Fast Modulation of Prefrontal Cortex Activity by Basal Forebrain Noncholinergic Neuronal Ensembles

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INTRODUCTION

The mammalian basal forebrain (BF) critically modulates cortical activity (Buzsáki et al. 1988; Riekkinen et al. 1991) during different wake–sleep states (Jones 2003; Zaborszky and Duque 2003). The BF also plays important roles in cortical plasticity (Dykes 1997; Kilgard and Merzenich 1998; Weinberger 2003), learning and memory (Everitt and Robbins 1997; Weinberger 2003), and attention (Chiba et al. 1995; Everitt and Robbins 1997). Although most BF functions have been attributed to its cholinergic neurons, however, the majority of cortical-projecting BF neurons are noncholinergic and their in vivo functions remain unclear. We investigated how BF modulates cortical dynamics by simultaneously recording ±50 BF single neurons along with local field potentials (LFPs) from the prefrontal cortex (PFCx) in different wake–sleep states of adult rats. Using stereotypical spike time correlations, we identified a large (roughly 70%) subset of BF neurons, which we named BF tonic neurons (BFTNs). BFTNs fired tonically at 2–8 Hz without significantly changing their average firing rate across wake–sleep states. As such, these cannot be classified as cholinergic neurons. BFTNs substantially increased the spiking variability during waking and rapid-eye-movement sleep, by exhibiting frequent spike bursts with <50-ms interspike interval. Spike bursts among BFTNs were highly correlated, leading to transient population synchronization events of BFTN ensembles that lasted on average 160 ms. Most importantly, BFTN synchronization occurred preferentially just before the troughs of PFCx LFP oscillations, which reflect increased cortical activity. Furthermore, BFTN synchronization was accompanied by transient increases in prefrontal cortex gamma oscillations. These results suggest that synchronization of BFTN ensembles, which are likely to be formed by cortical-projecting GABAergic neurons from the BF, could be primarily responsible for fast cortical modulation to provide transient amplification of cortical activity.

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recorded for the rat with a movable array in the BF. Electrodes were
patterns during the recording session. Rats were recorded for an
the novelty of the recording chamber and ensured normal sleep
SWS, and rapid-eye-movement sleep (REM)—were deter-
S-isonel–coated tungsten microwire electrode arrays (35- or 50-μm
diameter wires with 1–2 MΩ impedance measured at 1 kHz, arranged
in a 4 × 8 grid with 250-μm spacing between wires) in the BF (n =
6) and PFCx (n = 4) under ketamine (50–90 mg/kg) and xylazine
(5–10 mg/kg) anesthesia. One rat was implanted with a custom-built
movable array in the BF where the electrode tips could be advanced
by a microdrive. The following coordinates (in millimeters) relative to
Bregma were used to center the arrays: BF (−0.5 anteroposterior
[AP], +2 mediolateral [ML], −7.5 dorsoventral [DV]); PFCx (+3.5
AP, +2.5 ML, −4.5 DV). Electrode tip locations were verified with
cresyl violet staining of histological sections after the end of the
experiment and compared with reference anatomical planes (Paxinos
and Watson 2005). All electrode arrays were found at expected
positions.

**METHODS**

**Surgery**

All procedures were approved by the Duke IACUC and performed
in accordance with National Institutes of Health guidelines. The
surgical procedure has already been described in detail (Nicolelis et al.
1997). Six adult female rats, 250–300 g, housed under a 6 AM to 6 PM
lights-on/lights-off cycle, were implanted with custom-built 32-wire
1997). It also allowed us to investigate the relationship be-
tween BF and PFCx activity during different wake–sleep states.

**Recording**

Rats were allowed 7–10 days to recover from the surgery and were
then habituated to the recording chamber for 6 h (10 AM to 4 PM) for
2–3 consecutive days before the recording session, with unrestricted
access to water and food pellets. This extended habituation reduced
the novelty of the recording chamber and ensured normal sleep
patterns during the recording session. Rats were recorded for an
average of 4.97 ± 0.17 h (n = 10) per session. Five sessions were
recorded for the rat with a movable array in the BF. Electrodes were
advanced 250 μm per session (−7.25 to −8.25 mm DV for the five
sessions) and rats were allowed 1 wk of rest between sessions. For
other rats implanted with a fixed array in the BF, one recording
session per rat (n = 5) was used in the final analysis.

