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Research report

Neuronal encoding of meaning: Establishing category-selective response patterns in the avian 'prefrontal cortex'

Janina A. Kirsch[a,c,*], Ioannis Vlachos[c], Markus Hausmann[b], Jonas Rose[a], Man Yi Yim[c], Ad Aertsen[c], Onur Güntürkün[a]

[a] Department of Biopsychology, Institute of Cognitive Neuroscience, Faculty of Psychology, Ruhr-University Bochum, 44780 Bochum, Germany
[b] Department of Psychology, Durham University, Durham DH1 3LE, England, United Kingdom
[c] Bernstein Center for Computational Neuroscience, Albert-Ludwigs University Freiburg, 79104 Freiburg, Germany

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ABSTRACT

Forebrain association areas interweave perceived stimuli with acquired representations of own actions and their outcome. Often, relevant stimuli come in a bewildering variety of shapes and sizes and we slowly have to learn to group them into meaningful categories. Therefore, the aim of the present study was twofold: First, to reveal how single units in the pigeon's nidopallium caudolaterale (NCL), a functional analogue of the mammalian prefrontal cortex (PFC), encode stimuli that differ in visual features but not in behavioral relevance. The second aim was to understand how these categorical representations are established during learning. Recordings were made from NCL neurons while pigeons performed a go–nogo categorization paradigm. Responses during presentation of the two S+ stimuli and non-responding during presentation of the two S− stimuli were followed by reward. We recorded from two pigeons at different learning stages. In the beginning of the learning process, neurons were active during and shortly before reward, but only in go trials. These data suggest that during the early phase of learning avian 'prefrontal' neurons code for rewards associated with the same behavioral demand, while ignoring feature differences of stimuli within one category. When learning progressed, (1) category selectivity became stronger, (2) responses selective for nogo stimuli appeared, and (3) reward-related responses disappeared in favor of category-selective responses during the stimulus phase. This backward shift in time resembles response patterns assumed by the temporal difference (TD) model of reinforcement learning, but goes beyond it, since it reflects the neuronal correlate of functional categories.

1. Introduction

In humans and other mammals, the prefrontal cortex (PFC) is the essential structure to organize goal-directed behavior [32,39,58]. It allows to deal efficiently with an ever-changing world and to adapt to new behavioral demands [33]. The mammalian cortex has a laminar organization, while the avian pallium is organized in nuclei. The absence of a laminated component within the avian cerebrum led to the assumption that birds have virtually no pallium, but an enormously hypertrophied striatum instead. From this it was followed that birds were not capable for higher cognition but followed an instinct-based repertoire. However, birds are, like mammals, able to flexibly adjust their behavior to changing demands. Indeed, birds possess a forebrain structure, that is functionally equivalent to the mammalian PFC: the nidopallium caudolaterale (NCL) of birds resembles the mammalian PFC in anatomical connectivity [27], neurochemical organization [2], electrophysiological properties [7,25,43], and control of cognitive functions [7,15,16,35]. Here, therefore, we investigate how complex, category based learning processes are established in the avian NCL and how they change when learning progresses.

Since reward plays a central role in shaping behavior [6], its representation reflects the basis of goal-directed actions [58]. Midbrain dopamine neurons show increased activation when a reward is received [55]. When reward is contingently preceded by a cue, activity shifts backward in time to the reward predicting cue [47], such that it coincides with the reward predictor, but not with the reward itself. The orbitofrontal cortex (OFC) in monkeys, a substructure of the PFC, shows activity tuned to delivery and expectation of reward [18,51,53]. Reward-related activity was already reported to be present in the avian forebrain [1,24,61]. Furthermore, it has been demonstrated that the NCL is crucial for response selection [29] and not for the representation or memorization of external cues. Thus,

* Corresponding author. Tel.: +49 761 203 9575; fax: +49 761 203 9559.
E-mail address: Janina.Kirsch@ruhr-uni-bochum.de (J.A. Kirsch).

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we should expect cellular response properties that primarily code for the correct response and not for detailed stimulus properties. To test this prediction is the first aim of this study.

Under natural conditions, reward is rarely associated with a single stimulus, but rather with object classes. Object classes require a grouping process into categories along several dimensions [20,62]. These dimensions can be perceptual (stimuli share perceivable features) or functional (stimuli share a common outcome). There is strong evidence that the PFC plays a key role in perceptual categorization of various objects [12,26,34,37,54]. However, the neural coding of functional categories has not yet been studied. In a comparative approach using the pigeon model we therefore used a go–nogo categorization task. Two arbitrarily selected, non-similar stimuli instructed the animal to perform a 'positive' (go) response; two other arbitrarily selected, non-similar stimuli required a 'negative' (nogo) response. Thus, not the perceptual similarity but the behavioral meaning was a valid cue for the pigeon to make a correct response. We expected to find NCL neurons that responded equally to members of the same category, although their visual appearance had virtually nothing in common. To distinguish between categorization processes and reward expectancy at a single-cell level, rewards were given for correct responses in the positive ("hits") and the negative ("correct rejections") category.

The second aim of the present study was to understand how coding properties of NCL neurons change during the process of category learning. It is possible that coding switches from a reward- to a category-centered type and, thereby, 'moves' backward in time to the reward predicting cue as proposed by Schultz and co-workers for the OFC [49,52,53].

2. Materials and methods

2.1. Behavioral task

Two pigeons (*Columba livia*) were trained on a visual go–nogo task. Water consumption was restricted to daily training sessions. Prior to training, a head-fixation block was mounted onto the skull under anesthesia (equithesin, 0.3 ml/100 g). All procedures were in compliance with the guidelines of the National Institutes of Health for the care and use of laboratory animals and were approved by a national committee (North Rhine-Westphalia, Germany).

During training and recording, the pigeons were restrained, fixed at the head and placed in front of a TFT-monitor (Fig. 1). Mandibulations were registered by a light barrier below the beak. A trial started with a cue light switching on for at least 1.5 s (cue phase) to raise the attention of the pigeons to the upcoming stimulus. Mandibulation during this phase restarted the trial. After the cue phase a stimulus (go: heart or lightning; nogo: triangle or cross; each white on black background, width: 4.5 cm, 18° viewing angle) was presented in the binocular visual field of the pigeon for maximally 5 s. In go trials, mandibulation during the first 2 s of the stimulus was detected, but reinforcement (1.5 s water delivery) was delivered after these 2 s. Mandibulation after that time interval resulted in immediate reinforcement (hit). This ensured that neuronal responses to the stimulus could be separated from responses to water delivery. The stimulus switched off together with the end of the reward. Mandibulation during the nogo stimulus (false alarm) and refraining from mandibulation during the go stimulus (miss) were punished by 3 s lights off. Refraining from mandibulation during nogo stimuli (correct rejection) was reinforced 500 ms after stimulus offset (nogo reward). Stimuli were presented pseudorandomly.

Early in learning, electrophysiological recordings began in one pigeon, when the performance was low (BEGINNER), in the other pigeon after reaching the criterion of three consecutive days with performance >70% (EXPERT).

