Synchronous Activity in the Hippocampus and Nucleus Accumbens

*In Vivo*

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The hippocampus is one of the brain regions involved in cognitive functions, including learning and memory. Extensive studies have unveiled how information is processed within this system. However, the mechanisms by which hippocampal activity is translated into action remain unsolved. One important target of hippocampal projections is the nucleus accumbens, which has been described as the motivation-to-action interface. Previous experiments indicate that these projections can control information processing in this region by setting neurons into a depolarized state. Here, we report that membrane potential transitions in nucleus accumbens neurons are correlated with electrical activity in the ventral hippocampus, suggesting that hippocampal neural activity can determine ensembles of active accumbens neurons.

**Key words: nucleus accumbens; hippocampus; electrophysiology; schizophrenia; membrane potential states; in vivo intracellular recordings**

The hippocampus, a brain region involved in cognitive functions (Zola-Morgan et al., 1986; Muller et al., 1996), projects extensively to the nucleus accumbens (NAcc) (Kelley and Domesick, 1982), a brain region at the “limbic–motor interface” (Mogenson et al., 1980). Previous experiments have suggested that these projections can control information processing in the NAcc by setting NAcc neurons into a depolarized state (O’Donnell and Grace, 1995). The electrical activity of NAcc and dorsal striatal medium spiny neurons is characterized by periodical shifts from a very negative resting membrane potential (DOWN state) to a more depolarized (UP) state (Wilson, 1993; O’Donnell and Grace, 1995), which depends on excitatory synaptic inputs (Wilson, 1993; O’Donnell and Grace, 1995). Transection or stimulation of the fimbria-fornix, the fiber system carrying hippocampal afferents to the NAcc, resulted in disappearance or induction, respectively, of the UP state in NAcc neurons (O’Donnell and Grace, 1995). Furthermore, recordings in slices do not yield neurons with a bistable membrane potential (O’Donnell and Grace, 1993). Because activation of inputs from the prefrontal cortex (PFC) can evoke action potentials in NAcc medium spiny neurons only during the UP membrane potential state, it has been hypothesized that hippocampal afferents gate PFC–NAcc information flow by setting NAcc neurons into this depolarized state. Such a gating mechanism may define the ensemble of neurons appropriate to be active in a given context (O’Donnell, 1999), and its alteration may be responsible for pathophysiological changes in psychiatric disorders, such as schizophrenia (O’Donnell and Grace, 1998).

A prediction that arises from this model is that electrical activity in the ventral hippocampus would be synchronized with UP states in NAcc neurons. For example, in hippocampal CA1 and ventral subicular (vSub) regions, local field potentials show periodical sharp potential shifts (sharp waves and accompanying high-frequency oscillation, or ripples) during sleep or awake resting conditions (Buzsáki, 1986; Ylinen et al., 1995). Sharp waves in the CA1 region are induced by synchronous discharge of CA3 neurons, resulting in depolarization of CA1 pyramidal neurons (Buzsáki, 1986). Therefore, outputs of such synchronous activity in the hippocampus may be the driving force of NAcc membrane potential fluctuations. To test this hypothesis, we performed *in vivo* intracellular recordings from NAcc medium spiny neurons simultaneously with local field potential recordings from the ventral hippocampus.

**MATERIALS AND METHODS**

*Animals.* Simultaneous *in vivo* intracellular and local field potential recordings were performed from 25 neurons in 19 rats. Male adult Sprague Dawley rats (230–400 gm) were obtained from Taconic Farms (Germantown, NY). All experimental procedures were performed according to the United States Public Health Service Guide for the Care and Use of Laboratory Animals and approved by the Albany Medical College Institutional Animal Care and Use Committee. Rats were initially anesthetized with chloral hydrate (400 mg/kg, i.p.), followed by continuous supplemental anesthesia (chloral hydrate, 24–30 mg/hr) during the recording session via a cannula inserted intraperitoneally.