Single-unit activity and LFPs were referenced to a common skull
crack and recorded using a multichannel acquisition processor (MAP,
Plexon, Dallas, TX) (Nicolelis et al. 1997). Single-unit activities with
±3:1 signal-to-noise ratio were sorted on-line and later confirmed
with an off-line sorting algorithm (Plexon) as previously described
(Nicolelis et al. 2003). Only single units with clear separation from
the noise cluster and with minimal spike collisions (spikes with <1.5-ms
interspike interval) were used for further analyses. If any single unit
was recorded over multiple electrodes, as demonstrated by a spurious
zero-lag peak in the pairwise correlation function, the duplicate unit
was discarded from further analyses. Single units entering the final
analyses all contained adequate sampling (≥10 min) of the three
major states.

LFP signals, recorded from the same electrodes, were filtered
(0.3–400 Hz) and digitized (500 Hz). This digitization rate allowed
for reliable estimation of spectral power ≤100 Hz without concerns of
signal aliasing. Behaviors were recorded by way of two CCD video-
cameras and a VCR. Video and neural recordings were synchronized
with a millisecond-precision timer (model VTG-55, For-A, Tokyo,
Japan).

**Data analyses**

The three major wake–sleep states—waking (WK), slow-wave
sleep (SWS), and rapid-eye-movement sleep (REM)—were deter-
mind using the state-space method we previously developed (Ger-
vasoni et al. 2004). Briefly, two spectral amplitude ratios (0.5–20/
0.5–55 and 0.5–4.5/0.5–9 Hz) were calculated for each 1-s segment of
LFP data. The resulting time series were then smoothed with a 20-bin
Hanning window to reduce within-state variability. The two spectral
features spanned a two-dimensional state space (y-axis and x-axis,
respectively), where each dot corresponded to 1 s of LFP signal.
Because the prevalence of the whisker-twitching state was <0.5% in
these recordings [compared with the median prevalence of 1.3%
reported in Gervasoni et al. (2004)], we opted to analyze only the
three well-separated clusters in the state space, which corresponded
to WK, SWS, and REM states. Points within cluster boundaries were
labeled as the three main states. Short trajectories (<20 s) leaving
and reentering a given cluster boundary without touching the boundaries
of other clusters were also assigned to that state. Other points outside
cluster boundaries were labeled separately as state-transition epochs.
This ensured that the three coded wake–sleep states included only
physiologically homogeneous epochs at the core of the clusters. The
state coding was validated by video analysis of behaviors.

The firing rate of BF single units at each state was calculated by
dividing the total number of spikes in a given state by the total
duration of that state. The coefficient of variation of interspike interval
(CV ISI) distribution at each state was calculated by dividing the SD of
ISIs by the mean of ISIs, considering only ISIs taken from a given
state for each single unit.

Single-factor repeated-measure ANOVA (α = 0.05) was used to
investigate the general main effect of state dependency. When there
was a main effect, we performed post hoc comparisons using a paired
t-test (α = 0.05).

Pairwise correlation of spike trains was calculated for all simulta-
aneously recorded BF single-unit pairs (n = 4,844). State-dependent
correlation was also calculated using spikes in the three wake–sleep
states. Correlation functions were binned at 1 ms and calculated for
lag intervals of [−3, 3] seconds. Each correlation function was
normalized by the mean of its values at [−3, −1]- and [1, 3]-s intervals,
which represented the baseline level of correlation expected
by the respective firing rate of the two single units. To ensure that
each correlation function contained sufficient observations to make statisti-
cal inference, pairs with mean values at [−3, −1]- and [1, 3]-s intervals
less than a cutoff value 2.4 count per bin at any state were
discarded. This cutoff value roughly corresponded to two neurons
simultaneously firing at 2 Hz for 10 min. A cross-correlation function
was considered statistically significant when more than 10 bins within
the [−0.3, 0.3]-s interval laid outside the [minimum, maximum]
bound of its values at [−3, −1]- and [1, 3]-s intervals (P = 0.000005).
This formulation of statistical significance tested whether the two
neurons were more tightly correlated around time 0 than what would
be expected by the rate covariation at the timescale of a few seconds.
This ignored gross state-dependent firing rate modulations that took
place over longer timescales. Furthermore, the upper and lower
bounds of the confidence interval were determined empirically from
the correlation function at [−3, −1]- and [1, 3]-s intervals, which
provided an accurate estimation of chance correlation expected by the
firing rates of the two neurons, while preserving the fine temporal
structure of the two spike trains in the estimation of chance correla-
tion. This empirical construction of confidence interval also resolved
the task of analytically estimating chance correlation when calculating
state-dependent correlations, where each state was constituted by
many temporally disjoint intervals. Changing the stringency of the
statistical test mattered little to our results.