2.2. Recording procedures

Single-unit activity was recorded using glass isolated Pt/Tungsten electrodes with a shaft outer diameter of 80 μm and metal core diameter of 25 μm. The tips of the electrodes were sharpened (diameter 4 μm) and the impedances of the electrodes were 1–4 MΩ. Electrodes were advanced through the NCL with a mechanical microdrive (Thomas Recording, Giessen, Germany) at an angle of 50–55° to the vertical plane at a position of L4-7 according to the atlas by Karten and Hodos [21]. We did not prescreen neurons for task-related activity such as visual responsiveness or stimulus selectivity. Rather, we randomly selected neurons for study by advancing each electrode until the activity of one or more neurons was well isolated and then data collection began. This procedure was used to ensure an unbiased estimate of NCL activity. Signals were amplified (1000–2500×), filtered (500–5000 Hz), monitored on an oscilloscope and a loudspeaker, and digitized using a computer software (Spike 2, CED) with a sampling rate of 20 kHz, 16 bit. Stimuli, beak movements, and water delivery were digitized at a sampling rate of 1 kHz.

2.3. Data analysis

2.3.1. Behavior

The performance (percentage of correct responses) in each stimulus condition and in go vs. nogo trials, percentage of response types (hits, misses, correct rejections, and false alarms) and the reaction time after stimulus onset in each stimulus condition and in go vs. nogo trials were analyzed. The mean performance and percentage of response types of the pigeons was calculated by averaging the performances obtained in the training and recording sessions. Mean reaction times were calculated by averaging the time span between stimulus onset and first mandibulation in the recording sessions. One sample *t*-test was used to test for differences between performance and chance level (50%). All within-subject statistical analyses were conducted using paired *t*-tests, all between-subject statistical analyses were done by using unpaired *t*-tests. Alpha-level was adjusted (Bonferroni) whenever multiple testing procedures were used. Significance level was 0.05 in all cases.

2.3.2. Neuronal responses

2.3.2.1. Spike detection and spike sorting. The procedure used for the detection and sorting of spikes consisted of several consecutive steps and was implemented mainly in Matlab. Initially, the median and standard deviation of the originally recorded signals were calculated. A threshold of five times the standard deviation was then applied to detect the action-potentials. The value of the threshold was in some cases manually adjusted, depending on the signal-to-noise ratio. Every time the signal crossed the threshold a frame was cut out of the original trace spanning a time window from −0.4 to +1 ms around the point of threshold crossing. The resulting events were called "wavemarks" in the following.

Subsequently, two spike sorting methods were used which differed in the way they extracted the most relevant information for the subsequent clustering. The first one was a principal component analysis (PCA) that reduced the number of dimensions to three, which essentially explained most of the variance of the initial data. The second one was a wavelet analysis (WL) that yielded ten components. The results of either method were then fed into KlustaKwik (KK) for the actual spike sorting to be performed. KlustaKwik is an application for unsupervised classification of multidimensional data based on a CEM algorithm [3]. It returns an index for each wavemark indicating the cluster it belongs to. The number of clusters is computed by maximum likelihood estimation. In some rare cases when the results where not satisfactory, the two dimension-reducing methods were skipped and all data-points were fed into KlustaKwik. However, results were rarely better than those by using PCA or WL.

As a next step we adopted several criteria to further improve the sorting results: (a) The values of the probability density of inter-spike intervals (ISI) below 3 ms had to be below 0.01 (1%). (b) The isolation score, a value indicating the quality of isolation by computing the distance between clusters [22], was required to be above 0.8. The higher the isolation score, the better the classification, with a value of 1 indicating perfect isolation. In addition to this we demanded all clusters to contain more than 1800 wavemarks, as smaller clusters would not contain sufficient spikes for a meaningful rasterplot and histogram analysis. Depending of how well they satisfied to the above criteria, PCA + KK or WL + KK was chosen. All clusters by either combination that did not satisfy any of the criteria were dismissed from further analysis.

2.3.2.2. Classification of neuronal responses. Only spike data from correct trials were used since there were insufficient incorrect trials in the EXPERT to permit statistical analysis. Histograms (binwidth 50 ms) and raster diagrams were calculated under each stimulus condition (MATLAB). To classify the response types we determined the mean inter-trial interval (ITI) activity by averaging the neuron's discharge rate in all 10 s intervals before cue onset. Subsequently, a response threshold to 95% confidence interval based on mean ITI activity was calculated.

First, neurons were classified as "responsive" or "unresponsive", depending on whether in at least one stimulus condition the activity surpassed the response threshold (called "neuronal response" in the following). The "responsive" neurons were further classified as "categorical" when the neuronal response was restricted to the stimuli within one category (go *or* nogo) or as "non-categorical" when the neuronal response occurred in all stimulus conditions to equal extent (go *and* nogo), or only in a single stimulus condition (e.g. only in "heart trials"). The categorical neurons were then further subclassified depending on whether (1) the neuronal response occurred in a specific trial phase (after stimulus onset: STIM; before reward: PRE-REW; after reward: REW), (2) the neuronal response occurred in two separated trial phases, or (3) the neuronal response was more generally distributed over the whole trial and could therefore not be allocated to a specific trial phase. For the "non-categorical" cells, the trial phase of the neuronal response was determined in the same way (after stimulus onset: STIM; before reward: PRE-REW; after reward: REW).

