Distinct behavioural and network correlates of two interneuron types in prefrontal cortex

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Neurons in the prefrontal cortex exhibit diverse behavioural correlates1–4, an observation that has been attributed to cell-type diversity. To link identified neuron types with network and behavioural functions, we recorded from the two largest genetically defined inhibitory interneuron classes, the perisomatically targeting parvalbumin (PV) and the dendritically targeting somatostatin (SOM) neurons5–8 in anterior cingulate cortex of mice performing a reward foraging task. Here we show that PV and a subtype of SOM neurons form functionally homogeneous populations showing a double dissociation between both their inhibitory effects and behavioural correlates. Out of several events pertaining to behaviour, a subtype of SOM neurons selectively responded at reward approach, whereas PV neurons responded at reward leaving and encoded preceding stay duration. These behavioural correlates of PV and SOM neurons defined a behavioural epoch and a decision variable important for foraging (whether to stay or to leave), a crucial variable for foraging (whether to stay or to leave), a crucial variable important for foraging (whether to stay or to leave), a crucial variable important for foraging (whether to stay or to leave), a crucial variable important for foraging (whether to stay or to leave). Furthermore, PV neurons could fire in millisecond synchrony, exerting fast and powerful inhibition on principal cell firing, whereas the inhibitory effect of SOM neurons on firing output was weak and more variable, consistent with the idea that they respectively control the outputs of, and inputs to, principal neurons12–16. These results suggest a connection between the circuit-level function of different interneuron types in regulating the flow of information and the behavioural functions served by the cortical circuits. Moreover, these observations bolster the hypothesis that functional response diversity during behaviour can in part be explained by cell-type diversity.

To investigate whether distinct interneuron types can encode specific behavioural variables we recorded the activity of inhibitory neurons expressing parvalbumin and somatostatin markers (Supplementary Fig. 1a). PV basket cells are thought to control the spiking output of pyramidal neurons12–14, whereas most SOM interneurons, known as Martinotti cells (Supplementary Fig. 1c, d), target distal dendrites, gating the inputs arriving onto pyramidal cells15,16. To target these interneuron types for recordings, we used PV-Cre and SOM-Cre21,22 driver mouse lines in combination with adeno-associated viruses to deliver channelrhodopsin-2 (ChR2)23, rendering neurons light sensitive (Supplementary Fig. 1a, b). Miniature microdrives housing 6 moveable tetrodes and an optical fibre were implanted in deep layers of the anterior cingulate cortex (ACC) (Fig. 1a and Supplementary Fig. 1e–g). We recorded well-isolated single units (n = 1,339 from 6 PV-Cre and 6 SOM-Cre mice) and delivered brief pulses (1 ms) of blue light to elicit short-latency action potentials in ChR2-expressing neurons that served as a physiologic tag24 (Fig. 1b, c). To identify directly light-activated units we developed an optical-tagging test based on a statistical measure that yields a P value testing whether light-activation induced significant changes in spike timing (Fig. 1d and Supplementary Fig. 2, see Methods). Significantly activated units (P < 0.01) showed similar spontaneous and light evoked waveforms (correlation coefficient, r > 0.85, Fig. 1b and Supplementary Fig. 2c), low-latency light-induced response (< 4 ms), and low first-spike jitter (Fig. 1e, f), signatures of direct light-activation.

Extracellularly recorded units are traditionally classified based on spike width and firing rate, with narrow-spiking and fast-firing neurons categorized putatively as PV interneurons23–25. Indeed, most identified PV neurons were narrow-spiking with high firing rates (219 ± 10 ms, 31 ± 3 Hz, n = 23, Fig. 1e), whereas the spike-width distribution for SOM units was bimodal (Fig. 1e, bottom): a third of neurons had narrow spikes (‘NS’, < 270 ms) and high firing rates (212 ± 7 ms, 16 ± 4 Hz, n = 13) and the rest showed markedly wider spike waveforms and lower firing rates (‘WS’, 327 ± 7 ms, 4 ± 1 Hz, n = 22).