Principal component analysis (PCA) was used to determine the
dominant correlation patterns among all 4,844 pairs in the [−0.3,
0.3]-s interval. All bins except the three bins around zero time lag
(−1, 0, 1) ms were submitted to PCA, for a total of 598 bins. The
three middle bins were excluded from this analysis because correla-
tion at these bins could not be reliably estimated for single units
recorded from the same electrode. The first principal component

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BIAS/H11005 test for circular uniformity (significant if was then tested for significant phase locking by applying the Rayleigh resultant theta-phase distribution of BFTN synchronization events of the angle between the two vectors, a measure bounded by \[ \text{correlation using all spikes and correlation using only spike bursts, we results.}

The distance measure (d) was defined as \( d = \frac{1}{\text{CS}^* + \text{BIAS}} \), where BIAS = 0.5 and CS* = CS if CS \( \geq 0 \) or CS* = 0 if CS < 0.

Intuitively, this ensured that a high CS would lead to a small distance, whereas a small CS would lead to a large distance. The BIAS term was introduced to set an upper bound for the distance measure such that it would not go to infinity with a small CS. Negative CS was replaced with zero to avoid negative distance. A hierarchical cluster tree was computed on this distance matrix using the unweighted average distance method (Statistics toolbox, MATLAB). Intuitively, this ensured that a high CS would lead to a small distance, whereas a small CS would lead to a large distance. The BIAS term was introduced to set an upper bound for the distance measure such that it would not go to infinity with a small CS. Negative CS was replaced with zero to avoid negative distance. A hierarchical cluster tree was computed on this distance matrix using the unweighted average distance method (Statistics toolbox, MATLAB).

The hierarchical cluster tree, the dendrogram, was then rearranged by the ascending order of the minimum average distance in each subtree. This neuronal rank was then applied back to the matrix of correlation scores (Fig. 4B) such that neurons were ordered by their average correlation score and their similarities with each other. Guided by the dendrogram, BF tonic neurons (BFTNs) were defined for each correlation score matrix as the core set of neurons that were highly correlated with each other. Specifically, for a BF neuron to be labeled as a BFTN, more than half of its correlations with other BFTNs should have a correlation score >1.

Spikes within each spike train were identified as all the spikes with the preceding or following ISI in the [10, 30]-ms range. Spike bursts accounted for 13.6 ± 0.5% (n = 223) of BFTN spikes. The choice of ISI upper bound up to 100 ms mattered little to our final results.

To compare similarities between correlation score matrices for correlation using all spikes and correlation using only spike bursts, we transformed each matrix into a linear vector and computed the cosine of the angle between the two vectors, a measure bounded by \([-1, 1]\). Pearson’s correlation coefficient between the two vectors gave similar results (average correlation coefficient 0.73 ± 0.03, n = 10).

The BFTN population firing rate trace was calculated by combining the time stamp of all spikes from all BFTNs within each session, binned at 10 ms, and smoothed with a 10-bin Hanning window. To determine the timing of each BFTN transient synchronization event, we first generated the BFTN population firing rate trace using only spikes within spike bursts. BFTN transient synchronization events were defined as the top 20% local maxima in this population firing rate trace, which provided a conservative selection of BFTN transient synchronization events. Relaxation of our criteria up to the top half local maxima mattered little to our conclusions in Figs. 6 and 7.

To determine the theta phase of BFTN transient synchronization events, we first identified the local peaks and troughs of prominent theta oscillations (with an amplitude cutoff) during WK and REM states. Phase values were linearly interpolated by assigning theta peaks at phase zero and theta troughs at phase π and −π. The resultant theta-phase distribution of BFTN transient synchronization events was then tested for significant phase locking by applying the Rayleigh test for circular uniformity (significant if Z >5).

If the BFTN population firing rate trace was calculated in the three major states. All LFP averages were calculated at the \([-3, 3]\)-s interval. The LFP average was considered statistically significant when ≥20 bins within the \([-0.3, 0.3]\)-s interval laid outside of the \([\text{minimum, maximum}]\) bound of its values at \([-3, -1]\)- and \([1, 3]\)-s intervals (\( P = 0.000025 \)). Because all but one of the LFP averages were statistically significant using this criterion, relaxing the criterion did not change our conclusions.

