### Learning-dependent dynamics of beta-frequency oscillations in the basal forebrain of rats

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Learning-dependent dynamics of beta-frequency oscillations in the basal forebrain of rats

Laleh K. Quinn¹, Douglas A. Nitz¹, Andrea A. Chiba¹

¹University of California at San Diego, Department of Cognitive Science
Abstract
Cholinergic, GABAergic, and glutamatergic projection neurons of the basal forebrain (BF) innervate widespread regions of the neocortex and are thought to modulate learning and attentional processes. While it is known that neuronal cell types in the BF exhibit oscillatory firing patterns, whether or not the BF as a whole shows oscillatory field potential activity, and whether such neuronal patterns relate to components of cognitive tasks, has yet to be determined. To this end local field potentials (LFPs) were recorded from the BF of rats performing an associative learning task wherein neutral objects were paired with differently-valued reinforcers (pellets). Over time, rats developed preferences for the different objects based on pellet-value, indicating that the pairings had been well-learned. LFPs from all rats revealed robust, short-lived bursts of beta-frequency oscillations around the time of object encounter. Beta-frequency LFP events were found to be learning-dependent, with beta-frequency peak amplitudes significantly greater on the first day of the task when object-reinforcement pairings were novel than on the last day when pairings were well-learned. The findings indicate that oscillatory bursting field potential activity occurs in the basal forebrain in freely behaving animals. Furthermore, the temporal distribution of these bursts suggests that they are likely relevant to associative learning.

Introduction
GABAergic, glutamatergic, and cholinergic neurons of the basal forebrain (BF) together form a prominent projection to widespread regions of the neocortex (Zaborzsky et al., 1999; Gritti et al., 1997; Price and Stern 1983). As the major source of cholinergic innervation of the neocortex, the BF is well positioned to play an important role in cortical plasticity (Kilgard and Merzenich 1998; Weinberger, 2003; Conner et al. 2005) and in the processing of sensory information (Sarter et al., 2003; Linster et al., 2003). Accordingly, lesions of this region impair performance on tasks designed to test attention, learning, and memory. (Vanderwolf et al., 1993; Sarter et al., 2003; Chiba et al., 1995, 1999; Voytko et al., 1994).

One physiological function played by the basal forebrain system is to produce the continuous global neocortical EEG desynchronization which accompanies all waking states and is necessary for normal cognitive function on a relatively long timescale (Buzsaki et al 1988; Steriade et al., 1993; Szymusiak et al., 1995; Lee et al., 2004). However, the function of this system may not be limited to this role. The recent discovery of phasically-occurring, oscillatory firing patterns in several
different neuronal subtypes of the BF supports the view that basal forebrain processing may include a periodic temporal component as well. (Lee et al., 2005; Detari et al., 1999). Cholinergic, glutamatergic and gabaergic neurons which intrinsically oscillate at theta, beta, and gamma frequencies are each represented in the substantia innominata/nucleus basalis magnocellularis (SI/Nbm) region of the basal forebrain (Manns et al. 2003). As the SI/Nbm region projects to much of the cortical mantle, oscillatory activity arising from such a region may also allow for synchronous temporal coding across widespread regions of cortex.

In the present work, we sought to determine whether distinct patterns of neural activity, in the form of local field potential (LFP) oscillations, arise in the BF during performance of a cognitive task. LFPs were recorded from the SI/Nbm during performance of an associative learning task wherein rats learned the motivational significance of previously neutral objects. Discrete LFP events in the form of beta-frequency oscillations were found to occur during performance of the task, with robust bursts occurring around the time of object encounter. Beta-frequency LFP events were found to be learning-dependent, with amplitudes significantly greater on day one of the task, when object-reinforcement pairings were novel, than on subsequent days, when the pairings were well-known. Together, these findings support the hypothesis that associative learning processes may be driven by oscillatory bursts within cortically-projecting basal forebrain neuronal populations.

**Materials and Methods**

All procedures were performed in accordance with NIH and local IACUC guidelines. Ten adult male Long-Evans rats were used as subjects. The rats were housed individually, and maintained on a 12 hr light/dark cycle. They were acclimated to the colony room for three days and handled daily for at least one week prior to beginning the experiment, during which time they were placed on food restriction until they reached 85-90 % of ad libitum weight. Rats were 5-6 months old at commencement of recording. Weight ranged from 450-550 gms. Water was available at all times. All behavioral testing occurred during the rats’ light cycle.

