JOURNAL OF NEUROPHYSIOLOGY
Vol. 67, No. 1, January 1992. *Printed in U.S.A.*

Responses of Monkey Dopamine Neurons During Learning of Behavioral Reactions

TOMAS LJUNGBERG, PAUL APICELLA, AND WOLFRAM SCHULTZ
Institut de Physiologie, Université de Fribourg, CH-1700 Fribourg, Switzerland

SUMMARY AND CONCLUSIONS

1. Previous studies have shown that dopamine (DA) neurons respond to stimuli of behavioral significance, such as primary reward and conditioned stimuli predicting reward and eliciting behavioral reactions. The present study investigated how these responses develop and vary when the behavioral significance of stimuli changes during different stages of learning. Impulses from DA neurons were recorded with movable microelectrodes from areas A8, A9, and A10 in two awake monkeys during the successive acquisition of two behavioral tasks. Impulses of DA neurons were distinguished from other neurons by their long duration (1.8–5.0 ms) and low spontaneous frequency (0.5–7.0 imp/s).

2. In the first task, animals learned to reach in a small box in front of them when it opened visibly and audibly. Before conditioning, DA neurons were activated the first few times that the empty box opened and animals reacted with saccadic eye movements. Neuronal and behavioral responses disappeared on repeated stimulus presentation. Thus neuronal responses were related to the novelty of an unexpected stimulus eliciting orienting behavior.

3. Subsequently, the box contained a small morsel of apple in one out of six trials. Animals reacted with ocular saccades to nearly every box opening and reached out when the morsel was present. One-third of 49 neurons were phasically activated by every door opening. The response was stronger when food was present. Thus DA neurons responded simultaneously to the sight of primary food reward and to the conditioned stimulus associated with reward.

4. When the box contained a morsel of apple on every trial, animals regularly reacted with target-directed eye and arm movements, and the majority of 76 DA neurons responded to door opening. The same neurons lacked responses to a light not associated with task performance that was illuminated at the position of the food box in alternate sessions, thus demonstrating specificity for the behavioral significance of stimuli.

5. The second task employed the operant conditioning of a reaction time situation in which animals reached from a resting key toward a lever when a small light was illuminated. DA neurons lacked responses to the unconditioned light. During task acquisition lasting 2–3 days, one-half of 25 DA neurons were phasically activated when a drop of liquid reward was delivered for reinforcing the reaching movement. In contrast, neurons were not activated when reward was delivered at regular intervals (2.5–3.5 s) but a task was not performed.

6. With established task performance, neurons lost responses to primary reward and instead were activated in their majority by the conditioned light. Thus the response to primary reward was transferred during learning to the conditioned stimulus that predicted reward and had the capacity to elicit arm and eye movement reactions.

7. Subsequently, each animal was overtrained with 30,000 arm movements. This resulted in automated task performance with shortened reaction and movement times. Responses of 165 neurons to the light were progressively reduced in terms of responding neurons (46 and 34% in 2 successive phases, respectively) and overall response magnitude.

8. DA neurons in areas A8 and A10 showed responses similar to those in A9, where most neurons were recorded. In particular, there was no regional preference for neurons responding to a particular stimulus during any learning phase. Thus the populations of A8, A9, and A10 DA neurons showed homogeneous responses during each phase of experimentation. Each neuron either responded to a particular stimulus during each learning phase or lacked responses to any stimuli.

9. These data suggest that, during acquisition of simple behavioral tasks, DA neurons respond to unconditioned and conditioned salient stimuli that attract the attention of the animal, induce behavioral activation, and are associated with reward. Effective stimuli include *1*) novel, unexpected stimuli eliciting orienting reactions; *2*) primary reward, when delivered as reinforcer during conditioning; and *3*) conditioned incentive stimuli, which predict reward and have the capacity to elicit behavioral reactions. The decreased neuronal responsiveness after overtraining parallels the reduced attentional and incentive processes that occur when the task is performed as a habit and stimuli serve merely as temporal reference for automatic task performance. These data provide further evidence for the involvement of DA neurons in arousing, motivational, and behavioral activating processes that determine behavioral reactivity without encoding specific information about the behavioral reaction.

INTRODUCTION

The widespread human disease Parkinsonism is associated with degeneration of dopamine (DA) neurons projecting from the midbrain to striatum and frontal cortex. In an attempt to find neuronal relationships to movements that are deficient in this disease, researchers have studied the impulse activity of single DA neurons in normal behaviorally conditioned primates during performance of motor tasks. It was soon found that DA neurons lack phasic changes during the execution of movements (DeLong et al. 1983; Freeman and Bunney 1987; Steinfels et al. 1981) or show slow activations unrelated to specific parameters of individual movements (Nishino et al. 1987; Schultz et al. 1983). However, DA neurons were found to respond to particular environmental stimuli, such as intense or novel auditory and visual stimuli eliciting orienting reactions (Freeman and Bunney 1987; Steinfels et al. 1983; Strecker and Jacobs 1985) and conditioned stimuli triggering immediate behavioral reactions (Miller et al. 1981; Schultz 1986). Convergent responses to visual, auditory, and somatosensory stimuli demonstrated a lack of sensory specificity (Schultz and Romo 1990). Responses of DA neurons were time locked to the stimulus rather than to onset of the

triggered movement (Schultz 1986). When monkeys were engaged in a specific task, DA neurons responded to stimuli independent of arm or eye movement reactions (Schultz and Romo 1990). Thus DA neurons do not encode specific movement parameters, but respond to stimuli that have the capacity to elicit behavioral reactions.

Responses of DA neurons to external stimuli are related to specific behavioral contexts. Responses to visual and auditory stimuli disappear when subjects are distracted (Strecker and Jacobs 1985). Trigger stimuli are effective only in the context of a behavioral task. The identical stimulus fails to activate DA neurons when animals lack specific reactions outside of a task. Even during task performance, DA neurons lack responses to trigger stimuli when an immediately preceding stimulus instructs the animal not to react (Schultz and Romo 1990). Neurons respond when the animal touches a morsel of food reward during self-initiated movements in the absence of predictive trigger stimuli. The same neuron responds to the trigger stimulus during the performance of externally triggered movements, and the response to touch of food is no longer present (Romo and Schultz 1990). It appears that the propensity of an environmental stimulus to activate DA neurons is determined both by the context of presentation and the elicited behavioral reaction. These aspects are, to an important degree, determined by previous experience. Neutral stimuli, which do not elicit attention or particular behavioral reactions from the subject, do not activate DA neurons. Thus the phasic activation of DA neurons appears to be related to the attentional, arousing, or activating properties of salient stimuli encountered during appetitive behavior. Stimuli effective for driving DA neurons apparently have been associated with certain goal objects by prior learning and consequently elicit behavior directed toward reaching these goals.

In the present experiments, we investigated how changes in the appetitive properties of salient external stimuli during learning of behavioral tasks would influence the responses of DA neurons. The adaptation of behavior to changing requirements would involve a particularly pronounced participation of attentional and motivational mechanisms. We investigated responses to stimuli the behavioral significance of which changed according to the progressing experience of the animal over successive stages of acquisition of different reaction time tasks. One of the tasks, in which an intrinsically neutral stimulus acquired appetitive properties through the conditioning procedure, employed natural reaching movements for food reward. Using a second task, we investigated whether responses of DA neurons to conditioned appetitive stimuli could be established by an operant conditioning procedure, in which a visual stimulus was delivered at a spatially distinct position and with a temporal delay from primary liquid reward. This situation allowed us to separate responses to the unconditioned stimulus of primary reward before task acquisition from responses to conditioned stimuli after learning. The behavioral meaning of primary reward changes when task contingencies are acquired and a conditioned stimulus becomes a reliable predictor of primary reward. This task was also used for studying overtraining and automated task performance. Parts of these data were presented in preliminary form (Ljungberg et al. 1990, 1991; Schultz et al. 1990).

METHODS

The activity of single DA neurons was recorded with movable microelectrodes in two male *Macaca fascicularis* monkeys (*A* and *B*, both 3.5 kg) before, during, and after they were conditioned to perform two closely related behavioral tasks. Electromyographic (EMG) activity and eye movements were monitored through chronically implanted electrodes. On termination of recording, animals were killed for histological reconstruction of recording sites. Before we recorded from these animals, the details of conditioning of the behavioral tasks were developed in a third monkey without collecting electrophysiological data.

Behavioral procedures

The behavioral apparatus was positioned in the right part of the frontal wall of the completely enclosed primate chair (Fig. 1). It contained a touch-sensitive, immovable resting key on which the monkey kept its right hand relaxed, the elbow joint being at ~90°. A panel mounted above the key held either a food box with a frontal opening of 40 × 40 mm or a lever (15 × 20 mm) positioned 40 mm below a yellow light-emitting diode (11 × 11 mm). Both food box and light were placed at eye level, at reaching distance, and at 27° lateral to the midsagittal plane in front of the animal. The door of the food box opened vertically upward in 20–22 ms and, in rewarded trials, revealed a small morsel of apple (~1 g). A drop of diluted apple juice (0.2 ml) was delivered by an electronically driven solenoid valve at a spout in front of the animal's mouth. Liquid arrived at the animal's mouth with an average delay of 55 ms after the electric pulse. A Plexiglas table was placed above the animal's waist with a vertical divider to prevent arm movements across the midline. Monkeys were deprived of food and fluid during weekdays. They were released into their home cages after each daily experiment of 3–4 h and received monkey cubes and water ad libitum during the subsequent 1 h. Before conditioning of the two reaching tasks, animals were habituated to the experiment with regularly timed drops of apple juice (1 every 2.5–3.5 s) for keeping their hands relaxed on the resting key.

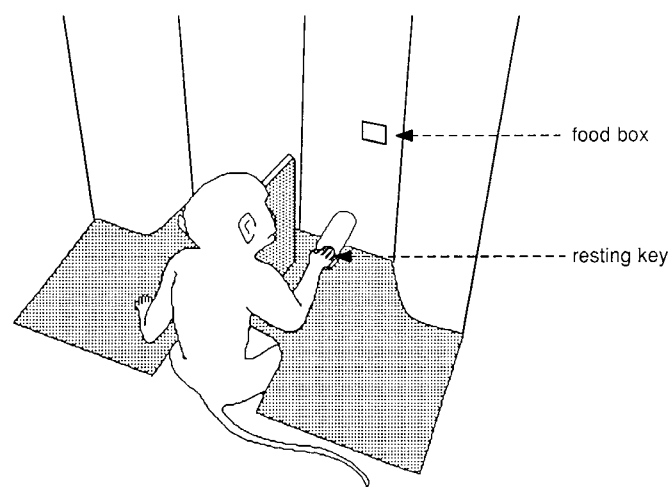

FIG. 1. Behavioral task. In the food box task, the animal sits with its muscles relaxed in a completely enclosed primate chair and faces a response panel with a touch-sensitive immovable resting key and a food box with a frontal opening of 40 × 40 mm. Door of the box opens rapidly upward; its movement is visible and audible to the animal. When the box contains a small morsel of apple, the animal releases the key in reaction to door opening, reaches into the box to collect the reward, brings it to the mouth, and consumes it. Door opening, key release, and entering and leaving the food box are monitored electronically. In the operant task (not shown), the food box is replaced by a panel holding a light and a lever. Animal releases the resting key on light illumination and depresses the lever to receive a drop of apple juice at its mouth.

FOOD BOX TASK. Neuronal recordings were done for 1 mo during the acquisition of natural reaching movements toward the food box. During the initial "no task" phase, the door of the empty food box opened once every 5–10 s. In the subsequent "intermittent task" phase, the box contained a morsel of apple on about every sixth door opening and opened empty in the remaining trials. In the final "full task" phase, a morsel of apple was present every time the door opened. The light-emitting diode was illuminated for 2 s as control stimulus in sessions separated from food box opening during all three task phases with *monkey B*. Animals were not required to fixate any particular spot with their eyes. Door opening and light stimulation were applied only while a DA neuron was being recorded.

With *monkey A*, the door was opened while the animal kept its hand on the resting key. This allowed separate measurements of reaction time (onset of door opening to key release) and movement time (key release to box entry). To condition this behavior, *monkey A* received regularly timed drops of juice not contingent on door opening during the no task and intermittent task phases. To exclude an influence of liquid delivery on neuronal responses to door opening, the door was opened without the resting key present with *monkey B*, and arm movement reactions were assessed only in terms of response time (from door opening to box entry). However, regular delivery of liquid did not significantly affect neuronal responses to door opening, and data from the two animals were pooled.

OPERANT REACTION TIME TASK. Subsequent to acquisition of the food box task, animals were conditioned to perform a similar reaching movement in response to light illumination. In the initial "no task" phase, the yellow light-emitting diode was illuminated for 2 s every 6–10 s. This was studied during 5 days with *monkey A* after the food box task had been terminated. With *monkey B*, light illumination and door opening were applied during 12 days in separate sessions. Data differed insignificantly between the two situations and were treated together. In separate sessions, animals received regularly timed drops of juice for staying on the resting key.

