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Neuronal synchronicity in the pigeon "prefrontal cortex" during learning

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Abstract

In general electrophysiological studies focus on the investigation of changes in discharge rate of neuronal responses which are related to sensory or behavioral events. However, equally important for explanation of higher cognitive functions, learning, memory storage and complex behavior is the interaction between neurons that are connected in cell assemblies. Synchronized inputs onto a neuron are much more effective at eliciting the following activity than uncorrelated inputs. The goal of the present study was to determine either the changes in discharge rate of neurons in the pigeon nidopallium caudolaterale as well as the synchronicity of these neurons during a discriminatory learning task. We found rate modulation effects as well as modulation of synchronization during the learning process.

Keywords: Pigeon; Forebrain; Nidopallium caudolaterale (NCL); Eyeblink conditioning; Multiple single-unit activity; Synchronization

1. Introduction

Activity alterations of neurons are coded by changes in the firing rates of neurons (cf. [6,7,27]) and/or by the synchronicity between cells within functional networks [1,3,11,20,24,28]. These two aspects should be approached independently from each other. A synchronized input onto a neuron is much more effective in eliciting actionpotentials compared to inputs which arrive in an uncorrelated way [2,29,30]. Due to the non-linear effectiveness of synchronicity, small correlation strengths are able to cause strong effects. These correlations are dynamic since neurons have to participate in different cell assemblies [15] at different times, depending on stimulus context and behavioral demands [25]. Indeed, such predicted context dependent modulations of spike synchronization were shown in different sensory cortical areas [4,5,9,22].

The nidopallium caudolaterale (NCL) of birds is a neuronal network, that is involved in higher cognitive processes as working memory [8,17], choice behavior [16], organization of action sequences [12] and reversal learning [14]. Anatomical studies have shown that the NCL receives input from all sensory association areas [19]. These studies make it probable that the avian NCL is functionally equivalent to the prefrontal cortex of mammals.

In this study we investigated discharge rates and synchronicity of single units in the pigeon NCL while the pigeon learned a discriminatory delayed eyeblink conditioning. This procedure is an established conditioning task for which the mammalian prefrontal cortex is known to be involved [21,23,26]. We present data, which show for the first time near-synchronous activity in the pigeon NCL during the early acquisition phase of the conditioning procedure.

2. Materials and methods

2.1. Subjects

We recorded single unit activity in the Nidopallium caudolaterale (NCL) of one pigeon (*Columba livia*) during a discriminatory delayed eyeblink conditioning paradigm. The pigeon was prepared for recording by implanting a head-fixation block and a recording chamber under anesthesia with equithesin (0.3 ml/100 g bodyweight). The recording chamber was fixed to the posterior-lateral skull at a location directly above the NCL according to coordinates obtained from the Karten and Hodos [18] stereotaxic atlas of the pigeon brain,

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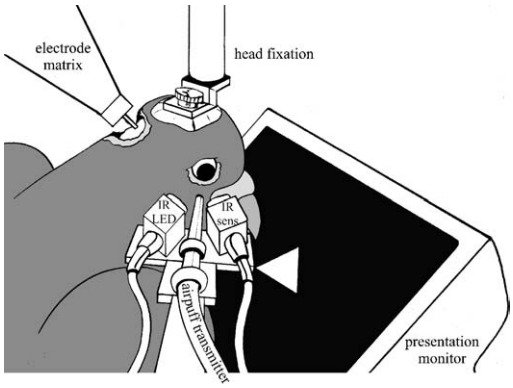

Fig. 1. Diagram of the pigeon in the test situation. Abbreviations: IR LED: infrared light emitting diode, IR SENS: infrared sensor.

the head-fixation block was fixed to the anterior part of the skull at the level of the eyes with dental acrylic. Additionally, the bone above the NCL was removed with a dental drill, so that the dura mater was visible. The opening was closed with tissue paper and paraffin wax. After a recovery period of 7 days, the animal was ready for the electrophysiological sessions.

2.2. Apparatus and procedure

During recording sessions, the pigeon was restrained by a loose cloth bag and placed on a foam couch in front of a TFT monitor. Its head was fixed into stereotaxic coordinates by the head holder (Fig. 1). Two different stimuli were depicted successively on the monitor: a white triangle on black background (3.7 cm, view angle 15°) was the CS−, a white heart on black background (3.7 cm, view angle 15°) was the CS+. The duration of CS+ and CS− was 2 s each. CS+ was overlapping and ending with the associated airpuff (duration 100 ms) which was applied to the right eye of the animal. Eyelid movements were detected by measurement of the reflection of an infrared light, which pointed on the eye (Fig. 1). The output of the detector was fed into the recording computer. CS+ and CS− were presented quasi-randomly, where not more than three equal stimuli were presented consecutively. Interstimulus interval was 15 s. One block consisted of 20 CS+ and 20 CS− stimuli. Interblock interval was 10–20 min. The number of blocks depended on the time a single unit could be held at an electrode.