Having identified PV and SOM interneurons, we first examined their effect on local circuit activity. Synchronous photostimulation of ChR2-expressing PV or SOM neurons had markedly different network effects, with PV neurons imposing brief uniform inhibition on nearby neurons26, and SOM neurons exerting longer and more variable inhibition (Fig. 2a, b and Supplementary Fig. 3a, b). These differences cannot be accounted for by systematic differences in the number of photo-activated neurons (Supplementary Fig. 4) and indicate that SOM and PV neurons exert distinct inhibitory footprints on network activity.

Optogenetic identification of many individual interneurons, in combination with simultaneous recording from a large number of their neighbours, allowed us to investigate the physiological impact of different inhibitory subtypes during behavioural epochs without light stimulation. To identify possible functional connectivity between neurons, we computed cross-correlograms (CCGs)—counts of spike co-occurrences in the putative pre- and postsynaptic neuron pairs at different time lags27 (Fig. 2c). Significant short-latency interactions were rare among pairs of unidentified ACC neurons (3.2% inhibitory, 5.2% excitatory, 1.3% both, out of 2,945 pairs, bootstrap test with P < 0.001 used for all CCG significance testing). Remarkably, 5/7 pairs of PV neurons showed interactions with 3/7 firing in millisecond zerolag synchrony, and 4/7 inhibited each other (trough at 2.25 ± 0.5 ms, Fig. 2c and Supplementary Fig. 5a, c). PV neurons also showed a high prevalence of short-timescale correlations with unidentified neurons (38/152 pairs, P < 0.001, Fig. 2c and Supplementary Fig. 5c), often with detectible inhibition (trough at 2.39 ± 1.3 ms, 18/152 pairs, P < 0.001). These results demonstrate that the PV population is capable of millisecond synchronization with fast and precise inhibitory effect on local neural activity.

In contrast to PV pairs, we found no short-timescale correlations between SOM pairs (0/11, 7 WS–WS and 4 NS–WS pairs, Fig. 2c and Supplementary Fig. 5b), and the influence of both NS-SOM and WS-SOM on unidentified neurons was sparser and more diverse (15/169 pairs, inhibitory in 2/169, P < 0.001, Fig. 2c and Supplementary Fig. 5c). The weak observable effect of SOM neurons on the firing output of their neighbours could be due to dendritic inhibition generating input suppression, which is expected to be more difficult to detect using a cross-correlation approach. Thus, PV and SOM interneurons

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form distinct inhibitory networks: a fast, synchronous PV network generating strong, transient inhibition and an asynchronous SOM network with weaker effect on firing output.

We explored whether these cell-type differences in network function are also reflected in specific behavioural correlates. To engage neural ensembles in the ACC we used a task that incorporated cue-based prediction, temporal control of actions and reward foraging decisions (Fig. 3a). Mice were trained to run back and forth on a linear track between two platforms to collect water rewards; entering one platform (‘trigger zone’) enabled reward availability at the other (‘reward zone’). As mice ran back to the reward zone platform, reward size was cued by an auditory signal. This task mimics the self-paced timing of foraging behaviours and exploits the natural tendency of mice to trade staying in a rewarded, safe area with running on an elevated open track to enable future reward collection. Behavioural performance was sensitive to anticipated reward outcomes because on a subset (15%) of trials in which the cue and reward were omitted, mice slowed their speed during reward zone approach.

We examined the responses of a population of 1,034 neurons (from 4 PV-Cre and 6 SOM-Cre mice) in the task. Neurons responded at several behavioural events and modulated their firing by different behavioural variables. For instance, as expected of neurons in the ACC3,9,11, we found single neuron correlates of reward prediction, staying time, and reward outcome and size (Supplementary Fig. 6a). The firing of many individual neurons was selective for single as well as combinations of task variables without any apparent clustering of response properties (Supplementary Fig. 6b–e). Therefore, we used an unbiased approach to determine firing rate modulation patterns for the PV and SOM neurons, which focused our analysis on two behaviourally relevant events, reward approach and leaving (see Methods and Supplementary Fig. 6f). Similar to the example neuron (Fig. 3c), most recorded PV neurons (11/14) phasically increased their firing as mice left the reward zone (Fig. 3d and Supplementary Figs 7c and 8a, b). To test the homogeneity and specificity of these event-related response profiles we used a resampling approach and compared PV interneurons to the unidentified population (see Methods). We found that the temporal response profiles of the PV interneurons were homogeneous ($P < 0.01$, bootstrap test) and distinct ($P < 0.001$) compared to randomly selected groups of neurons (see also Supplementary Fig. 9d). Moreover, knowledge of PV identity carried approximately twice the information about the time course of responses than knowledge that a neuron is narrow-spiking, despite the fact that PV neurons tend to be narrow-spiking ($P < 0.05$, Supplementary Fig. 7b).

