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Nucleus Basalis Stimulation Stabilizes Attractor Networks and Enhances Task Representation in Prefrontal Cortex

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Abbreviated Title: Nucleus Basalis stimulation enhances PFC

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ABSTRACT

The action of acetylcholine in the neocortex is critical for executive function. Cholinergic drugs can improve cognitive function in patient populations and normal adults. How endogenous cholinergic action affects neuronal activity in higher cortical areas is unknown. Here we tested the effects of electrical stimulation of the cortical source of acetylcholine in primates, the Nucleus Basalis of Meynert, on neural activity while monkeys performed working memory tasks. Stimulation delivered in an intermittent fashion improved behavioral performance and increased neuronal activity during the delay period of the task but not to the phasic responses of visual stimuli. Selectivity of neuronal responses broadened, rendering the bump of activity in an attractor network more stable, and filtering distracting stimuli more effectively. These neural results show that acetylcholine has effects on neural activity and selectivity in the prefrontal cortex opposing those of dopamine and fine tuning aggregate neural ensemble activity based on neuromodulatory tone.

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INTRODUCTION

The forebrain cholinergic system tightly regulates higher cognitive function (Sarter and Bruno, 1997; Bartus, 2000). Losses in cognitive function with aging and Alzheimer's disease (AD) occur in parallel with degeneration of the brain's cholinergic systems, and cholinergic deficits correlate well with the degree of cognitive decline (Terry and Buccafusco, 2003). Cholinesterase inhibitors, which prolong the neurotransmitter's ability to stimulate post-synaptic receptors and amplify the natural pattern of acetylcholine release, are frontline medications for treating Alzheimer's disease (Sabbagh and Cummings, 2011). Improvement in cognitive functions can also be achieved by stimulation of the Nucleus Basalis (NB) of Meynert, the sole source of neocortical acetylcholine in primates and humans (Mesulam et al., 1983; Hendry et al., 1987). Recent studies in primates suggest that intermittent NB stimulation is equally or more effective in improving cognitive performance task as high doses of acetylcholinesterase inhibitors, with further effects that aggregate over time (Blake et al., 2017; Liu et al., 2017, 2018).

The effects of cholinergic activation on the activity of neurons implicated in working memory and executive function have been less understood. Phasic electrical stimulation of cholinergic forebrain neurons has been used extensively in sensory cortex studies to transiently boost acetylcholine levels in studies of neuroplasticity (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998). More recent, optogenetic phasic stimulation has been used to test the effects of acetylcholine on a perceptual task (Pinto et al., 2013). Muscarinic and nicotinic cholinergic antagonists have been shown to decrease firing rate specifically during the delay periods of working memory tasks in the prefrontal cortex of nonhuman primates; nicotinic agonists enhance it (Zhou et al., 2011; Yang et al., 2013).

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We were therefore motivated to examine the effects of intermittent NB stimulation on neuronal activity during execution of a working memory task. We focused specifically on the prefrontal cortex, an area critical for working memory (Riley and Constantinidis, 2016), which receives innervation from a dedicated sub-region of the Nucleus Basalis (Gielow and Zaborszky, 2017). We implanted monkeys with NB stimulating electrodes and determined the effects of stimulation on performance and neural activity.

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RESULTS

We recorded behavioral performance and neural activity from the dorsolateral prefrontal cortex in two monkeys implanted unilaterally with electrodes that targeted the Nucleus Basalis of Meynert (Fig. 1A-B). Electrode placement was guided by MR imaging and verified with CT scanning after implantation (see Methods). Electrode location was finally visualized with ChAT immunohistochemistry, post mortem. One monkey was implanted in the left, and one in the right hemisphere. To obtain functional confirmation of the targeting, we collected LFP recordings from the implanted electrode at rest, with and without stimulation (Bjordahl et al., 1998). Continuous stimulation at 80 Hz produced LFP desynchronization (Fig. 1C and S1). Power in the 5-15 Hz range was significantly lower during stimulation than control (paired t-test, t_{17}=3.14, p=0.006 and t_{33}=2.5, p=0.02 for the two subjects, respectively).

Stimulation effects on behavioral performance

The monkeys performed a working memory task that required them to remember either the first or second of two sequential stimuli, as instructed by the color of the fixation point – white or blue, respectively, and perform an eye movement towards the remembered stimulus (Fig. 1D). Daily sessions without stimulation were interleaved with sessions during which intermittent electrical stimulation of the Nucleus Basalis was performed. Stimulation was applied during the inter-trial interval of the task for 15 s, at a frequency of 80 pulses per s (Fig. S2). Then the monkeys performed the task for 45 s (typically 4-5 completed trials), without stimulation. At the end of the trial that exceed the 45 s threshold, stimulation was applied anew and the cycle was repeated. This pattern of stimulation was elected based on recent results demonstrating the

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intermittent stimulation improves working memory and attention performance (Blake et al., 2017; Liu et al., 2017, 2018).

Behavioral performance in our task generally improved with intermittent stimulation (Fig. 1E-H). The effects of stimulation were generally greater for Subject GR. We also observed however, some instances when stimulation produced some lower overall performance compared to control e.g., for Monkey HE in the remember-second task (Fig. 1G). It is important to emphasize that stimulation was unilateral, and the task required selective maintenance of some stimuli in memory and filtering of others, that may appear at ipsilateral or contralateral locations. When we considered performance for two stimuli appearing at different locations with respect to the hemifield of the stimulus to be held in memory relative to the hemisphere of the implanted electrode, a clearer picture emerged. For monkey GR, stimulation improved performance for all conditions. A 3-way ANOVA on performance with factors stimulation (on or off), task (remember-first or remember-second) and location of stimuli (contralateral or ipsilateral stimulus to be remembered) revealed a significant main effect of stimulation ($F_{1,128}$=19.6, p=2.06x10^{-5}). The effect of task was also significant for this animal ($F_{1,128}$=12.9, p=4.66x10^{-4}**)**. The effect of stimulus location as well as interaction terms failed to reach significance. For monkey HE, this analysis revealed that stimulation improved performance specifically when the stimulus to be remembered was at a location contralateral to the site of the stimulation electrode (Fig. 1E, H – congruent conditions). Stimulation was ineffective when the stimulus was ipsilateral to the stimulation (Fig. 1F, G – incongruent conditions). Performing the same 3-way ANOVA analysis revealed no net effect of stimulation, precisely because of this opposing effects in the two hemifields ($F_{1,204}$=0.27, p=0.6**)**, but now a significant three way interaction between task, stimulation, and side of stimulus ($F_{1,128}$=6.04, p=0.015). Considering the congruent conditions

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alone (Fig. 1E,H), the effect of stimulation was highly significant (3-way ANOVA with factors monkey, and stimulation: $F_{1,173} = 14.14$, p= 0.0002 for remember-first task; $F_{1,173}= 5.61$ p=0.02 for remember-second task). The results of this analysis demonstrated that stimulation improved performance in the task, particularly for stimuli to be remembered appearing contralateral to the site of stimulation.

