

# Divisions of Identified Parvalbumin-Expressing Basket Cells during Working Memory-Guided Decision Making

## Highlights

- PV+ basket cells do not fire homogenously during a delayed cue-matching-to-place task
- Their firing differentiates between distinct task episodes or choice behavior
- Firing of individual basket cells is correlated with their amount of VIP+ input
- Firing patterns are impaired during task performance without memory content

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## In Brief

Lagler et al. (2016) recorded from identified parvalbumin-expressing basket cells in prefrontal cortex while rats performed a delayed cue-matching-to-place task. They show that these interneurons segregate into neuronal ensembles with different firing patterns and synaptic connectivity differentiating task sequences and choice behavior.

# Divisions of Identified Parvalbumin-Expressing Basket Cells during Working Memory-Guided Decision Making

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## SUMMARY

Parvalbumin-expressing basket cells tightly control cortical networks and fire remarkably stereotyped during network oscillations and simple behaviors. How can these cells support multifaceted situations with different behavioral options and complex temporal sequences? We recorded from identified parvalbumin-expressing basket cells in prefrontal cortex of freely moving rats performing a multidimensional delayed cue-matching-to-place task, juxtagullularly filled recorded neurons for unequivocal histological identification, and determined their activity during temporally structured task episodes, associative working-memory, and stimulus-guided choice behavior. We show that parvalbumin-expressing basket cells do not fire homogenously, but individual cells were recruited or inhibited during different task episodes. Firing of individual basket cells was correlated with amount of presynaptic VIP (vasoactive intestinal polypeptide)-expressing GABAergic input. Together with subsets of pyramidal neurons, activity of basket cells differentiated for sequential actions and stimulus-guided choice behavior. Thus, interneurons of the same cell type can be recruited into different neuronal ensembles with distinct firing patterns to support multi-layered cognitive computations.

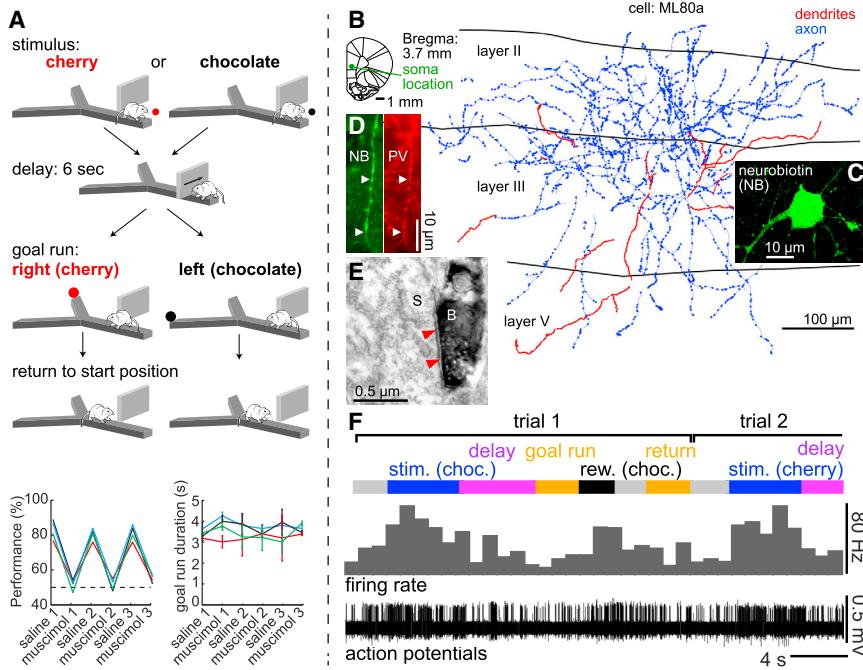
## INTRODUCTION

Environmental factors of rat habitats are constantly changing (Feng and Himsworth, 2014). Consequently, evolutionary pressure has favored cognitive flexibility, allowing rats to dynamically update strategies (Hamilton and Brigman, 2015). However, only few environmental changes are relevant and should induce cognitive flexibility, while the majority of changes are irrelevant, potentially represent noise, and should be ignored (Dayan et al., 2000; Granon et al., 1998; Otazu et al., 2009; Wimmer et al., 2015). Therefore, successfully directing behavior to a cur-

rent goal relies on an adequate trade-off between cognitive stability and flexibility (Cools, 2012). A brain region that has been frequently associated with cognitive control, balancing between stability and flexibility, is the prefrontal cortex (Cohen et al., 1996; Miller, 2000). Particularly, active stabilization of goal-relevant representation, a fundamental process of working memory, is one of the best-described functions of the prefrontal cortex (Goldman-Rakic, 1995; Jung et al., 2008).

In the prefrontal cortex of rats, memory-guided representations of goal-directed choice behavior are present in the form of temporally organized sequences of transiently active neurons (Fujisawa et al., 2008). Task-associated synchrony between prefrontal cortex, hippocampus (Jones and Wilson, 2005; O'Neill et al., 2013), and ventral tegmental area (Fujisawa and Buzsáki, 2011), as well as modulation of synaptic interaction-strength between pairs of prefrontal neurons (Baeg et al., 2007; Fujisawa et al., 2008), has been proposed to foster the formation of goal-representing cell assemblies. Remarkably, most of the working memory task-related short-term plasticity in the prefrontal cortex is composed of increased synaptic excitation converging onto putative interneurons (Fujisawa et al., 2008). Together with their well-documented role in synchronizing and gating information flow between distant brain regions including prefrontal cortex and hippocampus (Adhikari et al., 2010; Brockmann et al., 2011; Buzsáki et al., 2004; Hartwich et al., 2009; Tierney et al., 2004), this might point to the importance of GABAergic interneurons in supporting the stable formation of choice representation in the prefrontal cortex (Rao et al., 1999; Constantinidis et al., 2002).

The existence of a large diversity of distinct types of GABAergic interneuron is a hallmark of the cerebral cortex. Parvalbumin-expressing (PV+) basket cells provide precisely timed inhibition for controlling the output of pyramidal cells (Hu et al., 2014). Their firing patterns are highly stereotyped during different network oscillations (Klausberger et al., 2003; Lapray et al., 2012; Varga et al., 2014). Using transgenic mouse lines, it has been shown that neurons expressing PV (likely to contain multiple interneuron cell types) fire also with stereotyped firing patterns during initiation of actions (Kvitsiani et al., 2013) or during freezing in mice subjected to fear conditioning (Courtin et al., 2014). We asked how unequivocally identified PV+ basket cells in the prefrontal cortex might contribute to a multidimensional behavioral paradigm involving different behavioral



(D) Immunofluorescence micrographs showing neurobiotin-labeled dendrite (NB) positive for PV.

(E) Electron micrograph showing a labeled bouton (B) forming a type II synapse (arrowheads) onto a soma (S) of a putative pyramidal neuron.

(F) (Top) Each trial of the task was divided into seven consecutive episodes: pause, stimulus, delay, goal run, reward, post-reward, and return. Pause (waiting period before stimulus presentation) and post-reward (licking period after reward delivery has ended) are shown in gray. (Bottom) Stimulus-modulated firing of the labeled basket cell ML80a during the task. Note the cell's increased firing during stimulus presentation and reward consumption.

options, temporally structured task episodes, associative working memory, and stimulus-guided choice behavior.