The firing of many SOM neurons was strongly suppressed at the time of reward zone entry (13/21, suppression index $< 0$, $P < 0.01$, permutation test), like the example neuron (Fig. 3e). Similarly, most NS-SOM neurons were suppressed upon entry into the reward zone (9/10, Fig. 3f, bottom, and Supplementary Fig. 8a, b). In contrast, WS-SOM neurons were activated at different moments in time, around the entry into the reward zone (Fig. 3f, top). These profiles were different from both the PV and the unidentified population (Supplementary Fig. 7a, d). Together with their local-circuit effects described above, these observations support the idea that SOM neurons comprise at least two functional subtypes18,19,20, a narrow-spiking, more...
simultaneously recorded neurons without significant short-term interactions with PV or SOM neurons (Fig. 3g, h). These results reveal that functional connectivity, as identified by millisecond cross-correlations indicative of anatomical connections, also predicts post-synaptic neural responses on the time scale of seconds as relevant for behaviour.

Finally, we sought to better understand the behavioural functions of the PV population. We wondered whether the phasic recruitment of PV neurons is related to a specific movement or reflects a more abstract behavioural variable (Supplementary Fig. 9c). Specifically, the ACC has been implicated in foraging decisions9,10—whether to stay or to leave. Therefore, we trained mice on a task version in which they were rewarded at a water port after a fixed 1 s delay from entry. In this task variant, the motor program required for the leaving action was a backward movement (Fig. 4a, cartoon), distinct from the forward movement corresponding to the reward zone exit in the original task version. In addition, this enabled more precise measurements of behavioural timing. Mice stayed inside the port for varying durations (2.0 ± 2.2 s) to consume water reward then exited to initiate the next trial. We found that PV neurons responded with a large phasic firing rate elevation around the time of exit from the reward port (Fig. 4d, Supplementary Fig. 8a, b; 11/12 neurons with activation index > 0, \( P < 0.05 \), permutation test). Because mice could freely exit at any time, we wondered if the activity of these neurons was modulated by the duration of their stay inside the reward port. Indeed, we observed that the firing rate of PV neurons parametrically increased with longer staying times on a trial-by-trial basis (Fig. 4a–d). A similar representation of stay duration has been found in monkey ACC during a foraging task, which was shown to signal the negative value of staying or equivalently the likelihood of leaving during foraging decisions5. This suggests that the graded phasic response of PV neurons in ACC is related to a foraging decision, to leave the reward consumption area and initiate a new run.

Our findings demonstrate that two major classes of interneurons not only provide distinct modes of inhibition but also display unique behavioural correlates, with temporal and functional specificity comparable to principal neurons. Out of the many behavioural events in the task, the homogeneous responses of PV and NS-SOM interneurons bracketed a defined epoch: from reward approach to leaving, and represented a specific behavioural variable, staying time at the reward zone, critical for foraging decisions, a central function attributed to ACC9,10. How can this temporal and behavioural specificity be

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**Figure 3 | Distinct behavioural correlates of PV and SOM interneurons.**

- **a.** Cartoon of mouse performing the reward foraging task. **b.** Average run duration for rewarded (Rew) and omission (Omit) trials (\( n = 63 \) sessions, \( P < 0.05 \), Mann–Whitney U-test). **c.** Spike raster and peri-event time histogram (PETH) for an identified PV interneuron aligned to time of reward zone exit. **d.** Top, z-scored PETHs of 14 PV neurons sorted by latency to half-peak firing (colours from blue to red indicate low to high normalized firing rate of neurons, respectively); bottom, mean z-scored response (shaded area indicates s.e.m.). **e.** Spike raster and PETH for a NS-SOM interneuron aligned to the time of reward zone entry. **f.** Top, z-scored PETHs of 21 SOM neurons. NS-SOM and WS-SOM neurons are separated. Bottom, mean responses for NS-SOM and WS-SOM neurons (shaded area indicates s.e.m.). **g.** Average PETH for PV interneurons (red, \( n = 4 \)) with significant inhibitory cross-correlations, (CC-partners, black, \( n = 5 \)) and non-CC partners (grey, \( n = 76 \)). **h.** Average PETH for SOM interneurons (blue, \( n = 3 \), CC-partners (black, \( n = 3 \)) and non-CC partners (grey, \( n = 34 \)).

