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## REVIEW

# Embedded ensemble encoding hypothesis: The role of the “Prepared” cell

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## Funding information

This work was supported by NIH grants R01MH063503, U01MH109091, R01NS11613, R01EB022903, U01EB017695 and R01MH086638

## Abstract

We here reconsider current theories of neural ensembles in the context of recent discoveries about neuronal dendritic physiology. The key physiological observation is that the dendritic plateau potential produces sustained depolarization of the cell body (amplitude 10–20 mV, duration 200–500 ms). Our central hypothesis is that synaptically-evoked dendritic plateau potentials lead to a prepared state of a neuron that favors spike generation. The plateau both depolarizes the cell toward spike threshold, and provides faster response to inputs through a shortened membrane time constant. As a result, the speed of synaptic-to-action potential (AP) transfer is faster during the plateau phase. Our hypothesis relates the changes from “resting” to “depolarized” neuronal state to changes in ensemble dynamics and in network information flow. The plateau provides the *Prepared* state (sustained depolarization of the cell body) with a time window of 200–500 ms. During this time, a neuron can tune into ongoing network activity and synchronize spiking with other neurons to provide a coordinated *Active* state (robust firing of somatic APs), which would permit “binding” of signals through coordination of neural activity across a population. The transient *Active* ensemble of neurons is embedded in the longer-lasting *Prepared* ensemble of neurons. We hypothesize that “embedded ensemble encoding” may be an important organizing principle in networks of neurons.

## KEYWORDS

binding, dendritic, glutamate, plateau potential, rate code, UP state

## 1 | INTRODUCTION

How do neurons and neural networks interact to solve complex tasks? Despite major advances in cellular and molecular neuroscience (Grosenick, Marshel, & Deisseroth, 2015; Tao, Zhang, Xiong, & Zhou, 2015; Zhang, Cudmore, Lin, Linden, & Hugarir, 2015), and in human brain structural and functional imaging (Lee, Kreitzer, Singer, & Schiff, 2017), the basis of neural computation is not completely understood (Dayan, Feller, & Feldman, 2011; Olshausen & Field, 2004; Sompolinsky, 2014; Vogels, Rajan, & Abbott, 2005). In some ways, these major experimental advances and new data sets produce major strains on theories and viewpoints that had seemed clearly delineated in a time of simpler data. A primary challenge for neuroscience in general, and for theoretical neuroscience in particular, is to fit together existing theories with new findings, reconciling *top-down* concepts, models, frameworks, and theories (Palmer, Marre, Berry, & Bialek, 2015; Sompolinsky, 2014)

with the changing *bottom-up* landscape of neuroscience discoveries (Bittner et al., 2015; Grosenick et al., 2015; Seibt et al., 2017). Here we review recent experimental evidence of glutamate-mediated dendritic

## Significance

We explain how electrical signaling endows the central nervous system with the capacity to form a large number of neural ensembles coding for objects obtained from sensory perception or retrieved from memories. We review recent experimental evidence of glutamate-mediated dendritic plateau potentials, capable of bringing neurons into sustained depolarized state (*Prepared* state), which favors action potential firing and formation of new neural ensembles. Our hypothesis, “embedded ensemble encoding,” pulls together two concepts (rate coding vs. temporal coding) that are typically seen to be in opposition.

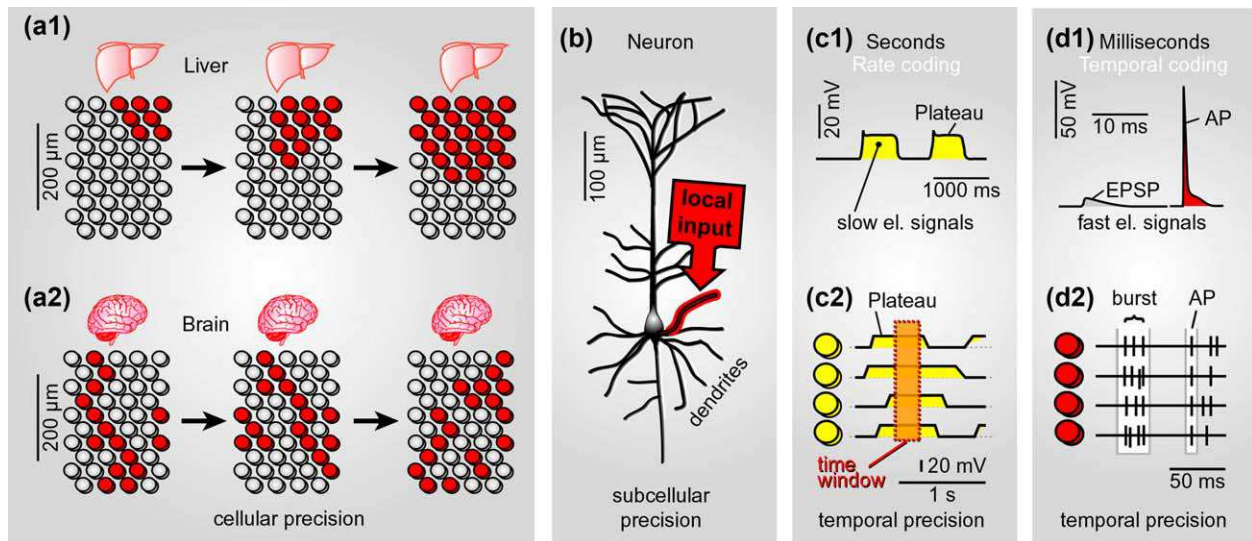
plateau potentials, capable of bringing neurons into a sustained depolarized state, which favors action potential (AP) firing and formation of new neural ensembles. Our hypothesis is based on the idea that synaptically-evoked dendritic plateau potentials lead to a prepared state that favors spike generation. The plateau both depolarizes the cell towards spike threshold, and provides faster response to inputs through a shortened membrane time constant. The speed of synaptic-to-AP transfer in individual cells is faster during the neuronal plateau depolarization. In this way, dendritic plateau potentials occurring in a fraction of cells are poised to affect the dynamic response of the whole network. The sustained depolarization of the cell body provides neurons with a time window of 200–500 ms, during which neurons can tune into ongoing network activity and synchronize spiking with other neurons. The group of actively spiking cells (ensemble “2”) is recruited from the group of cells (ensemble “1”) brought into sustained depolarized state by ongoing dendritic plateau potentials. The group of actively spiking cells is thus embedded in the group of prepared (depolarized) cells. The transient ensemble “2” of spiking neurons is embedded in the longer-lasting ensemble “1” of neurons in sustained depolarized state. Besides trying to fit together existing theories with new experimental findings, the “embedded ensemble encoding” hypothesis, also pulls together two concepts, rate coding versus temporal coding, that are typically seen to be in opposition.

## 2 | PART 1: ELECTRICAL SIGNALING

In this section, we argue that the theory of neural ensembles and the “embedded ensemble encoding” hypothesis are both critically dependent on electrical signaling (Grewe et al., 2017; Pinotsis, Brincat, & Miller, 2017). Researchers accept that electrical signaling is the key mechanism for information transmission and processing in the brain. Why are brain theory and computational models of brain function based on the electrical signal, and not on some other physical signal (optic or magnetic)? We identify six cardinal features of electrical signaling that are essential for a theory of the brain and cannot be replicated by other signaling mechanisms.

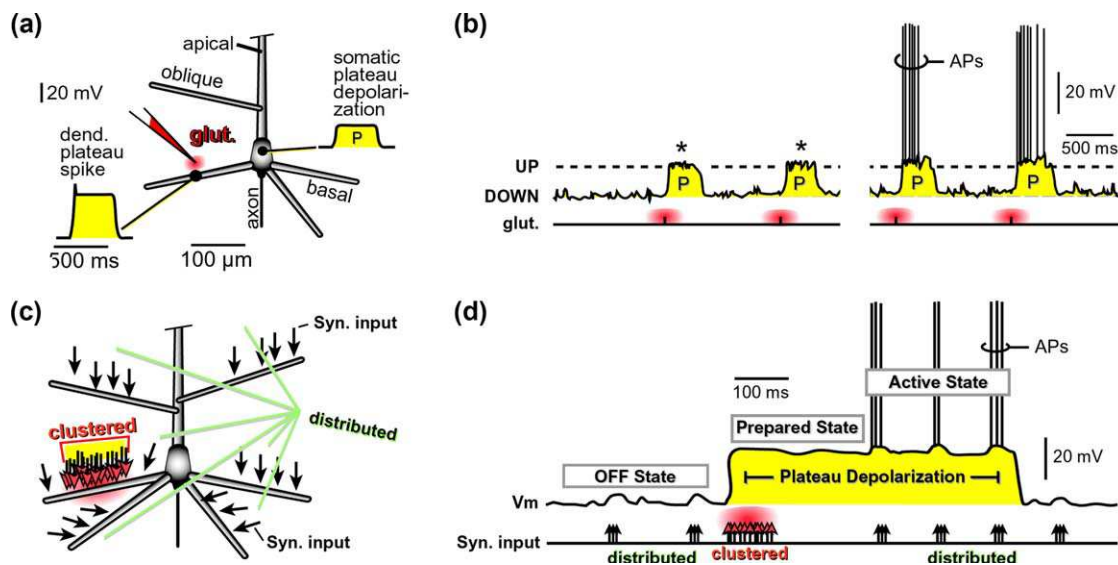
### 2.1 | Electrical signaling: Cellular specificity

A humoral signal emitted from a blood vessel or a liver cell will indiscriminately activate a large set of neighboring cells in one region of a large organ like the liver (Figure 1a<sub>1</sub>, Liver, red cells). The bottom line of the liver signaling scheme is that all activated cells are more or less grouped in one location (Figure 1a<sub>1</sub>, red cells). The number of potential ensembles of activated liver cells is low, because these cells are activated in large sections of adjacent cells via body fluids. Also, the structural complexity of a potential liver cell ensemble (Figure 1a<sub>1</sub>, red cells) is low for the same reason.