Effects on neural activity

We recorded from a total of 246 neurons (102 and 144 in the two monkeys, respectively) in areas 8 and 46 of the dorsolateral prefrontal cortex, as the monkeys performed the working memory task of Fig. 1D with and without Nucleus Basalis stimulation. Recording cylinders were implanted on the same side as the stimulation electrode. Blocks of 20 correct trials without stimulation were interleaved with blocks involving intermittent stimulation. Stimulation was always delivered in the inter-trial interval, in the same fashion as described above. Of those neurons, 109 (67 and 42 from the subjects, GR and HE respectively) responded to visual stimuli (evaluated with a paired t-test, at the $p<0.05$ significance level during the stimulus presentation or delay period) and had sufficient numbers of trials for comparisons between conditions. Most analyses that follow were based on these neurons; data from all neurons are shown in the supplementary material.

Nucleus Basalis stimulation had a predominantly excitatory effect (Fig. 2A). The distribution of firing rate differences computed in blocks of trials with or without 15 s of stimulation between them deviated significantly from a normal distribution (KS test for normalized rate differences, compared to normal distribution, $p=8.74\times10^{-6}$). For a total of 54 neurons, firing rate was significantly higher after stimulation (evaluated with a t-test at the

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$p<0.05$ level). An example is shown in Fig. 2B-E. However, stimulation also produced a significant decrease in firing rate in the fixation period for 16 neurons. To facilitate analysis of these opposing effects, we grouped neurons into those with a significant increase in rate, and those without an increase (which included neurons with significant decreases, and no significant effect). We separately analyzed responses of neurons in these two groups.

For the neurons with an overall increase in activation, stimulation had no effect during the inter-trial interval (Fig. 3A, 2-way ANOVA with factors tasks and stimulation: main effect of stimulation F_1 =2.6, p=0.113). After the fixation point turned on, whose color signified the remember-first or remember-second rule, stimulation increased firing rate (Fig. 3A and Fig. S2, paired t-test, 2-way ANOVA with factors tasks and stimulation: main effect of stimulation F_1 =8.186, p=0.006). In blocks of stimulation trials, the effects were stable over the time course of ~1 min between cycles of repeated stimulation (see Fig. S2). The firing rate was elevated in the first trial following stimulation and no further increase was present in successive trials (Fig. 3H, red line). No systematic effects were present, either, in the sequential trials after an intertrial interval that did not contain stimulation, in blocks of trials when no stimulation was present (Fig. 3H, blue line).

The phasic response to the best stimulus itself was largely unchanged between the control and stimulation conditions (Fig. 3A, 2-way ANOVA with factors tasks and stimulation: main effect of stimulation F_1 =0.397; p=0.53). The absence of an enhancement to the stimulus response (also visible in Fig. 3C-D) was evident in both the remember-first and remember-second tasks (Fig. S4, S5), and for both the first and second presentation of a stimulus in the receptive field (third and fourth plot in Fig. 3A). On the other hand, the shift in firing rate

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baseline in trials with stimulation persisted during the delay periods after each of the stimuli and in the saccade period.

Although it did not improve responses for the best stimulus location, NB stimulation enhanced responses to stimuli at non-optimal locations, which resulted in an apparent broadening of receptive fields during the cue presentation and delay period (Fig. 3B-E). Stimuli that appeared away from the peak of the response in the receptive field and elicited little or no response without stimulation generated a much stronger response under stimulation. Such examples in the remember-first task are the second stimulus in Fig. 3B and the first stimulus in Fig. S4G, H, I, J. The broadening of the receptive fields in the cue and delay periods was also evident in the remember-second task (see first stimulus in Fig. S5G, H, I, J). In order to quantify differences in responsiveness to sub-optimal stimuli, we relied on a selectivity index (SI) defined as (Max-Min)/(Max+Min) where Max and Min represent the firing rate to the best and worst stimulus location for each neuron. The NB stimulation condition produced a significantly lower SI value (2-way ANOVA with factors tasks and stimulation: main effect of stimulation $F_{1,53}$ = 25.4, $p = 5.7\text{x}10^{-6}$ for cue period, and $F_{1,53} = 24.2$, $p = 8.7\text{x}10^{-6}$ for delay period (Fig. S6). A time-resolved Receiver Operating Characteristic analysis revealed that the difference between best and worst stimulus responses declined with stimulation, for all task conditions (Fig. S7).

The decrease in neuronal spatial selectivity we observed under stimulation can be conceptualized as broadening of the bump of activity in the population of prefrontal neurons, which behaves as an attractor network during working memory (Wimmer et al., 2014). This model makes interesting predictions for behavioral performance under different combinations of remembered stimulus and distractor. Whereas a narrow bump in the control network may occasionally be interrupted by appearance of a distractor at a distant location (Fig. 4A), a wider

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bump under stimulation will generally be more stable due to activation of a larger number of neurons, and therefore more resistant to the activation induced by a subsequent distractor (Fig. 4B). An exception to this pattern of behavioral enhanced under stimulation involves stimuli placed near the peak of the initial bump (Fig. 4C). Under such a scenario, it is more likely that the bumps of activity corresponding to the stimuli will "merge", resulting in more errors at the end of the delay period. We therefore reanalyzed the pattern of behavioral responses shown in Fig. 1E-H, based on the distance between the initial and second stimulus (Figure 4D-E). In the remember-first condition, stimulation improved performance in the conditions involving distance distractors (Fig. 4D), however stimulation markedly *decreased* performance for stimuli appearing at adjacent locations (two-tailed t-test, t_{66}=2.83, p=0.006). Importantly, this pattern of responses was observed only for the remember-first condition. In the remember-second condition, stimulation improved performance for a second stimulus appearing at a close distance to the initial distractors (45° condition in Fig. 4E), as in this task it is beneficial to "pull" the initial bump of activity towards the second stimulus (Fig. 4C).