Oscillation phase at time 0 of the LFP averages was determined by linear interpolation using local peaks (phase zero) and local troughs (phases π and −π) in the LFP averages as reference points. LFP averages were first smoothed with a 30-bin Hanning window (60-ms duration) to smooth out small fluctuations.

The BFTN synchronization–triggered PFCx LFP spectral perturbations were calculated as follows: First, the log-power spectrogram was calculated for the \([-3, 3]\)-s interval around each BFTN synchronization event, with a 0.2-s window and a 0.01-s step. Second, these log-power spectrograms were averaged within each animal for all synchronization events (and also separately for events within WK, SWS, and REM states). Results from all eight sessions with PFCx

![FIG. 1. A: cresyl violet staining of histological sections at the level of basal forebrain (BF, left) and prefrontal cortex (PFCx, right). Locations of electrode tips are indicated by red arrows. CPu, caudate putamen; GP, globus pallidus; LO, lateral orbital cortex; LPO, lateral preoptic area; M1, primary motor cortex; MCPo, magnocellular preoptic nucleus; Pir, piriform cortex; PrL, prelimbic cortex; VO, ventral orbital cortex. B: an example of single-unit isolation showing the waveforms of the single unit (left), the interspike interval (ISI) distribution (middle) and all waveforms from the same channel projected into a 3-dimensional space (right). Four clusters were clearly separable and sorted as single units in this channel. Red dashed line (middle) indicates 1.5-ms ISI. C: state-space map of a recording session using local field potentials (LFPs) from the PFCx and BF. Points within the 3 main clusters were labeled as the 3 major states, whereas points in between clusters were labeled as state transitions.](http://jn.physiology.org/doi/10.1152/jn.00398.2006)
LFPs were averaged to give the final result. The spectrogram at the \([-3, -1.8]\)-s interval (100 time steps) was used as the baseline and the mean spectrum of the baseline period was subtracted from each time window to reveal spectral power fluctuations. The minimum, maximum amplitude during the baseline period (per frequency bin) was used as the threshold for statistical significance \((P < 0.01)\). Nonstatistically significant spectrogram bins were plotted with zero amplitude (green) in Fig. 7. In other words, all nongreen bins were statistically significant.

RESULTS

Six rats were implanted with multielectrode arrays in the BF and the PFCx (Fig. 1A). Single-unit activity from the BF and LFPs from both the BF and PFCx were recorded for, on average, 4.97 ± 0.17 h \((n = 10\) sessions, mean ± SE) during the light cycle when the rats were naturally sleeping and waking without being disturbed. A total of 327 single units were sorted off-line (Fig. 1B) (Nicolelis et al. 2003). An average of 32.7 ± 3.0 \((n = 10)\) BF single units were simultaneously recorded during each session. Epochs of the three major wake–sleep states—waking (WK), slow-wave sleep (SWS), and rapid-eye-movement sleep (REM)—were identified based on spectral features of LFPs using the state-space method we previously developed (Gervasoni et al. 2004) (Fig. 1C), which was behaviorally validated by classical sleep scoring methods. On average, 49 ± 11 min of WK, 156 ± 9 min of SWS, and 28 ± 3 min of REM epochs \((n = 10)\) were identified per recording session. State transition epochs (i.e., points connecting the three main clusters in the state space) were not coded toward the three major wake–sleep states.

State-dependent firing patterns of BF neurons

We first characterized the firing rate modulation of BF neurons in relation to wake–sleep states. As a population, BF neurons fired more during WK and REM relative to SWS [Fig. 2A, right; \(F_{(2,325)} = 7.709, P = 0.0005\), pairwise comparisons WK vs. SWS, \(P = 0.0001\); REM vs. SWS, \(P = 0.001\); WK vs. REM, \(P = 0.246\)]. It was expected that BF cholinergic neurons would have much higher firing rates during WK and REM and...
virtually cease firing during SWS, as recently demonstrated using juxtacellular recording and labeling in head-restrained rats (Lee et al. 2005). Consistent with this description of cholinergic neurons, roughly 10% (33/327) of our recorded BF neurons increased their firing rate 1.5-fold during WK or REM relative to SWS. However, in contrast to the state-dependent firing rate modulation of cholinergic neurons (Lee et al. 2005), the majority of our recorded BF neurons had similar firing rates across states (along the diagonal line of Fig. 2A with firing rate 2–8 Hz). This strongly suggests that these BF neurons were not cholinergic neurons.

