and ensure the well-being of the animals in our head-fixed behavioral setup. Additionally, we excluded from analysis the two minutes that lasted the i.c.v. injection because this procedure occasionally produced instability in the electrophysiological recordings.

Behavioral recordings in freely moving mice

Data in Figures 1, S1, and S2 was pooled from cohorts of mice that served as control (non-treated) across different experiments (for example, injected with EGFP in the IC) to avoid repetition and to minimize the number of animals used.

Before behavioral recordings, mice were food-restricted for 24 hours. On the day of the experiment, mice were transferred in their home cage to the behavioral room and were allowed to acclimatize for approximately 2 hours before any further procedures. After the injection of PGE2/vehicle or LPS/saline, mice were returned to their home cage, where their behavior was monitored using a USB camera (2K Webcam Auto Focus Ultra HD). The top of the home cage was replaced by an acrylic frame to gain better visual access. Camera acquisition was controlled using Bonsai (<https://bonsai-rx.org/>). Behavior was monitored for up to 2hs (PGE2 injections) or 5hs (LPS injections). After video recordings, mice were transferred back to the holding room.

LPS/saline injections

Mice were injected intraperitoneally with ~200 μl of 0.02 mg/kg of LPS in sterile saline¹¹⁸ or an equal volume of saline. Immediately after injection, animals were returned to their home cages, where locomotor activity and body temperature were continuously monitored. To control for circadian variations in body temperature, feeding behavior, locomotor activity, and environmental conditions, all LPS and corresponding control injections presented in Figures 1, S1, and S2 were administered between 11:00 and 13:00 h (typically at 12:00 h). All procedures were conducted under identical conditions for both experimental and control injections, in the same behavioral testing room equipped with the same recording setup.

PGE2/vehicle injections

Mice were restrained using a disposable restrainer (DecapiCones; Braintree). The pedestal of the guide cannula was pulled out of the restrainer using a small hole cut in the upper part of the restrainer. The cannula's cap was unscrewed, and a sterile i.c.v. injection needle (O.D.0.30mm-with M3.5 G1:0.5MM; cat#62203; RWD) was lowered through the guide cannula. 2 μ l of a sterile solution of PGE2 2 nmol/ μ l (cat#72194 and 72192; STEMCELL Technologies) in DMSO 1:1 (cat#D2650-5X5ML; Sigma Aldrich) or vehicle (sterile saline and DMSO 1:1)³³ were injected in the lateral ventricle at 1 μ l/minute using a Hamilton syringe (cat#HAM80075; Hamilton) connected with PE tubing (cat#62329; RWD). The final i.c.v. injection coordinates were: AP 0.0 mm, ML $\pm$ 0.8 mm, and DV 3.0 mm. The injection needle was kept in place for two minutes before retraction to prevent liquid backflow. After infusion, the cannula cap was screwed back, and mice were released from the restrainer and returned to their home cage where locomotor activity and body temperature were continuously monitored. The i.c.v. injection procedure took approximately 5 min per mouse. To control for circadian variations in body temperature, feeding behavior, locomotor activity, and environmental conditions, all PGE2 and corresponding control injections presented in [Figures 1](#) and [S1](#) were administered between 11:00 and 13:00 h (typically at 12:00 h). All procedures were conducted under identical conditions for both experimental and control injections, in the same behavioral testing room equipped with the same recording setup.

Histology Fos iDisco

Mice of both genders were used in this experiment. I.c.v. cannulas and body temperature loggers were implanted, as mentioned above. Treated mice received 4 nmol PGE2 in DMSO 50% i.c.v. or an equal volume of vehicle (i.c.v. injection procedures detailed above). 1.5h after i.c.v. injection mice were transported to an euthanasia chamber and sacrificed by CO₂ inhalation. Euthanized mice were transcardially perfused with PBS followed by PFA 4% in PBS (cat#30525-89-4; ThermoFisher). Brains were collected and postfixed in PFA4% at 4°C overnight. Brains were rinsed in PBS and stored in PBS with azide 0.05% (cat#RTC000068-1L; Merck) at 4°C until further processing.

