The roles of NMDA- and GABA receptors for working memory activity in primate prefrontal cortex

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Statement of contributions according to § 9 (2):

The thesis at hand is based on a paper that is in the process of publication and is titled "Blockage of NMDA- and GABA(A) receptors improves working memory selectivity of primate prefrontal neurons". Stephanie Westendorff and Andreas Nieder are co-authors of the paper, Paul Rodermund is the first author. The manuscript of the paper was used as a template for the thesis at hand, rewritten and vastly expanded concerning data analysis and topics covered. With regard to the paper Paul Rodermund and Andreas Nieder de-signed experiments; Paul Rodermund carried out experiments; Paul Rodermund and Stephanie Westendorff analysed data; Paul Rodermund, Stephanie Westendorff and An-dreas Nieder wrote the paper. The order of names mentionend reflects the significance of their respective contribution.

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As this doctoral thesis also marks the end of my academic career, I would like to thank my family and friends for all their personal support throughout the last years. Special thanks go to Karl, Friedrich and Wladimir for reliable guidance. I dedicate this thesis to my first born daughter, Leyla Hashemi, and my significant other Roxana Hashemi.

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Abstract

The persistent activation of prefrontal neurons after a stimulus has disappeared is considered a neuronal correlate of working memory. Current explanations suggest that persistent activation during a delay depends on a delicate but poorly understood interplay between excitatory glutamatergic and inhibitory GABAergic receptor effects. We addressed the roles of these receptor systems directly by iontophoretically applying the NMDA receptor antagonist MK-801 and the GABA(A) receptor antagonist bicuculline methiodide while simultaneously recording extracellular activity in prefrontal cortex of monkeys performing a working memory task. Following a delay period monkeys had to decide whether they had previously seen a stimulus that was either absent or present at intensities close to perceptual threshold. The blockade of GABA(A) receptors strongly improved the stimulus selectivity of the neurons' delay activity, causing an increase in signal to noise ratio during working memory periods as well as an enhancement of the neurons' coding selectivity. The blockade of NMDA receptors resulted in a slight enhancement of stimulus selectivity and encoding capacity of the neurons. Inactivation of both NMDA- and GABA(A) receptors in the same individual neurons showed a similar enhancement of the neurons' coding selectivity. Our findings emphasize the delicate and more complex than expected interplay of excitatory and inhibitory transmitter systems in modulating working memory coding in prefrontal circuits.

Introduction

Working memory

Working memory refers to the capacity to hold and manipulate information for brief periods of time (Baddeley, 1992). Fuster (2015), in agreement with Baddeley (1993), proposed that working memory can be best understood as sustained attention focused on an internal representation. Using information whose sensory trace has ceased for a goal-directed action is a vital cognitive function for everyday behaviour. It is central to many cognitive functions, such as perception, attention, and inhibitory control. Not surprisingly, working memory deficits often accompany more general cognitive deficits and are a hallmark of severe psychiatric disorders such as schizophrenia (Benes, 1995).

Abstractly speaking, working memory mediates contingencies between information from the past and consecutive actions in the future across short periods of time. To investigate the working memory period in between sensory evidence and subsequent actions many studies use variants of delay tasks, pioneered by Hunter (1913). Delay tasks are widely considered as the hallmark of working memory on the behavioural level. They require the subject to keep information of a stimulus online in working memory across a delay phase, in which the respective sensory information is absent, until it is needed for an appropriate response (Shettleworth, 2010). Delay tasks thus at least comprise three phases, a sample phase where the stimulus is presented, a delay phase, where sensory evidence is absent, and a response phase, where an appropriate motor action is executed. This task layout affords that the stimulus is perceived and remembered anew in each trial and a subsequent action,

contingent on the previous event, is executed while alternative actions are rejected (Fuster, 2015). Performance in delay tasks is dependent on many factors, amongst others on the richness of cues, the duration of stimulus presentation and the duration of the delay phase (Shettleworth, 2010; Fuster, 2015). In general, performance improves with saliency, unambiguousness and duration of the sensory evidence and deteriorates with longer delay periods.

Memory neurons

In delay-paradigms similar to those described above, current neuronal evidence suggests that working memory is physically instantiated as stimulus selective persistent spiking of neurons in the prefrontal cortex (PFC) and other cortical areas during the delay period (Goldman and Rosvold, 1970; Fuster and Alexander, 1971; Kubota and Niki, 1971; Funahashi et al., 1989; Goldman-Rakic, 1991). These neurons selectively elevate their firing rate during the retention of a specific task feature and appear central to short-term maintenance of task information (**Fig. 1**). In accordance with Fuster (2015) I will henceforth refer to these neurons as memory neurons or delay-selective neurons. Sustained neuronal delay activity is thought to reflect sustained representation of working memory content. The persistent activity of memory neurons is stimulus specific, commonly leading to elevated responses to a preferred stimulus and unchanged or inhibitory responses to non-preferred ones (Goldman-Rakic, 1999). By definition their firing rate is higher during mnemonic periods than in non-mnemonic task phases (Goldman-Rakic, 1996; Dash et al., 2007; Fuster, 2015).

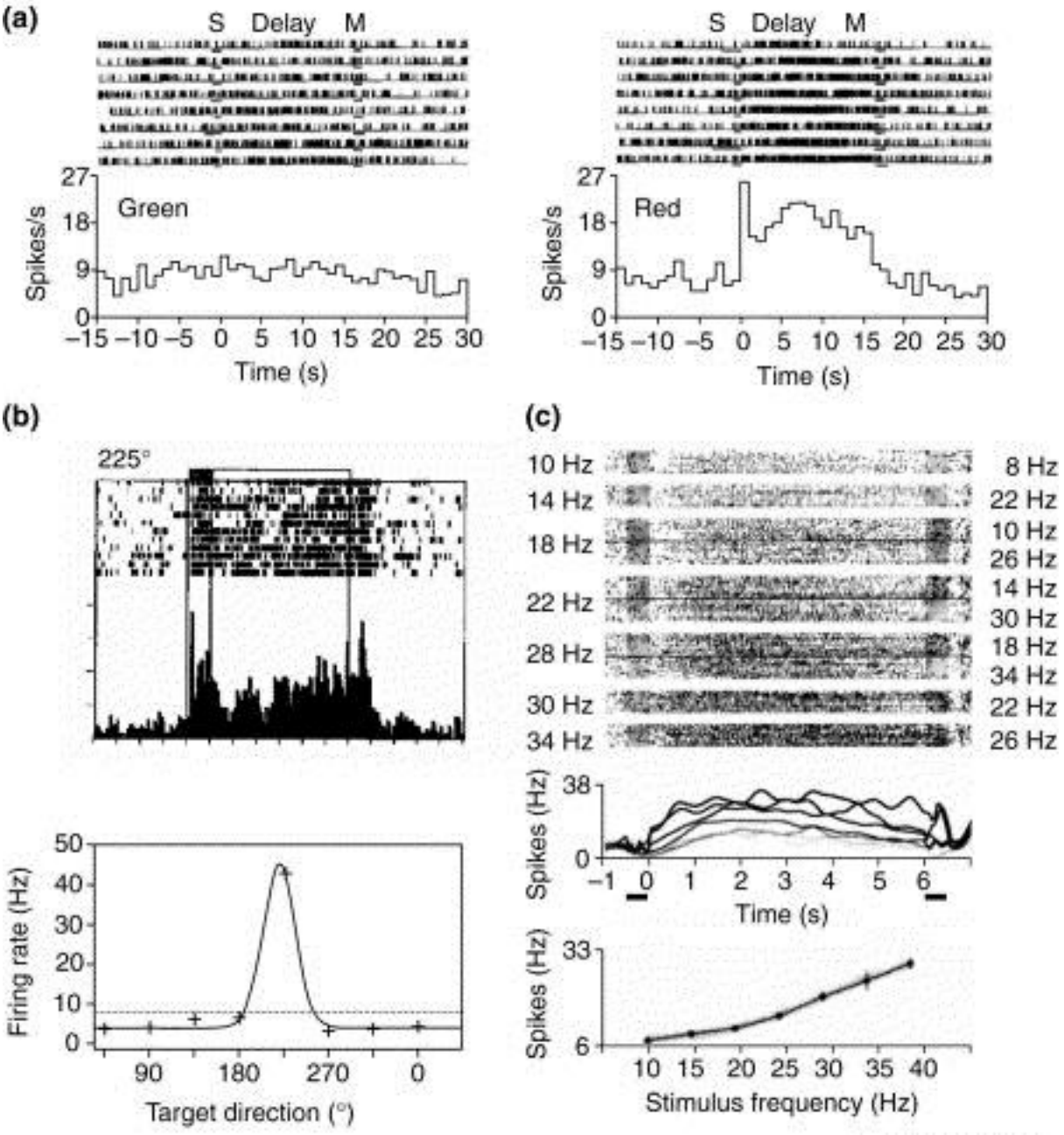

Figure 1. Three types of memory neurons. **a**, Discrete working memory. In a delayed match to sample experiment, an inferotemporal neuron shows sustained high activity for the colour red (but not green) of a visual cue, during a delay period of 16 s. Redrawn from Fuster and Jervey (1981). **b**, Spatial working memory. In a delayed saccade experiment, a prefrontal neuron shows persistent activity that is tuned to a preferred location of a visual cue. Upper panel: rasters and cumulative spike histogram for a preferred cue; lower panel: spatial tuning curve of delay period activity. Redrawn from Funahashi et al. (1989). **c**, Parametric working memory. In a delayed somatosensory discrimination task, a neuron in the inferior convexity shows persistent activity with a firing rate proportional to the cue frequency. Upper panel: rasters. Cue stimulus frequency indicated on the left, comparison stimulus frequency indicated on the right. Middle panel: trial-averaged firing rates as a function of time. Lower panel: mean firing rates, averaged across the entire delay period, as a

function of the cue frequency. Redrawn from Romo et al. (1999). *Figure and modified description from Wang (2001)*.

Importance of PFC for working memory

Specifics of memory neurons in the PFC

The neuronal network involved in working memory depends on the task, sensory modality and memory content (Christophel et al., 2017). Memory neurons are most numerous in the PFC, but are also found in association cortices like the posterior parietal cortex or the inferior temporal cortex, sensory and subcortical areas (**Fig. 2**) (Wang, 2001; Constantinidis and Wang, 2004; Riley and Constantinidis, 2016; Christophel et al., 2017). An extensive review on brain areas displaying sustained activity however suggests that only the association cortices exhibit robust persistent spiking suitable for working memory (Leavitt et al., 2017). The PFC stands out as it is involved in virtually all working memory tasks and memory cells can be found irrespective of the specifics of the task (Fuster, 2015). Working memory related spiking in the PFC has amongst others been found for natural images and objects (Miller et al., 1996), spatial information (Goldman-Rakic, 1995), frequency of tactile vibrations (Romo et al., 1999), colour (Buschman et al., 2011), visual motion (Zaksas and Pasternak, 2006), or numerosities (Nieder, 2002). Persistent, feature-specific delay activity in the PFC also occurs for task-irrelevant features as well as when animals are learning or only passively fixating, implying that feature-specific representations during a delay may arise spontaneously and unconsciously (O Scalaidhe et al., 1997; Constantinidis et al., 2001; Lauwereyns et al., 2001; Meyer et al., 2011; Meyers et al., 2012; Donahue and Lee, 2015).

Further highlighting the distinctiveness of PFC in working memory, neurons in the posterior parietal and inferior temporal cortex were prone to interference by a distractor, while neurons in the PFC commonly continued to code for the to be remembered item (di Pellegrino and Wise, 1993b, 1993a, Miller et al., 1993, 1996; Constantinidis and Steinmetz, 1996; Powell and Goldberg, 2000; Qi et al., 2010; Suzuki and Gottlieb, 2013). Depending on the precise circumstances, PFC neurons however also coded for the distractor (Jacob and Nieder, 2014; Cavanagh et al., 2018).

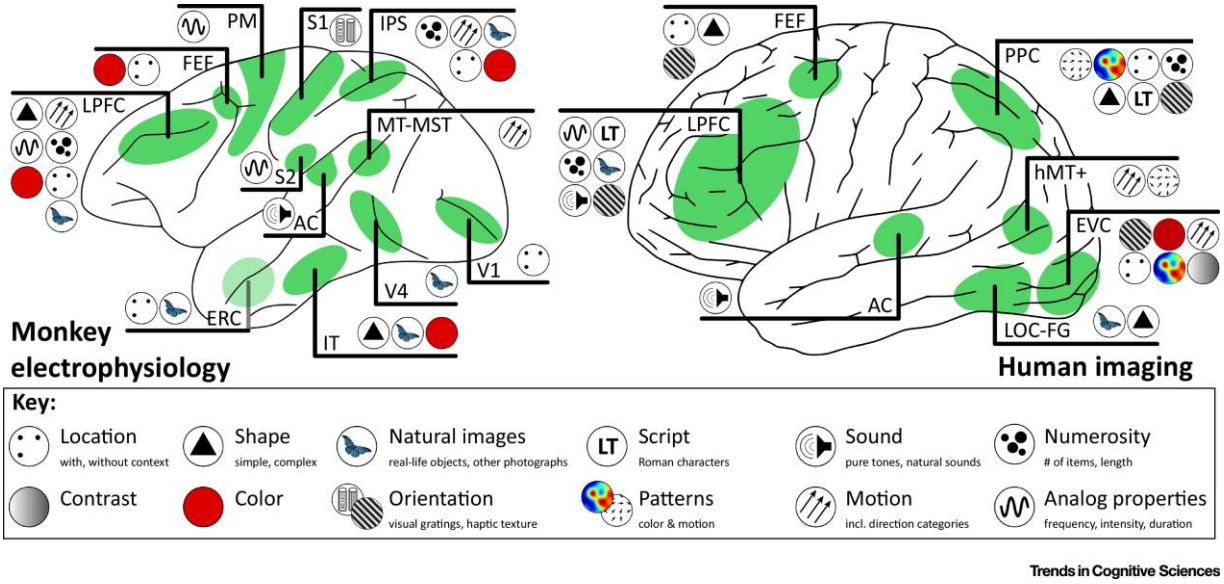

Figure 2. Overview of content-specific activity during working memory delays in the macaque (left) and human (right) brain. Icons indicate persistent stimulus-selective activity for each stimulus type indicated by the icon (see legend) at the respective locations. Brain areas are identified by abbreviations. AC, auditory cortex; ERC, enthorinal cortex; EVC, early visual cortex; FEF, frontal eye fields; FG, fusiform gyrus; hMT+, human analog to MT/MST; IPS, intraparietal sulcus; IT, inferior temporal cortex; LOC, lateral occipital complex; lPFC, lateral prefrontal cortex; PM, premotor cortex; PPC, posterior parietal cortex. *Figure and modified description from Christophel et al. (2017).*

Memory neurons in the PFC predict working memory performance

The memorandum (short for working memory content) is stored in the PFC in a way that is suitable to guide behaviour and thus not only contains retrospective information about the

stimulus but also prospective features relevant for the goal-directed action (Christophel et al., 2017). In line with this, persistent delay activity in the PFC is a good predictor of performance in working memory tasks. The discriminability of memoranda is positively correlated with the activity level of memory neurons and in error trials the persistent firing is suppressed (Funahashi et al., 1989; Constantinidis et al., 2001; Zhou et al., 2013). This means that animals are more likely to successfully solve a trial in a delay task when the neurons encoding the relevant task-feature are more active during the delay. Importantly, this close link of PFC memory neurons to behaviour has not been found for other brain areas or other neuronal parameters that have been suggested to underlie working memory.

PFC lesions cause working memory deficits

The PFC lies at the apex of the cortical hierarchy, underlies all executive functions and serves the purpose of representing and organizing information for execution of goal-directed actions (Fuster, 2015). Neuroanatomical properties of the PFC make the area well suited for exhibiting persistent activity. The expanded dendritic trees of layer III pyramidal neurons of monkeys have the highest number and density of spines in the cortex, as well as a wide ranging connection network, providing an anatomical substrate for reverberate and continuous excitations of the neurons (Riley and Constantinidis, 2016; Datta and Arnsten, 2018). The PFC further exhibits strong dopaminergic innervation, which has been shown to modulate working memory activity, and γ-Aminobutyric acid (GABA) interneurons specialised for stabilizing sustained activity (Riley and Constantinidis, 2016; Datta and Arnsten, 2018).

Indeed, in addition to electrophysiological studies, frontal ablations or lesions have accumulated evidence for the prominent role of PFC for working memory. Both, permanent and reversible lesions cause profound deficits in working memory performance of non-human primates as well as humans (Jacobsen, 1935; Spaet and Harlow, 1943; Blum, 1952; Mishkin, 1957; Milner, 1963; Glick et al., 1969; D'Esposito and Postle, 1999; Castner et al., 2004; Datta and Arnsten, 2018). The deficits mainly manifest in longer learning intervals and especially impaired performance with increasing delay intervals (Fuster, 2015). Tasks that only required maintaining information rather than monitoring task progression or manipulating the information, e.g. mental rotation, were however not severely affected by frontal lesions (Dash et al., 2007).

Visual processing is said to separate into a dorsal- and ventral visual stream, with the former stretching from V1 via the occipital- to the parietal cortex, mainly processing spatial information and the latter one reaching to the temporal cortex processing feature-based information (Mishkin and Ungerleider, 1982; Goodale and Milner, 1992). Amongst others lesion studies confirmed this well-studied separation of a dorsal "where" and a ventral "what" stream, as the posterior parietal cortex and the dorsolateral PFC were mainly involved in spatial working memory tasks, the inferior temporal cortex and the ventrolateral PFC are obligatory for non-spatial visual working memory (Müller and Knight, 2006; Fuster, 2015). Other than that the dorsolateral PFC is involved in the integration of spatial and temporal information and is thus involved in most working memory tasks, while ventral areas of the PFC are largely concerned with response control (Mishkin, 1964; Mishkin et al., 1969; Rushworth et al., 1997). The principal sulcus in the lateral PFC seems to be the most

delicate spot with regard to working memory, as most memory cells have been found here and lesions at this locus in turn most prominently impaired working memory (Castner et al., 2004; Fuster, 2015).

Is the memorandum stored in the PFC?

While there is no doubt that the PFC plays an important role in working memory, the idea that the PFC is also the site where the memorandum is stored has been challenged (Lara and Wallis, 2015). PFC lesions cause deficits beyond working memory and studies of PFC lesion patients find that not all kind of tasks which utilize working memory are disturbed (Malmo, 1942; Janowsky et al., 1989; D'Esposito and Postle, 1999). This led to the suggestion that the PFC exerts more general cognitive control functions which are necessary for attention, selection, organization and manipulation of working memory (Müller and Knight, 2006; Lara and Wallis, 2015; Pasternak et al., 2015).

It has also been proposed that the posterior association or sensory cortices are involved in actual storage of perceptual information while the PFC exhibits top-down executive control, causes focussed attention on the internally represented memorandum and holds memory related to the cognitive control of behaviour (Fuster, 2015). More specifically, the PFC might help selecting the relevant neural representation when different memoranda are competing, e.g. in a distractor task (Sreenivasan et al., 2014). This idea is in line with the PFC exhibiting top-down executive attention on upstream areas in other contexts, e.g. for inhibitory control (Shallice, 1982; Duncan, 2001; Braver et al., 2008). Findings of reversible lesion studies are partially conflicting on this matter. Fuster and colleagues (1985) for example

found that cooling either the PFC or inferior temporal cortex in a visual working memory task resulted in reduced spiking and a loss of selectivity in previously stimulus selective memory neurons in the other brain area (inferior temporal cortex or PFC) respectively, showing the interconnectedness of different brain areas involved in working memory. In other studies reversible inactivation of the PFC, however, impaired memory performance, but reversible inactivation of the posterior parietal cortex did not (**Fig. 3**) (Fuster and Bauer, 1974; Bauer and Fuster, 1976; Chafee and Goldman-Rakic, 2000). While these findings highlight the importance of the PFC for working memory, they partially also hint at the distributed nature of working memory, leaving the question of the locus of information storage unanswered.

To date, direct evidence for PFC sustained activity as a marker of PFC's top-down control of posterior areas is sparse and the susceptibility of posterior areas to distractors argues against the memorandum being stored there. Delay activity in sensory areas was often absent in working memory tasks and prone to interference in association areas other than the PFC. Nevertheless, on a population level it was often possible to decode sufficient information related to working memory from upstream areas like the visual cortex even in the absence of persistently spiking neurons (Harrison and Tong, 2009; Serences et al., 2009; Albers et al., 2013; Ester et al., 2013; Xing et al., 2013).

Similarly, human functional Magnetic Resonance Imaging (fMRI) studies have often argued against persistent delay activity in the PFC as a neuronal correlate of working memory, as they were also able to decode the memorandum from the Blood-oxygen-level-dependent (BOLD) signal in the visual and posterior parietal cortex (Todd and Marois, 2004, 2005; Xu and Chun, 2006; Harrison and Tong, 2009). FMRI however does not measure neuronal

activity per se, but links changes of blood flow to neuronal activity, disregarding whether neurons are inhibited or excited. Nevertheless, these findings have further challenged the notion that sustained delay activity is the neuronal correlate of working memory which will be discussed more thoroughly later.

Christophel and colleagues (2017) in turn argue that delay activity in early sensory areas reflects simple features of the memorandum while delay activity in PFC reflects a more abstract representation of the memorandum or even the plan for a subsequent goal-directed action. Sreenivasan and D'Esposito (2019) similarly suggest that delay activity in different cortical areas might represent different representational formats that are used dependend on strategies or task demands. This is in agreement with observed different roles of the PFC and other cortical areas in delay tasks. Memory neurons in the posterior association areas were more precisely tuned to the physical appearance of a stimulus than memory neurons in the PFC (Fuster and Jervey, 1982; Fuster, 1990; Zhou et al., 2007). Other than memory neurons in the posterior parietal cortex and the inferior temporal cortex, memory neurons in the PFC generalized across categories and tasks and thus exhibited more abstract representations of a memorandum (Freedman et al., 2001, 2003; Sarma et al., 2016). PFC neurons may additionally code for memoranda, even as abstract as the number of countable elements, from different sensory modalities (Romo et al., 1999; Nieder, 2012, 2016; Vergara et al., 2016).

These findings argue in the direction of specialized roles of the different association cortices during working memory and other cognitive functions (Katsuki and Constantinidis, 2012; Riley and Constantinidis, 2016). The idea that the memorandum is stored in one specific

brain area is thus probably oversimplified. Rather different aspects of working memory content are echoed by different brain areas, with the PFC exerting more abstract representations used for subsequent actions.

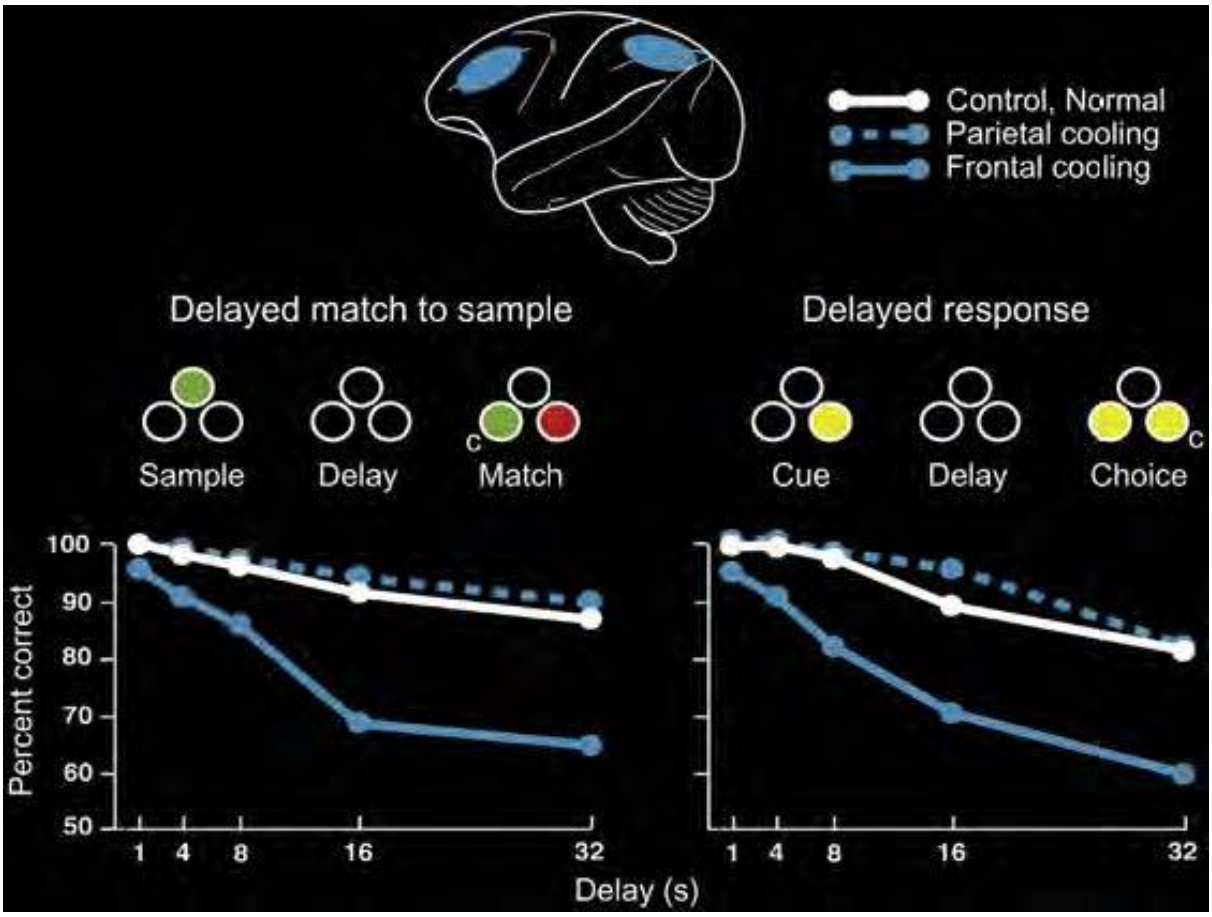

Figure 3. Effects of bilaterally cooling (to 20°C) parts of lateral prefrontal cortex or posterior parietal cortex (blue areas) on the performance of two delay tasks. Abbreviation: c, correct response. Cooling was applied throughout blocks of trials (sessions) with delays of varying length. Cooling sessions alternated with control sessions at normal cortical temperature. Note that prefrontal, but not parietal, cooling induces in both tasks deficits in correct response that increase with the length of intratrial delay. *Figure and modified description from Fuster (2015).*

Mechanisms of sustained activity

As reviewed earlier, memory neurons in the PFC exhibit elevated firing during a delay and are widely considered as the neuronal correlate of working memory. Before diving into more

current debates on whether sustained activity can be regarded as the veridical neuronal correlate of working memory we will take a look at potential underlying mechanisms.

