A category-free neural population supports evolving demands during decision-making

David Raposo1–3, Matthew T Kaufman1,3 & Anne K Churchland1

The posterior parietal cortex (PPC) receives diverse inputs and is involved in a dizzying array of behaviors. These many behaviors could rely on distinct categories of neurons specialized to represent particular variables or could rely on a single population of PPC neurons that is leveraged in different ways. To distinguish these possibilities, we evaluated rat PPC neurons recorded during multisensory decisions. Newly designed tests revealed that task parameters and temporal response features were distributed randomly across neurons, without evidence of categories. This suggests that PPC neurons constitute a dynamic network that is decoded according to the animal’s present needs. To test for an additional signature of a dynamic network, we compared moments when behavioral demands differed: decision and movement. Our new state-space analysis revealed that the network explored different dimensions during decision and movement. These observations suggest that a single network of neurons can support the evolving behavioral demands of decision-making.

Individual neurons are often seen as members of highly specialized categories, with response properties making them suitable for particular classes of computations1,2. This view has been fruitful for understanding early sensory areas, where single neurons can be strongly tuned for task parameters, such as direction of motion3 or disparity4.

The assumption of neural categories is reflected in many experimental designs and analysis methods, even those focusing on neural structures far downstream of early sensory areas. This assumption can be evident in the way neurons are sampled: sometimes, neurons must meet certain response criteria to be included for study, such as responsiveness to certain stimuli or activity during a delay period5–8. Implicit in this approach is the idea that the cell’s response during one stimulus identifies it as a member of the category being examined. The assumption of categories can also be evident during analysis: pie charts, a common way of summarizing population data9–11, explicitly assign neurons to categories. Another way of summarizing a population response, averaging over many neurons, likewise reflects the assumption that each neuron is an exemplar of a category, different from other category members mainly because of noise.

An alternative hypothesis is that neurons reflect random combinations of parameters, leading to neural populations in which neurons’ responses defy categorization. Theoretical work suggests a major advantage for category-free populations: when parameters are distributed randomly across neurons, an arbitrary group of them can be linearly combined to estimate the parameter needed at a given moment12–14. This obviates the need for precisely prepatterned connections between neurons and their downstream targets and also means that all information is transmitted. This latter property could allow the same network to participate in multiple behaviors simply by using different readouts of the neurons. Experimental work has not tested directly whether neural populations are category free, but many observations are broadly consistent with this possibility. Specifically, recent studies have demonstrated that neurons in parietal15–18 and frontal19,20 areas have mixed selectivity: individual neurons are modulated by multiple task parameters. Mixed selectivity would be expected if neurons reflect random mixtures of parameters, but it also might exist under other assumptions. Other experimental work has probed for the existence of neural categories defined by the timing of a neuron’s response21. That work argued against categories, but it tested only for categories defined by response sequence. A more general test is thus required. Further, because neurons in that study responded sparsely, it was not possible to test whether the same neurons participated statically or dynamically in the network as the behavioral demands evolved from decision to movement.

Here, we developed a multisensory decision task rich enough to expose the functional organization of a neural population, both at a single moment and over the course of a complex choice with evolving behavioral demands. Our data suggest that in the PPC, the population is category free: response features are randomly distributed across neurons. A possible explanation for this configuration is that it confers flexibility, allowing the brain to use the same neurons in different ways, depending on the needs of the animal. In keeping with this explanation, we found that the population can be decoded instantaneously to estimate multiple task parameters and that the population activity explored different dimensions as the animal’s needs evolved from decision formation to movement.

**RESULTS**

**Multisensory decision-making behavior**

We trained rats on an established decision-making task22,23 in which animals reported a judgment about a 1-s series of auditory clicks and/or full-field visual flashes (Fig. 1a–c). We refer to this 1-s period as “decision formation” because previous studies have demonstrated that stimuli presented throughout the 1-s period influence the...
animals’ decisions\textsuperscript{22,23}. Once the stimulus terminated, animals reported whether the event rate of the stimulus was above or below an experimenter-imposed category boundary. They reported decisions via movement to one of two choice ports. Rats were mostly stationary during stimulus presentation (Supplementary Fig. 1a) and did not typically move toward or away from the direction of the port they ultimately chose (Supplementary Fig. 1b). Rats mastered the ability to categorize the stimulus and report the decision regardless of whether stimuli were unisensory (visual-only or auditory-only) or multisensory (Fig. 1c). As in other studies\textsuperscript{22–24}, when auditory and visual stimuli were presented together (multisensory trials), performance for inactivation versus control days and observed shallower slopes of the psychometric functions revealed that inactivation did not reduce the animal’s motivation or introduce confusion about the stimulus-response contingency.

Further, we observed sparing of the multisensory enhancement, the improved sensitivity for multisensory relative to unisensory decisions (Supplementary Fig. 2a,b). This spared enhancement implies that PPC likely does not drive multisensory enhancement and leaves open the possibility that PPC may process visual inputs before they are integrated with other modalities. Under this scenario, we would predict only a small change in multisensory performance even when visual inputs are impaired. This is because multisensory mechanisms can still use the remaining, weak visual signal to improve their estimation alongside the spared auditory signal. The framework for multisensory integration therefore predicts a very minor change in enhancement during inactivation of one modality, even if that modality is clearly impaired (see simulation, Supplementary Fig. 2e–g).

One possible explanation for impaired visual decision-making is that the muscimol might have spread to portions of neighboring visual areas, posterior to PPC. This seems unlikely to have driven the effect because the retinotopic organization of visual areas means that restricted spread of muscimol would only have affected a portion of the visual field. Since our stimulus was full field, the unaffected parts of the visual field could likely have supported the behavior\textsuperscript{27}. Nevertheless, we wished to determine the spread of the inactivation.
Ipsilateral Contralateral

Figure 2  PPC neurons show mixed selectivity for choice and modality. Plots display visual or auditory trials; for multisensory trials, see Supplementary Figures 4 and 5. (a–d) Per-stimulus time histograms for four single neurons. Mean spike counts were computed in 10-ms time windows smoothed with a Gaussian (\(\sigma = 50\) ms). Error trials were excluded. Trials grouped by stimulus rate. Solid line, low-rate stimulus; dashed line, high-rate stimulus. Auditory trials, green; visual trials, blue. Shaded fills, s.e.m. Responses aligned to the time the visual or auditory stimulus began (Stim). (a) A neuron reflecting mainly categorical choice (392 trials). (b) A neuron reflecting mainly stimulus modality (414 trials). (c) A neuron mixing categorical choice and modality (586 trials). Arrow highlights ambiguous moment in which high-rate visual and low-rate auditory stimuli gave rise to the same firing rate. (d) A neuron mixing categorical choice and modality and displaying complex temporal dynamics (440 trials). (e) Choice divergence (Online Methods) for auditory trials (green; average of 262 neurons) and visual trials (blue; average of 268 neurons), and modality divergence (black; average of 266 neurons). Shaded fills, s.e.m. (bootstrap). (f) Histogram of choice preference for auditory trials, measured 200 ms before decision end. Filled bars indicate neurons for which index was significantly different from 0 (\(P < 0.01\), 1,000 bootstraps). (g) Same as f but for visual trials. (h) Same as f,e, but for modality.

