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Differential increase of extracellular dopamine and serotonin in the 'prefrontal cortex' and striatum of pigeons during working memory

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Abstract

Monoamines, such as dopamine (DA) and serotonin (5-HT), play a central role in the modulation of cognitive processes at the forebrain level. Experimental and clinical studies based on dopaminergic pathology, depletion or medication indicate that DA, in particular, is involved in working memory (WM). However, it is unclear whether DA is indeed related to WM, whether its function is specific to the prefrontal cortex (PFC), and whether other modulators, such as 5-HT, might have similar functions. Therefore, the aims of this study were threefold. First, we analysed whether increased prefrontal DA release is related to WM in general or only to its short-term memory component. Second, we examined whether the DA release during cognitive tasks is specific to prefrontal areas or also occurs in the striatum. Third, we analysed whether prefrontal or striatal 5-HT release accompanies working and short-term memory. We approached these questions by using *in vivo* microdialysis to analyse the extracellular DA and 5-HT release in the pigeons' 'PFC' and striatum during matching-to-sample tasks with or without a delay. Here, we show that DA has no unitary function but is differentially released during working as well as short-term memory in the pigeons' 'prefrontal' cortex. Striatal DA shows an increased efflux only during WM that involves a delay component. WM is also accompanied by a 'prefrontal' but not a striatal release of 5-HT, whose efflux pattern is thus partly different to that of DA. Our findings thus show a triple dissociation between transmitters, structures and tasks within the avian 'prefronto'-striatal system.

Introduction

The prefrontal cortex (PFC) orchestrates cognitive functions that flexibly link sensory events, internal states and actions (Fuster, 2003). One of these functions is working memory (WM), which is the ability to manipulate and temporally hold an active representation of goal-related information. WM is modulated by dopamine (DA) via the dense dopaminergic innervation of the PFC (Robbins, 2000). Depletion of prefrontal DA or blocking of D1 receptors impairs WM in patients (Mattay *et al.*, 2002) and monkeys (Brozoski *et al.*, 1979; Sawaguchi & Goldman-Rakic, 1991), while systemic or local administration of D1 receptor agonists increases WM performance (Müller *et al.*, 1998; Granon *et al.*, 2000). Many PFC neurons show increased activity during WM (Kubota & Niki, 1971; Goldman-Rakic, 1996), which is modulated via D1 receptors (Williams & Goldman-Rakic, 1995).

But it is still unclear whether DA plays a role in different components of WM (Durstewitz & Seamans, 2006). One component of WM is short-term memory, which is the temporally holding of a stimulus during its physical absence. Other components are executive processes that operate on the transiently held information. Although prefrontal extracellular DA is increased during short-term memory performance in monkeys (Watanabe *et al.*, 1997), recent data indicate that this is primarily related to reward expectation and not to maintaining a memory trace (Rossetti & Carboni, 2005). Thus, the first question addressed by this study is whether an increase in prefrontal DA release is specifically related to short-term memory.

A second question is the prefrontal specificity of DA efflux. The mesencephalic DA cell groups A8–A10 are not strictly organized into separate mesostriatal and mesofrontal systems, but partially overlap (Metzger *et al.*, 1996; Williams & Goldman-Rakic, 1998). Because prefrontal and striatal subcomponents are innervated by all three DA cell groups and are activated during WM tasks (Lewis *et al.*, 2004), it is possible that also striatal DA efflux is increased during delay tasks.

The third question is that of modulator specificity. The PFC is substantially innervated by serotonergic fibers and characterized by an abundance of serotonin $(5\text{-HT})_{2A}$ receptors (Smiley & Goldman-Rakic, 1996; Jakab & Goldman-Rakic, 2000). Because iontophoresis of 5-HT_{2A} antagonists and agonists results in reduction or enhancement of PFC cellular delay activity, respectively (Williams *et al.*, 2002), it is conceivable that also 5-HT release is increased within the PFC during short-term memory episodes.

We approached these questions by analysing DA and 5-HT efflux during matching-to-sample (MTS) tasks with or without a delay component in the pigeon's striatum and nidopallium caudolaterale (NCL). The PFC and NCL are considered analogous structures, as they share similar connectivity, chemoarchitecture, neurochemistry, behavioral relevance and single-cell properties (Güntürkün, 2005).

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In pigeons, NCL lesions result in delay-specific deficits (Diekamp *et al.*, 2002a). Because of the high degree of functional and structural convergence, catecholaminergic processes involved in the different aspects of the WM task are assumed to be highly similar in the avian NCL and mammalian PFC. Here we show that DA and 5-HT modulate diverse components of working and short-term memory at striatal and 'prefrontal' levels in a differential mode.

Materials and methods

Subjects

Five adult pigeons (from local breeders) were used in the experiments. They were housed in individual cages (30 × 30 × 45 cm) in a temperature- and humidity-controlled room with a 12 h light : dark cycle. All subjects were kept at a restricted diet and maintained at 80% of their free-feeding weight throughout the experiment with free access to grit and water. All experimental procedures were approved by national authorities (NRW, Germany) and conducted in accordance with German Guidelines for the Care and Use of Animals in Laboratory Research.

Apparatus and stimuli

Animals were trained and tested in an operant chamber (34 × 34 × 36 cm), which was controlled by a computer via a digital I/O board (CIO-PDISO8; Computer Boards). The back panel was equipped with a feeder, a light placed above it and three operant keys (2 cm diameter), which were arranged horizontally, 22 cm above the floor and spaced 10 cm apart. Colored light bulbs (80 mA) mounted behind each pecking key were used to transilluminate the keys with white, red or green light. The colors were not matched for brightness.

