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Single forebrain neurons represent interval timing and reward amount during response scheduling

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Abstract

Climbing activity, the gradual increase of neural discharge rate across a delay, has been suggested to play a crucial role in interval timing. However, most previous studies demonstrated climbing activity only in conjunction with tasks that involved a passive tracking of the passage of time, but that did not necessitate to actively time an event, e.g. a motor response. To demonstrate the significance of climbing activity for action timing, we trained pigeons in a self-control task requiring either immediate responding to a key after the onset of a light cue ('rapid-response' trials), or waiting for a fixed interval after cue presentation before responding to the key ('wait' trials). The cue also indicated whether a correctly timed response would be rewarded with a large or a small reward. Single-cell recordings in the Nidopallium caudolaterale, the avian prefrontal cortex, revealed that some neurons showed climbing activity between cue onset and response. Their increase in firing rate was flatter and reached the peak later in wait compared with rapid-response trials. An error analysis confirmed that, relative to correct responses, premature responses were accompanied by steeper, and tardy responses by flatter ramps. In addition, the climbing discharge pattern was modulated by the amount of the anticipated reward, suggesting that timing is an intrinsic property of neurons encoding other task-related information. These results demonstrate the behavioural and motivational significance of climbing activity in prospective information encoding. Our study supports a recent paradigm shift in our understanding of the vertebrate brain evolution, and it provides further evidence for the similarity between the mammalian cortex and the avian pallium.

Introduction

Unlike former psychological theories that refer to a centralized pacemaker or clock (Gibbon, 1977; Matell & Meck, 2000; Church & Meck, 2003), recent computational models propose that interval timing is an intrinsic property of distributed cells or cell assemblies (Buonomano & Karmarkar, 2002; Durstewitz, 2003, 2004; Miller *et al*., 2003; Reutimann *et al*., 2004; Staddon, 2005). Many of these models are based on the empirical observation that neural climbing activity, i.e. the gradual and steady increase in the discharge rate, reflects the length of the delay between two events. Crucially, the slope of the neural ramp is adjusted to interval duration, i.e. it is flatter and peaks later in long compared with short intervals. Such time-dependent climbing activity has been found in various structures, including posterior thalamus (Komura *et al*., 2001), posterior parietal cortex (Leon & Shadlen, 2003; Janssen & Shadlen, 2005), inferotemporal cortex (Reutimann *et al*., 2004), dorsolateral prefrontal cortex (Kojima & Goldman-Rakic, 1982; Rainer & Miller, 2002; Brody *et al*., 2003; Sakurai *et al*., 2004), cingulate cortex (Kojima & Goldman-Rakic, 1982), ventral striatum (Izawa *et al*., 2005), primary visual cortex (Shuler & Bear, 2006), frontal and supplementary eye fields (Schall, 2004; Roesch & Olson, 2005a), and premotor and supplementary motor cortex (Roesch & Olson, 2005a).

In these experiments, the beginning and the end of the to-be-timed intervals were indicated by external cues, regardless of behavioural performance and, in most tasks, it was not necessary for the animals to actively time their responses to receive a reward. This then raises the question in how far climbing activity might be merely a perceptual, input-driven phenomenon, caused by the passage of time between two sensory stimuli, as opposed to being internally generated to control the timing of future actions. If climbing activity is indeed used to plan and time actions, it should be possible to link it directly to self-paced responses in a motor timing task.

Along the same lines, an equally important question is how timing information, reflected in climbing activity, interacts with other prospectively encoded information that is relevant for action planning and self-control. Two studies have shown that, in the delayed-reward version of a self-control task, neurons in the forebrain integrate temporal information with the expected properties of the upcoming reward (Kalenscher *et al*., 2005b; Roesch & Olson, 2005b). In connection with climbing activity, such integration could change the slope of the climbing activity or the activation amplitude (as suggested by Komura *et al*., 2001), or both, reflecting different neural output signals whose impact on motivation and behaviour is elusive. To understand the functional significance of climbing activity, and of prospective information encoding in general, it would therefore be

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important to manipulate both climbing activity and expected reward properties in a timed response task.

To address this issue, we trained pigeons in a response scheduling task in which subjects were required to time a motor response in order to obtain either a large or a small reward, as indicated by a colour cue. The end of the interval, and hence the correct time point of responding, was not externally signalled, thus the response moment had to be internally planned. We recorded climbing activity in the Nidopallium caudolaterale (NCL) – a structure functionally comparable to the mammalian prefrontal cortex (Mogensen & Divac, 1982, 1993; Kröner & Güntürkün, 1999; Kalenscher *et al*., 2003, 2005a,b; Güntürkün, 2005). We were interested particularly in trials in which pigeons responded too early or too late, to infer the neural signal on which their response was based.

Materials and methods

Subjects

Nine naïve pigeons (*Columba livia*) were used in this experiment. For training and recording, they were put on a food deprivation schedule at approximately 80% of their free-feeding body weight. All subjects were kept and treated according to the German guidelines for the care and use of animals in neuroscience, and the European Communities Council Directive of 24 November 1986 (86/609/EEC). The research was approved by the ethics committee of the State of Nordrhein Westfalen, Germany.

Task

Pigeons were trained and tested in a cubic aluminium box (35 × 35 × 35 cm) that was equipped with one round pecking key, one feeder and one houselight. The key and feeder were arranged on the horizontal centre of the front wall of the box. The key had a diameter of 2.5 cm and was positioned 21 cm above the floor, or 13 cm above the feeder. The key could be illuminated in the four colours: red, green, white and blue. The white houselight was in the centre of the back wall of the box, 28 cm above the floor. The animals performed a two-factorial cued delayed response task (sometimes called differential reinforcement of long latencies task), with the factors 'required response latency' and 'reward amount'.