## RESULTS

We recorded the activity of 11 identified PV+ basket cells in the prefrontal cortex of freely moving rats performing a delayed cue-matching-to-place task using glass electrodes. Rats received a stimulus (chocolate- or cherry-flavored solution) in the start arm of a Y-maze and, according to the stimulus, had to choose one of the side arms of the Y-maze to receive reward. In each trial, the execution of choice was delayed by 6 s with a moveable door (Figure 1A; Fujisawa et al., 2008). We confirmed the involvement of prelimbic cortex in this task with muscimol-inactivation experiments (Figures 1A and S1). For neuronal recording experiments, all rats performed the task with >85% correct choices. After recording neurons for >20 trials ( $25 \pm 2$  trials), the glass electrode was moved into a juxtacellular position for filling the recorded cell with neurobiotin (Lapray et al., 2012). This allowed unequivocal identification of the cell type as well as detailed assessment of the axo-dendritic arborisation and synaptic connectivity. The recording locations were within the rostro-caudal and medio-lateral axis of the prefrontal cortex ( $n = 10$ ), with the exception of one cell located within the anterior cingulate cortex. Somata of the identified PV+ basket cells were located in prefrontal layer II/III ( $n = 5$ ; Figures 1B, 1C, 2B, 2C, 3B, and 3C) or layer V/VI ( $n = 5$ ; Figure 4). The cells displayed smooth, multipolar, and radially oriented dendrites (Figures 1B, 2B, and 3B). All identified

**Figure 1. Stimulus-Modulated Firing Activity of an Identified PV+ Basket Cell in Layer III of Prelimbic Cortex during a Delayed Cue-Matching-To-Place Task**

(A) (Top) Delayed cue-matching-to-place task. Rats were trained to associate a stimulus (chocolate- or cherry-flavored solution) with a reward location (left or right) on an elevated Y-maze. After stimulus presentation, rats had to stay in the start arm for 6 s (delay) before they were allowed to execute their decision. (Bottom) Bilateral muscimol injection in prefrontal cortex reversibly impaired task performance ( $n = 4$  rats, colored lines). Probability of observing impaired task performance during three muscimol injections, each interleaved with 1 day of normal performance after saline injection in four independent experiments is expected as  $p = 1/(20^4) = 0.00000625$ . Goal run duration was unaffected by muscimol injection.

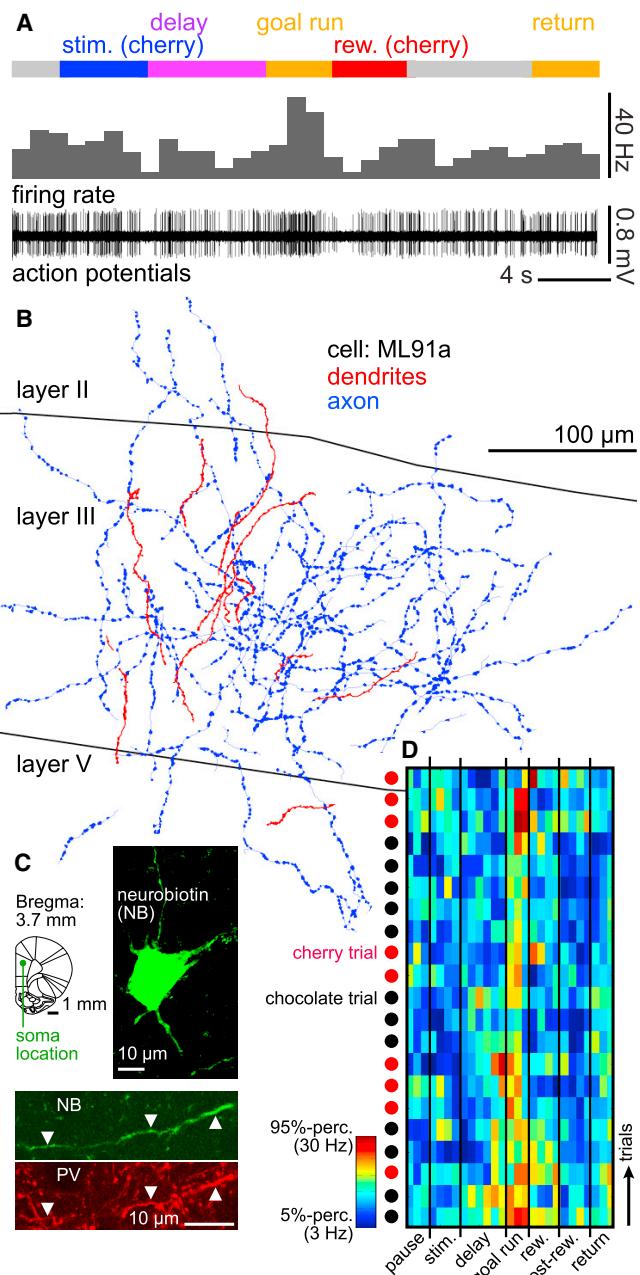
(B) Shown are dendrites (red) of the labeled cell spanning into layers II, III, and V (without the section containing the cell body). Axonal branching (blue) covers layers II, III, and V, with highest density in layer II (reconstructed from one 50 μm section adjacent to the section containing the cell soma).

(C) Labeled cell body was located in layer III of prefrontal cortex (confocal scan).

basket cells were tested PV+ on dendrites or somata (Figures 1D, 2C, and 3C). Their axonal arborisation was mostly restricted to the layer of the cell soma and neighboring layers (Figures 1B, 2B, and 3B). All interneurons presented in this study displayed a characteristically dense bouton distribution. Further light microscopic evaluation showed that the axon frequently formed boutons apposing cell somata and proximal dendrites ("basket-like" structures; Figure S2). We confirmed this observation by subcellular target identification using electron microscopy for the labeled cell ML80a (Figures 1E and S2).

## Individual PV+ Basket Cells Are Differently Recruited or Inhibited during Sequential Episodes of the Delayed Cue-Matching-to-Place Task

To compare the firing activity of different PV+ basket cells within and across animals, we defined seven consecutive, behaviorally distinct task episodes during each trial: pause, stimulus, delay, goal run, reward, post-reward, and return. The firing rate of each cell was calculated in 1 s windows, which can be allocated to different task episodes; each trial consisted of 27 time windows; goal runs and returns were divided into three windows, as they lasted on average 3 s. Investigating the firing patterns of PV+ basket cells during task performance, we observed that they did not fire at constant rates throughout the task, but instead strongly increased (Figures 1 and 2) or decreased (Figure 3) firing activity during one or more preferred task episodes, and they maintained such firing patterns during consecutive trials (Figures 2D and 3D). However, PV+ basket cells did not fire homogenously as a group,