Delay spiking may last for several seconds, which exceeds the time scales of synaptic integration and suggests that intrinsic neuronal properties or network dynamics are maintaining the elevated firing rate (Zylberberg and Strowbridge, 2017). The main theories regarding mechanisms underlying persistent firing in PFC neurons can be subdivided according to their locus of interest. The most focal theories try to explain sustained activity by intrinsic mechanisms of the respective neuron. They commonly regard neurons as bistable units with a default resting state and a memory state of persistent firing. Next are explanations that take neighbouring neurons into account, prominently this comprises the notion of recurrent excitation. On a larger scale are mechanisms that highlight long ranging interconnections of brain areas and distributed neuronal networks.

Bistable neurons and attractor states

Models incorporating persistently spiking delay neurons commonly rely on dynamic attractor states (Amit and Brunel, 1997; Compte et al., 2000; Druckmann and Chklovskii, 2012). An attractor state signifies a specific firing pattern that a neuronal network is drawn towards. The underlying idea is that neurons within a network are bistable and exhibit a default spontaneous resting state of low firing rate and an active state of persistently sustained activity that codes for the memorandum (Hopfield, 1982; Zipser et al., 1993; Constantinidis and Wang, 2004).

Bistability of single neurons, i.e. two equilibrium states with regard to their firing rate, could, in principle, be generated by ion channels. Calcium currents, for example, provide a strong neuronal signal modulating the neuron's membrane potential and triggering downstream intracellular signalling cascades (Wang, 2001). With regard to intrinsic mechanisms that may underlie persistent firing three major classes are currently investigated: voltage-gated Na^+ and Ca^{2+} currents, nonselective cation currents tracking Ca^{2+} concentrations, as well as state changes in K^+ or cation currents (Zylberberg and Strowbridge, 2017).

N-Methyl-D-aspartate (NMDA) receptors could be of great importance for generating bistability of neurons. Conductance of NMDA receptors is strongly voltage dependent, with reversal potentials, i.e. membrane potentials at which there is no net flow of ions inside and outside the cell, below -100 mV and around 0 mV, depending on the specific subunit composition, and a peak in cation influx in between (Hansen et al., 2017). In combination with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and GABA(A) receptors, the two reversal potentials of NMDA receptors could underlie two stable fixed points with the lower one corresponding to the resting state of the neuron and the higher one corresponding to the spiking state (Durstewitz et al., 2000a).

Cellular bistability could be especially important when novel information needs to be maintained in working memory. In these cases synaptic learning, which is thought to underlie the construction of neuronal networks involved in recurrent excitation, would be too slow (Domjan and Burkhard, 1993). These novel stimuli could in principle be represented by previously generated networks, which would correspond to the observed mixed selectivity of prefrontal neurons, i.e. neurons simultaneously coding for different task-

related aspects (Warden and Miller, 2010; Rigotti et al., 2013). Nevertheless, assuming two stable fixed spiking states may be too simple. Models incorporating bistability for example struggle with representing continuous quantities and reproducing the complex temporal dynamics observed in memory neurons (Major and Tank, 2004).

On a larger scale, different spike patterns within a network of activated neurons correspond to different attractor states, each coding for a specific memorandum (Wang, 2001). This may however be a very error prone way of coding for a memorandum, as attractor states are highly susceptible to interference and small changes in a subset of neurons would already correspond to a different attractor state (Miller et al., 2018). Further complications arise as attractor state models could not yet account for simultaneous storage of more than one item in working memory. Several models have thus proposed non-overlapping attractor states by assuming sparse coding of each memorandum by a small subset of the neuronal population in order to reduce interference (Amit et al., 2003). With regard to the PFC, this does not, however, corroborate with neurophysiological findings, as representations are largely overlapping and even a single neuron may code for different memoranda (Warden and Miller, 2010; Rigotti et al., 2013). While the underlying idea of attractor states seems reasonable, to date these models are immature and yet cannot account for the complexity observed in working memory tasks on a behavioural and neurophysiological level.

Recurrent excitation

The widest accepted theory regarding the mechanisms of sustained activity is recurrent excitation. It proposes that persistent spiking of memory neurons is generated by

reverberant activation from horizontal excitatory connections of clusters of glutamatergic pyramidal neurons tuned to the same memorandum (**Fig. 4**) (Goldman-Rakic, 1995; Riley and Constantinidis, 2016). It was mainly the groundbreaking work of Goldman-Rakic and her colleagues that put forward the idea that the PFC is organized columnarly, with neurons within one column coding for a specific memorandum (Goldman-Rakic, 1995). Within the lateral PFC of nonhuman primates memory cells were mainly found in the depth of cortical layer III (Goldman-Rakic, 1995).

Stimulus selectivity of persistently spiking neurons is thought to be mainly created by recurrent inhibition of interneurons from columns coding for another memorandum. Within and, importantly, across clusters of memory cells, lateral inhibition, i.e. suppression of activity of neighbouring neurons, mediated by fast spiking parvalbumin containing interneurons, preserved response specificity of the memory cells by inhibiting responses to non-preferred or irrelevant memoranda (Goldman-Rakic, 1995; Arnsten et al., 2012). Often these interneurons were oppositely tuned to the pyramidal cells they innervate, e.g. exhibiting antipodal stimulus preferences in spatial working memory tasks (Wilson et al., 1994). Counterintuitively, the pyramidal cells that were mainly innervated by interneurons may not have been the ones closest to them, as neighbouring pyramidal cells and interneurons were usually similarly tuned, constituting microcolumns of isodirectionally tuned pyramidal cells and interneurons (Rao et al., 1999; Constantinidis and Goldman-Rakic, 2002; Constantinidis and Wang, 2004).

These findings correspond well with a model put forward by Hopfield (1982) where cell assemblies coding for the same memorandum have strong excitatory connections, whereas

there are weak or inhibitory connections between cell assemblies coding for different memoranda. Anatomical and neurophysiological studies to date have gathered vast support for local recurrent excitation (Levitt et al., 1993; Kritzer and Goldman-Rakic, 1995; Melchitzky et al., 1998; Rao et al., 1999; Funahashi and Inoue, 2000; Gonzalez-Burgos, 2000). While it has become evident that this mechanism is underlying working memory processes, the data does not show whether it is also sufficient (Durstewitz et al., 2000a).

Thalamocortical and Corticocortical loops

Another notion is that persistent activity of PFC neurons could arise via long ranging cortical loops, such as reciprocal excitatory connections between cortical areas and the thalamus (Wang, 2001). These Thalamocortical loops would be in agreement with the observed sustained activity in thalamic neurons during the maintenance phase of working memory (Fuster and Alexander, 1973). Disruption of thalamic delay activity in rodents disturbed working memory performance as well as delay activity in frontal areas (Bolkan et al., 2017; Guo et al., 2017; Schmitt et al., 2017). However, it remains unknown whether thalamic mnemonic activity simply reflects persistent activity in cortical areas or whether working memory critically depends on reciprocal feedback of cortical and subcortical areas. Furthermore, it is also unclear whether sustained activity can be found in the thalamus in all contexts involving working memory (Constantinidis and Wang, 2004; Sommer and Wurtz, 2004).

An alternative mechanism by which persistent neuronal spiking during a delay could arise are the long-range Corticocortical loops between the PFC and posterior association cortices (Goldman-Rakic, 1987; Chafee and Goldman-Rakic, 1998; Sarnthein et al., 1998). However, disruption of mnemonic activity in the posterior parietal cortex by distractors did not affect performance in working memory tasks, arguing against a critical dependence on the posterior parietal cortex in the maintenance of working memory (Constantinidis and Steinmetz, 1996; Compte et al., 2000). Furthermore, several of the earlier mentioned reversible lesion studies argue against working memory being strongly dependent on posterior association cortices (Fuster and Bauer, 1974; Bauer and Fuster, 1976; Chafee and Goldman-Rakic, 2000). Thus, while the PFC is highly connected to other cortical and subcortical areas and these areas may be involved in processing working memory information, they do not seem to critically underlie persistent firing of PFC neurons during a delay.

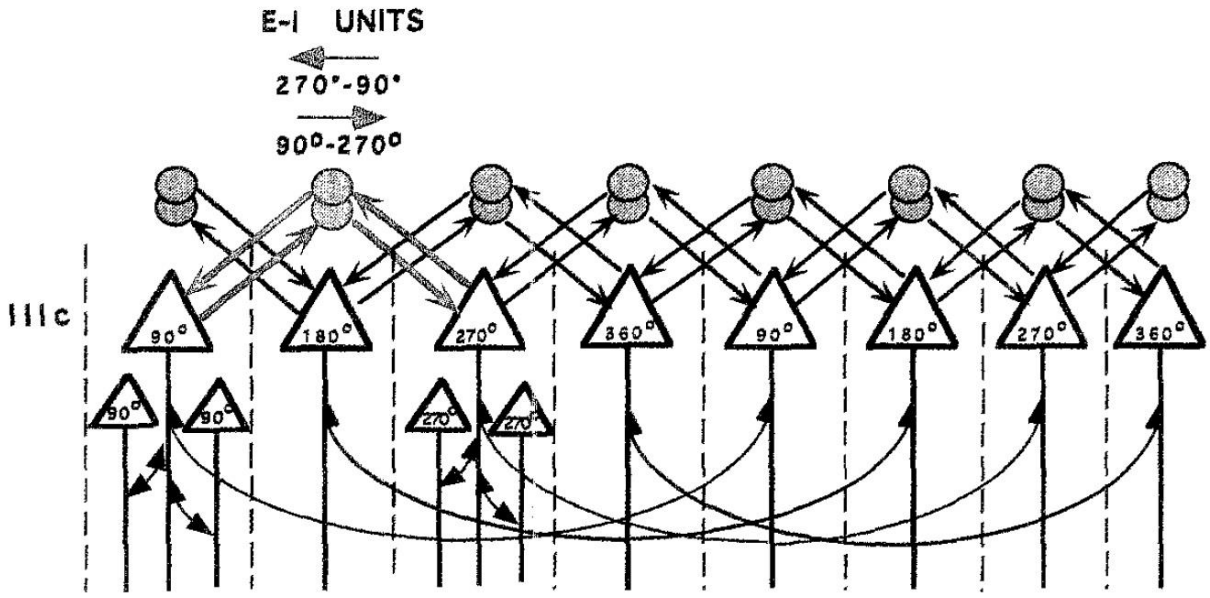

Figure 4. Hypothetical Model of Working Memory Modules in Prefrontal Cortex. Model of working memory modules consisting of clusters of tuned pyramidal neurons (triangles) arrayed by target location and directly interconnected with each other by their local excitatory axon collaterals (long, thin, curved arrows). Clusters of pyramidal neurons with like best directions are interconnected in a manner similar to iso-orientation columns in visual cortex. Two inhibitory interneurons (gray circles; presumed basket cells in the diagram) provide the reciprocal interconnections (straight arrows) between pyramidal cells with opposite best directions. For simplicity, only the 90°-270° and 270°-90° ensemble is illustrated. *Figure and modified description from Goldman-Rakic (1995).*

Do memory neurons show persistent spiking?

There remains a robust debate as to whether persistent delay activity is a necessary prerequisite to working memory function (D'Esposito and Postle, 1999; Sreenivasan et al., 2014; Stokes, 2015; Riley and Constantinidis, 2016). To date there is no model that coherently accounts for all neurophysiological findings (Sreenivasan and D'Esposito, 2019).

Several studies suggest that persistent activity of memory cells is central for working memory function. Fuster (1973) for example found that not rewarding correct choices in a

delay task and thereby removing the contingency between stimulus and response, thus also the necessity to memorize the stimulus, leads to disappearance of delay firing.

Persistent activity of single neurons could only be regarded as a veridical representation of working memory if it fitted behavioural data. On first sight sustained activity of PFC neurons should thus be robust to interference and last over the whole delay period (Brody et al., 2003). It has, however, become evident that many memory cells only show an elevated firing rate for a preferred memorandum throughout parts of a delay (Lundqvist et al., 2018). Besides, it has been found that the retained memorandum can often still be decoded from population data when persistently spiking neurons are absent (Stokes, 2015).

Rather than exhibiting selectively elevated discharge rates throughout the whole delay, stimulus selective PFC neurons commonly show modest elevations, variable onsets and durations in their firing profile (Naya et al., 1996; Rainer and Miller, 2002; Shafi et al., 2007). Some studies have argued that persistent activity is actually an artefact of averaging across trials and spiking is usually sparse within single trials (Lundqvist et al., 2016; Cavanagh et al., 2018). The selectivity and thus the coded information, often seems to disappear and reappear throughout the delay, arguing in favour of a dynamic (time-varying) coding framework (Rainer and Miller, 2002; Barak et al., 2010; Jun et al., 2010; Cavanagh et al., 2018).

The temporal-activation profiles of delay-active, stimulus selective neurons vary within and across studies. For example, these neurons may exhibit a stable (time-constant), elevated firing rate, any non-monotonic firing profile, ramping or decaying activity (Naya et al., 1996;

Rainer and Miller, 2002; Shafi et al., 2007). In addition, the spike rate of PFC neurons is comparably low, and a continuously elevated firing rate often only emerges as an average of spike rate over trials, but not on every single individual trial (Miller et al., 2018). Regarding the mechanisms underlying sustained activity recurrent feedback mechanisms are an appealing and very prominent idea, but there is little definite evidence from in vivo recordings. This may be due to the difficulty to record many neighbouring neurons simultaneously and to precisely manipulate their voltage and neurotransmitter systems, respectively (Major and Tank, 2004). The observed variance in firing profiles suggests that single neurons alone can hardly ever suffice as the neuronal representation of working memory.

These findings have fed several alternative suggestions on how working memory information might be stored at times where the spiking of individual neurons cannot account for it, most prominently activity-silent and synaptic mechanisms (Mongillo et al., 2008; Sugase-Miyamoto et al., 2008; Barak and Tsodyks, 2014; Stokes, 2015). Support for this notion comes from studies which show that intracellular depolarization can lead to persistent delay firing (Fransén et al., 2006; Pressler and Strowbridge, 2006; Navaroli et al., 2012; Jochems and Yoshida, 2013; Knauer et al., 2013). Some studies argue against persistent activity underlying working memory because they failed to find persistently spiking, delay-active neurons (Zaksas and Pasternak, 2006; Hussar and Pasternak, 2012, 2013). This is however not a strong argument, as null results do not rule out any hypotheses and other studies exploiting similar task designs and stimuli did find persistently spiking neurons (Riley and Constantinidis, 2016). Furthermore, to date no one has been able to demonstrate that the

level of complexity for working memory coding may be driven by intrinsic cellular mechanisms alone, e.g. channel- and intracellular signalling dynamics (Major and Tank, 2004). And for those neurons that maintain an elevated persistent firing rate over several seconds, internal mechanisms alone are an insufficient explanation, as these time scales are too long for immediate maintenance of sustained activity in single cells (Constantinidis and Wang, 2004). In fact, persistent activity models classically argue that the memorandum can be read out at any time from neuronal networks and not necessarily from single cells (Constantinidis et al., 2018). In general, it is thought that small patches of neurons in the PFC code for a specific memorandum and thus a failure to detect persistently spiking delay neurons may simply convey that recordings did not capture the relevant spot (Lafer-Sousa and Conway, 2013; Constantinidis et al., 2018).

Note that persistent-activity models suggest that a memorandum cannot be read out unambiguously from a single neuron but from clusters of neurons that are tuned to the same memorandum (Christophel et al., 2017; Constantinidis et al., 2018). In general, a population code for working memory suggests that the memorandum can be decoded by different activation patterns of a set of neurons, but not by one neuron alone (Stokes et al., 2013). The memory information could for example be stored in a cluster of neurons if these neurons would spike asynchronously, i.e. one neuron spikes while the other is silent, causing a persistent rate on the scale of the cluster (Lundqvist et al., 2018). It is methodologically challenging to find evidence for or against asynchronous spiking, because a large population of neurons within local networks would need to be recorded. Nevertheless, Miller and colleagues (2018) argue that recent findings of their lab, measuring spiking activity and local

field potentials across several frontal cortical areas, argue against asynchronous spiking but rather in favour of sparse coordinated bursting on the level of single neurons as well as local networks (Lundqvist et al., 2016; Bastos et al., 2018).

It is a matter of current debate whether the code of neuronal clusters is dynamic, i.e. time-varying, or not. Lundqvist and colleagues (2018) argue that the population code is dynamic and may not rely on the same set of neurons (Meyers et al., 2008; Barak et al., 2010; Stokes et al., 2013; Sreenivasan et al., 2014). Dynamic coding would have the advantage that elapsed time is tracked on the go and the memorandum should be less prone to interference, but it also complicates the readout of the memorandum by downstream brain regions (Stokes, 2015; Tiganj et al., 2018; Sreenivasan and D'Esposito, 2019). Constantinidis and colleagues (2018) in turn claim that recent evidence rather suggests that the population code is stable, i.e. time-constant, and generalizes across time (Murray et al., 2017; Spaak et al., 2017). While the latter view does not neglect that firing patterns of prefrontal delay neurons are often changing, especially when the length of delay windows are fixed, Constantinidis and colleagues (2018) suggest that neuronal activity might simply drift in the population and a stable readout of this varying firing pattern is still sufficient to decode the memory information (Machens et al., 2010; Druckmann and Chklovskii, 2012). Furthermore, no working memory model exploiting dynamic coding has yet found parameters comparable to persistent spiking models with regard to behavioural performance measures, such as error rate, accuracy or reaction time (Riley and Constantinidis, 2016).

As for now, there is agreement that single cells often do not exhibit a stable sustained firing profile across the whole delay. The remaining controversy is mainly centred on how firing

profiles of neurons within a neuronal network relate to one another (asynchronous spiking vs. coordinated bursting) and whether or not this changes in time (dynamic- vs. stable coding).

Sustained activity combined with synaptic facilitation

Lately, combinations of different mechanisms have been suggested as the neuronal correlate of working memory. A prominent idea is that synaptic facilitation may take over when neurons are not persistently firing and the spike rate can no longer account for memory storage, i.e. the memorandum cannot anymore be decoded from the spike rate (Barak and Tsodyks, 2014). Synaptic facilitation is an umbrella term for short-term plasticity facilitating synaptic efficiency, for example by paired pulse facilitation, increased vesicle release, or presynaptic calcium (Jackman and Regehr, 2017).

Neuronal spiking in the PFC may quickly induce short-lasting synaptic facilitation (Wang et al., 2006). This change in synaptic weights in combination with sparse, bursty spiking could thus account for working memory (Sandberg et al., 2003; Mongillo et al., 2008; Lundqvist et al., 2011, 2012; Stokes, 2015; Fiebig and Lansner, 2017). More precisely, residual calcium levels at the presynaptic terminal might serve as a neuronal correlate of working memory in the absence of persistent spiking, as removal of calcium is relatively slow with kinetics in the order of a second (Bertram et al., 1996; Zucker and Regehr, 2002; Mongillo et al., 2008).

In neurons in the entorhinal cortex it has been shown that a brief depolarization can cause persistent spiking mediated by calcium activated cation influx into the cell (Siegelbaum and

Kandel, 2013). This idea is also appealing because sparse spiking together with modulated synaptic weights is less prone to interference than persistent spiking alone and action potentials are metabolically costly (Attwell and Laughlin, 2001; Miller et al., 2018). It is however unlikely that synaptic mechanisms alone may account for working memory, as one hallmark of working memory is flexible manipulation of information online and synaptic mechanisms are simply too slow to account for rapid updating of working memory content (Constantinidis et al., 2018).

Even though direct evidence is yet missing, a combination of recurrent excitation and synaptic facilitation in PFC neurons seems to be a plausible mechanism underlying working memory. If this was the case, the memorandum would actually be stored at the level of single neurons, but could hardly be read out regarding one parameter alone. Rather, the neuronal trace of the memorandum would switch between an elevated firing rate and elevated presynaptic calcium levels. It is of course also possible that it is not solely recurrent excitation and synaptic facilitation that are underlying working memory, but a more complex combination of even more neuronal mechanisms.

Importance of NMDA- and GABA(A) receptors for working memory

Working memory relies on NMDA- and GABA(A) receptors

Irrespective of the precise neuronal mechanisms underlying working memory there is compelling evidence that at the level of neurotransmitters and their respective receptors glutamate and GABA are of great importance to functioning working memory. Glutamate and GABA are the most prominent excitatory and inhibitory neurotransmitters, respectively,

in the neocortex and the PFC (Krogsgaard-Larsen et al., 1997; Dash et al., 2007). Both are amino acids, with glutamate being a metabolic precursor for GABA. GABA is not only the major inhibitory neurotransmitter but also the most abundant of all neurotransmitters (Fuster, 2015). GABAergic neurons comprise about 20% of cortical neurons, the other 80% are excitatory (Somogyi et al., 1998). Nevertheless, inhibitory currents are thought to outshine excitatory ones as GABAergic interneurons contact almost every pyramidal cell nearby (Ferguson and Gao, 2018). Glutamate in turn is the major excitatory neurotransmitter of the nervous system and of pyramidal cells in the neocortex. Like the other main neurotransmitters, except for GABA, it originates in brainstem nuclei and projects to the PFC (Fuster, 2015). Cortical functions strongly depend on precisely tuned excitatory and inhibitory currents. Glutamate and GABA can thus be regarded as the counteracting cortical neurotransmitters mediating balance of excitation and inhibition. Recurrent excitation relies on an interplay of pyramidal cells and interneurons, respectively on glutamate and GABA with regard to the involved neurotransmitters.

At a behavioural level, systemic and local blockage of NMDA- receptors and GABA(A) receptors commonly disrupted working memory performance in rodents, non-human primates and humans (Ghonheim et al., 1985; Sawaguchi et al., 1989; Cole et al., 1993; Krystal, 1994; Verma and Moghaddam, 1996; Romanides et al., 1999; Baron and Wenger, 2001; Chrobak et al., 2008; Smith et al., 2011; Wang et al., 2013; Driesen et al., 2013; Auger and Floresco, 2015). As a potential neuronal mechanisms of this effect, NMDA- and GABA(A) receptors play a major role in sustained working memory activity (D'Esposito and Postle, 1999; Sreenivasan et al., 2014; Stokes, 2015; Riley and Constantinidis, 2016).

Further evidence for the delicate role of NMDA- and GABA receptors comes from studies with patients. For example, schizophrenic patients have deficits in working memory that are proposed to originate from deficiencies in NMDA- and GABA receptors (Benes, 1995; Coyle, 2004; Gonzalez-Burgos and Lewis, 2012; Tse et al., 2015; Datta and Arnsten, 2018).

NMDA receptors, but especially parvalbumin containing GABAergic basket cells are necessary for frontal oscillations in the gamma range (25-100 Hz), which have been suggested to be related to working memory load, encoding, maintenance of sensory information and importantly spiking related to information about the memorandum (Howard, 2003; Cardin et al., 2009; Buzsáki and Wang, 2012; Carlén et al., 2012; Ferguson and Gao, 2018; Lundqvist et al., 2018). However, rather than being coupled to one particular frequency band, all kind of oscillations may occur during working memory tasks, hinting at the distributed nature of working memory (Buschman et al., 2012; Liebe et al., 2012; Fuster, 2015).

Coupling of PFC neurons with neurons in more posterior areas is said to be modulated by working memory load (Pinotsis et al., 2019). Prefrontal oscillations in the beta (12.5 – 30 Hz) and gamma ranges are assumed to reflect communication with visual areas, whereas the alpha (8 – 12 Hz) and beta bands are often associated with inhibition and regulation of top-down information (Benchenane et al., 2011; Roux and Uhlhaas, 2014). Gamma oscillations are important for the maintenance of working memory information and theta oscillations (4 – 7 Hz) are thought to reflect relations to the hippocampus and underlie the generation of gamma bursts (Benchenane et al., 2011; Roux and Uhlhaas, 2014; Miller et al., 2018). While there is still little agreement on the precise functions of different oscillatory bands in

working memory, it is evident that synchronous spiking within the PFC as well as between the PFC and more posterior areas occurs during working memory and NMDA- and GABA receptors may be obligatory for this (Constantinidis and Wang, 2004). In turn, neuronal oscillations modulated membrane potentials of single neurons and may even evoke spikes, especially in the depolarizing phases of oscillations (Siegel et al., 2009).

NMDA receptors

Throughout the central nervous system, glutamate is the major excitatory neurotransmitter. When a respective nerve terminal is depolarized, glutamate is released into the synaptic cleft in a calcium dependent manner and inactivated metabolically, mainly by transformation into glutamine (Fuster, 2015). Glutamate acts via ionotropic and metabotropic receptors. The ionotropic glutamate receptors can be subdivided into three main classes, AMPA-, kainate and NMDA receptors, with AMPA- and NMDA receptors being the most abundant ones, mediating the effects of the majority of excitatory neurotransmission in the central nervous system (Traynelis et al., 2010; Paoletti et al., 2013).