**FIGURE 1** Unique advantages of electrical signaling. (a<sub>1</sub>) Schematic representation of a liver tissue. Each circle represents one hepatocyte. Color indicates “activated” cells. Cellular activity is synchronized among neighboring hepatocytes by diffusing chemical signals. (a<sub>2</sub>) Schematic representation of a brain tissue section. Each circle represents one neuron. Color indicates “activated” cells. Cellular activity is synchronized between particular neurons (not all neurons) by electrical signaling. (b) Synaptic inputs impinge on one basal dendrite of a cortical pyramidal cell. Synaptically-induced depolarization is strongest in one cellular compartment (red halo). (c<sub>1</sub>) Slow electrical signals in the form of sustained plateau depolarizations; amplitude ~20 mV, duration ~500 ms. (c<sub>2</sub>) Electrical activity is synchronized among four cells on a slow temporal scale (seconds). Dashed rectangle indicates a period of time when all four cells are in depolarized state. (d<sub>1</sub>) Fast electrical signals, excitatory postsynaptic potential (EPSP), and action potential (AP), are shown on a fast time scale. (d<sub>2</sub>) AP firing is simultaneously recorded in four neurons. Each vertical tick represents one AP, as in “single-unit” recordings. Synchronization can be based on the temporal relation between groups of APs (AP bursts) or individual APs (spikes). Synchronized bursts of APs or synchronized single APs are marked by white background rectangle





**FIGURE 2** Dendritic plateau potentials—somatic plateau depolarizations. (a) Cartoon of a pyramidal neuron showing classes of dendrites: basal, oblique, and apical trunk. Voltage waveform of dendritic plateau potential and the resulting somatic depolarization (“P”), in response to glutamatergic stimulation of one basal dendrite. “Glut.” marks the glutamate iontophoresis site. (b) Cartoon of four glutamate iontophoresis pulses (each pulse = 5 ms) delivered on one basal dendrite. Glutamate stimuli trigger somatic plateau depolarizations (P), which resemble neuronal UP states. The somatic P without APs (\*) is termed a “Prepared state” of a neuron (Pr). The somatic P accompanied by action potentials (APs) is termed “Active state” (Act). The somatic DOWN state is termed an “OFF state” of a neuron (off). (c) Cartoon of a pyramidal neuron—black arrows mark glutamatergic inputs of approximately identical weight. Two spatial patterns of synaptic inputs are shown: *distributed* (everywhere) and *clustered* (on one basal dendrite). Clustered inputs produce glutamate spillover (red cloud). (d) At the low levels of incoming synaptic inputs, the somatic membrane potential ( $V_m$ ) is dwelling near resting potential (OFF state). Clustered glutamatergic inputs onto one basal dendrite (“clustered”) produce spillover glutamate (red cloud), which triggers dendritic plateau potential, which in turn brings the cell body into a plateau depolarization. During the plateau depolarization (*Prepared state*), the membrane potential is  $\sim 20$  mV closer to the AP threshold and the membrane time constant is shorter due to a glutamate-mediated drop in membrane resistance. As a result, the neuron is more responsive to incoming synaptic inputs distributed across the dendritic tree (black arrows). The same-size synaptic input (three arrows) fails to initiate AP in the OFF state, but successfully drives AP firing in the *Prepared state*

The brain signals are a combination of bioelectrical and specialized humoral signals. Long-range brain signals are predominantly electrical, and many humoral signals are restricted by synapses so as to be point-to-point rather than broadcast. Nervous systems combine signal speed and signal specificity through precise targeting. Brain signals are carried great distances by electrical impulses without crosstalk via specific conduits—the axons. The enormous abundance and complexity of axonal paths allows for activation of specific individual cells separated by specific inactive cells (Figure 1a<sub>2</sub>, red cells). The targeting mechanism based on axons and electrical impulses permits activation of distinct groups of selected cells in and among brain regions (neural ensemble). Brain signaling therefore permits activated cells to be scattered in space (Figure 1a<sub>2</sub>, red cells). That is, any cell in the brain can potentially become a member of a functional ensemble. The number of distinct ensembles that can potentially be formed out of the total number of brain neurons (100 billion) is very large. If each neural ensemble is made of approximately 1,000 cells per functional ensemble, the number of distinct ensembles of this size that can potentially be formed out of the total number of living brain cells is astronomical, allowing for tremendous number of percepts and memory traces over the entire life span. In summary, electrical signaling permits large number of

combinations and permutations of individually-activated cells (Figure 1a<sub>2</sub>, red cells).

## 2.2 | Electrical signaling: Subcellular specificity

Histoanatomical and physiological data both show that electrical signals target specific subcompartments of neurons (Feldmeyer, Lubke, Silver, & Sakmann, 2002; Markram et al., 2015); down to the level of individual dendritic branches; for example, thin dendrites of cortical pyramidal neurons (Figure 1b). If synaptic inputs are restricted to one dendritic branch, then the resulting membrane depolarizations can be spatially restricted to that input site (Mel, 1993). At the same moment of time, one dendritic branch experiences strong membrane potential transient, while the neighboring dendritic branch is without voltage transients (Milojkovic, Radojicic, Goldman-Rakic, & Antic, 2004, their figure 7). Local dendritic depolarizations and local dendritic summation of synaptic inputs assure that nerve cells process afferent synaptic inputs in two stages (two-layered model, Poirazi, Brannon, & Mel, 2003; Polsky, Mel, & Schiller, 2004). In the first processing stage, incoming synaptic inputs are summed within thin-dendrite compartments or subunits, each of which is governed by timing-based, regenerative thresholding, or other I/O functional forms—for example, the sigmoidal thresholding

form proposed by Poirazi et al. (2003). “Thresholding nonlinearity” simply means that upon reaching a certain voltage threshold the dendrite is no longer behaving as a linear summator of potentials, but instead it is producing additional regenerative potential, disproportional to the received input. The examples of local regenerative potentials in thin branches of cortical pyramidal neurons (basilar, oblique, and tuft) include dendritic sodium spikelet, dendritic  $\text{Ca}^{2+}$  spikes, dendritic N-methyl-D-aspartate (NMDA) spikes, and glutamate-mediated dendritic plateau potentials (Antic, Zhou, Moore, Short, & Ikonomu, 2010; Ariav, Polsky, & Schiller, 2003; Grienberger, Chen, & Konnerth, 2015; Milojkovic, Wuskell, Loew, & Antic, 2005b; Schiller, Major, Koester, & Schiller, 2000). In a second stage of the synaptic input processing, the subunit outputs are combined linearly in the cell body to determine the overall response of the cell—the neuron generates an axonal AP, or spike which is selectively transmitted to the group of cells determined by the connectome. In other words, the neuron output signal, the final result of intracellular integration, is broadcast to all “prepaid” members of the neuronal network. A key prediction of the two-layer model, but not of the global summation model, is that summation should obey different rules for inputs delivered to the same versus different thin branches of the nerve cell (Koch & Segev, 2000; Rall et al., 1992). In essence, the AP output and the computational task of a given brain cell, both depend on the exact location of the synaptic input on its dendritic tree (Branco & Hausser, 2011; Briggman, Helmstaedter, & Denk, 2011; Smith, Smith, Branco, & Hausser, 2013; Vlasits et al., 2016). The final neuronal computations depend not only on the type of dendritic operation and the dendritic and axosomatic thresholds, but also on the *global* mapping of input features throughout the dendritic tree (Tran-Van-Minh et al., 2015). The necessary level of subcellular input specificity is exclusively accomplished by axons and electrical signals flowing through neural circuits and arriving at a precise dendrite (Figure 1b) or even dendritic segment (i.e., distal versus proximal dendritic segment) (Jadi, Behabadi, Poleg-Polsky, Schiller, & Mel, 2014). One exciting theme emerging in the field of dendrite physiology is the notion that dendritic regenerative potentials are essential for the organization of neural networks. According to this theme, glutamate-mediated dendritic spikes are the key determinants of synaptic plasticity (Brandalise, Carta, Helmchen, Lisman, & Gerber, 2016; Golding, Staff, & Spruston, 2002; Gordon, Polsky, & Schiller, 2006) and in the long run dendritic nonlinear responses build a high-performance connectome (Bono & Clopath, 2017; Legenstein & Maass, 2017). In summary, electrical signaling underlies not only function but also the development and structure of the complex brain.