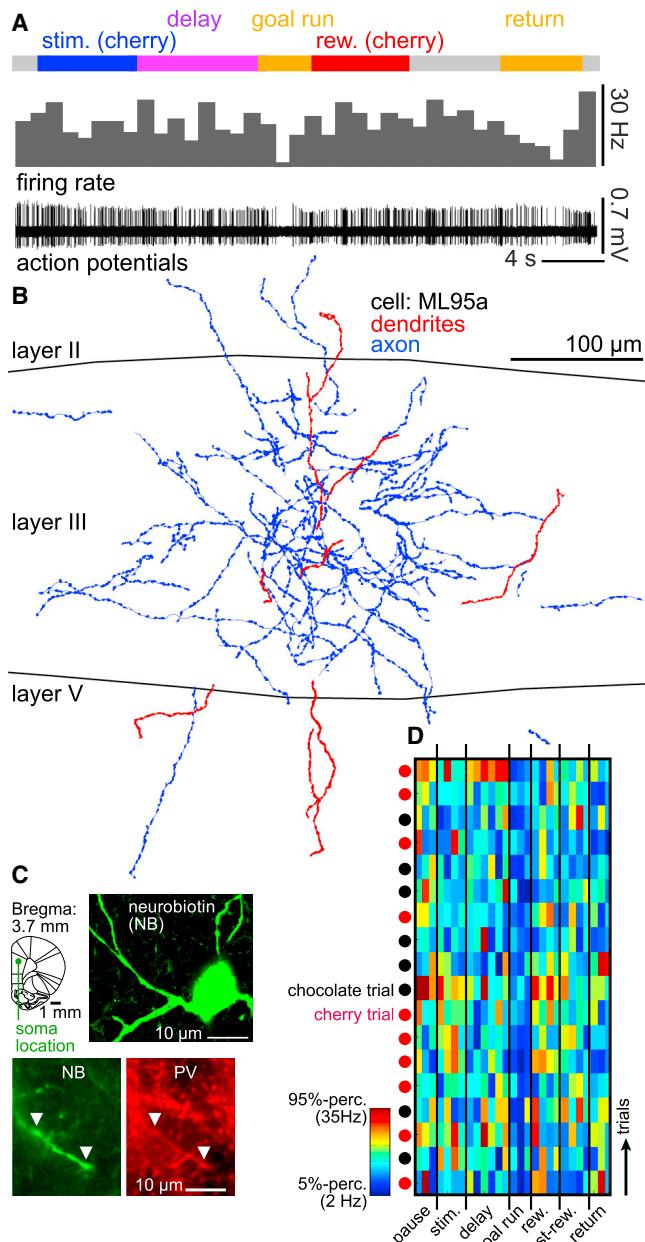


**Figure 2. Goal Run-Modulated Firing Activity of an Identified PV+ Basket Cell in Layer III of Prelimbic Cortex during a Delayed Cue-Matching-to-Place Task**

(A) Firing of a recorded and labeled PV+ basket cells during a trial of the task. Note the activation of the cell during goal run.

(B and C) Axo-dendritic arborization covering most of layer III, while sparsely entering layer II and V; dendrites (red), axon (blue) reconstructed from one section adjacent to the section containing the cell soma. Cell body was located in layer III of the prelimbic cortex (confocal scan). Neurobiotin-labeled dendrite (NB) tested immunopositive for PV.

(D) Increased firing of this PV+ basket cell during goal run is observed during each consecutive trial of the recording session.

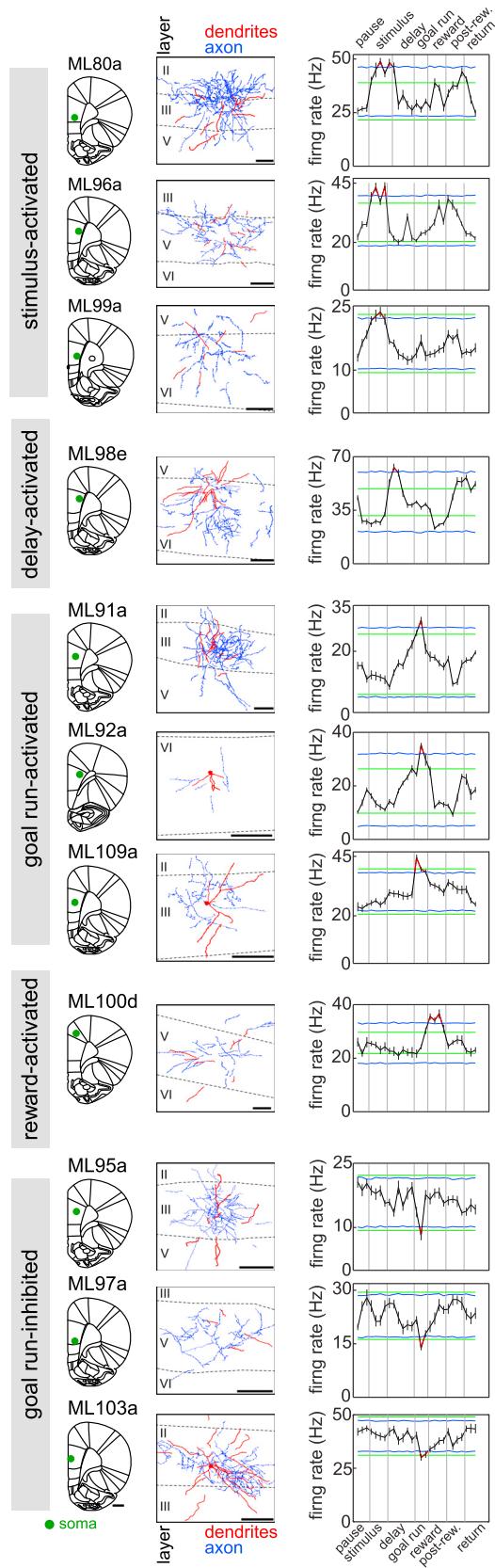


**Figure 3. Firing Activity of an Identified PV+ Basket Cell in Prelimbic Layer III Was Inhibited during Goal Run of the Working Memory Task**

(A) Firing of the labeled PV+ basket cell ML95a remained stable during most episodes of the working memory task but transiently decreased during goal run.

(B and C) Dendrites (red) spanning from layer II to layer V. Axon branching (blue) was most concentrated in layer III and sparsely entered layer II and V (reconstruction from one section adjacent to the section containing the cell body). Cell body was located in prelimbic layer III of the prelimbic cortex (confocal scan). Fluorescence micrographs showing neurobiotin-labeled dendrite (NB) was immunopositive for PV.

(D) Decreased firing of this PV+ basket cell during goal run is observed during each consecutive trial of the recording session.