While NMDA receptors are found throughout the nervous system, a high proportion resides within cortical layers II and III, the starting and endpoint of most corticocortical connections (Cotman and Iversen, 1987). Within the human cortex, the mRNA of NMDA receptor subunits was most abundant in the prefrontal cortex, and, to the extent that PFC activation underlies WM and executive function, suggests that NMDA receptors may be critical for these functions (Scherzer et al., 1998).

NMDA receptors are ionotropic receptors gating cations (e.g. Ca^{2+}, $Na^{+,}$ or K^{+}) upon activation, with the highest permeability for Ca^{2+}, contributing to the slow kinetics of the receptor (Bourne and Nicoll, 1993; Seeburg et al., 1995). Depending on the amount and duration of Ca^{2+} influx, NMDA receptors play a key role in mediating long term potentiation as well as long term depression, i.e. strengthening or weakening of respective synapses, regulating synaptic efficacy underlying learning and memory on short and long time scales (Citri and Malenka, 2008; Hunt and Castillo, 2012; Granger and Nicoll, 2013; Morris, 2013).

NMDA receptor activation depends on the simultaneous binding of glycine and glutamate (or respective agonists) and that the neuronal membrane is sufficiently depolarized to dislodge a Mg^{2+} ion which blocks the central pore (**Fig. 5**) (Hansen et al., 2017). The latter is usually achieved by glutamatergic activation of AMPA- / kainate receptors, resulting in a fast depolarization and a slow but long lasting activation of NMDA receptors (Mayer et al., 1984; Nowak et al., 1984; Hestrin et al., 1990a; Sah et al., 1990). NMDA receptors are thus coincidence detectors, requiring presynaptic glutamate release in combination with postsynaptic depolarization. Glycine is generally thought to be omnipresent and thus binding of glutamate is the critical ligand for receptor activation (Hansen et al., 2017). Glutamate is rapidly removed from the synaptic cleft after release but, once bound to an NMDA receptor, may remain bound for several hundred milliseconds (Lester et al., 1990; Clements et al., 1992; Lester and Jahr, 1992). While activated, NMDA receptors regularly undergo conformational changes, transitioning between open and closed conformational states (Hansen et al., 2017).

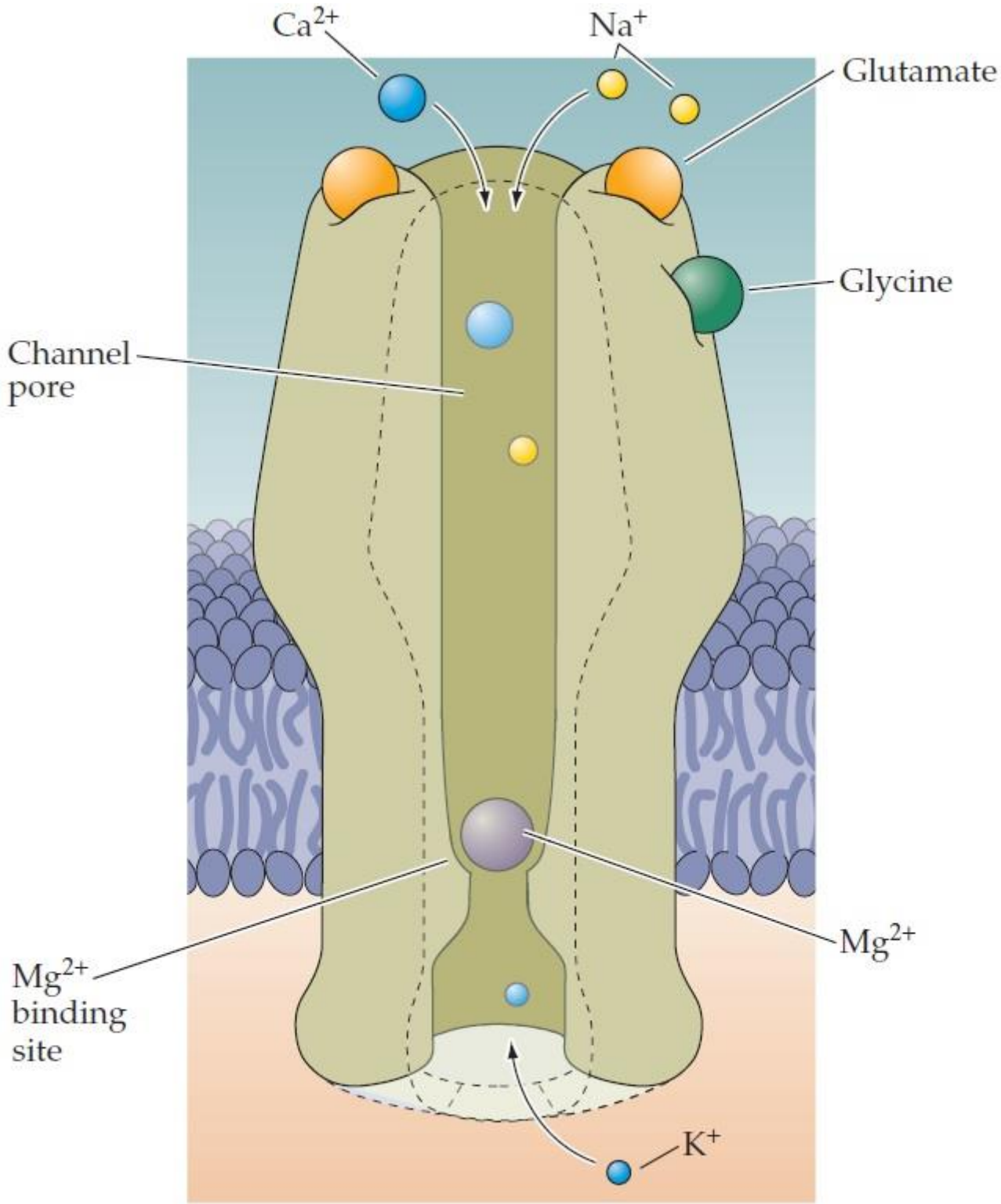

Figure 5. Illustration of a NMDA receptor. NMDA receptors contain binding sites for glutamate and the co-activator glycine, as well as an Mg^{2+}-binding site in the pore of the channel. At hyperpolarized potentials, the electrical driving force on Mg^{2+} drives this ion into the pore of the receptor and blocks it. *Figure and modified description from Augustine et al. (2004)*

A functioning NMDA receptor is tetrameric, meaning it comprises four subunits. To date, seven different NMDA receptor subunits have been identified, GluN1, GluN2A-D and GluN3A-B (Traynelis et al., 2010; Paoletti et al., 2013). The respective subunit composition of NMDA receptors in turn gives rise to different physiological properties, among them importantly the duration of mediated excitatory postsynaptic currents upon activation (Monyer et al., 1992; Vicini et al., 1998). All NMDA receptors comprise two GluN1 subunits that bind glycine and most NMDA receptors contain two GluN2 subunits that bind glutamate (Vyklicky et al., 2014; Hansen et al., 2017). NMDA receptor diversity is thus mediated by the glutamatergic binding sites, commonly GluN2A-D.

Glutamate is most potent at GluN1/2D receptors, i.e. NMDA receptors with GluN1 and GluN2D subunits, and least potent at GluN1/2A receptors. Channel conductance was highest in GluN1/2A and GluN1/2B receptors. The probability of the channel being in the open conformational state upon activation is highest for GluN1/2A receptors, followed by GluN1/2B receptors and lowest for GluN1/2C and GluN1/2D receptors (Erreger et al., 2004; Traynelis et al., 2010; Paoletti et al., 2013; Wyllie et al., 2013; Glasgow et al., 2015). Receptor deactivation was at around 50 ms for GluN1/2A, 290 ms for GluN1/2C receptors, 400 ms for GluN1/2B receptors and took over one second for GluN1/2D receptors (Monyer et al., 1992; Vicini et al., 1998; Yuan et al., 2009). In rodents, the GluN2B subunit is expressed throughout the brain during embryonic development; however, upon reaching adulthood, this receptor's expression becomes restricted to frontal areas (**Fig. 6**). The GluN2A subunit in turn was expressed all over the central nervous system in adults (Watanabe et al., 1992, 1993; Ishii et al., 1993; Akazawa et al., 1994; Monyer et al., 1994;

Wenzel et al., 1997). GluN2B subunits were further only weakly expressed in parvalbumin and somatostatin containing interneurons, but stronger on cholecystokinin containing interneurons which in turn project to other interneurons (Matta et al., 2013; Pfeffer et al., 2013; Harris and Shepherd, 2015). Taken together, NMDA receptors containing GluN2B subunits seem to be especially well suited for maintenance of persistent delay firing, with regard to their distribution and kinetics.

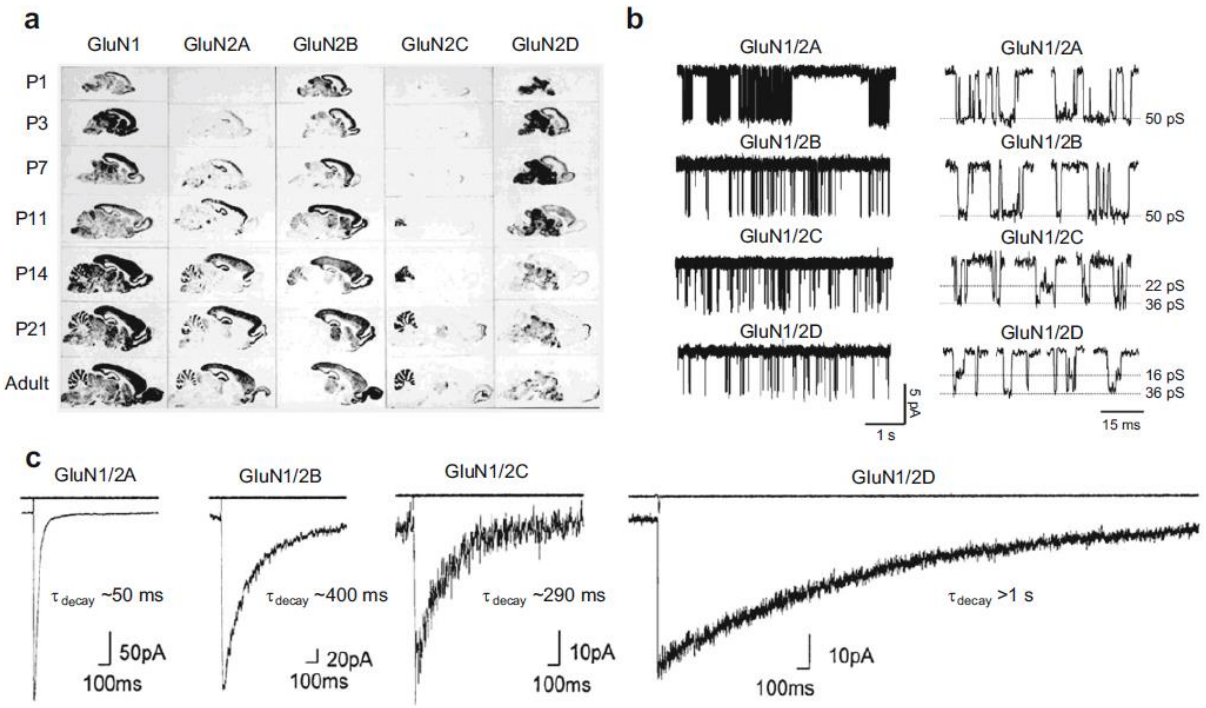

Figure 6. GluN2 subunit-specific expression and functional properties of recombinant NMDA receptor subtypes. **a**, Regional and developmental expression of GluN2 subunits in rat brain revealed in autoradiograms using in situ hybridizations of oligonucleotide probes for the relevant mRNAs to parasagittal sections. Modified from Akazawa et al. (1994). ***b***, Single-channel recordings of currents from diheteromeric NMDA receptor subtypes expressed in HEK293 cells (outside-out membrane patches). Open probability is ~0.5 for GluN1/2A, ~0.1 for GluN1/2B, and <0.05 for GluN1/2C and GluN1/2D. Highlights of individual openings are shown on the left. GluN1/2A and GluN1/2B have higher channel conductance (~50 pS) compared to GluN1/2C (~22 and ~36 pS) and GluN1/2D (~16 and ~36 pS). Redrawn from Yuan et al. (2008). **c**, Whole-cell patch-clamp recordings of responses from brief application of glutamate (1 ms of 1 mM glutamate) to recombinant diheteromeric NMDA receptor subtypes expressed in HEK293 cells. The open tip current indicating the duration of the drug application is shown in the upper trace. Redrawn from Vicini et al. (1998). *Figure and modified description from Hansen et al. (2017).*

Neurons commonly express at least two different types of NMDA receptors. Often the expressed receptors are triheteromers, meaning they comprise three different types of subunits, with GluN1/2A/2B receptors being the most common NMDA receptor in the cortex (Chazot et al., 1994; Luo et al., 1997; Al-Hallaq et al., 2007; Rauner and Köhr, 2011; Hansen et al., 2017). This further complicates the differentiation of the respective subunit contribution of NMDA receptors in vivo. Indeed, iontophoretic blockage of NMDA receptors with GluN2A subunits reduced delay cell firing in monkey PFC neurons just like blockage of NMDA receptors with GluN2B subunits and experiments with rodents suggest that GluN2A subunits may even be more central to working memory than GluN2B subunits (Wang et al., 2013; McQuail et al., 2016).

GABA(A) receptors

GABA receptors can be subdivided into two main receptor types, the ionotropic GABA(A)- and the metabotropic GABA(B) receptor (Enna, 2007). GABA(A) receptors are ligand gated chloride channels (Möhler et al., 1997). They are the most common postsynaptic receptors and are present throughout the nervous system (Fuster, 2015). The amino acid GABA is classically regarded as the major inhibitory neurotransmitter of the central nervous system (Enna, 2007). GABA(B) receptors mainly serve as presynaptic autoreceptors, regulating GABA concentrations (Deisz, 1997; Fuster, 2015).

Cortical expression of GABA receptors is highest within layers II to IV and, interestingly, on non-pyramidal neurons (Hirsch and Robins, 1962; Houser et al., 1983; Hendry et al., 1987; Gabbott and Bacon, 1996). Although the effects of GABA are mainly local, providing lateral inhibition and thereby sharpening different excitatory patterns, there are also long-range GABA neurons which project to and from the PFC (Fuster, 2015). In the PFC, GABA receptors are as common as they are in most other cortical areas (Emson and Lindvall, 1979; Hendry et al., 1987; Oishi and Kubota, 1990).

Upon binding of GABA, GABA(A) receptors increase Cl^- and HCO_3^- conductances by prolonged channel opening, commonly resulting in Cl^- flowing into the postsynaptic neuron and hyperpolarizing it (**Fig. 7**) (Bormann, 1988; Macdonald and Twyman, 1992; Farrant and Nusser, 2005). In some cases, e.g. during development or in some hippocampal synapses, activation of GABA(A) receptors caused a depolarization mediated by efflux of Cl^- because intracellular Cl^- was elevated compared to standard conditions (Cherubini et al., 1991; Payne et al., 2003; Krnjević, 2004; Rivera et al., 2005). GABA is cleared from the synaptic cleft by reuptake into neurons and glia cells (Fuster, 2015).

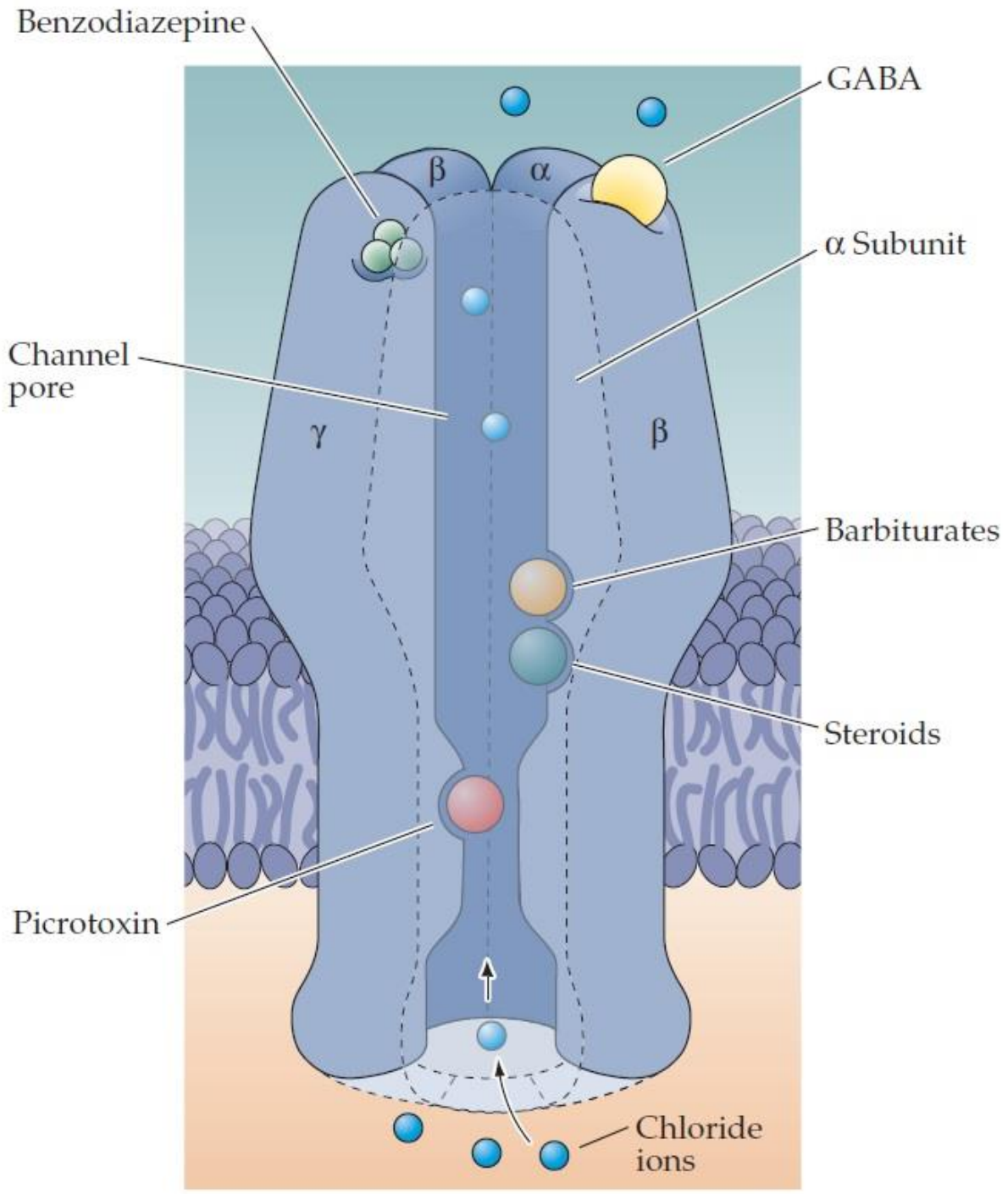

Figure 7. Illustration of a GABA(A) receptor. Ionotropic GABA receptors contain two binding sites for GABA and numerous sites at which drugs bind to and modulate these receptors. *Figure and modified description from Augustine et al. (2004)*

GABA(A) receptors are pentamers, i.e. composed of five subunits, made up from a selection of 19 potential subunits, 6α, 3ß, 3γ, 1ε, 1θ, 1δ, 1π and 3ρ (Enna, 2007). Despite the high

potential diversity of receptor subtypes, only about a dozen are functional in so far as they respond to GABA (Möhler et al., 1997; Kittler et al., 2002; Lüscher and Keller, 2004). Most GABA(A) receptors are composed of two α-, two γ-, and one β subunit, with a combination of two α1 and γ2 subunits each and one β2 subunit making up almost half of all GABA(A) receptors in the brain (Backus et al., 1993; McKernan and Whiting, 1996; Möhler et al., 1997). GABA binding occurs at parts of the α- and β subunits (Amin and Weiss, 1993; Smith and Olsen, 1995). Subunits comprise an N-terminal suited for extracellular ligand binding, four transmembrane domains and an intracellular loop that is prone to phosphorylation (Betz, 1990; Galzi and Changeux, 1994; Macdonald and Olsen, 1994).

ρ-subunit-containing GABA(A) receptors display some unique characteristics, which has previously led to grouping them as GABA(C) receptors (Enna, 2007). GABA(A) receptors are usually heteromers, but homomers containing only ρ1 subunits have been found, too. Additionally, ρ-subunit-containing GABA(A) receptors are insensitive to certain GABA(A) receptor agonists and antagonists, including bicuculline methiodide (Bic) (Bormann, 2000; Johnston, 2002).

GABA receptors mainly reside on three types of interneurons, parvalbumin expressing interneurons that largely project to pyramidal neurons and other parvalbumin interneurons, somatostatin expressing interneurons projecting to distal dendrites of pyramidal neurons and regulating the overall excitatory input of postsynaptic neurons, as well as serotonin receptor expressing interneurons, among them cholecystokinin containing ones, commonly projecting to other interneurons causing a net disinhibition (Tremblay et al., 2016). Parvalbumin expressing interneurons can be further subdivided into basket cells projecting

onto the soma and proximal dendrites of pyramidal cells and chandelier cells projecting onto the axon initial segment of pyramidal cells (Tremblay et al., 2016). Less is known about chandelier cells, but it has been suggested that basket cells play a greater role in establishing persistent activity because 90% of cortical inhibition occurs at dendrites (Kubota et al., 2016; Ferguson and Gao, 2018). Somatostatin containing interneurons are more susceptible than parvalbumin containing ones to changes in NMDA receptor signalling, as their ratio of NMDA to AMPA receptors is higher and vice versa NMDA receptor activation is modulated by activation of somatostatin containing interneurons (Kanemoto et al., 2011; Chiu et al., 2013; Krystal et al., 2017). The notion that parvalbumin expressing interneurons are more important than somatostatin expressing ones in establishing sustained activity has hence been challenged (Kim et al., 2016).

NMDA- and GABA(A) receptors underlie persistent activity

Biologically inspired models of persistent neuronal spiking usually rely on AMPA-, NMDA- and GABA(A) receptors (Compte et al., 2000; Durstewitz et al., 2000a; Barak and Tsodyks, 2014). Glutamate is thought to mediate recurrent excitation via AMPA- and NMDA receptors whereas GABA preserves stimulus specificity via local inhibition. With regard to the glutamatergic receptors NMDA receptors have gained most attention as they exhibit slow gaiting kinetics which may serve as a mechanism of maintaining stable sustained activity at low firing rates, as seen in the PFC (Wang, 1999).

Any persistent spiking model must assume at least two stable states: a spontaneous resting state and a persistent spiking state. Achieving stability depends on mediated excitation

being slower or at the range of mediated inhibition, otherwise neurons could never reach a stable plateau of persistent spiking and spiking would be bursty (Wang, 1999). While the exact receptor kinetics depend on the respective subunit composition, neuronal inhibition is typically slower than AMPA mediated synaptic transmission (Wang, 2001). GABA(A) receptor mediated inhibition in rodent hippocampal pyramidal neurons have a decay constant of 0.6 – 10 ms, while AMPA receptors are at the order of 0.2 ms, suggesting that GABA(A) receptor mediated inhibition outlasts AMPA receptor mediated excitation (Hestrin et al., 1990b; Abbott, 1997; Tsodyks and Markram, 1997; Banks et al., 1998; Barak and Tsodyks, 2014). GABA- and AMPA receptors alone would thus not be able to generate a stable state of persistent firing. Prefrontal NMDA receptor currents, which typically lasted from tens to hundreds of milliseconds, appear necessary to obtain stability in sparse spiking PFC neurons (Wang, 1999; Durstewitz et al., 2000a; Hansen et al., 2017). Taken together NMDA receptors thus critically contribute to the robustness of the neuronal correlate of working memory.

Stable, persistent activity arises from NMDA receptors quickly saturating at low firing rates and thus being able to maintain present synchronized network dynamics (Wang, 1999). The voltage dependency of NMDA receptors offers another feature well suited for establishing a resting state and an active state of neurons, as inactive NMDA receptors mediate robustness to small voltage fluctuations preventing persistent spiking. Additionally, this voltage dependency offers the possibility to filter out irrelevant information when NMDA receptors are inactive and thereby exhibit stimulus selectivity (Lisman et al., 1998; Compte et al., 2000; Brunel and Wang, 2001). The underlying idea is that a subgroup of neurons is more depolarized for a specific stimulus and subsequently the magnesium block of NMDA

receptors is only removed in this subset of neurons, which in turn contribute to code for the stimulus by recurrent excitation when the stimulus needs to be maintained in working memory (Wang, 2001). Unbinding of glutamate from NMDA receptors is comparatively slow. This mechanism could support receptor saturation during persistent spiking by providing a constant synaptic drive and preventing an undamped positive feedback loop with steadily rising firing rates (Wang, 1999, 2001).

The inhibitory effects of GABA also likely play a role in coding working memory load, as well as attention and stimulus selectivity of neurons (Funahashi et al., 1989; Compte et al., 2000; Yoon et al., 2016; Auger and Floresco, 2017; Bast et al., 2017). Cognitive functions such as working memory not only require attention to the relevant information, but also filtering of irrelevant information. GABAergic inhibition is commonly regarded as the main underlying neuronal mechanism of this process (Fuster, 2015). This was confirmed by studies by Rao and colleagues (1999, 2000), who found that GABA was of utter importance for inhibiting responses to non-preferred or irrelevant stimuli. In working memory tasks, sustained activity is thought to be mainly regulated by parvalbumin expressing interneurons (Sawaguchi, 2001; Lewis et al., 2002).