### 2.3 | Electrical signaling: Temporal specificity

Human brain areas, involved in complex behavioral responses, are separated by considerable path distances often exceeding 5 cm (~2,500 cell body diameters) and the flow of information in the brain is not unidirectional. Nearly all brain regions send signals into hierarchically higher brain areas and receive immediate feedback from these areas, in real time. For example, the cortical primary sensory areas (visual, auditory, somatosensory, etc.) receive feedback (top-down) projections

from secondary and tertiary sensory areas, providing them with preprocessed pieces of information pertaining to the object in the perceptual field (Gilbert & Wiesel, 1983a; Larkum, 2012; Shipp & Zeki, 2002). In this way, the outcome of the sensory process at the primary sensory area (which is supposed to process the simplest fragments of the perceptual object such as color, orientation, etc.) is strongly influenced (Bayesian biased) by the higher cortical areas that have already been informed about the complex features of the perceptual object including size, class, emotional valence, etc. (Dura-Bernal, Wennekers, & Denham, 2012; Ro, Breitmeyer, Burton, Singhal, & Lane, 2003). As the uninterrupted flow of information forth (from lower to higher) and back (from higher to lower) is critical for ongoing Bayesian expectation information processing and critical for the success of a complex information processing task, one can also assume that an exchange of just one forward and one feedback “message” would not suffice. Rather, several iterations may be required to achieve desirable results. If a psychophysical reaction takes ~200 ms (Wu, Crouzet, Thorpe, & Fabre-Thorpe, 2015), then a communication signal underlying this reaction should be capable of shuttling several times between two regions (couple of centimeters apart) in a period of time shorter than 200 ms. Among all known cellular and molecular signals operating in the brain, only electrical signals have sufficient speed to allow rapid exchanges between cells in the network. Very brief durations of electrical signals (Figure 1d1) allow neural networks to operate at relatively high frequencies and accomplish tasks very quickly (Greene & Oliva, 2009). Fast network communication at high frequency could in principle be achieved between connected neurons through radiation of photons, fluctuations of magnetic field, and passage of electricity. Since photons and magnetic fields have not yet been documented as the means of neuron-to-neuron communication, there is only one type of biological signal suited for the rapid transfer of information within a neural network: electrical signal (Kopell & Ermentrout, 2004; Salkoff, Zaghera, Yuzgec, & McCormick, 2015).

### 2.4 | Electrical signaling: Arithmetic integration

Electrical signals provide brain cells with a capacity to integrate signals—add and subtract signal intensities rapidly. In rough terms, the subthreshold depolarizing synaptic potentials add and hyperpolarizing synaptic potentials subtract from the “total score” in real time, and on a very fast time scale measured in milliseconds. Other signaling mechanisms that operate in the brain are also capable of positive and negative regulation, but these actions occur on a much slower time scale, seconds and minutes, and they do not follow precisely the rules of arithmetic summation and subtraction, such as linear summation of two excitatory postsynaptic potentials (EPSPs) arriving into the cell body from two dendritic branches (Cash & Yuste, 1998).

### 2.5 | Electrical signaling: Sustained depolarization

Cortical and hippocampal pyramidal cells, and medium spiny neurons of the striatum, nucleus accumbens and amygdala, comprise approximately 85% of neurons in telencephalon, and are the principle neurons

responsible for sensory perception, cognition, emotions, and motor output. Interestingly, all of the aforementioned neuron types exhibit glutamate-mediated dendritic plateau potentials (Milojkovic et al., 2004; Milojkovic, Radojicic, & Antic, 2005a; Oikonomou, Singh, Sterjanaj, & Antic, 2014; Plotkin, Day, & Surmeier, 2011). Dendritic plateau potentials produce sustained depolarizations of the cell body, amplitude  $\sim 20$  mV and duration  $\sim 300$ – $500$  ms (Figure 1c<sub>1</sub>, Plateau). Upon the cessation of the dendritic plateau potential, the cell body returns to resting membrane potential or a hyperpolarized inactive state (Milojkovic et al., 2005a). Cortical and hippocampal pyramidal cells, medium spiny neurons of the striatum, nucleus accumbens and amygdala, all exhibit two distinct physiological states, depolarized (UP) state and hyperpolarized (DOWN) state (Wilson, 2008). Such slow oscillatory activity (Engel, Fries, & Singer, 2001; Neymotin, Lee, Park, Fenton, & Lytton, 2011; Petersson & Fransen, 2012; Yu & Ferster, 2010;) can facilitate synchronous interactions by biasing neurons to discharge within the same time frame. Coincident depolarized plateau states may help the recruitment of neurons into the functional ensemble. The critical time window for the formation of a neural ensemble is the one over which the individual member cells are in depolarized states (Figure 1c<sub>2</sub>, dashed rectangle). Principal cells of cortex and striatum are not spontaneously active. An oncoming barrage of glutamatergic projections can move a group of cortical or striatal cell from resting into sustained depolarized state (UP state). A second group of glutamatergic inputs can then initiate APs. Only cells which are already in the depolarized (UP) state will respond to the second set of inputs, because it is difficult to initiate firing in cortical and striatal cells during the hyperpolarized DOWN state (but see Petersen, Hahn, Mehta, Grinvald, & Sakmann, 2003). In our view, a group of neurons in sustained depolarized state constitutes one neural ensemble. From this group, the second set of excitatory inputs can recruit neurons into an ensemble of a higher order; an ensemble comprised of cells engaged in the firing of APs. Thus, the group of spiking cells is embedded in the group of depolarized cells. We think that such “ensemble embedding” may be an important organizing principle in neural networks.

## 2.6 | Electrical signaling: Synchronization and binding

Representation of a physical or mental “object” during sensory perception, as well as sensorimotor coordination, requires binding together of many brain areas to produce coordinated activity. Binding requires some type of coordination of neural activity across populations of neurons on various temporal scales. Most popular in discussions of binding over the past decades, has been rapid binding through fast temporal coordination at rates corresponding to gamma oscillations in the range of 40 Hz (*binding theory*, Singer & Gray, 1995). Variations of this scheme include coding based on precise spike synchrony and coding based on timing relative to a slower wave envelope provided by theta or alpha oscillation. In the present account, we will focus on one type of synchrony coding which implies multiple cells are firing within a short period of time (like Figure 1d<sub>2</sub>). Synchronized spiking activity has been found in different species and different cortical areas (Bair, 1999; Buzsaki & Silva, 2012; Salinas & Sejnowski, 2001). For the same level

of firing, a synchronous input is more effective on postsynaptic neurons than asynchronous input (London, Schreiber, Hausser, Larkum, & Segev, 2002; Schneidman, Freedman, & Segev, 1998). Large-scale models predict that synchrony occurs due to the reciprocal connectivity and loops between clumps of neurons (Compte et al., 2003; Durstewitz, Seamans, & Sejnowski, 2000; Tononi, Sporns, & Edelman, 1992).

In summary, electrical signaling (Galvani, Helmholtz, Adrian, Eccles, etc.) endows neurons with the means for achieving communication between specific cells and avoiding their neighbors (Figure 1a<sub>2</sub>, red cells), for delivering inputs on specific subcellular compartments, individual dendrites, and avoiding neighboring dendrites, for rapid exchange of messages between two remote areas (a millisecond scale), and for arithmetic operations (additions and subtractions) using rapid afferent signals. Electrical signaling gives neurons the ability to shift quickly between hyperpolarized and depolarized states, back and forth, remain in depolarized state for several hundred milliseconds, and achieve binding through slow oscillations at  $\sim 1$  Hz (UP states), or through synchronized or precise spike timing activity which can reach frequencies of up to 40 Hz (Singer & Gray, 1995). There are no other biological signals capable of supporting the aforementioned features, and hence computational modeling of neuronal electrical signaling is currently the most promising approach towards understanding how the brain “walks” and “talks.”

## 3 | PART 2: SINGLE NEURON SIGNIFICANCE

The classical point neuron has simple dynamics and no spatial extent. A more modern concept (alternative abstraction) endows neurons with large nonisopotential dendritic trees, where individual dendritic branches behave as semi-independent units (Koch, Poggio, & Torre, 1983; Mel, 1993). The dendritic tree effectively increases the total number of processing units in the network. The cost of not having good single-neuron models is potentially high. In studying a neural circuit, computational neuroscientists naturally draw their concept of the neuron from the example of transistors used in computers, leading to imperfect assumptions regarding the capabilities of the various neuron types that make up the circuit. The large investment of time and money required to design the integrated large-scale systems performing multiple computations may be wasted if critical capabilities of individual neurons are omitted from the design (Agmon-Snir, Carr, & Rinzel, 1998; Euler, Detwiler, & Denk, 2002; Lavzin, Rapoport, Polsky, Garion, & Schiller, 2012; Legenstein & Maass, 2017; Rall & Shepherd, 1968).

### 3.1 | Spiny neurons

*In vivo* intracellular recordings have documented UP and DOWN transitions in cortical L5 pyramidal neurons, cortical L4 stellate cells, striatal medium spiny neurons, and spiny neurons of the amygdala (Brecht & Sakmann, 2002; Padival, Quinette, & Rosenkranz, 2013; Steriade, Timofeev, & Grenier, 2001; Volgushev, Chauvette, Mukovski, & Timofeev, 2006; Wilson & Kawaguchi, 1996). UP states in striatum and UP states in cortex use different network organizations to achieve the

same thing: sustained somatic depolarization. Recurrent excitation between neighboring pyramidal cells and corticocortical projections are dominant in cortex. Thalamic and cortical efferents, on the other hand, supply excitation to the striatum. In some nomenclatures, UP and DOWN states are terms reserved for specific membrane polarization levels occurring during slow wave sleep, occurring in a rhythmic fashion ( $\sim 1$  Hz) and involving nearly all cells in a block of tissue (Wilson, 2008). In the present article, we borrow the term "UP state" from the sleep studies to describe sustained somatic depolarization 200–500 ms in duration and  $\sim 20$  mV in amplitude ("neuronal UP state"). There is a conceptual difference between "cortical UP state" and "neuronal UP state." In cortical UP state occurring during slow wave sleep, almost all pyramidal cells in the area are synchronously depolarized. In the neuronal UP state (proposed here as a cellular mechanism for the recruitment of pyramidal cells into functional ensembles), only a selected group of neurons are depolarized, and all members of this depolarized group code for similar or related objects.