To achieve this, we used a second inactivation strategy: DREADD (designer receptor exclusively activated by designer drug), a pharmacogenetic inactivation method that permits visualization of the agent to determine its spread\(^{28}\). These effects were similar to the effects of muscimol inactivation in a second set of 2 rats: impairment of visual decisions (\(\text{Fig. 1f, } \sigma_{\text{saline}} = 3.89 \pm 0.64; \sigma_{\text{CNO}} = 5.29 \pm 0.71\)) and sparing of auditory decisions and multisensory integration (\(\text{Fig. 1g and Supplementary Fig. 2c,d};\) visual trials impaired: \(P = 0.011\); auditory trials spared: \(P = 0.91\), pooled across animals). Histological examination (Supplementary Fig. 3a–h) revealed that DREADD expression was minimal beyond the posterior border of PPC, defined as 5.0 mm posterior to bregma\(^{29}\). For one rat, expression was less than 0.12% of maximal expression; for the second rat, expression was 20.3% of maximal expression (Supplementary Fig. 3a–h; Online Methods). The more posterior expression was apparently not the source of the impairment because the rat with more expression posterior to PPC had weaker visual impairment compared to the other rat. We did not detect DREADD expression in other areas (Supplementary Fig. 3i). Overall, results were similar for all animals with both muscimol and DREADD inactivation. Effects were individually significant in 3 of 4 cases for visual trials and 0 of 4 cases for auditory trials.

Impairments on visual decision making might be driven by a change in the reliability of incoming visual signals or by a change in the animal’s decision-making strategy. Changes in decision-making strategy could include making ‘snap judgments’ that relied only on evidence presented at the beginning of the trial or ‘leaking’ evidence causing decisions to be made using only evidence presented late in the trial: using only evidence presented late in the trial. To distinguish changes in stimulus reliability from changes in strategy, we performed an analysis of animals’ decisions that took advantage of the ongoing fluctuations in visual and auditory rates that occur throughout the 1-s decision formation period (Online Methods)\(^{22,23,30}\). The analysis measures whether fluctuations at any given moment influence the eventual choice. The analysis generates a quantity termed “excess rate” that measures how strongly each moment in the stimulus influences the animal’s eventual choice. This analysis revealed no evidence of snap judgments or evidence leak. Instead, the analysis indicates that inactivation reduced the signal-to-noise of incoming evidence (Fig. 1h). No effects were observed on auditory trials (Fig. 1i and Supplementary Fig. 2h–m). The reduced excess rate on visual trials and unchanged excess rate on auditory trials also confirms the outcome of the previous analysis using a model-free approach that does not rely on fitted parameters.

The reduced excess rate over the entire course of visual trials suggests that inactivation reduced the reliability or signal-to-noise of visual signals. Behavioral experiments in which we reduced the brightness of visual flashes affected psychometric functions and excess rate nearly identically to these inactivations\(^{23}\). Taken together, our inactivation experiments and analyses suggest that PPC is required for accurate visual decision-making, perhaps by converting incoming visual signals into evidence for a decision. These observations point to PPC as causal for visual decision-making, laying the foundation for subsequent recording experiments that probe the functional organization and dynamics of cortical networks within PPC.

Choice and modality both modulate neural responses

To evaluate whether PPC neurons demonstrate mixed selectivity, we recorded from single, well-isolated neurons in the left PPC of five trained rats. Trials were grouped by modality and by the animal’s choice. Rare neurons had pure choice selectivity (Fig. 2a) or pure modality selectivity (Fig. 2b). However, most neurons mixed information about modality and choice (Fig. 2c,d and Supplementary Fig. 4). For such neurons, the mixing sometimes resulted in identical firing rates for different conditions (Fig. 2c).
and visual stimuli (Fig. 2c; multisensory in Supplementary Fig. 5a). Stronger choice divergence was evident on multisensory trials at many points during the trial (Supplementary Fig. 5b,c); stronger choice divergence was also evident on easy versus more difficult trials (Supplementary Fig. 5d–f).

Responses on multisensory trials were usually well predicted by a linear combination of auditory and visual responses. Simple linear regression revealed that 80.1% of neurons (218 of 272 units) had a multisensory response that was better predicted by the auditory and visual responses than by the multisensory mean (assessed on left-out data). Across all neurons, a linear combination of visual and auditory responses accounted for a median 68.2% of the multisensory variance.

An additional index, choice preference, captured not only the magnitude of the choice divergence but also whether it was in favor of a high-rate or low-rate choice (Online Methods). Choice preference was significant 200 ms before movement in over a third of individual neurons for both auditory and visual trials (Fig. 2f,g: 35.5% and 37.3% of neurons were significant on auditory and visual trials, respectively; for multisensory, see Supplementary Fig. 5g). Strong choice preferences for both ipsi- and contralateral decisions were observed. For both auditory and visual decisions, a slight majority of neurons fired more in advance of ipsilateral as compared to contralateral choices (two-sided sign test; auditory, median choice preference = −0.061, P = 0.0011, N = 262 neurons; visual, median choice preference = −0.038, P = 0.0502, N = 268 neurons). Choice preferences computed during visual and auditory trials were strongly correlated (Supplementary Fig. 5h, N = 262 neurons, r = 0.74, P < 10^{-4}); preferences during each unimodal stimulus were also correlated with preference during multisensory stimuli (Supplementary Fig. 5i, N = 236 neurons; auditory, r = 0.668, P < 0.001; visual, r = 0.807, P < 0.001).

We next assessed the effect of stimulus modality on responses during decision formation. “Modality divergence” measured how strongly a neuron’s responses diverged for auditory versus visual trials (for example, Fig. 2b). Compared to choice divergence, modality divergence increased earlier and faster during the stimulus presentation but was weaker overall (Fig. 2e). “Modality preference” captured not only the magnitude of the modality divergence but also whether it was in favor of visual versus auditory stimuli. A third of the neurons (33.8%) had significant modality preference (Fig. 2h). Visual-prefering and auditory-prefering neurons were observed in nearly equal numbers (Fig. 2h; median modality preference was 0.017 and did not differ significantly from 0; P = 0.888; N = 269 neurons). For both choice and modality, similar results were achieved when we assessed selectivity using a rate-based rather than ROC-based analysis (data not shown).

**PPC is category-free**

The data thus far indicate that many individual neurons are strongly modulated by modality or choice. We next investigated how frequently individual neurons had mixed selectivity for modality and choice. If mixed selectivity is common, many neurons should have a nonzero choice preference and a nonzero modality preference. This is exactly what we observed (Fig. 3a). Neural responses were not restricted to pure selectivity. Instead, most neurons had mixed selectivity for modality and choice. Moreover, a major component of the mixed selectivity was linear: that is, when predicting the neuron’s response to a given choice and modality (for example, a high-rate choice for visual stimuli), linear sensitivity to each task parameter alone was more important than a nonlinear interaction between parameters (Supplementary Fig. 6a). The nonlinear component we observed in
Figure 4 Choice and modality can be decoded from population activity. (a) Weighted sums of neural responses; weights were chosen by the classifier. Blue, visual; green, auditory; dashed lines, high-rate trials; solid lines, low-rate trials. Data from rat 4, N = 94 neurons. (b) The choice decoder could correctly classify responses as left versus right on trials where the rat was successful (bright red traces, one per rat), but is at chance for auditory versus visual (blue traces). On trials where the rat chose the incorrect port, the decoding tracked the rat’s choice (brown traces). Traces reflect the average of 1,000 classifications. (c) Same as b for all five rats, correct trials only. Each animal has one trace for modality and one for choice. (d) Bars, values of the weights used to generate the traces in a, ordered by magnitude. Purple lines, values of randomly generated 94-dimensional vectors ordered by magnitude. (e–h) Same as a–d but for the modality decoder. The modality decoding was nearly identical whether the rats chose the correct or incorrect port (f, light versus dark blue).