NB stimulation in the remember-second task yielded another unexpected finding: responses in anticipation of a stimulus, even when no stimulus was presented at all (e.g. first stimulus in Fig. 3F, G). Our behavioral task involved a fixed duration of the fixation interval that the monkey could time. A stimulus was presented after this interval, however in 20% of the trials no stimulus was presented and the trial continued with the presentation of the "second" stimulus at the time that was expected. We refer to that as the "null" condition. NB stimulation elicited elevated firing rate in the time interval that the first stimulus would have been expected in null trials (2-way ANOVA with factors tasks and stimulation: main effect of stimulation F_1=26.9, p=3.4x10^{-6}). Such an anticipatory signal was absent from the control trials, although presumably

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the monkey was anticipating a stimulus in these trials too. Appearance of such "phantom" bumps of activity in the absence of a real stimulus is also a consequence of a more stable attractor network.

Unlike the decrease in selectivity for stimulus location in prefrontal neuronal activity, the representation of task information (remember-first vs. remember-second) was not compromised by stimulation. We quantified this change by relying on a 2-way ANOVA analysis, plotting the p-value for the main effect of task, and, for comparison, the main effect for first and second stimulus location at each time point, for each neuron (Fig. S8). The consequence of the preservation of task information under stimulation was also evident when we compared conditions that were congruent or incongruent in terms of the significance of stimuli appearing in the receptive field, in the context of the task. The effect of stimulation in the delay period firing rate following the first stimulus was greater in the remember-first task, which required the stimulus to be actively remembered (Fig. S9A, paired t-test, t_{53}=1.67, p=0.025). This difference between stimulation and control conditions was diminished in the context of the remember-second task, when the first stimulus did not need to be remembered, and did not reach statistical significance (Fig. S9B). The task-effect of stimulation was even more pronounced in the second delay period, following a second stimulus in the receptive field. When the monkeys did not need to remember it for the purposes of the remember-first task (Fig. S9C) stimulation was not-significantly different than control (paired t-test, t_{53}=0.59, p=0.554). When they did need to remember the second stimulus for the purposes of the remember-second task (Fig. S9D), stimulation was significantly greater (paired t-test, t_{53}=4.0, p=1.8x10^{-4}).

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Stimulation effects beyond increased firing rate

We also examined effects of stimulation beyond the dominant pattern of increase in firing rate. Neurons that responded to stimuli but for which stimulation produced decreased activity were characterized by suppressed firing rate for both the remember-first (Fig. S10A-B) and remember-second tasks (Fig. S10C-D). Firing rate was reduced in the fixation period, but also in the stimulus presentation period and the delay period that followed it (Fig. S10A, C). Firing rate remained at low levels when the stimuli to be remembered were presented out of the receptive field (Fig. S10B, D).

Among neurons that did not respond significantly to visual stimuli a general increase in firing rate was observed during NB stimulation, similar to the effects of stimulation on task-responsive neurons. This was evident in both the remember-first and remember-second tasks, and throughout the duration of the trial (Fig. S11).

Although we emphasize firing rate differences that could account for the behavioral improvements in performance we observed under stimulation, alternative mechanisms of working memory have also been proposed, some identifying power in the gamma band of LFP as the critical variable predictive of maintenance (Constantinidis et al., 2018; Lundqvist et al., 2018). We therefore examined the LFP potentials recorded from the prefrontal cortex. Stimulation during task execution generally lowered power in the alpha range and increased power in the beta-frequency band (Fig. S12).

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DISCUSSION

Our study demonstrated that intermittent NB stimulation improves performance of monkeys in a working memory task that requires selective maintenance of a stimulus in memory, in agreement with recent studies that showed similar effects for other memory and attention tasks (Blake et al., 2017; Liu et al., 2017, 2018). We additionally show that the effect was associated with changes in the firing rate of neurons in the dorsolateral prefrontal cortex, most often increasing it. This increase was specific for task intervals, including the interval after the appearance of the fixation point, which signified the rule in our task and during the delay intervals over which a stimulus was needed to be maintained in memory. Stimulation also brought about changes in stimulus selectivity, suggestive of a broader peak of activation and more stable attractor network. Our results demonstrate the neural mechanisms through which NB stimulation affects neural activity and improves of cognitive performance.

Behavioral Effects of Stimulation

We have recently demonstrated that NB stimulation can improve cognitive performance in healthy adult monkeys, but only when administered in an intermittent fashion. Optimal stimulation parameters involve stimulation for 15-20 seconds per minute, and at a rate that delivers approximately a total of 1200 pulses of stimulation per minute (Liu et al., 2017). The current results expand the list of cognitive tasks that benefit from this protocol of stimulation. The behavioral effects of stimulation we report are also consistent with the known impairment of working memory caused by acetylcholine depletion in the prefrontal cortex (Croxson et al., 2011). Recent work established that the behavioral improvement induced by NB stimulation depends on acetylcholine release as cholinergic inhibitors abolish the performance benefits (Liu

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et al., 2018). This is not to say that non-cholinergic projections do not play a role; it is well understood that GABAergic ascending projections are critical (Walker et al., 1989; Kim et al., 2015) and our protocol of stimulation is likely to activate them, in contrast to systemic cholinergic drug administration.

Neural effects of NB Stimulation

The effects of NB stimulation we uncovered were generally consistent with the neural changes effected by systemic or microintophoretic administration of cholinergic agents in the prefrontal cortex of primates. Systemic administration of the muscarinic antagonist scopolamine generally depressed prefrontal firing rate during the baseline, had little effect on peak stimulus responses, and depressed delay period activity (Zhou et al., 2011), effects essentially opposite to those of stimulation we report here. Micro-iontophoresis of muscarinic and nicotinic-α7 inhibitors also depress prefrontal activity, particularly in the delay period of working memory tasks (Yang et al., 2013; Major et al., 2015), whereas cholinergic agonists increase activity in the prefrontal cortex (Yang et al., 2013; Sun et al., 2017). We should note that the effects of cholinergic agents in sensory areas are markedly different than those to the prefrontal cortex. Agonist administration in the primary visual cortex specifically enhances responses during stimulus presentation, and particularly attended over unattended ones (Herrero et al., 2008).

Our results stand in contrast to the effects of dopamine agonists, which “sculpt” neuronal activity to improve spatial selectivity (Williams and Goldman-Rakic, 1995). We saw the opposite effect by NB stimulation. The apparent size of neuronal receptive fields expanded, and spatial selectivity decreased during stimulation. This effect is reminiscent of cholinergic

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overstimulation with high doses of carbachol and M1R allosteric inhibitors administered iontophertically, which reduce prefrontal selectivity in the context of working memory tasks (Major et al., 2018; Vijayraghavan et al., 2018). Computational models (Compte et al., 2000; Wimmer et al., 2014) predict that activation of a larger population of neurons by a single stimulus resulting in a broader bump of activity render the network more resistant to distracting stimuli, although performance may be compromised in conditions involving distracting stimuli appearing in nearby locations. This was precisely the pattern of behavioral changes we observed (Fig. 4). We also observed prefrontal responses in anticipation of stimuli that did not appear, which is consistent with more stable attractors in which spurious activation may sometimes create "phantom" bumps. Neuronal responses in the Nucleus Basalis often signal novelty or surprise (Zhang et al., 2019), and in view of our results, such endogenous NB stimulation would have the effect of preferentially stabilizing activity elicited by unexpected stimuli.