Despite the lack of firing rate modulation across states, we observed a clear state-dependent modulation in the firing pattern among most BF neurons recorded: the variability of the firing rate trace increased prominently during WK and REM relative to SWS. However, in contrast to the state-dependent firing rate modulation of cholinergic neurons (Lee et al. 2005), the majority of our recorded BF neurons had similar firing rates across states (along the diagonal line of Fig. 2A with firing rate 2–8 Hz). This strongly suggests that these BF neurons were not cholinergic neurons.

FIG. 3. Many BF neuron pairs were correlated, especially during WK and REM. A: a typical pairwise correlation function of a significantly correlated BF neuron pair. Black dotted lines indicate confidence intervals. Inset: plot of autocorrelations for the 2 neurons. B: first principal component (PC1) (blue) of all correlation functions was overlaid on the pairwise correlation function shown in A (zoomed-in). C: histogram of correlation scores of all BF neuron pairs, grouped by their statistical significance. Example traces (blue) shown on top visually indicate the shape and amplitude of correlation functions that would give the corresponding levels of correlation score. Orange arrow indicates the correlation score of the example shown in A. D: same pairwise correlation shown in A calculated separately for each state, smoothed with a 10-ms Hanning window. E: average correlation score for all significantly correlated BF neuron pairs is significantly higher (*) during WK and REM relative to that during SWS.

Pairwise correlation of BF neurons in different states

To test our hypothesis, pairwise correlation of spike trains was used to quantify how well transient firing rate fluctuations among all simultaneously recorded BF neurons were correlated
across behavioral states. Among 4,844 simultaneously recorded BF neuronal pairs, 33% of them were significantly correlated in the [-300, +300]-ms interval. Many correlated pairs had a broad (about 150 ms) zero-lag peak (see Fig. 3, A and B). This observation was confirmed when we used principal component analysis (PCA) to determine the dominant correlation patterns among all pairwise correlation functions. This analysis revealed that the first principal component (PC1) (Fig. 3B), which overlapped almost completely with the example in Fig. 3A, explained 38% of the variability among all pairs and 42% of the variability among significantly correlated pairs. The second PC explained only 3–4% of variance and therefore does not reflect the dominant correlation patterns.

To give a quantitative measure of how strongly each pairwise correlation behaved like the PC1, a correlation score was defined for each pairwise correlation by projecting the individual correlation function (baseline subtracted) onto the PC1 (Fig. 3C). Correlation functions with a shape similar to that of the PC1 and high amplitude would result in high correlation scores (>1), as illustrated by example traces above Fig. 3C, whereas nonsignificantly correlated pairs had correlation scores close to zero (Fig. 3C). This confirmed that statistically correlated pairs had shapes similar to the PC1 and that PC1 successfully captured the most prominent correlation pattern.

We further tested whether wake–sleep states modulated the strength of correlation. Significant correlations were present in all three major wake–sleep states, with higher correlation peaks during the WK and REM states (see example in Fig. 3D). Correlation scores were significantly higher during WK and REM compared with that of SWS for all significantly correlated pairs [Fig. 3E; F(2,167) = 435.798, P = 4 × 10−152, pairwise comparisons WK vs. SWS, P = 2 × 10−124, REM vs. SWS, P = 1 × 10−114, WK vs. REM, P = 0.307]. These results confirmed our predictions that the spiking variability of BF neurons was indeed correlated, especially during WK and REM, and could potentially be used to modulate cortical activity without significantly modulating the average firing rate across wake–sleep states.

BF tonic neurons form functional assemblies

Because not all BF neuron pairs had high correlation scores, we further asked whether the pairs with high correlation scores were formed by a subset of BF neurons. For this purpose, we constructed a matrix of correlation scores for all BF neuron pairs recorded simultaneously within a session (Fig. 4A). If a subset of BF neurons accounted for most of the correlation, we should be able to reorder the neurons using a hierarchical cluster analysis and have high correlation scores clustered together. We observed that, in all recording sessions, a subset of neurons accounted for most of the high correlation score pairs (see example in Fig. 4B and population results in Fig. 4C) and constituted 67.8 ± 3.7% (n = 10) of all recorded BF neurons.