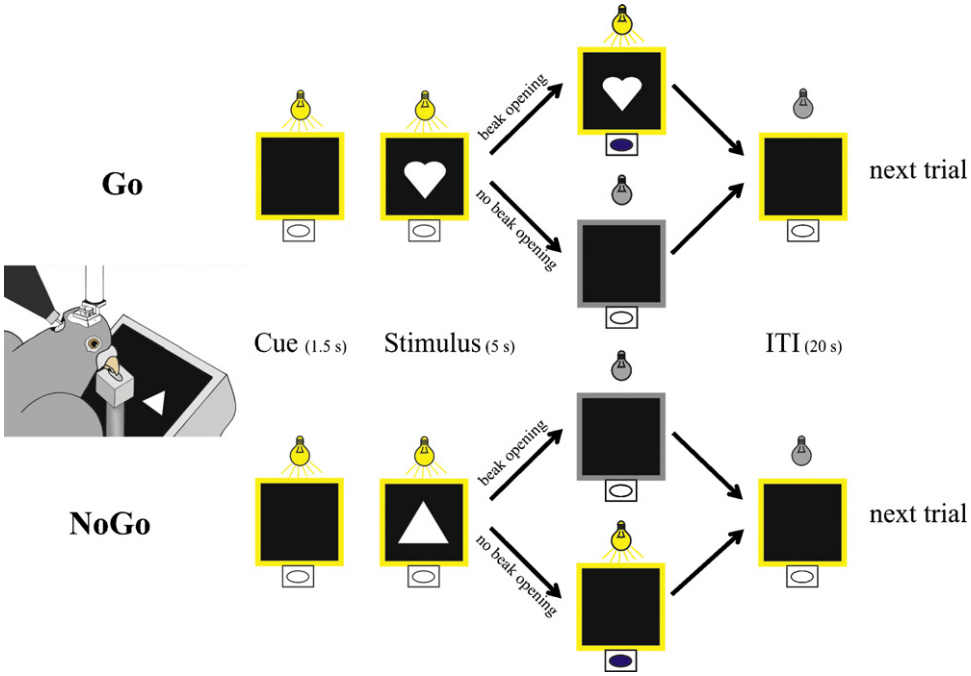

Fig. 1. Behavioral task. Inset on the left side: The pigeon sat in a foam couch looking on a monitor. The head was fixed and the reward could be delivered into a small plastic container below the beak of the pigeon. Beak movements (mandibulations) were detected by an infrared light barrier. The task consisted of two trial types (go and nogo), each containing two different stimulus types (go: heart or lightning; nogo: triangle or cross). Main figure: All trials began with a cue phase of 1.5 s where the cue light switched on. Afterwards one out of four possible visual stimuli were presented, instructing the animal about the type of trial (go vs. nogo). The stimulus phase took maximally 5 s. Mandibulation during the presentation of one of the go stimuli or rejection from mandibulation during the presentation of one of the nogo stimuli resulted in delivery of water for 1.5 s. Misses of go trials and mandibulation during nogo trials caused a mild punishment (all lights and stimuli off for 3 s).

Neurons were classified as STIM when the time between stimulus onset and the timepoint the threshold was passed was shorter than the time between passing threshold and reward onset. Neurons were classified as PRE-REW when the time between stimulus onset and threshold crossing was longer than the time between threshold crossing and reward onset, and when the threshold crossing occurred before reward delivery. Finally, neurons were classified as REW when the neuronal response reached threshold after reward delivery.

2.3.2.3. ROC analysis of categorical neurons. To examine the time course and strength of category selectivity, a sliding ROC analysis was performed. This was done for the categorical neurons only. Plotted are each neuron's area under ROC curve (AUROC) values for two time epochs: (1) around stimulus onset and (2) around reward delivery. The area under curve values were derived as follows: Bins of 200 ms were used to calculate the distribution of firing rates within the same bin across trials for both go and nogo stimuli. The ROC curve was then generated by plotting for each observed firing rate the proportion of one distribution lying above this value against the proportion of the second distribution lying above the value. Those proportions represent the true positive and false positives rates, respectively. The resulting ROC curve gives a measure of how well separated the two distributions are. To quantify the results, the area lying under the ROC curve was computed. The values for the AUROC lie between 0.5 and 1. A value of 0.5, for example, suggests that the two distributions completely overlap, whereas the maximum value of 1 indicates that the two distributions are completely separated.

2.4. Histology

In the last recording session, the tip location of one of the electrodes was marked with a small electrolytic lesion by passing a 3.3–4.65 μA AC current (50 kHz; 30 min) through the electrode tip. The next day, the pigeons were deeply anesthetized and perfused intracardially with 0.9% saline, followed by 4% paraformaldehyde solution in 0.1 M phosphatebuffer. The brains were embedded, cut into 40 μm slices in the sagittal plane parallel to the electrode penetrations, stained with cresylviolet, and microscopically analyzed to reconstruct recording positions.

3. Results

3.1. Behavior

The pigeon labeled BEGINNER received a total of 147 behavioral sessions. Of those, 97 sessions comprised a pre-training in which the pigeon learned to mandibulate to obtain reward and became familiar with the stimuli. Afterwards, the pigeon was subjected to the final training procedure. The first electrophysiological recording was conducted after 10 training sessions. Of the following 40 sessions, 25 were dedicated to training only and 15 were conducted together with electrophysiological recordings. In one training, and five electrophysiology sessions, the pigeon responded in less than 25% of the trials. These data were excluded from further analyses.

The pigeon labeled EXPERT received a total of 197 behavioral sessions. Of those, 118 sessions comprised the pre-training (see BEGINNER). Afterwards, the pigeon was subjected to the final training procedure. The first electrophysiological recording was conducted after 15 training sessions. Of the following 64 sessions, 37 were dedicated to training only and 27 were conducted together with electrophysiological recordings. In nine electrophysiology sessions, the pigeon responded in less than 25% of the trials. These data were excluded from further analyses.

The performance calculated over training and electrophysiology sessions was $56.56 \pm 0.6\%$ (mean $\pm$ SE) for the BEGINNER (44 sessions) and $80.13 \pm 0.86\%$ for the EXPERT (65 sessions). Table 1 (upper row) shows the performances of the two pigeons for the four different stimulus conditions (go: lightning, heart; nogo: triangle, cross).

Both for the BEGINNER and the EXPERT, performances were significantly above chance level of 50% (one sample *t*-tests; BEGINNER: $t(43) = 11.2$; $p < 0.001$, EXPERT: $t(64) = 35.1$; $p < 0.001$). For the BEGINNER, performance between stimulus conditions within categories were significantly different between heart and lightning stimuli (alpha-adjusted multiple paired *t*-tests (Bonferroni); $t(43) = 3.76$; $p < 0.05$), but did not differ between triangle and cross ($t(43) = 1.41$; n.s.). Performance between stimulus conditions between categories differed significantly between heart and triangle stimuli (alpha-adjusted multiple paired *t*-tests (Bonferroni); $t(43) = 3.16$; $p < 0.05$) and between heart and cross stimuli

Table 1
Upper row: mean percentages with standard error of correct responses in the four trials types. Lower row: mean response times with standard error of correct responses (hits) in go trials and of incorrect responses (false alarms) in nogo trials.

		Go		Nogo	
		♥	🗲	▲	✚
Correct responses (%)	BEGINNER	69.38 ± 2.99	57.95 ± 4	50.74 ± 3.18	48.18 ± 3.41
	EXPERT	86.61 ± 1.97	91.5 ± 1.4	74.8 ± 1.44	67.58 ± 1.61
Response time (ms)	BEGINNER	1968 ± 192	1956 ± 170	1060 ± 250	1152 ± 247
	EXPERT	831 ± 107	1047 ± 162	2172 ± 173	2248 ± 188

($t(43) = 3.55$; $p < .0.5$), but did neither differ between lightning and triangle nor between lightning and cross stimuli (all $t(43) < 1.3.5$; n.s.). For the EXPERT, all comparisons between stimulus conditions differed significantly from each other (alpha-adjusted multiple paired t-tests (Bonferroni); all $t(64) > -4.29$; $p < 0.05$).