Fos iDISCO+ immunolabeling was performed as previously described,⁷¹ using a rabbit monoclonal anti-Fos antibody at 1/2000th dilution from the stock (Synaptic Systems, cat#226-008). Brains were imaged on a Blaze light sheet microscope.

Neuropixel track reconstruction

Brains were post-fixed overnight in PFA 4% in PBS (cat#30525-89-4; ThermoFisher) at 4°C, rinsed in PBS, and cryoprotected in sucrose 30% in PBS for a minimum of 24 hs. Brains were embedded and frozen in a sectioning matrix (Tissue-Tek; Sakura). Coronal brain sections were cut at 30 μ m in a cryostat (CM 3059S; Leica). Brain sections were collected in glass slides (cat#11976299; Epre-dia SuperFrost Plus Gold; ThermoFisher) and kept at -20°C until further processing. Slides were rinsed in PBS for 10 min, mounted in DAPI-containing mounting medium (VectaShield Plus; Vector Laboratories), and placed at 4°C in the dark until picture acquisition.

Images were acquired at 4x in an epifluorescence scope (TiE; Olympus) equipped with a Hamamatsu Orca Flash 4 V2 (pixel size:6,5 μ m /2048 x 2048 pixel). Neuropixel tracks were reconstructed using the SHARP-Track pipeline.¹¹⁹

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral analysis of freely-moving mice

Movement data was acquired using pose estimation on video recordings of mouse behavior, recorded at 30 fps. A LightningPose model¹²⁰ was trained to track 26 anatomical landmarks across the animal's body, including points along the tail, spine, hips, sides, shoulders, ears, eyes, nose, and head center -similar to Ye et al. (2024).¹²¹ After training, videos were processed to generate CSV files containing pose keypoint coordinates and corresponding confidence values. For post-processing, the "movement" package was used.¹²² The pose data was filtered by confidence threshold (>0.6), the data interpolated over time (with a maximum gap of 1000 frames), and smoothed with a median filter of 0.1 seconds. We then used the integrated tools of the "movement" package to calculate displacement and velocity.

To correct for perspective distortion and standardize measurements across different recording sessions, we manually annotated the arena boundaries for each recording and applied a perspective transformation using OpenCV. The arena boundaries were mapped to a standardized rectangle coordinate system (320 $\times$ 160 pixels), with this transformation applied to all keypoint positions.

Stasis episodes, defined as periods of minimal movement, were identified through position data processing. First, position data was low-pass filtered with a Butterworth filter with a cutoff frequency of 12 Hz. Position change was calculated as the Euclidean distance between consecutive frames, and a rolling sum of movement was computed using a 5-second window. Frames with rolling movement below a threshold of 25 units were classified as stasis. Continuous stasis periods were identified, and episodes shorter than 5 seconds were excluded from the analysis. Importantly, stasis episodes were adjusted to exclude periods of food interaction to avoid misclassification.

Food interaction periods were detected using a deep learning model trained with DeepEthogram.¹²³ The model achieved an accuracy of 0.88, with an F1 score of 0.705 and AUROC of 0.938 [Figures S1A](#) and [S1B](#). Using an optimal threshold of 0.633, the model provided a precision of 0.611 and recall of 0.834 for food interaction detection. The mean average precision (mAP) was 0.707. Food interaction prediction confidence scores were thresholded at this optimal value to generate binary classifications. The cumulative time spent interacting with food was calculated for each recording and used for subsequent analysis.

Statistical comparisons between treatment and control groups were performed using multiple approaches, with all analyses conducted separately for the LPS ($n = 17$ treated, $n = 22$ saline) and PGE2 ($n = 11$ treated, $n = 11$ vehicle) experiments. All models were fitted using the lme4 and lmerTest packages in R.

Treatment effects on time-series variables

For body temperature over time, linear mixed-effects models (LME) with a treatment $\times$ time interaction were used to estimate the rate at which the treatment altered the variable over time, with individual mice as a random intercept (modeled as: $\text{temperature} \sim \text{injection_consolidated} \times \text{duration} + (1 \mid \text{individuals})$). The interaction coefficient represents the difference in slope ($^{\circ}\text{C}/\text{min}$) between treatment and control groups.