### 3.2 | Neuronal up state

Neuronal UP state in sleep and neuronal UP state in wake occur in the same cell: spiny telencephalic neuron. As a rule, spiny central nervous system (CNS) neurons do not fire APs from a DOWN state. Spiny neurons only fire APs when their cell body is in a depolarized, UP state (Brecht & Sakmann, 2002; Padival et al., 2013; Steriade et al., 2001; Volgushev et al., 2006; Wilson & Kawaguchi, 1996). A successful synchronization of the firing activity among spiny neurons in cortex, striatum, and amygdala would thus absolutely require that members of a neuronal ensemble have overlapping UP states (Figure 1c<sub>2</sub>, dashed rectangle). Synchronization can be achieved at two levels of precision: [A] neurons burst within the same window of time (100 ms precision), or [B] there is individual spike synchrony (1 ms precision). To become eligible for inclusion into a functional neuronal ensemble, a spiny neuron must quickly and reliably switch from a DOWN to an UP state, remain in the depolarized UP state as long as necessary, and quickly abort the UP state when a percept is formed or expired.

Dendritic plateau potential may not be the only cellular mechanism of sustained electrical activity in individual CNS neurons. Persistent firing which outlasts the original stimulus can be sustained via two traditional mechanisms: (i) Recurrent network excitation within microcircuits of CNS neurons (Goldman-Rakic, 1995). (ii) Intrinsic membrane conductances of neurons which do not require fast synaptic transmission (Egorov, Hamam, Franssen, Hasselmo, & Alonso, 2002). Activation of (iii) cholinergic, (iv) noradrenergic, and (v) dopaminergic afferents, as well as (vi) metabotropic glutamate receptors have also been implicated with different forms of sustained neuronal activity (Zhang, Cordeiro Matos, Jegu, Adamantidis, & Seguela, 2013). It is important to emphasize that the majority of the abovementioned cellular mechanisms (i–vi) are fundamentally based on the glutamatergic transmission on spiny dendrites. Glutamate-mediated plateau potentials may occur in dendrites of neurons engaged in persistent activity via recurrent excitation (Goldman-Rakic, 1995). Glutamate-mediated plateau potentials may occur upon a norepinephrine surge causing activation of alpha-1

adrenoceptors facilitating glutamate release and subsequent activation of postsynaptic mGluR5 receptors (Zhang et al., 2013).

Neuronal UP states are known to play an important role in the consolidation of memory (Marshall, Helgadottir, Mølle, & Born, 2006), but the exact cellular mechanism by which UP states control storage of information in the brain is poorly understood. A recent study showed that UP state-induced synaptic weakening of subthreshold inputs, while preserving synaptic strength of strong inputs (Bartram et al., 2017). This cellular mechanism causes a near-global synaptic downscaling during slow wave sleep. Subthreshold inputs, which comprise a majority of inputs, are downsized but a group of selected inputs (strong enough to bring cell to firing) are preserved unaltered.

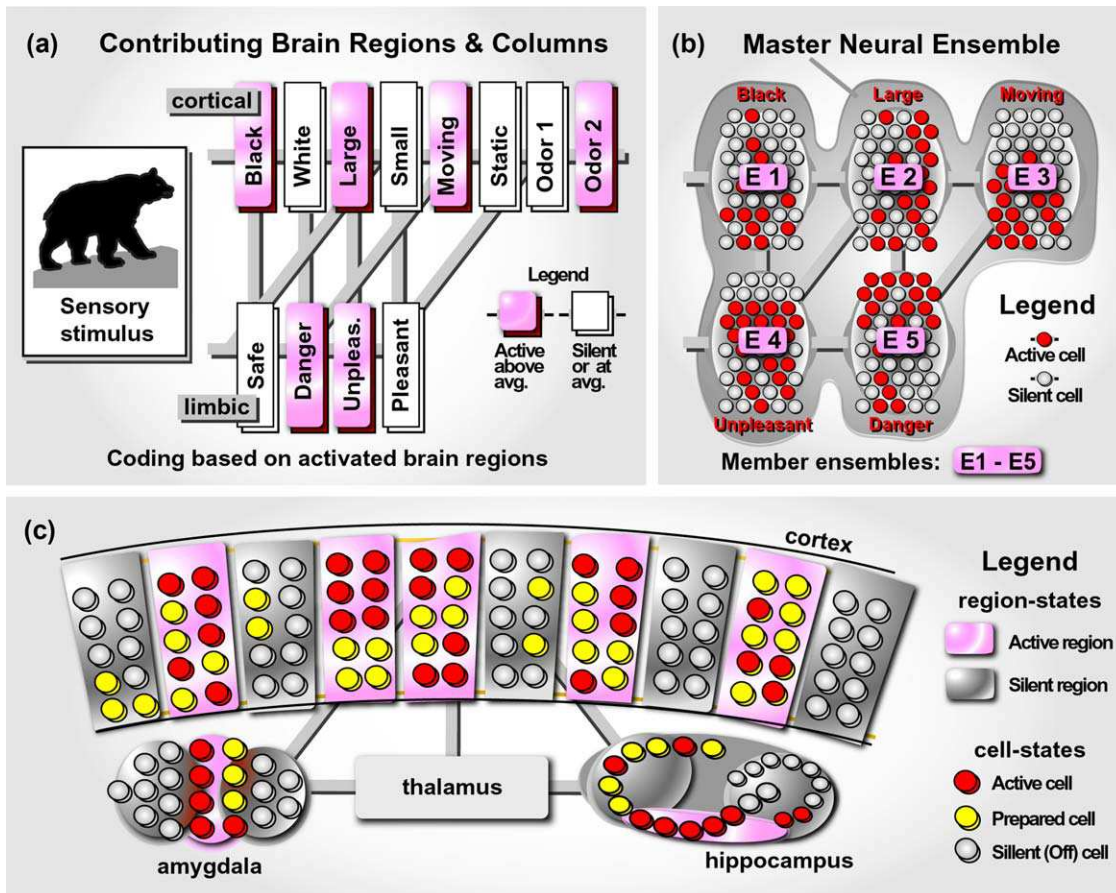
### 3.3 | Dendritic up state

Synaptically-evoked somatic plateau depolarization can be the consequence of glutamate-mediated dendritic plateau potential/spike, also known as "dendritic UP state" (Antic et al., 2010; Milojkovic et al., 2005a; Plotkin et al., 2011). The voltage waveforms of dendritic plateau potentials (Figure 2a, basal) were characterized using voltage-sensitive dye imaging (Milojkovic et al., 2004, 2005a). The somatic plateau rises a few milliseconds after the onset of the dendritic voltage transient and collapses with the breakdown of the dendritic plateau depolarization (Milojkovic et al., 2005a). In other words, the cell body shifts from DOWN to UP state after the generation of the dendritic plateau potential. It stays in the UP state as long as the dendritic plateau lasts, and it collapses with the collapse of the dendritic plateau potential. The slow component of the somatic depolarization accurately mirrors the duration of glutamate-evoked dendritic plateau potential (Figure 2a, compare voltage signal in dendrite "P" and voltage signal in soma). This observation is most apparent in experiments in which a gradually increasing intensity of glutamatergic input was delivered onto a basilar dendritic branch. At subthreshold glutamate input intensities the dendritic and somatic depolarizations are both subthreshold. As soon as the dendritic membrane develops a regenerative dendritic plateau potential (threshold reached in one dendrite), the somatic compartment of this neuron reports a neuronal UP state (Oikonomou, Short, Rich, & Antic, 2012, their figure 3). In summary, the relation between dendritic plateau potential and somatic UP state is reliable (Milojkovic et al., 2005a; Milojkovic, Zhou, & Antic, 2007).

#### 3.3.1 | Glutamate

Glutamate processing in neuronal and astrocytic processes (uptake, packaging, and release) occurs rapidly and vigorously. Spiny neurons in cortex and striatum are exposed to confluent and repetitive excitatory drives during intense neural activity. For example, neurons experience strong barrages of glutamatergic inputs every other second of the slow-wave sleep period; 1 Hz oscillation (Brecht & Sakmann, 2002; Padival et al., 2013; Steriade et al., 2001; Volgushev et al., 2006; Wilson & Kawaguchi, 1996). Whole-cell somatic recordings show that dendritic membrane responds robustly to repetitive glutamatergic stimulations with no signs of glutamate receptor desensitization or fatigue (Figure 2b), see also (Milojkovic et al., 2004 their figure 1, Oikonomou





**FIGURE 3** Object coding by neural ensembles. (a) An object in the perceptual field (bear) activates pockets of cells in many brain regions. Each rectangle represents a column, or a segment of a nucleus, devoted to one simple attribute of the object. Brain regions are connected with axonal projections (gray stripes). The overall activity of a column increases above average if an attribute were detected (pink) – the column is “net-active.” If an attribute was not detected, the net electrical activity remains at or below the average level (white) – the column is “net-silent.” (b) Schematic depiction of net active brain regions at cellular resolution. The net silent brain regions (white rectangles in the previous panel) are omitted from this presentation, for simplicity. Active neurons = red circles. Inactive neurons = gray circles. Multiple neural ensembles (E1–E5) are embedded in the master neural ensemble, which codes for a large, dark and dangerous moving object detected by sensory system. Member ensembles (E1–E5) may reside in separate brain regions (e.g., cortex, striatum and amygdala). Together the member ensembles form a master neural ensemble (gray contour). (c) Schematic depiction of four brain areas: cortex, hippocampus, thalamus, and amygdala. Each brain area is divided into smaller functional units/segments/columns. Units can be either net-active (pink) or net-silent (gray). In respect to electrical activity at single-cell level, neurons can be found in three functional states: OFF (gray circle), Prepared (yellow circle), and Active (red circle)

et al., 2012 their figure 7). Spiny neurons seem properly equipped to convert a surge of extracellular glutamate into a sustained depolarization episode, and to do so repeatedly (Figure 2b). Dendritic voltage imaging showed that sustained somatic depolarizations (Figure 2b, “P”) are caused by local dendritic plateau potentials (Milojkovic et al., 2005a,2007). Therefore, the glutamate-mediated plateau is a plausible membrane mechanism for placing CNS cells in a sustained depolarized state during intense cognitive operations.