PPC was smaller and more variable than for neurons in the prefrontal cortex\(^19\), perhaps suggesting that nonlinear mixed selectivity emerges gradually across cortical areas or depends on the nature of the task.

The existence of individual cells with mixed selectivity would be expected under two scenarios: response features might be randomly distributed across PPC neurons, or particular response features might cluster together, defining categories of neurons that are specialized for particular computations. The data (Fig. 3a) hint that choice and modality selectivity are randomly distributed across neurons. For example, choice and modality preferences were uncorrelated \((N = 268\) neurons, \(r = 0.074, P = 0.23\)). This is in keeping with studies from monkey PPC in which selectivity for spatial versus category parameters were likewise unrelated\(^15\).

However, lack of correlation does not conclusively rule out the existence of functional categories; selectivity for task features could still define categories. This could be the case if, for instance, the points in Figure 3a formed an “X” or formed clusters that were symmetrically arranged around the origin. We therefore wished to test whether neurons formed categories (broadly construed) or whether, instead, tuning for one feature was independent of tuning for others. To do so, we examined each neuron’s “feature vector”: the pair of values describing how strongly the neuron contributed to decoding choice and modality (see next section and Fig. 3b). Each neuron’s feature vector was compared with its nearest neighbors in this feature space. If some neural responses fell into categories, these neurons would tend to have closer neighbors in feature space than if there were not categories\(^31\). The distribution of nearest-neighbor angles for the population can thus distinguish the presence or absence of such neural categories. We used these nearest-neighbor angles to compute a statistic indicating whether the population had an excess of small nearest-neighbor angles. We term this statistic “PAIRS”: projection angle index of response similarity (Online Methods).

The PAIRS test did not indicate categories (Fig. 3c). The distributions of nearest-neighbor angles were statistically indistinguishable from a control distribution generated by randomly oriented two-dimensional vectors (for rat 5, PAIRS index = –0.052; \(P = 0.632\); Online Methods). No evidence for clear categories was present in any animal (PAIRS indices for rats 1–4: –0.135, 0.117, –0.080, –0.142; \(P\) values from Monte Carlo simulations: 0.236, 0.253, 0.399, 0.004; the one significant \(P\) value indicated less clustering than expected by chance). This observation is critical: it argues that neurons with pure selectivity (for example, Fig. 2a,b) are exceptions and occur about as often as would be expected by chance.

This analysis argues that choice and modality selectivity do not define categories. However, this leaves open the possibility that there are categories defined by other features of the data. More generally, a category might be defined by a shared pattern of firing rates across conditions and time. To test for this, we used principal component analysis to identify a set of neural response features that were not imposed by us. This version of the test is thus quite general because it captures whatever features of the responses were strongest and is sensitive to numerous such features (Supplementary Fig. 6b). The PAIRS test again pointed to a category-free population (Fig. 3d,e and Supplementary Fig. 6c). The overall lack of categories was not simply because neural variability caused our analysis to miss structure: when we introduced synthetic categories into the population with noise derived from the real neural data, a strikingly different PAIRS distribution was evident (Fig. 3e; Online Methods). Relatively
close neuron pairs were occasionally observed, but these differed from chance in only 1 of 5 cases (PAIRS index for rats 1–5: −0.011, 0.108, −0.038, 0.011, −0.007; P values from Monte Carlo simulations: 0.621, 0.001, 0.209, 0.491, 0.857; Supplementary Fig. 6c). These deviations from the random distribution indicate that a small fraction of neurons do have pairs in feature space. However, such neurons are rare; most neurons reflect a unique combination of response features. Most individual neurons participate in random combinations of response patterns: that is, they randomly mix task parameters and temporal response features.

Decoding choice and modality from a mixed population

Here we evaluate whether the mixed selectivity of PPC neurons poses any problem for decoding the key task variables. We first tested whether the animal’s choice could be decoded from the population response during decision formation. To achieve this, we used a machine learning classifier (support vector machine, SVM) as our decoder, trained with single-trial population responses for correct high-rate versus low-rate choices (Online Methods). The decoder successfully identified neural weights so that a weighted sum of the neural population was strongly choice dependent but mostly modality independent (Fig. 4a).

We trained the decoder using a portion of the stimulus epoch (500–700 ms after stimulus onset), then tested the decoder over the entire epoch. Time windows outside the training window test the generality of the decoder and probe the consistency of the population response throughout decision formation. We first examined both correct and incorrect choices for animals with sufficient error trials (2 rats). For both rats (Fig. 4b), PPC activity tracked the animal’s choice. As expected, decoder performance grew over the course of the trial. For all 5 rats (Fig. 4c), we examined correct-choice trials at the time point 700–800 ms after stimulus onset (outside the decoder’s training epoch). Decoder performance was significantly better than chance for all animals tested (Fig. 4c; decoding performances were 68.9%, 61.2%, 59.4%, 80.2%, 70.1%; 4 rats P < 0.001, rat 3 P = 0.002). The same decoder did not perform significantly better than chance when estimating stimulus modality (Fig. 4c; performance of 48.0%, 51.0%, 54.3%, 48.8%, 48.6%, all rats P > 0.2). Our decoding of choice was not perfect, but this is unsurprising: decoding was performed on a sample of tens of neurons out of many thousands, on a task where the animal’s performance is likely noise limited. Further, since the decoder indicated the opposite choice for error trials, this implies that correct choices could be distinguished from errors. Moreover, unlike in typical monkey experiments, the stimulus was not optimized for each neuron’s preference and neurons were not selected on the basis of tuning properties.

The analysis above was restricted to auditory and visual trials. We next tested the ability of the same decoder, trained only on unsensory trials, to classify performance on multisensory trials. Decoder performance was significantly better than chance for all animals tested.
Perhaps the neural state explores yet other dimensions (patterns of neural covariance) when the animal’s brain needs to perform a substantially different function. This could permit PPC to control what signals are routed to different areas at different times. As shown recently, exploiting additional dimensions can be particularly useful to control when movement should be produced. We searched for a signature of the neural states either aligning (using the same dimensions at different times) or exploring different dimensions during different epochs. To do so, we compared two moments in the trial where the animal’s behavior differed: during decision formation, when animals remain still to integrate sensory signals (Supplementary Fig. 1) and during movement, when they rapidly reorient their bodies to harvest a reward. Examination of PSTHs (Fig. 5a,b) shows that neural activity can differ substantially during decision formation and movement. For example, the neuron in Figure 5b has an elevated response during trials preceding a leftwards choice but is then suppressed during the leftwards movement used to report the choice. Such switching of preference from decision formation to movement was observed frequently (Fig. 5c).

To quantify the alignment of state spaces during decision and movement, we developed an analysis that we term variance alignment (Online Methods). The intuition behind this analysis is that if neurons’ firing rates co-vary in similar ways during decision and movement, then the dimensions that best capture the variance for one epoch will also capture much of the variance for the other epoch. The alternative is that the dimensions that account for much variance in one epoch will account for little variance in the other epoch: that is, that neurons will co-vary in completely different patterns from one epoch to the other. In this latter scenario, the neural state spaces for the two epochs can be described as misaligned.