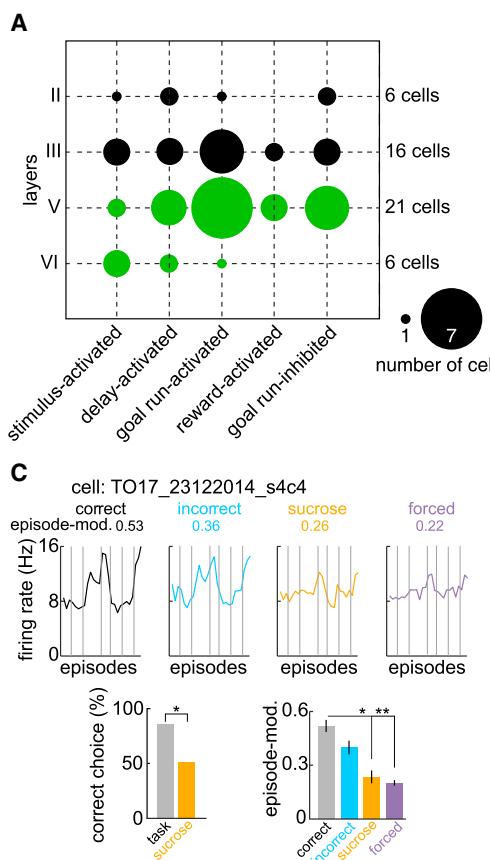


**Figure 4. Firing of Individual PV+ Basket Cells Is Differentially Modulated by Distinct Task Episodes**

Recorded and labeled PV+ basket cells were categorized by their firing during different task episodes. PV+ basket cells (dendrites, red; axon, blue; partial reconstructions) were located in different layers of prelimbic cortex; PV+ basket cell ML100d was recorded in anterior cingulate cortex. Scale bars represent the following: left, 1 mm; middle, 100  $\mu$ m. (Right) Firing of PV+ basket cells was strongly modulated by distinct task episodes. Significant (marked red) peak firing of different cells occurred during the entire sequence of task episodes; significantly reduced firing was observed only during goal run. Significance thresholds were calculated using a shuffling procedure performed on all correct trials followed by Bonferroni-correction of alpha values (blue lines) or were assumed as the mean firing rate  $\pm$  3 SD (green lines); see [Results](#) for further details. Data are shown as mean  $\pm$  SEM.

but individual neurons increased or decreased their firing during different task episodes ([Figures 1, 2, and 3](#)). To test these observations quantitatively, we employed three complementary statistical tests. First, we performed a shuffling procedure, and the measured firing rates of a cell during each 1 s time window were randomly assigned to any 1 s window of the same trial, a surrogate firing histogram was calculated from all trials, and the procedure was repeated to construct 10,000 surrogate histograms. Because of 27 windows used and to test for a possible activation or inhibition of firing rate, we accepted the firing rate of a window to be significantly activated or inhibited if it was larger or smaller than 99.9074% or 0.0925%, respectively, (Bonferroni correction for 27 windows and two-tailed) of the surrogate histograms in this window. We found that eight of the labeled PV+ basket cells significantly increased their firing rates during different task episodes, while the other three PV+ basket cells significantly decreased their firing rates, all during goal run ([Figure 4](#)). The p values for each cell and 1 s time window are stated in [Table S1](#). In a different type of analysis, we calculated the mean firing rate and standard deviation from the individual trials for each cell. A threshold for significant activation or inhibition was considered if firing above or below mean  $\pm$  3 SD (delineates 99.97% of a normal distribution) was observed. These thresholds resulted in similar observations compared to the shuffling procedure ([Figure 4](#)), supporting the robustness of these tests. In a third type of analysis, we tested whether the firing rates of each cell were different in the 27 time windows with a non-parametric and paired Friedman two-tailed test. All 11 PV+ basket cells were significantly different in their firing rates across the time windows ( $p < 0.0001$ ). A post hoc Dunn test indicated that those time windows, which were significantly increased or decreased with the shuffling procedure and the mean  $\pm$  3 SD thresholds, were most often tested significantly different from the firing rate of other time-windows ([Table S2](#)). Overall, three independent types of statistical analysis indicate that PV+ basket cells were activated or inhibited during distinct task episodes. Individual PV+ basket cells showed activation/inhibition during different episodes, suggesting sequential contributions to different task episodes by subsets of prelimbic PV+ basket cells.

When comparing firing activity of PV+ basket cells within and across layers of prelimbic cortex, we observed that cells with similar laminar position can have different firing patterns and cells with different laminar location can have similar firing patterns ([Figure 4](#)). To test this hypothesis, we recorded additional, putative



**Figure 5. Different Firing Patterns of PV+ Basket Cells Are Distributed within and across Prelimbic Layers**

(A) Layer location and firing patterns during the working memory task of 11 identified PV-basket cells and 38 putative fast-spiking cells. Note that in each cortical layer different firing patterns are present (horizontal rows). In addition, each firing pattern is found in different cortical layers (vertical columns).

(B) Comparison of the proportion of cells in superficial (II and III) layers with those in deep (V and VI) layers for activation of firing during stimulus and delay, activation during goal run and reward, and inhibition during goal run no statistical difference is observed ( $p = 0.934$ , Fisher exact test,  $n = 49$  cells).

(C) (Top) Firing rate of a putative fast-spiking interneuron shown over different task episodes and separated by different control conditions (incorrect trials, trials with sucrose instead of stimulus, and forced runs). Note the decrease in episode modulation during different control conditions. (Bottom) Choice performance dropped to chance level when stimulus was replaced by sucrose ( $\text{Chi}^2$ -test,  $p < 0.001$ ,  $n = 112$  trials with chocolate or cherry stimulus,  $n = 125$  trials with sucrose instead of stimulus). Interneurons significantly decreased episode-modulation during control conditions. (Friedman test, Dunn's post hoc test,  $*p < 0.05$ ,  $n = 5$ .)

(D) Simultaneous recording of an identified PV+ basket cell (glass electrode) and a putative fast-spiking interneuron (tetrode) with different episode-related firing. Note that different firing patterns occur simultaneously. Data are shown as mean  $\pm$  SEM.

PV+ basket cells (action potential width  $<0.3$  ms, firing rate  $>10$  Hz) for which the location in a cortical layer could be determined. These cells were recorded with a glass electrode in close proximity to another labeled cell and in the same electrode track, or with a silicon probe for which the location of the shanks and recording sites could be determined with histology (Figure S3A), or from tetrode experiments in which only a single tetrode was inserted into the prelimbic cortex and was not moved after recording the interneuron, allowing a reliable allocation of the recording site (Figures S3B and S3C). Firing patterns from these cells supported our observation that fast-spiking cells within the same cortical layer have different firing patterns and cells with the same firing patterns are observed in different cortical layers (Figures 5A and 5D). Comparing the proportion of cells in superficial (II and III) layers with those in deep (V and VI) layers for activation of firing during stimulus and delay, activation during goal run and reward, and inhibition during goal run, no statistical difference was observed (Figure 5B;  $p = 0.934$ , Fisher's exact test,  $n = 49$  cells). This suggests that different firing patterns are distributed within and across prelimbic layers rather than a laminar distinction of task-related firing patterns among prelimbic PV+ basket cells.