In line with the importance of NMDA receptors, increasing the relative ratio of AMPA to NMDA receptors in working memory models commonly makes persistent spiking networks less stable and vulnerable to noise (Wang, 1999; Compte et al., 2000; Durstewitz et al., 2000a, 2000b). Importantly, it is not the physical AMPA to NMDA receptor ratio per se that is vital to obtain stability, but their relative recruitment during persistent firing (Wang, 2001). The voltage dependency of NMDA receptors for example increases their recruitment

during a persistent firing compared to a spontaneous resting state (Collingridge et al., 1988; Wang, 2001). An increase in intracellular sodium during postsynaptic depolarization additionally increases NMDA mediated currents, but not AMPA currents (Yu and Salter, 1998). Notably, the affinity of NMDA receptors to glutamate is 100-fold higher than that of AMPA receptors (Kullmann and Asztely, 1998). Besides, the ratio of NMDA mediated synaptic currents may be enhanced by dopamine modulation. It has been shown that activation of D1 and D5 receptors enhances NMDA receptors currents (Seamans et al., 2001). In sum, during persistent firing, recruitment of NMDA receptors should be higher than that of AMPA receptors, irrespective of their physical ratio (Wang, 2001). While the best ratio of AMPA to NMDA receptors depends on the precise model parameters, Compte and colleagues (2000) found that NMDA receptors may contribute to 65% or more of excitatory postsynaptic currents.

While NMDA receptors are of great necessity for persistent spiking, they alone are not enough. It is the delicate interplay of excitation and inhibition that gives rise to precisely tuned neurons and stabilizes the spontaneous resting state as well as the persistent firing state. Localised networks exhibiting sustained activity can be modelled by inhibitory currents alone, mere excitatory currents are not sufficient however (Constantinidis and Wang, 2004). Consequently, persistent firing models commonly assume a domination of synaptic inhibition over excitation (Compte et al., 2000). In the persistent firing state excitatory and inhibitory currents are both enhanced compared to the spontaneous resting state, but their relative contribution remains roughly constant, establishing a dynamic balance between excitation and inhibition (Compte et al., 2000).

Effect of blocking NMDA- and GABA(A) receptors

While blocking NMDA receptors disrupts persistent neuronal activity, blocking AMPA receptors does not (Wang et al., 2013). The effects on NMDA receptors could be mediated by GluN2B subunits of NMDA receptors, as they are numerous within the PFC and exhibit comparatively slow gaiting kinetics and saturation properties well suited for causing sustained elevated firing rates (Wang, 1999, 2001, 2002; Compte et al., 2000; Wang et al., 2013; Wang and Arnsten, 2015).

However, the effects of general NMDA antagonists, such as MK-801 (MK), are controversial. MK is a general NMDA receptor antagonist. It is an open channel blocker, exclusively blocking activated NMDA receptors with open channels. More precisely it is a so called trapping blocker, being trapped inside the pore when agonists unbind and the channel closes (Sobolevsky and Yelshansky, 2000). Iontophoretic administration of MK in the PFC of behaving monkeys reduced task related firing, but not spontaneous activity per se (Wang et al., 2013). While studies with awake rodents found that MK preferentially blocked receptors on interneurons, others found stronger effects on pyramidal cells in vitro (Jackson et al., 2004; Homayoun and Moghaddam, 2007; Rotaru et al., 2011). MK had a net excitatory effect on prefrontal pyramidal cells by inhibiting fast spiking GABAergic interneurons in the former mentioned case (Jackson et al., 2004; Homayoun and Moghaddam, 2007). In contrast, MK had a net inhibitory effect on pyramidal cells on mice PFC slices in the latter, supposedly because drug effects were stronger for pyramidal cells than for interneurons (Rotaru et al., 2011). As the excitatory effects of MK were only seen after systemic but not

after local injection of NMDA antagonists in the rodent PFC, one interpretation is that the excitatory effects occurred due to blockage of inhibitory interneurons in other brain areas that project to the PFC (Suzuki et al., 2002; Lorrain et al., 2003; Skoblenick and Everling, 2012).

With regard to GABA(A) receptors, bicuculline methiodide (Bic) is a competitive GABA(A) receptor antagonist binding to the GABA recognition site of the receptor (Enna, 2007). Iontophoretic application of Bic reduces the tuning of task related neurons in primary sensory areas as well as the PFC by elevating the firing rate to non-preferred stimuli and decreasing signal to noise ratio (Rao et al., 2000; Constantinidis and Goldman-Rakic, 2002). However, the behavioural effects elicited by GABA antagonists are not consistent. For example, studies with rodents have shown that GABA antagonists in some cases do not impair working memory, memory acquisition or retention learning and may have a supportive effect on memory consolidation (Luft et al., 2004; Kim et al., 2012; Auger and Floresco, 2015; Farahmandfar et al., 2017).

Summary and aim of the study

Evidence suggests that neurons in the PFC that maintain an elevated firing rate across a delay when working memory is needed can be regarded as the neuronal correlate of working memory. It remains a matter of debate whether sustained activity in PFC neurons represents storage of the memorandum or rather top-down attention. Several mechanisms underlying sustained activity have been proposed. The most compelling, however perhaps

not exclusive, one is recurrent excitation. Recurrent excitation is thought to be established by reverberant excitation of pyramidal neurons together with local inhibition by parvalbumin containing interneurons. Excitatory effects are mediated via the glutamatergic AMPA- and NMDA receptors and inhibitory effects via GABA(A) receptors. With regard to the glutamatergic receptors NMDA receptors seem to be of great importance for stable maintenance of a persistent firing state. More specifically, NMDA receptors containing GluN2B subunits, which are restricted to frontal areas in adult rodents and exhibit slow decay constants, may play a major role. It has been proposed that NMDA receptors are mainly relevant for the persistent firing state of memory neurons, while GABA(A) receptors are in charge of the spontaneous resting state, when neuronal responses to non-preferred or irrelevant stimuli are suppressed. In agreement with this notion NMDA receptor antagonists have mainly diminished the previously elevated firing rate for preferred memoranda, while GABA(A) antagonists have mainly increased the previously low firing rate for non-preferred memoranda (Rao et al., 2000; Wang et al., 2013). Blocking NMDA- and GABA(A) receptors has however caused conflicting effects and their precise role in establishing sustained activity as a neuronal correlate of working memory remains elusive for now.

The aim of the study at hand was to investigate the effects of glutamate and GABA on neuronal response properties in the PFC. To do this, we iontophoretically blocked NMDA- and GABA(A) receptors while recording from PFC neurons of macaques performing an abstract perceptual decision task. In this task, the monkeys had to decide whether or not they had seen a stimulus displayed with different intensities clustered around perceptual

threshold (Merten and Nieder, 2012). We hypothesized that both MK and Bic would decrease delay selectivity in PFC memory neurons, albeit in different ways. To the extent that NMDA is responsible for the maintenance of an elevated firing rate when the preferred stimulus is maintained in working memory, we expected to see a relative stronger suppression of firing rates to the preferred stimulus condition and thus also a decrease in stimulus selectivity with iontophoretic administration of MK. Under the assumption that GABA suppresses the firing rates of tuned neurons when a non-preferred stimulus is shown, we hypothesized to see a relatively stronger increase of firing rates to the non-preferred stimulus condition after iontophoretic application of Bic, thereby decreasing stimulus selectivity. However, we observed the opposite effect.

Methods

Subjects and surgery

We trained two male rhesus monkeys (Macaca mulatta) on a delayed perceptual decision task. Monkey Q was 7 years old and weighed 8.5 Kg, Monkey Z was 9 years old and weighed 8.1 Kg. Both monkeys were water deprived and housed in small social groups of two to three animals.

Monkeys were implanted with titanium head posts for head fixation and a recording chamber above the right lateral prefrontal cortex, centred over the principal sulcus (**Fig. 8d**). Surgery was conducted using aseptic techniques under general anaesthesia. Structural magnetic resonance imaging was performed before implantation to locate anatomical landmarks. All procedures were authorized by the relevant authority, the Regierungspräsidium Tübingen, Germany.

Experimental set-up and behavioural protocol

Before every recording session monkeys were transported from single cages to the experimental set-up via custom-made primate chairs. Monkeys were head fixed and placed in front of a computer screen in a darkened chamber, with a distance of 57 cm between the screen and the eyes of the monkeys. Throughout a trial monkeys fixated on a centrally located small, white square (0.1° of visual angle) and kept their gaze within 3° of visual angle. Eye movements were monitored with an infrared eye-tracking system (ISCAN Inc.; Woburn, Massachussets, USA). The CORTEX program (National Institute of Mental Health;

Bethesda, Maryland, USA) was used for stimuli presentation, experimental control and behavioural data acquisition. Neuronal signals were simultaneously recorded with the PLEXON MAP system (Plexon Inc.; Dallas, Texas, USA).

Monkeys were trained to report the presence or absence of a visual stimulus by responding to an associated set of response rule-cues varying in colour or shape (**Fig. 8a**). The sample stimulus consisted of a grey circle (1.5° of visual angle) presented at six intensity (brightness) levels. Intensity levels were individually adapted to the monkeys such that three sample stimuli were salient (easily detectable) and three sample stimuli were around the perceptual threshold (~50% correct) of the monkey (**Fig. 8c**). Sample stimuli threshold intensities for monkey Q were slightly reduced after 14 of 70 recording days to ensure they remained near the perceptual threshold. Stimulus intensities are depicted in RGB values, stimulus intensity levels in ordinal numbers, with lower values representing lower stimulus intensities. Note that the given values do not represent actual physical intensity levels of the presented stimuli.

The animals initiated each experimental trial by grasping a lever and fixating a central target (fixation period) for 500 ms. Then, a visual stimulus (grey circle) was displayed for 500 ms in half of the trials (stimulus period), in the other half no stimulus was shown. The probability that a given trial would or would not contain a sample stimulus was 50%. The sample stimulus disappeared for the consecutive delay period (2000 ms). After the delay period a response rule-cue was presented. Two response rule-cues were associated with the presence of a sample stimulus (red square or grey triangle) and two response rule-cues with

its absence (blue square or grey cross). If a sample stimulus had been presented, a red square or a grey triangle as response rule-cue required the monkey to release the lever within 1200 ms to receive a fluid reward, whereas a blue square or grey cross as response rule-cue required the monkey to keep fixation and hold on to the lever for another 1200 ms. The associated response rule-cues applied in the reverse way if no sample stimulus had been presented (**Fig. 8b**).

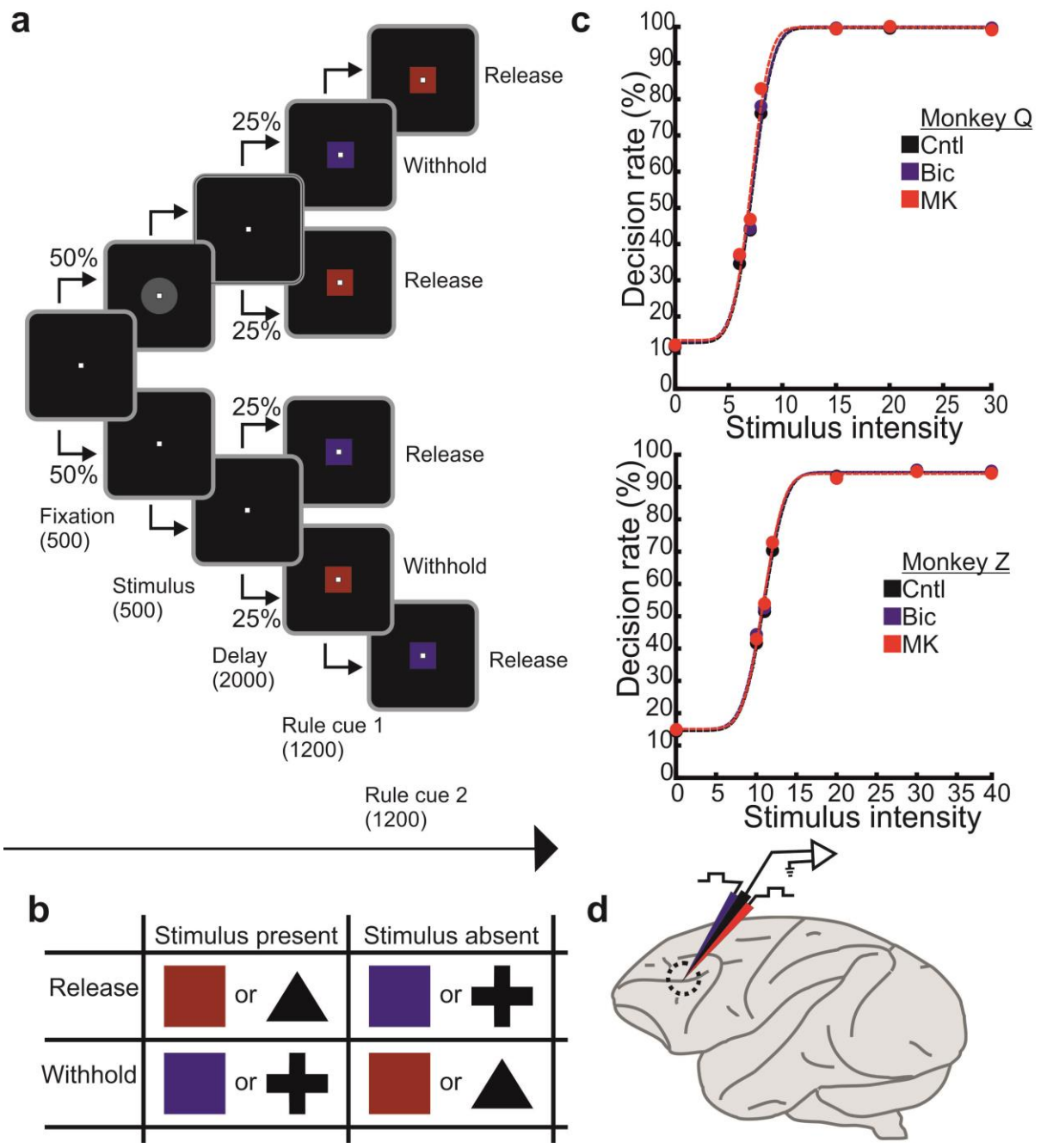

Figure 8. Behavioural protocol, performance and recording site. ***a***, After grasping a lever and holding fixation for 500 ms, a stimulus of varying contrast was flashed for 500 ms in 50% of the trials (top branch) and no stimulus was shown in the remaining 50% (bottom branch). After a delay of 2000 ms one of four response rule cues was shown and, depending on whether or not a stimulus was presented, either instructed the monkey to release the bar or to withhold a response. ***b***, Instructed response rules (left column) with respect to stimulus condition (middle and right column) by the respective response rule cues varying in either colour or shape. ***c***, Psychometric detection curves for monkey Q (70 sessions) and monkey Z (59 sessions) subdivided in control- (Cntl), Bicuculline methiodide- (Bic) and MK-108 (MK) trials. ***d***, Lateral view of a macaque monkey brain. The circled area depicts the area of extracellular recording and iontophoresis targeting GABA(A)- and NMDA receptors at the principal sulcus.

Electrophysiology

We performed extracellular recordings in the right lateral PFC centred on the principal sulcus. In each recording session, up to three custom-made electrode-pipette combinations (**Fig. 9**) were inserted transduraly using a modified electrical microdrive (NAN Instruments; Nazareth Illit, Israel) (Jacob et al., 2013). Neurons were recorded at random; no attempt was made to preselect neurons according to particular response properties. Signal acquisition, amplification, filtering, and digitalization were accomplished with the MAP system (Plexon Inc.; Dallas, Texas, USA). Waveform separation was performed offline (Offline Sorter; Plexon Inc.; Dallas, Texas, USA).

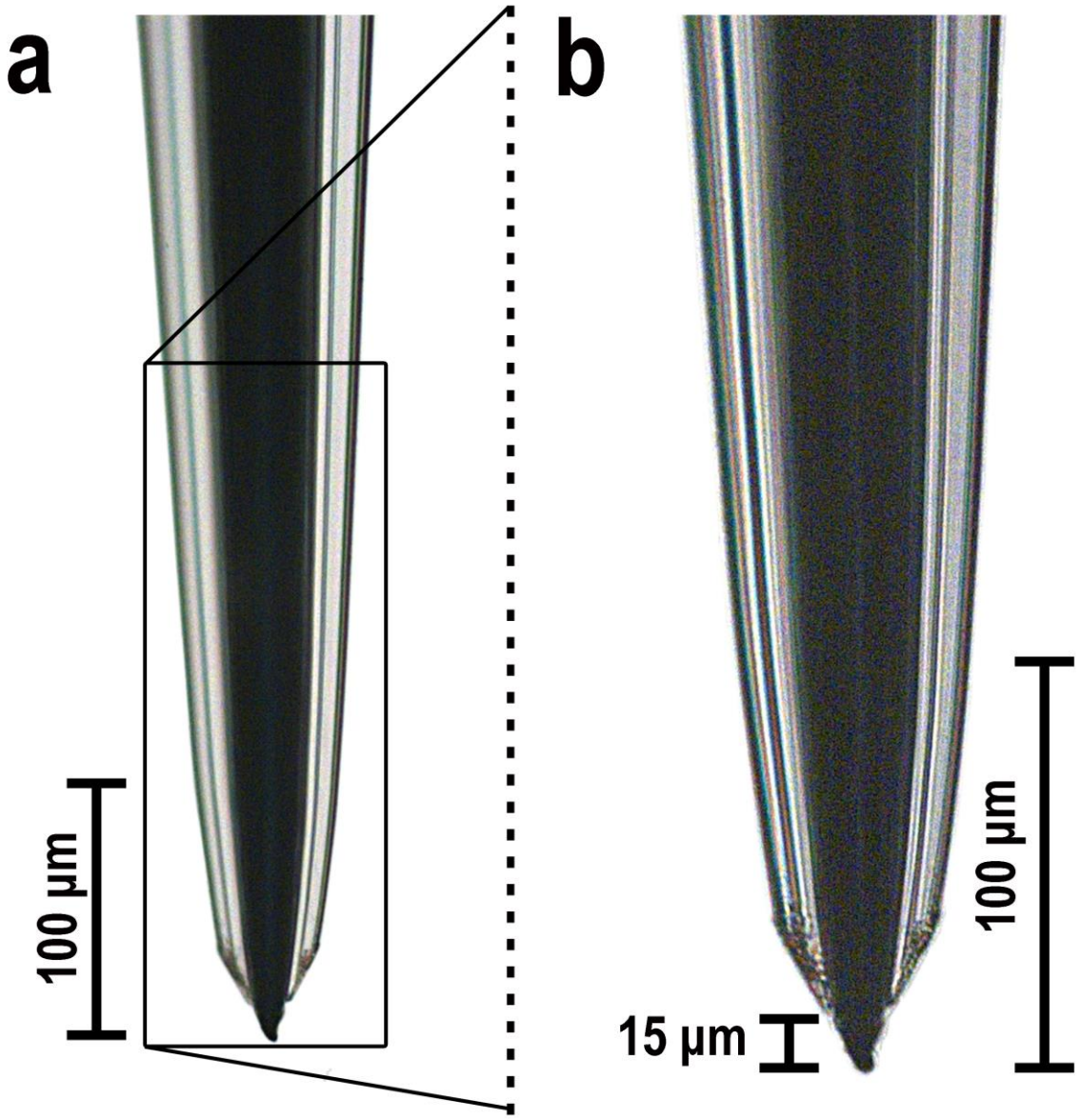

Figure 9. Microscope photography of electrode–pipette combination after grinding. ***a***: The flanking pipettes are open by few micrometers (magnification 10x). ***b***: Detailed view of ***a***. Electrode tip that protrudes the pipettes by about 15 µm (magnification 40x). *Figure and description from Ott (2018).*

Iontophoresis

MK and Bic were applied iontophoretically (MVCS iontophoresis system from NPI Electronics; Tamm, Germany) using custom-made tungsten-in-glass electrodes flanked by two drug-containing pipettes each (Thiele et al., 2006). Electrode impedances were usually around 1 MΩ, full range 0.2–6.4 MΩ (measured at 500 Hz; Omega Tip Z from World

Precision Instruments; Sarasota, Florida, USA). Pipette resistances depended on the pipette opening diameter, drug, and solvent used. Typical resistances were between 15 and 60 MΩ and the full range was 10–168 MΩ.

Retention currents of -7 nA were used for both drugs. Ejection currents for MK (0.01 mol/l in double-distilled water, pH 3.8 with HCl; Sigma-Aldrich; St. Louis, Missouri, USA) were usually at +25nA, full range +15-25nA. Ejection currents for Bic (0.002 mol/l in double-distilled water, pH 3.9 with HCl; Sigma-Aldrich; St. Louis, Missouri, USA) were usually at +15nA, full range +15-25nA. If only one drug was administered per electrode the other flanking pipette was filled with 0.9% NaCl with a pH of 7, but no current was applied on the saline-containing pipette. Electrode impedances and pipette resistances were measured after each recording session.

Trial blocks without drugs alternated with trial blocks during which drugs were continuously applied. Both control and drug blocks lasted between 8–26 min, depending on the time the monkeys needed to reach a sufficient number of correct trials. The first block and all odd-number blocks were control conditions; in the even-number blocks the drug was administered. The order of the administered drugs was counterbalanced across recording sessions. In the subset of neurons that were tested with both MK and Bic in sequence, the wash-out period was the duration of the control blocks, i.e. between 8–26 min.

Previous iontophoretic experiments using the same apparatus and methods demonstrated that changes in neuronal firing rate are not caused by positive ejection currents (Jacob et al., 2013; Ott et al., 2014). In these control experiments with 0.9% physiological NaCl and

ejection currents of +25 nA (as used here), or even higher values of +50 nA, none of the tested neuronal responses, neither spontaneous activity nor any of the selective responses, were affected by ejection currents alone (Jacob et al., 2013; Ott et al., 2014). In addition, we show here that application of MK and Bic resulted in opposite effects on the neurons' spontaneous firing rates, even when both drugs were ejected by the same amounts of positive currents. Taken together, this confirms that the effects observed in the present study were caused by the pharmacological agents and are not simply artefacts of iontophoretic current.

Data analysis

Monkey Q completed 70 recording sessions, monkey Z completed 59 sessions. In the first 18 recording sessions of monkey Q only one drug was applied, in all other recording sessions both drugs were used. Data analysis was performed using custom-written MATLAB code (MathWorks, Natick, Massachusetts, USA). All significance levels were evaluated at $\alpha = 0.05$. Behavioural performance was assessed via signal detection theory, classifying the monkeys' responses as correct (hits and correct rejections) or wrong (misses and false alarms) (Green and Swets, 1966). Behavioural data were pooled over all recording sessions. Psychometric detection curves were derived as the ratio of correct to wrong responses for each stimulus intensity, respectively. We compared psychometric performance using two-way repeated measures ANOVAs with main factors drug condition and stimulus intensity. The amount of aborted trials were compared across drug conditions using Wilcoxon signed-rank tests for paired data.

For neuronal analyses, we sorted spikes offline and studied the responses of all well-isolated neurons. Our criterion for inclusion of a neuron in subsequent analyses were: a mean firing rate above 1 Hz and a minimum of 8 trials each when the stimulus was present and absent during control and at least one drug condition. For spike density population plots, spike rates were normalized by subtracting the mean baseline firing rate in control trials and dividing by the respective standard deviation.

Neurons were classified as either broad- or narrow-spiking cells, i.e. putative pyramidal cells or interneurons, with a linear classifier (k-means, k = 2, squared Euclidean distance) (Diester and Nieder, 2008). Only cells that had a downward deflection in voltage before an upward deflection were classified. More precisely, the minimum of the extracted waveform had to occur between 200 and 400 µs and the maximum after more than 300 µs. 49 of 281 units did not fulfil these criteria. Waveforms were normalized by their difference between maximum and minimum voltage deflection and aligned to their minimum.

To examine drug effects on spontaneous firing rates, we compared firing rate of these neurons during the fixation period between control and respective drug condition using a Wilcoxon signed-rank test for paired data, because data was not normally distributed, as obtained by a Kolmogorov-Smirnov test. In order to investigate modulation of spontaneous firing rates at the transition from control to drug phase, we normalized baseline firing rates by dividing with the mean firing rate during control condition. All reported neuronal analyses are based on correct trials only.

We calculated spike densities by smoothing the spontaneous firing rate in each trial with the spike rate of its neighbouring trials in a weighted manner with a Gaussian kernel. For each trial we calculated a one-sample Wilcoxon signed-rank test to investigate whether the smoothed spike rate for each of the relevant subpopulations significantly deviated from 1 Hz. For spike density histograms depicting the population of stimulus selective neurons spike rates were normalized by subtracting the mean baseline firing rate in control trials and dividing by the respective standard deviation.

As most neurons did not have an elevated firing rate throughout the whole delay period, we used a sliding-window approach. We calculated spike densities by convolving each spike with a Gaussian kernel (σ = 50 ms). Thus 95% of the area under the Gaussian correspond to a window size of 196 ms. The obtained spike density functions were sampled with 10 ms resolution. Next, we calculated two-way ANOVAs with the main factors stimulus condition (present/absent) and drug condition (control/drug) for each 10 ms bin of the spike densities in the delay phase. In order to ensure that neurons were no longer responsive to presentation of the sample stimulus we excluded the first 100 ms of the delay. Cells that selectively responded to the stimulus condition for at least 300 consecutive milliseconds entered further analyses. In other words, the null hypothesis had to be rejected for at least 30 tests in a row. The longest selective time span was used as the analysis window for the respective neuron.

We quantified selectivity of the stimulus selective neurons (defined by the procedure described above) using receiver operating characteristic (ROC) analysis on the firing rates of the neuron for stimulus absent and present trials (Green & Swets, 1966). The area under the

ROC curve (AUROC) depicts the discriminability of two distributions, where 1 indicates perfect discriminability and 0.5 signals no separation. Stimulus present and absent conditions were labelled as preferred or non-preferred based on the respective AUROC values. If a neuron fired more strongly to the stimulus present condition (resulting in an AUROC value > 0.5), the stimulus present condition was the preferred condition. However, if a neuron responded more strongly to the stimulus absent condition, the stimulus absent condition was the preferred condition. For drug trials we kept the same analysis window and labels regarding stimulus preference as for control trials and calculated AUROC values again. AUROC values in control and drug condition were compared with a paired t-test.