*Recordings.* Intracellular and extracellular electrodes were made from 1 mm outer diameter Omegadot borosilicate glass tubing (World Preci-
If the peak exceeded three SDs (sumed for the histogram, and the significance of correlation was defined events within the time intervals analyzed. Normal distribution was as-
were filled with 2 M NaCl and 2% Pontamine Sky blue. Intracellular
cells measured at 2 kHz) were filled with 2 M NaCl and 2% Pontamine Sky blue. Intracellular
electrodes (impedance of 3–8 MΩ) were filled with 2 M NaCl and 2% Pontamine Sky blue. Intracellular
electrodes were lowered into the NAcc [anteroposterior (AP), bregma +1.4 to +2.0 mm; lateral, 1.0–2.0 mm; vertical, −5.8 to −8.4 mm] and extracellular electrodes into the ipsilateral CA1 (AP, bregma −6.0 mm; lateral, 5.3 mm; vertical, −7.8 mm) or vSub (AP, bregma −6.3 mm; lateral, 5.2 mm; vertical, −7.0 mm). Once intracellular electrodes yielded stable recordings, simultaneous baseline recordings were performed for 5 min. Only neurons showing at least −50 mV resting membrane potential and 40 mV spike amplitude measured from threshold were analyzed and included in the study. All data were stored using custom-made software (Neuroscope) and analyzed off-line.

**Histology.** After completion of the experiments, extracellular recording sites were marked by ejection of Pontamine Sky blue. Neurobiotin was injected into the intracellularly recorded neurons by passing positive current (1.0 nA, 200 msec pulses at 2 Hz) for at least 5 min. Animals were killed by a lethal dose of pentobarbital (100 mg/kg) and transcardially perfused with ice-cold saline, followed by 4% paraformaldehyde. Brains were removed from the skull, cryoprotected in 30% sucrose, and sectioned using a freezing microtome. Serial 50-μm-thick sections were cut coronally. Either dye markers or neurons were identified and localized according to the atlas of Paxinos and Watson (1998).

**Cross-correlation analysis.** Cross-correlograms were constructed based on the methods described previously (Perkel et al., 1967). The cross-correlation strength, $S_{ccr}$, was defined as:

$$S_{ccr} = \frac{N_{cc} - N_0}{\sqrt{N_{mp} \times N_{gp}}}$$

where $N_{cc}$ is the number of potential shifts detected simultaneously in intracellular and extracellular recordings within a ±50 msec interval, $N_0$ is the chance level of coincident events given by either shuffled cross-correlogram or baseline events, and $N_{mp}$ and $N_{gp}$ are the total number of events within the time intervals analyzed. Normal distribution was assumed for the histogram, and the significance of correlation was defined if the peak exceeded three SDs ($p < 0.013$).

**RESULTS**

*In vivo* intracellular recordings were performed from 25 NAcc neurons, located in the core region. Seventeen of 25 (68%) neurons showed a bistable membrane potential (Fig. 1a). The presence of UP and DOWN membrane potential states was determined when a histogram of time spent at different membrane potential values showed a bimodal distribution fitting to a dual Gaussian curve (Fig. 1b). The DOWN state, or resting membrane potential (O'Donnell and Grace, 1995), was −78.1 ± 10.5 mV (mean ± SD), and the UP state was −66.8 ± 10.4 mV. Transitions to the UP state occurred at 0.55 ± 0.21 Hz. In all four bistable neurons injected with Neurobiotin, histochemical procedures revealed that these were medium spiny neurons (Fig. 1c).

Local field potentials in the hippocampus, either CA1 ($n = 6$) or vSub ($n = 7$), were recorded simultaneously with intracellular NAcc recordings from cells exhibiting a bistable membrane potential. These local field potentials exhibited large periodical negative shifts that occurred simultaneously with UP membrane potential states in NAcc neurons in all pairs recorded ($n = 13$) (Fig. 2a). Cross-correlograms indicated that hippocampal field potential shifts and transitions to the UP state in NAcc neurons occurred within <100 msec (Fig. 2b). Cross-correlation strength ($S_{ccr}$) was defined as the ratio between coincident number of events and total number of events. CA1–NAcc and vSub–NAcc recordings resulted in $S_{ccr}$ of 0.27 ± 0.08 and 0.30 ± 0.07, respectively. These values were significantly higher than the strength of shuffled cross-correlograms (0.05 ± 0.01 and 0.06 ± 0.01; paired $t$ test; $p < 0.00001$ for both of them). Because there was no difference in the strength and pattern of correlation between NAcc and CA1 or vSub, the data were pooled. Half of the recordings obtained from the CA1 region exhibited ripples ($n = 3$), which were also synchronized with UP states in NAcc medium spiny neurons (Fig. 2c). Ripples were defined as oscillations of >100 Hz (Buzsáki, 1986; Ylinen et al., 1995). An association was found between the location of NAcc neurons intracellularly recorded and their cross-correlation strength with hippocampal activity. $S_{ccr}$ values were higher for NAcc neurons located more medial and dorsal, in the medial core territory (Fig. 2e). Neurons located in the lateral core exhibited lower $S_{ccr}$ with hippocampal activity. A statistically significant association was found between $S_{ccr}$ and the mediolateral location of the NAcc neurons (nonparametric Spearman rank order correlation; $r = -0.554; p < 0.05$).