In one response latency condition, the wait condition (Fig. 1A), a trial began with the illumination of the response key in a unique colour indicating the response condition and the reward amount that would be delivered for a correct response. Upon key illumination, the pigeons had to refrain from responding on the key for at least 1500 ms (no-go period, see Fig. 1A), and then had to peck on the key within a time window of another 1500 ms (go-period). A correctly timed response was rewarded with a large (3 s access to food) or a small reward (1.5 s access to food) following a pre-reward delay of 500 ms; an early response within the 1500 ms no-go period was punished (5 s light off); no response had no consequence. The only purpose of the pre-reward delay was to facilitate contingency learning, as a delay of 500 ms is known to promote the formation of response–reward associations in pigeons. The lapse of the no-go interval and the on- and offset of the response window were not indicated to the animal and had to be internally estimated. Trials were separated by intertrial intervals (ITI) of varying duration. The ITIs were adjusted to compensate for differences in response latency as well as reward and punishment durations, so that all trials across all conditions were of identical length. In the other latency condition, the rapid-response condition (Fig. 1B), the pigeons had to respond as quickly as possible after key illumination to obtain large or small rewards. Responses with a latency of longer than 1500 ms were punished; no responses, again, had no consequence. Every latency-reward combination was associated with a unique colour cue: red and green pecking keys always indicated wait trials with large or small reward amounts, respectively, while blue and white keys always indicated rapid-response trials with large or small reward amounts, respectively. The colour–reward amount associations were counterbalanced across pigeons, but consistent within the pigeons, so that each colour cue unambiguously predicted the upcoming reward amount and the latency condition.

Each session consisted of 120 trials. In half of the training and recording sessions, the first 60 trials of a session were wait trials, followed by 60 rapid-response trials. In the other half of the sessions, the order of the latency conditions was reversed. The sequence of the latency conditions was counterbalanced across and within the animals. Within each of the 60 wait or rapid-response trials, large and small rewards were delivered in blocks of 30 trials. The sequence of large- or small-reward blocks within a response latency condition was also counterbalanced across and within the animals.

Surgery and electrophysiological recording

The pigeons were prepared for extracellular single-cell recordings in awake, freely moving animals with a procedure described in greater detail elsewhere (Colombo *et al*., 2001; Bilkey *et al*., 2003; Kalenscher *et al*., 2005b). For surgery, pigeons were anaesthetized with a mixture of ketamine (Ketavet, Pharmacia & Upjohn, Erlangen, Germany; 40 mg/kg i.m.) and xylazine (Rompun, Bayer, Leverkusen, Germany; 8 mg/kg i.m.). They were allowed to recover fully from surgery for at least 1 week before continuing the training.

During surgery, one microdrive per animal was chronically implanted at a lateral position within the borders of NCL, as defined by Kröner & Güntürkün (1999). The tips of the electrodes were inserted to reach the following coordinates (all dorsal–ventral coordinates relative to brain surface and according to the pigeon brain atlas by Karten & Hodos, 1967): A 4.5–7.5, L 7.5, D 1.0–3.0. Each microdrive housed eight 25-μm formvar-coated nichrome wires. In each session, only two of the eight wires were used for recording. During recording, neural activity was measured from the difference between one of the wires carrying a neural signal vs. another wire with minimal activity that served as the indifferent electrode. Although sometimes several wires carried neural signals within a recording session, only one of these wires was used for recording and, after the session, the microdrive was advanced to exclude double recording from the same unit. Every recording day and in each pigeon, the electrodes were advanced by 40 μm, and the animal was returned to its home cage. The next recording session started at least 14 h after advancement of the electrode to allow the compressed brain tissue to expand and ensure stable recordings. The minimum accepted signal-to-noise ratio was 2 : 1.

During recording, the signals were continuously monitored with an oscilloscope and a speaker. All signals were first amplified and impedance matched through a field effect transistor (FET)- and preamplifier-headstage (MPA-8-I, Multichannel Systems, Tübingen, Germany), and then amplified and filtered online and stored on computer using standard biosignal amplifiers (DPA-2FX, npi electronics, Tamm, Germany), AD converters and Spike2 software (Micro 1401 system, Cambridge Electronic Design, Cambridge, UK).