2.3. Recording procedure

Before each recording session the scar tissue above the dura was removed with small tweezers. Single unit activity was recorded with the Eckhorn multielectrode system (Thomas Recording, Giessen, Germany) using Quartz-Platinum/Tungsten electrodes with an outer diameter of 80 μm an impedance of 2–6 MΩ at 1000 Hz. In this experiment we simultaneously recorded with four electrodes arranged in a square which could be positioned in different depths of the brain. The horizontal distance between the electrodes was 305 μm. Electrodes were caudally inserted onto the brain and advanced through the intact dura mater with the Eckhorn specific microdrive at an angle of 40°. The head of the pigeon was fixed at an angle of 60° ventrally (reference is the line between ear canal and beak). In different sessions the insertion site of the electrodes as well as the recording depths differed slightly. We noted the relative position of the insertion sites and the recording depths to reconstruct the recording sites in the histological slides. The mediolateral insertion position varied between L5 and L6 according to the atlas by Karten and Hodos [18]. Signals were amplified (Thomas Recording, Giessen, Germany) 1000-fold, bandpass filtered (500–5000 Hz) and continuously monitored on an oscilloscope and an audiomonitor. Single units were AD converted and stored on a computer (Spike 2, sampling rate 10 kHz, 16 bit) along with events of the conditioning paradigm (CS+, CS−, airpuff) and the trace with the signals from the eyeblink detector. After a session the pigeon was removed from the electrophysiological setup and the skull opening was closed with a cotton ball and a layer of laboratory tissue, which was fixed to the recording chamber with paraffin wax.

2.4. Data analysis

Spike activity was separated offline with a spike sorting feature of the program Spike 2 (CED) from background noise. Subsequently, the time of spike events were used for analysis. Raster diagrams and Peristimulus time histograms (PSTHs) were calculated summing the activity recorded under stimulus condition CS+/airpuff and CS−. Furthermore, we calculated the synchronicity of the spikes from the four electrodes using a cross-correlation analysis (MatLab) to check whether the spike timing of NCL neurons was correlated to each other. We also correlated the spike timing with the eyeblinks to determine eventual motor activity of NCL neurons. We counted the number of spikes occurring in a time interval of 300 ms after airpuff onset. Additionally we computed cross-correlations using spike times of simultaneously recorded neurons (window: 100 ms).

2.5. Histology

On the last day of recordings, the last recording track of one electrode was marked by electrolytic lesions (7 μA, 15 kHz sine wave for 10–15 min) in two different depths to reconstruct the insertion angle. The following day, the animals were given an overdose of equithesin (0.55 ml/100 g bodyweight) and perfused intracardially with saline followed by 4% formalin. The frozen brains were sectioned sagittally at 40 μm and stained with cresyl violet. The slices were controlled under a microscope to reconstruct the electrode penetration tracks. Using the coordinates and noted depths of the previous recordings we could reconstruct the recording sites of all electrophysiological sessions.

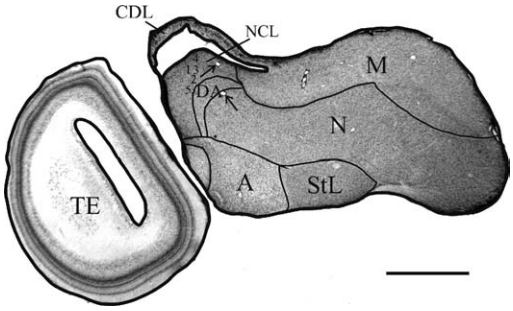

Fig. 2. Sagittal section of the brain including the lesions (arrow) made to reconstruct the recording sites and the penetration angle (1–5: recording sites of neurons 1–5). A: arcopallium, CDL: area corticoidea dorsolateralis, DA: tractus dorso-arcopallialis, M: mesopallium, N: nidopallium, NCL: nidopallium caudolaterale, StL: lateral striatum, TE: tectum. Scale bar = 2 mm.

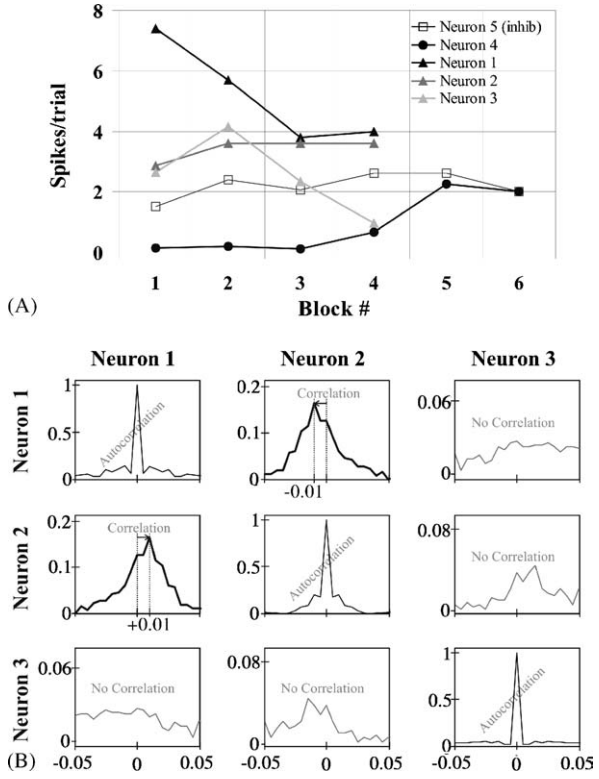

Fig. 3. Results of the discharge rate and synchronicity analysis. (A) Changes in discharge rate to the airpuff stimulus in a time interval of 300 ms after stimulus onset in five neurons. (B) Cross-correlation analyses for spike times of the neurons 1–3. Note the cross correlation between neurons 1 and 2 with a lag of about 10 ms. *y*-axis: cross-correlation coefficient, *x*-axis: time (s). Analysed window: 100 ms, binwidth 5 ms.