In addition to the hippocampal input, the NAcc receives excitatory afferents from the PFC (Phillipson and Griffiths, 1985). In a previous study, PFC stimulation failed to evoke transitions to the UP state in NAcc neurons (O'Donnell and Grace, 1995). It was then predicted that simultaneous recordings of intracellular NAcc activity and PFC local field potentials would yield a lower degree of cross-correlation compared with NAcc–hippocampus recordings. Although PFC local field potentials also exhibited slow negative potential shifts, they were not well correlated with transitions to the UP state in NAcc neurons ($n = 4$) (Fig. 3a). Cross-correlograms showed significant peaks in only two of four pairs (Fig. 3b). Comparison of correlation strengths among PFC–NAcc, shuffled PFC–NAcc, and hippocampus–NAcc pairs indicates that the cross-correlation between PFC local field potentials and NAcc membrane potential shifts was weaker than the cross-correlation between hippocampal local field potentials and NAcc membrane potential transitions (ANOVA; $F_{(2,30)} = 41.1; p < 5.0 \times 10^{-7}$) (Fig. 3c).

**DISCUSSION**

Hippocampal local field potentials were tightly correlated with transitions to the UP state in NAcc neurons, suggesting that...
Hippocampal inputs are important for the onset of UP membrane potential states in NAcc neurons. In contrast, the correlation between PFC local field potentials and NAcc UP transitions was weak. Such difference between excitatory inputs to the NAcc is consistent with previous anatomical and physiological findings. Five to 10% of hippocampal terminals in the NAcc contact cell bodies or proximal dendrites, whereas PFC axons are exclusively observed at distal dendrites (Meredith et al., 1990). This anatomical arrangement would result in hippocampal afferents having a stronger input on somatic membrane potential in NAcc neurons. Indeed, our finding of a cross-correlation strength between NAcc neurons and hippocampal local field potentials higher than that of NAcc–PFC pairs indicate that the PFC–NAcc projection is less important in driving the UP state than the hippocampal projections. Furthermore, the NAcc–hippocampal cross-correlation strength was higher when NAcc neurons were recorded from the medial aspect of the NAcc core. This may reflect the ventral hippocampus projecting primarily to the medial NAcc (Groe newegen et al., 1999).

UP and DOWN membrane potential states can be affected by...
the anesthetic agent used. Because the UP state is dependent on excitatory synaptic inputs, it cannot be seen in the presence of GABA-enhancing agents, such as barbiturates. On the other hand, these plateau depolarizations have been observed in chloral hydrate- or urethane-anesthetized animals (Wilson, 1993; O'Donnell and Grace, 1995) and even in locally anesthetized animals (Wilson and Groves, 1981).

Increasing attention is being paid to the role of neuronal ensembles in CNS information processing. Synchronous activity defining neural ensembles has been observed in the striatum (Jog et al., 1999), cerebellum (Thier et al., 2000), motor cortex (Laubach et al., 2000), PFC (Schoenbaum and Eichenbaum, 1995), and hippocampus (Deadwyler et al., 1996). These studies focused on spike firing in a population of simultaneously recorded neurons in behaving animals. Subthreshold membrane potential variations, however, were not considered. Because the UP membrane potential state brings NAcc neurons very close to their firing threshold, UP events can be seen as periods during which neurons are in a “ready-to-fire” state. Therefore, it would be important to consider the membrane potential states in the definition of active neuronal ensembles in systems exhibiting these oscillations (O’Donnell, 1999). Indeed, synchronous bistable membrane potential fluctuations in pairs of neurons have been reported recently in the dorsal striatum (Stern et al., 1998) and visual cortex (Lampl et al., 1999).

The results presented here support the idea of the hippocampal output gating corticostriatal information flow in the NAcc. The synchrony reported here is likely to be the result of hippocampal activity driving NAcc UP membrane potential states (O’Donnell and Grace, 1995). Thus, the activity of hippocampal neural ensembles may determine the ensembles of NAcc neurons that are appropriate to be set in the UP state, according to contextual cues (Fig. 4). This mechanism may provide a basis for the incorporation of hippocampal functions into motor planning and attentional mechanisms within the ventral basal ganglia circuits.
REFERENCES