During the subsequent "conditioning" phase, maintaining the hand on the resting key was no longer rewarded with liquid. Instead, monkeys were required to perform a reaching movement and depress a small lever after light illumination to obtain a drop of juice. The light was extinguished either after the lever was pressed or, in the absence of movement, after a preset time of 2–4 s. Reward was delivered only when the lever was depressed while the light was still illuminated. The upper limit of response time was shortened in steps from 4 to 2 s during the course of conditioning. To separate the events that could conceivably activate DA neurons, a 500-ms delay was introduced between lever press and delivery of liquid. Conditioning of the task lasted 2 and 3 days in *monkeys A* and *B*, respectively, and was performed in successive steps in which reward was given for releasing the resting key; touching the frontal enclosure of the primate chair; and, finally, pressing the lever. Whereas *monkey A* always began the reaching movement from the resting key, *monkey B* kept its hand on the Plexiglas table in front of it before reaching to the lever and required further training for starting the movement from the key. Therefore response times instead of reaction times were obtained in *monkey B* during this phase.

The "postconditioning" phase began when animals readily performed the reaching movement by releasing the key and depressing the lever within 1,100 ms after light illumination in >95% of trials. Reaction times were <500 ms, and saccadic eye movements were regularly directed toward the lever after light illumination unless the eyes were already on target. This phase lasted 4–6 days.

The subsequent "first overtraining" phase consisted of 10 training days and ~10,000 movements for each animal. For automatizing task performance, the latency of lever press after light illumination was limited to 1.0 s, and trials were instantaneously restarted after the preceding trial when the monkey's hand was on the resting key. Intertrial intervals were shortened so that the light was illuminated every 6–7 s. In spite of improved task performance, animals usually interrupted task performance after every 150–200 trials. For maintaining maximum performance, pauses were introduced after each 100–150 rewarded movements. The resting key was removed and animals were given small pieces of cookies or raisins. In the "second overtraining" phase, each animal performed another 20,000 movements during 20 days, thus totaling 30,000 movements over 30 days. DA neurons were recorded during the total duration of the no task, conditioning, and postconditioning phases and during 4–7 days at the end of each overtraining phase.

Data acquisition

The light and the solenoid delivering reward were driven by output pulses from a suitably interfaced laboratory computer that also monitored behavioral performance. Key release was detected by a frequency-sensing circuit that reacted to a change in electrical capacity induced by the touch of the animal's hand. Behavior was electronically monitored from standard electronic pulses generated from door opening, light illumination, key release, lever touch, entering the food box, and delivery of liquid reward.

Animals underwent implantation after 3–4 mo, when they were habituated to the primate chair and kept their hands relaxed on the resting key for extended periods of time. Under deep pentobarbital sodium anesthesia and aseptic conditions, cylinders for head fixation and a stereotaxically positioned stainless steel chamber were fixed to the skull to permit vertical access with microelectrodes to the left substantia nigra (SN). The dura was left intact. Teflon-coated multistranded stainless steel wires were implanted into the extensor digitorum communis and biceps muscles of both arms and led subcutaneously to the head. The extensor digitorum communis and biceps are prime mover muscles of the arm in the reaching task (Schultz et al. 1989). Ag-AgCl electrodes were implanted into the outer, upper, and lower canthi of both orbits. All metal components, including plugs for the muscle and periorbital electrodes, were embedded in several layers of dental cement and fixed to the skull with surgical-grade stainless steel screws. The area of SN was localized 1 wk after implantation under pentobarbital anesthesia by taking lateral and coronal radiographs with a guide cannula installed at a known coordinate in reference to the implanted steel chamber (Schultz et al. 1983). The ventroposteromedial thalamus overlying the lateral SN was electrophysiologically explored for trigeminal input on the same occasion and occasionally in the waking animal.

The activity of single neurons was recorded extracellularly with glass-insulated, platinum-plated tungsten microelectrodes (exposed tips of 9- to 16-μm length and 2.5- to 3.5-μm diam), which were passed each day together with and inside a rigid guide cannula of 0.6-mm OD into the brain. Microelectrodes were moved in parallel tracks vertically in the stereotaxic plane, conforming to a 1-mm grid. Signals from the microelectrodes were conventionally amplified, filtered (100-Hz lower cutoff at −3 dB), and monitored with oscilloscopes and earphones. Full waveforms of impulses from each neuron were displayed on a digital oscilloscope with the use of the pretrigger viewing facility and subsequently were stored on computer disks. Somatodendritic discharges were distinguished from those originating from fibers with the use of earlier established criteria, in particular the very short duration of fiber impulses (0.1–0.3 ms) (Schultz and Romo 1987). A conventional storage oscilloscope, triggered by door opening, was used to monitor neuronal activity. Neuronal discharges were also converted into standard digital pulses by means of an adjustable

Schmitt trigger, the output of which was continuously monitored on the digital oscilloscope together with the original waveform. Every recorded neuron that fulfilled the criteria for being dopaminergic (see RESULTS) was tested. It was included in the study when its histologically assessed position was in areas A8, A9, or A10, as defined previously (Felten and Sladeck 1983).

EMGs were collected during all neuronal recordings through the chronically implanted electrodes. EMG activity was filtered (10- to 250-Hz bandpass; −12 dB at 1 kHz), rectified, monitored on a digital oscilloscope with the use of the roll mode, and passed through an adjustable Schmitt trigger. Limb and mouth movements were continuously supervised by a closed-circuit video system. Horizontal and vertical electrooculograms (EOGs) were collected during all neuronal recordings from the implanted periorbital electrodes. The gain of ocular electrodes and positions of the eyes were calibrated by having the food-deprived animal fixate small morsels of food presented at several known horizontal and vertical eccentricities while the frontal enclosure of the primate chair was kept open. Direct current offset needed to be adjusted every 3–4 wk.

All behavior-related digital signals, pulses from neuronal discharges and EMG activity, and analog-to-digital converted EOGs were sampled on line at a rate of 2 kHz by a laboratory computer. Eight consecutive EOG values were averaged to obtain a temporal resolution of 4 ms (0.25 kHz) for data storage. Behavioral relationships of neuronal discharges, EMG activity, and EOGs were displayed in each trial on line on the computer screen in the form of dot displays and analog curves. All data were stored uncondensed on computer disks. Only neurons recorded with ≥10 trials of a given test are reported, except for the intermittent phase with ≥6 rewarded trials.

Data analysis

The temporal characteristics of the stereotyped responses of DA neurons to external stimuli in similar behavioral situations (Schultz 1986; Schultz et al. 1990) allowed us to develop a standard time window procedure to statistically assess and compare responses between different task phases. First, onset and offset times of activating responses to external stimuli were determined for each neuron showing a suspected change. Onset time was defined by the first of three or more consecutive bins in which activity deviated from control, as indicated by an inflection in the cumulative frequency distribution averaged over all trials (time resolution 4 ms) (Fig. 2). Offset time was defined by the first of three or more consecutive bins with activity back to control. A specially implemented two-tailed Wilcoxon matched-pairs signed-rank test served to assess the significance of increase between onset and offset on a trial-by-trial basis for each neuron ($P < 0.01$, Schultz 1986). Then a common, standard time window that included 80% of onset and offset times in significantly responding neurons was defined for each stimulus. Time windows for door opening, light presentation, and delivery of liquid reward were 80–216 ms, 92–228 ms, and 176–312 ms, respectively, after onset of each event. In a final step, the magnitude of change was determined in every neuron, independent of its response, by comparing the number of impulses between the standard time window and a control period of 500 ms preceding the first stimulus of each trial. The number of neurons responding was determined with the Wilcoxon test, comparing activity between the time window and control period in each neuron ($P < 0.01$, 2-tailed). All neurons used for determining response latency and duration also showed significant activations in the standard time window.

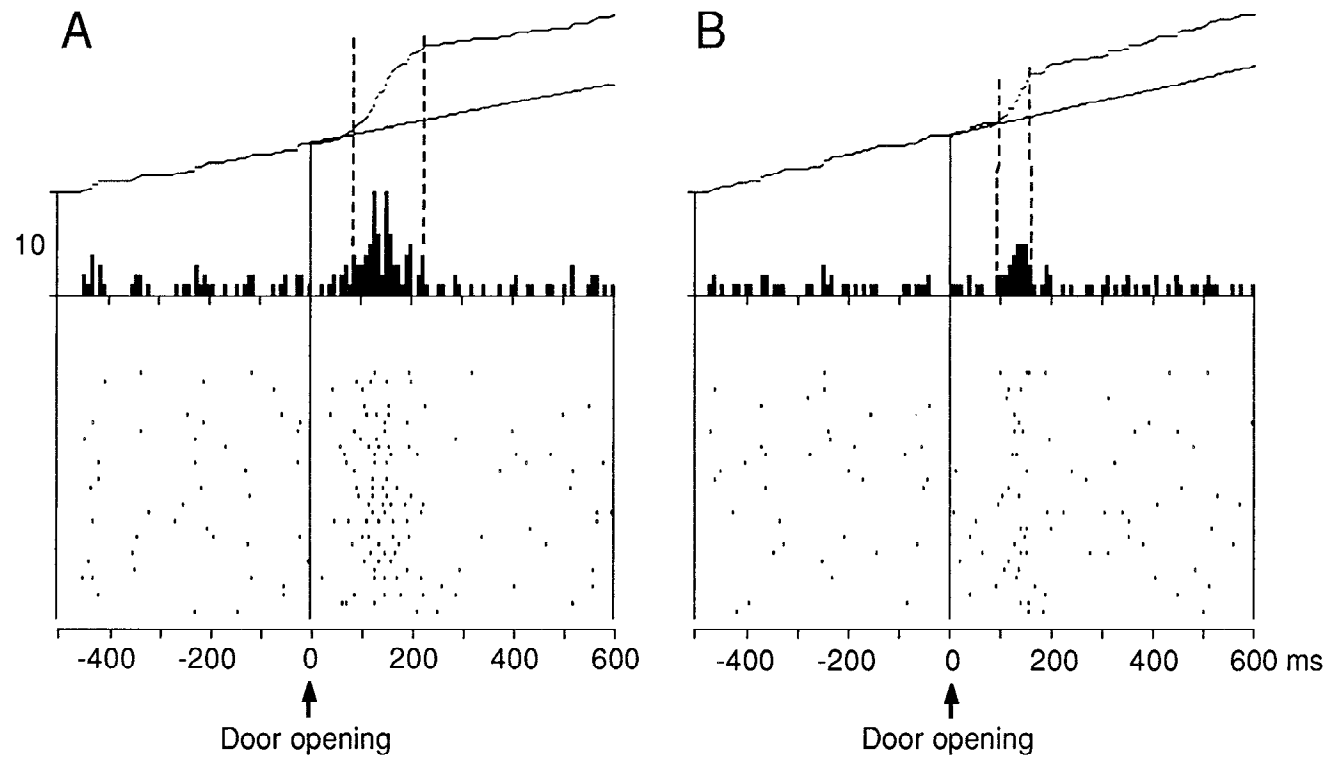

FIG. 2. Method for quantitative assessment of neuronal responses. Strong (*A*) and weak responses (*B*) of 2 DA neurons to door opening in the reaching task. From *top*: cumulative frequency distribution, perievent time histogram, and raster display of neuronal impulses. Each dot represents the time of a neuronal impulse, and each line of dots represents 1 trial. Histograms are composed of neuronal impulses shown as dots *below* them. Interrupted vertical lines show onset and offset of neuronal responses determined from inflections in cumulative frequency distributions. Oblique lines indicate average baseline activity during control period before the stimulus. Latency and duration data obtained with this method for each neuron were used for determining the standard time window.

Latencies of saccadic eye movements were determined off line by single-trial analysis using a movable cursor on a computer screen. Occasional spontaneous saccades were excluded (latencies <52 or >300 ms). Arm movements were evaluated in terms of reaction time (from door opening or light illumination to release of resting key), movement time (key release to box entry or lever press), and response time (door opening or light illumination to box entry or lever press). The median was chosen as single numerical value for each session because of skewed distributions during early stages of experimentation.

Magnitudes of changes of DA neurons in the standard time windows after external stimuli were normally distributed, indicating that responses were homogeneous and not separated into distinctive groups ($P > 0.05$, Kolmogorov-Smirnov 1-sample test; Siegel 1956). Session medians of behavioral parameters in each task phase were equally normally distributed, which allowed us to employ parametric statistics for all further evaluations. Means ± SE were used for description. Differences in distributions were assessed with 1- and 2-way analyses of variances (ANOVAs; with task phases and task phases and monkeys as factors, respectively), and with one-sample and paired or unpaired two-sample Student's t tests ($P < 0.001$). Differences in distributions of frequencies were tested with the χ^2 test ($P < 0.001$). Changes of neuronal responses or behavioral performance as a consequence of training were assessed for each task phase by correlating data from single sessions against order of sessions with the use of the Spearman rank correlation test ($P < 0.01$).