Principal neurons of cortex, hippocampus, neostriatum, and amygdala are highly branched structures hosting numerous glutamatergic synapses on every thin dendrite (Figure 2c, black arrows). In respect to glutamatergic synapses, there is a one-to-one relation between donor (presynaptic axon terminal) and the acceptor (postsynaptic dendritic spine). This means that in order to map synaptic inputs on a spiny

neuron, it is not necessary to label and visualize presynaptic terminals (axons). By simply counting and recording the locations of dendritic spines, one can arrive at a precise density of excitatory glutamatergic synapses on a given neuron (Larkman, 1991). One distinguishing feature of all principal spiny CNS neurons involved with cognitive tasks (cortex, striatum, and hippocampus) is the tendency to make as many synapses as possible, and extract information from as many neural circuits as possible (Chen, Rochefort, Sakmann, & Konnerth, 2013). The number of excitatory glutamatergic contacts on any given CNS spiny neuron is directly proportional to the complexity of its computational task. For example, layer five pyramidal neurons in the occipital lobe (primary visual cortex) have 2–3 times fewer synapses than the equivalent (L5) neurons in the prefrontal cortex (higher association cortex). The dendritic spine count per cell increases gradually from occipital to



parietal, to prefrontal cortex, in the same way the number of the sensory modalities increases from primary, to secondary, to tertiary association cortex (Elston, 2003).

Prolonged depolarizations, long decay time-constants and efficient temporal summations of synaptic inputs on pyramidal neurons are attributed to glutamate activation of NMDA receptors (Lisman, Fellous, & Wang, 1998; Wang, Stradtman, Wang, & Gao, 2008). Sustained depolarizations are in part driven by glutamate spillover (Figure 2a, red cloud), which keeps dendritic NMDA receptors in the open state for several hundred milliseconds (Chalifoux & Carter, 2011; Milojkovic et al., 2007; Oikonomou et al., 2012).

We think that astrocytes are essential for triggering of dendritic plateau potentials. We envision three synaptic scenarios for dendritic EPSPs, NMDA Spikes, and Plateau Potentials, respectively. [EPSPs] EPSPs are generated when glutamatergic inputs are sparse on spiny dendritic branches. Astrocytic processes, positioned in between dendritic spines, have ample capacity to clean up synaptic glutamate during sparse and scattered synaptic transmission (Oikonomou et al., 2012, their figure 1A). [NMDA Spikes] NMDA spikes are generated when inputs are clustered in a small dendritic segment (Figure 2c, clustered). Input clustering facilitates summation of the spill-over glutamate, which can partially overcome the local glutamate uptake in astrocytic process, resulting in a dendritic NMDA spike (Oikonomou et al., 2012, their figure 1B). [Plateau Potentials] Plateau potentials are generated when glutamatergic inputs are both clustered and repetitive, which brings a lot of glutamate molecules into a restricted space, where synaptic inputs are functionally clustered. Facing the excessive amounts of glutamate, the astrocytic processes not only stop the glutamate uptake, but they reverse glutamate uptake and spill out glutamate into the space surrounding spiny dendritic segment, resulting in a “glutamate clamp” of the dendritic membrane potential. Recall that the plateau phase of a local dendritic plateau potential is  $\sim 50$  mV above the resting potential (Milojkovic et al., 2004), and this voltage is at or near the glutamate reversal potential. In summary, repetitive synaptic stimulation overcomes the ability of astrocytic processes to clear glutamate from the extracellular space, allowing some dendritic segments to become submerged in a pool of glutamate, for a brief period of time. This dynamic arrangement activates extrasynaptic NMDA receptors located on dendritic shafts. Strong and sustained activation of AMPA and NMDA conductances keep the selected dendritic segments in a 50 mV depolarized dendritic UP state. The aforementioned synaptic scenarios are still in the domain of speculations. A solid experimental evidence for the astrocytic role in dendritic plateau potentials awaits methodological improvements in measuring glutamate transients inside astrocytic processes and in the extracellular matrix surrounding dendritic segments.

### 3.4 | Two maps of glutamatergic inputs

Glutamatergic inputs can be spatially segregated on one dendritic branch, one dendritic segment (Figure 2c, *Clustered*), or they can be sparsely distributed across the dendritic tree (Figure 2c, *Distributed*).

Both spatial organizations, *Clustered* and *Distributed*, could play important roles within the neuron's computational task.

#### 3.4.1 | Clustered

Theories based on the functional clustering of glutamatergic synapses on developing and mature dendrites, are gaining momentum (Bono & Clopath, 2017; DeBello et al., 2014; Kastellakis, Cai, Mednick, Silva, & Poirazi, 2015; Koch et al., 1983; Larkum & Nevian, 2008; Poirazi et al., 2003; Ujfalussy, Makara, Branco, & Lengyel, 2017). In parallel with hypotheses and neuron-modeling, experimentalists try to address this issue in the laboratory. New experimental results mostly support the theory of synaptic clusters (DeBello et al., 2014; Fu, Yu, Lu, & Zuo, 2012; Hill, Varga, Jia, Sakmann, & Konnerth, 2013; Kleindienst, Winnubst, Roth-Alpermann, Bonhoeffer, & Lohmann, 2011; Makino & Malinow, 2011; McBride & DeBello, 2015; Palmer et al., 2014; Pinchas & Baranes, 2013), but see (Chen et al., 2013) for an alternative perspective. We proceed by listing several studies in support of clustering.

- Glutamate uncaging experiments determined that approximately 50 synaptic inputs clustered within 100  $\mu\text{m}$  of a thin dendritic branch need to be activated within 3 ms in order to trigger a regenerative dendritic potential (Gasparini, Migliore, & Magee, 2004), with similar ionic composition to glutamate-mediated dendritic plateau potential. The exact size of synaptic cluster necessary for initiation of local dendritic plateau potential will vary depending on the branching order, distance from the cell body and proximity to large or complex dendritic bifurcations (Larkum, Nevian, Sandler, Polsky, & Schiller, 2009).
- Calcium imaging in neonatal brain slices during spontaneous bursts of activity show that synapses located near each other on the same dendritic branch exhibit a higher degree of temporal correlation than synaptic pairs on different dendrites (Kleindienst et al., 2011). This highly correlated activity of neighboring synapses could not be attributed to individual axons forming multiple synapses on short stretches of dendrite. Furthermore, Kleindienst et al. (2011) showed that repetitive spontaneous activity in developing neural networks causes spatiotemporal clustering of functional synapses on dendrites, and that this process is dependent on the intact function of glutamatergic NMDA receptors.
- Experience-dependent formation of synaptic input clusters can occur in juvenile brains and synaptic clusters formed early in life can be preserved to adulthood (McBride & DeBello, 2015).
- Anatomical studies demonstrate that excitatory connections in cortex are not uniformly distributed across a network but instead exhibit clustering into groups of highly connected neurons. Individual pyramidal and spiny stellate neurons in the cat primary visual cortex project their axons horizontally (up to 4 mm) but the axon collaterals are often distributed in repeating clusters, with an average periodicity of 1 mm. The clustering pattern is most apparent when the cells are viewed parallel to the cortical surface (Gilbert & Wiesel, 1983b).
- While sensory deprivation causes homeostatic synaptic enhancement globally on all dendrites, meaningful sensory experiences

preferentially produce synaptic potentiation onto nearby dendritic synapses. Such clustered synaptic potentiation is thought to bind behaviorally relevant information onto dendritic subcompartments (Brandalise et al., 2016; Bono & Clopath, 2017; Makino & Malinow, 2011).

- Different motor tasks trigger calcium spikes in largely nonoverlapping distal apical tuft branches of the same L5 pyramidal neurons (Cichon & Gan, 2015). The apparent specialization of individual branches for a particular motor task (Cichon & Gan, 2015), suggests that ensembles of neurons specific for one motor task cluster on one branch, while other task-specific ensembles cluster on other branches of the same neuron.
- Computational modeling studies suggest that neurons with correlated activities will tend to form clusters of synapses close together on the dendrites of a target neuron, whereas neurons with unrelated activities will tend to form synapses that are further apart (Ujfalussy et al., 2017). Synaptic clustering may increase storage capacity of CNS neurons (Poirazi & Mel, 2001; Sheffield & Dombeck, 2015). Information storage in neural tissue could reside primarily in the selective addressing of synaptic contacts onto dendritic subunits (clustering), as opposed to the traditional view stating that memories are primarily encoded in the overall connection strengths between neurons.