Behavioral paradigm

Pigeons were trained to perform a delayed MTS task (DMTS) following the procedures outlined in Diekamp *et al.* (2002a). Each trial started with the presentation of a sample stimulus (e.g. red) on the center key (Fig. 1). The sample stimulus remained on, until 15 pecks were made to the center key. With the termination of the sample stimulus, the delay period began, which was either 0 s in MTS trials, or 4 s in DMTS trials. The delay period was followed by the presentation of two choice stimuli (red and green), which were presented on the left and right lateral pecking keys. The colors of the sample and left and right choice keys were randomized. Thus, pigeons could not learn an established sequence. One peck on the choice stimulus matching the color of the previously shown sample stimulus was considered a correct response. This was rewarded with 3 s access to food. One peck on the choice stimulus not matching the color of the previous sample stimulus was punished with a 10 s time-out period during which all lights in the operant chamber were turned off. No correction trials were introduced. The next trial started after a 15 s intertrial interval. Each session consisted of an initial 60 min resting period in the conditioning chamber and a subsequent 20 min test period composed of either MTS trials or DMTS trials. All pigeons were trained and tested in this task until they performed the DMTS task at a level of 80% correct responses in three subsequent sessions. Without the initial instrumental conditioning training, pigeons received on average 66.00 DMTS sessions ± 12.7 SEM (range: 29–89 sessions) over a period of about 6 months to reach this criterion.

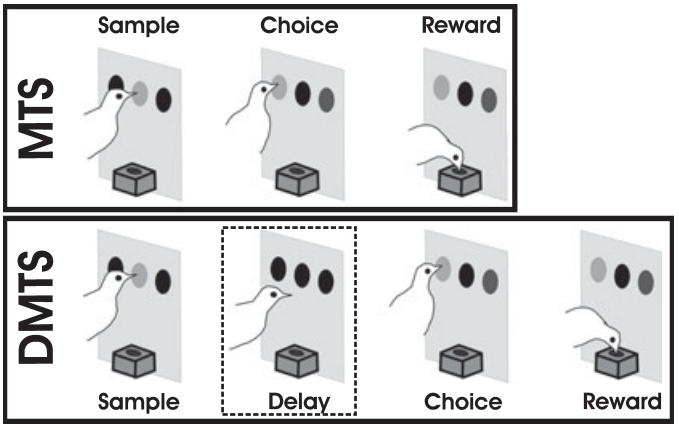

FIG. 1. Schematic diagram of the behavioral design. Both in the matching-to-sample (MTS) and in the delayed-matching-to-sample (DMTS) experiments, each trial started with the presentation of either a green (here depicted as light gray) or red stimulus (dark gray) as the SAMPLE on the central key. Fifteen pecks onto this stimulus immediately activated the side keys in MTS experiments. During this CHOICE phase, the pigeons had to peck the side key that matched the color of the sample. A single correct peck started the REWARD phase with 3 s food access. In the DMTS experiment, pecking on the sample key started a 4-s DELAY period during which the animals had to memorize the sample color. Then, the side keys lit and started the CHOICE period which, after a correct choice, was followed by the REWARD phase. Thus, all cognitive and motor aspects between MTS and DMTS were equal, with the exception of the 4-s delay period within each trial (framed in dotted lines).

They were then prepared in a brief surgery for the microdialysis experiments.

Surgery

For the implantation of the guide cannulae (stainless steel, OD 0.75 mm) that later held the microdialysis probes, pigeons were anesthetized with Ketamin/Rompun (40 mg Ketavet®, Upjohn, and 8 mg Rompun®, Bayer, per kg body weight, i.m.). The guide cannulae were aimed towards the NCL and striatum. Note that the avian and mammalian striatum are homologous to their mammalian counterparts (Reiner *et al.*, 2004), whereas the avian NCL and mammalian PFC are considered evolutionary convergent (homoplasic) structures (Güntürkün, 2005) but not homologous structures by topographical (Medina & Reiner, 2000) and genetic (Puelles *et al.*, 2000) criteria. However, the PFC and NCL show an astonishing degree of resemblance in terms of anatomical (Durstewitz *et al.*, 1998; Kröner & Güntürkün, 1999), neurochemical (Bast *et al.*, 2002; Karakuyu *et al.*, 2003), electrophysiological (Kalt *et al.*, 1999; Diekamp *et al.*, 2002b; Kalenscher *et al.*, 2005; Rose & Colombo, 2005) and cognitive (Mogensen & Divac, 1982; Hartmann & Güntürkün, 1998; Diekamp *et al.*, 2002a; Kalenscher *et al.*, 2003; Lissek & Günürkün, 2005) characteristics.

Stereotaxic coordinates for the tip position of the cannulae (Fig. 2) were A 5.0, L 7.5 and V 1.0 (from the surface of the brain) for the left NCL, and A 11.0, L 2.5 and V 5.7 for the right medial striatum, according to the atlas of Karten & Hodos (1967). The guide cannulae, which had a screw cap, were secured to the skull with dental cement. Birds were allowed to recover for 3–4 days after surgery before they were retrained. Animals reached an 80% correct performance level in DMTS sessions within 8.20 ± 2.75 sessions. However, to reach a stable level of performance and the previous criterion of 80% correct responses in three subsequent DMTS sessions, it took 17.00 ± 6.26 DMTS sessions (range: 7–41 sessions).