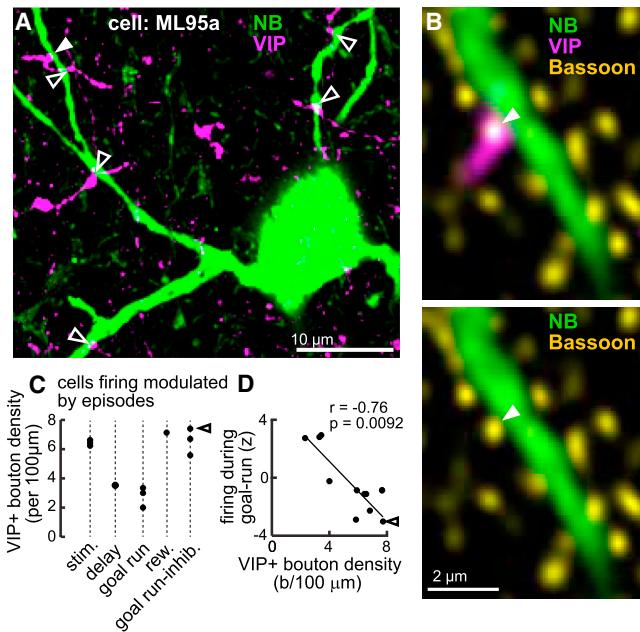
### Episode-Modulated Firing of Prefrontal Neurons Is Related to Task Content

In order to assess task dependence of the observed firing patterns in the prelimbic cortex, we performed a series of control ex-

periments, which were repeated on two recording days. First, we obtained a session of unaltered delayed cue-matching-to-place behavior with performance  $>85\%$ . Next, we omitted presenting information of stimulus by providing sucrose solution in the home arm (sucrose trials). Behavioral performance immediately dropped to random decisions (Figure 5C). However, during sucrose trials the rat still executed its own intentions. Therefore, in another control condition we prevented the rat from executing its own intention by blocking one arm and forcing the animal into randomly assigned reward locations (forced trials). We recorded the firing of prelimbic neurons throughout the different conditions with a silicon probe. We found significant reduction of task episode modulation of firing during sucrose and forced trials for putative interneurons (Figure 5C). Task episode modulation was calculated as maximum difference of z-scored firing from the mean. This indicates that observed firing patterns of prelimbic interneurons differentiating consecutive task episodes are related to the cognitive content of the working memory task rather than reflecting navigation along the maze only.

### Firing Patterns of Individual PV+ Basket Cells Are Correlated with the Amount of Their Presynaptic VIP+ Inputs

Next, we tested the hypothesis that the local connectivity profile of individual PV+ basket cells correlated with their firing patterns during task performance. GABAergic interneurons expressing



**Figure 6. Density of VIP+ Input onto Dendrites of PV+ Basket Cells Correlates with Their Firing during Goal Run**

(A) Confocal maximum intensity projection (35 optical sections, 12.66  $\mu\text{m}$  thick z stack) of VIP+ boutons apposing dendrites of neurobiotin-filled (NB) PV+ basket cell ML95a (arrowheads).

(B) VIP+ synaptic input to a dendrite of prefrontal PV+ basket cell ML95a. A puncta immunopositive for bassoon apposing a neurobiotin-filled dendrite (NB) co-localized with VIP (filled arrowhead, single optical section).

(C) Density of VIP+ boutons innervating PV+ basket cells sorted by the episode of significant modulation of firing (arrowhead indicates cell ML95a).

(D) Density of VIP+innervation onto dendrites of prefrontal PV+ basket cells and their firing during goal run were correlated. Cells that received enriched input from VIP+ boutons displayed lower firing during goal run (Spearman rank correlation,  $r = -0.76$ ,  $p = 0.0092$ ).

VIP have been described as exclusively innervating other interneurons (Hajos et al., 1996), and their importance for controlling activity of other interneurons in vivo has been suggested (Donato et al., 2013; Fu et al., 2014; Pfeffer et al., 2013; Pi et al., 2013). Most cortical PV+ interneurons receive VIP+ innervation; the degree, however, is highly variable (Dávid et al., 2007). Accordingly, we used immunofluorescence microscopy on the labeled neurons and found that prefrontal PV+ basket cell dendrites receive VIP+ (Figures 6A and 6B) input at various levels (Figure 6C). We observed that density of VIP+input onto dendrites of PV+ basket cells was negatively correlated with their z-scored firing during goal run, when different subsets of PV+ basket cells are either activated, inhibited, or do not change their activity (Figure 6D). These data suggest that the local connectivity of individual PV+ basket cells is related to their different firing patterns during the delayed cue-matching-to-place task.

#### Coupling of PV+ Basket Cells to Hippocampal Theta Oscillations during Goal Run

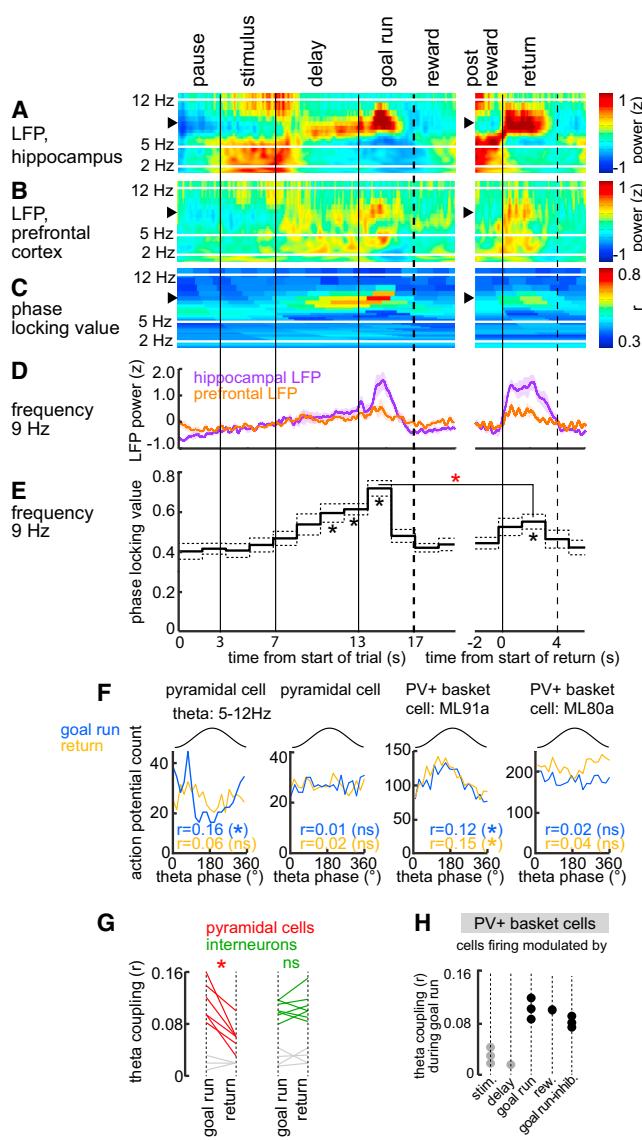
During cognitive behavior, when spatial information is integrated into a decision-making network, synchrony between hippocampus and prefrontal cortex is enhanced (Benchenane et al., 2010;

Jones and Wilson, 2005). Among prefrontal GABAergic interneurons, PV+ cells are preferentially targeted by hippocampal projections (Gabbott et al., 2002), and their firing is phase coupled to hippocampal theta oscillations in vivo (Courtin et al., 2014; Hartwich et al., 2009).