**Behavioral testing**

Six adult male Long-Evans rats were trained to leave a start box, traverse a space 25 cm in length, and push aside one of three distinct Lego objects in order to obtain a food pellet from a hole underneath. Each object covered one of three differently-valued pellets (100% sucrose, 25% sucrose,
or .02% quinine, Noyes/Research Diets). The task consisted of the rats learning to associate Lego objects with particular reinforcers. An object set consisted of three different Lego objects, each one consistently paired across sessions and across days, with one of the three types of pellet, resulting in a “best” object (100% sucrose), a “good” object (25% sucrose) and a “bad” object (.02% quinine). The rats began in an opaque Plexiglas start box with the experimenter tapping lightly on the doors of the box to orient the rat. Once the rat was oriented the doors would be opened and the rat would leave the box, head towards the Lego object that was placed a set distance away from the door of the start box, always in the same location for every object, and push it over in order to obtain the pellet in the food well underneath. As soon as the rat turned away from the object the rat was returned to the start box to begin a new training trial with a different Lego object. Intertrial intervals were somewhat variable, generally lasting as long as it took the experimenter to place the new object or objects on the board. The three Lego objects were presented a total of 18 times (6 times per object) during the initial training period of the daily experiment.

Subsequently rats were required to undergo test, or performance trials. Each test trial involved a presentation of two of the three objects. Both objects in the test trial, picked according to a set schedule which changed daily, were placed on the board 12 holes away from the start box door, equidistant from the center of the start box and covering adjacent holes where the appropriate food pellets were placed. The rat was oriented to the door of the start box in the same manner as in the training trials, and then had to approach the two objects and pick one of the two to push over and examine or eat the pellet underneath. The rat was consistently prevented from pushing over the other object during the pre-surgery training for the experiment, and by the onset of data collection the rats generally did not try to push over both objects. As soon as the rat turned away from the object that was pushed, it returned to the start box.

The first test trial was preceded by the sixth training trial. Each subsequent test trial was preceded by another set of three training trials (an encounter with each object one time). A total of 12 test trials with their interspersed training trial sets were performed each day, resulting in a total of 17 training trial encounters per object. (Fig. 1). Behavioral testing on an object set was carried out over the course of six days.
As it was of great importance that the rat not rely on olfactory cues to determine the associative relationship between Lego object and pellet type, the holes under the objects were saturated with the three types of pellet to mask any olfactory cueing. In addition to this measure, each rat was tested at the end of the experiment for olfactory cuing by placing the wrong pellet under the object and testing choice behavior. The rats consistently chose on the basis of object association as opposed to pellet type, indicating the rats do not use olfactory cues from the pellets underneath or tactile information to guide their behavior.

Surgery
Once rats were well trained on performance of the task, consistently pushing over the objects and choosing between two objects on test trials (typically 3-4 weeks), they underwent surgery for the implantation of wires for recording of LFPs. All surgeries were performed under aseptic conditions and under strict accord with animal care guidelines. Each rat was anesthetized using isoflurane anesthesia (4-5% induction, 1-2% maintenance) and was placed in a stereotaxic apparatus (Kopf Instruments). A burr hole was made through the skull, and the dura was carefully reflected to allow passage of the electrodes. Divets were made in several places along the skull for the insertion of anchoring screws. Twisted pairs of 50-micrometer, polyimide-coated, tungsten wire were implanted stereotaxically in the substantia innominata/nucleus basalis magnocellularis. (Target coordinates, relative to bregma, were posterior 0.8 mm, lateral 2.8 mm, and ventral 6.5-7.0 and 8.1 mm) Wires were led to a circular 12-pin connector that was fixed to the animal’s skull with dental acrylic. Post-surgically, animals were treated with buprenorphine (0.05 mg/kg) for analgesia and allowed to recover for 1 week before retraining.

Histology
Histological verification of the recording sites was performed subsequent to the end of experiment. All rats had electrodes placed in SI/Nbm of the basal forebrain (Figs. 2 and 3).