3. Results

We recorded from 24 neurons (four neurons in six sessions) in the pigeon forebrain. One neuron responded with a decrease in discharge rate to the airpuff applied to the eye, five neurons responded with a phasic increase in discharge rate to the same stimulus. For one neuron we only acquired data from the first block, and therefore excluded this cell from further analysis. The recording sites of the neurons are shown in Fig. 2. The remaining neurons were unresponsive to the airpuff and to the stimuli depicted on the monitor.

In the following, results from neurons 1–5 are presented in detail. We recorded from neurons 1–3 simultaneously for four blocks of 40 trials (20 CS+ and 20 CS− stimuli), from neurons 4 and 5 in separate sessions for six blocks. The number of spikes occurring 300 ms after airpuff onset were counted, plotted as mean spike counts per trial in a diagram (Fig. 3A) and computed using regression analyses. Neurons 1 and 3 decreased their discharge rates significantly ($p < 0.05$) with increasing numbers of airpuffs, whereas neuron 2 and 4 significantly increased their discharge rate ($p < 0.05$). The responsiveness of neuron 5 remained constant.

Subsequently we determined the temporal interactions between neurons. Therefore, a total of 36 cell–cell interactions were analysed by means of cross-correlations. Spike trains of neurons 1 and 2 showed clearly a near-synchronous activity (Fig. 3B) (near-synchronous activity is generally defined as a spiking time correlation within a time window of ±10 ms). The analysis shows that neuron 2 was active about 10 ms before neuron 1. This correlation analysis was taken from block 2.

4. Discussion

The aim of the present study was to analyse simultaneous recorded single unit data from the pigeon forebrain during acquisition of a classical conditioning learning paradigm under the aspect of, (1) changes in discharge rate over time and, (2) temporal cell–cell interactions resp. synchronicity.

Regarding the aspect of rate coding, we found that 25% of our neurons recorded in the NCL responded with rate changes to an airpuff applied to the eye. Additionally, most of these neurons showed systematic alterations of their spiking level that probably reflects the ongoing pace of the learning procedure. Kröner and Güntürkün [13,19] have shown that the NCL receives projections from secondary sensory areas of all modalities including from the somatosensory and the trigeminal domain. Units responding to the airpuff therefore could code for the tactile event evoked by the airpuff. During classical conditioning procedures, the UCS is the only stimulus that is able to ignite a specific stimulus-response link. The activation of a substantial percentage of NCL-neurons to this stimulus, while being unresponsive to the others, therefore resembles activity patterns in the PFC. Everling et al. [10] could clearly show that neurons in the PFC are able to discriminate between targets and non-targets during a focused attention task, with strong responses to targets and less responses to non-targets. This indicates that PFC-neurons are able to focus on stimuli of relevance and may constitute a

filter for less relevant stimuli. In the present study we could show, that the units in the NCL, at least during the early phases of a conditioning task, selectively responded to the UCS (airpuff), and not to other stimuli (CS+, CS−) that initially seemed to bear less relevance. As shown by Kalt et al. [17] and Diekamp et al. [8], after prolonged training NCL-units start to code also for various other stimuli once their predictive power for reinforcement is established. Regarding the second aspect (synchronicity) we could for the first time describe near-synchronous cell–cell interactions in the NCL. In one case the neurons did not only synchronize their spike timing, but also responded to the airpuff. In another pair we found an increase in discharge rate elicited by the airpuff without a correlated spike timing between the two neurons. Although all three neurons responded to the airpuff with an increase in discharge rate, they need not unavoidably synchronize their spike timing. In monkeys, Riehle et al. [25] found a positive correlation between the growing stimulus expectancy and the occurrence of synchronous activity. They could also show, that in cognitive events like planning, expectation, and attention-guidance, neurons preferentially synchronize their spike occurrence without changing their discharge rate, whereas in externally triggered behaviorally relevant events (presentation of visual stimuli) neurons tend to synchronize their spike occurrence and modulate their firing rates at the same time. Under this aspect the observed synchronicity and the increase in discharge rate to the airpuff stimulus of neuron 1 and 2 could code for a behaviorally relevant external stimulus. Synchronization between neurons is a strong tool, with which also weak stimuli can activate an assembly of neurons [2,30].

This study shows for the first time near-synchronous spike timing combined with a modulation in discharge rate in the pigeon NCL. Although preliminary, these data clearly show that rate and time coding contributes to the learning procedure that alters the activity patterns of avian forebrain neurons.

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