Moreover, the percentages of response types were analyzed: correct responses (go trials: hits, nogo trials: correct rejections) and incorrect responses (go trials: misses, nogo trials: false alarms) for BEGINNER an EXPERT (Fig. 2). Within-subject comparison revealed that for the BEGINNER the hit-rate was significantly higher than the miss-rate (paired t-test; $t(43) = 4.29$; $p < 0.001$), but the difference between correct rejections and false alarms was not significant (paired t-test; $t(43) = -0.17$; n.s.). In the EXPERT, both the hits–misses and the correct rejections–false alarms differences reached significance (paired t-tests; all $t(64) > 15.34$; $p < 0.001$). Between-subject comparisons revealed significant differences between BEGINNER and EXPERT in all response types (unpaired t-test; all $t(107) > -7.02$; $p < 0.001$). These data show that the BEGINNER already learned to some extent to mandibulate in the presence of go stimuli, but failed to learn not to respond in the presence of nogo stimuli. In the EXPERT, the difference between hits and misses in go trials increased, compared to the BEGINNER, and additionally, the difference between correct rejections and false alarms was significant, which was not the case in the BEGINNER. However, in the EXPERT the difference between hits and misses was still bigger than the difference between correct rejections and false alarms, indicating that both EXPERT and BEGINNER learned better to respond to go stimuli than to not respond to nogo stimuli.

Reaction times (time between stimulus onset and first mandibulation) were obtained in electrophysiology sessions only and are shown in Table 1 (lower row). For the BEGINNER, the reaction times calculated from go onset (hits) were significantly longer than reaction times calculated from nogo onset (false alarms) (paired t-test; $t(9) = 2.29$; $p < 0.05$), the inverse pattern was observed in the EXPERT with longer reaction times in nogo trials (false alarms) than in go trials (hits) (paired t-test; $t(16) = -12.12$; $p < 0.001$). Additionally, the EXPERT responded significantly faster in lightning trials than in heart trials (paired t-test (Bonferroni-adjusted); $t(16) = 3.08$; $p < 0.05$). Between-subject comparison revealed that in go trials (hits), the EXPERT responded significantly faster than the BEGINNER (unpaired t-test; $t(25) = 6.56$; $p < 0.001$) whereas in nogo trials (false alarms) the BEGINNER responded significantly faster than the EXPERT (unpaired t-test; $t(25) = -4.09$; $p < 0.001$).

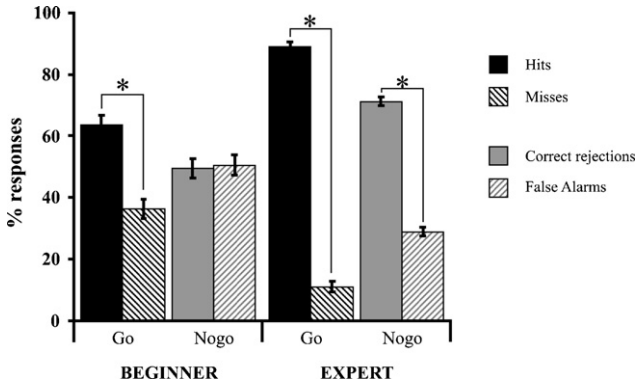

Fig. 2. Frequency of response types of the BEGINNER (left) and the EXPERT (right) for go trials (hits: solid black bars; misses: black/white striped bars) and nogo trials (correct rejections: solid grey bars; false alarms: grey/white striped bars). The error bars represent standard error of the mean, significance level was 5%.

3.2. Electrophysiology

A total of 96 single neurons were analyzed: 42 from the BEGINNER and 54 from the EXPERT. Recordings obtained in sessions in which the pigeons responded in less than 25% of the trials or recordings that could not be stabilized for the entire session were not analyzed. Fig. 3 depicts the classification of the neurons. In the BEGINNER, 20 neurons (48%) and in the EXPERT 30 neurons (56%) were responsive during the task, with 15 (75%) and 22 (73%) being categorical in the BEGINNER and EXPERT, respectively. Electophysiological recordings were performed after pigeons revealed fundamental differences in performance levels as a result of differently intensive training (the EXPERT received 21 pre-training sessions and 5 regular training sessions more than the BEGINNER). Thus, we are inclined to believe that the present findings are a result of differences in training and do not reflect general individual differences.

3.2.1. Responses of categorical neurons

Nine categorical neurons in the BEGINNER responded in a specific phase of the trial with seven neurons responding to the reward in go trials (example given in Fig. 4A_1) and one to the reward in nogo trials. One neuron responded at the end of the trial with showing increased activity in go and depressed activity in nogo trials. Four categorical neurons had a generally different activity level between go and nogo trials that could not be assigned to a specific trial phase (Fig. 4A_2). Two neurons responded in two temporally separated trial phases. In all trial types, both exhibited a short activity depression before the first mandibulation and an activation response to reward in go trials (Fig. 4A_3).

Sixteen categorical neurons in the EXPERT responded in a specific phase of the trial. Four neurons responded to reward in nogo trials, but not to reward in go trials. However, eight neurons responded before the go (Fig. 4B_1) and one before the nogo reward. Furthermore, two neurons responded shortly after stimulus onset in go trials only (Fig. 4B_3). An intermediate pattern was exhibited by five neurons, which responded in two different trial phases: three neurons responded before the reward in go trials, but after the reward in all trial types, two neurons responded after the stimulus in go trials and after the reward in all trial types (Fig. 4B_2).

3.2.2. Responses of non-categorical neurons

In the BEGINNER, non-categorical neurons were distributed over all trial phases: STIM ($n = 2$), PRE-REW ($n = 1$), REW ($n = 1$), and before the first mandibulation in a trial. In the EXPERT, one neuron