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METHODS

Two male, rhesus monkeys (*Macaca mulatta*) weighing 7-10 kg were used in this study. The monkeys were trained to perform working memory tasks and baseline neurophysiological recordings were obtained from the prefrontal and posterior parietal cortex (Qi and Constantinidis, 2015; Qi et al., 2015). All experimental procedures followed guidelines by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Research Council's Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the Wake Forest University Institutional Animal Care and Use Committee.

Surgery and neurophysiology. A 20-mm-diameter recording cylinder was implanted over the dlPFC (Fig. 1). A second cylinder was also implanted over the PPC of each monkey at the same time, but this was not used in the current experiment. Extracellular activity of single units was recorded from areas 8a and 46 of dlPFC. The anatomic location of electrode penetrations was determined on the basis of MR imaging. Recordings were obtained with arrays of two to four microelectrodes in the cylinder. These were epoxylite-coated tungsten electrodes with a 250 µm diameter and 1-4 MΩ impedance at 1 kHz (FHC, Bowdoin, ME). The electrical signal from each electrode was amplified, bandpass filtered between 500 Hz and 8 kHz, and recorded with a modular data acquisition system at 25-µs resolution (APM system; FHC, Bowdoin, ME). Waveforms that exceeded a user-defined threshold were sampled at 25 µs resolution, digitized, and stored for off-line analysis.

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Deep Brain Electrode Implantation and Stimulation. Once the head-cap and recording cylinders had been implanted, a second surgery was performed to implant the stimulating electrode. Based on MR imaging, stereotaxic coordinates were obtained for target implantation. The animals were implanted unilaterally (one in the left, and one in the right hemisphere) at 8mm lateral, 16 mm anterior inter-aural, and 29 mm below the cortical surface in a vertical penetration. The lateral and anterior coordinates, and depth, were chosen to correspond to the center of the anterior portion of the Nucleus Basalis of Meynert, which would contain the highest density of projections to the prefrontal cortex (Mesulam et al., 1983; Gielow and Zaborszky, 2017). A small cylindrical titanium chamber (5-mm inner diameter and 7-mm outer) was mounted on the cranium and chamber was encased in bone cement, in continuity with the existing head-cap. A 26 ga. sharp hypodermic guide tube was lowered and the tip advanced 5 mm below the dura mater. The electrode was inserted into the guide tube, and a stylus was used to push it to the appropriate depth. The guide tube was then raised while the stylus depth maintained. The chamber was evacuated of fluid, flushed with ceftriaxone, and thereafter fluid evacuated a second time. Silicone was poured into the chamber to seal the fenestrations in the skull and the inside of the chamber. The electrode was fixed in depth with a drop of cyanoacrylate, and its rear wire was stripped and soldered to a connector that was fixed on the chamber outer wall. One week after the surgery, the animals returned to behavioral studies. Placement of the electrode was verified with CT scanning, after implantation, in one animal. The rear end of the electrode could be continuously visualized to confirm proper depth.

The stimulation pulses were created by an isolated pulse stimulator (Model 2100, A-M Systems, Sequim WA), which was controlled by custom programed software, written on the MATLAB platform. Impedances of electrodes were checked monthly during experiments.

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Intermittent stimulation was applied for 15 seconds at 80 pulses per second, followed by approximately 45 seconds with no stimulation. Stimulation was applied in the inter-trial interval, after a trial had completed, and a new trial began after stimulation had elapsed.

Electrodes were custom manufactured in our laboratory based on published specifications (McCairn and Turner, 2009). Conductors were 50 μm Pt/Ir, Teflon-insulated wire (A-M systems, Seattle, WA) embedded within a 30 ga. hypodermic tube, which was encased in a 28 ga. polyimide sheath. The wire extended from the end of the sheath into the brain tissue by roughly 1 mm, and the last 0.7 mm of insulation was stripped to achieve impedances of 5-10 kOhm at 1 kHz. The far end of the electrode was soldered to an extracranial connector fixed on the chamber outer wall. Preliminary experiments on electrode placement in the two pilot animals tested the effects of short periods of stimulation on EEG desynchronization. Stimulation was delivered with biphasic, negative first, unipolar 200 μA pulses with 100 μs per phase, and 10 pulses were delivered in 100 msec. This resulted in LFP desynchronization when the electrode was at a depth corresponding to the atlas position of Nucleus Basalis. In pilot experiments, an electrode movement vertically in either direction of more than 1 mm was adequate to make desynchronization not possible using the same protocol (Liu et al., 2017).

Behavioral tasks. The monkeys faced a computer monitor 60 cm away in a dark room with their head fixed. Eye position was sampled at 240 Hz, digitized, and recorded with an infrared eye position tracking system (model RK-716; ISCAN, Burlington, MA). The visual stimulus presentation and behavior monitoring were controlled by in-house software (Meyer and

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Constantinidis, 2005) implemented in the MATLAB computational environment (Mathworks, Natick, MA), using the Psychophysics Toolbox (Brainard, 1997).

The tasks used in the present study were variations of the Oculomotor Delayed Response task (Funahashi et al., 1989), but involving two stimuli appearing in sequence, requiring the monkey to remember and make an eye movement to either the first or the second stimulus (Fig. 1D). The monkeys were trained to saccade to the location of the remembered stimulus according to the color of fixation point. After the animals fixated at a white/blue square (0.2° in size) located at the center of the monitor for 1 second, two white squares (1.5° in size) were displayed sequentially for 0.5 s, with a 1.5 s intervening delay period. The first stimulus was displayed pseudo-randomly at one of eight locations arranged along a circular ring of 12° eccentricity, with a 45° angular separation between neighboring stimuli. The second stimulus appeared at a variable location relative to the first stimulus. After a second delay period of 1.5s, the monkeys were required to saccade to the location of the first stimulus if the fixation point was white in color (remember-first condition), and to the location of the second stimulus if the fixation point was blue (remember-second condition). To minimize the uncertainty about the stimulus to be remembered, the remember-first and remember-second conditions were presented in blocks of trials. The animal was required to perform ten correct trials of the remember-first task, involving all stimulus locations, before the task alternated to the remember-second condition. The monkeys were rewarded with fruit juice after making a correct saccade. Breaking fixation led to the immediate termination of the trial without reward.