The overall response of all neurons tested during each task phase was assessed by calculating the population histogram. For each neuron, a normalized peristimulus time histogram was obtained by dividing the content of each bin by the number of trials. A population histogram was obtained by averaging normalized histograms referenced to the same behavioral event.

Histological reconstruction

Toward the end of recording, small marking lesions were placed by passing negative currents (10–20 mA for 10–20 s) through the microelectrode immediately after recording from a neuron in SN, whereas larger lesions (20 mA for 20 or 60 s) were positioned at a few locations above in the same track. This produced distinct patterns of vertically oriented histological marks. Animals were deeply anesthetized with pentobarbital sodium and conventionally perfused with formaldehyde through the heart. Guide cannulas were inserted into the brain at known coordinates of the implant system to delineate the general area of recording. The tissue was cut in 50-μm-thick serial coronal sections on a cryotome and stained with cresyl violet. All histological sections were projected

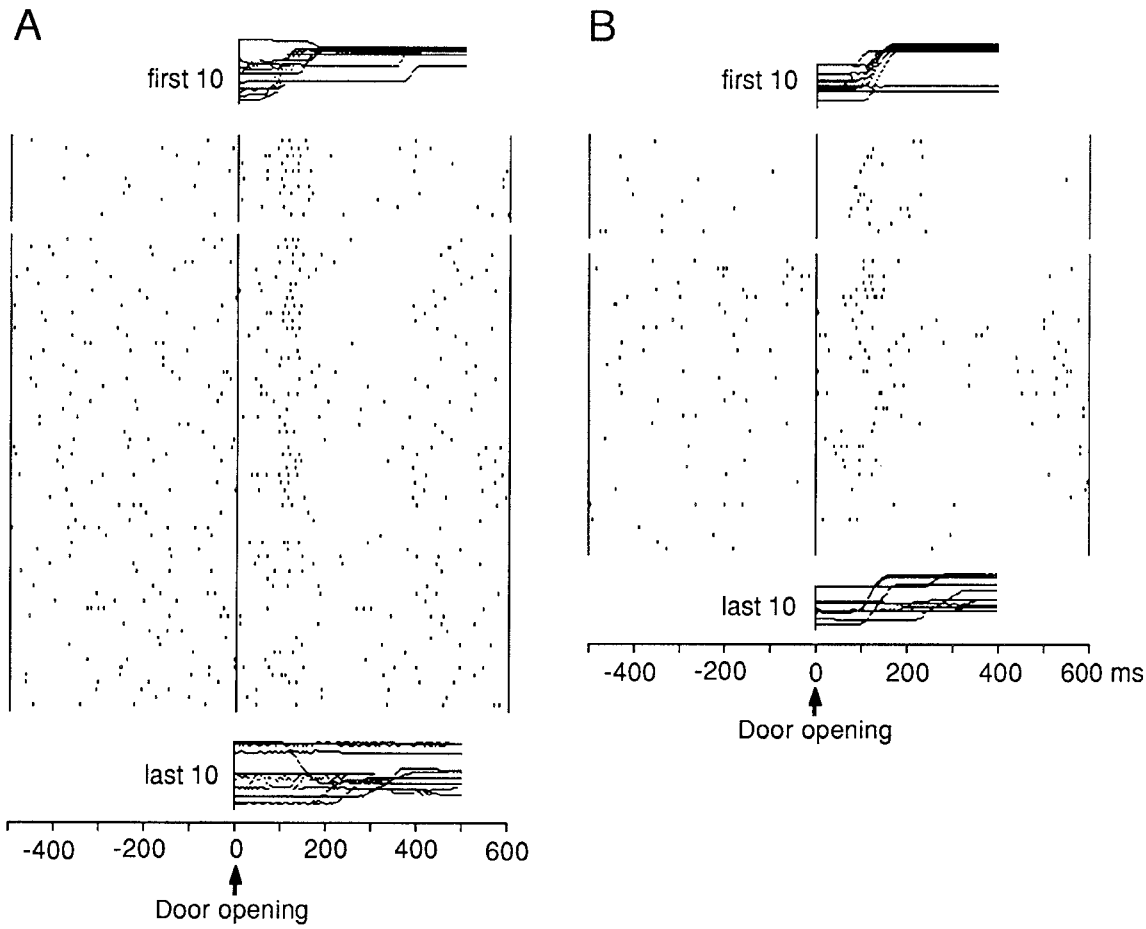

FIG. 3. Responses to novel door opening stimulus in 4 DA neurons. Box was empty in all trials. *A* and *B* refer to the 2 monkeys, respectively. Raster displays from the 1st and 2nd neuron recorded with door opening in each monkey are separated. Each dot represents the time of a neuronal impulse, and each line of dots represents 1 trial. Natural sequence of trials is shown downward. Intervals between subsequent door openings were 7–8 s; intervals between 1st and 2nd neuron recorded were 15–20 min in each monkey (after 11 and 13 trials in *A* and *B*, respectively). Superposed horizontal components of simultaneously recorded electrooculograms are shown *above* and *below* rasters from the first and last 10 trials, respectively, demonstrating that animals initially reacted to door opening. Saccades toward the right are shown by upward deflections.

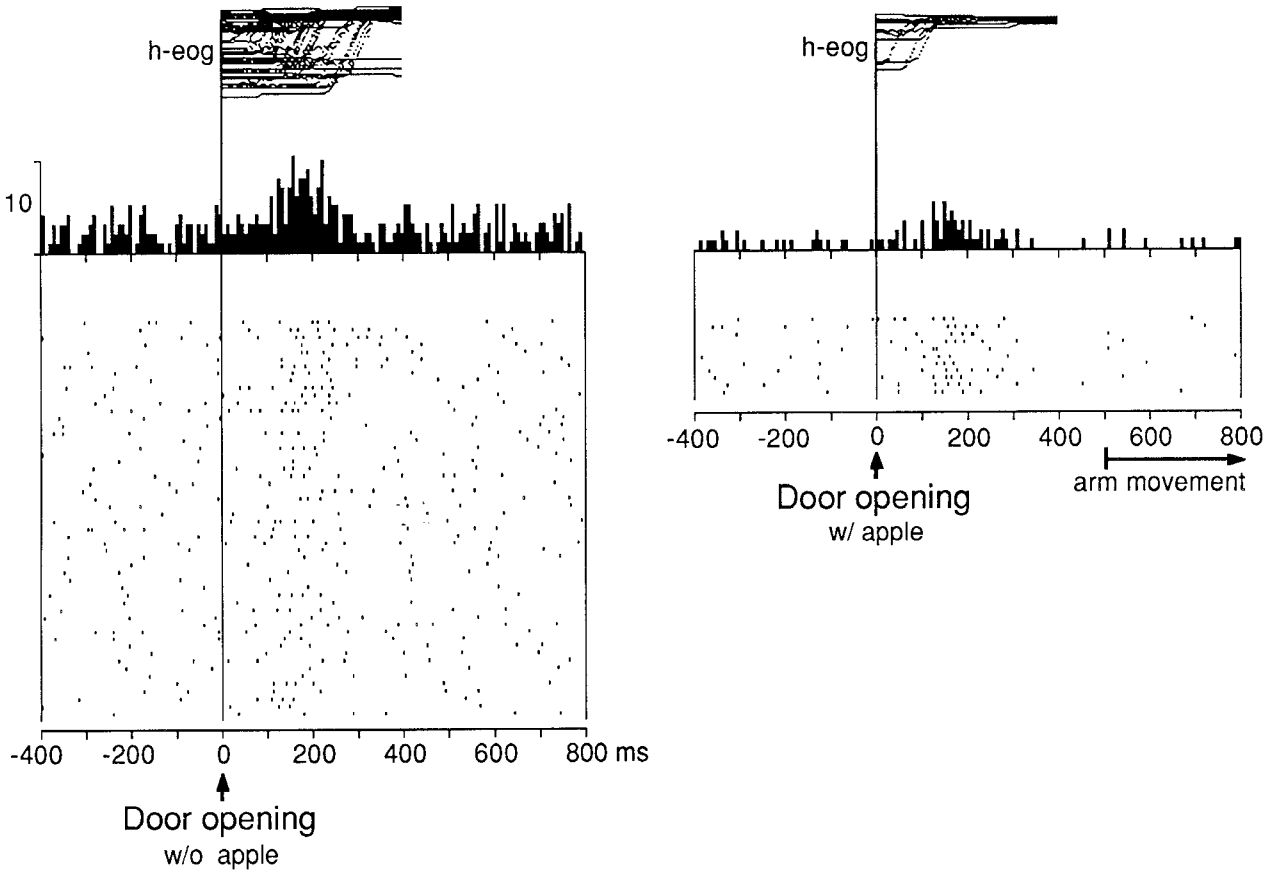

FIG. 4. Response of a DA neuron to door opening in the intermittent task. *Left*: response in trials without food and, consequently, in absence of arm reaching movement. *Right*: response in trials with food in box and reaching movement. Superposed traces from eye movements recorded simultaneously with neurons are shown *above* histograms and reveal frequent saccadic reactions. Horizontal components of saccades (h-eog) toward the right are shown by upward deflections. Because of absence of a visual fixation spot, eyes were at varying positions when the door opened. Key release (onset of movement) is indicated by vertical line below *right* raster display. Trials shown to *left* and *right* were intermingled during the experiment and separated off-line.

on paper, and the outlines of brain structures and the marks from lesions and recent electrode tracks were drawn. Recording positions in tracks marked by electrolytic lesions were reconstructed by using the distances to the lesions according to protocoled micrometer readings. Positions in parallel neighboring tracks at 1-mm distance were reconstructed at comparable vertical levels.

RESULTS

A total of 552 DA neurons were recorded before, during, and after the successive acquisition of the two behavioral tasks. During the course of experimentation, neurons responded to different extents with phasic activations to primary reward and to intrinsically neutral stimuli that, during learning of the tasks, acquired behavioral significance. A few neurons showed short depressions that either followed the activation or occurred alone after a stimulus. All responses lasted for <300 ms, and their latencies and durations were parametrically distributed. They thus followed the criteria underlying the constant time window procedure for data evaluation.

DA neurons of SN (A9), and adjoining groups A8 and A10, discharged initially negative or positive impulses at low frequencies (0.5–7.0 imp/s) and with polyphasic waveforms of relatively long durations (1.8–5.0 ms), in agreement with previous experience (Romo and Schultz 1990; Schultz 1986; Schultz and Romo 1987). In these characteristics, DA neurons contrasted with reticulata neurons of SN discharging impulses of <1.1-ms duration at median rates of 70–90 imp/s, with a few neurons discharging short impulses (<1.0 ms) at low rates and with presumptive fibers discharging very short impulses (0.1–0.3 ms).