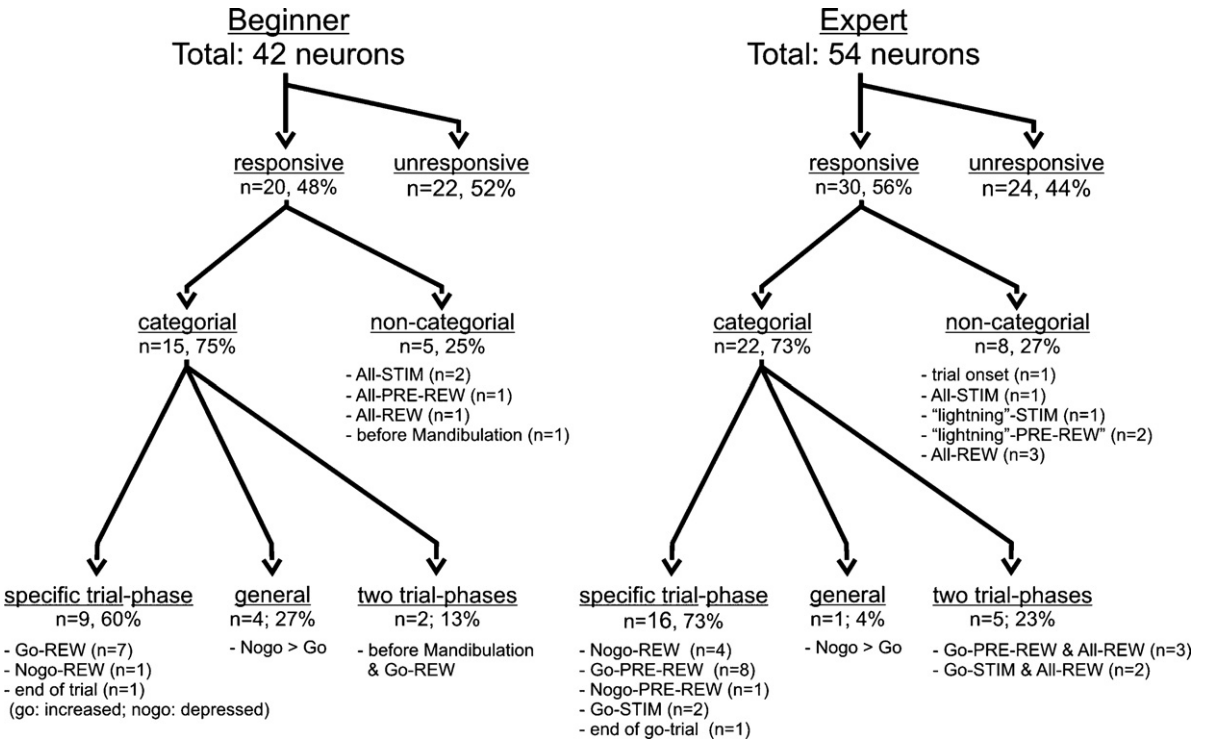

Fig. 3. Response classes of the neurons recorded from the BEGINNER (left) and the EXPERT (right). The fractions given in percentage were based on the total number of the level above, respectively. First, the neurons were divided in "responsive" and "unresponsive" neurons, depending on whether they responded to events within the task. Second, they were classified in "categorical" and "non-categorical", depending on whether they responded to stimuli within on category only or not. Finally, they were analyzed regarding the trial phase in which the neuronal response occurred.

responded to all stimuli, three neurons responded to all rewards. One neuron responded to the cue light indicating the start of the trial and three neurons were responsive to only on specific stimulus, the "lightning" (one after stimulus onset, two before the reward).

3.2.3. Histological reconstruction of the recordings sites

Histological reconstruction of the recording sites of task-related neurons is illustrated in Fig. 5. All neurons were within the borders of the NCL. There was no regional clustering concerning the response properties of the neurons.

3.2.4. ROC analysis of categorical neurons

To examine the time course and strength of category selectivity, a sliding ROC analysis was applied. This was done for the categorical neurons only (14 in BEGINNER, 21 in EXPERT). The results are shown in Fig. 6. Plotted are each neuron's area under ROC curve values for two time epochs: (1) around the stimulus onset (−2 to 2.5 s) and (2) around the reward delivery (−2.5 to 5 s). The area under curve values were derived as follows: Bins of 200 ms were used to calculate the distribution of firing rates within the same bin across trials for both go and nogo stimuli. The ROC curve was then generated by plotting for each observed firing rate the proportion of one distribution lying above this value against the proportion of the second distribution lying above the value. Those proportions represent the true positive and false positives rates, respectively. The resulting ROC curve gives a measure of how well separated the two distributions are (cf. Section 2). To quantify the results, the area lying under the ROC curve was computed. The values for the AUROC lie between 0.5 and 1. Values were sorted according to each neuron's mean ROC value across the respective phase. These plots suggest that the effect of category was stronger in the EXPERT with highest ROC values after about 800 ms. To quantify the strength of the category effect we calculated a Wilcoxon rank sum test over the mean AUC and maximal AUC values in the stimulus and reward phase between BEGINNER and EXPERT. In the stimulus phase, mean AUC values were 0.52 (BEGINNER) and 0.54 (EXPERT) ($p < 0.01$), maximal ROC values were 0.61 (BEGINNER) and 0.69 (EXPERT) ($p < 0.01$). In the reward phase, mean AUC values were 0.51 (BEGINNER) and 0.53 (EXPERT) ($p < 0.01$), maximal AUC values were 0.56 (BEGINNER) and 0.69 (EXPERT ($p < 0.01$).

4. Discussion

The present study shows that neurons in the pigeons' NCL are able to categorize stimuli according to their functional meaning, thereby neglecting their visual properties. Thus, cellular coding at the level of the avian 'prefrontal cortex' is primarily goal-centered and less input-oriented. Additionally, our data reveal that neuronal activity patterns in an experienced animal occur mostly during stimulus delivery, i.e. during an early time point within a trial. In contrast, NCL neurons of a novice animal were activated after reward onset, and thus, towards the end of a trial. Taken together, these findings indicate that functional categories are learned at 'prefrontal' level in pigeons by a switch from reward- to categorized stimulus-coding that establishes goal-related neuronal activity patterns.

In our design, four different stimuli arbitrarily constituted two categories (go and nogo). The two stimuli within each category were physically different in shape and pigeons had to learn a common response strategy by rote. Our data show that the two intracategorical stimuli elicited comparable neuronal response patterns. Thus, functional categorization seems to establish a category-selective coding at 'prefrontal' level in pigeons. Different types of responses could be distinguished: responses occurring directly after stimulus presentation (STIM), responses occurring shortly before reward delivery (PRE-REW), and responses occur-