Importantly, this novel correlation-based characterization revealed a physiologically homogeneous subset of BF neurons. These neurons were exclusively tonic-firing neurons (4.23 ± 0.10 Hz, n = 223) that showed little change in average firing rate across states [Fig. 4D; F(2,221) = 0.462, P = 0.631] but had robust increases in the spiking variability during WK and REM relative to SWS [Fig. 4E; F(2,221) = 185.473, P = 5 × 10−48, pairwise comparisons WK vs. SWS, P = 5 × 10−56; REM vs. SWS, P = 3 × 10−49; WK vs. REM, P = 0.395]. We refer to this set of neurons as the BF tonic neurons, or BFTNs.

Transient synchronization among BFTNs using spike bursts

Given that BFTNs formed functional assemblies and their spike timings were highly correlated, we further investigated the output of the BFTN ensembles. The population firing rate of BFTNs fluctuated more during WK and REM relative to SWS (pop-FR traces in Fig. 5A). Prominent peaks in the BFTN population firing rate traces were clearly visible in all three major states (Fig. 5, A1–A3), indicating that the output of BFTN ensembles was not temporally uniform and varied on a fast timescale.

When the spike trains of BFTNs were plotted using the new neuron rank derived from the hierarchical cluster analysis (Fig. 4B), most BFTNs fired brief spike bursts (spikes with 0- to
Among BFTNs (see METHODS), which was more prevalent during WK (see example in Fig. 5B; correlation score paired much higher correlation peaks than correlation using all spikes bursts (referred to hereafter as spike burst correlation) showed were correlated. Pairwise correlation using spikes within spike synchronization events through brief spike bursts.

These observations indicated that BFTNs engaged in transient population firing rate traces (pop-FR) were drawn at the bottom for all spikes (black) and for spike bursts only (red). Local peaks in the red pop-FR trace indicated transient synchronization events among BFTNs (see METHODS), which was more prevalent during WK (A1) and REM (A3) than during SWS (A2). B: an example of pairwise correlation between 2 BFTNs using only spike bursts (red) and all spikes (blue). C: correlation scores of spike burst correlations plotted in the same matrix configuration as in Fig. 4B. Notice the different color scale.

50-ms ISI, labeled as red spikes in Fig. 5A) at the peaks of BFTN population firing rates. Conversely, BFTN spike bursts accounted for most of the peaks in the BFTN population firing rates (compare the two pop-FR traces in Fig. 5A). These observations indicated that BFTNs engaged in transient population synchronization events through brief spike bursts.

We further quantified how strongly spike bursts of BFTNs were correlated. Pairwise correlation using spikes within spike bursts (referred to hereafter as spike burst correlation) showed much higher correlation peaks than correlation using all spikes (see example in Fig. 5B; correlation score paired t-test, t_{2416} = 58.29). The spike burst correlations were similar in width with correlations using all spikes because the PC1 of spike burst correlations was almost identical with that in Fig. 3B (correlation coefficient 0.9869). The PC1 explained 51% of the variability among all spike burst correlations. Furthermore, the matrices of correlation scores using all spikes (Fig. 4B) and spike burst correlations (Fig. 5C) were also highly similar (average similarity 0.82 ± 0.02, n = 10). They differed only in scale (20-fold). Notice that only spike burst correlations between BFTNs showed high correlation scores, indicating that these correlations represent a unique property of BFTNs.

These observations indicated that most of the observed pairwise correlations can be accounted for by transient population synchronization among BFTNs, consisting of brief spike bursts. BFTN transient synchronization events, defined as the local peaks of the BFTN population firing rate trace (see METHODS), were more prevalent during WK (0.52 ± 0.07 Hz) and REM (0.72 ± 0.07 Hz) than during SWS (0.26 ± 0.03 Hz) \( F(2,8) = 34.386, P = 0.0001 \). The average duration of BFTN transient synchronization, represented by the width of the PC1 (Fig. 3B) at half-peak, was roughly 160 ms, which is the same time window for one cycle of exploratory behaviors such as whisking, licking, and sniffing (Davis and Smith 1992; Welker et al. 1964) and also the duration of one theta oscillation cycle. Taken together, the BFTN ensemble output seems to be organized into transient synchronization events, which could have a substantial impact on the modulation of cortical activity.