To detect possible coupling between firing of PV+ basket cells and network oscillations during the delayed cue-matching-to-place task, we placed a tetrode and a single wired metal electrode into layer II/III of prefrontal cortex and hippocampus, respectively, in addition to the glass electrode for juxtaglomerular recording. Hippocampal theta oscillations (5–12 Hz) were observed during the delay period and peaked during the execution of choice (goal run). After reward consumption, hippocampal theta oscillations were present when the animal returned to start location (Figure 7A). Theta oscillations recorded in layer II/III of prefrontal cortex were smaller in amplitude but followed the same time course during task episodes (Figures 7B–7D). Consistent with previously published observations (Benchenane et al., 2010; Jones and Wilson, 2005), we observed synchronization of theta phase between hippocampus and prefrontal cortex selectively emerging during goal run (Figure 7C). Hippocampal-prefrontal coherence was significantly higher during goal run compared to return (repeated-measurement ANOVA, Tukey's post hoc test,  $p < 0.05$ ; Figure 7E). Accordingly, we observed that prefrontal pyramidal cells were stronger coupled to hippocampal theta oscillations during goal run compared to return. However, the strength of theta phase coupling of PV+ basket cells was similar during goal run and return (Figure 7G). Interestingly, not all PV+ basket cells were coupled to hippocampal theta oscillations, but significant coupling was observed only in those cells which were activated or inhibited during goal run or reward (Figures 7F and 7H).

#### Neuronal Ensembles Consisting of Prefrontal Pyramidal and PV+ Basket Cells Differentiate between Task Episodes as well as Stimulus-Guided Choice Selection

For a delayed cue-matching-to-place task it has been reported that subsets of putative pyramidal cells differentiate their firing between right and left choices before the decision is executed (Fujisawa et al., 2008). While in monkeys such “memory” cells differentiate their firing for future choice throughout the trials (Goldman-Rakic, 1995), in rats sequential cell assemblies carry the information of future choices from stimulus to execution of decision (Fujisawa et al., 2008). We tested if PV+ basket cells contribute to representations of choice and observed that 6 out of 11 PV+ basket cells fired differentially during chocolate and cherry trials (Figure 8A; Table S3). This differentiation in firing rate between two choices occurred during different task episodes for individual PV+ basket cells. To test differential firing for chocolate and cherry trials statistically, we used a shuffling procedure, and each trial was randomly re-assigned as a chocolate or cherry trial. A significant difference in firing rate between chocolate and cherry trials in a 1 s time window of the measured data was accepted if this difference was larger than that of 99.8148% of the surrogate histograms in this time window. To account for 27 time windows, the alpha value was set to 0.05/27 = 0.00185 (Bonferroni correction). In a complementary statistical analysis, we used a non-parametric two-tailed Mann-Whitney U test to assess whether firing rates during chocolate



**Figure 7. Spike Coupling of Prelimbic PV+ Basket Cells to Hippocampal Theta Oscillations**

(A) Powerspectrogram of hippocampal local field potential (LFP) measured in the pyramidal layer of the dorsal CA1 region and calculated across different task episodes. Note pronounced theta oscillations (5–12 Hz) emerging during the delay period and reaching their peak during goal run and return.

(B) Powerspectrogram of prefrontal LFP measured in layer II/III of the prelimbic cortex.

(C) Hippocampal-prefrontal coherence measured as phase-locking value indicating transient epochs of interaction between the two regions emerging during delay, goal run, and return.

(D and E) LFP power and phase-locking value for a single frequency (9 Hz, black arrowhead in Figures 7A and 7B). Phase coherence between hippocampus and prefrontal cortex was significantly increased during the second half of delay, goal run, and return as compared to baseline/pause (black asterisk, Friedman test, Dunn's post hoc test,  $*p < 0.05$ ,  $n = 7$ ) and was significantly higher during goal run than during return (red asterisk, Friedman test, Dunn's post hoc test,  $*p < 0.05$ ,  $n = 7$ ). All spectra of power and phase locking value display averages over seven rats (one session each, only correct trials included).

(F) Histograms showing individual prelimbic PV+ basket and pyramidal cells with and without phase coupling to hippocampal theta oscillations during

and cherry trials were significantly different. To correct for 27 time windows, the alpha value was set  $0.05/27 = 0.0019$ . With this analysis, all neurons, which were tested significantly different with the shuffling procedure, were significantly different with the Mann-Whitney U test as well, supporting the robustness of the observation. Overall, these data show that the firing rate of some recorded and labeled PV+ basket cells differentiates between chocolate and cherry trials.

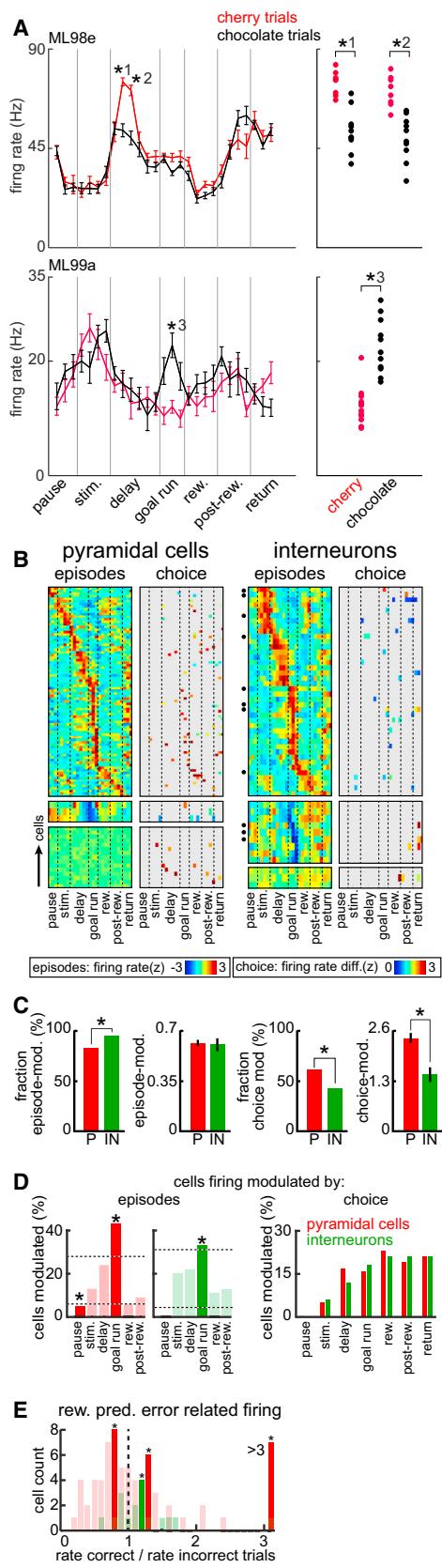
In addition to 11 identified PV+ basket cells, we recorded 41 fast-spiking, putative interneurons (action potential width  $<0.3$  ms, firing rate  $>10$  Hz) and 102 putative pyramidal neurons (action potential width  $\geq 0.3$  ms, firing rate  $\leq 10$  Hz) in the prelimbic cortex with a silicon probe, tetrodes (Figure S3), or glass electrodes (Figure 8B). The majority of pyramidal neurons ( $n = 87$  out of 102) and interneurons ( $n = 49/52$ ) exhibited firing rates that were significantly modulated by episode. Neurons with peak firing during different task episodes were often recorded simultaneously (Figure 5D). Nine out of 52 interneurons and 3/102 pyramidal cells had significantly reduced firing rates during part of the goal run, and 3/52 interneurons and 15/102 pyramidal cells did not fire modulated by episodes. Differential firing rates during chocolate and cherry trials, reflecting different stimuli and arm choices, were observed in 61/102 pyramidal neurons, and 22/52 interneurons. We found groups of putative pyramidal cells and interneurons differentiating their firing activity for all of the task episodes and arm choices (Figure 8B), including stimulus presentation and delay, when the animal has not executed its decision yet. We sorted cells with significant episode modulation of firing based on their episode of peak activity and observed that differential firing between chocolate and cherry trials often coincided with the episode of peak activity (Figure 8B).