Recordings
Twisted wire pairs were staggered 1-2 mm in the dorsal/ventral plane in order to obtain LFP recordings local to the region of interest. In two animals, the reference electrode was instead a screw implanted in the skull overlying the cerebellum. No differences were found between these two groups of animals. LFP signals were first passed to an array of field effect transistors built into the connector assembly of a recording cable (NBLabs, Denison, TX). The same assembly housed LEDs used in tracking the animal’s position in the environment (DragonTracker, Boulder, Co). Signals were then amplified 4,000X and bandpass filtered between 1 and 475 Hz (Neuralynx, Tucson, AZ). Discovery software (Datawave, Boulder, Co.) was used to acquire and store data at a sampling rate of 512 Hz.

*Behavioral analysis*

Performance on choice behavior across days was tested with repeated measures ANOVA. Post hoc Sheffe analysis was utilized to determine effect of day on choice.

*Behavioral event timing analysis:*

Timing of object encounters was established by three methods. First, during the behavioral task, keystroke markers were inserted by an observer at the points of object encounter. Second, piezoelectric strips were placed directly underneath the objects so that immediately upon the rats’ touching of the object a signal would be registered by the Datawave acquisition system. Finally, a custom-made graphical user interface (GUI) created in Matlab and showing the rats’ pixel to pixel position was utilized. This data was used offline to precisely step through the data to mark encounters with objects for each rat on a trial by trial basis. A comparison of the three methods showed consistent reliability. Ultimately the GUI was utilized for the final analysis, which permitted the unambiguous determination of the start and end points of all trials. The start of each trial was taken as the time at which the animal crossed a line corresponding to the threshold of the start box. The arrival of the animal at an object was taken as the time that the animal crossed a line drawn just prior to the point at which tracking data indicated a full stop. LFP analyses were performed for data collected from between 2 seconds prior to the start times and 2 seconds after object arrival times. For test trials, choices were considered correct if the object associated with the pellet of higher value was chosen (i.e. the pellet containing the highest percent of sucrose).
Localization of Field Potentials

As field potential activity can be detected in one region through volume conduction from another region, having both reference and recording electrode within the brain area of interest suggests that the potential recorded is likely to be intrinsic to the SI/Nbm. However, for greater assurance, two separate animals were implanted with wires in both SI/Nbm and piriform cortex, a region that shows beta-frequency activity and is in close proximity to the SI/Nbm (Bressler, 1984). Local field potentials were obtained simultaneously from the two regions in the same way as described above.

Behavior during field potential recording was simple foraging on an open field. Periods in which beta frequency bursts were observed in the SI/Nbm were compared to the piriform cortex during the same time period in order to check for overlapping beta activity.

If the field potentials recorded are local in their origin, there should be some coincident firing rate differences in single unit activity during the bursting periods. To determine this, recordings were obtained from two additional rats with single unit wires placed in the SI/Nbm. Both single unit recordings and LFP recordings were obtained simultaneously from the two rats. Field potentials were obtained in the same way as stated above. For single unit recordings, a drivable bundle of five 25µm insulated tungsten stereotrodes was used. A small current was passed through each stereotrode to reduce final impedance to 250–500 kΩ measured at 1 kHz (Impedance tester IMP-1; Bak Electronics, Germantown, MD). A tripod-shaped mounting plate held the drive body containing a 28-gauge cannula through which wires were passed (modified from Kubie, 1984). The signal was band-pass filtered from 600- 6K for single units. Field potentials were obtained from the same wires with band pass filtering at 1Hz to 475Hz. The single unit and field potential data was acquired through the Cheetah data acquisition system (Neuralynx, Bozeman, MT.) Classification of cells was obtained through Offline Sorter (Plexon, inc). Each electrode bundle was implanted immediately above the left substantia innominata at coordinates relative to bregma of -.8mm posterior, 2.6 mm lateral and 5=-6.5 mm ventral. Recording sites in SI/Nbm were verified histologically in all animals.