### 3.4.2 | Distributed

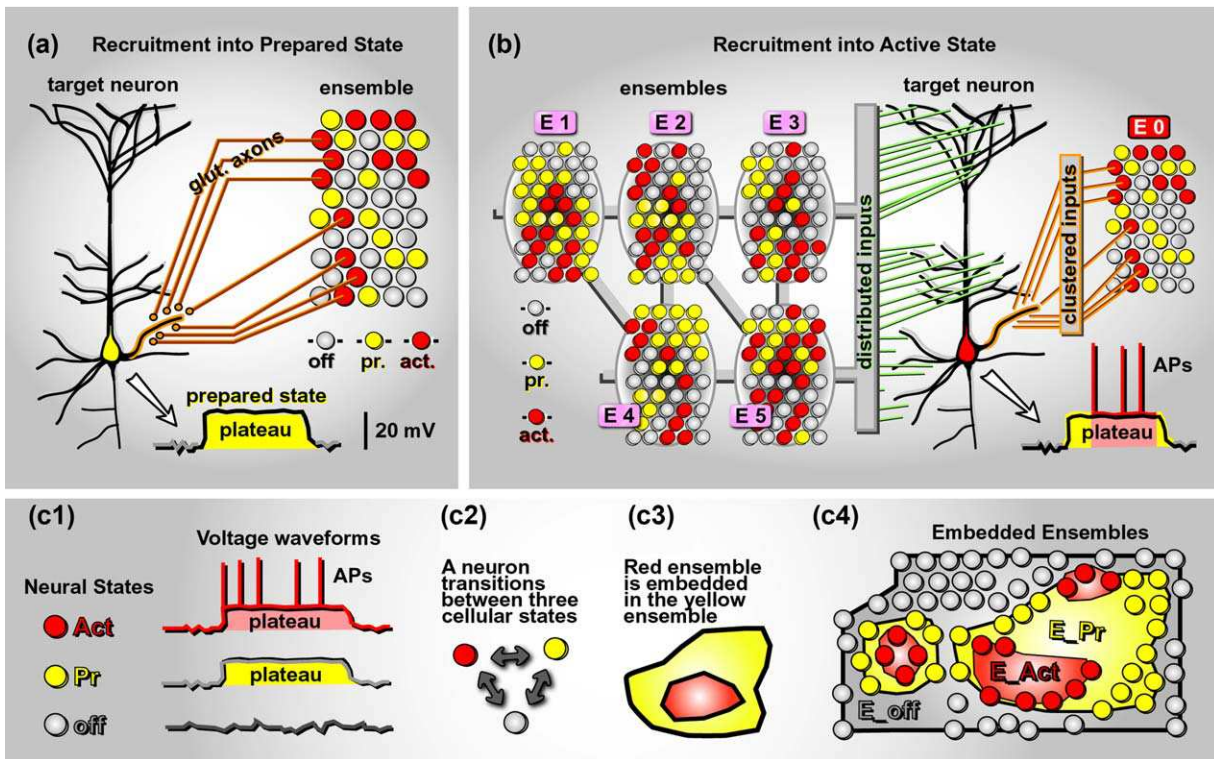
Excitatory synaptic inputs distributed on dendritic branches contribute little depolarization to the AP initiation zone located in the axon initial segment (Gulledge, Kampa, & Stuart, 2005). In our view, the most basic job of the distributed synaptic assortment (Figure 2c) is to extract the background patterns of network activity. In the schematic representation, black arrows depict distributed glutamatergic inputs arriving onto three classes of dendrites in a pyramidal neuron (Figure 2c, tuft, oblique, and basal). On the time axis (Figure 2d, synaptic input), black arrows indicate the timing of synaptic triplets arriving in regular time intervals, as being driven by a hypothetical rhythmic network activity. A triplet of synaptic inputs (triplet of black arrows) arriving during the neuronal *OFF* state is unable to bring the cell to fire an AP (Figure 2d). An identical triplet of inputs occurring during a responsive, *Prepared*, state successfully drives the AP firing (Figure 2d). Glutamate-mediated dendritic plateau potentials depolarize neuronal membrane potential ( $V_m$ ), decrease neuronal membrane resistance ( $r_m$ ) and shorten the membrane time constant ( $\tau$ ). EPSPs with faster rising times have better chance of reaching the AP voltage threshold because voltage transients with faster rising times are more powerful activators of the voltage-gated sodium current (which constitutes AP) than slow voltage transients (Hodgkin & Huxley, 1990). Due to the voltage dependence of the sodium channel inactivation gate, a slow ramp potential may reach very depolarized levels without ever triggering an AP (Hodgkin & Huxley, 1990). This why faster rising EPSPs are more effective triggers of neuronal spiking. A second important factor that renders distributed synaptic inputs more efficient in the neuronal *Prepared* state is depolarization itself. The glutamate-mediated dendritic plateau potential effectively

shifts the somatic membrane potential by approximately +20 mV (Figure 2d). At this new depolarized membrane potential (*Prepared* state), small voltage transients from distributed synaptic inputs have a better chance of reaching the AP voltage threshold, compared to the *OFF* state, in which the somatic and axonal membranes were 20 mV more hyperpolarized (Figure 2d). In summary, during *Prepared* state, small synaptic inputs, previously ineffective, now gain the ability to drive the initiation of APs. The *Prepared* state allows a pyramidal neuron to faithfully convert the incoming patterns of synaptic activity into the outgoing patterns of AP firing (McCormick et al., 2003), but see (Petersen et al., 2003).

The plateau phase of a glutamate-mediated dendritic plateau potential (Figure 2c, *Prepared* state) may serve to provide spiny neurons with a window of opportunity, for tuning into the ongoing network activity more robustly (increased firing) and more accurately (faster membrane response; Figure 2c, *Activated* state). For example, in hippocampal “place cells,” the generation of plateau potentials notably elevates AP firing frequency (Bittner et al., 2015). CA1 pyramidal neuron synapses are facilitating in nature, hence dendritic plateau potential initiation appears to be a powerful gain modulation of place cell's output improving both the firing rate and short-term synaptic efficacy of its axon terminals. Dendritic plateau potentials effectively change the state of a CNS neuron. Dendritic plateau potentials create a context for a sharp increase in neuronal output (Grienberger et al., 2015; Lavzin et al., 2012; Schmidt-Hieber et al., 2017). In hippocampus, the context will be “place.” In cortex, depending on the area, the context may be a class of external objects (Figure 3a). The cellular mechanism for bringing spiny neurons into receptive *Prepared* state is most likely based on synaptic clustering (Kastellakis et al., 2015; Larkum & Nevian, 2008; Magee, 2011; Mel, 1993; Shai, Koch, & Anastassiou, 2014). The job of a clustered synaptic assortment is to bring the target CNS neuron into a “receptive” *Prepared* state (Figure 2d). This transition from the inert and silent *OFF* state to a depolarized and responsive *Prepared* state occurs when the neuronal network fulfills three requirements: (1) Glutamate-releasing axons converge anatomically onto the dendritic tree (Figure 4a); (2) These axons become activated synchronously, within 10–20 ms (Branco & Hausser, 2011; Gasparini et al., 2004), and (3) The synchronized activation of axons is repeated several times in a relatively short period of time (100 ms) (Milojkovic et al., 2004; Polsky, Mel, & Schiller, 2009; Suzuki, Kodama, Hoshino, Izumi, & Miyakawa, 2008). Clustered and repetitive glutamatergic activity is necessary for buildup of glutamate levels in the extracellular space (Figure 2c, red cloud) (Chalifoux & Carter, 2011; Oikonomou et al., 2012; Suzuki et al., 2008).

## 4 | PART 3: NEURAL ENSEMBLES

In this section, we adopt a notion that brains process and store information using neuronal ensembles. A neuronal ensemble is a dynamic structure composed of synchronously activated neurons engaged in the same task (Eichenbaum, 1993; Engel & Singer, 2001; Hebb, 1949). At one instant of time a neuron is a member of one ensemble, while in



**FIGURE 4** Recruitment of neurons into neural ensembles. (a) A pyramidal neuron is receiving glutamatergic projections from a brain region comprised of neurons in three characteristic states of activity (OFF, Pr and Act). A fraction of spiking cells (red) sends their glutamatergic projections (glut. axons) onto the target pyramidal neuron, clustering on one particular basal branch. A computational task engaging the neural ensemble (red cell ensemble activity) is poised to recruit the target neuron into a *Prepared* state (somatic plateau depolarization, amplitude  $\approx 20$  mV, duration  $\approx 300$  ms), via the generation of a local dendritic plateau potential (basal dendrite-orange halo). (b) A target pyramidal cell is receiving two types of glutamatergic inputs, clustered and distributed. “Clustered inputs” arrive from ensemble E0. Thus, ensemble E0 has the capacity to recruit our neuron into *Prepared* state, by triggering local dendritic plateau potential (same as in A). “Distributed inputs” arrive from various active brain regions (E1–E5) and they scatter across the entire dendritic tree, including basal, oblique and apical tuft branches. Distributed inputs have the capacity to drive AP firing, but only if E0 had been successful in recruiting this cell into a *Prepared* state. (c1) A characteristic somatic voltage waveform applies to each neuronal state, *OFF*, *Prepared*, and *Active*. (c2) Each neuron transitions between three basic states, depending on the ongoing pattern of excitatory glutamatergic inputs. (c3) A group of *Active* cells (red contour) are recruited from a group of *Prepared* cells (yellow contour). (c4) Cartoon of a brain region shown at “cellular resolution,” each circle represents one neuron. The ensemble made of neurons in *Active* state ( $E_{Act}$ , red) is just a subset of the ensemble of neurons in *Prepared* state ( $E_{Pr}$ , yellow), which itself is a subset of neurons in the *OFF* state ( $E_{off}$ , gray)

the next instant of time the same neuron participates meaningfully in the function of another neuronal ensemble (Desimone, Albright, Gross, & Bruce, 1984; Eichenbaum, 1993; Engel & Singer, 2001; Legenstein & Maass, 2017; Wilson & McNaughton, 1993). This “time-sharing” feature of the ensemble-organization principle assures a very high number of neuronal ensembles in the mammalian brain that can be assigned to a very high number of specific objects, including perceptual and mental objects—just as the pixel on your screen is only a tiny piece of an image and will be lit up for many other images as well.