On a population level, the firing rate of a larger number of interneurons was modulated by episode compared to pyramidal cells. Choice modulation of firing rate was observed more often in pyramidal cells than in interneurons, and choice modulation of significant cells was larger in pyramidal cells compared to interneurons (Figure 8C). Furthermore, we found that episode modulation of firing was not homogeneously represented by prelimbic neuronal ensembles. During goal run, significantly enriched fractions of putative pyramidal neurons and interneurons were recruited with significantly modulated firing (Figure 8D). Differences in firing rate during distinct arm choices were observed throughout the trial except for the pause when the animal has not yet been instructed by the stimulus (Figure 8D).

goal run (blue) and return (orange). Phase coupling was measured in a 5–12 Hz band. Vector length ( $r$ ) indicating coupling strength (Rayleigh test, ns, not significant;  $*p < 0.05$ ).

(G) For PV+ basket cells, coupling strength to theta oscillations did not differ between goal run and return (Wilcoxon signed ranks test [exact, two-tailed],  $n = 7$ ,  $p > 0.05$ ). Pyramidal cells were stronger coupled during goal run than during return (Wilcoxon signed ranks test, exact, two-tailed,  $n = 6$ ,  $p < 0.05$ ). Gray lines indicate that coupling of individual cells was not significant ( $p > 0.05$ ) during goal run and return. Cells that were not significantly coupled were not included in the statistical comparison between goal run and return.

(H) Theta coupling of PV+ basket cells during goal run sorted by the episode of significant modulation of firing. Gray and black indicate not significantly and significantly coupled cells, respectively.



**Figure 8. Neuronal Ensembles of Prelimbic Pyramidal and PV+ Basket Cells Differentiate Firing during Distinct Task Episodes or Choice Arm Selections**

(A) Firing patterns of two PV+ basket cells differentiating their firing for chocolate (black) and cherry (red) trials during periods of the working memory task. Significance was tested with a shuffling procedure and Bonferroni-correction;  $p = 0.000001$ ,  $p = 0.000001$ , and  $p = 0.00028$  for \*1, \*2, and \*3, respectively. (Right) Dots indicate firing rates during individual trials.

(B) Episodes: z-transformed firing rates (color coded) of putative pyramidal cells and fast-spiking interneurons, including identified PV+ basket cells (black circles), during different task episodes and sorted by the episode of significant modulation of firing. Choice: color indicates significant z score difference of firing rates during trials with left and right arm choice, with same order of cells as for episodes. (Upper block) Cells with significantly increased firing; (middle block) cells with significantly decreased firing during goal run; (lower block) cells lacking significant episode modulation. Note that on a population level the entire task episode sequence was densely covered, and the largest fraction of cells increased firing during the goal run.

(C) Comparison of the fraction of pyramidal cells (P) and putative interneurons (IN) with significant modulation by task episodes or choice of arm (Chi2 test, episode  $p = 0.0422$  and choice  $p = 0.0394$ , respectively); also, the magnitude of modulation is compared between significantly modulated pyramidal cells and interneurons (Mann Whitney U test, episode  $p = 0.8359$  and choice  $p = 0.00004$ ).

(D) (Left) Enriched populations of interneurons and putative pyramidal neurons have increased firing during goal run (shuffling analysis, dashed line represents 99.58% confidence interval, alpha value was corrected for multiple comparisons and two-tailed). (Right) Percentages of cells with differentiating firing for distinct arm choices are distributed over various task episodes except pause. (E) Firing of putative pyramidal cells and fast-spiking interneurons with reward prediction error. The ratio of firing rate during correct and incorrect trials was calculated within the first 500 ms after the rat poked into the reward port (shuffling analysis using 2.5%/97.5% confidence interval).

Additionally, we analyzed the firing rates of prelimbic pyramidal cells and interneurons during short time periods (500 ms) at the beginning of reward consumption (correct choices) or reward anticipation (incorrect choices). We observed significant fractions of prelimbic pyramidal cells and interneurons, whose firing patterns indicated reward prediction error (RPE). While the firing of pyramidal neurons reflected the presence as well as the absence of reward, firing of interneurons reflected only the presence of reward (**Figure 8E**).

Overall, these data suggest that sequential neuronal ensembles consisting of pyramidal and PV+ basket cells differentiate between task episodes as well as stimulus-guided choice-selection during task performance.

## DISCUSSION

How distinct types of neurons contribute with their temporal activity and synaptic connections to cortical network operations underlying cognitive behavior represents a key question in systems neuroscience. With the advent of novel tools, it has recently become possible to study how genetically defined subgroups of neurons fire during behavioral tasks. Because of the current shortage of cell-type-specific promoters, genetically defined subgroups often contain several distinct types of neuron. Therefore, we endeavored and succeeded in recording and labeling of PV+ interneurons with glass electrodes from freely moving rats performing a working memory-guided decision-making task. Despite the moderate number of juxtacellularly identified PV+

basket cells recorded in this study ( $n = 11$ ), we consistently show that individual PV+ basket cells fire with different firing patterns during different episodes and choices, an observation we found statistically supported by numerous statistical comparisons based not only on identified cells but also on a larger sample of putative PV+ basket cells.

### Different Computational Roles of Prelimbic PV+ Basket Cells during Working Memory

Evidence from various recent studies covering a wide range of behavioral paradigms supports the hypothesis that distinct types of cortical interneuron may serve specific computational roles (Gentet et al., 2012; Isomura et al., 2009; Lee et al., 2013; Letzkus et al., 2011; Pi et al., 2013; Pinto and Dan, 2015; Wilson et al., 2012). In line with Kvitsiani et al. (2013) and Isomura et al. (2009), we observed that firing patterns of some PV+ basket cells were associated with choice-related actions. However, by using a complex task paradigm consisting of a succession of distinct and outcome-relevant episodes, we observed that not all PV+ basket cells fired in an action-related manner. We rather found that increased firing of subsets of PV+ basket cells provides a complete coverage of all outcome-relevant episodes, thus illustrating the potential to undergo task-associated specialization of computational function within a single cell type.

Together with prelimbic pyramidal neurons, subsets of PV+ basket cells sequentially increased their firing rate along different tasks episodes. In addition, both pyramidal and PV+ basket cells differentiated their firing between trials with different stimuli and goal locations during various task episodes. Although we show similarly sized fractions of putative interneurons and pyramidal neurons with differential firing during task episodes and choice behavior, on a network level, firing of interneurons was more strongly modulated by task episodes, whereas activity of pyramidal cells was stronger linked with choice behavior. This observation is compatible with most prefrontal pyramidal neurons displaying choice-related activity, which is often time-locked to short behavioral events, task-associated local excitatory interactions strongly converging onto interneurons (Fujisawa et al., 2008), PV+ cells being highly interconnected (Fukuda et al., 2006; Galarreta and Hestrin, 1999; Hu et al., 2011; Pfeffer et al., 2013; Tamás et al., 1998), and mediating synchronization at different time scales (Amilhon et al., 2015; Sohal et al., 2009; Stark et al., 2013). Thus, subsets of prelimbic PV+ basket cells may act as local frameworks for episode monitoring via selective sampling from presynaptic pyramidal cells (Glickfeld et al., 2013; Lee et al., 2014; Monier et al., 2003; Velez-Fort et al., 2014; Yoshimura and Callaway, 2005).