Food box task

BEHAVIOR. Before task acquisition, monkeys sat with their arm muscles relaxed in the chair. *Monkey A* usually kept its

TABLE 1. *Behavioral performance in the food box task*

	Intermittent Task	Full Task
Reaction time*	518 ± 26 (17)	318 ± 8 (34)
Movement time*	294 ± 8 (17)	266 ± 7 (34)
Response time†	825 ± 19 (43)	598 ± 6 (76)
Saccadic latency†		
With apple	107 ± 3 (33)	105 ± 2 (76)
Without apple	140 ± 5 (33)	

Values are means ± SE in ms, with numbers of sessions in parentheses. Reaction, response, and movement times are significantly longer in the intermittent than in the full task phase. Saccadic latencies are significantly longer in trials without apple in the box than in trials with apple during the intermittent task. **Monkey A* only. †Both monkeys.

right hand on the resting key, for which it was rewarded, whereas *monkey B* had both hands on the Plexiglas table or the vertical divider. Both animals reacted, but only during the first few trials, with saccadic eye movements to opening of the empty food box (Fig. 3). In the intermittent task phase, the food box contained a small morsel of apple on about every sixth opening. Within the first session, animals reached out for the food and consumed it. Saccadic ocular reactions to door opening occurred in both rewarded and unrewarded trials (Fig. 4). They were seen in 87 and 56% of rewarded trials in *monkeys A* and *B*, respectively. Eye positions were already on target at the time of door opening in most remaining trials, because the eyes were free to move in the absence of a particular fixation spot. Arm movement reactions were slow (Table 1). Saccadic latencies differed according to the content of the food box. In the full task, the food box contained a morsel of apple in all trials. All arm movement measures, particularly reaction and response times, were significantly shorter than during the intermittent phase. Saccades occurred in 75 and 58% of trials in

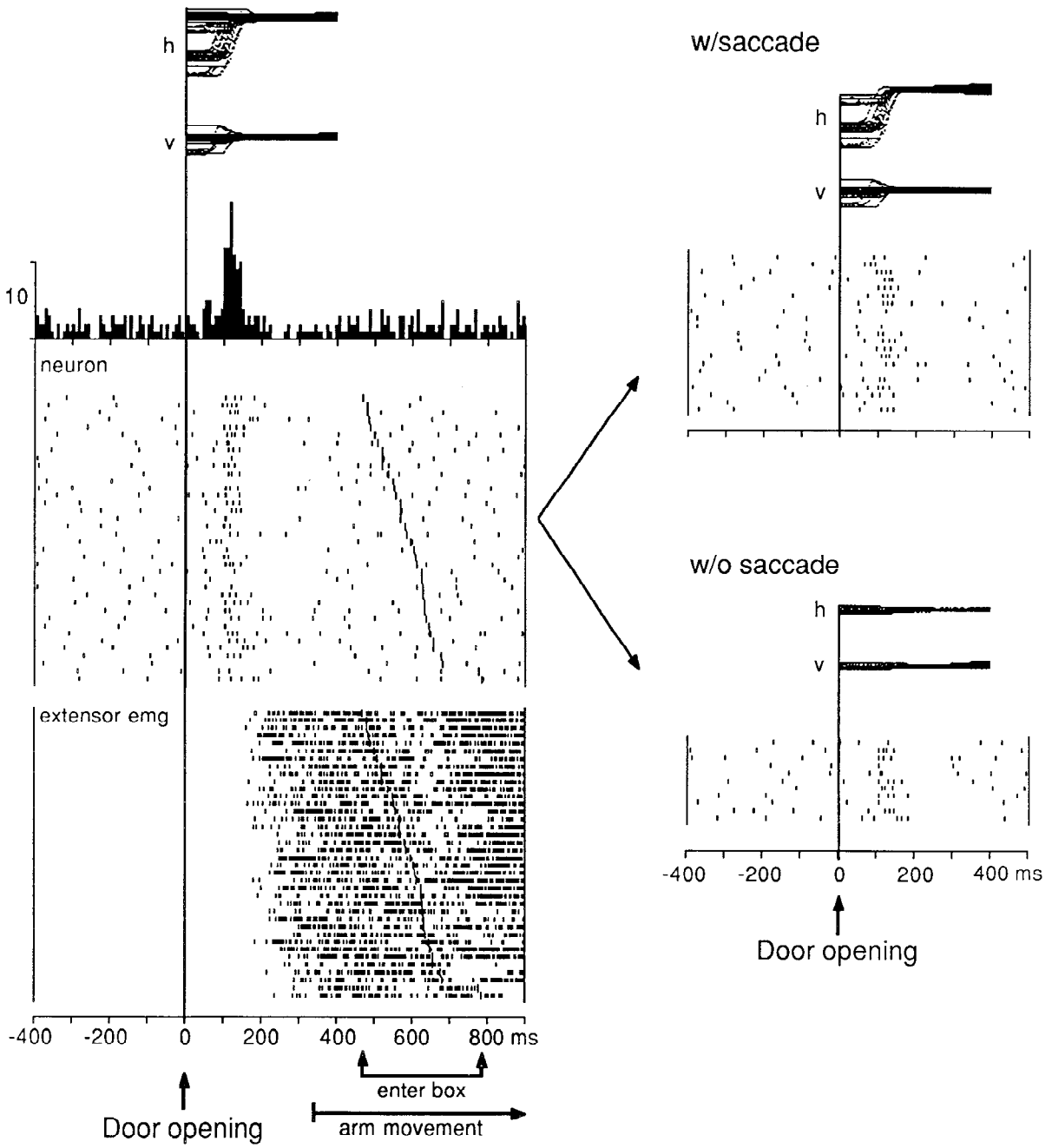

FIG. 5. Response of a DA neuron to door opening during performance of the full food box task. *Left*: door opening triggered an arm reaching movement and a saccadic eye movement toward the food box. Small bars in raster displays indicate when the animal's hand arrived at the food box. Raster displays of neuronal impulses are shown *above* rasters of EMG activity in the extensor digitorum communis. Sequence of trials is rearranged according to intervals between door opening and arrival at food box. *Right*: lack of relation of neuronal response to saccadic eye movements. Same trials as shown to the *left*, but separated according to presence or absence of eye movements (h, horizontal and v, vertical component of electrooculogram).

monkeys A and *B*, respectively, the eyes being on target in all trials (Figs. 5 and 6). Ocular and skeletal reactions to light illumination were absent (Fig. 6).

NO TASK. The first two DA neurons recorded in each monkey after introduction of door opening of the empty box responded to this stimulus (Fig. 3). At the same time, animals reacted with a target-directed saccade to door opening. Afterward, the neuronal response faded away together with the ocular reaction. None of 79 DA neurons recorded thereafter were significantly activated by opening of the empty box (Fig. 3). Eleven neurons (13%) were significantly depressed by door opening, most of them recorded immediately after the 4 activated cells. Magnitudes of changes after door opening in the population of the 83 neurons tested were insignificantly different from 0% change (Table 2). None of 11 neurons tested showed significant responses to light illumination.

INTERMITTENT TASK. A total of 17 of 49 DA neurons (35%) responded with significant phasic activation to opening of the food box during intermittent task performance (Fig. 4). Three neurons (6%) were depressed by door opening. Activating and depressant responses occurred typically both in the presence and the absence of food. Activating responses to opening of empty and filled boxes began in *monkey A* after 34 trials of intermittent task performance while recording the second DA neuron in this task phase. In *monkey B*, activating responses to opening of the filled box began after 20 trials of the intermittent task while recording the second DA neuron and to opening of the empty box after 162 trials while recording the fourth DA neuron. Magnitudes of changes were significantly higher for trials in which door opening revealed the sight of food, compared with empty box trials (Table 2). None of 11 neurons activated by door opening responded to light illumination.

FULL TASK. A total of 41 of 76 DA neurons (54%) responded with phasic activation to door opening (Figs. 5 and 6). Four neurons (5%) were depressed by door opening. Neuronal responses were time-locked to stimulus presentation, were temporally unrelated to the following onset of arm muscle activity or movement, and occurred independently of saccadic eye movements (Fig. 5), thus closely resembling the responses seen previously (Schultz 1986; Schultz and Romo 1990). With one exception, neurons activated by door opening lacked responses to light illumination (Fig. 6).

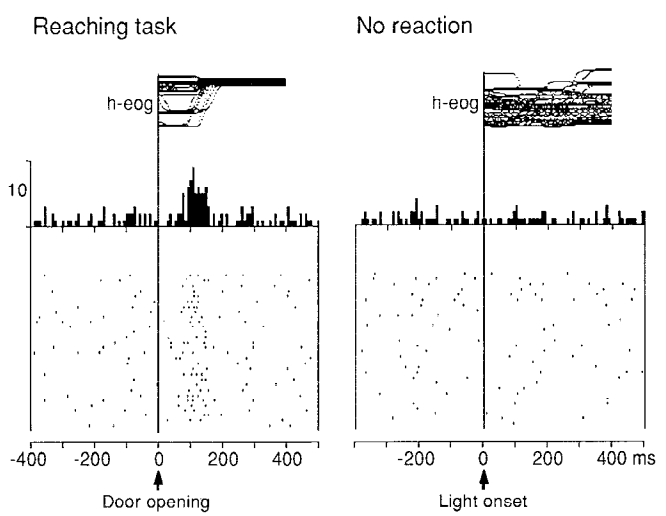

FIG. 6. Activity of 1 DA neuron after 2 phasic stimuli in different behavioral contexts during performance of the full food box task. *Left*: response to door opening used for triggering arm reaching movement and eye movement. *Right*: lack of response of same neuron to light illuminated outside of a specific behavioral task. Traces *above* histograms demonstrate the presence of horizontal ocular reactions to door opening (*left*), whereas only spontaneous eye movements are observed after light illumination (*right*).

TABLE 2. *Magnitudes of neuronal changes after door opening in the food box task*

	Magnitude	*n*
No task	14 ± 10 (NS)	83
Intermittent task		
Empty box	60 ± 13*	49
Full box	134 ± 25*	49
Full task	166 ± 18*	76

Magnitudes were assessed in the standard time window for the door opening stimulus and are given as means ± SE of %above-control activity from all neurons recorded during the learning phases indicated, independent of showing significant responses. All changes lacked significant differences between animals and were pooled. *$P < 0.001$ against a 0% change; NS, not significant. Differences in magnitudes between task phases were significant (taking empty box for intermittent phase) (1-way ANOVA). The difference between empty and full box trials in the intermittent task was significant (*t* test).

COMPARISONS BETWEEN TASK PHASES. Frequencies of responding neurons increased significantly over the three consecutive task phases. Magnitudes of changes in the time window after door opening differed equally significantly among the three consecutive task phases but not between monkeys (Table 2). Thus the proportions of responding neurons and the response magnitudes demonstrate the development of neuronal responsiveness with acquisition of the reaching task. This is directly shown by the population histograms constructed from all DA neurons recorded during the no task and full task phases, respectively (Fig. 7).

The latency of neuronal activations after door opening during the intermittent and full task phases was 92 ± 3 (SE)

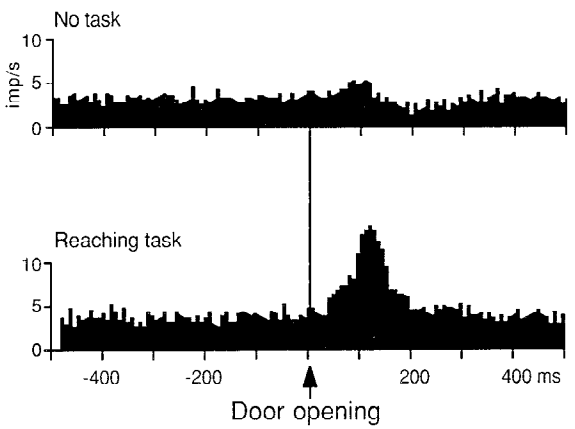

FIG. 7. Development of population response of DA neurons with acquisition of food box reaching task. Histograms contain data from all neurons tested in both monkeys during no task (*top*, 83 neurons) and reaching task (*bottom*, 76 neurons). For *top* and *bottom*, histograms from each neuron normalized for trial number were added and the resulting sum divided by the number of neurons. Vertical calibration indicates mean impulse rate.

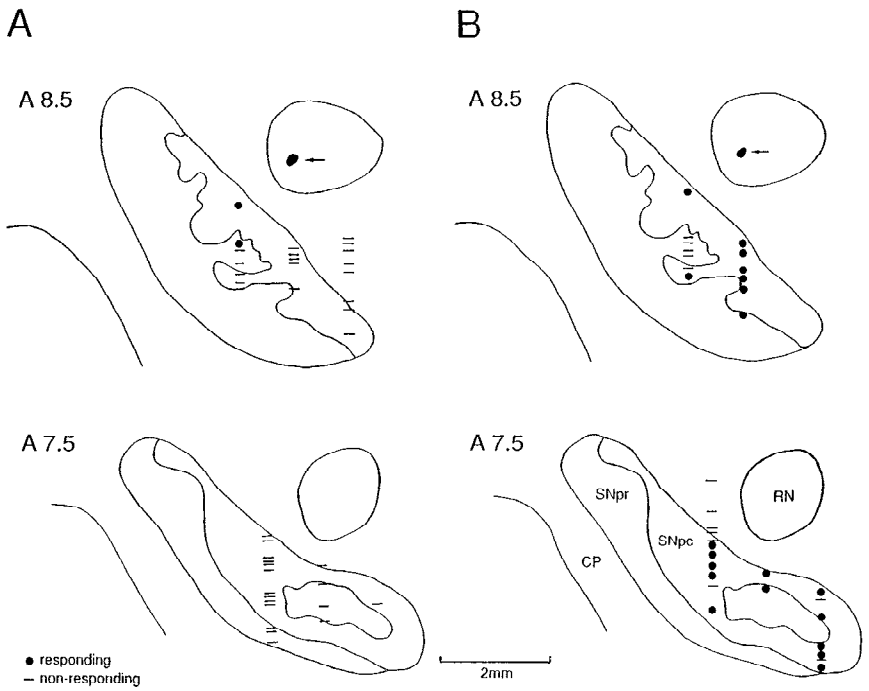

FIG. 8. Histological reconstruction of recording positions of DA neurons. Two representative coronal sections are shown from 1 monkey for no task (*A*) and full task phases (*B*) with the food box. Approximate anteroposterior levels are shown in millimeters rostral to interaural line according to an atlas (Shanta et al. 1968). Dots, DA neurons activated by door opening; lines, unresponsive DA neurons. Arrow points to marker lesion placed above neuronal recording sites for track identification. SNpc, pars compacta of substantia nigra; SNpr, pars reticulata of substantia nigra; RN, red nucleus; CP, cerebral peduncle.

ms; their duration was 85 ± 5 ms (n = 58). Both measures varied insignificantly over task phases. Statistically significant responses to door opening in the full task phase consisted of a mean increase from 3.92 imp/s during control to 19.95 imp/s, using the response durations determined individually for each neuron (409 ± 29%; n = 41).