Analysis of single unit rate changes during beta bursts

Changes in single unit firing rates, in the form of firing rate increases or decreases, during beta peak activity were then determined. For 219 single neurons, firing rates within a 500 ms time period surrounding the peak of individual beta-frequency oscillations were compared, by t-test, to firing
rates within equal-length time periods at points preceding and following beta oscillations by 10 s. This procedure minimizes the possibility that firing rate increases associated with beta-frequency oscillations merely reflect slower (minutes-long) variations in mean firing rate and, at the same time, maintains approximately equal variance about the calculated firing rate means. Only those neurons exhibiting statistically significant increases or decreases (at p<0.05) for both comparisons were considered to have activity rates altered during beta-frequency oscillations. Thus, the expected proportion of neurons expected, by chance, to exhibit firing rate changes is 0.05^2, or 0.0125. Comparisons of beta peaks with firing rate activity of single units indicated that cells within the SI showed increases or decreases in firing rate coincident with beta activity.

Phase relationship analysis
In order to determine whether there was a phase relationship between single units and beta oscillations in the basal forebrain, a Rayleigh test for uniformity of phase distribution was performed on spikes that occurred during the beta bursts. Portions of the record containing robust bursts were determined as described below, and filtered between 15 and 30 hz. For all spikes that occurred between two peaks of the signal the phase (45 degree bins), mean angle and vector length was determined.

LFP signal processing and analysis
Power spectral density analysis of LFPs was utilized to determine the precise frequency at which oscillatory bursts occurred and to determine whether the incidence of robust examples changed across days of the experiment. Although robust examples of beta-frequency oscillatory bursts were observable in raw LFP recordings of all animals across all days, the duration of such bursts varied continuously. That is, basal forebrain beta-frequency oscillatory bursts often subjectively appeared as discrete all-or-none events, but, as is the case with hippocampal 200-Hz ‘ripples’ and cortical ‘sleep spindles’, power in the associated frequency band (15-30 Hz) was found to vary continuously as opposed to bimodally. Thus, to determine the precise frequency at which prominent beta-frequency oscillations occur, particularly robust examples were identified by computing the power spectral density for all 0.5-second epochs of all trials and finding all epochs for which power in any single 1 Hz bin of the beta-frequency range exceeded 0.8% the total spectral power between 1 and 256 Hz.
Though this procedure was used primarily to determine precise beta-range frequency, a natural consequence of the analysis is that the proportion of trials containing particularly robust beta-frequency oscillatory bursts could be determined. As such, we also examined, by repeated-measures ANOVA, whether the proportion of such bursts of beta-frequency oscillation varied according to experimental day, trial type (training versus test), or object type (high, medium, or low value).

A different set of LFP analyses were designed to enable the precise determination of the peak times, amplitudes, and number of LFP oscillatory events. To this end, LFPs were digitally bandpass-filtered in the beta-frequency range (15-30 Hz). The filtered signal was then rectified and low-pass filtered (10 Hz). Trial to trial fluctuation in the timing and amplitude of peak beta-frequency oscillatory activity was obtained by finding the maximum value of this signal. The final output of this process was a signal which faithfully represented, with full temporal resolution, increases and decreases in the amplitude of beta-frequency LFP activity (Fig. 4). From this signal, the time, number, and amplitude of peak beta-frequency oscillatory activity for each trial could be determined and compared within and across days of task performance. Repeated measures ANOVA was used to determine significance of changes in timing and amplitude across days, trial type, and object type.

Results

Behavioral performance

The rats reliably learned to choose the Lego object of higher value (100% sucrose over 25% sucrose, 25% sucrose over 0.02% quinine, 100% sucrose over 0.02% quinine) across days, as is illustrated in Figure 5 (a). A main effect of “day” on a two-way, repeated measures ANOVA demonstrates learning, by indicating that choice performance improved across days of training trials, [F=7.52; p<.0003]. Post hoc Scheffe analysis demonstrated that choice behavior on day 1 was significantly different from days 3, 4, 5 and 6 (p<.05). No other comparisons between days were found to be statistically significant.

Beta-frequency Oscillations
LFPs from the SI/Nbm region of the basal forebrain were successfully recorded in six animals during performance of the visual associative learning task (Fig. 1). Recordings commenced when animals had returned to pre-surgical levels of task performance (typically 21 days). Animals were then recorded over the course of six days during which time a novel set of objects was paired with the high-sucrose, low-sucrose, and quinine pellets.