### Diversity of Prelimbic PV+ Basket Cells Supports Computational Specialization

In the neocortex, PV+ basket cells are not only the most numerous (Kubota et al., 2011) but also most extensively studied interneuron type (Hu et al., 2014). We show that firing patterns of individual prelimbic PV+ basket cells during task performance were linked with their synaptic connectivity.

In several cortical areas, VIP+ interneurons have been directly linked to disinhibitory control of pyramidal cells via suppression of somatostatin+ and a fraction of PV+ interneurons (Lee et al.,

2013; Pfeffer et al., 2013; Pi et al., 2013). In the auditory cortex, VIP+ interneurons mediate disinhibition upon reinforcement signals, providing a circuit mechanism that might use long-range cortical or sub-cortical input to modulate local neuronal representations (Pi et al., 2013). The observation that individual PV+ interneurons are differentially affected by activation of VIP+ cells (Pi et al., 2013) is consistent with VIP+ cells targeting PV+ cells to various degrees in the somatosensory cortex (Dávid et al., 2007). We detected such diversity in VIP+ input onto our sample of prelimbic PV+ basket cells. Strikingly, we found that PV+ basket cells, which significantly reduced their firing during goal run, received a larger amount of VIP+ input. It is tempting to speculate that inhibition of those basket cells during goal run might be brought about by increased GABAergic input from VIP+ terminals. Evidence for VIP-input-dependent cortical plasticity has been reported in conjunction with somatostatin+ (Fu et al., 2014) and PV+ interneurons (Donato et al., 2013).

We found that those subsets of PV+ basket cells, which exhibited increasing or decreasing firing rates during goal run, were coupled to hippocampal theta oscillations (Hartwich et al., 2009). Interestingly, coupling strength of those cells was similar during goal run and return, when theta power in prelimbic cortex was also indistinguishable. In line with previous reports (Benchenane et al., 2010; Jones and Wilson, 2005), we found hippocampal-prefrontal theta phase synchronization significantly increased during goal run as compared to all other task episodes including return. Considering that hippocampal projections to prefrontal cortex target also interneurons (Tierney et al., 2004), carry spatial information (Ciocchi et al., 2015), and support working memory (Spellman et al., 2015), subsets of prelimbic PV+ basket cells might support synchronization between hippocampus and prefrontal cortex in a task-contingent manner.

Altogether, our observations of heterogeneity among PV+ basket cells are in general agreement with recent reports describing a range of intrinsic and circuitry measurements (Jiang et al., 2015) subjected to experience-driven plasticity (Dehorter et al., 2015; Donato et al., 2013, 2015; Kuhlman et al., 2013) that might be triggered by molecular cues (Di Cristo et al., 2004), neuronal activity (Chattopadhyaya et al., 2004; Xue et al., 2014), or cellular identity of postsynaptic pyramidal cells (Ye et al., 2015). Alongside this, diversity in PV-basket cell activity might also contribute to improving the efficiency of population decoding by means of reduced noise correlations between individual cells as proposed for recurrent networks in the visual cortex (Chelaru and Dragoi, 2008). However, our data do not indicate that the observed heterogeneity of measured parameters can be explained by discreet subtypes within the group of PV+ expressing basket cells.

### Relevance of Working Memory for the Observed Firing Patterns

Several lines of evidence indicate involvement of the rodent medial prefrontal cortex in paradigms that require working memory, including lesion (Delatour and Gisquet-Verrier, 1996, 1999) and inactivation studies (Horst and Laubach, 2009; Urban et al., 2014) as well as studies demonstrating various neuronal correlates of working memory (Baeg et al., 2003; Fujisawa et al., 2008; Fujisawa and Buzsáki, 2011; Jones and Wilson, 2005; Liu et al., 2014; Spellman et al., 2015). Moreover,

post-mortem studies of patients with working memory deficits show reduced GAD67 mRNA levels predominantly affecting PV+ interneurons (Lewis et al., 2005). Consistently, cell-type-selective circuitry perturbations in rats revealed that prefrontal PV+ interneuron signaling is required for working memory behavior (Murray et al., 2015).

Addressing task specificity of differential firing patterns of prelimbic neurons along the delayed cue-matching-to-place paradigm, we found significant reduction in the differential modulation of firing for pyramidal cells and interneurons during trials, in which no guiding information was provided. Decline of episode-modulated firing displayed a progressive trend from correct, incorrect, and no stimulus to forced trials. Altogether, this indicates that during the delayed cue-matching-to-place task, the observed firing patterns in prelimbic networks were primarily linked to memory-guided choice behavior rather than to navigation along the maze or simple goal-directed behavior without association to different stimuli.

Thus, the observed diversity in firing patterns of identified PV+ basket cells might therefore support and temporally organize cell assemblies of the prefrontal cortex during working memory-guided decision making.

## EXPERIMENTAL PROCEDURES

All procedures on animals were performed under approved license by the Austrian Ministry of Science and Medical University of Vienna. Twenty-two male Long Evans rats (300–500 g) were trained in a prefrontal cortex-dependent delayed cue-matching-to-place task on an elevated Y-maze (Fujisawa et al., 2008). In this paradigm, rats had to sample a small amount of chocolate- or cherry-flavored solution that indicated the location of reward in one of the two goal arms of the Y-maze. Once rats reached accuracy of >85% correct choices on at least 3 consecutive days, juxtacellular glass electrode and tetrode recordings in prelimbic cortex (coordinates, AP +3 mm, ML +0.5–1 mm) and LFP recordings in dorsal CA1 hippocampus (coordinates, AP –3.5 mm, ML +2.3 mm) commenced during task performance. After juxtacellular recording and labeling of interneurons (Lapray et al., 2012), rats were deeply anaesthetised and perfusion fixed for 20 min. Labeled cells were identified based on expression of PV and synaptic targets and further analyzed for their input and output connectivity. Firing patterns of PV+ basket cells during the task were analyzed in relation to different task episodes, choice behavior, and outcome. In one rat, we recorded neuronal activity with an eight-shank silicon probe in prelimbic cortex during performance of a series of control experiments.

Significance of episode and choice modulation of firing was assessed by shuffling procedures or non-parametric tests followed by Bonferroni correction of alpha values to compensate for multiple comparisons. All values are displayed as mean ± SEM.

## SUPPLEMENTAL INFORMATIONS

Supplemental Information includes three figures, three tables, and Supplemental Experimental Procedures and can be found with this article at <http://dx.doi.org/10.1016/j.neuron.2016.08.010>.

## AUTHOR CONTRIBUTIONS

M.L., A.T.O., S.L., H.M.-V., Z.B., R.H., A.J., and T.K. contributed to experiments, analysis, and preparation of the manuscript.

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