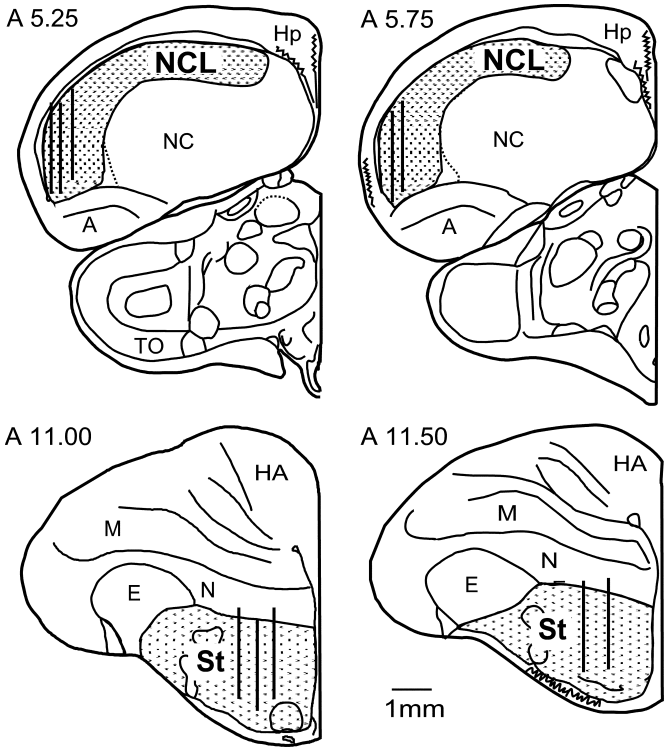

FIG. 2. Location of microdialysis probes in the left nidopallium caudolaterale (NCL) and right medial striatum (St) in schematic coronal sections of the pigeon brain (Karten & Hodos, 1967). The anterior distance from the zero point is labeled in each of the four sections. The areas of the active membranes of the dialysis probes are indicated by black lines in NCL and St. Abbreviations: A, arcopallium; E, ectopallium; HA, hyperpallium accessorium; Hp, hippocampus; M, mesopallium; N, nidopallium; Nc, nidopallium caudale; TO, tectum opticum.

Dual-probe in vivo microdialysis

On the day of the *in vivo* microdialysis, self-made concentric microdialysis probes (Boix *et al.*, 1995; Bast *et al.*, 2002) with an active membrane length of 2.5 mm were inserted into the guide cannulae. The dialysis capillary was made from regenerated cellulose (ID 215 μm, OD 251 μm, molecular weight cut off 6 kDa; Akzo, Wuppertal, Germany) glued to a 26 g stainless steel cannula. A fused silica capillary (ID 75 μm, Cluzeau, Sainte Foy la Grande, France) inside the probe served as the outlet. They were secured in place with the screw cap, and connected via a dual channel swivel (CMA/Microdialysis AB, Solna, Sweden) to the microdialysis pump (PHD 2000 Infusion, Harvard Apparatus). The swivel was equipped with two Eppendorf tubes, which were used to collect the dialysates. This swivel assembly was attached to a counterbalanced arm and the connection to the animal was made by a spring wire tether, which was connected to its counterpart on the pigeons' carrier cloth-band.

After insertion of the microdialysis probes into the guide cannulae, they were perfused with artificial cerebrospinal fluid (aCSF; in mM: Na^+, 147; K^+, 2.5; Ca^{2+}, 2.2; Mg^{2+}, 0.9; Cl^-, 155.7) at a rate of 1 μL/min.

Reversed phase high-pressure liquid chromatography (HPLC)

The NCL dialysates (20 μL/sample) and 5 μL of the internal standard dihydroxybenzylamin (DHBA) were injected manually into the HPLC system immediately after performing the experiments, while striatal dialysates were stored at 4 °C and analysed the following day. The mobile phase (pH 3.0) contained 14.14 g chloracetic acid ($ClCH_2COOH$), 4.66 g sodium hydroxide pellets (NaOH), 200 mg octylsulfate ($CH_3(CH_2)_7OSO_3Na$), 250 mg ethylenediaminetetraacetic acid (EDTA), 18 mL tetrahydrofuran (C_4H_80) and 50 mL acetonitrile (CH_3CN) in 1 L bi-distilled water. A nucleosil C18 column (ODS-AM, 120 Å, S-3 μm, 250 × 3 mm i.d., YMC, Europe GmbH, Schermbeck) was used as the stationary phase. Electrochemical detection was performed with a VT-03 flow cell (Decade, Antec-Leyden, Netherlands). The working electrode was set at +0.7 V against an Ag/AgCl reference electrode. The sensitivity of the HPLC system was 0.2 nA/V for both DA and 5-HT. The data were integrated by the Chromeleon® software data system (Dionex, Idstein, Germany).

Substances in the dialysates were quantified from peak areas using a calibration graph for each substance. Data were not corrected for the *in vitro* recoveries of the microdialysis probes because such an adjustment has been demonstrated to be disadvantageous for the determination of relative changes of extracellular DA levels (Glick *et al.*, 1994). The detection limit of DA and 5-HT were 0.15 pg/20 μL. All probes were corrected by using the internal standard DHBA.

Experimental procedure

Five pigeons were tested on two consecutive days during which microdialysates were collected. Because birds could not be housed with the microdialysis probes still attached, new microdialysis probes were inserted at the beginning of each testing day. The probes were inserted through the same guide cannulae, so that the active membranes of the probes were targeting precisely the same area in the NCL and striatum in both experimental days. Repeated probe insertions in the same brain site, especially when inserted the next day, cause few morphological changes and yield very reliable and comparable dialysate measurements (Georgieva *et al.*, 1993; Fumero *et al.*, 1994).

On each experimental day the sampling started 2 h after the insertion of the microdialysis probes and perfusion with aCSF. Samples were collected in 20-min intervals. All pigeons were tested under three different experimental conditions. During resting phases, each lasting 1 h (three microdialysis samples), birds were not involved in any operant task. During the MTS and DMTS task conditions, each lasting 20 min, birds had to perform in the matching, task paradigm in which the short-term memory load was either close to absent (0 s delay) or high (4 s delay).

The within-subject experimental design during each experiment was as follows: baseline samples 1–3; one sample during MTS or DMTS; resting phase samples 5–7; one sample during DMTS or MTS; resting phase samples 9–11. The experiments were performed for each bird twice on two successive days, with the reversed order of the MTS and DMTS task on the second day. Three birds were tested on Day 1 in the order of first the DMTS and then the MTS task, two birds were tested on the first day in reverse order. Overall, the data of five animals were collected in sessions in which they were first tested in a MTS and then DMTS task. In sessions in which the DMTS task was conducted first and then the MTS task, we were able to analyse the dialysates from only four of those five animals due to technical reasons.