Visual inspection of LFPs recorded during performance of the task revealed discrete oscillatory events in the beta frequency range having durations of between 0.5 and 1.0 seconds. Such events could easily be observed even in raw LFP recordings having wide bandpass filtering (1-475 Hz) and dominated power spectral density color maps (Fig. 4). In all animals a conservative criterion was adopted to identify the most robust examples of LFP oscillations. This was used primarily to determine the frequency at which beta frequency oscillations occurred. The overall mean oscillation frequency was 25.8 (+/-2.7) Hz with a range, across all animals, of 20-29 Hz.

Task-dependence of beta frequency activity

Having established that significant beta-frequency oscillatory activity in the basal forebrain occurs spontaneously in awake, freely-behaving animals, we next asked whether such activity could be related to task performance. To examine this possibility, we compared the peak amplitudes and peak times of beta-frequency oscillations: 1. between days where behavioral performance was significantly different (see Fig. 5a); 2. between training trials associated with different objects; and 3. between training trials and test trials.

Beta frequency oscillations were significantly lower in amplitude on the later days of training when the objects were well learned than on the first day of training (See Fig. 5b). [F=9.3; p<.0001] Repeated measured ANOVA] On average, Day 1 peak values were 74.6% greater than those on Day 6 when the lowest overall amplitudes were observed. Post hoc tests for significance by day were then performed. Day 1 values were significantly different from all days with the exception of day 4. Notably, however, comparison of day 1 and day 4 was on the
borderline of statistical significance ($p=.07$, Sheffe method). Finally, we observed a significant correlation between performance and beta frequency peak amplitude across days and animals. ($Pearson \, r = -0.53, p<0.01$).

Object type and associated reinforcer (pellet) value mildly affected the amplitude of beta-frequency oscillations. Peak amplitudes associated with object 3 (100% sucrose or “best”) were on average 16.4 % greater than those observed for both object 1 and 2 (0.02% quinine or “bad” and 25% sucrose or “good”) which themselves differed by only 1.00%. A significant effect of object type was observed across training trials, collapsed across days [$F=3.61, p=0.031$]. Post hoc analysis revealed that the combined mean of object 1 and 2 trials (bad and good) was significantly different from the mean of object 3 trials (best). Notably, despite the slight change in amplitude as a function of object type or reinforcer value, the decrease in beta frequency peak amplitudes across days was observed for training trials with each of the three objects as well as for test trials. [All F values $p < 0.005$]

Beta-frequency oscillation amplitudes were unaffected by whether the animal was forced to make a choice between objects. No statistically significant differences were found between beta-frequency oscillatory bursts on training trials where the animals were faced with only one object and test trials where a choice between two objects was forced [$F=1.295; p=0.2038$]

We also determined whether the most robust oscillatory activity (i.e. peaks of beta easily visible in raw LFP records) changed in number across days. To examine this question we utilized the same burst identification criterion as previously described for determining mean oscillation frequency. The proportion of such bursts per trials did not significantly vary across days either for test or training trials, and did not vary across the three types of training trials considered separately (See Fig. 6a). (All F values $p>0.1$). The proportion of bursts did not differ between training trials and test trials [$F=1.345; p=0.187$].

We then examined the time relative to object encounter at which the peak oscillations occurred. Oscillatory events tended to surround the time of object encounter, with peaks occurring both before and after the timepoint of encounter. (Fig.4) On average, beta
frequency oscillatory activity peaked 230 ms after object encounter, but occurred prior to object encounter on 32% of all trials. No change in peak time relative to object encounter was observed across days, object type, or trial type (test vs training) (See Fig. 6b). [All F values p>0.12] Similarly, the proportion occurring prior to vs following object encountered was unaltered [All F values p>.19].