Treatment effects on distribution variables

For non-cumulative distribution variables (temperature, velocity, log-transformed stasis episode length), LME models with treatment as a fixed effect and individual as a random intercept were used (modeled as: $\text{variable} \sim \text{injection_consolidated} + (1 \mid \text{individuals})$). The treatment coefficient represents the mean shift in the variable attributable to the treatment.

Treatment effects on cumulative variables

For cumulative displacement and cumulative food interaction time, direct modeling of the full time-series is inappropriate because cumulative variables are inherently autocorrelated, which can inflate significance. Instead, we used endpoint comparisons: the last observation per animal per session was extracted, and an LME was fitted on these endpoints with treatment as a fixed effect and individual as a random intercept (modeled as: $\text{endpoint} \sim \text{injection_consolidated} + (1 \mid \text{individuals})$). The treatment coefficient represents the difference in total accumulated displacement (cm) or food interaction time (s) at the end of the recording.

Peak temperature comparisons

Within-subject comparisons of maximum body temperature between treatment and control conditions were performed using paired t-tests.

Peak-aligned behavioral analysis

To assess the temporal relationship between fever and behavioral changes, all behavioral responses were aligned to the onset of peak body temperature for each animal. PGE2 injection day and the paired vehicle control day from the same animal were compared. At each 1-minute time bin relative to the temperature peak, the fraction of animals in stasis, the fraction engaged in food interaction, and the mean velocity were computed as group averages ($\pm \text{SEM}$), smoothed with a 5-minute centered moving average.

Sex differences

To test whether the treatment effect differed between male and female mice, three types of sex $\times$ treatment interaction models were fitted, with individual as a random intercept. For temperature time-series, a three-way interaction model was used ($\text{temperature} \sim \text{gender} \times \text{injection_consolidated} \times \text{duration} + (1 \mid \text{individuals})$), where the three-way interaction coefficient indicates whether the rate of the treatment response over time differs between sexes (in $^{\circ}\text{C}/\text{min}$). For non-cumulative distribution variables (temperature, velocity, log stasis length), a two-way interaction model was used ($\text{variable} \sim \text{gender} \times \text{injection_consolidated} + (1 \mid \text{individuals})$), where the interaction coefficient indicates whether the treatment effect on the measured variable differs between sexes. For cumulative variables (displacement, food interaction), the same endpoint comparison approach was applied: the last observation per animal was extracted, and the model $\text{endpoint} \sim \text{gender} \times \text{injection_consolidated} + (1 \mid \text{individuals})$ was fitted. This interaction approach controls for baseline sex differences by including both treated and control animals in each model. The reference level for gender was female; a positive interaction coefficient indicates that males exhibit a larger treatment effect.

Baseline body temperature

To compare baseline circadian temperature patterns between sexes, body temperature data from non-injection days were used. Per-animal mean temperatures were computed separately for day (10:00–18:00) and night (22:00–06:00) phases, excluding transition periods (06:00–10:00 and 18:00–22:00) to avoid circadian transition effects. Female versus male comparisons within each phase and experiment were performed using Welch's t-tests. Data processing and analysis were implemented using Python and R.

Behavioral analysis of head-fixed mice

Video data from head-fixed mice obtained during neuropixels recordings were analyzed with point tracking FaceMap¹²⁴ to quantify the % of eye opening as the difference between the y position of a point placed on the top eyelid and another at the bottom eyelid. Treadmill velocity was estimated from a contrasting bar pattern etched on the edge of the disk treadmill and captured in the videos. Treadmill velocity was calculated from simple kymographs of these moving bars obtained in ImageJ. Grooming events were evaluated on a per-second basis manually, assisted by BORIS (<https://www.boris.unito.it/>). Due to the sparse occurrence of grooming events, grooming frequency was estimated as the per-second number of grooming events averaged over 5 minutes.

Statistical comparisons of the head-fixed behavioral readouts typically consisted of 2-sample Kolmogorov-Smirnov tests between the pooled data from vehicle and PGE2-injected mice, performed using the kstest2 function for MATLAB.