Histology

At the end of the final experiment, pigeons were anesthetized with Equithesin (0.5 mL/100 g body weight) and perfused intracardially

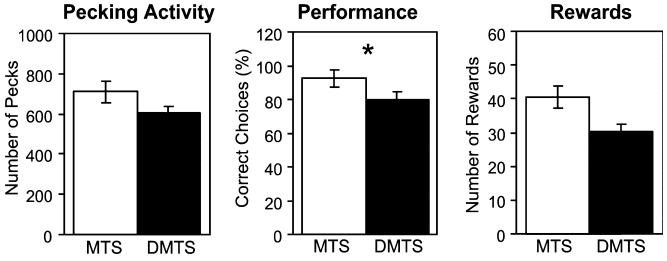

FIG. 3. Behavioral performance during the matching-to-sample (MTS) and delayed matching-to-sample (DMTS) tasks. Error bars indicate standard error means. The star denotes significance based on the Wilcoxon's matched pairs test at $P < 0.05$ between the MTS and DMTS condition. *$P < 0.05$.

TABLE 1. Basal levels of DA and 5-HT from the NCL and striatum

	NCL	Striatum	*t*-value	*P*-value
DA				
(pg/20 μL)	2.41 ± 0.49	3.10 ± 0.63	−3.991	0.016
(nM)	0.78 ± 0.16	1.01 ± 0.20		
5-HT				
(pg/20 μL)	1.71 ± 0.29	2.33 ± 0.59	−1.050	0.353
(nM)	0.48 ± 0.08	0.66 ± 0.16		

Data are presented as means ± SEM. The *t*-test for independent samples and its *P*-value indicate whether statistically significant regional differences between the NCL and striatum were found. 5-HT, serotonin; DA, dopamine; NCL, nidopallium caudolaterale.

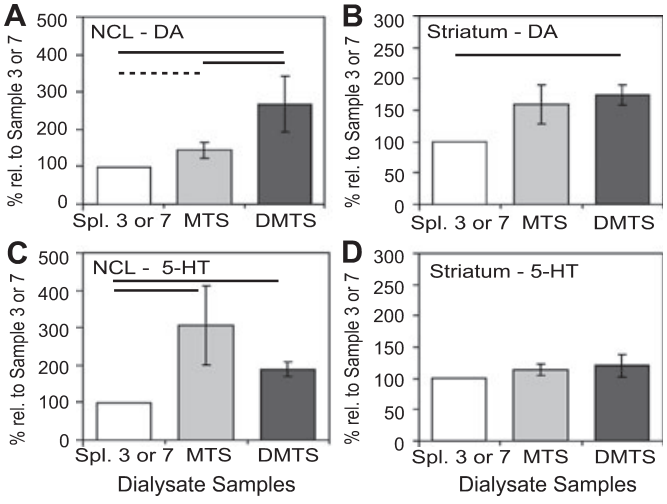

FIG. 4. Dopamine (DA) release in the nidopallium caudolaterale (NCL) in (A) and striatum in (B) and serotonin (5-HT) release in (C) the NCL and in (D) the striatum during the matching-to-sample (MTS) and delayed-matching-to-sample (DMTS) task. MTS and DMTS transmitter concentrations are shown as percent change relative to the preceding sample (i.e. sample 3 or 7), which was collected during a resting period and defined to be 100%. Horizontal lines represent significant differences at $P < 0.05$ (solid lines) or a trend at $P < 0.10$ (broken lines).

with 0.9% NaCl (40 °C) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (4 °C, pH 7.2). Standard histological techniques were used to prepare 40-μm coronal sections that were Nissl stained with Cresyl violet. These sections were used to verify the positions of the guide cannulae and the location of the active membrane (Fig. 2). Because of the larger diameter, guide cannulae caused large visible lesions in the brain tissue. The active membrane of microdialysis probes, which protruded into the brain tissue from the guide cannulae, caused much smaller tissue damage. In all cases, the location of the active membranes of the microdialysis probes could be visually identified in the histological sections, and the exact extent of the probe in the tissue could be reconstructed in combination with the lesions from the guide cannulae. All probes were located in the lateral aspect of the NCL or in the medial striatum (Fig. 2). Although gliosis and neuronal loss was evident around the guide cannulae, which were implanted at least 4 weeks prior to the microdialysis experiments, no clear glial boundary was seen surrounding the site of the active part of the microdialysis probes, which had been inserted only 2 days prior to the final perfusion.

Data analysis

Behavioral measures

During the MTS and DMTS tasks, the number of correct choices was counted and expressed as percent correct. The amount and number of rewards in each session was equal to the number of correct responses. The overall motor activity was assessed by the number of pecks made within each session. These behavioral measures were compared between the MTS and DMTS condition using Wilcoxon matched pair's tests. In addition, correlation analyses between the DA efflux in the MTS and DMTS task and the behavioral performance, the amount of reward and the pecking activity were performed and Pearson's correlation coefficients were calculated.

Microdialysis measures

Basal values of each transmitter were calculated from the average of the first three samples, which were defined as 100% baseline for each experimental day. For further analysis, DA and 5-HT concentrations were expressed as percentages of their respective baseline levels. Outliers (1.7% of all values) were identified using Grubb's test and replaced by the average of the two adjacent sample values. Friedman non-parametric analyses of variance (ANOVAs) were applied to test for overall changes in the release of the transmitters DA and 5-HT during the different sample periods (11 samples). These ANOVAs were run for samples from the NCL and striatum, and for each of the two experimental days (Fig. 5). If overall effects were significant ($P < 0.05$), planned Wilcoxon tests were run between the baseline value of sample 3 and the DMTS and MTS samples. As in other microdialysis/HPLC studies, we consider the value of sample 3 as being a baseline for all subsequent measurements taken during an experimental session. Friedman ANOVAs on the samples collected as baselines (samples 1–3) and during the resting periods (samples 5, 6, 7, 9, 10 and 11) revealed no significant differences in any of the tests (tests on DA, 5-HT and each experimental session: all $\chi^2 < 10.55$; $P > 0.228$). In addition to the comparison with sample 3, a planned Wilcoxon test was also run between the sample of the second task, i.e. sample 8, which was either a MTS or DMTS, and the preceding sample 7, which was collected during a resting period.