POSITIONS OF NEURONS. Histological reconstructions of recording sites in the ventroanterior midbrain revealed that the majority of DA neurons were recorded in catecholamine group A9 (pars compacta of SN: n = 165, 79%), whereas some neurons were found in groups A8 (n = 26, 13%) and A10 (n = 17, 8%), dorsal and medial to SN, respectively. There were no systematic differences in recording areas between the different task phases. Positions of responsive and unresponsive neurons recorded from one monkey during the no task and full task phases are shown on two representative sections in Fig. 8.

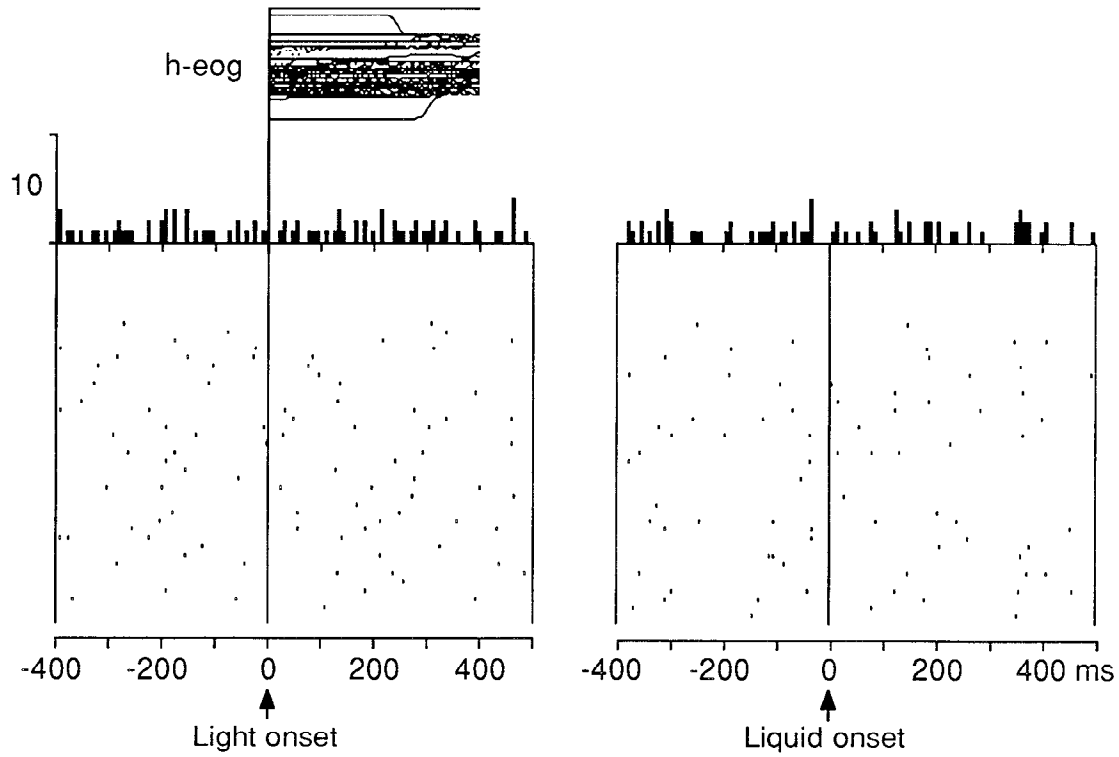

FIG. 9. Lack of response of 1 DA neuron to light illumination and liquid reward delivery before operant conditioning (no task). *Left*: absence of neuronal response to light is paralleled by lack of ocular reaction. *Right*: absence of response to liquid that was delivered in separate sessions once every 2.5–3.5 s when the animal kept its hand on the resting key.

Operant reaction time task

BEHAVIOR. During the no task phase, ocular and skeletal reactions to light illumination were absent (Fig. 9). During light illumination sessions, *monkey A* kept its right hand on the resting key without being rewarded, whereas *monkey B* had both hands on the Plexiglas table or the vertical midline divider. During the conditioning phase, arm movement reactions to light illumination appeared and were successively better initiated after the initial steps of learning (Table 3, Fig. 10). During the subsequent postconditioning phase, animals consistently showed >95% correct task performance. A training effect is demonstrated by progressively shorter and less varied reaction times, movement times, and response times (Fig. 11). These parameters showed significant negative correlations with session order during the conditioning and postconditioning phases in both monkeys. Arm movement parameters showed further, albeit insignificant, improvement during both overtraining phases.

During conditioning, saccades directed toward the light appeared within the first session and occurred in 57 and 46% of trials in *monkeys A* and *B*, respectively. Because the eyes often fixated the light immediately before it was illuminated, they were on target in 80 and 74% of all trials in *monkeys A* and *B*, respectively (at 300 ms after stimulus presentation). After conditioning, the frequency of saccades remained unchanged, but the eyes were on target in 83–98% of trials. Saccadic latencies lacked significant differences between task phases and showed inconsistent development with overtraining (Table 3).

NO TASK. Only 6 of 90 DA neurons (7%), most of which were found during the early part of this phase, responded with significant phasic activation to light illumination. None of the 90 neurons were depressed. Delivery of liquid was studied in 138 DA neurons while animals did not perform any specific task and kept their hand relaxed on the resting key (Fig. 9). Included in these tests were 48 neurons recorded in *monkey B* during the no task phase of the preceding food box task. Only 18 of the 138 neurons (13%) responded with significant phasic activation, and 6 (4%) were depressed. Thus most neurons lacked responses to light illumination and reward delivery (Fig. 9), and the

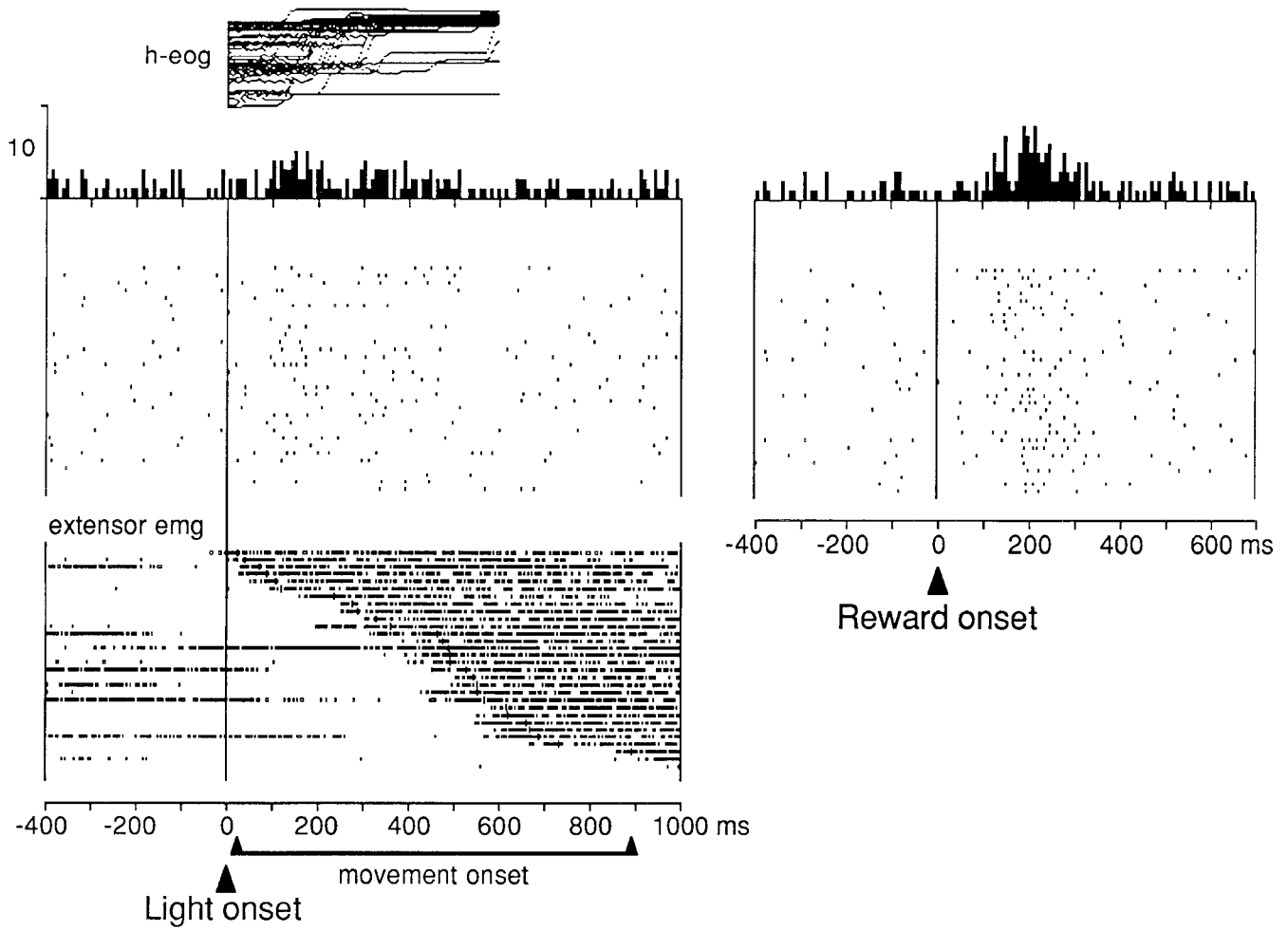

FIG. 10. Responses of 1 DA neuron during operant conditioning. *Left*: small response to light occurred when animal began to react with an arm reaching movement. This response was statistically significant in the standard time window. Small bars in EMG raster from extensor digitorum communis recorded simultaneously with neuron indicate when the hand left the resting key (movement onset). Sequence of EMG trials is rearranged according to time intervals between light illumination and key release. Saccades toward the light occurred in many trials in which eye position was not on target at the moment of illumination. A marked variability in skeletal and ocular reactions is apparent during this phase. *Right*: pronounced response of same neuron to liquid reward delivered on correct task performance (same trials as shown to the *left*).

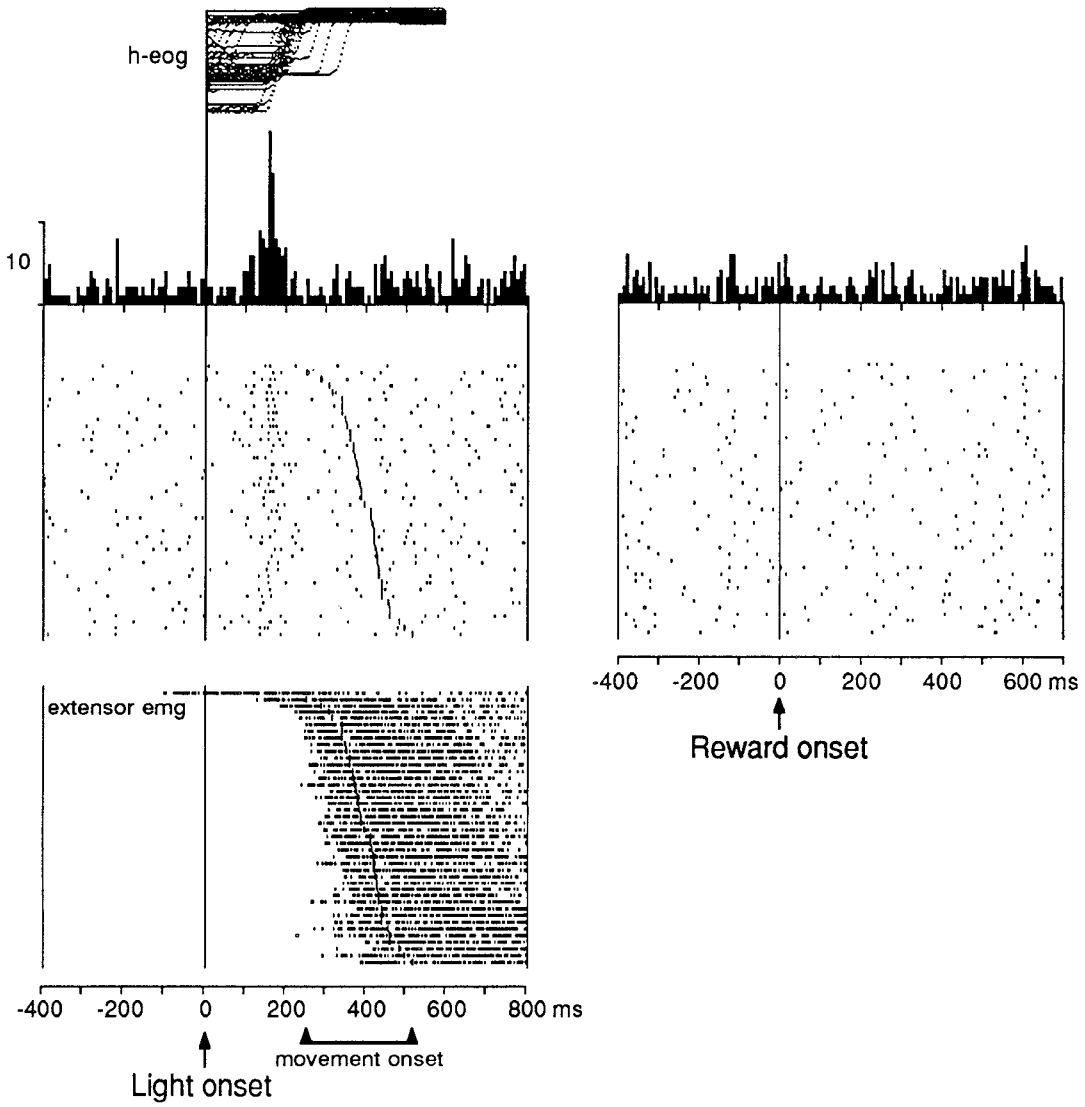

FIG. 11. Responses of 1 DA neuron after acquisition of the operant reaction time task (postconditioning phase). *Left*: pronounced neuronal response to the light triggering an arm reaching movement and an eye movement. Behavioral performance is more regular than during conditioning. Small bars in neuronal and EMG rasters indicate release of resting key (movement onset). Sequence of trials in both rasters is rearranged according to reaction time. *Right*: lack of response of the same neuron to liquid reward delivered on correct task performance (same trials as shown to the *left*).

mean magnitude of changes in all neurons tested was very small (Table 4).