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**Figure 4 | PV interneurons in the ACC signal stay duration at foraging decisions.**

- **a.** Mouse exiting the reward port (inset). Response of a PV neuron during reward port exit. Raster is sorted by staying time in the port and grouped into tertiles. Blue ticks denote water valve offset. PETH is shown for each tertile. **b.** Linear regression between firing rate of the neuron in a, (epoch indicated by a grey bar) and staying time is significantly positive (\( r = 0.16, \) slope, 3.63, \( P < 0.005 \)). **c.** Histogram of regression slopes for all PV neurons. Black bars indicate significant (\( P < 0.05 \)) regression. **d.** Top, z-scored PETHs of 12 PV neurons aligned to reward port exit sorted according to latency of half-peak firing. Bottom, average PETH for PV population grouped into staying time tertiles. L, M and S denote long-, medium- and short-staying times, respectively.
understood in the context of our current knowledge of interneurons? First, tuning specificity may arise from the dense, convergent local input these interneuron types receive, enabling them to ‘summarize’ local neural activity, which may be particularly high at the moments when a region is engaged in a task. Second, PV interneurons have been implicated in controlling pyramidal cell output, consistent with the synchronous firing and strong inhibitory coupling we observed. In contrast, SOM neurons are thought to gate long-range inputs to principal cells, and their asynchronous activation and weaker inhibitory impact on firing output is consistent with this role. In our behaviour, input and output regulation might be expected around the foraging decision, consistent with the observed suppression of NS-SOM interneurons during approach followed by the activation of PV interneurons at reward exit. Taken together, our findings suggest a conceptual model in which these interneuron subtypes specialize in temporally regulating the flow of information in a given cortical circuit during the behavioural events relevant to that area. In summary, these observations bolster the long-held hope that probing identified cell-types will reveal the intrinsic logic of cortical circuits under more natural behavioural settings.

METHODS SUMMARY

All procedures involving animals were carried out in accordance with National Institutes of Health standards as approved by the Cold Spring Harbor Laboratory Institutional Animal Care and Use Committee.

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

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Author Contributions D.K., S.R. and A.K. designed experiments, D.K. and S.R. set up and performed experiments. B.H. developed the optical tagging index. D.K., S.R., B.H. and A.K. analysed data and wrote the paper. H.T. and J.Z.H. generated SOM-Cre mice, discussed results and edited the paper.

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METHODS

Microdrive construction. We designed two microdrive models that enabled concurrent optical stimulation and recording of neuronal activity. The plastic frame for the drives was designed using AutoCAD Inventor (Autodesk) and 3D printed (Vista Technologies). In one microdrive model (3.5 g) the frame can house up to 10 independently moveable shuttles and is well suited to record and optically activate large population of neurons. Each shuttle has precision holes drilled to attach the tetrode and/or optical fibre and a miniature screw (0.6 mm outer diameter, 12 mm length) with a pitch of 160 µm. An electronic interface board (EIB32, Neurolynx) connects tetrodes to the preamplifier (HS366, Neurolynx). The fibre-optic probe for optical stimulation consists of a polyimide coated multimode fibre (60 µm diameter, Polymicro Technologies) glued into a fibre-optic ferrule (LC ferrule 80 µm, Precision Fibre Products). The ferrule end is polished using standard optical methods for efficient light coupling and the other end is precisely cleaved for insertion into the brain. The other microdrive model can house up to 5 independently adjustable shuttles and weighs 2.2 g when loaded with a single shuttle driving a bundle of 6 tetrodes and an optical fibre (Supplementary Fig. 1e).