Complex percepts, such as a black bear shown in Figure 3a, are composed of sensory details (fragments) including, color, size, orientation, odor, emotional valence, etc. Each fragment of the sensory experience is processed by a specific cortical column or specific segment of a brain region specialized for that fragment of experience, and the combination of active and nonactive regions thus represents a neural code for this class of objects (Figure 3a). Both activated and silent brain regions contribute important clues about the nature (class) and/or

identity of the object. Gathering information from as many regions as possible is a procedure that serves to reduce ambiguity. Since these attributes belong to different sensory modalities, and each modality is hosted by a different brain lobe (in two hemispheres simultaneously), it is inevitable that large neural ensembles (Figure 3b, master neural ensembles) are composed of active neurons residing in many remote brain regions, including the thalamus, primary-sensory, and association areas of the cerebral cortex, striatum, and amygdala. Hippocampus, entorhinal cortex, and midbrain are also activated, because the fundamental nature of sensory perception demands that current sensory percepts are compared with previously stored ones, as a measure of novelty (Lisman & Grace, 2005), while hypothalamus, amygdala, and nucleus accumbens promptly assign emotional valence (Damasio, 1994). Experimental measurements show that the distribution of neurons involved in one functional neural ensemble is not restricted to the cerebral cortex, but it is likely to include subcortical gray matter (Figure 3c, amygdala and hippocampus) (Brecht, Singer, & Engel, 1998; Ziaei,



Peira, & Persson, 2013). Cortex-wide  $\text{Ca}^{2+}$  imaging in mice performing decision-making behavior revealed robust activation of neurons distributed across the majority of dorsal cortex (Allen et al., 2017). In summary, these activated groups of cells are widely distributed across different areas of the brain, each specialized in signaling a different attribute of the object or different element within the scene (Mountcastle, 1997; Perrett, Rolls, & Caan, 1982; Singer, 1999; Yu & Ferster, 2010).

#### 4.1 | Master ensembles

The “master neural ensemble” is a functional construct (Figure 3b, gray contour) that is formed when several member ensembles (E1–E5) are activated simultaneously. Each member ensemble (subensembles E1–E5) combines some first-order features (such as two co-occurring visual orientations) into a second-order feature (such as checkered pattern). Detection of a checkered pattern on a given object is just one small step toward recognizing that object. This type of feature-binding is often performed locally in the primary sensory cortex (Muir, Molina-Luna, Roth, Helmchen, & Kampa, 2017), hence a subensemble can, in general, be restricted to a given cortical region. In contrast, master ensembles are not encapsulated by one brain structure even if the brain structure is very large and multifaceted (e.g., cerebral cortex). This idea has repercussions on the actual experimental strategies. For example, studying the cellular determinants of face recognition by measuring electrophysiological responses from the cortical area specialized for face recognition (fusiform gyrus) might be an inadequate strategy, because the face recognition task requires an interplay between several brain structures including at least four visual cortices (V1–V4), fusiform gyrus, amygdala (Pujol et al., 2009), and thalamic nuclei. It would be difficult to identify the cellular mechanism of any given computational task, without physiological data from all these regions. For this reason, the current trend in experimental neuroscience is development of experimental recording methods for sampling voltage transients from many brain cells in many brain regions, simultaneously (Antic, Empson, & Knopfel, 2016).

As a first approximation, the constellation of active brain regions is a code for one class of objects, or for one identifiable familiar object. However, the number of (a) object classes, (b) identifiable objects, (c) maps, (d) paths, (e) sequences, and (f) strategies that need coding in everyday life, surpasses the combinatorial power of a few known cortical areas. The new view proposed in the present account states that within each activated specialized brain area, there is further refinement of the assembles of active neurons. Not all neurons in the activated specialized brain region (Figure 3c, pink regions) are put into a *Prepared state* (Figure 3c, yellow cells). Then, among the set of neurons in *Prepared state*, only a selected few cells will go one step further and generate APs, *Active state* (Figure 3c, red cells). The question is what cellular process determines the switch of one cell from the OFF state to *Prepared state*, and what determines the switch from the *Prepared state* to the *Active state*. We propose that the switch from OFF to *Prepared state* is mediated by clustered glutamatergic inputs, while the

generation of APs (*Active state*) is predominantly the consequence of distributed synaptic inputs (see below).

#### 4.2 | Time window 200–500 ms

The majority of brain processes related to the feeling of awareness require that neural activity lasts for 200–500 ms (Wu et al., 2015). This window of time is perhaps a minimum amount of time needed to guarantee interactions among multiple brain regions. The 200–500 ms of sustained firing triggers the awareness of a stimulus either directly by producing significant glutamatergic output in target brain areas, or indirectly by allowing the feedforward stream from thalamus to interact appropriately with feedback streams from higher cortical areas (Cauller, 1995; Engel et al., 2001; Lamme & Roelfsema, 2000; Larkum, 2012; Ro et al., 2003). The 200–500 ms time window of sustained neuronal depolarization may be the consequence of reverberant activity closing the loop between past and present features of a moving object, or by closing the loop between long-term memory traces and the current sensory percept (reviewed in Tononi & Koch, 2008). Interestingly, the duration of synaptically-evoked somatic plateau depolarizations (Figure 2a) is also in the range of 200–500 ms (Milojkovic et al., 2004; Oikonomou et al., 2012).

#### 4.3 | Global neural workspace

Psychological cognitive experiments suggest that a brain process exists (best termed consciousness), which enables the unification of otherwise separate neuronal functions. The unification of separate neuronal functions may be a serial process in which information travels from a node to a node (less likely) or occurring simultaneously in multiple nodes (more likely). It was previously suggested that distinct subsystems (brain regions), are competing for consciousness in the global workspace (Baars, 2002). This type of “competition” implies the existence of specific structures or specific networks performing exclusive integrating functions to keep track of the global workspace (Smallwood, Brown, Baird, & Schooler, 2012). If there were local structures exclusively responsible for multinet network integration, they would be expected to locally mirror activity from all member ensembles that they integrate. In line with these predictions, electroencephalography (EEG) and fMRI recordings identified several brain regions containing a significant number of converging network signals, in contrast to other regions in the same brain with much simpler function and activity patterns (Braga, Sharp, Leeson, Wise, & Leech, 2013; John et al., 2001; Mittner, 2013). The neural underpinnings of this putative global brain architecture, in which specific structures or networks fulfill integrating functions associated with the global workspace, are currently unknown. The proposed theories (Baars, 2002; Dehaene et al., 2001; Engel & Singer, 2001; Llinas & Ribary, 2001; Tononi & Koch, 2008) are focused on identifying brain regions engaged in the process, but they do not offer cellular (synaptic and membrane) mechanisms utilized by individual neurons in this process. Here we propose the embedded ensemble encoding principle, which employs the ability of dendrites to trigger local glutamate-mediated regenerative membrane potentials

(Figure 2a, dend. plateau spike). In order to compete for access into the global workspace, the candidate brain structures (member ensemble) influence the responsiveness of cells in the higher-order processing area, structures involved in multinet network integration (Braga et al., 2013; John et al., 2001). The goal of synaptic integration in this context is to trigger a dendritic plateau potential in the target brain area, which switches the state of the host neuron from *OFF* to *Prepared*. This is best achieved by clustered synaptic activity (Figure 4a). A “cluster” is just a group of presynaptic axon terminals arriving on the same dendritic branch and carrying closely related information. Which presynaptic axons converge to one dendritic branch is determined by the “connectome.” The connectome is based on initial developmental wiring, then later on the life experiences, learning, and memory. The connectome dictates that some cells are better connected with a particular functional ensemble than others. A functional ensemble is made of neurons carrying the same informational content in a given unit of time. The hardwiring is in essence equivalent to the idea of engrams. The engram can be considered similar to a memory trace, whereas formation of transient neural ensembles by synchronized AP firing is similar to memory retrieval or ephory. The term “ecphory” applies to a process in which an engram stored in the brain is retrieved and expressed behaviorally through interactions with strong retrieval cues, such as sensory input, ongoing behavior, or voluntary goals (Josselyn, Kohler, & Frankland, 2015). Experimental evidence supports the idea that mental representations, memory storage, and memory retrieval all operate using neural ensembles. Multiunit electrophysiological recordings performed in rodents showed that patterns of neuronal activity that occurred during the time of encoding are detected hours or days later, and when they do repeat, they influence the animal's behavior. Modern studies based on neuron “tag-and-erase” methods have shown that silencing the so called “engram” neurons block the memory usage, and thus established that activation of “engram” neurons is necessary for successful retrieval of memories. Conversely, artificial stimulation of the “engram” neurons induces artificial memory recovery, providing strong evidence that activation of “engram” neurons is sufficient for retrieval (reviewed in Josselyn et al., 2015). The “connectome-favorite” target cells receive large number of inputs, spatially segregated in their dendritic tree (clustered inputs, Figure 4b). Only cells in *Prepared state* will be able to tune into synaptic activity constantly impinging across the entire dendritic tree (distributed inputs, Figure 4b) and convert these distributed synaptic inputs into AP firing output in order to join the network of active neurons. AP firing patterns are then used for binding (Engel & Singer, 2001).

## 5 | PART 4: EMBEDDED ENSEMBLE ENCODING

In this section, we combine: (a) clustered glutamatergic inputs, (b) dendritic plateau potentials, (c) sustained depolarization of the cell body, (d) distributed glutamatergic inputs, (e) temporal coding, and (f) frequency coding, into one unified principle for transient recruitment of CNS neurons into dynamic neural ensembles. We dubbed this principle: *embedded ensemble encoding*, EEE theory.