We calculated mean firing rates of stimulus selective neurons during their respective analysis window and compared drug effects on stimulus absence and –presence preferring neurons with two-way repeated measures ANOVAs with drug condition and stimulus intensity as main factors. Next, we compared mean firing rates and Fano factors for preferred and non-preferred stimulus conditions across drug conditions with paired t-tests (Fano, 1947). Mean firing rate differences between control and drug condition were calculated separately for preferred and non-preferred stimulus conditions and also compared with a paired t-test.

The ROC analysis was repeated for those neurons that were tested in control and both drug (MK and Bic) conditions (n = 53). We used a binomial test to examine whether the amount of neurons that increased their AUROC values with administration of either drug was expected by the amount of neurons that increased their AUROC values with one of the drugs.

Results

We investigated the effects of blocking NMDA- and GABA(A) receptors on prefrontal working memory activity of two rhesus monkeys trained on a delayed perceptual decision task. At the beginning of a trial either a sample stimulus of varying intensity was flashed, or no sample stimulus was shown (stimulus absent trials) (**Fig. 8a**). The monkeys had to decide whether or not they had seen a stimulus. Intensity levels of the sample stimuli were individually adapted to the monkeys such that three sample stimuli were salient (stimulus present trials) and three sample stimuli were around the perceptual threshold (stimulus threshold trials). This challenged the monkeys' stimulus present/stimulus absent judgments and forced subjective decisions under uncertainty. The monkeys reported their perceptual decisions based on a subsequent response-rule cue. Importantly, it was not until the response-rule cue that the monkeys knew whether or not a motor act was required depending on their decision (**Fig. 8b**). Neuronal activity in the preceding delay period was thus dissociated from potential motor preparation.

The monkeys' behavioural performance was classified according to signal detection theory. Hits and correct rejections were rewarded, while misses and false alarms were not reinforced. Not reinforcing misses of stimuli that were presented around perceptual threshold leads to a small bias of the monkeys to erroneously report the presence of a stimulus in some of the stimulus absent trials. Both monkeys were able to detect the salient stimuli in over 90% of the cases and correctly rejected over 80% of the trials in which no stimulus was shown. Psychometric detection curves are depicted in **Figure 8c**.

While the monkeys performed the task, we recorded the electrical activity of randomly selected neurons from the lateral PFC (**Fig. 8d**). 281 of the recorded neurons fulfilled our minimal criteria and entered further analyses (118 from monkey Q, and 163 from monkey Z). These neurons were classified into narrow-spiking (i.e. putative interneurons, NS) and broad-spiking (i.e. putative pyramidal cells, BS) neurons based on their waveform characteristics of the extracellularly-measured spikes (**Fig. 10a-b**) (Diester and Nieder, 2008; Viswanathan and Nieder, 2015). During recordings, trial blocks without pharmacological manipulation (control condition) alternated with blocks in which either MK or Bic was iontophoretically applied to the vicinity of the recorded cells (drug condition). A total of 186 neurons fulfilled the criteria in control and MK conditions, 193 neurons in control and Bic conditions, and 98 neurons in control and both drug conditions.

We first explored the potential effects of the drugs on the monkeys' behaviour. Administration of either drug improved psychometric performance for monkey Q ($F(2) = 3.75$, $p = 0.029$, two-way repeated measures ANOVA), but not for monkey Z ($F(2) = 0.49$, $p = 0.614$, two-way repeated measures ANOVA) (**Fig. 8c**). In addition, both monkeys aborted significantly more trials in both drug conditions, independent of the respective stimulus intensity. Specifically, monkey Q aborted 43.80% of all trials during MK administration, compared to 20.26% during respective control trials ($Z = -6.27$, $p < 0.001$, paired Wilcoxon signed-rank test), and 42.31% during Bic administration, compared to 20.55% during respective control trials ($Z = -6.27$, $p < 0.001$, paired Wilcoxon signed-rank test). Similarly, Monkey Z aborted 42.96% of all trials during MK administration, compared to 20.51% during

respective control trials (Z = -6.15, p < 0.001, paired Wilcoxon signed-rank test), and 41.84% during Bic administration, compared to 20.71% during respective control trials (Z = -6.21, p < 0.001, paired Wilcoxon signed-rank test).

NMDA receptor blockade reduced spontaneous firing rate, whereas GABA(A) receptor blockade increased it

We first evaluated the general effect of MK and Bic on the PFC neurons' spontaneous firing rates. Application of MK slightly decreased spontaneous firing rates (Z = -2.41, p = 0.016, paired Wilcoxon signed-rank test), while Bic significantly increased them (Z = 7.39, p < 0.001, paired Wilcoxon signed-rank test) (**Fig. 10c-d**). The effects of MK and Bic were similar for the BS and NS subpopulations: MK application tended to decrease the firing rates of BS (n = 106, Z = -1.87, p = 0.061, paired Wilcoxon signed-rank test), NS were not modulated by MK (n = 46, Z = -1.61, p = 0.107, paired Wilcoxon signed-rank test). Bic increased firing rates for BS (n = 115, Z = 6.35, p < 0.001, paired Wilcoxon signed-rank test) as well as for NS (n = 47, Z = 4.42, p < 0.001, paired Wilcoxon signed-rank test). Note that the number of BS and NS do not add up to the total of 186 neurons analysed for MK, and the total of 193 neurons analysed for Bic, because the waveforms of some neurons could not be classified as either broad or narrow.

Next, we examined the time course of firing rate modulations at the transition from control to drug phases, in order to see how quickly drug effects are detectable (**Fig. 10e**). In the pooled data, drug effects of MK are no longer significant. The continuously increasing firing

rate caused by Bic was stronger for BS as compared to NS. The normalized firing rate for each trial was smoothed with a Gaussian kernel, taking the two neighbouring trials into account (see Methods). We tested whether each of the obtained spike density histograms significantly deviated from the normalized firing rate of 1 Hz for every trial after drug on switch. BS treated with Bic exhibited a significantly elevated firing rate ten trials after drug on switch and stayed elevated for the rest of the trials (one-sample Wilcoxon signed-rank tests). NS treated with Bic reached significance after six trials, but some of the remaining trials did not significantly differ from a firing rate of 1 Hz (one-sample Wilcoxon signed-rank tests). For neurons treated with MK, the subpopulation of BS reached significance after 44 trials and the subpopulation of NS significantly differed from a firing rate of 1 Hz after 47 trials (one-sample Wilcoxon signed-rank tests).

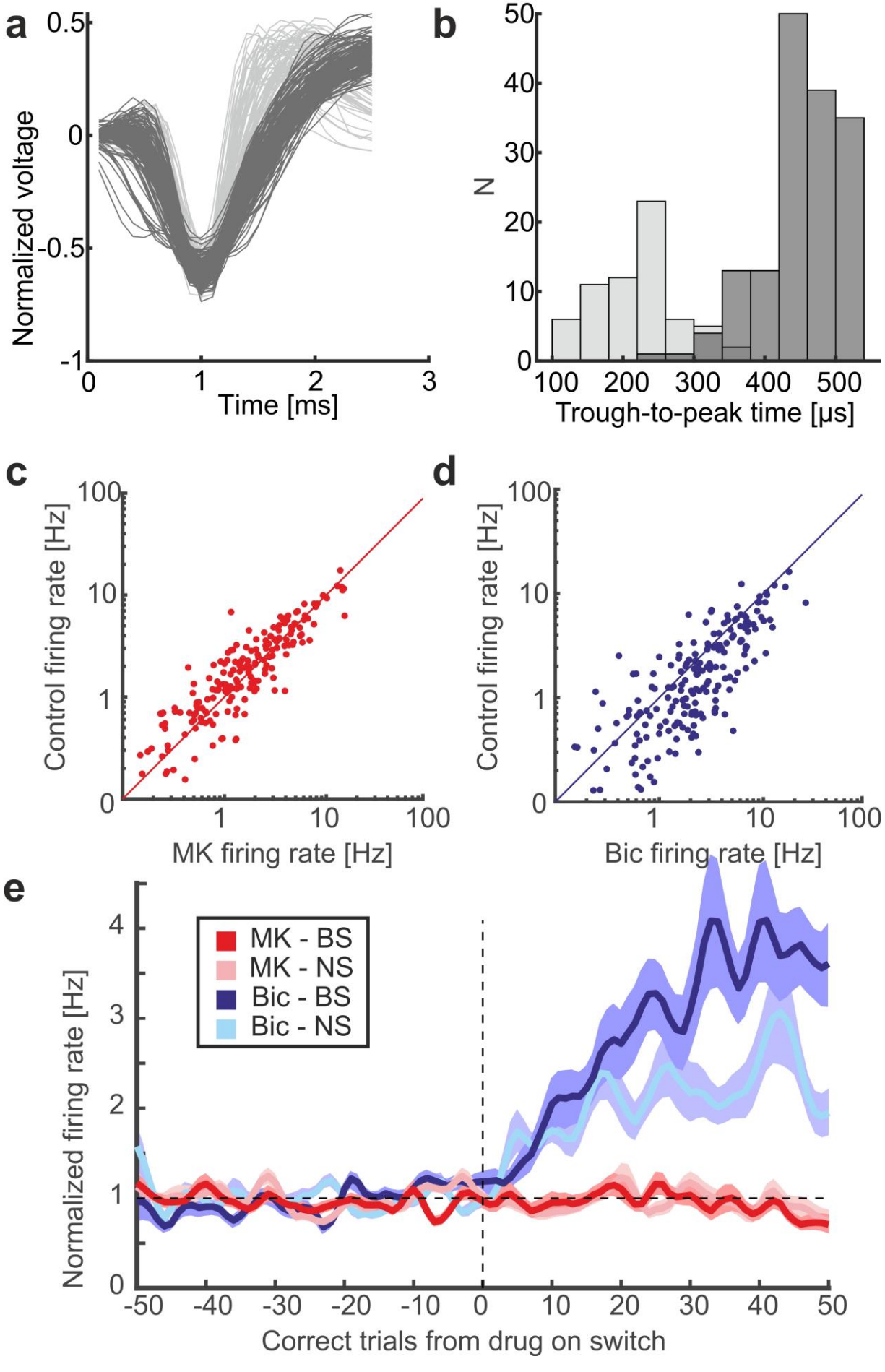
a
Normalized voltage
Time [ms]
b
N
Trough-to-peak time [µs]
c
Control firing rate [Hz]
MK firing rate [Hz]
d
Control firing rate [Hz]
Bic firing rate [Hz]
e
MK - BS
MK - NS
Bic - BS
Bic - NS
Normalized firing rate [Hz]
Correct trials from drug on switch

Figure 10. Waveform separation and drug effects on spontaneous firing rate. ***a***, Waveforms of recorded neurons. ***b***, Distribution of broad- and narrow-spiking neurons. ***c-d***, Spontaneous firing rate during the fixation period in control and drug condition (***c***: MK-108 (MK), 186 neurons; ***d***: Bicuculline methiodide (Bic), 193 neurons). ***e***, Normalized spontaneous firing rate for broad- (BS) and narrow-spiking (NS) neurons for the last 50 correct trials in control condition before drug on switch and the first 50 correct trials after drug on switch.

NMDA- and GABA(A) receptor blockade each improved selectivity to preferred stimulus condition

Many of our recorded neurons showed selective activity either for stimulus present trials or stimulus absent trials. We used a sliding window ANOVA to assess selectivity of the neurons to the stimulus condition in the delay period (see Methods) and refer to the stimulus condition that caused a significantly elevated firing rate as the preferred stimulus condition, whereas the other condition is referred to as the non-preferred stimulus condition. Of 186 neurons recorded in the MK condition, 72 were stimulus selective; of those, 46 preferred stimulus presence and 26 preferred stimulus absence. Likewise, of the 193 neurons recorded under the Bic conditions, 83 were stimulus selective; of those, 56 preferred stimulus presence and 27 preferred stimulus absence. Of the 98 neurons recorded in both drug conditions, 53 were stimulus selective, with 34 preferring stimulus presence and 19 preferring stimulus absence.

Next, we explored the neuromoteric functions with and without drugs for the stimulus-absent and stimulus-present neuron population separately. To that aim, we compared the mean firing rates of neurons preferring stimulus presence or absence in control and drug condition across all stimulus intensities (**Fig. 11**). Significant firing rate differences after drug

applications were observed for both, neurons that preferred the stimulus present condition and neurons that preferred the stimulus absent condition. Bic, in particular, caused a clear upwards parallel shift of the neurometric functions for both cell categories (**Fig. 11b**). Specifically, we found no effect of MK on the firing rate of stimulus presence preferring neurons ($F(1) < 0.01$, $p = 0.999$, two-way repeated measures ANOVA), and no interaction of firing rate with stimulus intensities ($F(6) = 1.11$, $p = 0.355$). We also found no effect of MK on the firing rate of stimulus absence preferring neurons ($F(1) = 2.30$, $p = 0.142$, two-way repeated measures ANOVA), but a marginal interaction of firing rate with stimulus intensities ($F(6) = 2.25$, $p = 0.042$). Post-hoc testing revealed significant differences at stimulus intensity levels 3 ($p = 0.036$) and 4 ($p = 0.022$). In contrast, we found significant differences in firing rate following Bic administration for stimulus presence preferring ($F(1) = 23.51$, $p < 0.001$, two-way repeated measures ANOVA), without significant interaction with stimulus intensities ($F(6) = 1.22$, $p = 0.294$). Similarly, Bic administration significantly changed activity for stimulus absence preferring neurons ($F(1) = 5.41$, $p = 0.028$, two-way repeated measures ANOVA), again without significant interaction ($F(6) = 0.78$, $p = 0.587$).

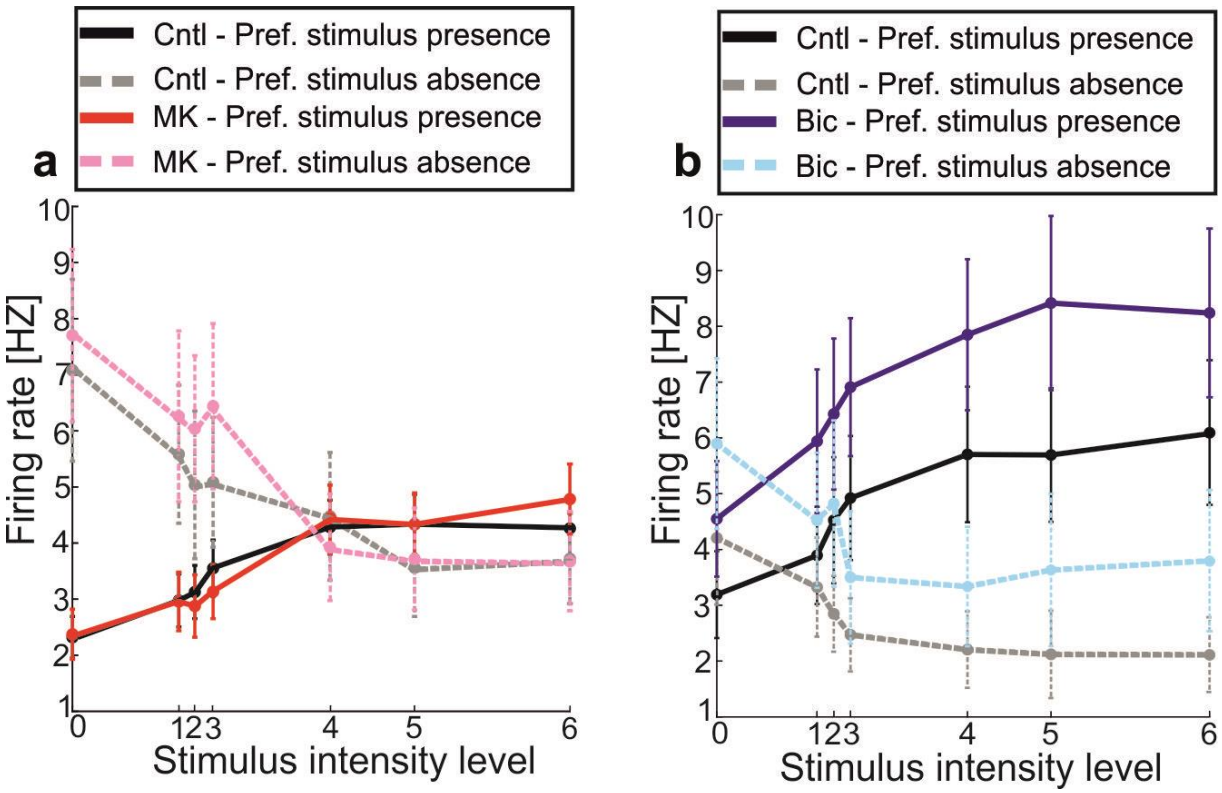

Figure 11. Drug effects on firing rate for neurons preferring stimulus absence or presence. Because the monkeys worked with different absolute intensity values, the data are plotted in relative intensity values according to the monkeys' individual psychometric functions (see **Fig. 8c**). Relative stimulus intensity levels 0 to 6 correspond to absolute stimulus intensities 0, 6, 7, 8, 15, 20, 30 for monkey Q and 0, 10, 11, 12, 20, 30, 40 for monkey Z. Firing rate means were derived during each neuron's respective analysis window. ***a***: Firing rates with MK are depicted in reddish colours relative to control discharges in black and grey. ***b***: Firing rates with Bic are depicted in bluish colours relative to control firing rates. Neurons preferring stimulus presence are depicted in solid lines and darker colours. Neurons preferring stimulus absence are drawn in dashed lines and lighter colours. Error bars indicate SE.

Next, we analysed whether the administered drugs affected the spontaneous firing rate of the subpopulation of stimulus selective neurons. Application of MK had no effect on spontaneous firing rates ($Z = -0.57$, $p = 0.571$, paired Wilcoxon signed-rank test), while Bic significantly increased them ($Z = 6.60$, $p < 0.001$, paired Wilcoxon signed-rank test). The effects of MK and Bic were similar for the subpopulations of BS and NS. BS firing rates were

not affected by application of MK ($n = 41$, $Z = -0.95$, $p = 0.341$, paired Wilcoxon signed-rank test), and NS firing rates were not modulated either ($n = 18$, $Z = -0.37$, $p = 0.711$, paired Wilcoxon signed-rank test). Bic increased firing rates for BS ($n = 50$, $Z = 5.36$, $p < 0.001$, paired Wilcoxon signed-rank test) as well as for NS ($n = 22$, $Z = 3.20$, $p = 0.001$, paired Wilcoxon signed-rank test).

We further analysed the impact of both drugs on the encoding of the stimulus condition of these stimulus selective neurons. **Figure 12** depicts example cells showing improved stimulus selectivity after blocking NMDA receptors (MK condition). For the neurons depicted in **Figure 12** the AUROC values increase from 0.87 in control condition to 0.97 in MK condition (panel **a**) and from 0.61 to 0.62 (panel **b**). **Figure 13** likewise shows example cells exhibiting improved stimulus selectivity after blockage of GABA(A) receptors (Bic condition). For the depicted neurons the AUROC values increases from 0.61 in control condition to 0.77 in Bic condition (panel **a**) and from 0.70 to 0.81 (panel **b**).

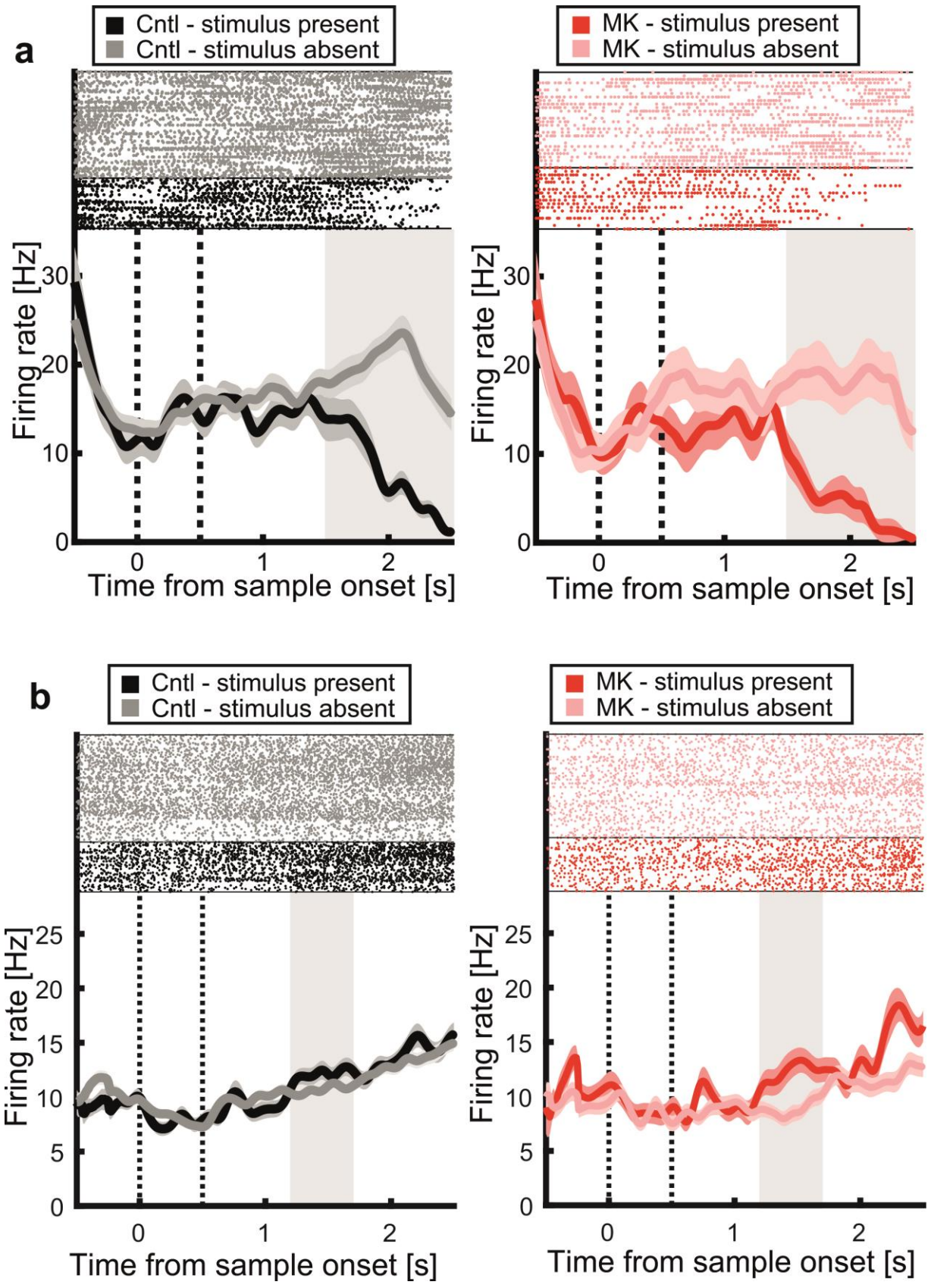

Figure 12. MK effects on selectivity for example neurons**.** Raster plots (every line stands for a trial, and every dot represents a spike) and spike density histograms (time-resolved average firing rates) depicting the activity of two example neurons with increased selectivity after MK administration (***a-***

b). Beginning of stimulus presentation and delay period are depicted with dashed vertical lines. Shaded backgrounds indicate the analysis window. Stimulus present trials are drawn in dark colours, stimulus absent trials in light colours. Shaded areas around the spike density histograms represent respective standard errors of the mean (SE). ***a*** shows a narrow-spiking cell from monkey Z in control (left panel) and MK (right panel) condition preferring stimulus absent trials. ***b*** shows a broad-spiking cell from monkey Z in control (left panel) and MK (right panel) condition preferring stimulus present trials.