Localization of Field Potentials
Field potential recordings were obtained from the SI and piriform cortex simultaneously in two animals. Prominent bursts at the beta frequency were found in the SI, but not concurrently in the piriform cortex in both animals. While there exist other possible sources of volume conduction, the piriform cortex may be considered the most likely source due to its proximity to the SI/Nbm and known beta frequency oscillatory activity, suggesting that the bursts were intrinsic to the basal forebrain (Fig. 7). Further, two animals were implanted with both field potential and single unit electrodes in the SI to determine whether single units within the region are altered during beta activity. Bursts of beta-frequency oscillations were found to impact local neural activity. 219 cells were recorded and 22 or roughly ten percent of the neurons significantly altered firing rates (p<.0125) by either increasing or decreasing firing rate during the burst times as compared with rates during time periods of the same duration preceding and following the identified LFP bursts (eight increased, 14 decreased). This activity appears broad in scope in that both increases and decreases in rate were observed and both slow-firing and fast-firing neurons were affected (19 slow firing <1.5hz, 3 fast firing >9 hz). Such correlative activity provides further evidence that the field potentials recorded were, indeed local to the basal forebrain.

Phase relationship of LFPs and single units
The Rayleigh test to determine mean phase angle and strength of phase-specific firing revealed significant phase-specific firing for 8 of the 36 (22%) neurons which exhibited firing rate changes associated with LFP bursts of beta-frequency activity (p=0.05, Rayleigh’s criterion). This provides some further support that the observed beta-frequency oscillatory LFP bursts are intrinsic to the basal forebrain.

Discussion
The present findings indicate that oscillatory activity patterns in the beta frequency range are indeed a spontaneous and significant feature of basal forebrain activity in the awake, behaving animal. As previous work in-vitro and in anesthetized animals has demonstrated, cortically-projecting BF neurons can exhibit rhythmic discharge in the theta, beta, and gamma frequency ranges (Manns et al. 2003; Lee et al, 2005). As such, it was essential to determine whether or not such patterns of activity are a significant feature of basal forebrain neurophysiology during performance of a cognitive task and, if so, whether such patterns are sustained for long periods of time or are generated as phasic, task-relevant events. We found robust, short-lived oscillatory bursts of neural activity within the basal forebrain during a task which required the rats to learn associations between previously neutral objects and different types of reinforcement (Fig. 1). The bursts occurred with the oscillation frequency centered in the beta frequency range and were predominantly observed near object encounter, the timeframe in which learning of stimulus-reinforcer associations most likely occurs. The peak amplitudes of beta-frequency oscillations were highly negatively correlated with learning of the object association pairings, with the timepoint of greatest increase in performance mirroring the largest drop in burst amplitude.

Some evidence suggests that the origin of beta-oscillations could be intrinsic to the basal forebrain region itself. In vitro recordings indicate that non-cholinergic basal forebrain neurons are capable of generating rhythmic discharge in the beta frequency range and that such rhythmic discharge sometimes takes the form of activity bursts (Manns et al., 2003). Thus, beta-frequency LFP oscillations could originate in the basal forebrain through synaptic activity derived from coherent, rhythmic discharge of interneurons and/or feedback connections from projection neurons. Alternatively, both the prefrontal cortex (PFC) and amygdala are potential extrinsic sources of basal forebrain LFPs, since each of these regions exhibit beta-frequency oscillatory activity and send efferents to the basal forebrain (Campbell and Feinberg, 1993; Zaborsky et al., 1997; Collins et al, 2001; Alheid and Heimer, 1988). As discussed below, the PFC and amygdala are also targets of SI projection neurons, suggesting that beta-frequency oscillatory events in the SI reflect a functional interaction between multiple brain regions.

Unlike more tonically existent oscillatory activity, such as the theta rhythm in hippocampus, the short-lived (.5-1 sec) nature of basal forebrain beta-frequency oscillations suggests that they are...
possible candidates for providing a relevance signal during important learning timepoints. Historically, basal forebrain stimulation in the presence of a sensory stimulus is permissive of plasticity in the constituent sensory cortices (Dimyan and Weinberger, 1999; Kilgard and Merzenich 1998). Such plasticity is believed to be dependent on the cortical release of acetylcholine resultant from this stimulation, as in many instances concurrent blockade of cortical acetylcholine receptors, also blocks this plasticity (Goard and Dan, 2009; Bakin and Weinberger, 1996; Fromke et al., 2007). Furthermore, the bursts occur across days, even when the objects are highly familiar, indicating that they are signaling relevance over time. This would differ from recent work showing increases in beta oscillatory activity during exploration of a novel environment. Here Burke et al. (2008) placed mice in a novel environment and found that there were bursts of beta oscillatory activity during early exploration of the environment, but that these bursts were greatly diminished after a few minutes of exploration. This fits nicely with the view of the hippocampus as a structure that encodes novelty. However, in the basal forebrain, the bursts we observe, while largest in amplitude on the first day of learning a novel object set, are yet very low in amplitude for the first five encounters with the novel objects, increasing with later encounters (Fig. 8).