CONDITIONING. While animals began to react to light illumination, 8 of 25 DA neurons (32%) responded with phasic activation to the light; none responded with depression. In contrast to all other learning phases, these responses were largely restricted to one animal (7 of the 8 neurons were in *monkey A*). Responses to light in general resembled those during regular task performance in the postconditioning phase, although they were more sluggish (Fig. 10, *left*). Magnitudes of responses (Table 4) varied insignificantly

TABLE 3. *Behavioral performance in the operant reaction time task*

	Conditioning		Postconditioning		1st Overtraining		2nd Overtraining	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Reaction time	569 ± 25		386 ± 13	384 ± 6	290 ± 5	311 ± 10	259 ± 3	278 ± 2
Movement time	656 ± 52		445 ± 14	449 ± 9	293 ± 3	382 ± 7	316 ± 4	300 ± 1
Response time	1,271 ± 59	1,461 ± 154	845 ± 25	857 ± 14	587 ± 8	702 ± 15	583 ± 5	584 ± 3
Saccadic latency	190 ± 4	166 ± 13	188 ± 3	141 ± 3	207 ± 2	147 ± 3	194 ± 2	136 ± 3
n	12	12	30	38	44	39	41	41

Values are means ± SE in ms. Data were obtained during sessions in which DA neurons were recorded (*n*, number of sessions). They are shown separately because of significant differences between monkeys. *A* and *B*, *monkeys A* and *B*, respectively.

TABLE 4. *Magnitudes of changes after light illumination and reward delivery in the operant reaction time task*

	Light				Reward	
	Monkey A		*Monkey B*			
	Magnitude	*n*	Magnitude	*n*	Magnitude	*n*
No task	24 ± 5*	45	7 ± 4 (NS)	45	15 ± 5*	138
Conditioning	118 ± 24*	12	7 ± 9 (NS)	13	110 ± 32*†	25
Postconditioning	164 ± 37*†	26	142 ± 22*†	38	39 ± 9*	64
1st overtraining	99 ± 19*†	44	75 ± 17*†	39	14 ± 5*	83
2nd overtraining	64 ± 13*	41	45 ± 13*	41	20 ± 5*	82

Magnitudes were assessed in the standard time window for the door opening stimulus and are given as means ± SE in %above-control activity from all neurons recorded during the learning phases indicated, independent of significant responses. Magnitudes of changes after light illumination differed significantly between monkeys and are shown separately for each animal, whereas changes after reward lacked significant differences between animals and were pooled. *$P < 0.001$ against a 0% change; ns, not significant. Changes between phases were statistically significant (1-way ANOVA). †Significant difference against no task magnitudes (Dunnett's test).

between correct and unrewarded trials (14 neurons with ≥10 trials in each situation).

The most prominent responses during conditioning occurred with the delivery of primary liquid reward. Statistically significant phasic activations after reward were seen in 13 of 25 DA neurons (52%; Fig. 10, *right*). Only six of these neurons were also activated by the light. None of the 25 neurons were significantly depressed by reward. Magnitudes of changes after reward of all 25 neurons during conditioning were significantly higher than when animals kept their hands on the key without a task (Table 4).

POSTCONDITIONING. After task acquisition, 37 of 64 DA neurons (58%) responded with a significant phasic activation to light illumination; none responded with depression (Fig. 11, *left*). Responses were time-locked to onset of the trigger stimulus and not to onset of the following arm movement. They remained present when animals occasionally failed to react. Responses occurred independently of individual saccadic eye movements (eye positions were occasionally on target at the time of light illumination). Magnitude of changes after the light was significantly higher than during the no task phase (Table 4).

In parallel with the increased responsiveness to the conditioned light, responses to delivery of primary reward were largely reduced (Fig. 11, *right*). Only 11 of 64 neurons (17%) responded with significant phasic activation to liquid reward; none responded with depression. Magnitudes of changes after reward were insignificantly higher than when the monkeys kept their hands on the key without a task.

FIRST AND SECOND OVERTRAINING. A total of 38 of 83 DA neurons (46%) responded with significant phasic activation to light illumination after the first overtraining, and 28 of 82 (34%) after the second overtraining (Fig. 12). Two neurons (2%) were depressed in each phase. In agreement with the reduced number of responding neurons, the mean magnitudes of changes after the light decreased successively over the two overtraining phases (Table 4).

Only 9 of 83 neurons (11%) responded with significant phasic activation to liquid reward after the first overtraining, and, equally, 9 of 82 (11%) after the second overtraining. Four and two neurons, respectively, were depressed. Magnitudes of changes after reward after the first and second overtraining differed insignificantly from magnitudes measured when the monkeys kept their hands on the key without a task.

COMPARISON BETWEEN TASK PHASES. Differences in magnitudes of time window changes between task phases were significant for both light illumination and reward delivery. Performance of Dunnett's test subsequent to ANOVA revealed that magnitudes of changes after light illumination during the postconditioning and first overtraining phases were significantly higher than during the no task phase (Table 4). By contrast, magnitudes of reward-related changes

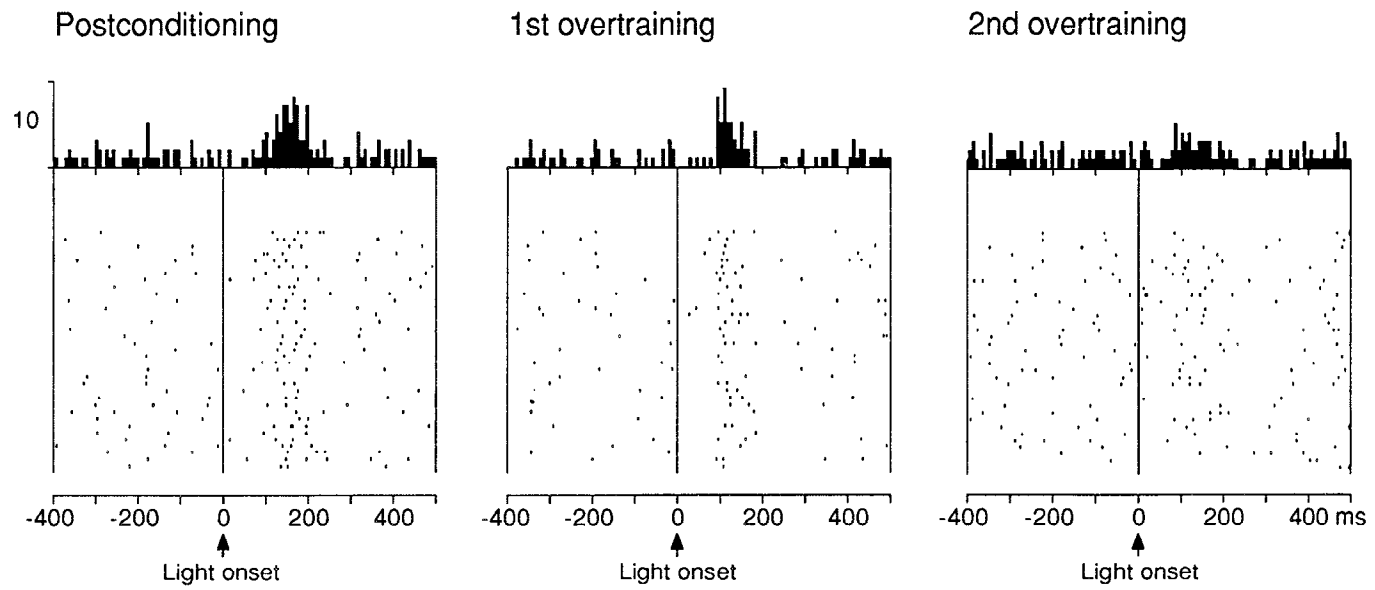

FIG. 12. Decrease of neuronal responsiveness after overtraining. Representative responses to light illumination are shown for 3 DA neurons recorded during performance of reaching task during postconditioning and 1st and 2nd overtraining phases.

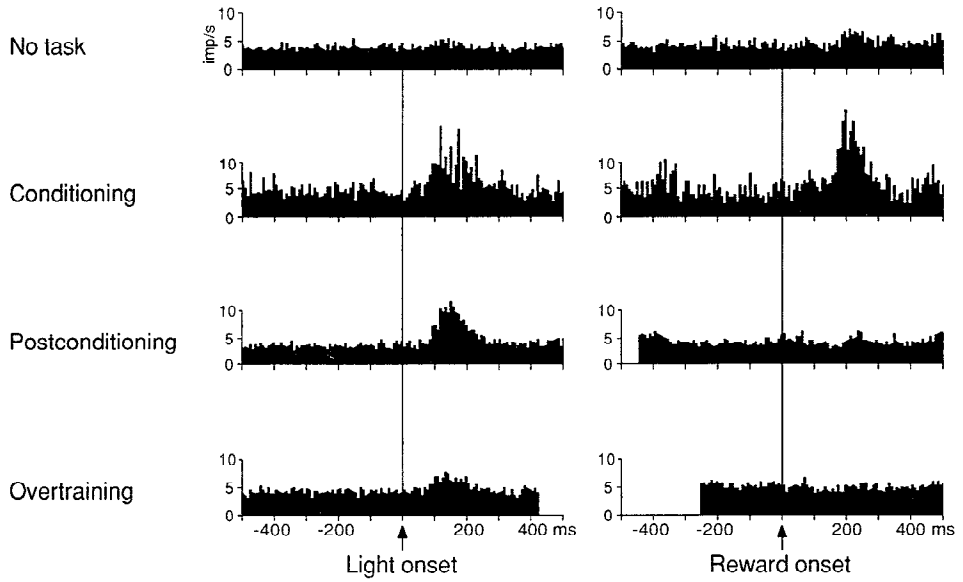

FIG. 13. Development of population response of DA neurons during operant conditioning. *Left*: light illumination. *Right*: liquid reward delivery. Overtraining refers to 2nd overtraining phase. Population histograms were calculated from all neurons tested in both monkeys, independent of individual neuronal responses. These were 90, 25, 64, and 82 neurons in no task, conditioning, postconditioning, and 2nd overtraining phases, respectively. For each population histogram, histograms from each neuron normalized for trial number were added and the resulting sum divided by the number of neurons. Vertical calibration indicates mean impulse activity.

were significant during the conditioning phase. Thus responses to light illumination became prominent with completed task acquisition (postconditioning and first overtraining phase) and decreased successively after each step of overtraining. Reward responses were restricted to conditioning before full task acquisition. Population responses of DA neurons recorded during the five phases are shown in Fig. 13.

Latency of activation after light illumination in all responding neurons was 106 ± 2 ms; their duration was 95 ± 4 ms (Fig. 14). Activations began at 185 ± 4 ms after reward and lasted for 92 ± 6 ms. Reward latency corrected for the 55-ms delay of liquid delivery after the electronic pulse was 130 ms. The differences in latencies, but not durations, of responses were significant among light illumination, liquid reward delivery (uncorrected and corrected), and door opening. Light and reward latencies differed insignificantly between monkeys or task phases.