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Behavioral Performance. We calculated behavioral performance as the percentage of trials that resulted in correct saccades into the target window. Trials that were aborted prior to end of the second delay period (due to premature saccades, or blinks) were not included in this analysis.

Neural Data Analysis. All data analysis was implemented with the MATLAB computational environment (Mathworks, Natick, MA). Recorded spike waveforms were sorted into separate units using an automated cluster analysis relying on the KlustaKwik algorithm (Harris et al., 2000), which relied on principal component analysis of the waveforms. Mean firing rate was then determined in each task epoch. Neurons selective for the stimuli either the cue period or the delay period, evaluated with a 1-way ANOVA, at the $p < 0.05$ significance level were used for most analyses. Most analyses relied on these neurons, and on data from correct trials. The effect of different stimulus conditions on firing rate was evaluated by computing the average firing rate during the period under study and using a repeated-measures, 2-way ANOVA to compare responses of the same neurons to different stimuli, under control and stimulation conditions.

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REFERENCES

Bakin JS, Weinberger NM (1996) Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. Proc Natl Acad Sci U S A 93:11219-11224.

Bartus RT (2000) On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol 163:495-529.

Bjordahl TS, Dimyan MA, Weinberger NM (1998) Induction of long-term receptive field plasticity in the auditory cortex of the waking guinea pig by stimulation of the nucleus basalis. Behav Neurosci 112:467-479.

Blake DT, Terry AV, Plagenhoef M, Constantinidis C, Liu R (2017) Potential for intermittent stimulation of nucleus basalis of Meynert to impact treatment of alzheimer's disease. Communicative & integrative biology 10:e1389359.

Brainard DH (1997) The Psychophysics Toolbox. Spat Vis 10:433-436.

Compte A, Brunel N, Goldman-Rakic PS, Wang XJ (2000) Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. Cereb Cortex 10:910-923.

Constantinidis C, Funahashi S, Lee D, Murray JD, Qi XL, Wang M, Arnsten AFT (2018) Persistent Spiking Activity Underlies Working Memory. J Neurosci 38:7020-7028.

Croxson PL, Kyriazis DA, Baxter MG (2011) Cholinergic modulation of a specific memory function of prefrontal cortex. Nat Neurosci 14:1510-1512.

Funahashi S, Bruce CJ, Goldman-Rakic PS (1989) Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61:331-349.

Gielow MR, Zaborszky L (2017) The Input-Output Relationship of the Cholinergic Basal Forebrain. Cell reports 18:1817-1830.

Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G (2000) Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. J Neurophysiol 84:401-414.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. Nature 454:1110-1114.

Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity [see comments]. Science 279:1714-1718.

Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, Chen L, Kocsis B, Deisseroth K, Strecker RE, Basheer R, Brown RE, McCarley RW (2015) Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations. Proc Natl Acad Sci U S A 112:3535-3540.

Liu R, Crawford J, Callahan PM, Terry AV, Jr., Constantinidis C, Blake DT (2017) Intermittent Stimulation of the Nucleus Basalis of Meynert Improves Working Memory in Adult Monkeys. Curr Biol 7:2640-2646.

Liu R, Crawford J, Callahan PM, Terry AV, Constantinidis C, Blake DT (2018) Intermittent stimulation in the nucleus basalis of meynert improves sustained attention in rhesus monkeys. Neuropharmacology 137:202-210.

Lundqvist M, Herman P, Miller EK (2018) Working Memory: Delay Activity, Yes! Persistent Activity? Maybe Not. J Neurosci 38:7013-7019.

Major AJ, Vijayraghavan S, Everling S (2015) Muscarinic Attenuation of Mnemonic Rule Representation in Macaque Dorsolateral Prefrontal Cortex during a Pro- and Anti-Saccade Task. J Neurosci 35:16064-16076.

Major AJ, Vijayraghavan S, Everling S (2018) Cholinergic Overstimulation Attenuates Rule Selectivity in Macaque Prefrontal Cortex. J Neurosci 38:1137-1150.

McCairn KW, Turner RS (2009) Deep brain stimulation of the globus pallidus internus in the parkinsonian primate: local entrainment and suppression of low-frequency oscillations. J Neurophysiol 101:1941-1960.

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Mesulam MM, Mufson EJ, Levey AI, Wainer BH (1983) Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. J Comp Neurol 214:170-197.

Meyer T, Constantinidis C (2005) A software solution for the control of visual behavioral experimentation. J Neurosci Methods 142:27-34.

Pinto L, Goard MJ, Estandian D, Xu M, Kwan AC, Lee SH, Harrison TC, Feng G, Dan Y (2013) Fast modulation of visual perception by basal forebrain cholinergic neurons. Nat Neurosci 16:1857-1863.

Qi XL, Constantinidis C (2015) Lower neuronal variability in the monkey dorsolateral prefrontal than posterior parietal cortex. J Neurophysiol 114:2194-2203.

Qi XL, Elworthy AC, Lambert BC, Constantinidis C (2015) Representation of remembered stimuli and task information in the monkey dorsolateral prefrontal and posterior parietal cortex. J Neurophysiol 113:44-57.

Riley MR, Constantinidis C (2016) Role of prefrontal persistent activity in working memory. Front Syst Neurosci 9:181.

Sabbagh M, Cummings J (2011) Progressive cholinergic decline in Alzheimer's Disease: consideration for treatment with donepezil 23 mg in patients with moderate to severe symptomatology. BMC neurology 11:21.

Sarter M, Bruno JP (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Brain Res Rev 23:28-46.

Sun Y, Yang Y, Galvin VC, Yang S, Arnsten AF, Wang M (2017) Nicotinic alpha4beta2 Cholinergic Receptor Influences on Dorsolateral Prefrontal Cortical Neuronal Firing during a Working Memory Task. J Neurosci 37:5366-5377.

Terry AV, Jr., Buccafusco JJ (2003) The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 306:821-827.

Vijayraghavan S, Major AJ, Everling S (2018) Muscarinic M1 Receptor Overstimulation Disrupts Working Memory Activity for Rules in Primate Prefrontal Cortex. Neuron 98:1256-1268 e1254.

Walker LC, Price DL, Young WS, 3rd (1989) GABAergic neurons in the primate basal forebrain magnocellular complex. Brain Res 499:188-192.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572-575.

Wimmer K, Nykamp DQ, Constantinidis C, Compte A (2014) Bump attractor dynamics in prefrontal cortex explains behavioral precision in spatial working memory. Nat Neurosci 17:431-439.