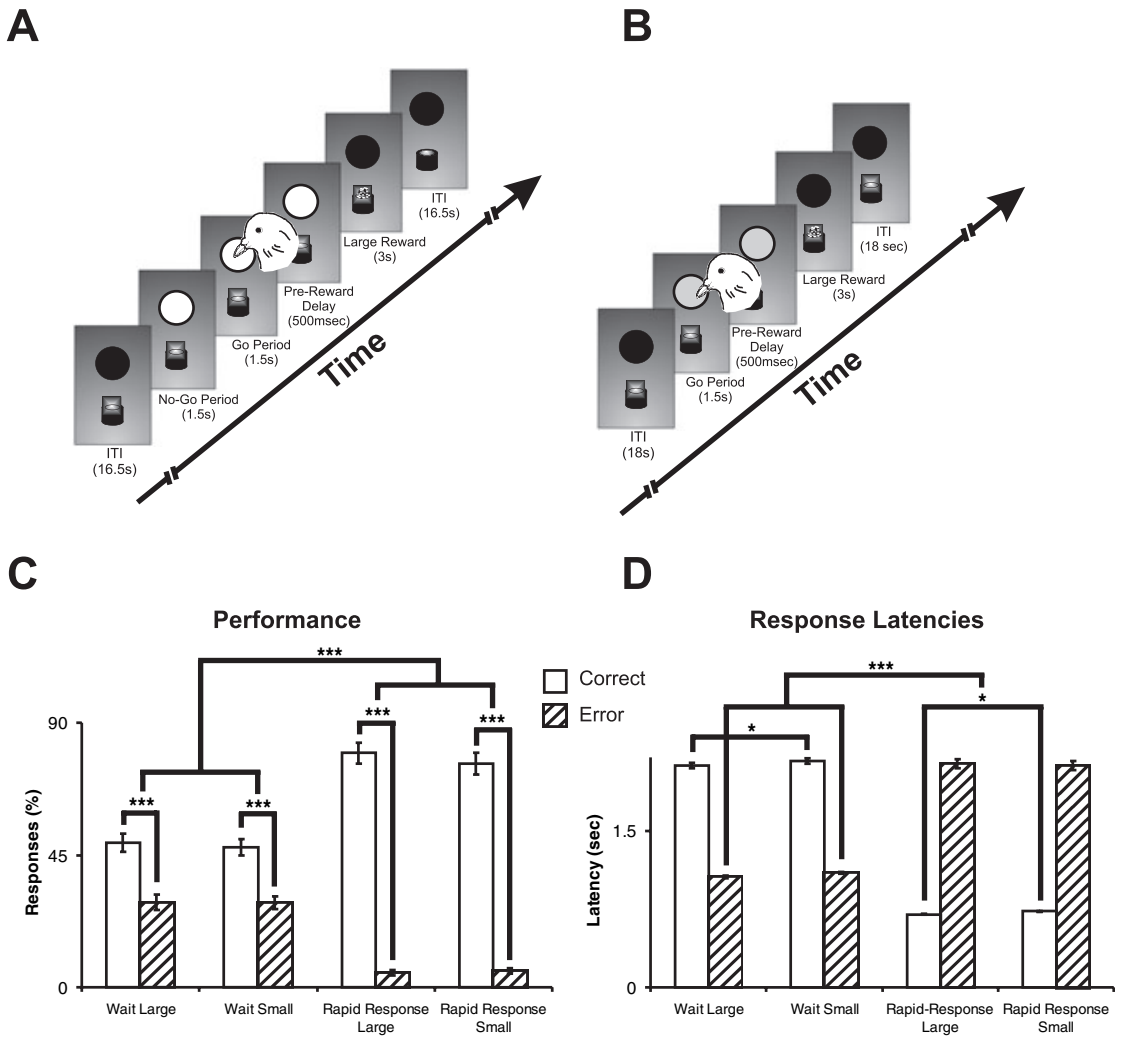

FIG. 1. Task and behavioural results after surgery. (A) The sequence of events within one trial in the wait condition. A trial was preceded by an intertrial interval (ITI) of varying duration, and began with the illumination of the response key in the colour indicating response condition and upcoming reward amount. Upon key illumination, the pigeons had to refrain from responding on the key for at least 1.5 s (no-go period), and then had to respond to the key within another 1.5 s (go period). Following a pre-reward delay of 500 ms, a large reward (3 s access to food, as displayed) or a small reward (1.5 s access to food, not displayed) were delivered. Early responses were mildly punished with a 5 s light-off period (not displayed). (B) The sequence of events in one trial in the rapid-response condition. The sequence was identical to (A), however, a different colour cue instructed the pigeons to respond within the first 1.5 s after key illuminations (go-period). The ITI was adjusted to compensate for differences in response latency as well as reward and punishment durations, so that all trials were of identical length. (C) The percentage of correct and error responses in each condition of the recording sessions (mean ± SEM). Blank bars correspond to correct trials, striped bars to error trials. (D) The response latencies for correct and error trials in each condition of the recording sessions (mean ± SEM). Same bar pattern as in (C). $*P < 0.05$; $***P < 0.001$.

Histology

To reconstruct the locations of the electrode penetrations, the pigeons were deeply anaesthetized with equithesin (0.5mL/100g i.m.) and transcardially perfused with 0.9% saline (40 °C) and a 4% (wt/vol) paraformaldehyde solution (4 °C). The brains were removed, post-fixed and cut into 40-μm frontal sections on a freezing microtome. Every third slice was kept and stained with Cresyl violet.

Results

Behaviour

The performance of all pigeons, averaged across the last six sessions preceding the surgery, was 71.85% correct responses (± 1.87 SEM). After surgery, the mean performance dropped to 63.2% correct per session (± 2.8 SEM, averaged across all subjects and sessions), but was still significantly above chance in all conditions as the average number of correct responses was significantly higher than the average number of errors (17% ± 1.2 SEM, $P < 0.001$, $t_{59} = 17.047$, paired samples *t*-test) or response omissions (17.8% ± 2.8 SEM, $P < 0.001$, $t_{59} = 10.133$).

An ANOVA for repeated measures revealed that the pigeons made significantly fewer errors in the rapid-response than the wait condition ($F_{1,59} = 72.9$, $P < 0.001$); however, there was no effect of reward size on performance ($F_{1,59} = 1.27$, $P = 0.26$), and no interaction between reward size and response condition ($F_{1,59} = 0.2$, $P = 0.66$). The numbers of correct and incorrect responses per condition are displayed in Fig. 1C. Furthermore, the ANOVA showed that the response latency (measured as the time between key-onset and response) was, as expected, significantly shorter in the rapid-response than the wait

condition ($F_{1,748} = 12330.5$, $P < 0.0001$; only correct responses, shown in Fig. 1D), and there was a small but significant effect of reward size on the response latency ($F_{1,748} = 6.25$, $P < 0.05$), but no significant interaction between reward size and response condition ($F_{1,748} = 0.53$, $P = 0.466$). When pigeons responded too early in the wait condition, their response latencies were still significantly longer compared with correctly timed responses in the rapid-response condition ($t_{1235} = 34.09$, $P < 0.001$, *t*-test for paired samples), although the difference between too late responses in the rapid-response condition and correct responses in the wait condition just failed to reach significance ($t_{228} = 1.62$, $P = 0.11$).