We tested for alignment by computing an index that describes whether the dimensions that capture most of the variance during movement likewise capture the variance during decision formation. Crucially, this measure describes whether the neural state moves through the same dimensions (that is, maintains the same patterns of covariance), not whether the trajectories are similar within those dimensions. Four two-dimensional projections of decision epoch data are shown in Figure 5d–g. A projection onto the first two principal components is shown in Figure 5d. For comparison, we can view different two-dimensional projections of the exact same data, with the projection chosen based on the movement-epoch activity (Fig. 5e), a random projection (Fig. 5f), or the smallest two principal components (Fig. 5g). The key element of these plots is the size of the ellipse representing the s.d.: if a space captures the decision-epoch variance well, then the s.d. should be nearly as large as in Figure 5d. Surprisingly, we found that dimensions that captured considerable variance during the movement captured the variance during decision formation far less well (Fig. 5e), arguing against aligned state spaces for decision formation and movement. Indeed, the amount of alignment was slightly less than would be expected by chance (Fig. 5h). This absence of alignment (index near zero) or even significant misalignment (index near −1) was present in all animals tested (rat 1, index = −0.498, P = 0.0012; rat 2, index = −0.145, P = 0.51; rat 3, index = −0.559, P = 0.003; rat 4, index = −0.230, P = 0.27; rat 5, index = −0.219, P = 0.20).

For comparison as a positive control, we repeated the variance alignment analysis on neural responses during decision formation, comparing two different stimulus conditions: visual and multisensory. This analysis, by contrast, revealed strong alignment (Fig. 5i, index near 1 for rat 4; index = 0.840, P < 10−4), present in all rats tested (indices were 0.644, 0.883 and 0.812 for rats 1, 2 and 3, P < 10−4 in all cases). This strong alignment indicates that the inherent noisiness of neural responses does not cause neural states to falsely appear.
neurons are shown to reflect random combinations of task parameters, for the existence of categories and design appropriate analyses if interpreting population data. Specifically, future studies can test directly defined categories are functionally diverse. By demonstrating ally specific, although many other studies report that connectivity—

As a caveat, we note that neural categories defined by other properties, challenges these assumptions about the organization of cortical structures. 

DISCUSSION

We used a multisensory decision task to understand the organization and dynamics of PPC, an area that we demonstrate to be causal for visual decisions. We found that PPC neurons have mixed selectivity for two task parameters: the animal's developing choice and the modality of the stimulus. We used a new test, PAIRS, to demonstrate that task parameters and time-varying response features are distributed randomly across neurons. This configuration does not pose a problem for decoding: a linear SVM could accurately estimate the modality of the stimulus and the rat's choice on the basis of single-trial responses. A final analysis further demonstrated the flexibility of the population: our variance alignment test revealed that the network explores different dimensions during decision and movement. This may allow PPC to translate the decision about rate into an abstractly related action. Taken together, these results point to PPC neurons as a category-free population that is combined dynamically as the behavioral demands of a complex decision evolve.

Theoretical motivations for functionally specialized neurons, and their existence in early visual areas, has driven a widespread assumption of categories that has influenced both experimental design and analysis. Our finding of a category-free neural population challenges these assumptions about the organization of cortical structures. As a caveat, we note that neural categories defined by other properties, such as cell type or connectivity, might reveal specialization. Indeed, a few studies have found projection-based categories that are functionally specific, although many other studies report that connectivity-defined categories are functionally diverse. By demonstrating here that cortical areas can lack categories defined by selectivity to task parameters or by time-dependent response features, our findings invite a new approach to interpreting population data. Specifically, future studies can test directly for the existence of categories and design appropriate analyses if neurons are shown to reflect random combinations of task parameters, as they are here. 

Although individual neurons reflected random combinations of task components (as predicted by theory), the observed responses were nonetheless structured. Specifically, we observed that most neurons that were driven by choice had ‘tolerance’ for modality: they retained their choice preference whether the stimulus was auditory, visual or multisensory. Neurons in monkey inferotemporal cortex are likewise tolerant: many neurons have a preferred stimulus identity that is stable, though modulated, across many retinal positions. Indeed, our task configuration is reminiscent of that used to study object recognition: just as a given object can be viewed from two different angles, a ‘low rate’ decision here can be informed by two different modalities. In inferotemporal cortex, the possibility of using the same linear decoder under many conditions, indicating tolerance, is taken as evidence that the neural data has been reformatted from an original, ‘tangled’ representation in earlier sensory areas. In PPC, analogously, a linear decoder was capable of reading out the animal’s choice independent of modality. Combined with evidence that PPC responses are nearly linear functions of choice and modality, this suggests that PPC may likewise be at an advanced stage of processing where representations have been untangled to guide decisions. To obtain such a representation, multiple stages of reformating may be required; this may explain the surprising prevalence of multisensory neurons in early sensory areas.

In our study, as in primate work, neural responses in PPC seem likely to reflect a process of transforming ambiguous sensory information into action. As in primate vision studies, responses gradually diverged according to the eventual decision outcome; the response divergence had a long latency, but was evident many hundreds of milliseconds before the animal reported the choice. This was true for both auditory and visual decisions. Primate PPC neurons are active in advance of movements driven by auditory stimuli as well. However, it was not known whether PPC neurons were causally involved. Our inactivation results were surprising in that auditory decisions were spared despite a clear signature of the developing choice in PPC neurons. Auditory responses in PPC, though apparently not necessary for this task, may be invoked by other decision tasks, such as those that require the animal to decide when to stop accumulating evidence, those that require a report of confidence or those that require temporally precise multisensory information.

Two methodological differences between our study and primate decision-making studies are notable. First, our stimuli were full-field rather than spatially restricted, and were related abstractly to their required movement (for example, low rate, move left). These features may explain why we found no bias for contralateral movements. Second, we recorded from all encountered neurons and used identical stimuli for each. The more traditional approach of using neuron selection criteria and customized stimuli is successful in identifying neurons with strong choice signals, but may leave unexamined neurons with subtler choice signals that nonetheless shape the evolving decision. This point is underscored by our observation that most of our neurons contributed to the choice and modality decoding, including neurons that were modulated only weakly by those parameters.

PPC thus represents multiple behaviorally relevant variables in the same population of neurons, with these representations structured in a way that could allow easy decoding by subsequent cortical areas—perhaps especially those that inform movements. These patterns of activity are dynamic and task dependent, and are determined by more than connectivity alone. This use of different patterns of activity could confer flexibility on PPC in converting stimuli into action, and it highlights the importance of understanding the population activity over the course of decision formation.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.
ACKNOWLEDGMENTS

We thank P. Znamenskiy, S. Jaramillo and T. Zador for technical advice, K. Rockland and A. Licata for help with histology, J. Sheppard and M. Ryan for help with electrophysiology and neural clustering, and M. Carandini and A. Rangel for providing input to early versions of the manuscript. Funding for this work was provided by US National Institutes of Health grants EY101972 and EY022979, the John Merck Fund, the McKnight Foundation, the Marie Robertson Memorial Fund of Cold Spring Harbor Laboratory, and a Swartz Foundation fellowship.

AUTHOR CONTRIBUTIONS

D.R. and A.K.C. designed the experiments. D.R. performed the electrophysiology and inactivations. M.T.K. developed the PAIRS, variance alignment and decode analyses. D.R. and M.T.K. analyzed the data. A.K.C. wrote the paper. All authors discussed the results and implications and commented on the manuscript at all stages.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprintsindex.html.