Viral injection. Adeno-associated virus (AAV) 2/9 serotype (8 × 10^12 genome copies per ml, UNC Vector Core Facility) carrying EF1a-DIO-ChR2-EYFP or EF1a-DIO-Arch-EYFP construct23 was injected into 1-month-old PV-Cre and SOM-Cre male mice. Mice were anaesthetized with an intraperitoneal injection of a mixture of high frequency and low frequency tones, 0.1 s duration). Mice also received a reminder auditory cue immediately after exiting the trigger zone (Fig. 3a). On a small fraction of trials (15%) reward was omitted. Mice performed 60–200 trials per session lasting 1–2 h. Animals were kept on a water restriction schedule to maintain 85–90% of free-drinking weight.

Recording and optical stimulation of genetically identified interneurons. Electrophysiological recordings were performed using a Neurolynx Cheetah 32 system. All units were split and separately amplified and filtered for local field potentials (LFPs) and single unit activity. The signal was band-pass filtered between 600–6,000 Hz and sampled at 32 kHz to record spiking activity, while LFPs were filtered between 0.1–400 Hz and acquired at 3 kHz. We used 6 tetrodes and one optical fibre to record a total of 1,339 single units from 12 mice. Of these, 1,034 neurons (from 4 PV-Cre and 6 SOM-Cre mice) were recorded during the foraging task and 305 neurons (from 2 PV-Cre mice) were recorded in the port variant of the same task. We recorded a total of 28 PV cells from ACC in 5 PV-Cre animals (5, 5, 6, 2 and 10 cells from each animal out of 15, 14, 19, 24 and 41 respectively) and 35 cells from 6 SOM-Cre animals (14, 4, 3, 10, and 2 cells per animal with 29, 12, 16, 17 and 12 sessions respectively). In addition we recorded one PV-Cre animal that gave no tagged neurons. Neurons that had baseline firing rate <1 Hz or showed no activity during perievent periods (window size was specific for each event, see below) were excluded from behavioural analyses. An optical multimode fibre (55 µm diameter NA = 0.7, Polymicro Technologies) was coupled via a modified LC-LC type connector to a multimode fibre (126 µm diameter, numerical aperture = 0.27, CablesPlus USA), which collected light from a blue laser (473 nm; 20 mW; CrystaLaser). Maximal power at the tip of the fibre ranged from 10% to 30% of power at the light source resulting in 2–6 mW of total output at the fibre tip.

To avoid photo-electric artefact due to light stimulation37, we positioned our tetrodes parallel to the fibre and in cases where we saw an artefact, we minimized it by lowering the light intensity. We verified the validity of optical tagging by comparing the average peak-aligned spontaneous waveform with average light evoked waveform using Pearson’s correlation coefficient (r > 0.85).

The light stimulation protocol (15–30 min) for optogenetic tagging was performed at the end of each recording session consisting of 1–2 s light pulses at 4, 10, 16, 40 and 100 Hz frequencies. The fibre and tetrodes were lowered 20–40 µm every day after each recording session. At the end of the experiments, electrolytic lesion was induced expression we applied the same light for 1 h in 1 ms pulses at 20 Hz. The spread of light in photobleaching experiments was ~1,000 µm (dorso-ventral axis) by 500 µm ( medio-lateral axis). Maximum c-Fos induction occurred within a 0.5 mm² area (Supplementary Fig. 4a–c). Because our tetrodes were well within 500 µm from the tip of the optical fibre, light reach is not expected to be a limiting factor for optical tagging.

To evaluate the spatial extent of light on brain tissue we conducted (1) photo-bleaching experiments to measure the area with bleached fluorophore and (2) c-Fos staining around the fibre tip. For photobleaching experiments, blue light (473 nm, 2–4 mW power) was applied continuously for 1 h, whereas for c-Fos induced expression we applied the same light for 1 h in 1 ms pulses at 20 Hz. The spread of light in photobleaching experiments was ~1,000 µm (dorso-ventral axis) by 500 µm ( medio-lateral axis). Maximum c-Fos induction occurred within a 0.5 mm² area (Supplementary Fig. 4a–c). Because our tetrodes were well within 500 µm from the tip of the optical fibre, light reach is not expected to be a limiting factor for optical tagging.