Neural responses

Classification of task-related activity

We recorded a total of 74 neurons. Out of these, 55 units (74.3%) showed significantly enhanced activity (spikes/s) during key illumination (between key-onset and response, or key-offset in missed trials) relative to baseline (baseline period: 5000–10 000 ms after ITI onset; all $Z > 3.2$, all $P < 0.005$, Wilcoxon test). Compared with baseline, 56 units (75.7%) showed significantly enhanced activity during reward delivery (between reward onset and offset; all $Z > 4.7$, all $P < 0.01$, Wilcoxon test); however, only 16 (21.6%) neurons were significantly more active during punishment (between houselight off and houselight on; all $Z > 2.7$, all $P < 0.01$, Wilcoxon test). The majority of neurons showing activity related to key illumination also showed reward-related activity. However, two neurons only showed key- but not reward-related activity, and three other neurons showed only reward- but not key-related activity. All neurons with punishment activity also showed enhanced key-related activity.

Only the 55 units exhibiting significantly enhanced activity during key activation were selected for further analysis. To determine whether these units showed climbing activity, we divided the interval between key-onset and response (or key-offset in trials without a response) into three equally long segments, and calculated the discharge rate (spikes/s) in each segment. Sixteen units (21.6%) showed a significant activity increase between the first and second segment, and also between the second and third segment (all $Z > 2$, all $P < 0.05$, Wilcoxon test). None of the neurons had a significant decrease in activity.

Response pattern of an exemplar climbing neuron

Figure 2 shows the normalized, averaged and smoothed peri-stimulus time histogram (PSTH) and the raster plots for a representative neuron with climbing activity. It displays the data for correct trials in the wait and rapid-response conditions, pooled across reward amount conditions and aligned to the onset of the response key (Fig. 2A) or peck (Fig. 2B). Figure 2 shows that the climbing function of this neuron was flatter and peaked later when the pigeon had to schedule long-response latencies (wait condition) compared with short latencies (rapid-response condition; Fig. 2A). When aligned to the response (Fig. 2B), the climbing function had an earlier onset and was more stretched out in wait than in rapid-response trials. An error analysis revealed that, when the pigeon accidentally responded prematurely in the wait condition, the climbing function was steeper and peaked earlier compared with correctly timed responses (Fig. 3A), and when the pigeon incorrectly responded too late in the rapid-response condition, the ramp was flatter and peaked later compared with correctly timed responses (Fig. 3B). When the animal expected large vs. small rewards in the wait condition, no clear difference in the slope of the climbing functions could be detected for this neuron (Fig. 3C).

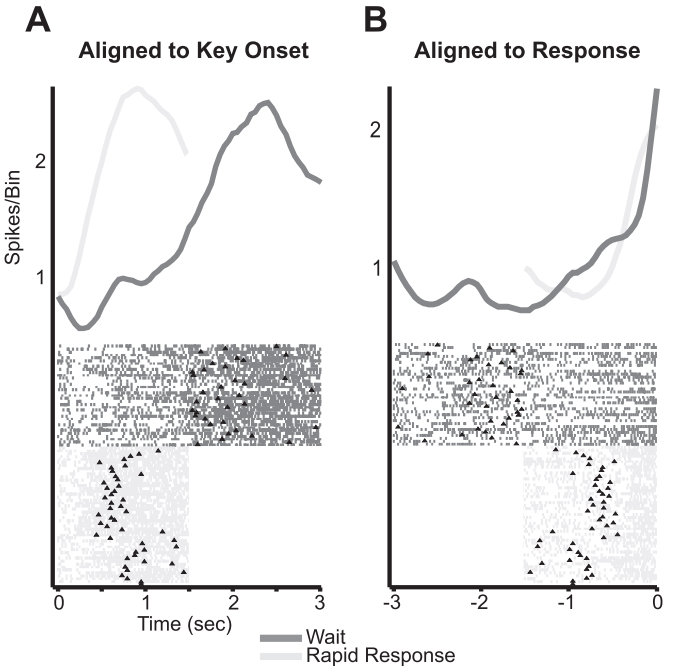

FIG. 2. Exemplar activity pattern of one neuron in the rapid-response and wait conditions. (A) PSTH and raster plots aligned to key-onset. Each row in the bottom parts of the panels corresponds to one trial, each line corresponds to one spike of the neuron. The upper part displays the averaged PSTH after key illumination (3 s for wait trials and 1.5 s for rapid-response trials; only trials with correct responses; bin size 50 ms). The PSTH is aligned to key-onset (time point zero on the *x*-axis) and smoothed with a zero-phase moving-average filter. Light grey lines correspond to data from rapid-response trials, dark lines correspond to data from wait trials. The triangles in the raster plots indicate the time points of responses. Data were pooled across large- and small-reward trials. The drop in the PSTHs following their peaks was presumably caused by the variation in the response latencies: towards the end of each PSTH, the pigeon had responded in the majority of the trials. Thus, towards the end of the interval, several trials with (lower) post-response/reward-related activity contributed to the computation of the PSTHs. (B) Data from the same neuron, aligned to peck (time point zero at the right-hand side of the *x*-axis). The *x*-axis shows the time before the peck in seconds. The triangles indicate the onset of the response key (key illumination on).

However, in the rapid-response condition, the slope of the climbing function in large-reward trials was somewhat steeper and the amplitude higher than the ramp in small-reward trials (Fig. 3D). Interestingly, this reward-dependency of the slope matched the response speed of this pigeon in the rapid-response condition, as this animal, like all other animals, responded significantly faster in trials with large relative to small rewards ($t_{20} = 3$, $P < 0.01$, *t*-test for paired samples).

Slope and time point of the climbing function

We quantified the steepness and the time point of maximum discharge rate of the climbing functions. For each of the 16 neurons showing climbing activity and for each trial the time point of maximum discharge rate in the interval between key-onset and response (response omissions were excluded from this analysis) was determined by binning the spikes into 50-ms bins, and accepting the bin with the highest value as the time point of highest activation. The slope of the climbing function was estimated for each trial by fitting a regression line into the binned normalized neural responses between key-onset and the time point of maximum activation (normalized in each trial to the maximum discharge rate during key activation), and taking the linear gradient as a measure for the steepness of the ramp.