1. Barlow, H.B. Summation and inhibition in the frog's retina. J. Physiol. (Lond.)119, 69–88 (1953).
2. Kuffler, S.W. Discharge patterns and functional organization of mammalian retina. J. Neurophysiol.16, 37–68 (1953).
3. Britten, K.H., Newsome, W.T., Shadlen, M.N.,Celebrenzi, S. & Movshon, J.A. A relationship between behavioral choice and the visual responses of neurons in macaque MT. Vis. Neurosci.13, 87–100 (1996).
4. Nienborg, H. & Cumming, B.G. Macaque V2 neurons, but not V1 neurons, show choice-related activity. J. Neurosci.26, 9567–9578 (2006).
5. Rottman, J.D. & Shadlen, M.N. Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task. J. Neurosci.22, 9475–9489 (2002).
6. Balan, P.F., Driscolg, J., Schneider, D.M. & Gottlieb, J. Neuronal correlates of the set-size effect in monkey lateral intraparietal area. PLoS Biol.6, e198 (2008).
7. Georgopoulos, A.P.,Kalaska, J.F.,Caminiti, R. & Massey, J.T. On the relations and inactivations. M.T.K. developed the PAIRS, variance alignment and D.R. and A.K.C. designed the experiments. D.R. performed the electrophysiology and neural clustering, and M. Carandini and A. Rangel K. Rockland and A. Licata for help with histology, J. Sheppard and M. Ryan for help with electrophysiology and neural clustering, and M. Carandini and A. Rangel for providing input to early versions of the manuscript. Funding for this work was provided by US National Institutes of Health grants EY101972 and EY022979, the John Merck Fund, the McKnight Foundation, the Marie Robertson Memorial Fund of Cold Spring Harbor Laboratory, and a Swartz Foundation fellowship.

AUTHOR CONTRIBUTIONS

D.R. and A.K.C. designed the experiments. D.R. performed the electrophysiology and inactivations. M.T.K. developed the PAIRS, variance alignment and decode analyses. D.R. and M.T.K. analyzed the data. A.K.C. wrote the paper. All authors discussed the results and implications and commented on the manuscript at all stages.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprintsindex.html.

1. Barlow, H.B. Summation and inhibition in the frog's retina. J. Physiol. (Lond.)119, 69–88 (1953).
2. Kuffler, S.W. Discharge patterns and functional organization of mammalian retina. J. Neurophysiol.16, 37–68 (1953).
3. Britten, K.H., Newsome, W.T., Shadlen, M.N.,Celebrenzi, S. & Movshon, J.A. A relationship between behavioral choice and the visual responses of neurons in macaque MT. Vis. Neurosci.13, 87–100 (1996).
4. Nienborg, H. & Cumming, B.G. Macaque V2 neurons, but not V1 neurons, show choice-related activity. J. Neurosci.26, 9567–9578 (2006).
5. Rottman, J.D. & Shadlen, M.N. Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task. J. Neurosci.22, 9475–9489 (2002).
6. Balan, P.F., Driscolg, J., Schneider, D.M. & Gottlieb, J. Neuronal correlates of the set-size effect in monkey lateral intraparietal area. PLoS Biol.6, e198 (2008).
7. Georgopoulos, A.P.,Kalaska, J.F.,Caminiti, R. & Massey, J.T. On the relations and inactivations. M.T.K. developed the PAIRS, variance alignment and
ONLINE METHODS

Behavior. Animal training and behavioral task. Adult, male Long-Evans rats (approximately 250 g) were trained following previously established methods. Briefly, rats were trained to wait in the center port while stimuli were presented and to associate stimuli with reward ports. Stimuli for each trial consisted of a series of events: auditory clicks, full-field visual flashes, or both together. Stimulus events were separated by either long (100 ms) or short (50 ms) intervals. For the easiest trials, all inter-event intervals were identical, generating rates that were 9 events/s (all long intervals) or 16 events/s (all short intervals). More difficult trials included a mixture of long and short intervals, generating stimulus rates that were intermediate between the two extremes and therefore more difficult for the animal to judge. Rats 1–4 were rewarded with a drop of water for moving to the left reward port following low-rate trials and to the right reward port following high rate trials. Rat 5 was rewarded according to the reverse contingency.

For rat 1, the stimulus began immediately when the rat’s snout broke the infra-red beam in the center port. For rats 2, 3, 4 and 5, a variable delay separated the time of entrance to the port and the start of the stimulus. The length of this delay was selected from a truncated exponential distribution (rats 2–4: λ = 30 ms, minimum = 10 ms, maximum = 200 ms; rat 5: λ = 33 ms, minimum = 0, maximum = 1,000 ms) to generate an approximately flat hazard function. The total time of the stimulus was usually 1,000 ms. For rat 1, the stimulus ranged in duration from 500 to 1,000 ms.

Trials of all modalities and stimulus strengths were interleaved. For multi-sensory trials, the same number of auditory and visual events were presented. Our previous work has demonstrated that rats make nearly identical decisions regardless of whether stimulus events are presented synchronously or independently. Most neurons here, with the exception of a few sessions for rat 1, were recorded using synchronous stimuli. No obvious effect of synchronous versus independent stimuli on the neurons was apparent.

Animals typically completed between 500 and 1,200 trials/d. Most experiments had 18 conditions (3 modalities x 6 stimulus strengths), leading to ~27–67 trials per condition per day.

Analysis of behavioral data. Four-parameter psychometric functions were fit to choice data using the psignifit version 3 toolbox for MATLAB (http://psignifit.sourceforge.net/), following the maximum likelihood methods described by Wichmann and Hill. Psychometric functions were parameterized as

\[ f(r, \mu, \sigma, \gamma, \lambda) = \gamma + (1-\gamma-\lambda) \left[ 1 + \text{erf} \left( \frac{\mu - r}{\sigma \sqrt{2}} \right) \right] \]

where \( r \) is the trial event rate, \( \mu \) and \( \sigma \) are the first and second moments of a cumulative Gaussian function, \( \gamma \) and \( \lambda \) are the guessing and lapse rates (constrained so that 0 \( \leq \) \( \gamma \), \( \lambda \) \( \leq \) 0.1), and erf is the error function. \( \sigma \) is referred to as the psychophysical threshold; smaller \( \sigma \) results in a steeper psychometric function. Standard errors for \( \sigma \) were computed via bootstrap analysis of the choice data (2,000 resamples). To assess the magnitude of inactivation effects, we took the ratio of \( \sigma \) values computed for pairs of inactivation versus control days. This ratio is termed the impairment ratio (Fig. 1c,e,g). Values > 1 indicate impaired performance on inactivation days relative to control days.

The excess rate analysis, described in detail elsewhere, complements the psychometric function as a means of quantifying the animal’s decision-making behavior. The idea is to relate momentary fluctuations in the instantaneous rate with the animal’s choice by computing a quantity termed “excess rate” in sliding 200-ms windows in the trial. Consider an example window from 0–200 ms after stimulus onset. Three steps are required. First, we select all trials in which the rate outside the window (for example, 200–1,000 ms) is neutral. The resulting group of trials differ only in the stimulus rate presented from 0–200 ms. Next, we separate trials into groups where the rat made a left versus right choice. Finally, we average the rate for each group and take the difference in rate between trials preceding right versus left choices. If the difference is zero, this indicates that trials preceding left and right choices were identical and that the time window under study did not influence the choice. Stimulus rates in excess of zero indicate that the window under study did influence the choice. This process is repeated for sliding 200-ms windows, generating excess rates for every moment in time. Excess rate for data is compared to a shuffled data set (thin black line on Fig. 1h,i and Supplementary Fig. 2h–m) in which trials are randomly assigned to a “left” and “right” pool.

Implants for electrophysiology. Custom implants were prepared in-house. Each assembly contained up to eight independently moveable tetrodes (nickel/chrome alloy wire, 12.7 µm, Sandvik–Kanthal). Tetrodes were connected to an EIB-36 narrow connector board (Neuralynx) mounted on the assembly. The assembly was secured within a plastic enclosure before implanting. Tetrodes were gold-plated to 300–700 kΩ at 1 kHz; one additional tetrode was used as an internal reference for electrophysiological recordings and plated to ~100 kΩ.