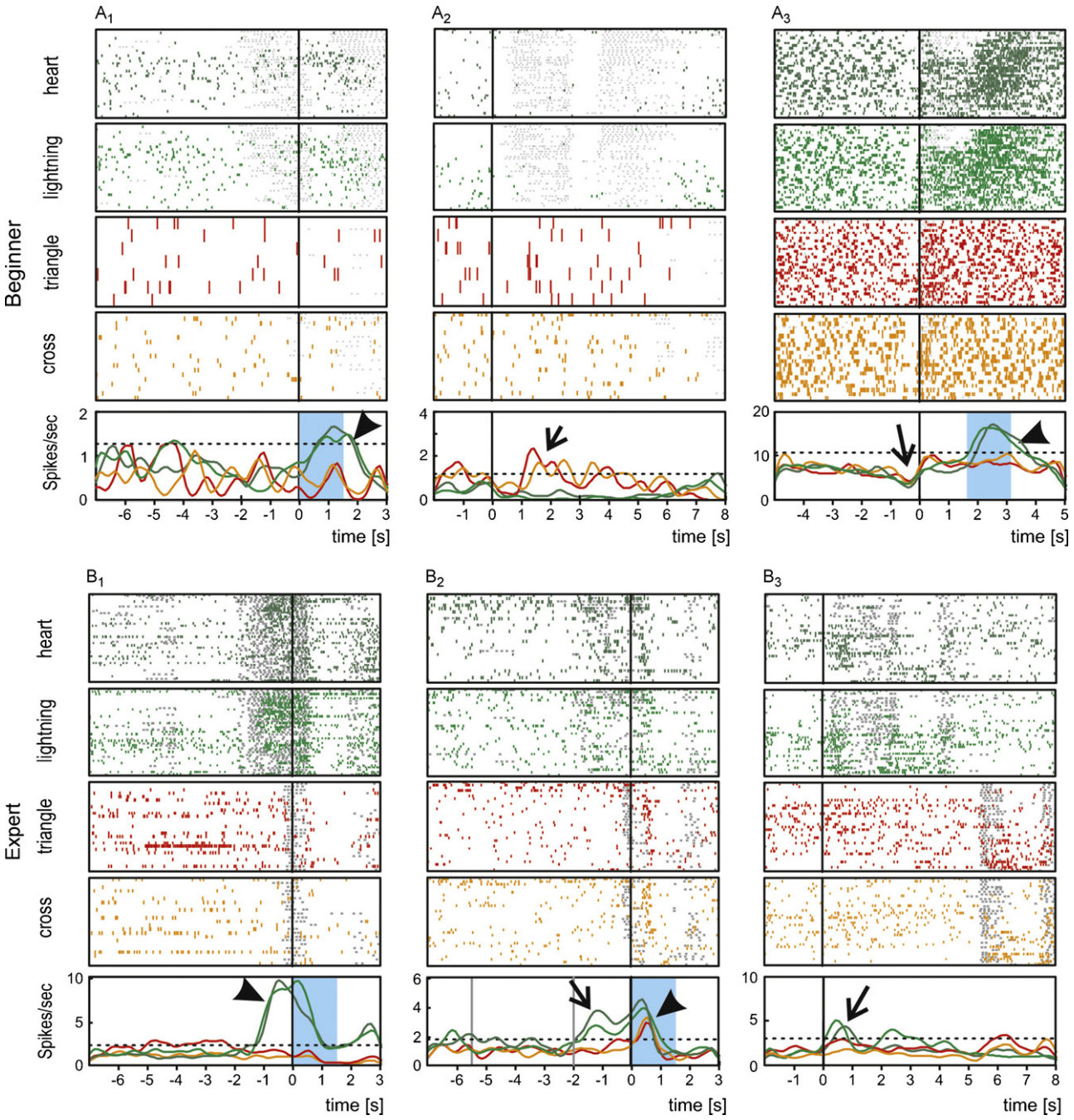

Fig. 4. Examples of neuronal responses collected from the BEGINNER (A) and the EXPERT (B). In each subplot the first four rows contain the raster diagram of the four different stimuli (from top to bottom: heart, lightning, triangle and cross; green: go, red: nogo). The grey dots within the raster diagram represent the mandibulations of the pigeon. The plot at the bottom of each subplot depict the histograms (binwidth 50 ms, filtered with a gaussian kernel) of the four raster diagrams above with the same color code. The dotted horizontal line represents the response threshold (mean ITI discharge rate +2 SD). In the subplots A_1, B_1, and B_2 the raster diagrams and the histograms were aligned to reward onset, in the subplots A_2 and B_3 to stimulus onset and in subplot A_3 to the first mandibulation after stimulus onset. The light blue area represents the time span of reward delivery. A_1 (Go-REW) shows a neuron which responded to the reward delivery in go trials (arrowhead). A_2 (Nogo > Go) shows a neuron which showed a general difference between go and nogo trials, without being triggered by a specific event (arrow). A_3 (before first mandibulation and Go-REW) shows a neuron the activity of which was suppressed about 500 ms before the first mandibulation occurred (arrow) and then responded to the reward in go trials only (arrowhead). Although in this plot the data for the nogo trials were obtained from incorrect trials (otherwise the mandibulation had not occurred), and therefore the blue reward area is not valid for the nogo trials, this neuron did not respond to the reward in correct nogo trials (not shown). B_1 (Go-PRE-REW) shows a neuron which increases discharge rate before reward onset (arrowhead) and peaked at reward delivery in go trials only. B_2 (Go-STIM and All-REW) shows a neuron which responded to the go stimuli onsets with a first peak (arrow) at about 800 ms after stimulus onset (depicted by the grey vertical line at timepoint −2 s) and peaked a second time after reward onset in all conditions (arrowhead). The grey vertical line at −5.5 s represents the nogo stimuli onsets. B_3 (Go-STIM) shows a neuron which responded to the stimulus onset on go trials only (arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ring after reward delivery (REW). Within each response type, responses were selective or non-selective to either category. The results of two other studies with chicks are comparable to the present study. The first investigated the arcopallium [1], the second the medial striatum [61]. Concerning the arcopallium, the authors described go- or nogo-selective activities prior to and during reward which mostly did not differ between the two go conditions, and thus, could encode the memorized association between colors

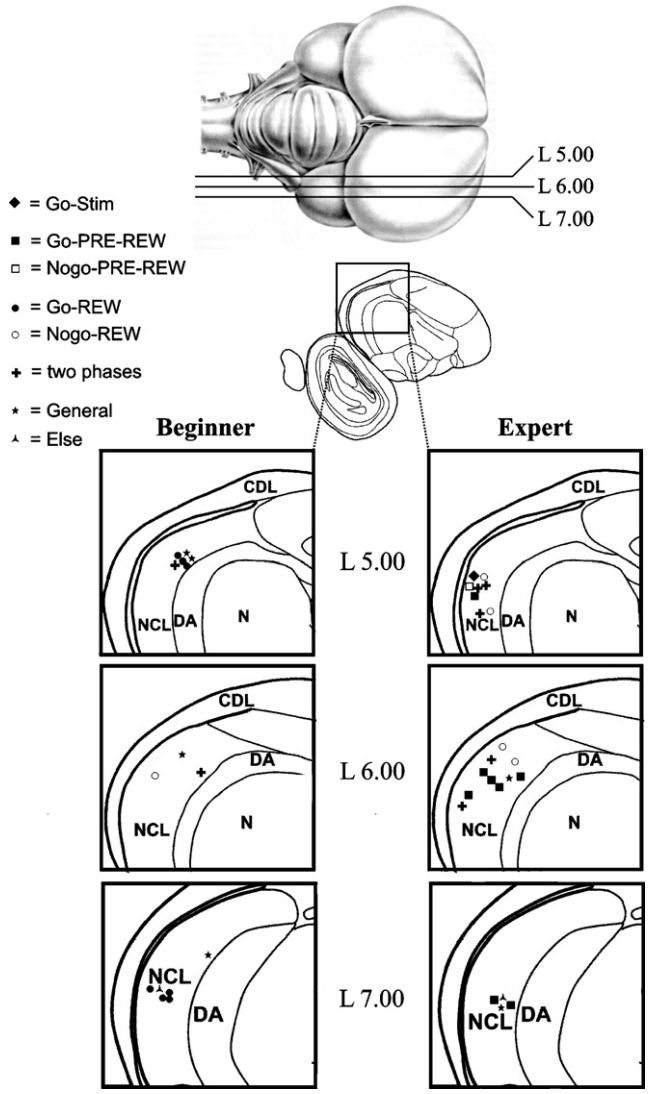

Fig. 5. Schematic sagittal sections of the pigeon brain recording sites. The uppermost drawing represents the dorsal view on a pigeon brain with caudal left and rostral right. Lines depict the position of section planes shown in the six subplots at the bottom. The line drawing in the middle represents a section at L5.00 to illustrate the region enhanced in the subplots. Each symbol represents the recording site of one neuron. CDL: area corticoidea dorsalis; DA: tractus dorso-arcopallialis; N: nidopallium; NCL: nidopallium caudolaterale. Figures adapted from graphs in the pigeon brain atlas [25].

and reward [1]. However, correct rejections were unrewarded, and therefore it is also possible that the activity patterns observed simply reflected reward expectancy. The same problem is present in studies reporting differential neural responding in go and nogo trials in pigeons [8,23,25] and monkeys [52,53]. In contrast, Yanagihara et al. [61] reported a medial striatal neuron type which responded only to rewarded go, but not to rewarded nogo stimuli. Thus, this cell type reflects a categorization-like coding at striatal level and is comparable to the present 'prefrontal' results.