Statistically significant responses to the light during postconditioning consisted of an increase in mean activity, from 3.66 imp/s during control to 14.42 imp/s during the individually determined response durations (+294 ± 19%, $n = 37$). Significant responses to reward delivery during the conditioning phase amounted to an increase from 4.80 to 17.23 imp/s (+259 ± 19%, $n = 13$).

POSITIONS OF NEURONS. The majority of DA neurons were recorded in catecholamine group A9 (pars compacta of SN), whereas fewer neurons were found in groups A8 and A10 (Table 5, *top*). When the area of DA neurons was divided into three equal mediolateral zones, recordings were obtained predominantly from the intermediate zone. There were no systematic differences in recording areas between monkeys or between different phases of conditioning. Fractions of neurons responding to light or reward were evenly distributed over groups A8–A10 and over the entire

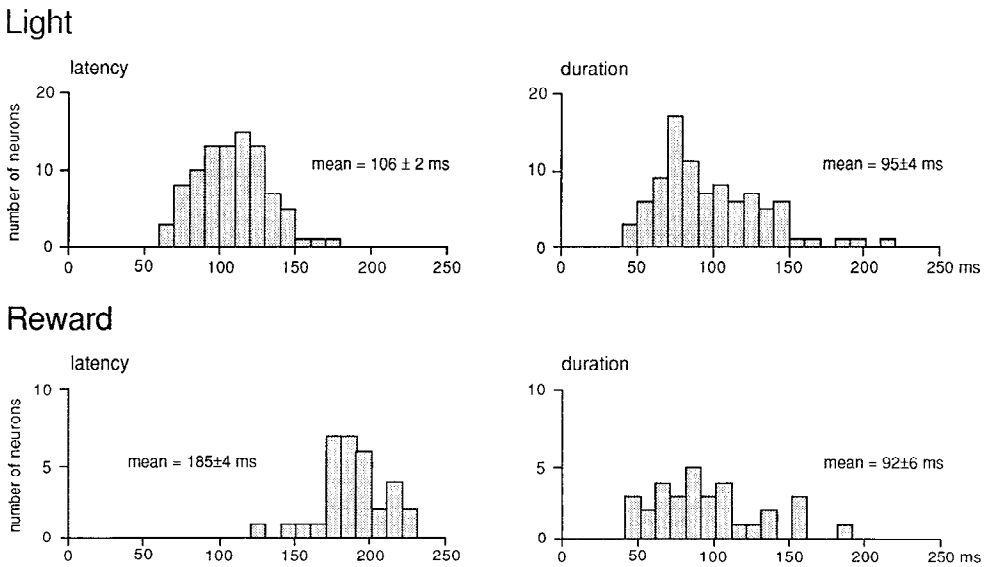

FIG. 14. Latency and duration histograms of responses of DA neurons to light illumination and liquid reward delivery. Only data from significantly responding neurons are included. All measurements were done with binwidth of 4 ms.

TABLE 5. *Regional distributions of DA neurons in monkey midbrain investigated during operant conditioning*

	DA Cell Group				Midbrain		
	A8	A9	A10	*N*	Lateral	Intermediate	Medial
	Numbers of neurons recorded						
No task							
Light	12	72	6	90	16	64	10
Liquid	21	99	18	138	9	104	25
Conditioning	8	17	0	25	0	25	0
Postconditioning	9	47	8	64	10	40	14
1st overtraining	5	71	7	83	7	69	7
2nd overtraining	7	63	12	82	6	64	12

	DA Cell Group				Midbrain		
	A8	A9	A10	Average	Lateral	Intermediate	Medial
	Percentage of neurons activated						
No task							
Light	8	7	8	7	0	9	0
Liquid	10	13	17	13	11	13	16
Conditioning + postconditioning							
Light	35	52	75	51	30	49	71
Liquid	29	25	29	26	20	28	31
1st + 2nd overtraining							
Light	42	42	26	40	15	44	26
Liquid	17	11	11	11	9	12	11

mediolateral extent (Table 5, *bottom*). As the only exception, responses to light illumination during conditioning and postconditioning phases appeared to be more frequent in medial parts of midbrain, although this difference did not reach the level of significance ($P > 0.05$). Positions of neurons responding to light illumination are shown for the conditioning-postconditioning phases and the two overtraining phases in Fig. 15, *A* and *B*, respectively. Recording sites of reward responses are shown in Fig. 16.

DISCUSSION

The present results demonstrate that responses of DA neurons to external stimuli are related to specific characteristics attached to different stimuli according to behavioral contexts attained during successive stages of learning. Several stimuli were effective for activating DA neurons, such as novel stimuli, which elicited orienting eye movements; sight of primary reward when the food box opened; delivery

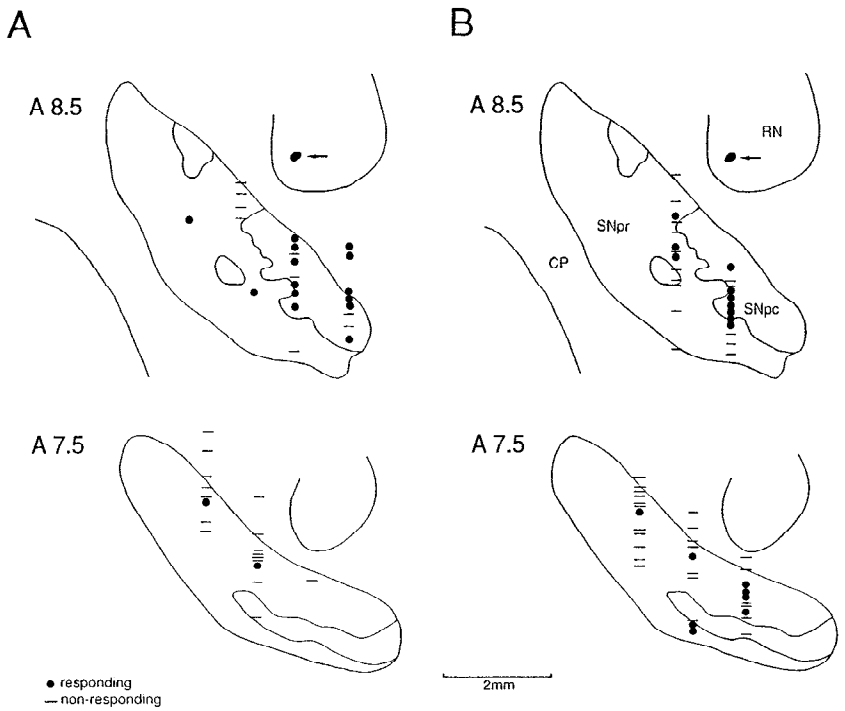

FIG. 15. Positions of DA neurons responding to light illumination. *A*: neurons recorded during conditioning and postconditioning phases. *B*: neurons recorded after 1st and 2nd overtraining in same monkey. Dots, DA neurons responding to light illumination; lines, unresponsive DA neurons. Arrow points to lesion for track identification. Same monkey and abbreviations as in Fig. 8.

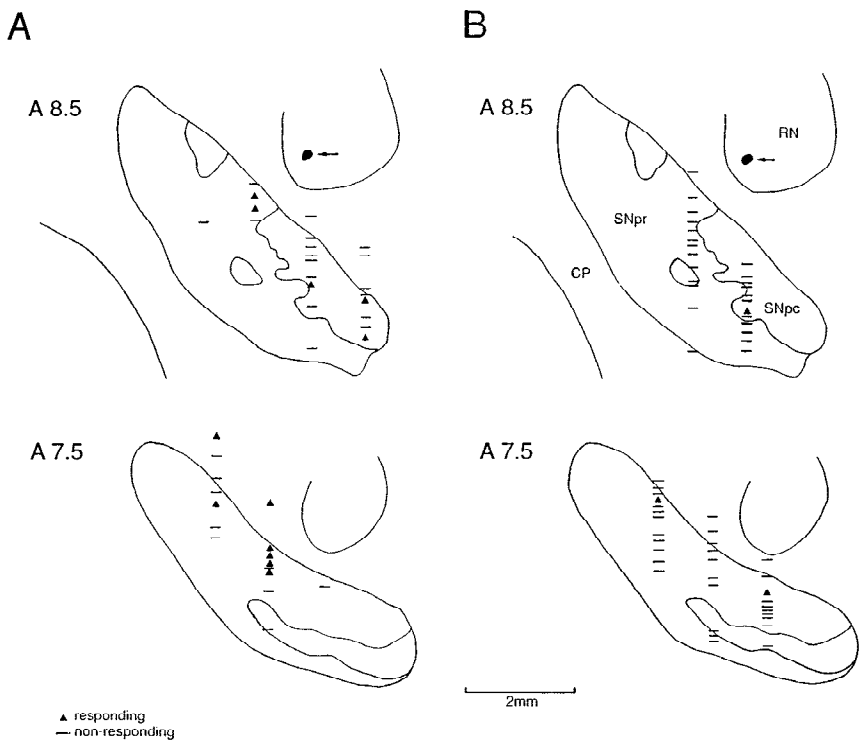

FIG. 16. Positions of DA neurons responding to delivery of liquid reward. *A*: neurons recorded during conditioning and postconditioning phases. *B*: neurons recorded after 1st and 2nd overtraining. Triangles, DA neurons responding to reward; lines, unresponsive DA neurons.

of primary reward during operant conditioning; and intrinsically neutral stimuli such as an opening box or a light, which—through association with primary reward—gained the capacity to elicit behavioral reactions. Responses were progressively reduced with automatic, routine task performance after overtraining. Neurons responded in a homogeneous fashion and were not separated into different classes. Within each learning phase, they either responded to the stimulus effective during that phase or lacked responses.

The dopaminergic nature of the neurons is suggested by characteristics emerging from the common experience of numerous research groups. DA cells histologically located in SN and areas A8 and A10 were distinguished from other neurons by the form, duration, and frequency of impulses. Neurons with these characteristics fulfill more stringent criteria that are usually unfeasible for work in awake monkeys, such as intracellular transmitter-specific staining (Grace and Bunney 1983) and depressant response to low doses of the DA autoreceptor agonist apomorphine (Bunney et al. 1973; Schultz 1986; Schultz and Romo 1987; Steinfels et al. 1983). Although final proof should be obtained by the use of a combination of these techniques, we feel confident that the chosen characteristics are sufficiently sensitive to ascertain the dopaminergic nature of recorded neurons.

Comparison of neuronal responses over different stages of behavioral conditioning during several weeks required standardization of methods. We attempted to study the same population of neurons during different task phases by recording from the same area of the ventroanterior midbrain and by studying every DA neuron encountered. The stereotyped, homogeneous responses of DA cells, together with the high proportion of responding neurons, allowed us to develop the standard time window method for assessing neuronal responses, which largely reduced subjective judgements and uncertainties derived from response variability.

Nature of changes in responsiveness

The first exposure to the door opening stimulus elicited target-directed eye movements and responses in DA neurons in each monkey, whereas repeated testing rapidly reduced behavioral and neuronal responses to this stimulus unrelated to reward. The less salient light stimulus introduced after box opening at the same spatial position did not elicit behavioral or neuronal responses. Obviously, the neuronal response to initial door opening was related to the novelty and salience of the unexpected stimulus. Subsequent reduction of neuronal responsiveness should be due to loss of novelty and not to habituation, because responses came back when the stimulus was associated with reward during the intermittent task. The sample of neurons showing novelty responses is very small because of the restriction to stimuli required for establishing a future behavioral contingency. Therefore the conclusions drawn at this time should not discriminate between possible attributes of these stimuli, such as novelty, unexpectedness, or salience. Similar low numbers of neurons were obtained in other studies in which a single neuron was recorded while an animal was engaged in learning a new contingency (e.g., Watanabe 1990). Responses of DA neurons to stimuli eliciting orienting reactions were previously observed in cats. They also were lost with behavioral habituation on repeated stimulus presentation (Steinfels et al. 1983).

Responses in the intermittent food box task were more pronounced in trials with food present than in opening of the empty box, suggesting that responses were partly due to the sight of primary food reward. Very quickly, neurons acquired responsiveness to door opening without food in intermingled trials, suggesting that door opening became effective through its association with food reward, induced by the temporal and spatial coincidence of the two stimuli. By contrast, the neutral light stimulus failed to activate DA

neurons during this period, demonstrating that DA neurons discriminated between different sensory stimuli and were driven only by the stimulus with behavioral significance.

Neuronal responses in food box and operant tasks developed concomitantly with target-directed eye and arm movements. However, during this period they also occurred in individual trials in which saccades were absent, because the eyes incidentally fixated the opening food box or the appearing light. Responses were also independent of individual arm movements, as seen during the intermittent task phase, in which animals reached out only when the box contained food. In agreement with previous results (Schultz and Romo 1990), this suggests that responses were not related to the initiation of individual eye and arm movements but were determined by the properties of stimuli that, through the association with primary reward, had gained the capacity to elicit immediate behavioral reactions.