Calculation of a sickness index

To produce an integrated, unidimensional readout summarizing body temperature and head-fixed behavioral dynamics positively correlated to sickness, consisting of $-(\text{treadmill speed})$, $-(\text{eye opening})$, and grooming frequency, we defined a sickness index. To calculate the sickness index, we first normalized the body temperature and behavioral readouts to be bounded between 0 and 1. Then, we defined the sickness index as the sum of these four normalized variables, which were normalized again to range between 0 and 1. Given no a priori reason to differentially weight these symptoms in the sick state, we normalized their sum to define the SI.

Neuropixels data processing and analysis

High-pass filtered and common average referenced spike recordings were produced with CatGT (<https://billkarsh.github.io/SpikeGLX/#catgt>) as described earlier.¹¹⁷ These preprocessed datasets were spike-sorted and drift-corrected using kilosort4.¹²⁵ The output of kilosort was subject to manual curation using Phy2 (<https://github.com/cortex-lab/phy>), obtaining single-units with somatic AP waveforms, good waveform consistency (amplitude) across spikes, showing few or no refractory period violations (2ms refractory period violation time window) in their correlogram plots and firing a minimum of 400 times in 60 min of recording.

Decoding analysis

For decoding analysis of population activity data we calculated the firing rates of the simultaneously recorded single unit populations as the spike count per 1s time bins. To avoid an influence of the i.c.v. injection manipulation, we excluded from the decoding analysis the 10 minutes pre-injection and the 2 minutes during injection, obtaining 48 minute long firing rate datasets (2880 time points).

To compare the recorded population dynamics in vehicle and PGE2-treated mice, we resorted to the multisession CEBRA-behavior algorithm (<https://github.com/AdaptiveMotorControlLab/cebra>)⁹⁹ to embed the aforementioned firing rates datasets recorded in each mouse (session) in a common coordinate system through supervised dimensionality reduction guided by accessory variables. The accessory variables we used to supervise the embeddings were: 1) body temperature, 2) % of eye opening, treadmill speed, and grooming frequency simultaneously, or 4) body temperature, % of eye opening, treadmill speed, and grooming frequency simultaneously. After this supervised embedding, we performed cross-validated binary classification of the embedded points by fitting support vector machine (SVM) classifiers to 80% of the embedded firing rate data points and predicting the corresponding treatment (PGE2 or Vehicle) on the 20% left out. We fitted the SVMs using the `fitsvm` function from MATLAB with default parameters and evaluated the hit rates as the proportion of correctly predicted labels. Chance-level hit rates were obtained by scrambling the treatment labels and repeating the decoding analysis as described.

Alternatively, we decoded the corresponding body temperature, behavioral readouts, or the sickness index by fitting generalized linear models (GLM) to the firing rate datasets, leaving out every 5th time point for prediction (80/20% cross-validation). We fitted the models to simultaneously acquired data from each mouse during one experiment session, avoiding pooling, using the `fitglm` function from MATLAB with default parameters. For decoding during body temperature up and down phases, we used a 67/33% cross-validation ratio (one every 3 time points was left out for prediction) to compensate for the roughly half-sized datasets and preserve statistical power for comparing decoding errors. With the fitted models, we predicted the body temperature, behavioral readouts, or sickness index from the time points left out and calculated the prediction error as the difference between the actual values and the predicted values. Since the number of single units simultaneously recorded with one electrode from SSp was typically much larger than in IC, for comparisons between decoding performance at IC and SSp we randomly subsampled the SSp units to decode SSp subsamples of equal size than the collected IC sample. This process was iterated 30 times to better represent the simultaneously recorded SSp population. Chance-level decoding performances were obtained by scrambling the temperature, behavioral, or SI traces and repeating the decoding analysis as described.

Statistical comparisons of the decoding errors or hit rates consisted of either Wilcoxon rank sum or signed rank tests, 2-sample Kolmogorov-Smirnov tests, performed using the `ranksum`, `signrank`, and `kstest2` functions for MATLAB, followed by Bonferroni's correction of the obtained *p* values.