To be able to combine the data collected during the two experimental sessions and to focus on the different task conditions, we calculated the change in transmitter concentrations during MTS and DMTS relative to sample 3 or 7 (whichever was preceding). Consistent with the non-significant difference between corresponding samples collected in the two sessions (all Wilcoxon matched pairs tests with $N = 4$, $Z < 1.461$, $P > 0.144$), values from the two sessions were averaged (Fig. 4). These values were analysed by non-parametric Friedman ANOVAs ($N = 5$, d.f. $= 2$) to test the overall effects of task conditions on the release of the transmitters DA and 5-HT in the NCL

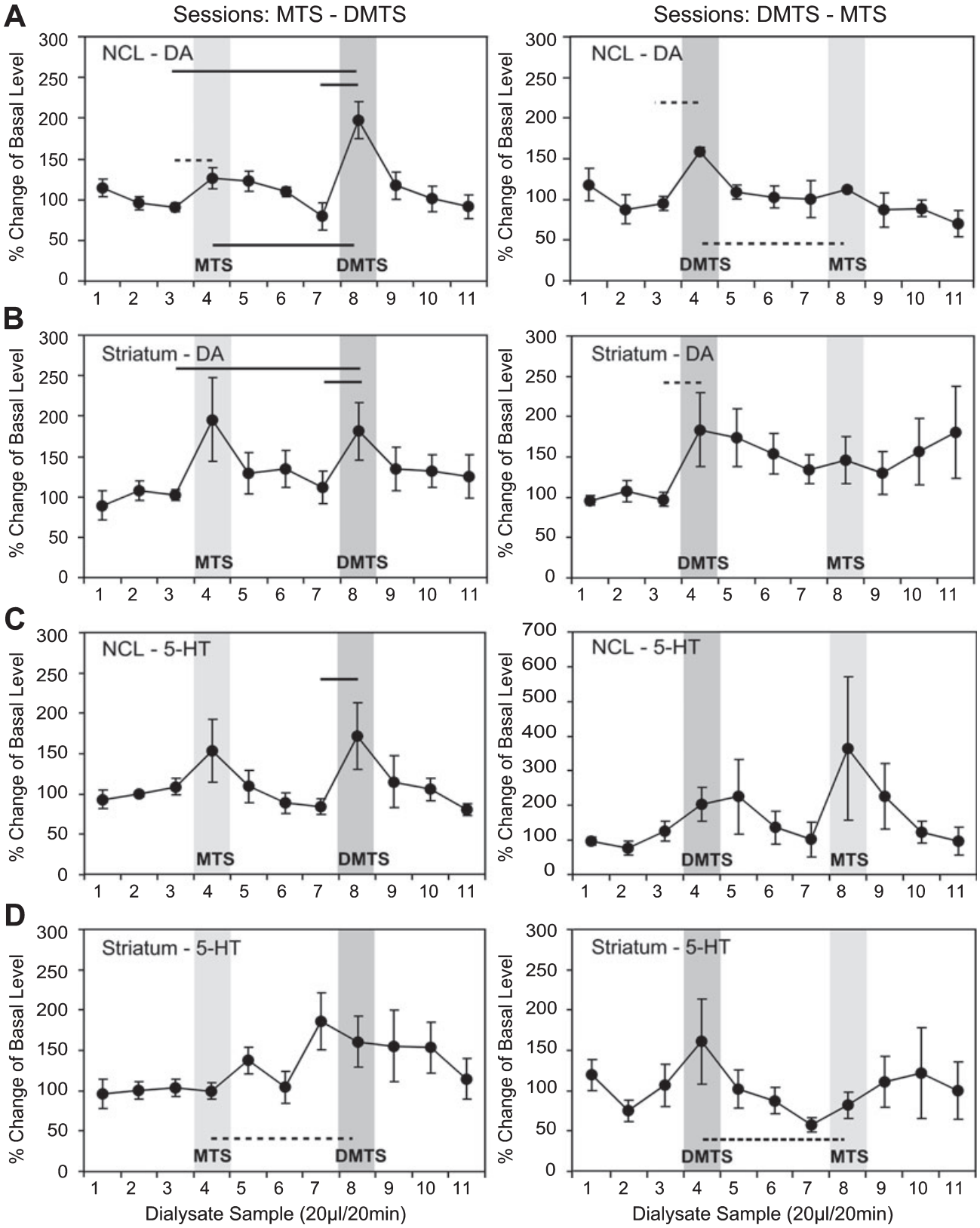

FIG. 5. Dopamine (DA) efflux in (A) the nidopallium caudolaterale (NCL) and (B) the striatum, and the serotonin (5-HT) efflux in (C) the NCL and (D) the striatum over the course of two testing sessions. In one testing session (left column) pigeons were first tested in the matching-to-sample (MTS) and then in the delayed matching-to-sample (DMTS) task, in the other session (right column) the order of the tasks was reversed. Eleven microdialysate samples were collected in each session, with sample 4 and sample 8 taken during the MTS or DMTS. All values are expressed as percent change relative to baseline (i.e. 100% representing the average concentration of samples 1–3). Error bars indicate standard error means. Horizontal lines between samples represent significance between MTS or DMTS and resting samples 3 or 7 at $P < 0.05$ (solid lines) or a marginal significance at $P < 0.10$ (broken lines).

and the striatum. If the overall effects were significant, planned Wilcoxon tests were applied as *post hoc* tests to compare the three values. All statistical analyses were performed using the software packages Statistica (Evanston, IL, USA) or SPSS (Chicago, IL, USA).

Results

Dialysates were collected from two forebrain regions, NCL and striatum, from five pigeons during their performance in the DMTS and MTS tasks. Measurements were repeated in a second test session in four of those birds. Reliable measurements of both extracellular DA and 5-HT for all experimental conditions were obtained.