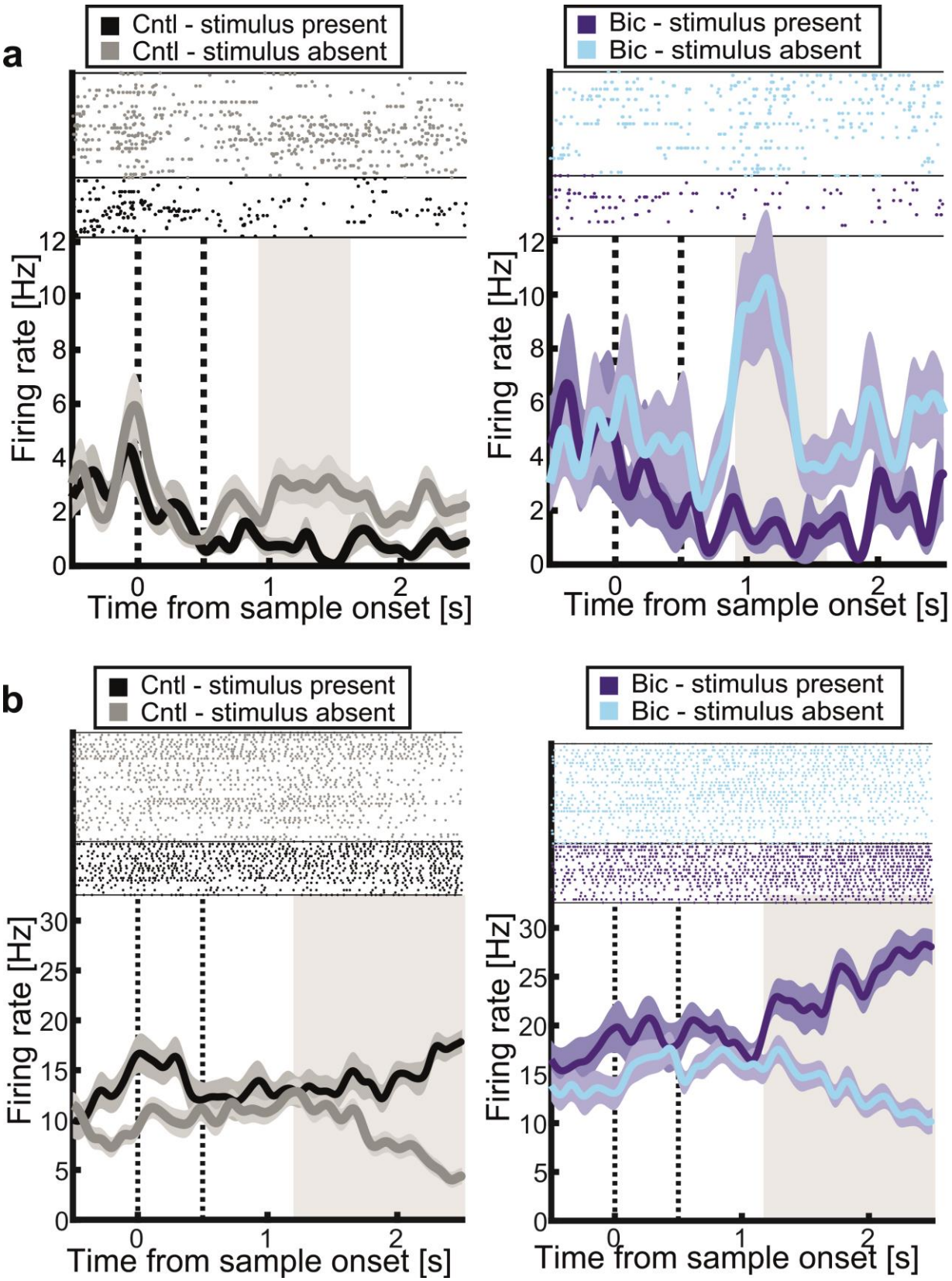

Figure 13. Bic effects on selectivity for example neurons. Raster plots and spike density histograms depicting the activity of two example neurons with increased selectivity after Bic administration (***a-b***). Beginning of stimulus presentation and delay period are depicted with dashed vertical lines.

Shaded backgrounds indicate the analysis window. Stimulus present trials are drawn in dark colours, stimulus absent trials in light colours. Shaded areas around the spike density histograms represent respective SEs. ***a*** shows a narrow-spiking cell from monkey Q in control (left panel) and Bic (right panel) condition preferring stimulus absent trials. ***b*** shows a broad-spiking cell from monkey Z in control (left panel) and Bic (right panel) condition preferring stimulus present trials.

To evaluate the drug effects on the population of PFC cells, we derived and compared the area under the ROC-curve (AUROC) as a measure of neuronal selectivity. Here, the AUROC is a nonparametric measure of the discriminability of two distributions of firing rates recorded in the stimulus present and stimulus absent conditions, respectively. Values of 0.5 indicate no separation, and values of 1 signal perfect discriminability. Comparing AUROC values during drug and control conditions for the population of stimulus selective neurons yielded significantly higher AUROC values in both drug conditions. Mean AUROC values increased from 0.71 (SE = 0.01) in control to 0.74 (SE = 0.01) with administration of MK ($t(71) = -2.20$, $p = 0.031$, paired t-test), and from 0.68 (SE = 0.01) in control to 0.74 (SE = 0.01) with administration of Bic ($t(82) = -4.19$, $p < 0.001$, paired t-test). The blockade of both NMDA- and GABA(A) receptors thus improved working memory selectivity to stimulus condition (**Fig. 14**). These stimulus selective neurons commonly lost their selectivity or were oppositely tuned in error trials. The distribution of analysis window durations during which neurons exhibited stimulus selectivity is depicted in **Figure 15**. For both MK (**Fig. 15a**) and Bic (**Fig. 15b**), neurons show a mixture of brief and sustained selectivity.

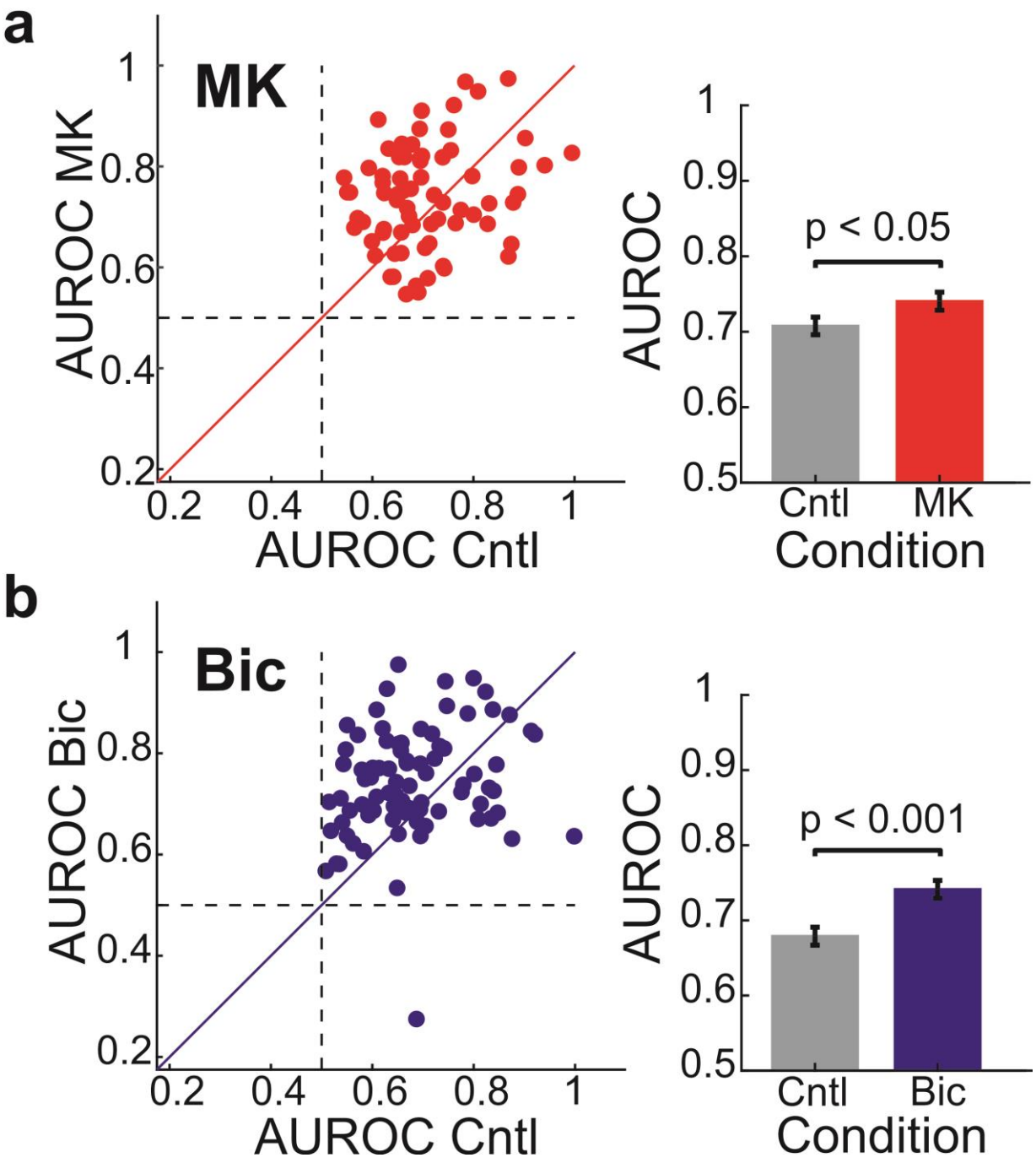

Figure 14. Drug effects on the selectivity for the population of stimulus selective neurons. AUROC values depict discriminability between preferred and non-preferred stimulus condition in control and drug condition (***a***: MK, 72 neurons; ***b***: Bic, 83 neurons). Left panels show AUROC values for each neuron, right panels show mean (and SE) for the respective population.

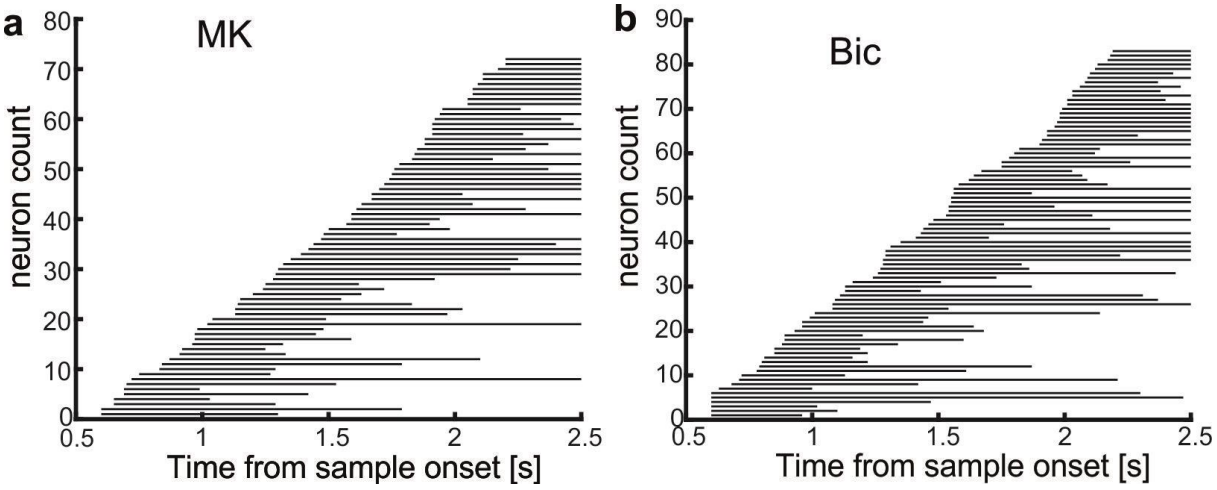

Figure 15. Distribution of analysis windows of stimulus selective neurons. Stimulus selective neurons in MK (***a***) and Bic (***b***) condition are sorted according to the beginning of their analysis window.

As every recording session started with a control block, we wanted to ensure that drug effects were not based on duration of recording time. Therefore, we compared AUROC values of the first and second control block separately for both drug conditions. AUROC values were comparable for neurons recorded in MK condition ($t(10) = 0.47$, $p = 0.647$, paired t-test) as well as for neurons recorded in Bic condition ($t(20) = -0.72$, $p = 0.478$, paired t-test).

In order to elucidate mechanisms that lead to enhanced selectivity, we further investigated drug effects on firing rates in the preferred and non-preferred stimulus condition separately. For stimulus selective neurons modulated by MK, firing rates in control trials were by definition higher for the preferred stimulus condition ($M = 5.92$, $SE = 0.79$) than for the non-preferred condition ($M = 2.88$, $SE = 0.43$) (**Fig. 16**). MK did not change absolute firing rates of these neurons, neither in the preferred stimulus condition ($M = 6.3912$, $SE = 0.77$, $t(71) = -0.60$, $p = 0.552$, paired t-test, **Fig. 16a**), nor in the non-preferred stimulus condition ($M =$

2.82, SE = 0.44, t(71) = 0.02, p = 0.981, paired t-test, **Fig. 16b**). Firing rate differences between drug and control trials for preferred (M = 0.47, SE = 0.85) and non-preferred (M = -0.06, SE = 0.47) stimulus conditions were comparable (t(71) = 0.97, p = 0.334, paired t-test, **Fig. 16c**). The absence of MK-effects at the population level is also depicted in **Figure 17** showing the normalized spike density histograms of the population of stimulus selective neurons treated with MK. The population spike density histograms look as if the selectivity is decreased with drug administration even though we observed improved selectivity on the level of individual neurons. This can be explained by the respective analysis windows of the neurons being distributed all over the delay (**Fig. 15**) and implies that the neurons' preference for one of the stimulus conditions is diminished or even inversed outside this window. An analysis of the Fano factor, a measure of firing rate dispersion and variability, revealed no significant difference between control and MK trials, neither for the preferred (3.44 in control trials, 3.33 in MK trials; t(71) = 0.287, p = 0.775, paired t-test), nor the non-preferred stimulus condition(2.98 in control trials, 2.56 in MK trials; t(68) = 1.44, p = 0.156, paired t-test). Taken together these results suggests, that the improved selectivity with MK arises from a combination of non-significant changes in the activity of selective neurons.

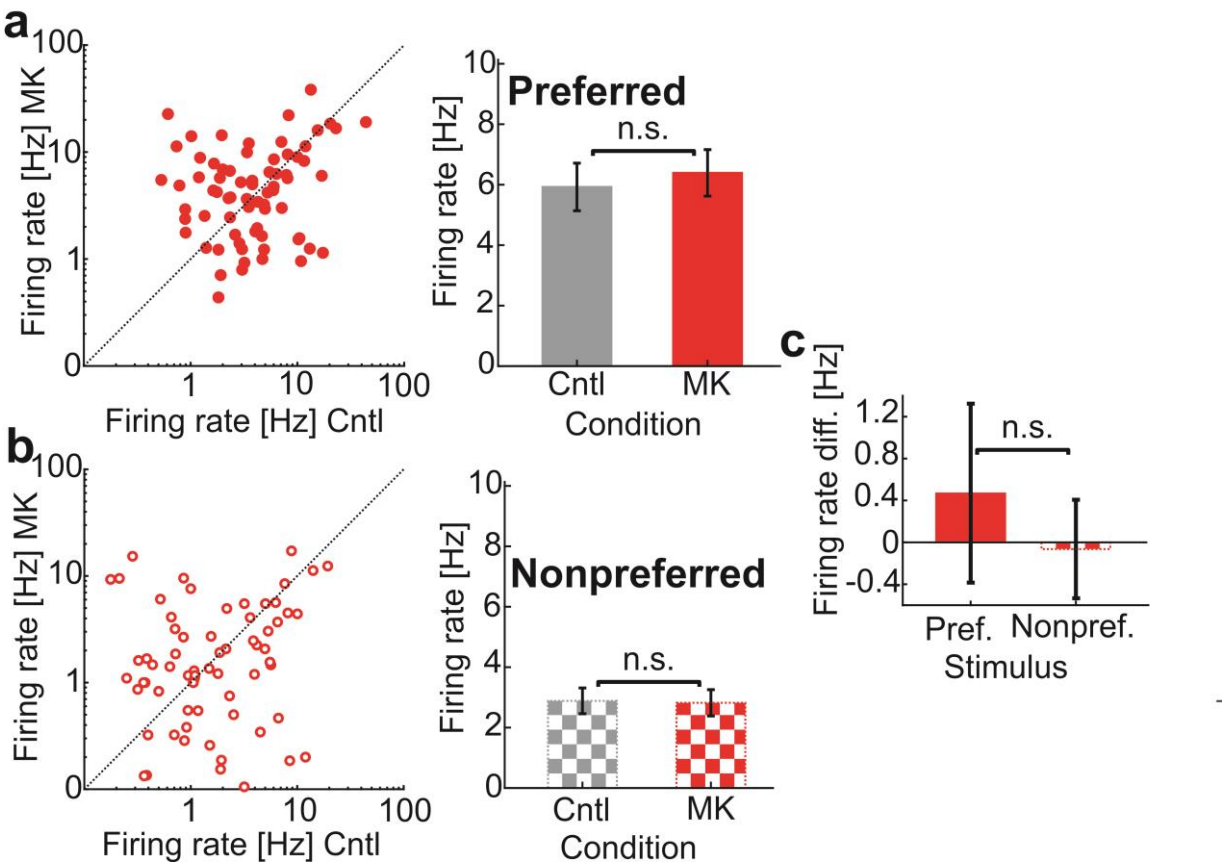

Figure 16. MK effects on the firing rate for preferred and non-preferred stimuli. ***a-b***, Firing rates for control and MK condition separated for preferred (***a***) and non-preferred (***b***) stimulus condition. Left panels show firing rates for each neuron, right panels show mean firing rates for the respective population. (***c***) Differences in mean firing rate between MK and control condition for the preferred and non-preferred stimulus condition. Error bars indicate SEs.

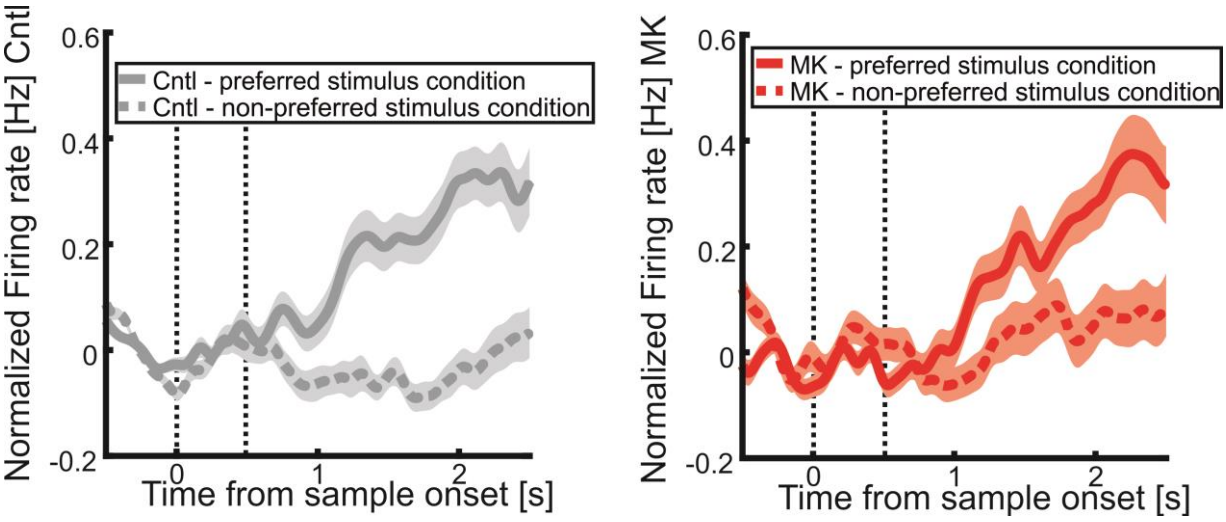

Figure 17. Time resolved effects of MK on the preferred and non-preferred stimulus condition. Spike density histograms depict the activity of the population of stimulus selective neurons in control (left panel) and after MK administration (right panel). Beginning of stimulus presentation and delay period are depicted with dashed vertical lines. Preferred stimulus conditions are drawn with solid lines, non-preferred stimulus conditions with dashed lines. Shaded areas around the spike density histograms represent respective SEs.

Next, we analysed how Bic achieved the improved selectivity reported above. Again, by definition, mean firing rates in control trials were higher for the preferred stimulus condition (M = 5.67, SE = 0.94) than for the non-preferred condition (M = 2.81, SE = 0.59) for stimulus selective neurons modulated with Bic (**Fig. 18**). Bic did not increase absolute firing rates, neither in the preferred stimulus condition ($t(82) = -1.61$, $p = 0.111$, paired t-test, **Fig. 18a**), nor in the non-preferred condition ($t(82) = -1.27$, $p = 0.207$, paired t-test, **Fig. 18b**). A comparison of firing rate differences between control and drug trials for preferred and non-preferred stimulus condition revealed that Bic tended to increase firing rates stronger for the preferred stimulus condition (M = 2.47, SE = 1.54) than for the non-preferred condition (M = 1.24, SE = 0.97) ($t(82) = 1.79$, $p = 0.077$, paired sample t-test).

Figure 19 depicts normalized spike density histograms of the population of stimulus selective neurons treated with Bic that illustrate these effects across time. The Fano factors for Bic were comparable between control and drug trials, both for the preferred condition (3.43 in control trials, 3.39 in Bic trials; t(82) = 0.10, p = 0.920, paired t-test), and the non-preferred condition (3.08 in control trials, 2.87 in Bic trials; t(79) = 0.61, p = 0.547, paired t-test). This implies that the variability of firing rates is not modulated via GABA(A) receptors.

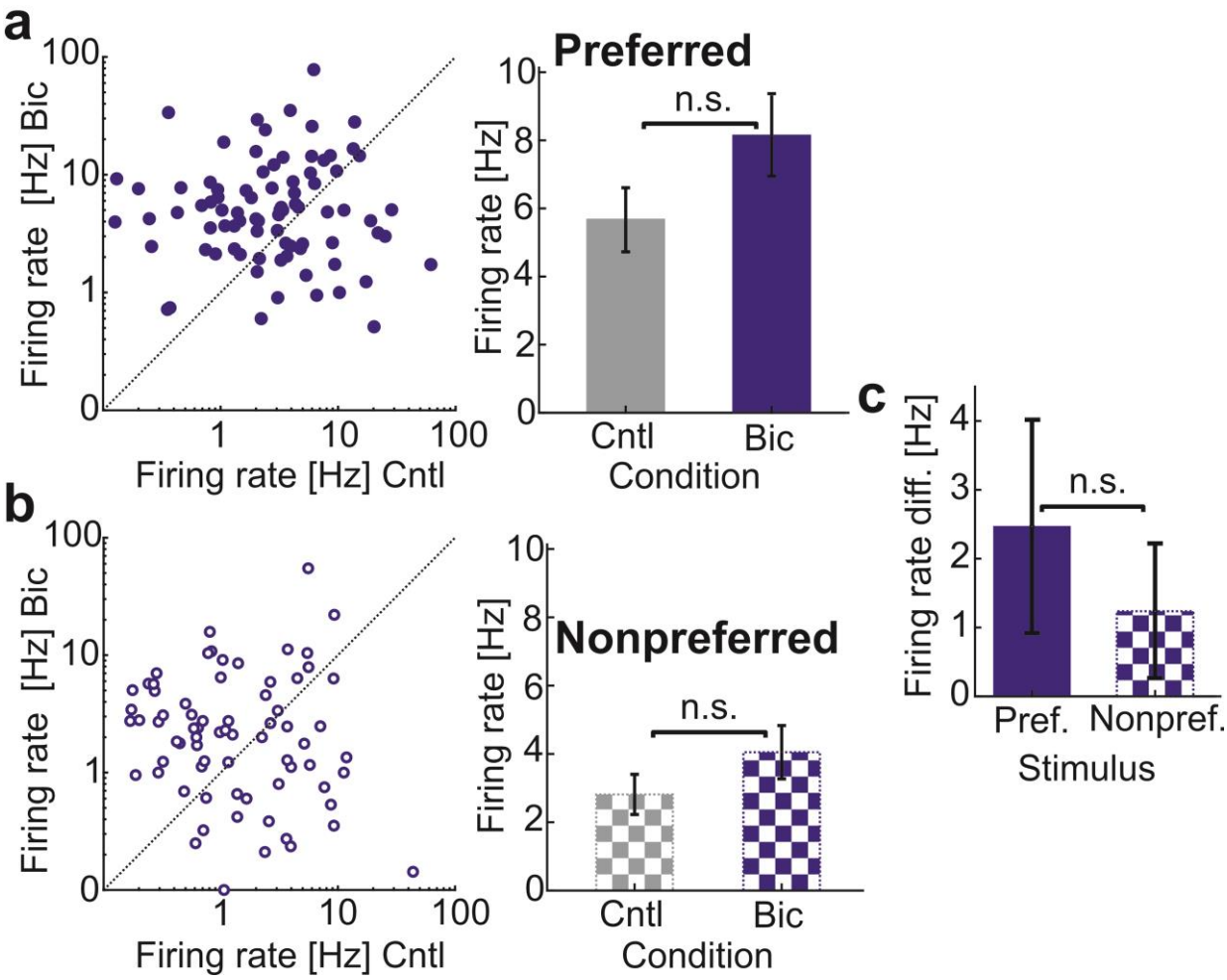

Figure 18. Bic effects on firing rate for preferred and non-preferred stimulus condition. ***a-b***, Firing rates for control and Bic condition separated for preferred (***a***) and non-preferred (***b***) stimulus condition. Left panels show firing rates for each neuron, right panels show mean firing rates for the

respective population. (***c***) Differences in mean firing rate between Bic and control condition for the preferred and non-preferred stimulus condition. Error bars indicate SEs.

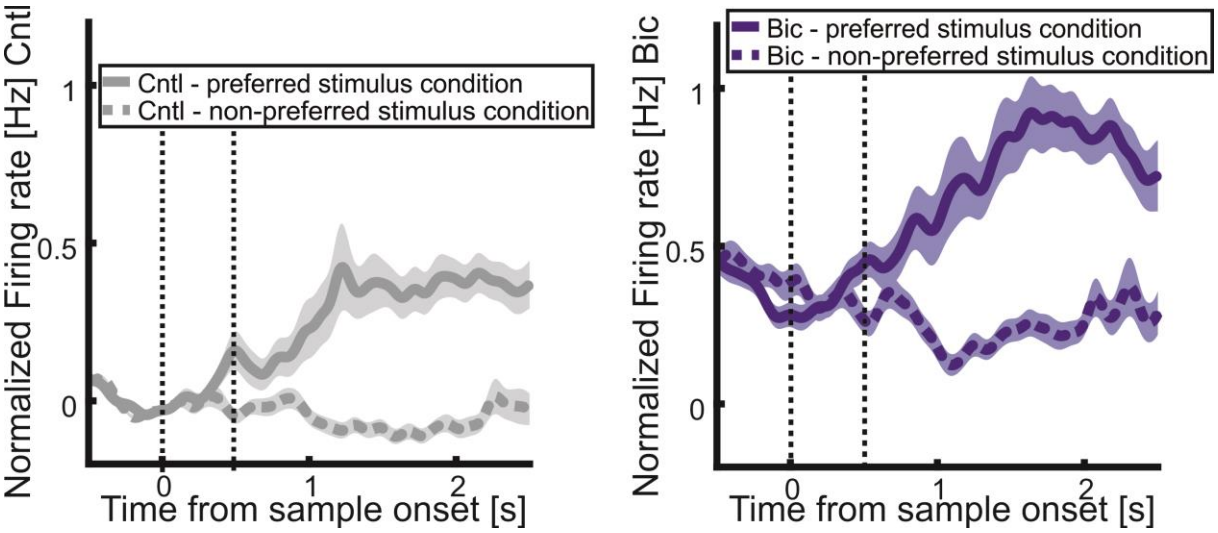

Figure 19. Time resolved effects of Bic on the preferred and non-preferred stimulus condition. Spike density histograms depict the activity of the population of stimulus selective neurons in control (left panel) and after Bic administration (right panel). Beginning of stimulus presentation and delay period are depicted with dashed vertical lines. Preferred stimulus conditions are drawn with solid lines, non-preferred stimulus conditions with dashed lines. Shaded areas around the spike density histograms represent respective SEs.