General surgical procedures. All rats subject to surgery were anesthetized with isoflurane and administered 5 mg/kg ketoprofen before surgery for analgesia. Isoflurane anesthesia was maintained by monitoring respiration and foot pinch responses throughout the surgical procedure. Ophthalmic ointment was applied to keep the eyes moistened throughout surgery. Lidocaine solution (~0.1 mL) was injected below the scalp to provide local analgesia before performing scalp incisions. 0.05 mg/kg buprenorphine was administered daily for postsurgery analgesia (usually 2–3 d). Animals that received implants or cannulae were trained before surgery and then recovered to normal performance. All surgical and behavioral procedures conformed to the guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Cold Spring Harbor Laboratory.

Injection surgery procedures. Two rats, 3–5 weeks of age, were anesthetized and placed in a stereotaxic apparatus (Kopf Instruments). Small craniotomies were made over PPC (3.8 mm posterior to bregma; 2.2, 3.2 and 4.2 mm left or right of midline). One of the rats was subject to unilateral injections (left hemisphere) and the second rat was subject to bilateral injections. Small durotomies were performed at each craniotomy and virus was pressure injected at depths of 400, 600 and 800 µm below the pia (140 nL per depth) using calibrated pipettes and a syringe (rate of ~1 nL/s). 2–3 min were allowed after injection at each depth to allow diffusion of virus. Adeno-associated virus expressing muscardin receptor hM4D-mCitrine under an hSyn (SYN) promoter (AAV5-hSyn-HAM4D-ires-mCitrine; construct provided by Bryan Roth, UNC; virus produced by UNC Gene Therapy Center) was used.

Cannula implant surgery. Rats were anesthetized and placed in the stereotaxic apparatus. Two craniotomies were made on each side of the brain; these were positioned to cover medial and lateral PPC on each side (4.0 mm posterior to bregma and extending from 2.0 to 3.6 mm left or right of midline). Durotomies were performed and a double guide cannula (PlasticsOne, C235G-1.2) was placed in the brain 100–200 µm below the pia at each craniotomy. The exposed brain was covered with 2% agarose solution and both cannulae were anchored to the skull with dental acrylic (Lang Dental).

Implant surgery procedure. After scalp shaving and incision, the skull was cleaned and anchoring screws were drilled into six locations on the skull. Dental cement (Parkell, Inc.) was applied to the skull surface and a craniotomy was made above the left PPC (4 mm posterior to bregma, 2.5 mm left of midline, –2.4 mm anteroposterior; –3.4 mm mediolateral in size). A durotomy was performed and the implant assembly was lowered until the tetrodes just penetrated the pial surface. 2% agarose solution was applied to cover the tetrodes and craniotomy, and dental acrylic (Lang Dental) was applied to secure the implant to the skull. The incision was closed around the base of the implant using Vetbond (3M). Following surgery, tetrodes were advanced in increments of 40–80 µm until action potentials were encountered.

Inactivation. Muscimol inactivation sessions. Muscimol was infused into PPC with a concentration of 0.5–1.0 mg/mL and a volume of 0.3 µL per site. A double internal cannula (PlasticsOne, C235I/SP), connected to 2-µL syringes (Hamilton microliter syringe, 7000 series), was inserted into each previously implanted guide cannula. Internal cannulae extended 0.5 mm below the guide (estimated 600–700 µm below the pia). Muscimol was delivered using an infusion pump (Harvard PHD 22/2000) at a rate of 0.1 µL/min. Internal cannulae were kept in the brain for 3–5 more minutes to allow diffusion of muscimol. Rats were removed from anesthesia and returned to cages for 30 min before beginning behavioral sessions. The same procedure was used in control sessions, in which muscimol was replaced with sterile saline.
DREADD inactivation sessions. Before DREADD inactivation and control sessions, clozapine N-oxide (CNO, 1 mg/kg) or sterile saline was injected intraperitoneally into rats expressing the muscarinic receptor hM4D. Animals were returned to their cages for 30 min after injection, before beginning behavioral sessions.

**Histology.** At the conclusion of physiological experiments, animals were deeply anesthetized with ketamine and medetomidine. To indicate the final positions of electrodes, electrolytic lesions were made at the tetrode tips by passing 30 μA current through each electrode for ~10–15 s. After lesioning, animals were perfused transcardially with 4% paraformaldehyde. Brains were extracted and postfixed in 4% paraformaldehyde for 24–48 h. After postfixing, 100-μm coronal sections of the brains were cut on a vibrotome (Leica).

At the conclusion of inactivation experiments, both animals that had been injected with DREADD were perfused transcardially with 4% paraformaldehyde. One of the brains was extracted, postfixed and sectioned following the protocol described above. The second brain was postfixed, then kept in 30% sucrose solution for 48 h, then frozen at −80 °C. 20-μm coronal sections were cut from this brain using a cryostat (Leica CM1850). In both cases, brain sections were mounted on slides with Vectashield mounting medium.

**Quantification of DREADD expression.** Brain sections were imaged using an epifluorescence microscope. The resulting images were analyzed with MATLAB software. A region of interest (ROI) was manually defined for each brain section that was analyzed. The ROI extended from ~1.5 to 5.0 mm lateral to the midline and −0.2 to 1 mm below the pia. A second, smaller ROI was defined near the first one, in a region that was not infected by the virus (which thus should have had no expression). This region was used as a measure for baseline pixel intensity. Average pixel intensity across columns of pixels was calculated inside the first ROI, then normalized by the average pixel intensity inside the second ROI (baseline). To quantify expression for a particular brain section, we calculated the area below the average pixel intensity curve for that section and above baseline (a flat line at unity). We used this measurement to compare the expression in two places: at the border between PPC and secondary visual cortex, and near the injection site in PPC (Supplementary Fig. 3a–h).

**Electrophysiological methods.** Electrophysiological data were collected daily while animals were engaged in the task. Spike-triggered waveforms were recorded from each tetrode using Digital Lynx SX hardware and Cheetah data acquisition software (Neuralynx, Bozeman, MT). Data were acquired with a sampling rate of 32 kHz, and spike waveforms were bandpass filtered at frequency ranges of 600–6,000 Hz. Tetrodes were moved 40–80 μm after each recording session to ensure that independent populations of neurons were sampled across sessions.

Monitoring of head and body orientation during recordings. We used two methods to monitor the animal’s orientation during electrophysiology sessions. First, we connected red and green LEDs to the animal’s implant and tracked head orientation throughout the behavioral session using Cheetah data acquisition software (Neuralynx, Bozeman, MT). LED positions were sampled at 30 Hz. Head angles were computed at each sample time and then smoothed with a Gaussian. For the second method, we used an open-source software package (BONSIA; G. Lopes, https://bitbucket.org/horizongr/bonsia/) to track the animal’s whole body orientation. Body angle was sampled at 100 Hz. The estimates produced using the implant LEDs and body tracking were generally in good agreement, although there tended to be more variability in body angle than head angle (for example, the rat’s head could remain stationary in the central port despite small body movements).

Analysis of electrophysiological data. Raw spike-triggered waveforms were manually clustered using MClust software (A.D. Redish, University of Minnesota) for MATLAB (MathWorks). Only isolated clusters corresponding to single neurons were included for analysis. Neural recordings were also trimmed or excluded if a portion of the recording had a strongly nonstationary mean firing rate over time, as based on automated criteria. In addition, neurons had to satisfy a signal-to-noise criterion. Specifically, the firing-rate range (over conditions and times) divided by the maximal s.e.m. (for all conditions and times) had to be greater than 3.3.