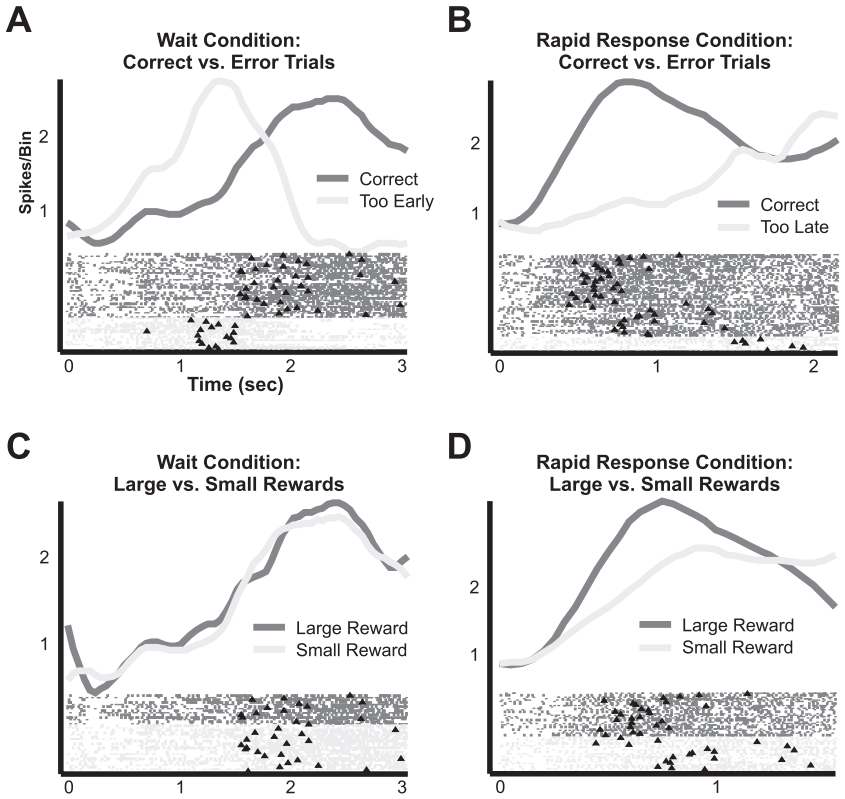

FIG. 3. Exemplar neural responses in error trials and large- vs. small-reward trials. (A) PSTH and raster plots in wait trials with correct (dark grey lines) and premature responses (light grey lines). Data are obtained from the same cell as in Fig. 2, and are aligned to key-onset. The axis and figure descriptions are the same as in Fig. 2. (B) Neural data in rapid-response trials with correct (dark lines) and too-late responses (light lines). Figs 2A and B show that the slope of the climbing function in error trials differed from the slope in correct trials. (C) PSTH and raster plots in large-reward trials (dark lines) and small-reward trials (light lines) in the wait condition. Only data from trials with correct responses are displayed. (D) PSTH and raster plots in correct rapid-response trials with large rewards (dark lines) and small rewards (light lines). The slope of the small-reward PSTH was flatter and peaked later than the slope of the large-reward PSTH. Interestingly, this pigeon also had a faster response latency when it expected large compared with small rewards.

Across all climbing neurons, the linear gradients correlated negatively with the pigeons' response latencies ($r = –0.71$, $P < 0.0001$, Spearman's non-parametric correlation), suggesting that the neural ramp was flatter in trials with longer-response latencies. Accordingly, the linear gradients in the wait condition were significantly smaller than the gradients in the rapid-response condition ($Z = 12.5$, $P < 0.0001$, Wilcoxon test; only correct trials). An error analysis revealed that, in the wait condition, the slope of the neural ramp in trials with premature responses was significantly steeper than the slope in trials with correctly timed responses ($Z = 9.7$, $P < 0.0001$, Wilcoxon test). This difference in the linear gradients in correct vs. late trials was also significant in the rapid-response condition ($Z = 4.4$, $P < 0.0001$, Wilcoxon test). A comparison of the linear gradients in trials differing in reward size (only trials with correct responses) revealed that the slopes were significantly steeper in large- compared with small-reward trials in the rapid-response condition ($Z = 2.2$, $P < 0.05$, Wilcoxon test), but no significant difference was found in the wait condition ($Z = 0.8$, $P = 0.4$, Wilcoxon test). The results are displayed in Fig. 4A.

A similar significance pattern was found in the analysis of the time points of maximum discharge rates (Fig. 4B). The climbing functions peaked significantly later in correct wait than correct rapid-response trials ($Z = 14.9$, $P < 0.0001$, Wilcoxon test). Also, in the wait condition, peaks occurred significantly earlier in premature compared with correct responses ($Z = 12.2$, $P < 0.0001$, Wilcoxon test), and they occurred significantly later in late compared with correctly timed responses in the rapid-response condition ($Z = 5.5$, $P < 0.001$, Wilcoxon test). A comparison of the peak time points in large- vs. small-reward trials (only correct responses) revealed a small, but significantly shorter, peak latency when the pigeons expected large rewards in the rapid-response condition ($Z = 2.0$, $P < 0.05$, Wilcoxon test), but no significant difference in the wait condition ($Z = 1.6$, $P = 0.11$, Wilcoxon test).