Yang Y, Paspalas CD, Jin LE, Picciotto MR, Arnsten AF, Wang M (2013) Nicotinic alpha7 receptors enhance NMDA cognitive circuits in dorsolateral prefrontal cortex. Proc Natl Acad Sci U S A 110:12078-12083.

Zhang K, Chen CD, Monosov IE (2019) Novelty, Salience, and Surprise Timing Are Signaled by Neurons in the Basal Forebrain. Curr Biol 29:134-142 e133.

Zhou X, Qi XL, Douglas K, Palaninathan K, Kang HS, Buccafusco JJ, Blake DT, Constantinidis C (2011) Cholinergic modulation of working memory activity in primate prefrontal cortex. J Neurophysiol 106:2180-2188.

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FIGURE LEGENDS

Figure 1. Localization and effects of stimulation. A. Schematic diagram of the monkey brain. The approximate location of the implanted electrode is indicated with the solid/dashed vertical line. The shaded area represents the cortical region sampled with neurophysiological recordings. Abbreviations, AS: arcuate sulcus; PS: principal sulcus. B. Anatomical MR scan from one monkey obtained prior to implantation, at the coronal level corresponding to the position of the electrode (red dashed line). **C.** Power Spectrum of Local Field Potential recorded from the implanted electrode during rest (solid line) and following 80 Hz stimulation. **D.** Successive frames illustrate the sequence of events in the behavioral task. Depending on the color of the fixation point, white or blue, the monkey has to remember either the first or the second of two stimuli presented in sequence, respectively. At the end of the trial, the fixation point turns off and the monkey needs to perform an eye movement towards the location of the correct stimulus in order to receive a liquid reward. **E-H**. Percentage of correct trials is shown for each of the two monkeys, for different stimulus types (n=18 sessions for stimulation, 17 for control for monkey GR; n=19 stimulation and 35 control for monkey HE). **E.** Mean performance (and sem) for trials in which first stimulus appears contralateral to the stimulation site, when the monkey is executing the remember-first task, and needs to remember the first stimulus. **F.** Performance in the remember-first task when the first stimulus appears ipsilateral to the stimulation site. **G**. Performance in the remember-second task when the second stimulus appears ipsilateral to the stimulation site. **H**. Performance in the remember-second task, when the second stimulus appears contralateral to the stimulation site.

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Figure 2. Distribution and example of stimulation effects. **A**. distribution of firing rate differences between stimulation and control conditions. Positive values indicate higher fixation period firing rate in the stimulation condition. Each neuron is represented twice in this diagram; once for the remember-first, and once for the remember-second task. Mean firing rate of neurons with significant increase in activation by NB stimulation (n=2x112 neurons). **B-E**. Raster plots and Peri-Stimulus Time Histograms represent responses of a single neuron in the remember-first (B-C) and remember-second task (D-E), under control and stimulation conditions. Trials are pooled from conditions when the first stimulus appeared inside the receptive field (B, D) or outside (C, E). Insets to the right of the PSTH represent schematically the location of stimulus relative to the receptive field; the actual locations and receptive field locations varied in each neuron.

Figure 3. Population responses under stimulation. **A**. Mean firing rate with and without stimulation is shown during the intertrial interval, fixation interval, first stimulus presentation involving the best stimulus of each neuron, second stimulus presentation involving the best stimulus of each neuron, and saccade towards best stimulus. Results from the remember-second task are shown, for neurons with significant increase in activation by NB stimulation (n=54 neurons with sufficient numbers of trials in all conditions). **B-E**. Mean firing rate in the remember-first task, in conditions involving presentation of the first stimulus in the receptive field, followed by a second stimulus at progressively less responsive locations. Insets to the right of PSTH represent location of the stimuli relative to each neuron's receptive field; results from neurons with different receptive field locations have been averaged together, but only one stimulus location is indicated. **F-G**. Mean firing rate in the remember-second task, in conditions

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involving no first stimulus, followed by a second stimulus in or out of the receptive field. **H**. Firing rate in sequential trials in blocks of trials when stimulation was applied or not. X axis represents time after the offset of stimulation, or sham inter-trial interval.

Figure 4. Attractor network behavior. A. Schematic diagram of bump of activity in the network of prefrontal neurons. Ordinate represents time after initial stimulus onset, abscissa neurons with preference for different stimulus locations, indicated by location varying between 0 and 360°. Activity of neurons with different preference is indicated based on color scale. The first stimulus appearance at 270° (indicated by horizontal line on top of the panel) elicits a bump of activity which is maintained during the delay period, after the stimulus is no longer present. Appearance of the second stimulus at the 90° location causes the initial bump of activity to terminate and a new bump to be maintained at 90°. The subject retrieves this location at the end of the delay period (black triangle, to the right of the panel). **B.** A network with neurons with broader selectivity results in a wider bump of activity, which is more resistant to the interference of the second stimulus, and the subject is able to retrieve the location of the original stimulus at 270°. **C**. When the second stimulus appears at location near the first, the bumps of activity are more likely to merge in the network with the broader bump. The recalled location (black triangle) is thus "pulled" towards the location of the second stimulus. **D.** Mean performance (and sem) in the remember-first task, for trials grouped by distance between the first and second stimulus (180, 90, or 45°), under stimulation or control conditions. Data from both monkeys pooled together (n=35 stimulation sessions with sufficient trials for this analysis, 51 for control). **E.** Mean performance (and sem) in the remember-second task, for trials grouped by distance.