Fos+ cell density analysis

ClearMap 2¹²⁶ was used for the segmentation and analysis of Fos+ cell distributions from the light sheet scans, as described in (Renier et al. 2016).⁷²

LPS i.p. and PGE2 i.c.v. Fos map comparison

To compare the brain-wide Fos activation patterns induced by LPS i.p. and PGE2 i.c.v., we performed a quantitative region-by-region comparison of Fos effect sizes across the two sickness models.

Data sources

For LPS, we used the whole-brain Fos dataset from Ilanges et al.,¹ which provides per-region Fos-positive cell counts from mice sacrificed 3 hours after injection of 0.5 mg/kg LPS i.p. (*n* = 3 LPS, *n* = 4 saline). For PGE2, we used the iDISCO Fos dataset generated in this study (*n* = 5 PGE2, *n* = 5 vehicle), with per-region counts summed across hemispheres.

Effect size computation

For each brain region in each dataset, we computed Cohen's *d* as: $d = (\text{mean}_{\text{treatment}} - \text{mean}_{\text{control}}) / \text{pooled SD}$ where $\text{pooled SD} = \sqrt{((n_{\text{control}} - 1) \times \text{SD}_{\text{control}}^2 + (n_{\text{treatment}} - 1) \times \text{SD}_{\text{treatment}}^2) / (n_{\text{control}} + n_{\text{treatment}} - 2)}$. Positive values indicate increased cFos expression in the treatment group relative to control.

Region mapping and filtering

Allen CCF region IDs from both datasets were mapped to standardized brain region acronyms using the IBL atlas (`iblatlas` Python package). Regions belonging to fiber tracts, ventricles, or the atlas root were excluded. To ensure reliable effect size estimates, regions were further filtered to include only those with a maximum group mean (across treatment and control) of at least 10 Fos-positive cells. After merging the LPS and PGE2 datasets on shared region acronyms and applying these filters, 473 brain regions were retained for analysis. For bivariate classification, each region was independently classified along each treatment axis based on its Cohen's *d*: suppressed ($d \leq -0.5$), unchanged ($-0.5 < d < 0.5$), or activated ($d \geq 0.5$). This produced a 3 × 3 bivariate classification (LPS effect × PGE2 effect).

Central Autonomic Network (CAN) annotation

Regions were annotated as belonging to the Central Autonomic Network based on their position in the Allen CCF hierarchy. A region was classified as CAN if it matched, or was a descendant of, any of the following parent structures (based on Benarroch⁶⁹; Saper, 2002): agranular insular cortex (AI), gustatory cortex (GU), anterior cingulate (ACAd, ACAv), infralimbic cortex (ILA), prelimbic cortex (PL), central amygdala (CEA), medial amygdala (MEA), bed nuclei of the stria terminalis (BST), paraventricular hypothalamus (PVH), lateral hypothalamic area (LHA, LH), dorsomedial hypothalamus (DMH), anterior hypothalamic nucleus (AHN), posterior hypothalamus (PH), median preoptic nucleus (MEPO), medial preoptic nucleus (MPN), medial preoptic area (MPO), lateral preoptic area (LPO), vascular organ of the lamina terminalis (OV), supraoptic nucleus (SO), suprachiasmatic nucleus (SCH), arcuate nucleus (ARH), ventromedial hypothalamus (VMH), periventricular hypothalamic nuclei (PVp, PVi, PVa), periaqueductal gray (PAG), ventral tegmental area (VTA), dorsal raphe (DR), parabrachial nucleus (PB, PBI, PBm), locus coeruleus (LC), Barrington's nucleus (B), nucleus of the solitary tract (NTS), area postrema (AP), dorsal motor nucleus of the vagus (DMX), raphe pallidus (RPA), raphe obscurus (RPO), raphe magnus (RM), parvocellular reticular nucleus (PARN), and intermediate reticular nucleus (IRN). Of the 473 retained regions, 77 were classified as CAN members.

Correlation analysis

The overall concordance between LPS and PGE2 activation patterns was assessed by computing the Pearson correlation coefficient (r) and Spearman rank correlation coefficient (ρ) between $d(\text{LPS})$ and $d(\text{PGE2})$ across all 473 matched regions.