Interestingly, the peak time points of the climbing functions seemed to be closely tied to the response latencies. Although the responses occurred significantly later than the maxima of the climbing functions (correct and error trials; $Z = 18.1$, $P < 0.0001$, Wilcoxon test), the time points of highest activation correlated significantly with response latency ($r = 0.74$, $P < 0.0001$, Spearman's non-parametric correlation). The actual mean difference between responses and time points of neural peak activity across all conditions was 0.2 s (± 0.01 SEM) or 0.29 s (± 0.02 SEM) in the wait condition, and 0.12 (± 0.01 SEM) in the rapid-response condition. This result indicates that, generally, the responses were executed shortly, but not immediately, after the climbing functions reached their peak. The time courses of response latencies and peak time points are displayed in Fig. 5A.

In summary, this analysis confirms the observations in Figs 2 and 3, namely that the neural ramps of the climbing neurons were scaled to the timing requirement. When the pigeons had to time and plan long-response latencies, the slope of the climbing function preceding their response was flatter and peaked later than in trials allowing short-response latencies. Moreover, compared with trials

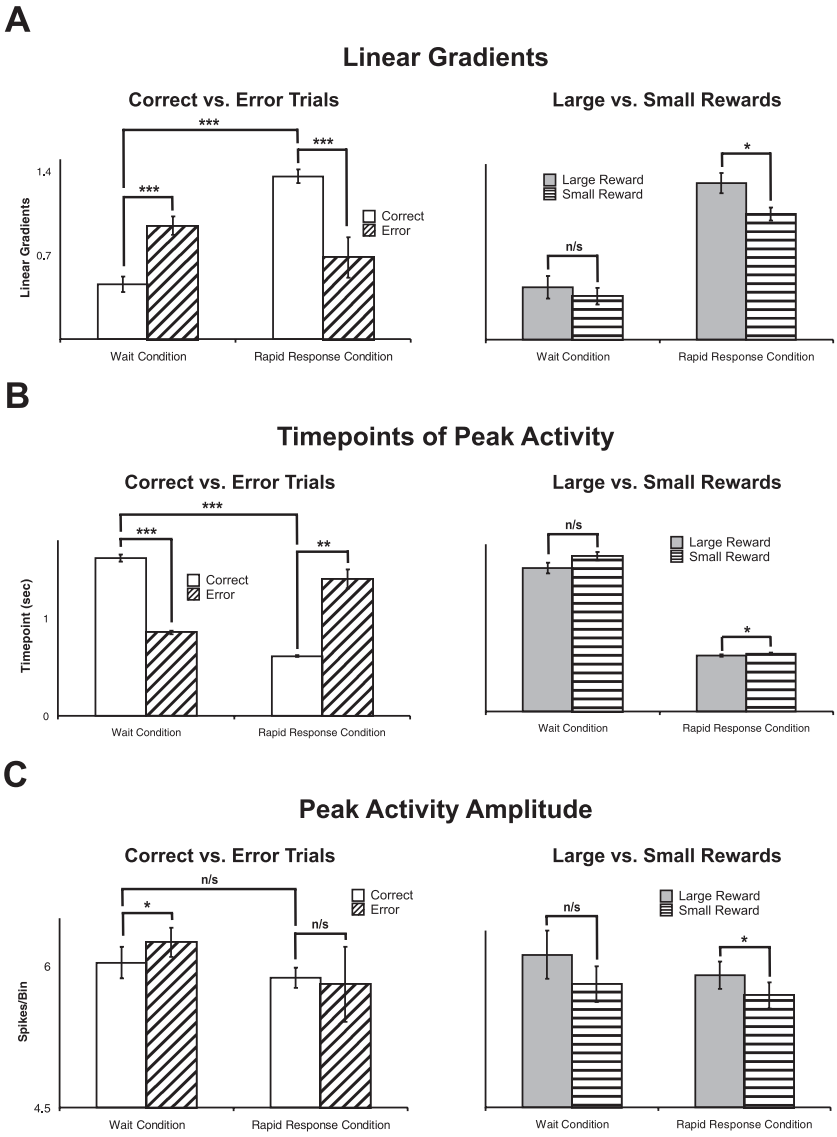

FIG. 4. Population data: time and reward differentially modulate the shape and the discharge rate of the climbing neurons. (A) Linear gradients as a measure for the steepness of the climbing function, averaged across all 16 climbing neurons (mean ± SEM). The left panel shows the linear gradients in both response latency conditions and for correct (blank bars) and error responses (striped bars). It illustrates that the steepness of the climbing function significantly differed between the response conditions, but also between correct and error trials. The right panel shows the linear gradients for both latency conditions for large (grey bars) and small rewards (striped bars). The units on the *y*-axis refer to the gradients of the linear fits. (B) Time points of peak activations (in s), averaged across all climbing neurons (mean ± SEM). The left panel displays the time points for both response latency conditions and for correct (blank bars) and error responses (striped bars). The right panel shows the time points for both latency conditions for large (grey bars) and small rewards (striped bars). This figure confirms that the peaks in the climbing functions were reached at significantly different time points, depending on the response latency requirement, the precision of the response timing and also reward amount. (C) Reward- and timing-related modulation of activation amplitude. This figure displays the peak discharge rate (spikes/bin in the bin with highest activation, mean ± SEM) in both latency conditions. The left panel shows data from correct (blank bars) and error trials (striped bars), the right panel shows data from correct large- (grey bars) and correct small-reward trials (striped bars). Although the neural activity did not differ between latency conditions, the anticipated reward amount affected the neurons' discharge rates (see text). $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$.

with correctly timed responses, the climbing function was steeper and peaked earlier when the pigeons responded too early in the wait condition, and was flatter and peaked later when the pigeons erroneously responded too late in rapid-response trials. When pigeons expected a large compared with a small reward, they responded slightly, but significantly, faster. Correspondingly, in large-reward trials in the rapid-response condition, the slope of the climbing function was steeper and peaked earlier than in small-reward trials. However, no such effect was found in the wait condition. Moreover, a peck was executed shortly, but not directly, after the climbing function reached its peak.