Behavioral data

Animals were retrained before the microdialysis sessions took place to a presurgical performance level, which was a stable performance of at least 80% in three subsequent DMTS sessions. Thus, there was no difference in performance during the last three sessions prior to surgery and the last three sessions prior to the experimental sessions (Wilcoxon tests, $N = 5$; MTS: $Z = 2.022$, n.s.; DMTS: $Z = 0.674$, n.s.). In addition, no difference in performance was detected between the last three sessions prior to microdialysis and during the microdialysis sessions (Wilcoxon tests, $N = 5$; MTS: $Z = 0.674$, n.s.; DMTS: $Z = 0.135$, n.s.).

The behavior during the MTS and DMTS conditions during microdialysis was assessed in terms of the overall pecking activity, percent correct responses and amount of reward (Fig. 3). On average, birds completed within a 20-min time period (the time period for collecting one microdialysis sample) 44.4 ± 3.3 SEM trials in MTS and 37.8 ± 1.9 trials in DMTS sessions (Wilcoxon test, $N = 5$, $Z = 1.483$, n.s.). It should be noted that the delay of 4 s in each DMTS trial amounts to 151 s within the 20-min interval. Taking this delay into account, birds performed at an almost equal rate in these conditions and needed on average 27.02 s per trial in the MTS task (20 min/44.4 trials) or 27.75 s per DMTS trial [(20 min − 151 s)/37.8 trials]. No differences were found between MTS and DMTS sessions in the overall pecking, i.e. motor activity (MTS: 710.4 ± 53.4, DMTS: 604.8 ± 30.2; $Z = 1.483$, n.s.). But, as expected, birds performed the MTS task significantly better, with 92.2 ± 5.0% correct responses, than the DMTS task, in which they only achieved 79.9 ± 4.6% correct responses ($Z = 2.023$, $P < 0.043$). Consequently, the total number of rewards was lower in DMTS sessions (30.4 ± 2.3) than in MTS sessions (40.9 ± 3.3), although this difference missed significance ($Z = 1.753$, $P < 0.079$).

Basal values of DA and 5-HT

The mean dialysate concentrations of DA and 5-HT during the first three intervals were taken as basal values (Table 1). Basal levels of DA and 5-HT in the NCL and striatum did not differ between the two experimental days [all t(d.f. = 3) < 1.135; n.s.], and therefore were averaged over the 2 days. In general, basal levels were higher for DA and 5-HT in the striatum as compared with the NCL.

DA release in the NCL and striatum

In the NCL, the two different task conditions had a significant effect on DA release (Friedman ANOVA: $\chi^2 = 8.40$, $P < 0.015$; Fig. 4A). During MTS and DMTS, DA release was significantly elevated compared with the sample from the resting period preceding the task ($P < 0.05$ for DMTS; marginal significance for MTS, $P < 0.08$). Thus, working in a MTS task increased the efflux of DA in the avian 'prefrontal' area. In addition, during the DMTS task the DA efflux was significantly higher than during the MTS task ($P < 0.05$). Thus, the addition of a short-term memory component further increased DA release.

Similar effects could be seen when analysing the two testing sessions of our balanced design separately (Fig. 5A). In sessions in which animals were first tested in the MTS and then in the DMTS task, overall significant differences in DA release were found between the 11 samples [Friedman ANOVA: χ^2 ($N = 5$, d.f. = 10) = 20.25, $P < 0.027$], which could be attributed to the significant increase during DMTS compared with samples 3 and 7 (both $P < 0.05$) and a trend between MTS and sample 3 ($P < 0.08$). This increase in DA efflux was fast, as it occurred within the 20-min time window during which pigeons performed the DMTS task. It also had a fast decline, as DA release was back to resting levels in the 20-min interval following the DMTS task. Changes in DA release were less pronounced, when pigeons were first tested in the DMTS and subsequently in the MTS task (χ^2 ($N = 4$, d.f. = 10) = 15.23, $P < 0.124$]. The principle pattern of the results was comparable, however, with the DA release during DMTS showing a strong trend to be increased relative to sample 3 ($P < 0.07$).

In the striatum, the effects of the WM task on DA release were less pronounced than in the NCL ($\chi^2 = 6.40$, $P < 0.041$; Fig. 4B). Here, extracellular DA was significantly increased during DMTS compared with the preceding sample ($P < 0.05$), while there was no increase during MTS. Overall, changes in DA efflux in the striatum over the course of the entire session were not significant [Fig. 5B; MTS followed by DMTS: χ^2 ($N = 5$, d.f. = 10) = 13.93, $P < 0.177$; DMTS followed by MTS: χ^2 ($N = 4$, d.f. = 10) = 11.41, $P < 0.327$]. However, a planned comparison analysis showed significantly elevated DA levels in DMTS dialysates compared with samples 3 and 7 (in MTS–DMTS sessions: both $P < 0.05$; in DMTS–MTS sessions: $P < 0.068$).

In principle, these effects could have been due to differences in activity or reward levels of the animals during the different task components. To investigate this possibility, correlations between behavioral measures (number of rewards, percent correct responses, number of pecks) and the DA efflux in NCL were calculated. Because individual animals showed different levels of behavior during the two experimental days, measures were not averaged but used as independent measures. The only significant effect in the correlations between the behavioral measures in either the MTS or DMTS and the corresponding microdialysate samples was a negative correlation between the number of pecks during MTS and DA efflux in NCL (Pearson's correlation coefficient $r = 0.803$, $P < 0.01$). The stepwise increased release of DA in NCL from baseline to MTS and thence to DMTS can thus not be explained with simple behavioral measures, but is probably related to the cognitive requirements.