**BFTN synchronization was coupled to PFCx activity**

If transient BFTN synchronization indeed modulated cortical activity, one would predict that it should be tightly coupled with fluctuations in cortical oscillation patterns. Indeed, we observed that during WK with prominent theta oscillations and during REM, BFTN synchronization events occurred preferentially between PFCx theta peaks and slightly lead theta troughs (see example in Fig. 6A). We linearly extrapolated the theta phase of each BFTN synchronization event relative to theta peaks (phase zero) and theta troughs (phase \( \pi \)). Across the eight sessions where PFCx LFPs were recorded, in 14/16 cases (7/8 for WK; 7/8 for REM) the theta-phase distribution of BFTN synchronization events was not uniform (Rayleigh test, \( Z > 5 \)). Overall, the preferred theta phase of BFTN synchronization was at the downslope of PFCx theta oscillations just preceding the trough (Fig. 6B).

Next, we calculated PFCx LFP averages triggered by BFTN synchronization events. All but one (23/24, three states \( \times \) eight sessions) of the LFP averages showed significant modulations in the [−300, 300]-ms interval around the time of BFTN synchronization (Fig. 6C). LFP averages showed prominent theta oscillation (5–9 Hz) coupling during WK and REM and oscillations at lower frequencies during SWS (Fig. 6C). Furthermore, consistent with the theta oscillation coupling during WK and REM (Fig. 6A), BFTN synchronization occurred preferentially at the downslope of cortical oscillations preceding the trough of LFP averages in all wake–sleep states (Fig. 6, C2 and D). Because cortical oscillation troughs likely correspond to increased neuronal activity in the cortex (Chrobak and Buzsáki 1998; Fries et al. 2001), these results suggested that the BFTN synchronization phase leads transient increases in cortical neuronal activity.

**FIG. 5. Transient population synchronization among BFTNs using spike bursts.** A: examples of spike rasters of BFTNs in the 3 states, aligned with PFCx and BF LFPs and the clustering dendrogram. Spike bursts (spikes with 0- to 50-ms ISI) were labeled red. BFTN population firing rate traces (pop-FR) were drawn at the bottom for all spikes (black) and for spike bursts only (red). Local peaks in the red pop-FR trace indicated transient synchronization events among BFTNs (see METHODS), which was more prevalent during WK (A1) and REM (A3) than during SWS (A2). B: an example of pairwise correlation between 2 BFTNs using only spike bursts (red) and all spikes (blue). C: correlation scores of spike burst correlations plotted in the same matrix configuration as in Fig. 4B. Notice the different color scale.
Because LFP averages could reveal only phase-locked oscillations, we further investigated whether BFTN synchronization was associated with non-phased-locked modulations of the PFCx LFP oscillations. We did that by calculating the mean log-transformed PFCx LFP power spectrum around BFTN synchronization events (Fig. 7A). Overall, BFTN synchronization was associated with transient increases in PFCx oscillation power, not only in the phase-locked low-frequency range (<10 Hz), but also in the gamma oscillation range (30–100 Hz). The most prominent increase in gamma oscillation power lasted about 200 ms (red in Fig. 7A), similar to the duration of a BFTN synchronization event (about 160 ms). The transient increases in gamma oscillation power persisted for about 1 s at lower amplitude, accompanied by a slight decrease in lower-frequency (<20 Hz) oscillations. The coupling between BFTN synchronization and PFCx gamma oscillations was observed in all wake–sleep states (Fig. 7B). The less-prominent coupling during WK and REM, mainly in the high gamma range (60–100 Hz), was probably a result of the fewer number of WK and REM epochs in our recording. These observations demonstrated that BFTN synchronization indeed leads to fast and transient increases in cortical activity at multiple frequency ranges.

**DISCUSSION**

Despite the physiological heterogeneity of BF neuronal populations in vivo (Aston-Jones et al. 1984; Lee et al. 2004), we uncovered a physiologically homogeneous subset of BF neurons, the BF tonic neurons, by using a novel neuronal correlation analysis. Although previous physiological characterizations of BF neurons have primarily focused on identifying neurons with significant firing rate modulations across wake–sleep states (Alam et al. 1997; Detari et al. 1987; Lee et al. 2004), our results demonstrate that BFTNs, unlike BF cholinergic neurons (Lee et al. 2005), maintain similar average firing rates across wake–sleep states (Alam et al. 1997; Detari et al. 1987; Lee et al. 2004). Therefore BFTNs are likely not cholinergic neurons.