Although most neurons in the EXPERT responded between stimulus onset and reward onset, we only found few neurons that coded for a single stimulus according to its perceptual properties, and thus independent of its functional association. This is similar to studies revealing a coding of behavioral significance in the PFC of monkeys [56,57]. Purely stimulus-driven responses are more likely to be found in the primary and secondary visual areas of birds, upstream to NCL [5,27,36]. Despite the fact that either the accuracy or the reaction times of the EXPERT differed between "lightning" and "heart" trials, this difference was only visible at neuronal level in the stimulus specificity of three neurons. This strongly suggests that neurons in the NCL do not primarily code stimulus appearance, but rather stimulus category. Studies investigating neuronal responses in rats performing a 2-odor of 4-odor go–nogo task showed that OFC neurons, comparable to the neurons in the present study, responded selectively to cues based on their associative significance [44–46]. Thirty percent of the OFC neurons exhibited selective activity during evaluation of the odor cues after learning had occurred, but they rarely exhibited selective activity during odor evaluation before the rats reached learning criterion, and far fewer reversed selectivity after reversal. The authors concluded that the OFC used the information from the basolateral amygdala, which encodes the motivational significance of the cues, in the selection and execution of an appropriate behavioral strategy. What makes those data different from that obtained in the present study is the relation between the positive cues, which predicted delivery of delicious reward as sucrose solution and the negative cues predicted a disgusting "reward" as quinine solution. Consequently, the rats stopped responding to negative cues. By contrast, in our design the pigeons had the possibility to receive identical rewards in both go and nogo trials. Therefore, the category association was determined only by the behavioral requirement. Our data, thus, reveal a cellular correlate of the ability of pigeons to categorize various stimulus patterns [13,14,17,20,28,30,59].

A major result of our study is the observation that in the BEGINNER all and in the EXPERT most of the neurons responded selectively in go trials. This is very similar to observations during recordings from the monkey OFC [52,53]. Why are go and nogo categories represented asymmetrically? Due to our design, in which water was delivered after hits (go) and correct rejections (nogo), the presence or absence of a reward can be ruled out as a reason for this difference. Another explanation could be the fact that only go trials required a discrete motor response, whereas nogo trials required the suppression of an action. Thus, the nogo trials could be irrelevant for the pigeons. This could imply that at cellular level, go selective activities serve motor preparation. If this would be true, then also neuronal activities to spontaneous mandibulations during ITI would be expected. However, this was not the case (Fig. 7).

One possible explanation for the asymmetric representation of go and nogo trials could be the single-code/default strategy of pigeons [4,23]. This strategy implies that pigeons only learn, and therefore code for, the go stimuli and respond by default (refrain) to other patterns. Accordingly, nogo stimuli should not be encoded by the network. However, some findings in the present study contradict this hypothesis. First, when performance increased during learning, as observed in the EXPERT, several neurons started responding after or prior to reward delivery. In the EXPERT only one single neuron responded exclusively after the delivery of the nogo reward. Contrary to this first finding several neurons in the BEGINNER exhibited a general increase in activity during nogo trials, whereas in the EXPERT only one single neuron of this pattern was detected. Although the number of nogo-selective cells was still small, their presence confutes a strong version of the default response strategy. Second, concerning the behavior, the distribution of percent correct responses in go and nogo trials shows that especially the EXPERT reached more hits in go trials than correct rejections in nogo trials. In other words, there were about 49 sessions in which the pigeon performed between 80 and 100% correct in go trials, whereas only in 16 sessions the pigeon performed at the same level in nogo trials. If the default response strategy would simply consist of a pure nogo-reaction, we should expect the inverse result pattern. In the BEGINNER, more neurons differentiated in a

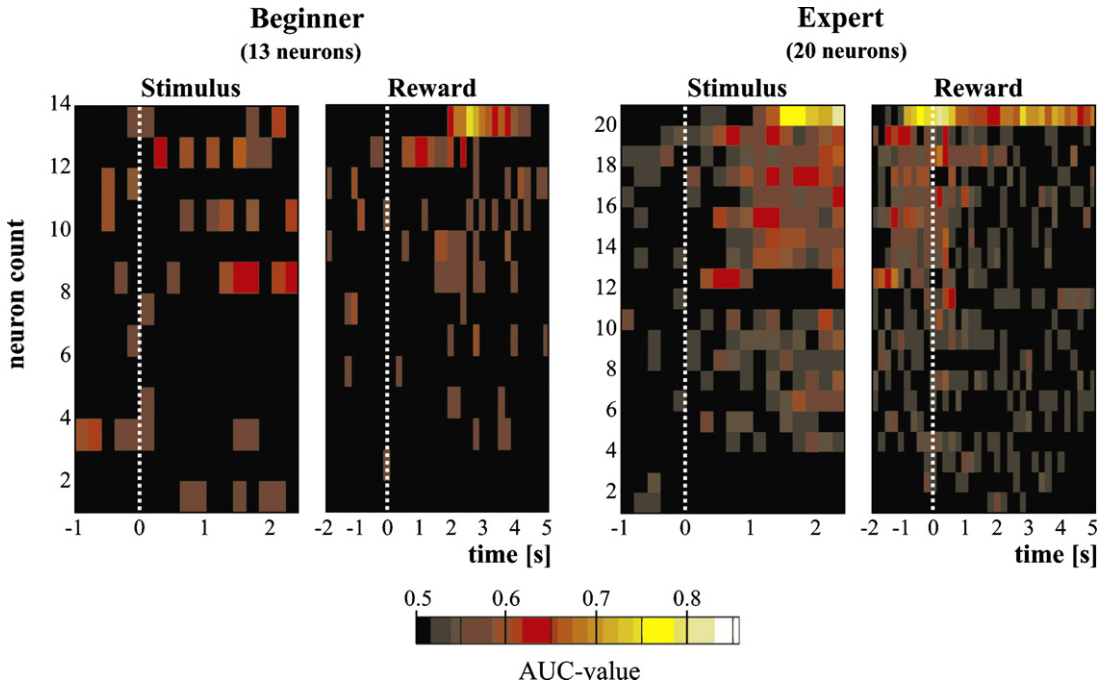

Fig. 6. Time course of category selectivity, using a sliding ROC analysis across all specifically responding categorical neurons in the BEGINNER (13 neurons, left column) and the EXPERT (20 neurons, right column) over two trial epochs: Around stimulus onset and around reward delivery. Each row represents the AUC time course of one neuron; the figures were constructed by sorting the neurons (*y*-axis) by their mean AUC value in the stimulus epoch. Category selectivity was stronger in the EXPERT than in the BEGINNER.

general fashion between go and nogo than in the EXPERT. This could provide some evidence that the BEGINNER learned the go trials first. At the stage the electrophysiological recordings were obtained, neurons just started to treat the nogo stimuli adequately. Neurons which code the nogo stimuli more generally could make the pigeons suppress the mandibulations in nogo trials. A third explanation is based on the finding that NCL [24] and OFC neurons [38,42] differentiated between different subjective reward values. Although go and nogo stimuli predicted the same amount of reward, the different time lags between stimulus onset and putative reward (go: 2–5 s; nogo: 5.5 s) might affect the subjective reward values of go and nogo rewards differently. Since nogo stimuli predict delayed water delivery, they were associated with a reduced reward value compared to the go stimuli. Thus, the asymmetry between the numbers of cells coding for go and nogo stimuli could result from the different subjective reward values of these cues.