5-HT release in the NCL and striatum

The overall pattern of 5-HT efflux differed from that of DA. 5-HT release in the NCL changed significantly during the different task conditions ($\chi^2 = 7.60$, $P < 0.023$; Fig. 4C), with a significant increase of 5-HT release during the MTS and DMTS tasks compared with baseline samples 3 or 7 (all $P < 0.05$). 5-HT efflux during MTS and DMTS, however, was not different. Thus, the MTS procedure resulted in an increase of 5-HT in NCL, irrespective of the presence or absence of a short-term memory component. But this result only emerged from

the averaging of the two experimental sessions. Separate analyses of the two sessions revealed no significant changes in 5-HT levels over the course of the experiment (MTS followed by DMTS, $\chi^2 = 14.51$, $P > 0.150$; DMTS followed by MTS, $\chi^2 = 8.90$, $P > 0.540$; Fig. 5C), although there was a significant difference between the DMTS compared with sample 7 ($P < 0.05$).

In the striatum, no significant differences were found in transmitter efflux during MTS and DMTS and the preceding samples 3 or 7 ($\chi^2 = 1.20$, $P > 0.548$; Fig. 4D). Similarly, no overall changes in 5-HT efflux were found during the two experimental sessions (MTS then DMTS, $\chi^2 = 13.58$, $P > 0.193$; DMTS then MTS, $\chi^2 = 15.23$, $P > 0.124$). The direct comparison between MTS and DMTS samples only revealed a trend towards increased values during DMTS compared with MTS ($P < 0.08$). No significant correlations were found between the behavioral measures in either the MTS or DMTS and the corresponding 5-HT dialysate.

Discussion

The present results reveal a triple dissociation between tasks, structures and transmitters within the avian forebrain. 'Prefrontal' DA seems to contribute to both the execution of a MTS procedure and to the maintenance of information during a delay. Striatal DA is significantly increased only during a DMTS task, which includes a delay. Thus, only the combination of a WM and short-term memory task sufficiently stimulates DA efflux within the striatum. 5-HT also displays a structure-dependent difference of release with an increase during MTS in NCL, while striatal efflux could not be correlated with the task demands of either a MTS or DMTS task.

DA in the NCL and striatum

The increase of DA in the NCL was task related. Relative to baseline and to between-task comparisons, MTS and DMTS resulted in successive increases of DA efflux. Thus, both components of WM were related to DA release: (1) the requirement to successfully hold a memory trace during the delays; and (2) the cognitive functions to perform the MTS. Thus, DA seems to play a role for both short-term memory (holding information) and executive processes (operating on the held information). Consequently, extracellular DA in the NCL was stepwise increased from baseline to MTS to DMTS.

The increase of NCL DA efflux from baseline to MTS could have several reasons. First, during MTS (but not during baseline) the animals were rewarded and cues associated with food consumption elicit PFC DA efflux (Bassareo & Di Chiara, 1997; Robbins, 2000; Ahn & Phillips, 2002). Second, the retrieval of trial-specific information very likely also increases DA release during MTS and DMTS (Phillips *et al.*, 2004). Third, tegmental DA neurons (Schultz, 1998) fire in response to reward prediction and could thus increase prefrontal DA release.

A further increase of DA efflux in the NCL was found with the addition of a 4-s delay to the MTS task. During such a 4-s delay, a memory trace of the relevant information needs to be held active. Some PFC and NCL neurons display a sustained activity during delay that could hold a memory trace for a subsequent response (Rainer *et al.*, 1999; Diekamp *et al.*, 2002b; Machens *et al.*, 2005). If this activity within the PFC/NCL breaks down spontaneously, the animal is likely to err (Fuster, 1973; Diekamp *et al.*, 2002b). If the animal is instructed to forget the sample stimulus, neuronal NCL activity is abolished, and behavioral performance drops to chance level (Rose & Colombo, 2005). Delay time-specific activations of PFC neurons are modulated by the dopaminergic system via D1-receptors (Williams & Goldman-Rakic, 1995). Consequently, blockade of dopaminergic D1-receptors in the NCL or PFC disrupts WM performance (Seamans *et al.*, 1998; Güntürkün & Durstewitz, 2001). Possibly, DA stabilizes active prefrontal neural representations against interfering input (Durstewitz *et al.*, 1999) by altering ionic and synaptic conductance, which enhance spike frequencies of preactivated assemblies (Durstewitz *et al.*, 2000; Seamans *et al.*, 2001; Seamans & Yang, 2004). Taken together, the increase of DA efflux in the NCL from the MTS to the DMTS task could result in a stabilization of delay time-specific activity patterns that are required for the subsequent response.

Our results resemble those of Watanabe *et al.* (1997), who showed that extracellular DA in the monkey PFC is increased in a delayed alternation task relative to a sensory-guided control experiment. Unfortunately, the two tasks used by Watanabe *et al.* (1997) differed not only with respect to the addition of a delay phase, but also in further cognitive components that could have triggered DA release. We therefore believe that the present result is the clearest demonstration that short-term memory is related to DA efflux. If delay time is extended to 30 min, memory probably shifts to hippocampal circuits (Floresco *et al.*, 1997), and DA prefrontal efflux drops during waiting time (Phillips *et al.*, 2004).

Rossetti & Carboni (2005) challenged the idea that DA efflux during delay is related to short-term memory. They measured prefrontal DA during a delayed alternation task in a T-maze with either one arm or both arms baited, such that the animals in the second condition did not need to recall the last trial. Because DA release was statistically equal between both procedures, the authors concluded that prefrontal DA release is related to reward anticipation and not to short-term memory. However, it is conceivable that the dopaminergic contribution to short-term memory was underestimated in this study. Rats quickly learn a delayed alternation paradigm with food reward and achieve high performance levels (Petrinovich & Bolles, 1954). Because DA release and utilization positively correlate with effort (Wang *et al.*, 2000; Cetin *et al.*, 2004; Denk *et al.*, 2005), the experimental task of Rossetti & Carboni (2005) may have triggered only a moderate DA release. Indeed, prefrontal DA efflux between both conditions just had missed significance ($P = 0.06$). Thus, it is possible that in the study of Rossetti & Carboni (2005), a moderate prefrontal DA efflux may have accompanied the introduction of a delay in the task.