The characteristics of the beta activity observed in our study imply that its role in learning may be two-fold. The presence of the bursts across days may signal the described role in stimulus relevance, serving to modulate the temporal properties of its projections towards facilitating processing of a relevant stimulus. The amplitude modulation of the bursts, however, may reflect the theoretical function of cholinergic neurons of the basal forebrain in signaling stimulus uncertainty or unpredictability. This is consistent with established findings from studies implementing selective lesions of basal forebrain cholinergic neurons and examining attention to stimuli in an associative learning framework. These studies indicate that cholinergic neurons are required for increasing cortical processing when stimulus uncertainty is high (Chiba et al., 1995, Bucci et al., 1998).

The uncertainty of a stimulus or context is a property that has been proposed to facilitate learning of predictive associations in the environment in both animal (Dickinson & Mackintosh, 1978, Hall & Pearce, 1992, Holland & Gallagher, 1993; Cordova, 2005) and computational learning theories
(Maybeck, 1982, Dayan et al., 2000). Interestingly, a formal theoretical model of acetylcholine in learning and memory proposes that acetylcholine release in cortex is maximal when predictive uncertainty is highest and decreased thereafter. (Yu and Dayan, 2005). This pattern is remarkably consistent with the pattern of peak amplitude in our study, in that the peak amplitude is high when the rats are learning an object set and decreases once they achieve accurate performance (Fig. 5). Thus, the beta-bursts observed in this study may serve to efficiently convey differential information from the heterogeneous population from which they arise, a topic of interest for future investigation.

Further, the beta-frequency peak amplitudes were only marginally modulated by the hedonic value of the stimuli. Decreases in beta-frequency amplitudes across days were roughly five times greater than differences between object types, and cross-day decreases in amplitude were significant for all object types. This focus on relevance and not hedonic value is corroborated by the recent finding of BF neuronal ensemble bursting during presentation of motivationally significant stimuli. (Lin and Nicolelis, 2008) Both reward and punishment-predicting stimuli elicited robust short-lived bursting of BF neurons. While the authors found no neuronal bursting response to neutral cues, we did see beta-oscillatory field potential responses during the early encounters with a novel cue (prior to learning its association). This is most likely due to the fact that despite the continual changing of object sets to be learned, the relevance of cues at that point in space had been well established. This suggests that animals were able to use position within the environment or to generalize object type to register relevance, in the form of beta-bursts, to new stimuli.

Oscillatory bursts of basal forebrain neurons reflecting a relevance signal may contribute to the process of forming stimulus-reward associations, perhaps through modulation of attention. The PFC and amygdala are likely to be important targets for such a signal in that they are each interconnected with the basal forebrain and have been implicated in the learning of stimulus-reward associations similar to those utilized in the present work (Schoenbaum et al., 1998; Quinn et al, Manuscript in Preparation). In fact, recent data indicate that the PFC inputs to the basal forebrain are capable of altering both acetylcholine release and electrophysiological responsiveness to stimuli in other regions of neocortex (Zaborzsky et al., 1997; Nelson et al., 2005).
Previous work has shown that alterations in beta-frequency oscillations are observed in other brain regions during behavioral tasks or repeated stimulus presentation (Boeijinga and Lopes da Silva, 1989; Vanderwolf and Zibrowski, 2001; Zibrowski and Vanderwolf, 1997; Chapman et al., 1998; Ravel et al. 2003; Martin et al. 2007). The existence of beta in multiple systems during learning lends support to the view that beta-rhythmicity across disparate regions of the brain may be important in the processing of behaviorally relevant stimuli. In this respect, it is notable that theoretical work suggests that beta-, as opposed to gamma-, frequency rhythms are particularly well-suited to the development of coherent neural activity between spatially-segregated, but interconnected brain regions (Bibbig et al., 2002). Through widespread projections to nearly all regions of cortex, rhythmic bursts of activity within the basal forebrain could impact activity patterns in many cortical structures, having a profound effect on stimulus processing. Such rhythms could have the effect of aligning unit activity patterns across a number of cortical and sub-cortical brain regions, thereby impacting the critical processes of synaptic plasticity underlying learning.