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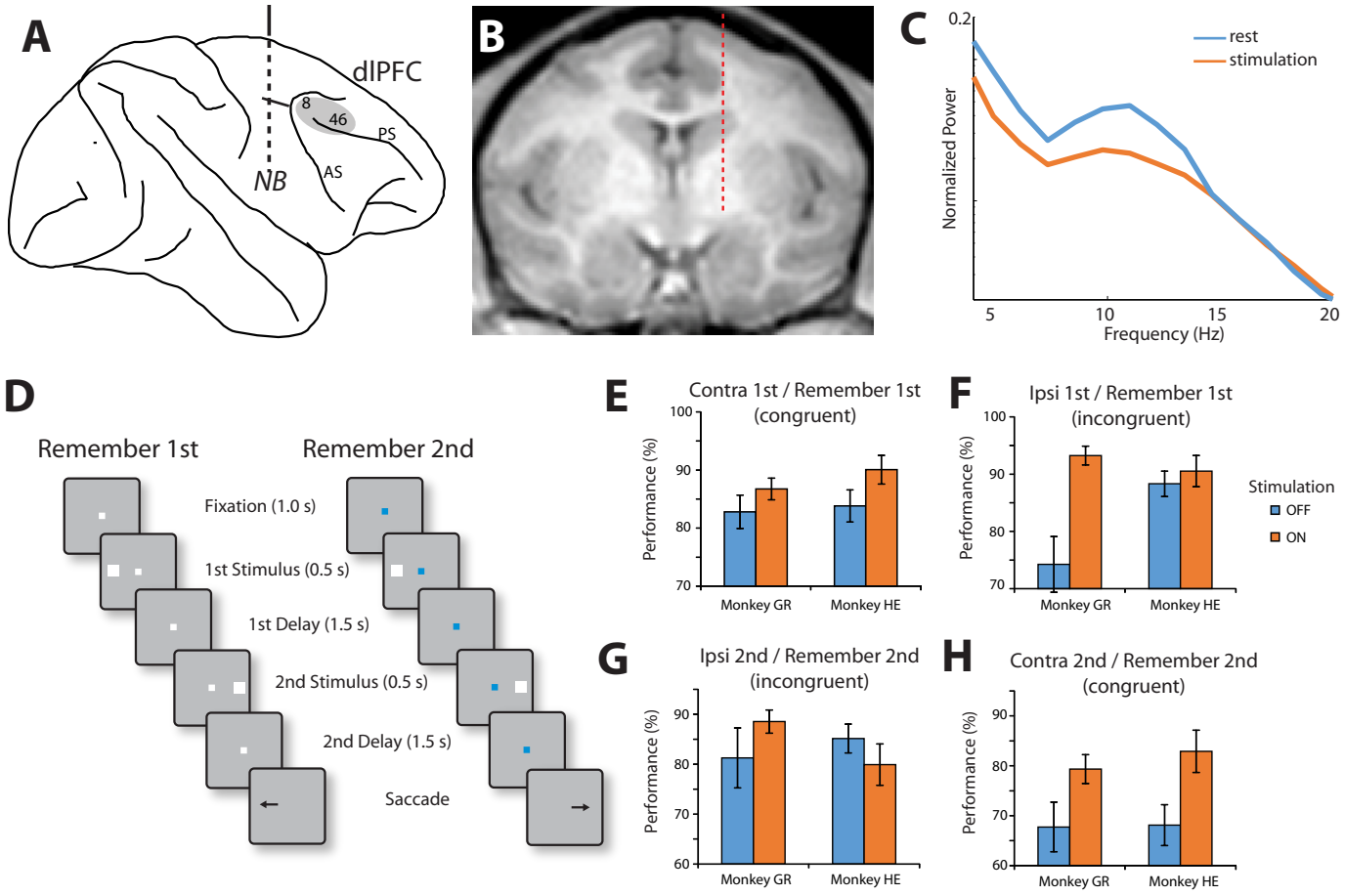

FIGURE 1

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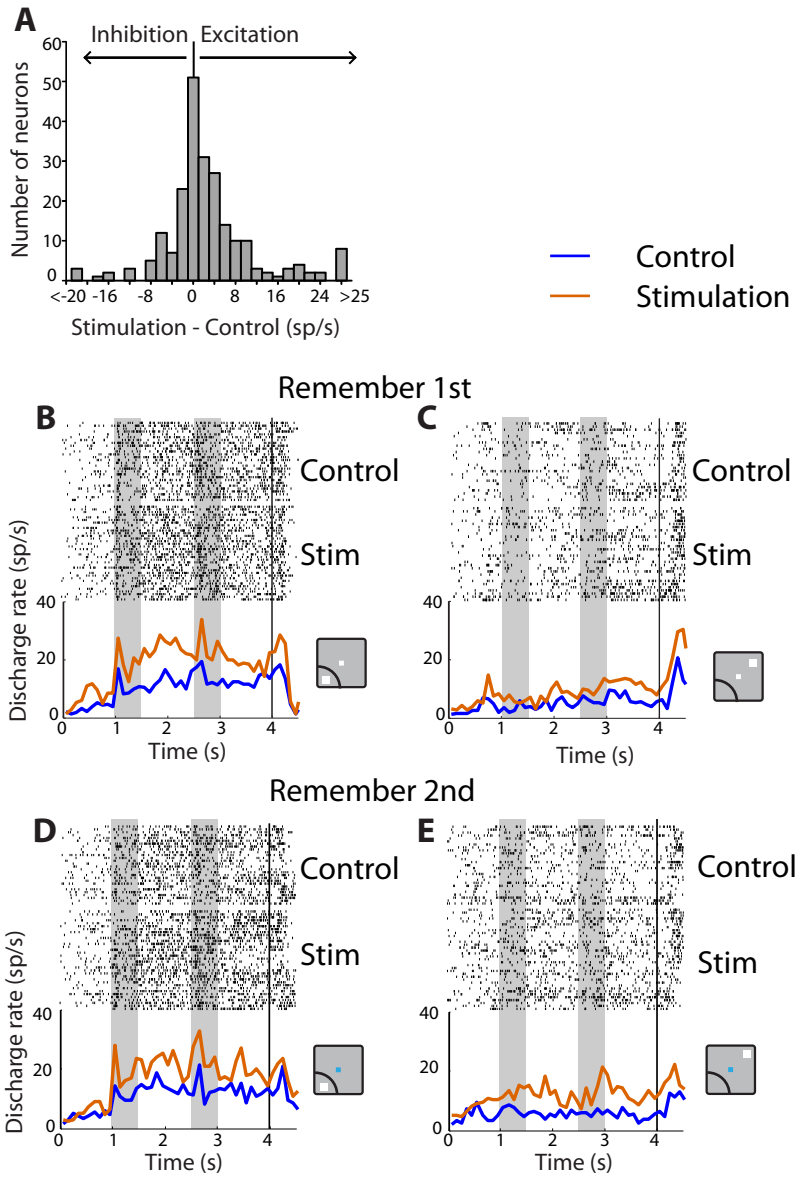