### 5.1 | Rate coding and temporal coding merge

Neural synchrony with a millisecond precision may be crucial for processing of information in mammalian brain, underlying many aspects of cognitive functions including the arousal, sensory perception, attentional selection, and working memory. Current models suggest that synchrony can occur through reciprocal connectivity among groups of neurons (Bair, 1999; Buzsaki & Silva, 2012; Salinas & Sejnowski, 2001). The EEE theory modifies this by hypothesizing that, on a particular cycle of ensemble of neurons in the *Active state* ( $E_{Act}$ ), this connectivity will only be effective among current members of  $E_{Act}$ . For the same level of firing, synchronous input is more effective on postsynaptic neurons than asynchronous input (London et al., 2002; Schneidman et al., 1998). Such synchronous firing might then not only signal cooperative binding during this period of an  $E_{Act}$ , but would also activate sets of cells that are not involved in the current  $E_{Act}$  to set up a new  $E_{Act}$  for the subsequent period. Additional oscillatory activity associated with alpha activation will facilitate synchronous interactions by providing a restricted time frame within the longer period of the plateau depolarization (neuronal *Prepared state*) that underlies  $E_{Act}$ . In our view, those oscillatory time intervals are superimposed on the glutamate-mediated dendritic plateau potentials (Figure 2d, *Prepared state*) to enhance spike and burst synchronization. EEE theory is innovative in that it postulates two different ways to define cell assemblies, rather than the one usually assumed. The dendritic plateau activation in multiple cells leads to the ensemble of prepared cells (Figure 4c<sub>4</sub>, Yellow). Spike generation is permissive in members of this ensemble only, which leads to the more transient production of multiple activity-synchronized embedded ensembles  $E_{Act}$  at different times during the duration of the plateau (Figure 4c<sub>4</sub>, Red). EEE theory proposes that larger “Master” ensembles (Figure 3b) are made of neural subensembles (member ensembles) simultaneously organized in two planes, space and time: (1) Subensembles as physically distributed entities (Figure 4c<sub>4</sub>); and (2) Subensembles as transient, temporal entities—temporal coding embedded in rate coding (Ainsworth et al., 2012; Fries, Nikolic, & Singer, 2007). These two related concepts are transformational because they pull together two concepts (rate vs. temporal coding) that are typically seen to be in opposition (Ahissar, Sosnik, & Haidarliu, 2000).

### 5.2 | Bayesian predictive coding

Depolarized prepared state provides bases for choosing one solution over another, which, in essence, is “Bayesian process.” In statistics, probability of some event is represented by its frequency. The more often some phenomenon occurs, the more probable it is. In a Bayesian process, probability is interpreted as reasonable expectation representing a state of knowledge, or as quantification of a personal belief. Knowledge is previous experience “recorded” by glutamatergic connections in the brain (e.g., long term potentiation (LTP)). Glutamatergic inputs trigger dendritic plateau potentials (Figures 2 and 4). Personal belief is comprised of knowledge and emotional state, both supported by the activity of hippocampus and limbic system, which project their glutamatergic inputs into the cerebral cortex, where we conceptualize dendritic plateau potentials

in this article (Figure 3). Gathering information from as many regions as possible (Allen et al., 2017) is a procedure that serves to reduce ambiguity in computational tasks. The neuronal depolarized state (Prepared state) could be conceived as the Bayesian expectation that is then ready to be played into by input from either: hierarchically higher brain areas, or hierarchically lower areas, depending on a specific task.

Bayesian predictive coding theory suggests “how information about the environment, the individual's needs, motivational states, and previous experience are represented” (Clifford, Mareschal, Otsuka, & Watson, 2015). In Bayesian theory, information streams are theorized to interact and provide information integration via projections between hierarchically-lower and hierarchically-higher cortical areas (as well as from amygdala, thalamus and other areas, Friston, 2008). Higher cortical areas provide predictions or internal models that are then used to interpret incoming sensory information. Ongoing Bayesian expectation information processing is nicely explained in the context of the problem of recognizing a coworker when you see them outside of work. At the work place, neurons that code for the list of coworkers are kept in a *Prepared* state each time a person enters our visual field. Outside the work place, this preparatory signal is missing, and a coworker may go unrecognized.

Another good example of the Bayesian expectation task is the need to identify the same object at almost any angle or perspective even if that object has never been seen before at that particular angle. In the multimodal association cortex, the neuronal depolarized state (*Prepared* state) could be conceived as the Bayesian expectation that is then ready to be played into by projections from the primary sensory areas. Presented with a multitude of choices and pressed to solve complex cognitive tasks, a healthy brain is in a permanent and dire need for: (a) context and (b) reasonable expectations. Chance favors the prepared mind/neuron. You will not recognize something that you are entirely unprepared for.

### 5.3 | Searchlight hypothesis

The “searchlight hypothesis” (Crick, 1984) shares many features with the present *embedded ensemble encoding hypothesis* (EEE). First, both theories are fundamentally grounded in the idea that neural ensembles are central for brain function. In the searchlight hypothesis, the searchlight appears to be focused on one important object, and it is controlled by reticular nuclei of thalamus. In the EEE theory, there is no focus on one object, but rather all (i) possible, (ii) similar, and (iii) reasonable object vectors are silently turned ON at the same time, waiting for sensory input or memory retrieval signal to pick the best active ensemble embedded in a much larger ensemble of cells waiting in a depolarized prepared state. Second, in the searchlight hypothesis the reticular thalamic nucleus is working through von der Malsburg synapses—strong synapses formed by previous experiences. The EEE theory equally depends on excitatory connections formed by previous life experiences and learning. One major difference is that EEE theory specifies that connections which carry similar information or closely related information must impinge on the same dendritic branch. Clustered together in space and time, the EEE synapses exploit both synaptic and intrinsic

dendritic excitabilities for amplifying afferent signals and converting excitatory postsynaptic potentials into dendritic plateau potentials, which, in turn, maintain the cell body in a sustained depolarized state for 200–500 ms (Milojkovic et al., 2004).

### 5.4 | Closing remarks

In summary, the same general type of cell recruitment process is used across the entire telencephalon (cortex, striatum, accumbens, amygdala, and hippocampus). In any given brain region at any given moment of time, nerve cells are found in one of the three basic states, *OFF*, *Prepared*, and *Active* (Figure 4c<sub>1</sub>). Each spiny neuron is capable of transitioning between three basic states, back and forth (Figure 4c<sub>2</sub>). For the reasons explained in Figure 2, the proper transition from the neuronal *OFF* to the *Active* state requires one obligatory step, a *Prepared* state. Since, the recruitment of neurons into the *Active* state is most easily accomplished from neurons in the *Prepared* state (Figure 4b), the group of *Active* cells is going to be a subgroup of *Prepared* cells (Figure 4c<sub>3-4</sub>). The ensemble of *Active* cells is embedded in the ensemble of *Prepared* cells. *Active* cell is the cell that is spiking, hence providing the substrate to two types of temporal coding: (1) *Rate coding*, which will allow binding with a subset of *Prepared* cells that reached AP firing threshold; and (2) *Spike-time coding*, which will allow binding with a subset of cells in the *Active* state (e.g., gamma oscillation).

Among several classes of dendritic spikes (Brandalise et al., 2016; Golding et al., 2002; Schiller et al., 2000), the glutamate-mediated plateau (Milojkovic et al., 2004) and apical calcium plateaus (Larkum, Zhu, & Sakmann, 1999) produce the strongest and longest depolarization at the AP initiation site and therefore have the most direct influence on cell firing via two mechanisms: (a) depolarization amplitude approaching the AP voltage threshold and (b) shortening of the neuronal membrane time constant. As a result, small synaptic inputs, previously ineffective, now gain the ability to drive the initiation of APs—“Chance favors the prepared cell.” Our central hypothesis is that the dendritic plateaus lead to a prepared neuronal state that favors spike generation, which results in the emergence of the embedded ensemble of prepared cells (Figure 4, yellow color). Dendritic plateau potentials and the resulting plateau depolarizations of the cell body (*Prepared* state) produce notable changes in the neuronal electrical behavior (Figure 2d), which are poised to spill over into the network dynamics, and are therefore too important to be left out. It would be interesting to examine in computational models, dynamic features of ongoing network activity that are gained when model pyramidal neurons, members of the large-scale network, are provided with the ability to produce dendritic plateau potentials (Antic, Acker, Zhou, Moore, & Milojkovic, 2007; Bittner et al., 2015; Gambino et al., 2014; Plotkin et al., 2011). We hypothesize that “embedded ensemble encoding” (Figure 4c<sub>4</sub>) may be the pivotal organizing principle in cortical networks of neurons.

### ACKNOWLEDGMENTS

We are grateful to Salvador Dura and other members of the Antic, Hines, and Lytton laboratories for critical comments. National Institute of Mental Health, R01MH063503, U01MH109091 and



R01MH086638; National Institute of Neural Disorders and Stroke, R01NS11613; National Institute of Biomedical Imaging and Bioengineering, R01EB022903 and U01EB017695; Associated Editor: Joshua Plotkin.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### AUTHOR CONTRIBUTIONS

All authors provided critical feedback and helped shape the manuscript.

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**How to cite this article:** Antic SD, Hines M, Lytton WW. Embedded ensemble encoding hypothesis: The role of the "Prepared" cell. *J Neuro Res*. 2018;00:1–17. <https://doi.org/10.1002/jnr.24240>