Peri-stimulus time histograms (PSTHs) were computed for two epochs in the trial: a decision formation epoch (the time during stimulus presentation and enforced central fixation) and a movement epoch. The spike trains for these were aligned to the stimulus onset or to the movement onset, respectively. Firing rates were averaged across like trials and smoothed over time with a Gaussian kernel (σ = 50 ms).

Data from 5 rats were analyzed. One animal in the cohort had stimulating fibers implanted alongside tetrodes. This animal was used as a control for a separate optogenetic study. For 9 of 18 electrophysiology sessions in this animal, laser stimulation (473 nm) was introduced through the fibers on 50% of trials. The animal expressed no light-activated ion channels in its brain, however, and laser stimulation had no effect on neural activity or behavior.

**Choice selectivity and modality selectivity.** PSTHs were constructed from spike trains by averaging firing rates in 10-ms bins and smoothing with a Gaussian kernel (σ = 50 ms). Correct trials were grouped according to two different aspects of the trials. The first way was based on the animal’s response: trials ending in a contralateral choice versus trials ending in an ipsilateral choice. The second way was based on the stimulus modality: visual trials versus auditory trials. We used ROC analysis to calculate the ability of an ideal observer to correctly classify the animal’s choice or the stimulus modality. This was done on each trial from the smoothed spike trains, at intervals throughout the trial. Choice and modality preference were derived from the area under the ROC curve (AUC) and defined for each time point as 2 × (AUC – 0.5); this value ranged from −1 to 1 (ref. 51). A choice preference of −1 indicates that a cell always fired more during trials ending in an ipsilateral choice; a value of 1 means that the cell always fired more during trials ending in a contralateral choice. Modality preference was computed separately for rightward and leftward trials and averaged. A modality preference of −1 indicates that a cell always fired more during auditory trials; a modality preference of 1 means that the cell always fired more during visual trials.

Choice divergence was computed the same way as choice preference except that each neuron was assigned a ‘preferred’ choice or modality based on its responses at the end of the trial (100–200 ms before movement onset). Choice divergence at other time points was computed on the basis of this preference. This is a closely related measure to the absolute value of choice preference, but this way of computing the index has the advantage that it prevents small fluctuations in selectivity due to noise (either positive or negative) at the beginning of the trial from giving the incorrect impression that the neuron is selective before stimulus onset52. For choice and modality preference, significance (P < 0.01, one-sided, Fig. 2f–h) was assessed via bootstrapping (1,000 iterations). A neuron was considered to have significant modality preference if this value was significant for either rightward or leftward trials.

**Analysis of response clustering.** To test for the presence of neural clusters, we developed the PAIRS (projection angle index of response similarity) analysis. To calculate the statistic, we first built a matrix of the trial-averaged neural data (the A matrix). This matrix had n rows by c columns, where n is the number of neurons, c is the number of conditions (6 conditions: 2 choices × 3 sensory modality conditions) and t is the number of time points in the decision epoch (including 300 ms before stimulus onset). We then reduced the dimensionality of this matrix using one of two methods. One method was to perform principal component analysis (PCA) on the A matrix, reducing the dimensionality (number of rows) to 8. This dimensionality was estimated from the data, and the results were not sensitive to the exact dimensionality used. For the alternative, ‘feature based’ method, we used the two dimensions specified by our choice decoder and our modality decoder. In this case, the two dimensions were orthogonalized using the Gram–Schmidt algorithm (since they were nearly orthogonal but not perfectly so) to ensure that they captured independent variance. The PCA method is assumed for further description below.

The coefficients matrix resulting from PCA is of size 8 × n; that is, each neuron received a single 8-element vector representing its response profile across conditions and over time. If a pair of neurons had similar response profiles, they would receive similar vectors (that is, the angle between the vectors would be small). For each neuron, we found the angle it made with each of its k most similar partners (for example, k = 3 partners). For each neuron, these k values were then averaged. This produced a distribution of near-neighbor angles, θ_data, with one angle per neuron. We took the median of this distribution, denoted θ_median, which will be small for data with strong clustering of neural responses or larger if there is little or no clustering.

For comparison, we generated 10,000 simulated data sets composed of n random vectors from a two- or eight-dimensional Gaussian distribution, as appropriate. For each simulated data set, we then computed the distribution of angles.
The neuron was related to four others. These faux neurons had exactly the same clusters introduced. To do so, we first selected one-fifth of our neurons at random. To avoid having overly large values of $P$ space was only two-dimensional and therefore the feature vectors were packed more tightly. To avoid having overly large values of $k$ (which would limit us to finding only large clusters), we chose a smaller value for the target angle: $\pi/8$. This produced values of $k$ from 9 to 24.

We also compared the neural data with synthetic data that had artificial clusters introduced. To do so, we first selected one-fifth of our neurons at random. For each neuron selected, we generated a quintet of ‘partner’ neurons related to the original. These partners were produced by resampling trials with replacement, then producing PSTHs as in the real data. In the resulting synthetic data, there were as many faux neurons as in the original data, but each faux neuron was related to four others. These faux neurons had exactly the same amount of noise as their originating neurons. This process was repeated 1,000 times. The distribution of the resulting $\theta_{\text{clusterrad}}$ values is plotted as the dashed line in Figure 3c.

Finally, we note that PAIRS is not only a test for clustering but also more generally for nonuniformity of the distribution of tuning across neurons. For example, if a strong majority of neurons ‘preferred’ the high-rate stimulus, then the neurons’ coefficient vectors would be mostly packed into half the space. The near-neighbor angles would therefore be reduced relative to fully random, and PAIRS would detect ‘clustering’ (properly, nonuniformity). While it is probably not possible to detect all conceivable ways in which the data might cluster, this method is a reasonably general test.

Decoding neural responses. The goal of the decoding analysis was to train a trial-by-trial classifier that could identify left versus right choices but was tolerant of modality or that separated auditory from visual trials but was tolerant of choice. That is, we looked for a weighted sum of neurons such that the result was high for one choice and low for the other regardless of modality, and a second weighted sum of neurons that was high for one modality and low for the other regardless of choice.

For a neuron to be included in this analysis, we required at least 20 successful trials each for all four choice/modality pairs (only visual and auditory modalities were used). Most of our neurons were not recorded simultaneously; we therefore constructed ‘pseudo-trials’ by choosing random trials of the desired condition (defined by choice and stimulus modality) for each neuron. The epoch from 500 to 700 ms after stimulus onset was used for training the classifier. We assembled as many pseudo-trials as possible by randomly sampling trials from each neuron without replacement; because the number of trials from each neuron was balanced across conditions when training the classifiers, the process was limited by the neuron with the fewest trials in any one condition (21–32). We then used a standard machine learning technique, the linear support vector machine (SVM), to train one classifier for choice and a second for modality. Training was repeated 1,000 times with different random samples of pseudo-trials, resulting in 1,000 trained classifiers each for choice and stimulus modality. These were combined using a standard technique called bootstrap aggregation (‘bagging’; ref. 53, described below).

Each of these classifiers is a vector consisting of a set of linear weights, with one weight per neuron. We averaged the 1,000 trained classifier vectors to obtain a final classifier orientation for choice and a final classifier orientation for modality. In addition, the classifiers required a threshold. To obtain the thresholds, we randomly sampled additional balanced sets of pseudo-trials, projected them onto our classifier vector and found the optimal threshold on the basis of Gaussian fits to the two classes (high versus low rate or visual versus auditory). Classifier thresholds were averaged across 25 iterations of this process.