Activity magnitude is modulated by the anticipated reward

To test whether reward amount and response latency also affected the peak activation amplitude, in addition to the slope of the climbing function, we compared the maximum discharge rate (highest bin per trial) in correct and error trials between all conditions. The mean peak firing rates (± SEM) are displayed in Fig. 4C. The peak firing rates did not correlate significantly with response latency ($r = 0.28$, $P = 0.28$, Spearman's non-parametric correlation). However, the Wilcoxon test revealed a small, but significant, difference between correct and error trials in the wait condition ($Z = 2.5$, $P < 0.05$), albeit not in the rapid-response condition ($Z = 0.34$, $P = 0.73$). The difference in peak

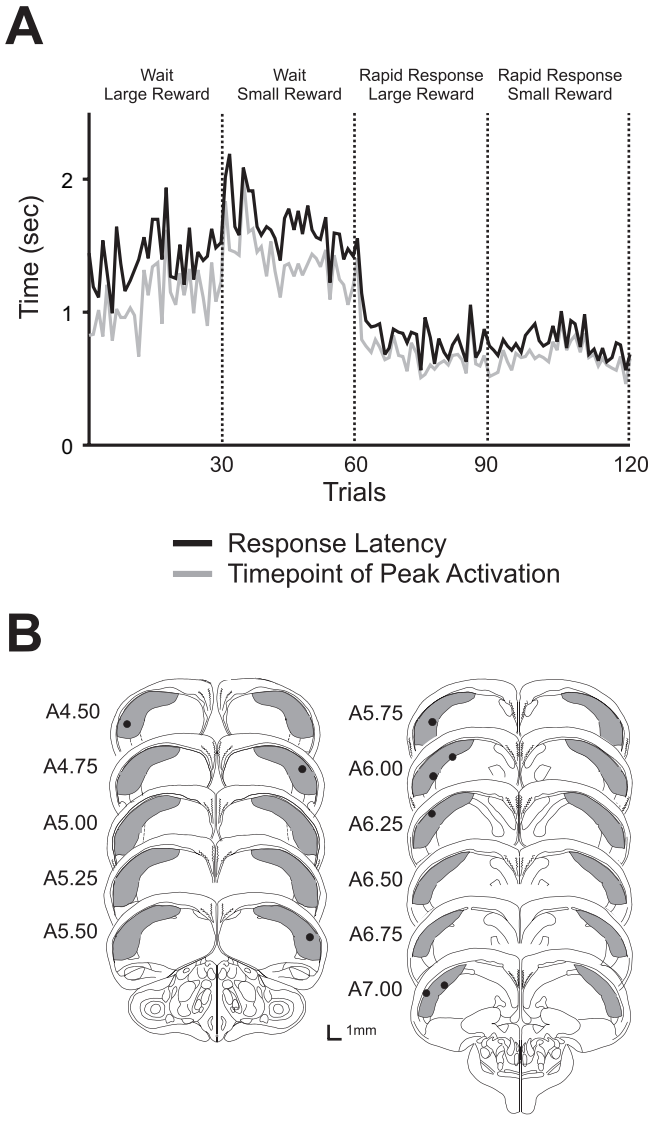

FIG. 5. Coupling of peak time point to response latency, and histological reconstruction of the electrode tracks. (A) Time course of time points of maximal neural activation (grey line) and response latencies (black line), for each trial averaged across all climbing neurons (incl. correct and error trials). The *x*-axis depicts trial number, the *y*-axis time in seconds. The conditions are indicated by the dotted vertical lines. (B) Histological reconstruction of recording sites. The grey areas depict the borders of the NCL, as defined by Kröner & Güntürkün (1999), the black dots indicate the location of the deepest point of the penetration track. All recordings were within the borders of the NCL.

activity between correct wait and rapid-response trials did not reach significance ($Z = 0.82$, $P = 0.41$). The peak activity was higher when the pigeons anticipated a large reward in the rapid-response condition ($Z = 2.3$, $P < 0.05$), although the test failed to reach significance in the wait condition ($Z = 1.7$, $P = 0.097$).

Climbing neurons do not encode the temporally discounted reward value

Animals prefer large over small rewards, and temporally proximal over temporally distant rewards. Accordingly, an abundance of behavioural studies on intertemporal choice behaviour indicate that the value of a reward is temporally discounted, i.e. it correlates positively with reward amount, but negatively with the delay between response and reward delivery (McDiarmid & Rilling, 1965; Ainslie, 1975; Green *et al*., 1981, 1994; Mazur, 1988). Because reward amount and time-to-reward were varied in the present task, we specifically tested whether the climbing neurons integrated reward amount and waiting time to reflect the temporally discounted reward value, as shown previously for neurons in the NCL (Kalenscher *et al*., 2005b). If this was the case, then there should be a difference in firing rate when the animals expected a large, immediate reward (large-reward trials in the rapid-response condition = highest reward value) compared with a small, delayed reward (small-reward trials in the wait condition = lowest reward value). However, the Wilcoxon test did not show any such difference ($Z = 0.1$, $P = 0.92$), indicating that the neurons' activity did not co-vary with the temporally discounted reward value.

Histology

As displayed in Fig. 5B, all recording sites were within the borders of the NCL, as defined by Kröner & Güntürkün (1999).