Individual neurons increased selectivity in response to both NMDA- and GABA(A) receptor blockade

To find out how individual neurons were affected by blockade of NMDA- and GABA(A) receptors, we further analysed the subset of 53 stimulus selective neurons in sequential MK- and Bic- conditions. **Figure 20a-b** depict example cells showing enhanced delay selectivity with administration of either drug. Note that in both depicted cells MK mainly increases selectivity by diminishing the firing rate for the stimulus absent condition.

A population analysis applying again an ROC analysis of selectivity to stimulus condition during the respective analysis windows in the delay revealed that MK tended to increase ROC values ($t(52) = -1.86$, $p = 0.069$, paired t-test) and Bic significantly increased selectivity of these neurons ($t(52) = -2.97$, $p = 0.004$, paired t-test). 33 of the stimulus selective neurons treated with both drugs enhanced their selectivity with administration of MK, 38 did so by administration of Bic and 28 neurons enhanced selectivity with either drug compared to control. Other possible combinations were infrequent, with ten neurons decreasing selectivity with MK but increasing it with Bic, five neurons increasing selectivity with MK but decreasing it with Bic, and ten neurons decreasing selectivity with either drug (**Fig. 18c**). Statistical testing revealed that the proportion of cells that improved their selectivity with both drugs (52.83%) was within the range expected by the proportion of cells that improved their selectivity with one of the drugs (44.64%) ($p = 0.143$, 1-sided binomial test). Overall, 85% (28 of 33) of the neurons that improved their selectivity with application of MK also improved their selectivity when Bic was applied. Likewise, 74% (28 of 38) of those neurons that improved their selectivity with application of Bic also improved their selectivity when MK was applied. The neuronal populations increasing their selectivity with application of Bic and MK respectively thus largely overlap.

Effects were comparable for the subset of BS and NS. Ten of the 53 neurons could not be classified as either NS or BS, 26 were BS and 17 were NS. Of the 26 BS 17 improved their selectivity with administration of MK, 19 did so with administration of Bic and 13 with either drug. The proportion of BS that improved selectivity with both drugs (50.00%) was within the range expected by the proportion of cells that improved their selectivity with one of the

drugs (47.78%) ($p = 0.489$, 1-sided binomial test). Of the 17 NS, eleven improved their selectivity with administration of MK, 13 did so with administration of Bic and nine with either drug. Again the proportion of NS that improved selectivity with both drugs (52.94%) was within the range expected by the proportion of cells that improved their selectivity with one of the drugs (49.48%) ($p = 0.483$, 1-sided binomial test).

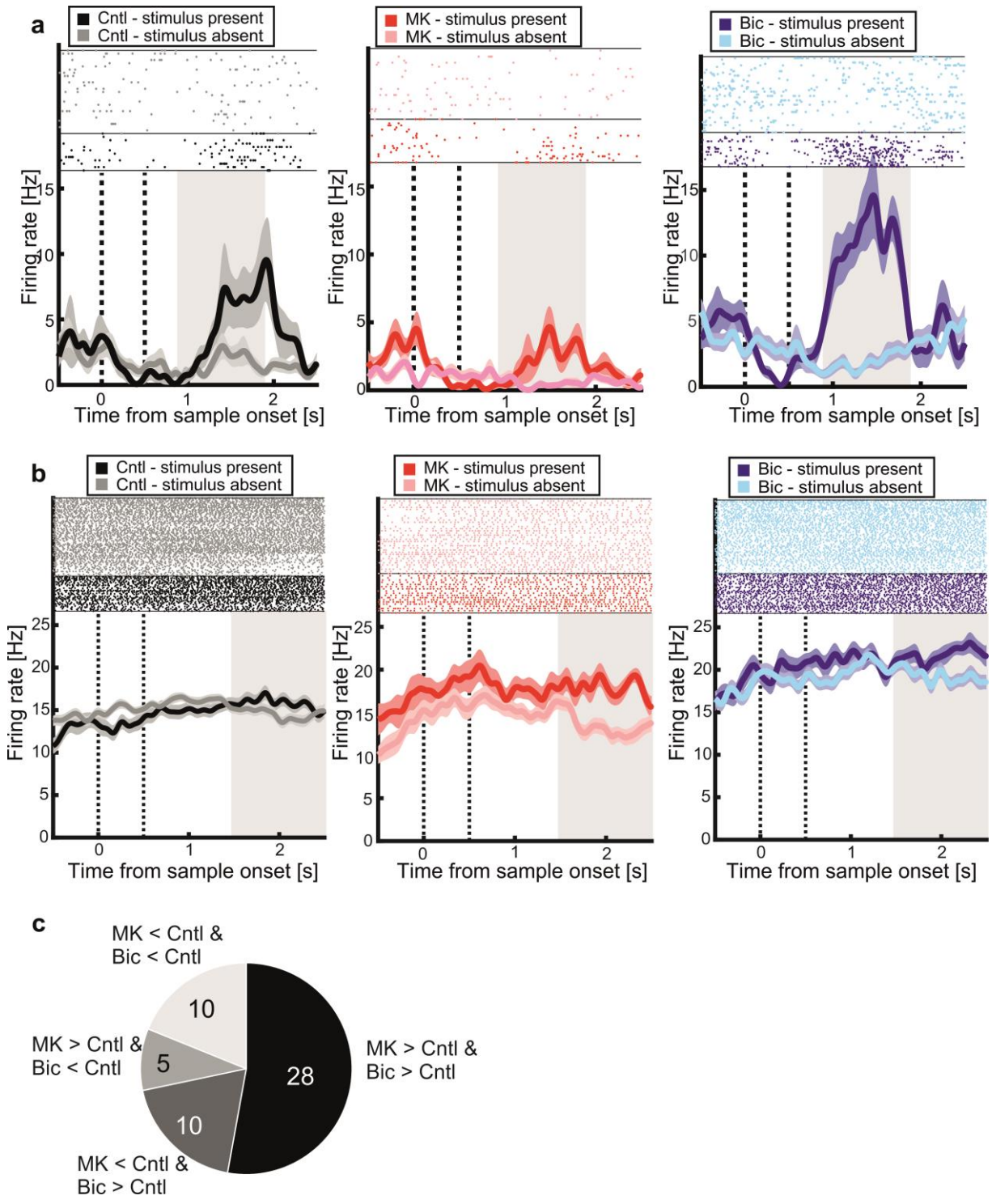

Figure 20. Stimulus selective neurons modulated by Bic and MK. ***a-b***, Raster plots and spike density histograms depicting the activity of a broad-spiking cell preferring stimulus-present trials from monkey Q (***a***) in control (left panel), MK (middle panel) and Bic (right panel) condition, as well as a broad-spiking cell preferring stimulus-present trials from monkey Z (***b***) in control (left panel), MK (middle panel) and Bic (right panel) condition. The beginning of stimulus presentation and delay period are depicted with dashed vertical lines. Shaded backgrounds indicate the analysis window. Stimulus-present trials are drawn in dark colours, stimulus-absent trials in light colours. Shaded areas around the spike density histograms represent respective SEs. ***c***, Amount of neurons that

increased/decreased their selectivity with drug administration. The less-than (<) and greater-than (>) signs show whether the AUROC value, signifying discriminability between preferred and non-preferred stimulus condition, was higher in the respective drug or control condition.

Discussion

We assessed the effects of the excitatory glutamatergic and inhibitory GABAergic neurotransmitter systems on single neurons of the lateral PFC contained in awake, behaving monkeys performing a delayed perceptual decision task requiring working memory by combining single-cell recordings and simultaneous micro-iontophoretic drug applications. The aim of this study was to investigate the role of glutamatergic NMDA- and GABAergic GABA(A) receptors in establishing sustained activity of PFC neurons, as the hypothetical neuronal correlate of working memory.

Performance changes by the drugs were only seen in one of the two tested monkeys; administration of either drug improved performance of monkey Q, but not monkey Z. Additionally, we observed that both monkeys significantly aborted more trials in drug conditions. Overall, these behavioural findings were rather unexpected, given that the effect of micro-iontophoresis is very focal (Herz et al., 1969). We thus assume that the drugs affected small clusters or micro-networks of neurons, rather than individual cells alone.

We found that PFC neurons of macaque monkeys elevated their spontaneous firing rate after GABA(A) blockade via Bic administration, in contrast, PFC neurons decreased their firing rate following NMDA blockade via MK administration. The main finding of this study was that the blockade of both excitatory glutamatergic synapses with NMDA-receptor antagonists MK-801 (MK), as well as the inactivation of inhibitory synapses by GABA(A)-receptor antagonist Bicuculline methiodide (Bic), respectively, increased stimulus selectivity of prefrontal memory neurons during the delay period, albeit only subtle for NMDA-receptors. Bic tended to improve selectivity by increasing the firing rate stronger for the

preferred stimulus condition than for the non-preferred one. No differences of firing rate effects with regard to the preferred and non-preferred stimulus condition were observed after MK administration. In a subset of neurons, we were able to apply both drugs (MK and Bic) and, consistent with the recordings where only one drug was applied, we found that most neurons (both putative pyramidal cells as well as inhibitory interneurons) increased their signal to noise ratio in response to both drugs, suggesting that the effects may be carried by a common population of neurons containing both NMDA- and GABA(A) receptors. While the drug effects on the spontaneous firing rate of PFC neurons were as expected, the improved selectivity was contrary to expectation for both drugs. These results contrast previous findings in behaving monkeys (Rao et al., 2000; Wang et al., 2013).

Most of the recorded stimulus selective neurons did not exhibit sustained activity throughout the whole delay, consistent with prior reports that memory neurons do not necessarily persistently fire throughout the whole delay (Miller et al., 2018). Approximately two thirds of our stimulus selective neurons preferred the stimulus present condition and a third of the neurons preferred the stimulus absent condition. This discrepancy was expected, because the more salient a stimulus, the higher its chance to be maintained in working memory (Barak and Tsodyks, 2014). Besides, the stimulus absent condition is absent from any sensory evidence and is solely coded as an abstract category (Merten and Nieder, 2012).

Blocking NMDA receptors increases spontaneous responses and improves stimulus selectivity

MK's effects on spontaneous firing rate

We found a significant, albeit mild reduction of spontaneous firing rate after administration of glutamatergic NMDA-receptor antagonist MK in awake behaving monkeys. Only prior studies done in-vitro and in anesthetized rats after previous NMDA administration have shown a decrease in spontaneous firing rate after application of MK (Huettner and Bean, 1988; Zhang et al., 1992; Rotaru et al., 2011). Tests on the subgroups of BS and NS suggest that the detrimental effects of MK on spontaneous firing rate of the whole population of neurons seemed to be mainly mediated by pyramidal cells. In turn this proposes that during spontaneous firing blockage of NMDA receptors on interneurons was less effective, probably because interneurons were generally less active than pyramidal neurons, masking the drug's supposed preference for interneurons (Jackson et al., 2004; Homayoun and Moghaddam, 2007).

Although focal in effect, iontophoretic drug application does not preclude local diffusion of the drug. Therefore, it is unlikely that our drug application affected only the particular neurons at the electrode recording tip and, likely our iontophoretic manipulations pharmacologically affected other neurons in the local microcircuit. Thus, the observed effects may not necessarily be due to direct effects on the respective receptors of the recorded neurons, but could also stem from secondary effects mediated via the local microcircuit. The effects of NMDA receptor blockage are thus further complicated when considering interactions of excitatory and inhibitory neurons at the level of the microcircuit.

For example, depending on whether NMDA-mediated excitatory input to pyramidal cells or inhibitory interneurons is blocked, effects on firing rates of postsynaptic neurons is opposite. Therefore, whether MK has a net excitatory or inhibitory effect on clusters of neurons should depend on the relative ratio of recruited pyramidal cells and interneurons. Given that many of these neurons are selectively active during task engagement, drug dosage and attentional engagement in the task at hand are likely important. In fact, systemic administration of high doses of MK in rodents commonly causes excitatory effects, whereas low dosages do not (Suzuki et al., 2002; Lorrain et al., 2003; Jackson et al., 2004; Homayoun and Moghaddam, 2007; Skoblenick and Everling, 2012). MK's excitatory effects are suspected to be mediated by an inhibition of glutamatergic input in inhibitory interneurons, which leads to a net disinhibition of local circuits (**Fig. 22**). The same is true for other NMDA receptor antagonists. Ketamine, for example, may increase or decrease net glutamate outflow in the PFC, as obtained by microdialysis and HPLC, depending on the dosage and also affects dopamine release (Moghaddam et al., 1997).

Echoing the conflicting findings of MK administration, we found that while blocking NMDA receptors lowered the spontaneous firing rate in the whole population of recorded neurons, it did not seem to affect the spontaneous firing rate in the subset of stimulus selective neurons. Extensive conclusions should not be drawn from this discrepancy, as effects of NMDA blockage are generally hard to capture and the population of stimulus selective neurons may have been too small to detect the mild drug effects. Besides, comparable literature on the iontophoretic effects of MK in the PFC of behaving monkeys is yet sparse. If our findings prove to be stable, however, they may imply that NMDA receptors serve

different roles in the resting state of memory neurons in the PFC compared to the overall population of PFC neurons. More precisely, this suggests that NMDA receptors of memory neurons, as well as nearby neurons synapsing with them, are commonly not open in the spontaneous resting state, while NMDA receptors are more likely to be open in the overall population of PFC neurons. Perhaps a subset of the recorded neurons that do not code for the memorandum were active during the fixation period in our task because they mediated focussed attention. Alternatively, these neurons may have served a purpose that was not related to our task.

Wang and colleagues (2013) found that iontophoretic administration of MK to PFC neurons recorded in awake behaving monkeys did not affect their spontaneous firing rates. However, when they considered MK's effect on delay selective neurons (n = 15), they found a marked reduction of firing rates in all task epochs. Assuming that these neurons were not simultaneously involved in task unrelated duties, there is no reason to suspect activation of their NMDA receptors in the spontaneous resting state. MK, however, only acts on open channels and its effectiveness thus depends on the presence of glutamate (Huettner and Bean, 1988). Consistent with this interpretation, we found that MK did not modulate the spontaneous firing rates of memory neurons, but decreased the firing rates of the overall population, where most of the neurons did not code for the memorandum.

MK's effects on delay firing rate

A key finding of the current study was that MK-induced NMDA receptor blockade improved neuronal selectivity during working memory phases. This improved working memory coding on a neuronal level should on larger scales also improve working memory performance, as the discriminability of memoranda on the behavioural and neuronal level are positively correlated (Funahashi et al., 1989; Constantinidis et al., 2001; Zhou et al., 2013). In partial agreement with our finding, Jackson and colleagues (2004) similarly found that low doses of intra-PFC infused MK improved working memory performance of rodents, whereas high doses decreased working memory performance.

In contrast to our findings, Wang and colleagues (2013) found impaired performance as well as reduced task related firing of delay selective cells of monkeys in an oculomotor delayed response (ODR) task after systemic administration of different doses of the NMDA antagonist ketamine. In the ODR task, monkeys have to make a saccade to a remembered spatial location after a memory delay period. In this ODR task, iontophoretic application of MK mimicked the neuronal effects of systemic ketamine administration by reducing the activity of delay selective cells, however more so for the preferred direction. This effect caused an impairment of stimulus coding during the ODR task, opposed to an enhancement of stimulus selectivity as observed in the current study.

The disparate findings on working memory activity in ODR tasks versus feature-based delayed decision tasks may also relate to anatomically distinct PFC neurons that have been described for spatial and feature-based working memory (Wilson et al., 1993), even though many single neurons represent both spatial and visual information (Rao et al., 1997). An

alternative explanation is that the delay activity in the ODR task might reflect mainly motor preparation signals or allocation of spatial attention rather than maintenance of signals in working memory (Lebedev et al., 2004; Takeda and Funahashi, 2004; Markowitz et al., 2015). This is because the monkeys know from the onset of the sample location where they have to make a saccade to in the subsequent test phase. In our delayed decision task, however, the monkeys lacked information during the delay period that would have allowed them to prepare an action. Thus, the ODR task might entail specific circuits engaged in preparatory motor signalling, which could be differentially modulated by glutamatergic and GABAergic receptors. Such disparate pharmacological findings on working memory activity in ODR tasks versus feature-based delayed response tasks have also been reported for the dopaminergic modulatory system (Vijayraghavan et al., 2016; Ott and Nieder, 2017, 2019).

In the subset of memory neurons, MK improved stimulus selectivity, however the mean and the variability of the firing rate were neither significantly affected in the preferred stimulus condition nor in the non-preferred stimulus condition. The precise mechanisms behind the increase in stimulus selectivity thus remain elusive. Nevertheless, these findings suggest that NMDA receptors were not only open for the preferred stimulus condition, but at least also partially for the non-preferred stimulus condition. We believe that it was an interplay between modest (but in themselves non-significant) changes in firing rates and firing variability that added up to significant changes in selectivity as detected by the ROC analysis (**Fig. 21, 22**).

As stated above, effects of iontophoresis are focal, but they exceed the locus of the electrode's recording tip, potentially causing small network effects (Herz et al., 1969).

Interneurons located in the vicinity of stimulus selective pyramidal neurons and within the same column are thought to code for the same memorandum (Rao et al., 1999; Constantinidis and Goldman-Rakic, 2002; Constantinidis and Wang, 2004). While these interneurons, most probably parvalbumin expressing cells, should preferentially inhibit memory neurons that are oppositely tuned, it has also been found that they contact nearly every neighbouring neuron (Ferguson and Gao, 2018). These interneurons may thus provide small inhibitory effects to pyramidal cells tuned to the same memorandum under physiological conditions. Effects are potentially stronger for the preferred memorandum compared to the non-preferred one, as more NMDA receptors are suspected to be open in the first case. This would also be in line with the observed stronger (positive) effect of Bic on coding strength for preferred memoranda.

Considering glutamate's role as the primary excitatory neurotransmitter, synaptic glutamatergic concentrations are higher during persistent activity for preferred memoranda and NMDA receptors are thus more likely to be in an open state. In our task, it is likely that during the delay epoch, when feature-encoding pyramidal neurons were most active, the NMDA receptors on both these neurons and on nearby interneurons were open. One possible explanation of our data is that interneuron NMDA receptor activation strongly supressed pyramidal neurons coding for a different memorandum and importantly also mildly supressed pyramidal neurons coding for the same memorandum in order to balance overall excitation and inhibition. This interpretation suggests that iontophoretic administration of MK preferentially blocked NMDA receptors on interneurons, providing net disinhibitiory effects on the recorded memory neuron and thereby enhanced

representational coding strength.

Bic is more potent than MK

Overall, effects of MK on firing rate were moderate, particularly when compared to Bic's effects. One possible explanation of this is that a reduction in firing rate is generally harder to capture than an increase in firing rate because the range by which the firing rate can be reduced (to a minimum of zero spikes) is considerably smaller than its potential increase. Considering that the spontaneous firing rate of neurons in the PFC is notoriously low (compared to upstream brain areas), further reducing the firing rate mimics the potential loss of the neuron during recording and heightens the difficulty in capturing firing rate decreases as compared to increases. Second, MK's efficacy depends on the presence of extracellular glutamate and a study with a comparable drug administration protocol to ours did not show any MK effects on spontaneous firing rate, suggesting extracellular glutamate concentrations in the PFC may have been rather low (Wang et al., 2013). Third, within the lateral PFC of adult monkeys, GluN2B subunits of NMDA receptors are exclusively found on dendritic spines in layer III, while they are also found extrasynaptically in other brain areas (Wang et al., 2013; Wang and Arnsten, 2015). NMDA receptors are mainly found on dendrites, whereas a high proportion of GABA(A) receptors targeted by parvalbumin containing interneurons are perisomatically localized (Chiu et al., 2019). It is likely that the limited distribution of NMDA receptors with GluN2B subunits along a neuron also confines the effectiveness of MK on delay cell firing as less open NMDA receptors are in the vicinity of the applied drug.

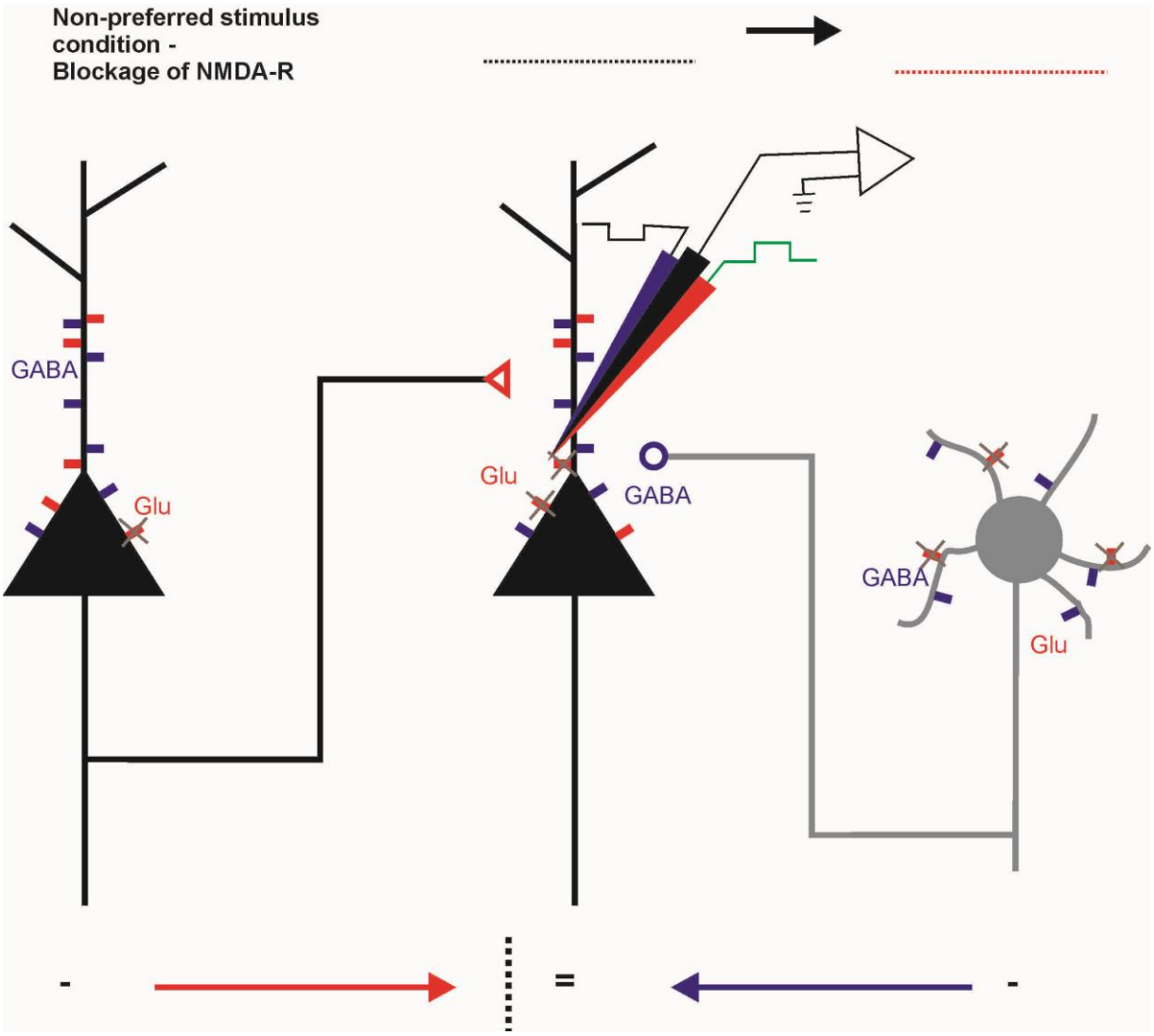

Figure 21. Model of MK effects in the non-preferred stimulus condition. The black line atop represents the firing profile of the recorded neuron in control condition and the red line in MK condition. The firing rate remains unchanged, if at all there is a slight detrimental effect. The electrode-pipette combination is drawn next to the recorded neuron, a pyramidal cell in this case. Blue and red boxes represent NMDA- and GABA(A) receptors, "Glu" and "GABA" signify extracellular glutamate and GABA concentrations respectively. Receptors that are crossed out are assumed to be blocked. For simplification we only consider one neighbouring pyramidal neuron (left) that is tuned alike the recorded neuron and releases glutamate upon activation and one interneuron (right) that is also tuned alike the recorded neuron and provides inhibition mediated by release of GABA upon activation. Symbols at the bottom summarize the supposed drug effects. MK diminishes the firing rate of both neighbouring neurons, indicated by the minus symbols on the left and right. Coloured arrows show whether the neuron has an excitatory (red) or inhibitory (blue) effect on the recorded neuron. The dashed vertical line indicates that the receptors for the released transmitter are partially blocked, reducing the effects of these neurons on the recorded cell. The equal sign in the middle shows that in sum the overall firing rate remains unchanged, because blockage of NMDA receptors on the recorded neuron and the neighbouring pyramidal cell has a net inhibitory effect and blockage

of NMDA receptors on the neighbouring interneuron has a net excitatory effects. Either effects are thought to be small because transmitter concentrations are low.