The fibre was connected to a slip-ring commutator (PSR-27, Neuralynx) and counterbalance assembly to enable mice to run more freely. The counterbalance consists of a 40-cm boom moving freely on air bearings with a spherical and counterbalance assembly to enable mice to run more freely. The counterbalance consists of a 40-cm boom moving freely on air bearings with a spherical

Microdrive implantation. After anaesthesia a ~1 mm diameter hole was drilled through the skull at the site of viral injection. Animals received supplementary dose of anaesthetic at 30–90-min intervals to maintain depth of anaesthesia. The microdrive was positioned with the help of a stereotaxic arm (David Kopf Instruments) above the craniotomy with protruding tetrodes. The optical fibre and tetrodes were gradually lowered to a depth of 500 µm from the brain surface. Two 0.25-mm diameter stainless steel wires (Alpha Wire Company) were stripped at the end and inserted into cerebellum and right parietal lobe to a depth of ~1 mm below dura to serve as reference and ground electrodes respectively. Two mini-ature watch screws (Micro-Mark) were fixed into the pia mater plates as anchors. The microdrive was secured to the skull with ultraviolet light curable dental cement (Vitrebond Plus) followed by a layer of black dental acrylic (Lang with lidocaine. A small craniotomy was made above the left dorsomedial prefrontal cortex to allow diffusion of the virus. Animals were allowed to recover for at least 2 weeks for optimal viral expression.

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To reveal the morphology of SOM interneurons we used MADM36 to visualize the arborisation of these interneurons at single cell resolution.

Data analysis. All data analysis was carried out using built-in and custom-built software in MATLAB (MathWorks).

Spike sorting. Spikes were manually sorted into clusters (presumptive neurons) offline based on peak amplitude and waveform energy using MC瞭st software (A.D. Redish). Cluster quality was quantified using isolation distance36 and L-ratio18. Clusters with isolation distance <18 or L-ratio >0.2 were excluded (median isolation distance, 29; median L-ratio, 0.033, see Supplementary Fig. 2a, b). Autocorrelation functions were inspected for all putative cells. In cases in which the autocorrelation showed absolute refractory period violations, we improved cluster separation, otherwise, the cluster was excluded.

SALT. We developed a statistical test to determine whether optogenetic activation caused a significant change in the timing of spikes after stimulation onset (Supplementary Fig. 11). The distribution of first spike latencies relative to the light pulse, assessed in a 10 ms window after light-stimulation, was compared to epochs of the same duration in the stimulus-free baseline period. The choice of a 10 ms window was motivated by the provided sufficient statistical power without limiting the number of detected neurons. To measure the distance between these distributions, we used an information theoretic measure (modified Jensen–Shannon divergence)37. Using this metric, we tested the hypothesis that the post-stimulus spike-latency distribution is different from a set of baseline distributions for low frequency

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light stimulation (4 or 10 Hz) yielding a P-value for significant short-latency light-activation. (See Supplementary Notes for a detailed description and http://kepecslab.cshl.edu/software/ for MATLAB implementation). Note that we also employed a spike shape correlation measure to ensure that our spike sorting was not compromised due to high laser intensities9. This is a complementary test as it pertains to spike sorting, whereas SALT tests light effects assuming that spike sorting is correct.

**Detection of light-induced inhibition.** To detect light-induced inhibition we first determined the spike suppression period using adaptive smoothing procedure and then evaluated the statistical significance of the firing rate suppression compared to a stimulus-free baseline. First, spike rasters were convolved with a variable kernel Gaussian function to provide a spike density function (SDF) estimate. The kernel width of the Gaussian window was adapted to the local estimate of spiking probability to implement stronger smoothing when information was sparse. Variance was mapped onto spiking probability between 0 (moving average, corresponding to probability of 0) and infinity (Dirac-delta, corresponding to probability of 1). Next, minimal firing was assessed as the minimum of the SDF within 100 ms from light pulse onset. The baseline firing rate was calculated from mean firing probability within a 100 ms window before the start of a pulse train. If the minimal firing after stimulation was lower than 50% of baseline firing rate, then we determined the putative suppression period as the epoch between the half-baseline crossings before and after the minimum. The statistical significance of this suppression was determined by comparing the spike count distribution within this suppression period with an equivalent baseline period using the Mann–Whitney U-test (P < 0.05). Note that we used a 50% baseline minimum to provide sufficient statistical power to the spike rate comparison and to avoid false detection of random fluctuations in firing rate.