A further central point of this study is the development of category selectivity during learning. As learning progressed, several changes occurred: (1) category selectivity was strengthened, (2) reward-related activity shifted backward in time to the time point of stimulus presentation. These observations will be discussed in the following.

ROC analysis revealed that the category selectivity of the neurons strengthened in the cause of learning. This presents strong evidence for the view that the NCL is responsible for selection of correct actions, irrespective of sensory stimulus features. Thus, at a neuronal level, category boundaries become sharper, concomitant with an increase in discrimination performance between categories. This indicates that neurons of the NCL code for the behaviorally relevant properties of cues, thereby neglecting their sensory properties. This is in agreement with a behavioral study, showing the NCL to be the essential structure for response selection

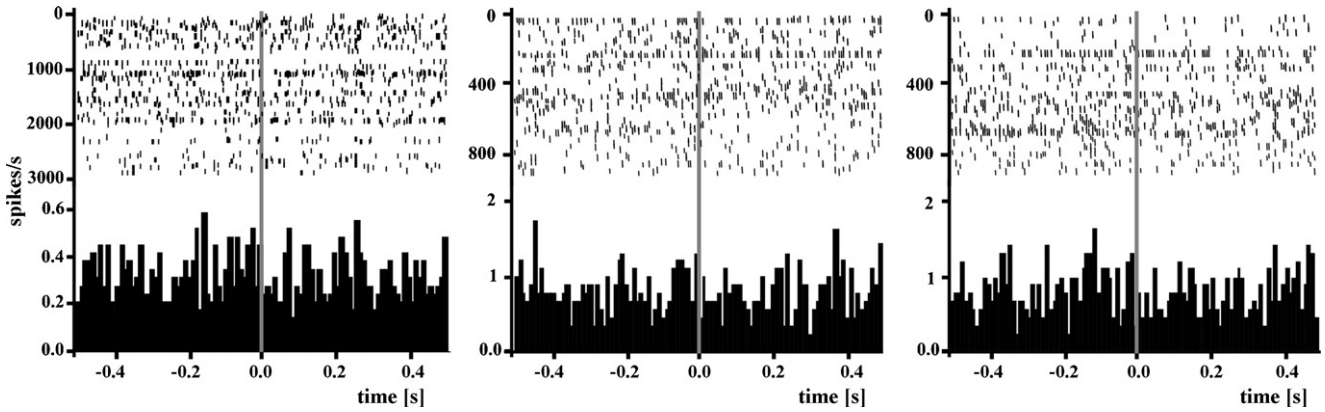

Fig. 7. Examples of the activity of three neurons which were classified as STIM-neurons correlated to motor behavior. Mandibulations take place at the origin of the abscissa (vertical grey line). An activity peak around 80 ms prior to beak movement would be characteristic of premotoric activity. Top: raster diagram, bottom: histogram (binwidth: 50 ms).

[29]. This kind of processing that is tuned to the functional properties of stimuli is reminiscent of a coding for the 'meaning' of cues.

The distribution of response types suggests that during learning the fraction of neurons responsive to reward delivery decreased, while the fraction of neurons responding prior to reward and also to stimulus onset increased. Thus, the feedback categorization which dominated in the BEGINNER, seems to develop into a stimulus categorization in the EXPERT. This conclusion is strongly supported by the neurons which responded prior to the go rewards and, additionally, to the reward itself in all conditions. The earlier, stimulus-activated cellular response could be responsible for the faster behavioral choices of the EXPERT. By contrast, the BEGINNER would behave more slowly in the categorization task, since the majority of its 'prefrontal' neurons were activated shortly before reward delivery and thus, later in the trial.

We have to explain which neuronal mechanisms contribute to categorization as such, resulting in the dominance of stimulus-driven responses during an advanced stage of learning. A theory which explains the generation of categories is the model of object recognition by Riesenhuber and Poggio [40,41]. This model is a hierarchical extension of the classical paradigm [19] of building complex cells from simple cells. It posits that categorization arises as inputs from feature selective neurons upstream to the prefrontal cortex (in our case the NCL) converge on neurons specialized for encoding behaviorally relevant variables. Additionally, the authors state that in principle, a similar division of labor may occur with any complex visual stimuli for which category membership must be learned. Thus, it might also serve as the force which leads to category selectivity without any need for differences in reward value. However, this model does not fit well to the categorization process investigated in the present study, since it requires categories defined by perceptual features and does not explain categorization determined by behavioral requirement.

An alternative mechanism could be the temporal difference (TD) [48] model which could more easily explain how pigeons learn to predict reward. This model stems from response properties of midbrain dopamine neurons and rests on the observation that the initial reward activity of midbrain dopamine neurons shifts back in time to the reward predicting cue when a reward consistently follows this cue. This event-driven activation of the dopaminergic system is also reflected in the frontostriatal system, mainly in the OFC [18,51,53]. In our view, three different arguments support the notion that the TD-model could explain the category selectivity of NCL neurons. First, the NCL receives a massive dopaminergic input from the midbrain [9–11,31,60]. Second, rewards obtained in nogo trials have probably a lower subjective value than rewards obtained in go trials [24]. Third, dopamine neurons in the midbrain are capable of coding different reward values, e.g. amount of reward [50]. Based on these arguments, it is possible that the category selectivity of NCL neurons stems from a simple threshold discrimination of dopaminergic input. That means that dopaminergic midbrain neurons which project to NCL neurons could respond stronger in go trials than in nogo trials and may, thus, reflect the different reward values between go and nogo. These expected reward value differences are transferred onto NCL via the dopaminergic projections. This could result in a simple contrast sharpening between strong and weak midbrain responses, finally establishing a functional category selectivity of NCL neurons. Although there might be additional alternative mechanisms also leading to a selectivity of functional categories, circumventing differential reward values, e.g. the association with a differential motor response, the described mechanism is the most parsimonious explanation. Taken together, our results imply that the beginning of a category learning process in pigeons is characterized by a categorization of feedback. Later on, when the animals are already able to solve the task at higher levels, the categorization of the stimuli is predominantly used to select the correct response.

Acknowledgements

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