Whereas the average firing rates for BFTNs remain unchanged across behavioral states (Fig. 4D), we observed that the firing patterns of BFTNs, both at the single-neuron level and at the population level, change substantially across behavioral states. Specifically, at the single-neuron level, the spiking variability (CV<sub>ISI</sub>) of BFTNs increased by 46 and 38% from SWS to WK and REM (Fig. 4E). Similarly, the proportion of spikes in spike bursts increased by 72 and 101% from SWS to WK and REM (spike bursts accounted for 10.8% spikes during SWS). At the population level, the frequency of BFTN synchronization events increased by 103 and 181% from SWS to WK and REM. Altogether, these results reflect a major shift in firing patterns of BFTNs across behavioral states despite the same average firing rate. The conserved average firing rate of BFTNs implies that the ephemeral BFTN transient synchronization would be difficult to detect using functional magnetic resonance imaging. Furthermore, the change in firing patterns at the population level will not be seen with single-electrode
recording and can be unraveled only by simultaneously recording a large population of BF neurons.

We showed that, despite minimal changes in the average firing rate across states, BFTNs maximized their impact on cortical dynamics by forming functional assemblies and engaging in transient population synchronization, which lasted on average 160 ms. We observed that BFTN transient synchronization was accompanied by brief increases in PFCx theta and gamma oscillations. Furthermore, BFTN transient synchronization preferentially occurred preceding the trough of cortical oscillations. Cortical LFP oscillation troughs represent epochs in which cortical neurons are maximally active (Chrobak and Buzsáki 1998; Fries et al. 2001) because, during LFP troughs, electrical currents flow away from extracellular recording electrodes and into neurons, thus leading to membrane depolarizations and increased excitability. Our observations thus suggest that BFTN synchronization was leading, and probably contributing to, the transient cortical activity increase.

Although our results clearly revealed a strong association between BFTN synchronization and theta oscillations during WK and REM, such association does not preclude the BF–PFCx coupling in the absence of theta oscillations, such as during SWS. Our data showed that during SWS, BFTN synchronization events are still present, although much less frequently than during WK and REM. Our analysis (Fig. 7B2) indicated that synchronization events during SWS are effectively coupled with PFC activity, with comparable or even stronger coupling than that during WK and REM (Fig. 7, B1 and B3). The potential behavioral significance of such coupling during SWS is unclear but might play a role in the off-line processing of information, a role of SWS many recent reports have supported (Ribeiro et al. 2004).

Several observations support our hypothesis that BFTNs are cortical-projecting BF noncholinergic neurons. The transient (about 200 ms) PFCx modulation, along with its fast onset and offset, associated with BFTN synchronization is incompatible with slow actions of muscarinic receptors, which could last for several seconds (McCormick and Prince 1986). The fast temporal dynamics, however, are consistent with the ionotropic actions mediated by BF, either GABAergic or glutaminergic, cortical-projecting neurons (Gritti et al. 1997). Furthermore, the transient increase of cortical gamma oscillations associated with BFTN synchronization is consistent with the anatomical data that cortical-projecting BF GABAergic neurons synapse on cortical GABAergic interneurons (Freund and Gulyas 1991; Freund and Meskenaite 1992) and thus could increase cortical excitability through disinhibition (Dykes 1997; Freund and Gulyas 1991). In addition, the majority presence of BFTNs (roughly 70%) in our recorded BF neurons is consistent with the predominance of GABAergic neurons among BF cortical-projecting neurons (Gritti et al. 1997), which have large cell body sizes that would be favorably sampled by our extracellular recording technique. These observations together strongly suggest that BFTNs are likely cortical-projecting BF GABAergic neurons.

Because the in vivo pharmacological characterizations of various BF neuronal types are not conclusive, to clearly validate the neurochemical identity of the BFTNs would require intracellular or juxtacellular recording and labeling simultaneously with chronic recording of a large population of BF neurons in naturally waking and sleeping rats. Such a combination of techniques is currently not feasible in freely behaving animals. However, our conclusion that BFTN transient synchronization is associated with fast PFCx modulation does not rely on the exact knowledge of the neurochemical identity of BFTNs.

Overall, our results reveal that transient synchronization of putatively BF noncholinergic neurons leads to fast cortical modulation. That is contrary to the traditional view that neuromodulatory actions are generally slow because of the use of metabotropic receptors (Kaczmarek and Levitan 1987). These observations support the hypothesis that BF noncholinergic systems are primarily responsible for fast cortical modulation.
to transiently amplify cortical activity and the processing of incoming stimuli, especially during WK and REM. Such a transient signal-amplifying mechanism could serve as a neural substrate for selective attention.

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