To test the performance of the classifier, we randomly sampled more sets of pseudo-trials. Spike trains were filtered with a 100-ms boxcar, then each time point was classified. This was repeated 1,000 times and performance was averaged. To assess statistical significance, we assessed generalization performance on the epoch from 700 to 800 ms after stimulus onset. Performance of the classifier on 1,000 pseudo-trials was compared with the performance of 10,000 random classifiers on the same number of pseudo-trials. To generate the random classifiers, we first chose a random vector with $n$ elements ($n$ being the number of neurons). Since different neurons have unequal variances and high-variance neurons will tend to be used more heavily by a trained classifier, we multiplied each element of the random vector by the s.d. of the corresponding neuron. $P$ values were two-sided. Additional cross-validation of the classifier was achieved by using classifiers trained on visual data and tested with multisensory data (Supplementary Fig. 7). This analysis also ensures that the success of the classifier outside the training window was not due to temporal correlations in the data because no temporal correlations will exist between data collected on different trials (multisensory versus visual).

To interpret the weights chosen by the classifier, we examined whether only a subset of neurons might be used heavily, or whether all the neurons were used. To do so, we compared the distribution of weights from the classifier found using the real data with the distribution of weights from the random classifiers described above (shown as purple lines in Fig. 4d,b). To evaluate whether the real classifier was significantly different from the random ones, we computed the kurtosis of the distribution of weights from the data and the kurtosis for each random classifier. To obtain a $P$ value, we could then compare the kurtosis for the real classifier’s weights to the distribution of kurtosis expected by chance. If the real classifier’s weight kurtosis differed from chance, this would indicate either that fewer neurons were strongly involved in the classifier as compared to a random classifier or that neurons contributed more uniformly to the classifier than expected by chance. Neither of these was observed (all $P > 0.1$, two-sided).

Variance alignment analysis. We initially reduced the dimensionality of the data as above to $k$ dimensions (chosen as 8) using PCA. This step de-noised the data. For this analysis, the $A$ matrix on which we performed PCA contained data from both the decision and movement epochs together; this ensured that the resulting space captured the structure of both epochs. We then determined the shape of the variance ellipsoid for the movement epoch alone (−200 to 800 ms from movement onset). That is, we rotated the data in the $k$-dimensional space so that the first dimension captured as much movement-epoch variance as possible, the second the next most, etc. This was accomplished using PCA on the ($k$-dimensional) movement-epoch data alone, retaining all components. The decision data were then rotated into this movement-determined orientation. For each dimension $d$ (1 to $k$, horizontal axis in Fig. 5h), we could then determine how much variance was present in the first $d$ dimensions of the decision data. These values were normalized by the maximum possible variance that could be captured in the same number of dimensions (that is, if the rotation were found using PCA on the decision–epoch data itself). Perfect alignment would produce a unity variance alignment value, while maximal misalignment defines the lower bound (that is, if the highest variance dimension during the movement epoch were the lowest variance dimension during the decision epoch). To determine the chance variance alignment, 10,000 randomly oriented orthogonal bases for the $k$-space were chosen. The confidence intervals shown in Figure 5h were derived from these random bases (not corrected for multiple comparisons).

To obtain a summary variance alignment index, we computed the area between the data curve and the chance curve. If the data curve was above the chance curve, the index was taken as positive and was normalized by the area between the perfect alignment curve and the chance curve. If the data curve was below the chance curve, the index was taken as negative and was normalized by the area.
between the chance curve and the maximally misaligned curve. The index thus ranges from −1 (maximally misaligned) to 1 (perfectly aligned). This index was also computed for each of the 10,000 random orientations. The resulting chance distribution was used to calculate a P value (two-sided).

For a control comparison, we repeated this analysis on two different modality conditions, visual and multisensory, during the decision epoch. This is a useful comparison because if activity patterns during the visual condition and multisensory condition are aligned, it demonstrates that the finding of chance-level alignment during decision formation and movement truly results from misaligned states and not noise. For this analysis, we determined the ordering of dimensions using data from multisensory trials. Then data from visual trials were rotated into the multisensory-determined orientation. This analysis was performed on rats 1–4; rat 5 was excluded because this animal had some neurons for which multisensory trial data were not collected.

To better interpret the result, we asked whether neurons that had strong stimulus-epoch modulation tended to have strong or weak movement-epoch modulation. To measure the depth of modulation for each neuron, we first created a vector containing the trial-averaged firing rate at each time point for each condition. For each neuron, there was one vector for the stimulus epoch (starting 300 ms before stimulus onset) and one vector for the movement epoch (−200 to 800 ms from movement onset). The variance of each vector was then taken. Since the resulting distributions were approximately log-normal, we took the log of these values before correlating them.

Testing for linear and nonlinear components of neurons' responses. We wished to test how much of neurons' tuning was a linear function of choice or stimulus modality and how much was a function of nonlinear interaction between choice and stimulus modality. To determine this, we considered one neuron at a time and analyzed only successful visual and auditory trials. Multisensory trials and failures were excluded. We first reduced the neuron's response on each trial to a single number: the spike count in a 200 ms window of time (600 to 800 ms after stimulus onset). The variance of each vector was then taken. For each row, the first element was always one, to capture the mean across trials. Each row summarized the conditions for the corresponding trial. For each row, the first element was always one, to capture the mean across trials. The second element captured choice preference, set to +1 for rightward trials and −1 for leftward trials. The third element captured modality preference, set to +1 for visual trials and −1 for auditory trials. The last element captured the interaction, set to +1 for visual rightward trials and auditory leftward trials, and −1 otherwise.

To find β, we performed a generalized linear model (GLM) regression with a Poisson noise distribution (since single-trial spike counts are typically assumed to have Poisson noise). In order to have orthogonal columns of X, it was necessary to have equal numbers of trials for each combination of left and right and visual and auditory. We therefore randomly downsampled trials to make these groups equal size before performing the regression. This was done 100 times for each neuron and the resulting β values were averaged. For analysis, we examined the two linear terms of β and compared them with the final, interaction term of β.

Statistics. The statistical tests used to evaluate each measure are listed in the corresponding sections above. A brief summary of the three types of tests is outlined here. First, nonparametric tests (Mann-Whitney U) were used to determine significance of the behavioral effects from inactivation. Second, bootstrap tests were used to measure the significance of the multisensory enhancement, the strength of choice and modality tuning, and decoder performance. Finally, Monte Carlo simulations were used to evaluate the PAIRS analysis and the variance alignment analysis. No statistical tests were done to predetermine sample size. The study was not blinded.

A Supplementary Methods Checklist is available.

49. Casarini, M. & Churchland, A.K. Probing perceptual decisions in rodents. Nat. Neurosci. 16, 824–831 (2013).
50. Wichmann, F.A. & Hill, N.J. The psychometric function: II. Bootstrap-based confidence intervals and sampling. Percept. Psychophys. 63, 1314–1329 (2001).
51. Feierstein, C.E., Quirk, M.C., Uchida, N., Sosulski, D.L. & Mainen, Z.F. Representation of spatial goals in rat orbitofrontal cortex. Neuron 51, 495–507 (2006).
52. Erlich, J.C., Bialek, M. & Brody, C.D. A cortical substrate for memory-guided orienting in the rat. Neuron 72, 330–343 (2011).
53. Breiman, L. Bagging predictors. Mach. Learn. 26 doi:10.1023/A:1018054314350 (1996).
54. Nelder, J.A. & Wedderburn, R.W. Generalized linear models. J. R. Stat. Soc. [Ser. A] 135, 370–384 (1972).