Discussion

The present study demonstrates the role of climbing activity for timing behavioural actions. Pigeons were trained to either respond immediately following the onset of a cue, or wait for a fixed delay before making the response. The observed slope of the climbing activity, and therefore the time point of peak activation, correlated with the response-timing of the pigeons. Furthermore, the slope reflected whether the responses were correctly timed, or occurred too early or too late, and it also co-varied with the reward amount-dependent variation in response latency. This means that the climbing activity followed the behaviour of the pigeons, not just the objective length of the delay interval, as could have been inferred from previous studies (Kojima & Goldman-Rakic, 1982; Komura *et al*., 2001; Rainer & Miller, 2002; Brody *et al*., 2003; Reutimann *et al*., 2004; Sakurai *et al*., 2004; cf. Renoult *et al*., 2006). In our task design, there was no external cue to indicate the end of the interval, hence pigeons had to internally estimate the interval length based on the outcomes of their previous behavioural actions. When the pigeons underestimated the length of the interval, as reflected by a steeper climbing ramp and earlier peak activation, they responded too early; conversely, when they overestimated the length of the interval, as reflected by a flatter ramp and later peak activation, they responded too slowly. We conclude that these errors resulted from genuine mistakes of response timing, not from confusions of the task conditions, as too-early responses in the wait condition occurred still later than correctly timed responses in the rapid-response condition. On the basis of these data we suggest that climbing activity is indeed used for timing actions and self-planned behaviours, in gross correspondence with previous observations, although these either did not manipulate interval length and the timing requirement (e.g. Niki & Watanabe, 1979), or did not necessitate interval timing for correct performance (e.g. Kojima & Goldman-Rakic, 1982; Komura *et al*., 2001; Rainer & Miller, 2002; Brody *et al*., 2003; Reutimann *et al*., 2004; Sakurai *et al*., 2004).

Importantly, the fact that our task design did require pigeons to time their responses in accordance with the instructive cue introduces a self-control component to the task, above and beyond mere time estimation. Specifically, pigeons had to withhold their responses in the wait condition, thereby delaying the expected reward delivery relative to the rapid-response condition. From this perspective, it seems plausible to assume that what pigeons did during the wait intervals, while climbing activity was slowly building up, is to increasingly inhibit an already prepared response that was released only at the peak

of the climbing activity. Some authors have argued in favour of this idea and suggested that climbing activity is indeed related to the suppressing of a response that intensifies as the delay progresses and the probability of the interval end increases (Genovesio *et al*., 2006; cf. also Schall *et al*., 2002). However, this explanation is somewhat difficult to reconcile with the climbing activity we found in the rapid-response condition where no response inhibition was necessary.

Another possibility is that the climbing activity could reflect primary motor activity, as it occurred whenever a motor response was executed. In fact, the animals' response latencies were highly correlated with the time points of maximum neural activation. However, there was a significant lag of about 200 ms between the peak of the climbing function and the response. If the units were primary motor neurons, one would expect them to be active until immediately before or during motor execution. The lag between neural peak amplitude and response suggests, however, that the peak activation of the climbing function, although crucial for the scheduling of the peck, was tied somewhat loosely to the execution of the response. This finding is more consistent with the idea that the observed climbing function – while tightly coupled to behavioural action – did not reflect the activity of primary motor neurons, but some form of premotor planning, cognitive preparation or decision-making processes instead, which are translated to pure motor execution commands at a subsequent stage.

We propose that response timing is realized via such preparatory activity that is temporally aligned to the anticipated event time, and read out as the peak activation is reached (cf. Durstewitz, 2003; Maimon & Assad, 2006). According to this idea, timing information is always co-encoded in any prospective information processing, e.g. motor planning, whether or not current task demands require this information for accurate response scheduling. The prospective timing of the anticipated event can then be implemented by adjusting the slope of the neural ramp so that it peaks at the required time point, and subsequent information processing (e.g. primary motor activity and response execution) is only triggered once this threshold is reached. Such a theory is consistent with recent computational models that conceptualize interval timing as an intrinsic property of distributed neural cells or ensembles that encode timing information in parallel with other task-relevant processes, and not necessarily via an independent clock or pacemaker (Durstewitz, 2003, 2004; Miller *et al*., 2003; Reutimann *et al*., 2004; Staddon, 2005; Shuler & Bear, 2006).

Additional support for the notion that timing is co-encoded with other preparatory processes comes from our finding that other task features, in this case expected reward amount, altered the peak activation amplitude of the climbing activity (cf. Komura *et al*., 2001), and also its slope and peak time point of the climbing activity, although this effect was less clear in the wait condition than it was in the rapid-response condition. These findings have important theoretical implications for the read-out mechanism of the climbing activity. If the mechanism is indeed based on a simple activation threshold (e.g. Durstewitz, 2003), the effect of reward amount on the slope of the climbing function would lead to systematic differences in response timing, as the threshold would be reached earlier for larger relative to smaller rewards, with the consequence that the animal would tend to respond earlier for large rewards. Our behavioural analyses confirmed this prediction as the pigeons indeed responded slightly, but significantly, faster when expecting large compared with small rewards.

In conclusion, the present study demonstrated that single neurons in the pigeon forebrain showed climbing activity that varied both with interval length and expected reward amount in a task that required self-paced, internally generated response timing. Our results are consistent with models assuming a distributed timing mechanism that is intrinsic to the neural ensemble that processes the to-be-timed event, and supports a threshold-based read-out mechanism of climbing activity.

At a broader theoretical scale, our data are coherent with a recent paradigm shift in the cognitive neurosciences in our understanding of vertebrate brain evolution (Reiner *et al*., 2004; Jarvis *et al*., 2005). Despite the anatomical dissimilarities, persuasive evidence suggests that the avian pallium shares a lot more features with the mammalian cortex than previously thought. The resemblance between the neurons' discharge patterns in NCL and prefrontal cortex in the present timing task, and in particular the fact that climbing functions were found in both the mammalian and avian brain, provides further support of the notion of universal, evolutionary convergent neural functions. The present work contributes to establishing pigeons, which are one of the most widely used species in the behavioural timing literature, as a suitable animal model to study the neuroscience of timing behaviour.

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Abbreviations

ANOVA, analysis of variance; FET, field effect transistor; ITI, intertrial interval; NCL, Nidopallium caudolaterale; PSTH, peri-stimulus time histogram.

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