FIGURE 2

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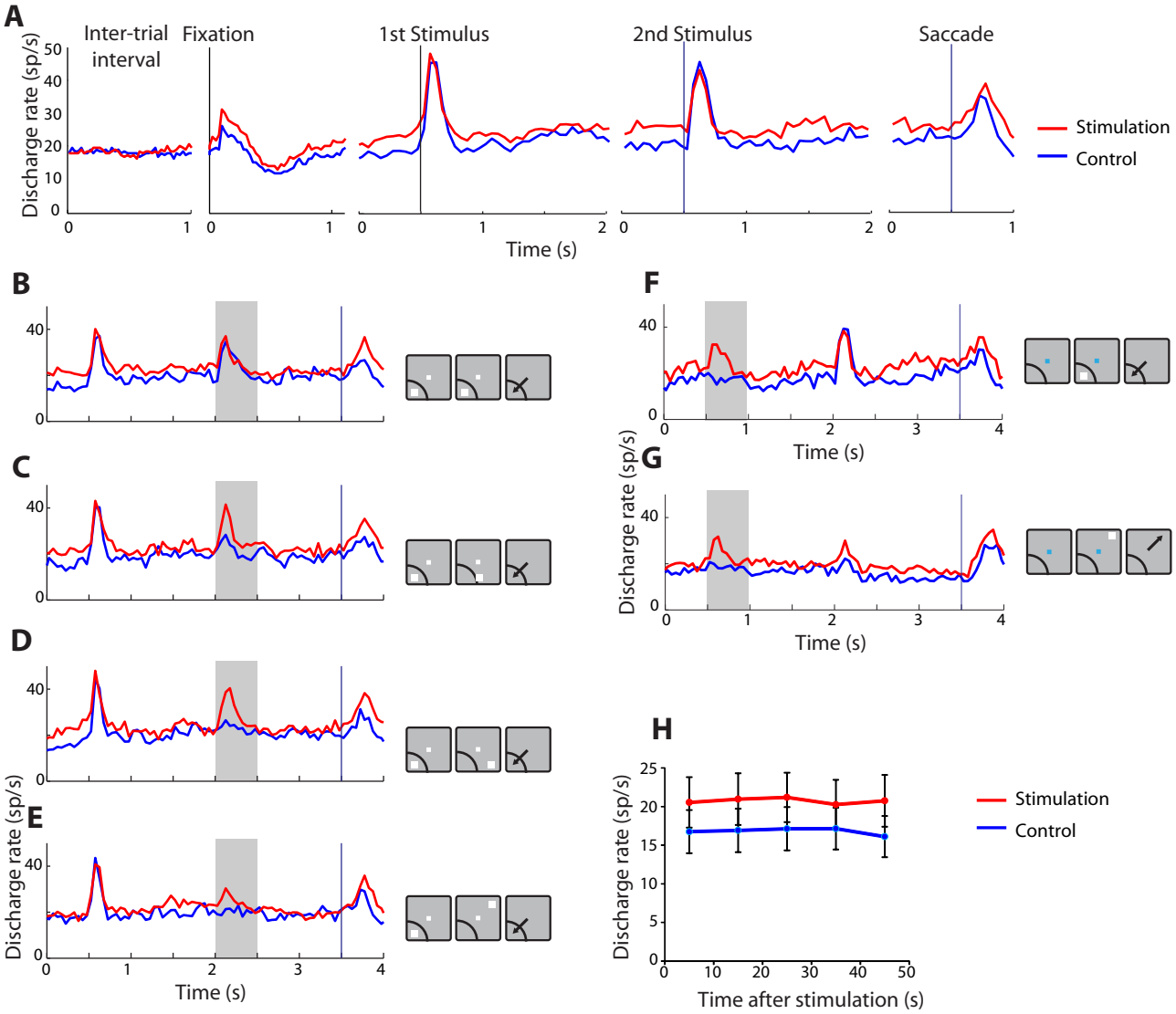

FIGURE 3

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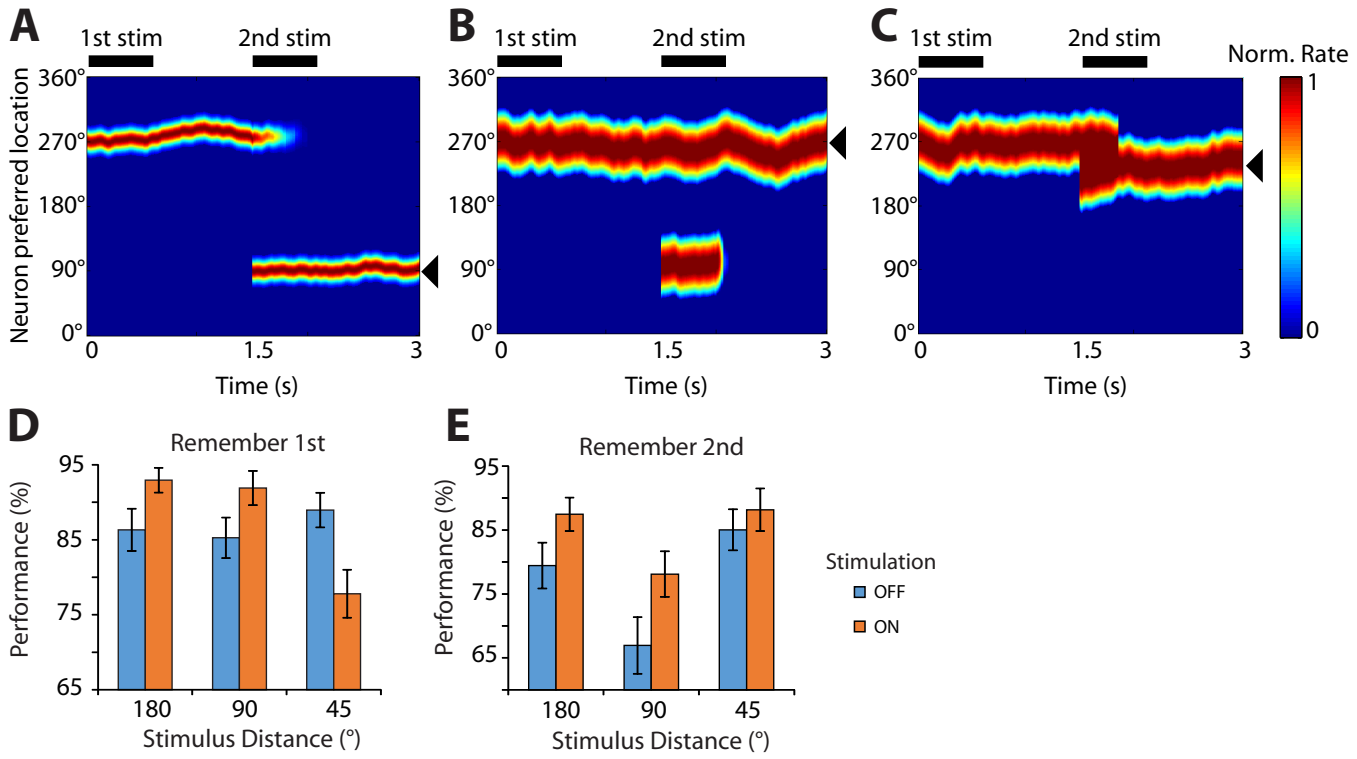

FIGURE 4