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**References**


Figure 1: Behavioral task. Opening the doors of a start box initiated each trial. On each trial, the animal traversed a short path to encounter a single object or pair of objects. Each object was consistently paired with one of three different food pellets positioned beneath the object and accessible by displacement of the object (A = 100% sucrose, B = 25% sucrose, C = 0.02% quinine). Presentation orders were randomized. During the training phase of the task (left panel), each of the three Lego objects are presented one at a time paired with their associated food pellet. During the test phase of the task (right panel), two of the Lego objects are presented simultaneously, and the rat is allowed to approach one of the two objects in order to obtain the desired food pellet. Each daily training session is comprised first of seventeen training trials followed by twelve blocks of training and test trials. Each of these blocks was comprised by one test trial followed by three training trials (one for each object-food pellet pairing).
Figure 2: Visible in this photomicrograph are two holes resultant from low threshold current passed through field potential wires prior to perfusion. This is a representative placement, demonstrating that the wires spanned the Ventral Pallidum and the Magnocellular Preoptic Nucleus, both of which contain corticopetal basal forebrain neurons. Abbreviations: Acp, anterior commissure posterior part; Vp, ventral pallidum; Sib, substantia innominata basal; Mcpoa, magnocellular preoptic area; HDB, horizontal limb of the diagonal band. Bregma level: 0.4 AP (Paxinos).

76x62mm (600 x 600 DPI)
Figure 3: Summary of histological analyses. Subsequent to recording experiments, small marker lesions at the recording site for each animal were made to allow identification of both wires used for differential recordings of basal forebrain LFPs. In each case, the more ventral placement was found to lie within the substantia innominata sub-region of the basal forebrain.
Figure 4: Robust beta-frequency oscillations occur during task performance. Raw and filtered LFP traces and power spectral analyses from 2 different animals are depicted. In each, the top trace corresponds to the raw LFP obtained during a single trial on day 1 (top plot: training trial; bottom plot: test trial). The times at which the animal exited the start box and encountered the object are given by green and red lines, respectively. Note that in each case a prominent, short-lived oscillatory event occurs near the time of object encounter. Raw LFP traces were filtered in the beta-frequency range (15-30 Hz, middle traces) and subsequently rectified (bottom trace in each). The rectified signal was then low-pass filtered (<10 Hz, yellow traces) for use in detecting the amplitude and timing of beta-frequency oscillatory events. Power spectral analyses of the raw LFP (color-mapped images below LFP traces) reflected the discrete nature of these events and were used to determine the mean frequency for each animal (range 20-29 Hz).
A. Behavioral Results: Average performance by block shows greater than 85% accuracy on all choice types by day 5. Error bars = standard deviation.

B. Choice accuracy increases were accompanied by decreases in peak amplitudes of beta-frequency oscillatory events (*, p<0.01). Error bars = standard deviation. There was a significant correlation between performance and beta frequency peak amplitude across days and animals. (Pearson r = -0.53, p<0.01)
A. Proportion of trials containing robust bursts of beta-frequency oscillations (see methods for definition). Mean proportions across animals (+/- SD) did not vary as a function of experimental day.

B. Peak time of beta-frequency amplitude peaks. Mean time of peak beta-frequency activity (+/- SD) did not vary significantly across experimental day.

219x155mm (96 x 96 DPI)
Peak beta-frequency amplitudes across 5-trial blocks of day 1 when object sets are most novel (values normalized to the mean across all blocks). Mean normalized amplitude (+/- SEM) on the first five trials was lowest and significantly different than values observed in the majority of subsequent blocks (T-test analysis of block 1 versus each subsequent block, *p<.05 **p<.01).
Oscillatory bursts in LFPs recorded in substantia innominata do not coincide with similar bursts in piriform cortex. In this animal, LFPS were recorded simultaneously in substantia innominata and piriform cortex. Shown are LFP traces, each 600 msec in length, for two different time points distinguished by oscillatory bursts in the substantia innominata LFP recording.