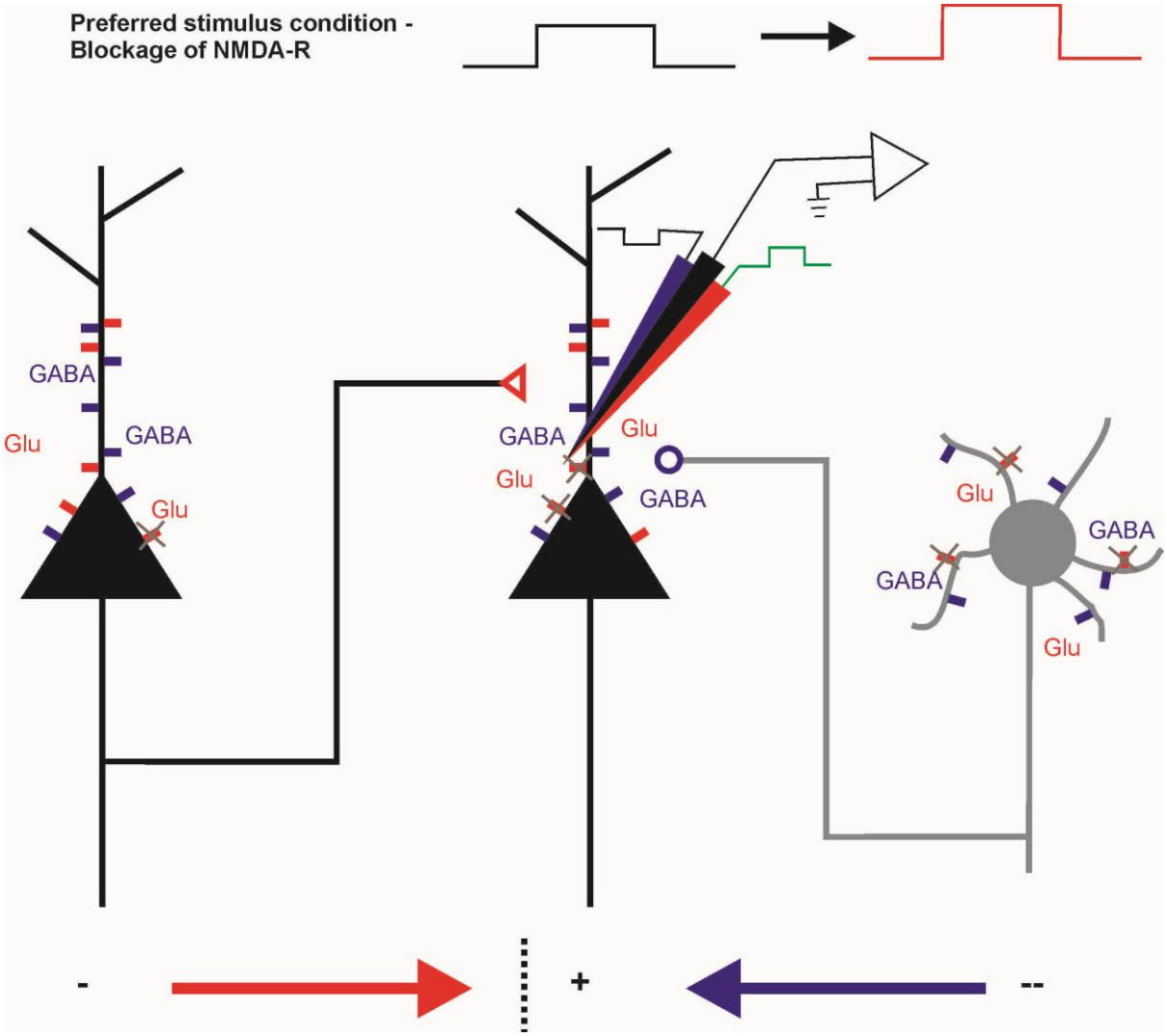

Figure 22. Model of MK effects in the preferred stimulus condition. The black line atop represents the firing profile of the recorded neuron in control condition and the red line in MK condition. The firing rate is slightly increased with drug administration. Symbols at the bottom summarize the supposed drug effects. MK diminishes the firing rate of both neighbouring neurons but has a stronger effect on the interneuron, indicated by two minus symbols. The plus sign in the middle shows that in sum the overall firing rate is increased because inhibitory inputs are strongly decreased. For conventions of colour and symbol usage refer to **Figure 21**.

Blocking GABA(A) receptors increases neuronal selectivity preferentially by disinhibiting preferred stimuli

Bic's effects on spontaneous firing rate

Consistent with prior studies, iontophoretic administration of the GABA(A) antagonist Bic generally increased the spontaneous- and task-related firing rates of both pyramidal cells and interneurons in lateral PFC (Rao et al., 2000; Sawaguchi, 2001). With regard to the spontaneous firing rate our results mirror earlier findings and are well in line with the notion that GABA(A) receptors commonly downregulate a neuron's spike rate by hyperpolarizing its membrane potential (Rao et al., 1999, 2000).

Bic's effects on delay firing rate

A major finding of the current study was that Bic-mediated blockade of GABA(A) receptors improved neuronal selectivity during working memory periods. Specifically, in the subset of memory neurons Bic tended to increase the firing rate stronger for the preferred than the non-preferred stimulus condition (**Fig. 23, 24**). As elaborated for MK, expanding the improved local selectivity of memory neurons, caused by administration of Bic, to many more PFC neurons should also improve working memory performance. This supportive effect of blocking GABA receptors on memory is in partial agreement with behavioural studies in rodents that found improved memory retention and consolidation after administration of GABA antagonists (Luft et al., 2004; Kim et al., 2012). In our study the blockade of GABA(A) receptors by Bic tended to increase the firing rate stronger for the

preferred than the non-preferred stimulus condition and thereby improved selectivity. The variability of the firing rate was not affected by Bic. This suggests that GABA downregulates memory processing on the neuronal level by diminishing coding of preferred stimuli and precisely blocking GABA receptors in space and time may improve stimulus processing (Bast et al., 2017).

Contrary to our findings, Rao and colleagues (2000) found that iontophoretic administration of Bic in PFC of macaque monkeys performing an ODR task diminishes spatial tuning of pyramidal cells and interneurons, especially in the delay phase. In their study, Bic impaired tuning by disinhibition of the non-preferred direction as well as directions neighbouring the preferred one, but also created tuning in a subset of previously untuned cells. Rao and colleagues (2000) proposed that this was due to an unmasking effect disinhibiting neuronal responses to stimuli adjacent to the preferred stimulus that were previously undetectable because of lateral inhibition. In accordance to this Sawaguchi (2001) found that iontophoretic administration of Bic unmasked task-related activity of previously silent PFC neurons.

This effect may have contributed to the improved selectivity following drug administration in our study. We selected stimulus selective neurons with a two-way ANOVA with stimulus condition as the main factor, pooling spike data for drug and control condition. This way some of our stimulus selective neurons passed the analysis because the neuron exhibited a strong selectivity in the drug condition, but were only weakly tuned to a stimulus condition during control trials. Additionally, in these cases the exact locus of the analysis window would mainly be determined by data in the drug condition and might not capture the

supposable optimal analysis window in the control condition. These neurons may not have been regarded as stimulus selective if we would have been using a fixed window for our ANOVA, like Rao and colleagues (2000). Indeed, of the 83 memory neurons recorded in Bic and control conditions, 58 were stimulus selective in the control condition when using a fixed analysis window over the whole delay with an offset of 100 ms. Likewise, 55 of the 72 memory neurons that were recorded in MK and control conditions were stimulus selective in control condition with the same fixed analysis window.

As discussed previously, the disparate findings in the study by Rao et al. (2000) compared to our study may also be based on differences in spatial versus feature-based task protocols, as well as different types of cognitive signals (premotor versus pure working memory aspects) activated during the delay periods. In addition, GABA most likely affects working memory in a dose-dependent manner, and possibly follows an inverted-U response curve. This idea is supported by the findings that too much as well as too little GABA impaired optimal behavioural performance (Pezze et al., 2014; Bast et al., 2017; Ferguson and Gao, 2018). Finally, one has to be aware that Bic also blocks calcium dependent potassium channels and may thus cause non GABAergic side effects, among them potentiated burst firing (Johansson et al., 2001).

The neuron's resting state is not dominated by GABAergic inhibition

As blocking GABA(A) receptors is more effective in the preferred stimulus condition, this suggests that GABA(A) receptors are more active in the preferred than in the non-preferred

stimulus condition under physiological conditions. In turn, this brings up the question how a low firing rate for non-preferred or irrelevant stimuli may be achieved apart from GABAergic inhibition and why GABA receptors are more active when the neuron is persistently spiking. Possibly the low firing rate during the resting state of the neuron is mainly achieved by NMDA receptors. Although seemingly counterintuitive, it has been proposed that the voltage dependency of NMDA receptors is well suited for maintaining a resting state (Lisman et al., 1998; Compte et al., 2000; Brunel and Wang, 2001). The Mg^{2+} block provides a physiological barrier to keep NMDA receptors in an inactive state, mediating an overall low firing rate of the neuron. Additionally, inhibitory and excitatory effects are said to both be lifted or lowered for the active and resting state of the neuron respectively, keeping an overall balance of excitatory and inhibitory currents (Compte et al., 2000). The elevated activation of GABA(A) receptors during sustained activity could thus serve mediating an overall balance of excitation and inhibition on the network level, eventually terminating sustained activation and preventing runaway activity (Isaacson and Scanziani, 2011; Chiu et al., 2019).

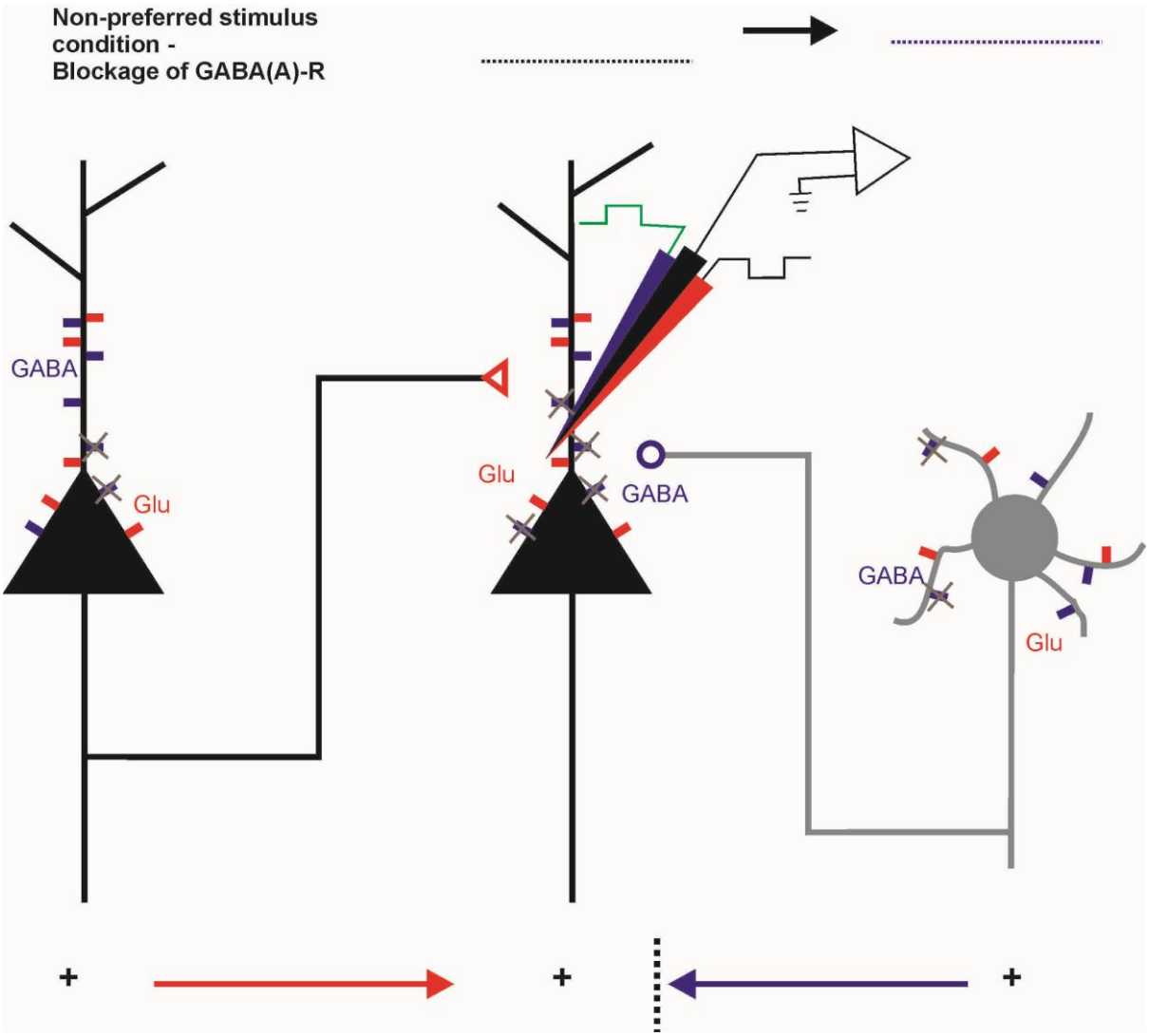

Figure 23. Model of Bic effects in the non-preferred stimulus condition. The black line atop represents the firing profile of the recorded neuron in control condition and the blue line in Bic condition. The firing rate is increased with drug administration. Symbols at the bottom summarize the supposed drug effects. Bic increases the firing rate of both neighbouring neurons. The plus sign in the middle shows that in sum the overall firing rate is increased. For conventions of colour and symbol usage refer to **Figure 21.**

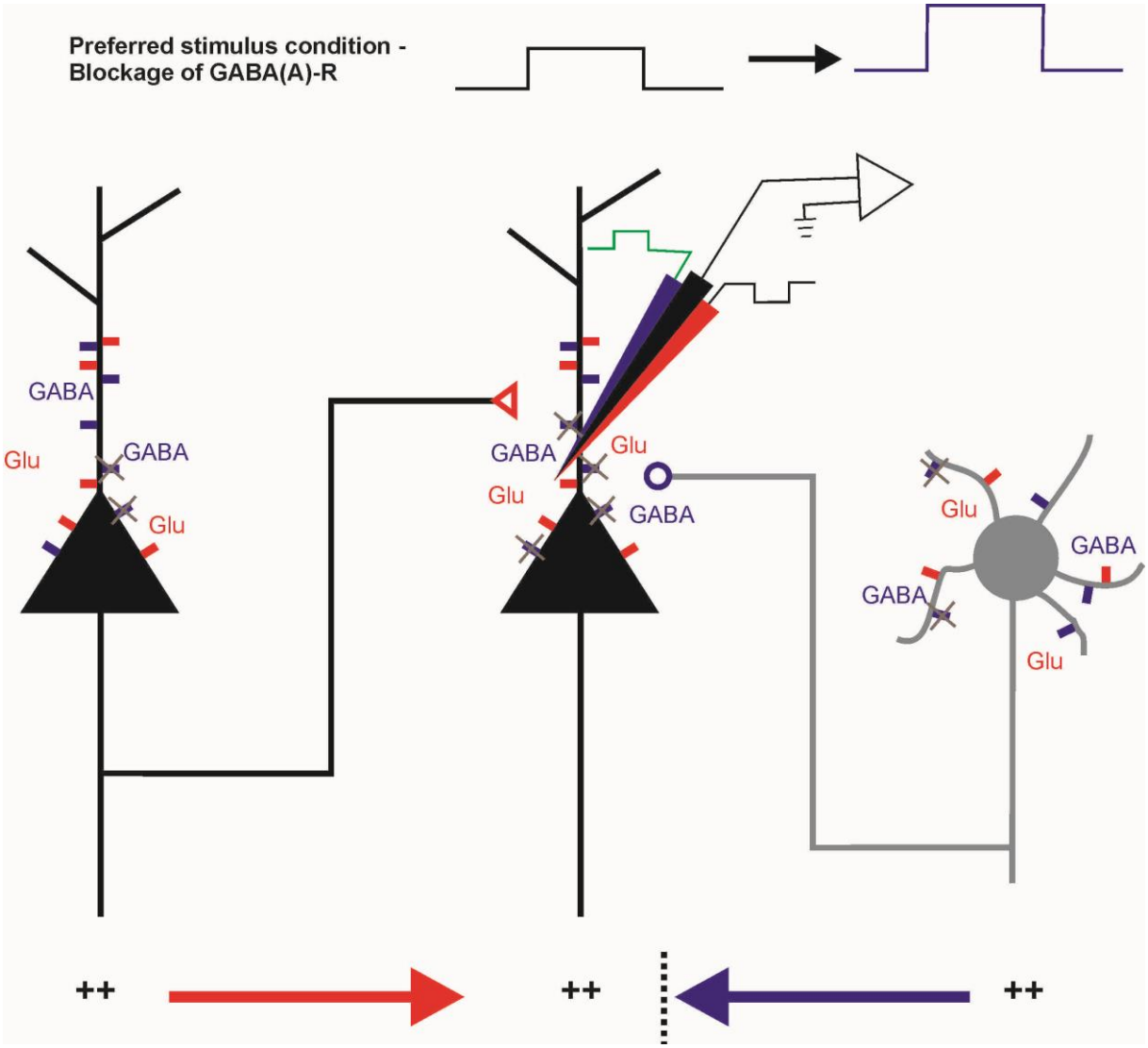

Figure 24. Model of Bic effects in the preferred stimulus condition. The black line atop represents the firing profile of the recorded neuron in control condition and the blue line in Bic condition. The firing rate is strongly increased with drug administration. Symbols at the bottom summarize the supposed drug effects. Bic increases the firing rate of both neighbouring neurons, but effects are stronger than in the non-preferred stimulus condition (**Figure 23**) because extracellular GABA concentrations are higher. The two plus signs in the middle show that in sum the overall firing rate is strongly increased. For conventions of colour and symbol usage refer to **Figure 21**.

NMDA- and GABA(A) receptors reside on the same neurons

In previous iontophoretic studies with monkeys, only one pharmacological substance was explored per neuron. We were therefore interested to find out if and how individual

neurons would react to application of both NMDA and GABA antagonists. We found that most neurons which improved their signal to noise ratio with one of the drugs also increased their selectivity with the other. This was true for both putative pyramidal cells and inhibitory interneurons (as classified on the basis of spike-width). Rather than finding one population of neurons increasing their selectivity with excitatory neurotransmitter antagonist, and another population with the inhibitory neurotransmitter antagonist, we found that these neuronal populations strongly overlapped, so that more than half of our recorded neurons increase their selectivity with either drug. In agreement with this, neuronal co-expression and reciprocal modulation of NMDA- and GABA(A) receptors has been found regularly (Hickmott and Constantine-Paton, 1993; Pettit and Augustine, 2000; Homayoun and Moghaddam, 2007; Carlén et al., 2012). Furthermore, NMDA receptors have been found at GABAergic synapses and GABA(A) receptors have been found at glutamatergic synapses (Nusser et al., 1996, 1998; Gundersen et al., 2004; Luján et al., 2005).

The drugs seemingly affected both broad-spiking, putatively pyramidal neurons, and narrow-spiking, putatively interneurons, similarly. Both neuron types increased their firing rate after administration of Bic, BS tended to decrease their spontaneous firing rate after administration of MK, NS were not affected by MK application. This similarity of BS and NS is to be expected when the input of a recorded neuron is affected. NMDA receptors commonly have an excitatory effect on both pyramidal cells and interneurons, whereas GABA receptors commonly have an inhibitory effect on both cell classes. However, the net output effect of both cell types on the postsynaptic neurons is expected to be in the opposite direction.

Given that iontophoretically applied drugs diffuse beyond the particular neuron(s) at the recording site, the found detrimental and supportive effects of MK-mediated NMDA receptor blockade likely reflect both the primary effect of the drugs binding to the recorded neurons and also secondary effects mediated via the local microcircuit. This could explain some of the variance found at the single-neuron level regarding effects of MK administration across studies and emphasizes the importance of larger neuron population analyses. While we mainly attribute the observed increase in working memory selectivity after iontophoretic administration of MK to network effects, the decreased spontaneous firing rate after MK-application, as well as the increased spontaneous firing rate and working memory selectivity following Bic-administration should largely be caused by blockage of receptors at the recorded neurons. This leads to the conclusion that both major classes of cortical neurons in the PFC contained both glutamatergic NMDA and GABA receptors.

Differentiating NMDA and GABA effects on firing rate is not straight-forward because glutamatergic transmission is controlled by GABA receptors and vice versa GABAergic transmission is controlled by NMDA receptors (Del Arco and Mora, 2002; Higley, 2014; Farahmandfar et al., 2017; Chiu et al., 2019). Because of this delicate interplay of excitation and inhibition in cortical networks, neuropsychiatric conditions like schizophrenia have also been associated with impairments in the NMDA system as well as the GABA system (Benes, 1995; Gonzalez-Burgos and Lewis, 2012; Datta and Arnsten, 2018). This suggests that a better understanding of these transmitter systems, also with respect to potential psychiatric therapies, requires an investigation of glutamatergic and GABAergic effects back-to-back.

An updated working memory model

Recurrent excitation may not be the only mechanism for maintaining information about a ceased stimulus online in working memory, but it is an important component nevertheless. Our findings have some implications on the supposed cellular mechanisms underlying recurrent excitation. The conflicting findings and dose dependent effects of Bic and MK suggest that optimal GABA and NMDA levels may follow an inverted U function where too little and too much may impair memory performance, but small changes may have an optimizing effect (Krystal et al., 2017).

Apart from the specifics of the respective drugs our findings have inferences on models of recurrent excitation underlying working memory. It seems reasonable to assume that PFC memory neurons exhibit a spontaneous resting state when a non-preferred or irrelevant stimulus was presented and a state of sustained activity when coding for the preferred memorandum. The low firing rate of pyramidal cells and interneurons during their spontaneous resting state seems to be mainly mediated by NMDA receptors that are inactive, potentially indicated by the ineffectiveness of MK in the non-preferred stimulus condition and Bic being less effective when compared to the preferred stimulus condition. NMDA receptors perhaps are inactive because the extracellular glutamate concentrations are too low to activate enough AMPA receptors that elevate the membrane potential to a level that allows removal of the Mg^{2+} block of the NMDA receptors. GABA receptors in turn are not very active either in the spontaneous resting state, mediating balance of excitatory and inhibitory currents, as indicated by Bic being less effective in the non-preferred stimulus condition than in the preferred one. Extracellular GABA is however not completely

diminished as interneurons from columns that prefer the respective memorandum release GABA at their axon terminals. In the active memory state NMDA receptors are very active, providing sustained activity due to their receptor kinetics, potentially indicated by seemingly stronger effects of MK in the preferred stimulus condition compared to the non-preferred one. GABA receptors are also more active, again providing a balance of excitation and inhibition and preventing an undamped recurrent excitation, as indicated by Bic being more effective in the preferred than in the non-preferred stimulus condition (Isaacson and Scanziani, 2011; Chiu et al., 2019). Extracellular GABA levels are elevated because nearby interneurons that mainly supress neurons from other columns coding for a different memorandum also synapse onto nearly every neuron in their vicinity.

Taken together, our findings hint at the importance of NMDA receptors not only for establishing sustained activity but also for stabilizing a spontaneous resting state. As inhibitory currents are stronger during persistent firing than in the resting state, it seems that the activity of GABA receptors more or less scales with the activity of NMDA receptors, highlighting the role of GABA in mediating balance of excitatory and inhibitory currents (Murray et al., 2017). Indeed, it seems that GABAergic inhibition is functionally coupled to glutamatergic excitation and action potential generation, mediating balance on short and long time scales (Chiu et al., 2019). It is thus the complex and delicate interplay of excitation and inhibition within microcircuits that underlies working memory.

As our findings are in partial conflict with earlier studies, more research is needed to further elucidate the cellular mechanisms behind persistent activity. Drugs acting on specific receptors should be administered locally at different dosages in monkey PFC to test the

proposed dose dependency. Whether MK preferentially blocks NMDA receptors on interneurons and whether this preference is dose dependent also remains unclear. Together with further investigations regarding the distribution and properties of the respective receptors in monkey PFC, it should be possible to get a better understanding of the mechanisms underlying sustained activity, working memory, cognitive deficits and their potential medical treatment.

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List of figures

List of abbreviations

AC: auditory cortex

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANOVA: analysis of variance

AUROC: area under the ROC curve

Bic: Bicuculline methiodide

BOLD: Blood-oxygen-level-dependent

BS: broad-spiking cell

Ca^{2+}: calcium ion

Cl^-: chloride ion

cm: centimetre

Cntl: control

ERC: enthorinal cortex

EVC: early visual cortex

FEF: frontal eye fields

FG: fusiform gyrus

GABA: γ-Aminobutyric acid

Glu: glutamate

HCl: hydrogen chloride

HCO_3^-: bicarbonate ion

hMT+: human analog to MT/MST

Hz: Hertz

i.e.: id est / that is to say

IPS: intraparietal sulcus

IT: inferior temporal cortex

Kg: kilogram

K^+: potassium ion

HPLC: high performance liquid chromatography

l: liter

LOC: lateral occipital complex

lPFC: lateral prefrontal cortex

M: mean

Mg^{2+}: magnesium ion

min: minutes

MK: MK-801 / dizocilpine

ms: milliseconds

mV: millivolt

MΩ: megohm

µs: microseconds

nA: nanoampere

Na^+: sodium ion

NMDA: N-Methyl-D-aspartate

NS: narrow-spiking cell

ODR: oculomotor delayed response task

PFC: prefrontal cortex

PM: premotor cortex

PPC: posterior parietal cortex

ROC: receiver operating characteristic

s: seconds

SE: standard error of the mean