Our data show no similar stepwise increase of striatal DA in the two tasks. Instead, an increase of striatal DA could be recorded only during DMTS. Two conclusions can be drawn from this observation. First, mesostriatal and mesopallial DA systems display a differential release pattern, making a functional and neurochemical specificity of these two ascending pathways likely. While both pathways are not as strictly separated as previously assumed, they still derive in part from different DA midbrain clusters (Wynne & Güntürkün, 1995; Metzger *et al.*, 1996; Williams & Goldman-Rakic, 1998). Second, the magnitude of DA increase in the striatum was especially evident when the animals had to perform the WM task with a delay. This could point to a selective increase of striatal DA efflux during short-term memory. Indeed, the role of striatal DA in delayed WM experiments had previously been demonstrated in imaging and clinical studies (Owen *et al.*, 1998; Jahanshahi *et al.*, 2002; Lewis *et al.*, 2004; Mehta *et al.*, 2005), as well as with behavioral experiments subsequent to striatal DA depletion (Collins *et al.*, 2000). Additionally, computational studies (Gruber *et al.*, 2006; Hazy *et al.*, 2006) suggest that DA leads to a bistable up or down state in striatal activity, which possibly prevents alterations of prefrontal activity due to internal noise. All of these effects could stabilize cellular sustained activity patterns during

delay periods. However, the data displayed in Fig. 4 also show that striatal DA release during MTS was close to that during DMTS, but just missed significance. Therefore, the difference between NCL and striatal DA efflux during MTS should be interpreted cautiously.

5-HT in the NCL and striatum

5-HT release in the NCL showed a significant increase both in MTS and DMTS without being correlated with motor behavior or the amount of obtained reward. Thus, 'prefrontal' 5-HT is probably related to executive functions but not specifically to short-term memory performance (Güntürkün, 1997). Several studies underline the importance of prefrontal 5-HT for various aspects of executive functions (Clarke *et al.*, 2004; Chamberlain *et al.*, 2006). Of special importance for the present task are the serotonergic effects on interference suppression (Boggio *et al.*, 2005) and attentional control (Carli *et al.*, 2006), which could increase the ability of the animal to focus on the relevant stimulus and to generate the appropriate response. Maintenance of stimulus/response representations during delay are probably not associated with prefrontal 5-HT, as both Jäkälä *et al.* (1993) and Ruotsalainen *et al.* (1997) demonstrated that 5-HT receptor blockade decreased delayed non-matching-to-position performance independent of delay length. Thus, prefrontal 5-HT and DA seem to be relevant for different aspects of executive functions, while only DA plays an important role for the maintenance aspect of short-term memory.

Our results seem to contrast with those of Williams *et al.* (2002), who showed that moderate iontophoretic stimulation of 5-HT_{2A} receptors in the monkey PFC accentuated the spatial tuning of recorded neurons during the delay component of a delayed-response task. This would imply an increase of short-term memory performance by prefrontal 5-HT. In contrast to Williams *et al.* (2002), other pharmacological studies showed no serotonergic effect on delay performance (Jäkälä *et al.*, 1993; Ruotsalainen *et al.*, 1997), or demonstrated an improvement of delayed MTS performance by 5-HT_{2A} receptor antagonists (Terry *et al.*, 2005), and an impairment of spatial WM by increased 5-HT levels (Luciana *et al.*, 1998; Fernandez-Perez *et al.*, 2005). A part of these contradictions might, however, be explained by the fact that Williams *et al.* (2002) used an iontophoretic application of tiny amounts of substance that only affects the very local network, while other studies applied systemic applications with widespread effects that also influence other neurochemical systems (Pehek, 1996; Friedman *et al.*, 1999).

Our results reveal no significant alterations of striatal 5-HT efflux during MTS or DMTS in pigeons. Midbrain raphe nuclei send serotonergic projections to the striatum in mammals and birds, which synapse with spiny neurons (Lavoie & Parent, 1990; Challet *et al.*, 1996). Van Bockstaele *et al.* (1993) revealed that only few raphe neurons bifurcate to project to both the PFC and the striatum. This anatomical segregation may promote a functional difference between 'prefrontal' and striatal release patterns, as demonstrated in the present study. Taken together, 5-HT efflux not only shows a complementary pattern between 'prefrontal' and striatal targets, it also is complementary to DA with respect to 'prefrontal' short-term memory.

Conclusions – monoaminergic release during WM within the 'prefronto'-striatal system

The present study shows that DA and 5-HT are released in the 'prefronto'-striatal system of pigeons during diverse cognitive tasks in a differential way. Several conclusions can be drawn from these observations.

First, DA efflux in the NCL is stepwise increased during working memory functions and during short-term memory requirements, whereas it is increased in both conditions in the striatum. Thus, within the NCL, DA has no unitary function but seems to be involved in multiple cognitive operations of which only one is to stabilize the memory trace during delay. In the striatum DA release is significantly increased during DMTS only and thus could play a role in the maintenance of short-term memory traces.

Second, 5-HT release is correlated with executive functions in the NCL, while it displays no consistent relation to the employed cognitive tasks within the striatum. Thus, 5-HT plays an area-dependent role during cognitive operations. This implies that the anatomical segregations within the midbrain raphe system that give rise to either striatal or prefrontal projections are differentially activated during diverse cognitive operations (van Bockstaele *et al.*, 1993).

Third, the analysed transmitters, structures and cognitive functions display a triple dissociation. The efflux of 'prefrontal' and striatal DA is related to short-term memory. Contrary, 'prefrontal' DA and 5-HT are also released during executive functions that imply no delay component.

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Abbreviations

5-HT, serotonin; aCSF, artificial cerebrospinal fluid; DA, dopamine; DHBA, dihydroxybenzylamin; DMTS, delayed matching-to-sample; HPLC, high-pressure liquid chromatography; MTS, matching-to-sample; NCL, nidopallium caudolaterale; PFC, prefrontal cortex; WM, working memory.

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