**Cross-correlation analysis.** Cross-correlations between spike trains were calculated using 1-ms bin size and their statistical significance was evaluated using a modified temporal shuffling method. To infer putative monosynaptic interactions from extracellularly recorded neurons it is critical to rule out co-firing arising from slow time-scale covariances, for instance due to common input or oscillatory modulations41. Under the assumption that spike trains are independent of one another, the shift predictor can be used to establish the expected level of firing co-occurrence. However, common input or other slow-time scale fluctuations can create trial-to-trial co-variations independent of synaptic interactions. We dealt with the issue of multiple time-scale effects in two ways. First, we used spectral filtering to remove slow time-scale interactions for which shuffle techniques are ill-equipped. This slow modulation was added back to the shuffled cross-correlations. The statistical significance of this slow modulation was added back to the shuffled cross-correlations to obtain estimates of cross-correlation that are not distorted by the filter on). Significance limits were assessed by upper and lower 0.005 percentiles of shuffled PETHs. Shuffling was performed with a similar method to cross-correlation analysis, with random shifts between the firing rates and the events ranging from 10 to 30 s, shuffling was performed 2,000 times. Response modulation index for each neuron and event was computed as the standard deviation of the PETH. To compute overall selectivity profiles, modulation indices for significantly modulated PETHs was averaged for different neuronal populations (PV, SOM and not tagged) for each event (Supplementary Fig. 6).

Hierarchical clustering was performed on response profiles with respect to three behaviourally relevant events (reward zone entry, reward zone exit and cue presentation) using squared Euclidean distance measure, averaged over the three events. Number of clusters was iterated ranging from 1 to 100 and the gap statistic was calculated to assess the number of clusters naturally present in the data set (see full description of this in Supplementary Notes).

**Activation and suppression indices.** The modulation indices (activation index for reward zone exit and suppression index for the reward zone entry events) were both calculated using receiver operating characteristic (ROC) analysis to provide a graded measure and a significance value associated with them46. These indices represent scaled version of ROC area (AUC) between two firing rate distributions before and after the event (window size, 0.4 s). We scaled the AUC so that it ranges from −1 to 1 with the sign denoting whether a neuron is activated or suppressed. Modulation index = 2 (ROCarea − 0.5) and ROCarea = \( \frac{1}{2} \left[ P(f<e) + P(f>e) \right] \) in which \( P(f<e) \) and \( P(f>e) \) refer to the firing rates before and after the relevant event. Statistical significance was evaluated using a permutation test, in which trial order was pseudo-randomly shuffled 1,000 times to yield a P value. Preference index. To compute preference index during various behavioural epochs, trials were divided into two groups according to cue (cue 1 and cue 2), staying time (shorter and longer than median staying time) and reward size (small and large reward). Firing rates within a fixed peri-event time window (200 ms for cue, 1 s for staying time, and 500 ms for reward preference) were compared using ROC analysis identical to activation and suppression indices. A significant cue preference index of less than 0 means that the neuron preferentially fires for cue 1, whereas more than 0 means preference for cue 2, similarly for staying time preference (long = −1, short = 1), and reward preference (small = −1, large = 1).

For the test of homogeneity, the within-group homogeneity of the PV population was computed by averaging pair-wise correlations (Pearson correlation coefficient) of z-scored PETHs aligned to the reward zone-exit event. This estimate of homogeneity was then tested against the average pair-wise correlation calculated for randomly selected groups of not tagged neurons with the same sample size (1,000 bootstrap samples).

For the test of firing rate modulation, the phasic positive modulation of PV neurons was quantified by the average correlation between PV PETHs aligned to reward zone exit and a template of event-locked firing rate increase. This template was computed as mean z-scored PETH of all positively modulated not tagged neurons (\( P(0.05 < e < 0.95, n = 107, \text{bootstrap test}) \)). This estimate of positive firing rate modulation for PV neurons was tested against a bootstrap sample of similar estimates for not tagged neurons in the same manner as for the ‘test of homogeneity’ (see above).

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