Pronounced responses to reward were found during acquisition of the operant task but were largely lost with established task performance. The decrease in reward response was paralleled by the appearance of the light response after conditioning with very similar temporal response characteristics, as though the neuronal response was shifted from primary reward to the conditioned light stimulus. Only a few neurons responded to the light during conditioning, and even fewer responded to both light and reward. A comparable, albeit instantaneous, shift of responsiveness was previously observed in those DA neurons that responded to primary reward during self-initiated arm movements without predictive stimuli and to the conditioned stimulus predicting reward during stimulus-triggered movements (Romo and Schultz 1990). This suggests that the presently observed shift occurring over several days reflects the modified behavioral significance of stimuli induced by conditioning and should not be due to sampling from different neurons.

Responses to the light were considerably reduced after overtraining. The response to the conditioned stimulus represented a very persistent activation of DA neurons, and its reduction required an excessive amount of overtraining. In earlier studies, we failed to see response reduction with prolonged use of the food box task over several months (Schultz 1986; Schultz and Romo 1990). The present result should be ascribed to the high degree of task automatization, facilitating stereotyped performance with minimal attention; the high number of trials; the low-impact visual stimulus; the single modality of the stimulus; the restriction to a single task without variations; and the delivery of liquid reward to the animal's mouth. Earlier reports of lack of task-related modulations of DA neurons might be attributed to similar factors (DeLong et al. 1983).

Responses to the light during established task performance were, in general, similar to responses to opening of the food box (Schultz 1986; Schultz and Romo 1990). They occurred in 58% of neurons and thus were in the same range of frequency observed with door opening presently and previously (54 and 55–75%, respectively). Response magnitudes were slightly lower after light illumination (294%) compared with door opening (409%). The lower magnitudes after the light may be explained by the stationary stimulus and its single sensory modality, compared with the composite visual-auditory nature of the door opening stimulus. Similar differences were previously observed within the food box task when individual sensory components instead of composite door opening were used (Schultz and Romo 1990).

Properties of stimuli effective for activating dopamine neurons

NOVEL STIMULI. Neurons responded to door opening when it was a novel stimulus not associated with reward. In these situations, door opening prompted the animal for attention and elicited an orienting reaction. Novel stimuli are important for an animal because they may conceal appetitive or aversive consequences and thus have the potential for demanding immediate approach or avoidance behavior. The animal can afford to neglect a stimulus only when its neutral nature for behavior has been established. The lack of neuronal responses to door opening during the no task phase should thus be related to the loss of novelty through repeated presentation, after which the stimulus failed to elicit orienting reactions.

PRIMARY REWARDS. Reward responses during operant conditioning occurred when animals were required to learn the task to find a way to obtain liquid. During learning, reward delivery after the casual touch of the lever after light illumination represented a particularly prominent event. Besides leading to consummatory behavior, reward served to direct the animal's behavior so that the appropriate reactions for reaching the goal could be established. The fact that responses of DA neurons to reward occurred only in this specific situation should be related to the attention-generating and goal-directing characteristics of this stimulus. These characteristics were lacking when DA neurons failed to respond when a conditioned stimulus preceded reward, or when reward was given in a routine manner with the animal's hand on the resting key. By contrast, striatal neurons respond unconditionally to reward in various situations of a well-established task (Apicella et al. 1991). This suggests that the responses of DA neurons were related to the salient character of reward during learning. An involvement of DA neurons in reward detection is suggested by the blocking effects of DA receptor antagonists on reinforcement processes (Beninger and Hahn 1983; Fibiger and Phillips 1986; Wise and Rompre 1989).

CONDITIONED INCENTIVE STIMULI. Once animals received reward from the food box or by the casual touch of the operant lever, they began to pay attention to the particular stimulus that directed the behavioral sequence toward the box or the lever, respectively, and thus led to reward. Through behavioral conditioning, intrinsically neutral stimuli, namely, door opening and light illumination, sustained the arm movement. Through association with primary reward, intrinsically neutral stimuli gain incentive value, attract the attention of the subject, elicit a state of expectation by predicting reward, and thus sustain a variety of goal-directed behavioral acts (Bindra 1968, 1974; Bolles 1972; Toates 1986). The development of incentive properties of door opening and light illumination was paralleled by the development of neuronal responses to these stimuli. The responses to conditioned trigger stimuli may be factors that

contribute to the general involvement of DA neurons in incentive motivation, as proposed on behavioral grounds (Beninger and Hahn 1983; Fibiger and Phillips 1986).

Reaching toward the food box occurred in a rather natural situation, in which the eliciting stimulus (door opening), target of movement, and reward were presented simultaneously and at the same spatial position. This may explain why responses of DA neurons to door opening appeared virtually instantaneously, suggesting the rapid development of incentive stimulus properties of door opening when the box contained a morsel of apple. In contrast, operant conditioning of the light-lever reaction required several days, during which neuronal responses gradually shifted from primary reward to the conditioned incentive light stimulus.

Very few DA neurons responded to both delivery of primary liquid reward and the conditioned light stimulus, and none responded to the touch of food after door opening. This demonstrates that DA neurons respond only to the first reward-associated stimulus encountered in a behavioral sequence, this being either primary reward without preceding predictive stimuli or the conditioned stimulus predicting reward, in agreement with earlier results (Romo and Schultz 1990). This suggests a common reward-related stimulus attribute that contributes to the neuronal response, which is attached to only one stimulus in a given situation of this task. Both primary reward and reward-predicting conditioned stimuli are salient stimuli that attract the attention of the animal. Conceivably, the first of two predictably linked stimuli elicits a higher degree of arousal than the second one. Being related to motivation, this form of arousal could be termed "motivational arousal" (Bindra 1974; Wise 1982). Thus, in the respective situations in which DA neurons responded to them, primary reward and the conditioned stimuli were the key signals capable of eliciting motivational arousal.

OVERTRAINING. The reduction of neuronal responses to the operantly conditioned light after overtraining requires that the stimulus property of the light in this particular behavioral situation be assessed. During acquisition, the individual stimuli and actions contributing to a behavioral reaction are internally represented in a declarative form, with a particular emphasis on incentive stimuli (Dickinson 1980). After extensive practice, the behavioral act is represented in a procedural form, in which the whole sequence and not individual events are modeled. Thus behavior becomes a habit and is executed in an automatic and routine fashion, with a minimum of attention generated by external stimuli (Pearce and Hall 1980) and without reference to a particular goal (Dickinson 1980).

In the present study, overtrained animals conceivably performed the task as a habit that was centrally represented in a procedural form. The light would have lost its prominent predictive value for reward and thus its incentive and salient character. It merely provided a temporal reference for task performance, in which the lever needed to be touched within a 1-s period after light illumination. Behavior was not driven by reward but by custom, which would agree with the repeated spontaneous task interruptions of the fluid-deprived animal. Thus the reduction of responsiveness of DA neurons after overtraining may be ascribed to the reduced incentive and attention-generating character of the stimulus.

Previous investigations in Parkinsonian and choreatic patients have suggested that the striatum is involved in procedural representations underlying habit formation and performance (Heindel et al. 1988; Saint-Cyr et al. 1988). Although specific experiments are lacking, it may be hypothesized that DA neurons respond to the most significant attention-generating and motivating signals during learning until the behavior is represented in the striatum in a procedural form, after which responses of DA neurons are considerably reduced. Thus DA neurons might influence striatal processes during the formation of habits.

Dopamine neurons, learning, and the adaptation of behavior

The common character of events driving DA neurons appears to be the salience of external stimuli attracting the attention of the subject. According to the present results, this character is shared by novel, unexpected stimuli; primary rewards; incentive stimuli; and stimuli in general associated with reward, all of which activate DA neurons. Reward-related events represent a particularly prominent class of salient stimuli. The selective responses to salient stimuli during specific periods of learning allow DA neurons to play a particularly prominent role in the acquisition of behavioral tasks and the adaptation to changing environmental conditions, rather than to be involved in routine or habitual performance. Interruption of dopaminergic transmission during this critical phase should be particularly effective. Indeed, establishment of operantly conditioned behavior was preferentially attenuated by blockade of DA receptors by neuroleptics, whereas well-trained behavior was less readily disrupted (Beninger and Hahn 1983; Tombaugh et al. 1979; Wise and Schwartz 1981). These comparisons suggest that the observed impulse responses may be involved in mediating the modification of behavior according to external demands.

The phasic activation of DA neurons may be related to the increased tendency to react to those stimuli that attract the animal's attention and provide a goal for its behavior. The animal would adapt its behavior according to the information contained in the stimulus. For example, exploratory tendencies would be encouraged by novel stimuli. When the animal is learning an operant task, the phasic neuronal activation elicited by reward would sustain exploratory tendencies by which the animal discovers the contingencies for obtaining reward. Eventually, animals learn that a lever has to be pressed after light presentation to receive reward. When the task is acquired, the conditioned incentive stimulus would activate DA neurons and lead to an increased probability of the appropriate behavioral reaction for acquiring the reward, the specific parameters of the reaction being determined by the postsynaptic neuronal network influenced by DA released. Responses of DA neurons are considerably reduced when stimuli lose their incentive value and behavior is driven by habit rather than by pursuit of a particular goal, as seen after overtraining. This suggests that the activation of DA neurons is related to goal-directed, purposeful behavior. In summarizing results from behavioral studies, several authors have suggested that acti-

vation of DA systems is associated with motivational arousal (Wise 1982), energizing of behavioral reactions (Fibiger and Phillips 1986), and psychomotor stimulation (Robbins and Sahakian 1983; Wise and Bozarth 1987). Excessive stimulation of DA receptors results in the well-known syndrome of behavioral hyperactivity and stereotypes (Randrup and Munkvad 1970).

Regional specificity of dopaminergic function

The responses to reward occurred during task acquisition, whereas the responses to the conditioned trigger stimulus were seen during the performance of the well-established task. Previous results suggest that mesolimbic DA projections from the ventral tegmental area to ventral striatum are particularly involved in learning and reinforcement processes (Fibiger and Phillips 1986; Wise and Rompre 1989). The ventral striatum, together with input from amygdala, appears to play a particular role in establishing stimulus-reward associations (Cador et al. 1989; Gaffan et al. 1988). In contrast, established stimulus-guided behavioral performance in trained animals may depend more on DA neurons projecting from SN to dorsal caudate and putamen (Amalric and Koob 1987; Carli et al. 1989). One might expect that such distinctions in function between DA systems would be reflected in preferential distributions of DA neurons responding to primary reward (ventral tegmental area) and to conditioned stimuli (SN). However, neurons responding to reward during task acquisition and to trigger stimuli afterward were presently found over the entire mediolateral extent. There may be several reasons for this. *1*) We may not have sampled enough neurons in each region. In particular, the ventral tegmental area (A10) has again yielded a relatively low number of neurons. *2*) The relatively short acquisition phase of the simple reaction time task may have precluded a sufficient number of neurons responding to reward. More complex tasks with longer conditioning periods, or tasks with a higher yield of reward responses even after acquisition, might better unravel a regional preference. *3*) Responses in mesolimbic and nigrostriatal neurons may not mutually exclusively be related to reward and conditioned incentive stimuli, respectively, but may reflect a common underlying salient, attention-generating, and incentive stimulus character. They would respond to whatever stimuli possess this character, both during acquisition and during performance of stimulus-guided behavior, and the selectivity of behavioral processes cited above would reflect the function of the innervated structures. Manipulations of DA neurotransmission would result in disturbances, according to the function of the structure influenced by dopaminergic neurotransmission, rather than reflecting a specific, mutually exclusive involvement of the two DA systems in learning and performance. An additional argument favoring this view is provided by the earlier reported finding that the same DA neurons respond both to primary reward without predictive stimuli and to conditioned incentive stimuli (Romo and Schultz 1990).

We thank F. Tinguely, A. Schwarz, and J. Corpataux for technical assistance.

The study was supported by Swiss National Science Foundation Grant 3.473-0.86, Fyssen Foundation, Fondation pour la Recherche Médicale, and United Parkinson Foundation.

Present addresses: P. Apicella, Laboratoire de Neurosciences Fonctionnelles, U3, CNRS, F-13274 Marseille, France; T. Ljungberg, Dept. of Pharmacology, Karolinska Institute, S-10401 Stockholm, Sweden.

Address for reprint requests: W. Schultz, Institut de Physiologie, Université de Fribourg, CH-1700 Fribourg, Switzerland.

Received 2 January 1991; accepted in final form 26 August 1991.

REFERENCES

AMALRIC, M. AND KOOB, G. F. Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reaction-time performance. *J. Neurosci.* 7: 2129–2134, 1987.

APICELLA, P., LJUNGBERG, T., SCARNATI, E., AND SCHULTZ, W. Responses to reward in monkey dorsal and ventral striatum. *Exp. Brain Res.* 85: 491–500, 1991.

BENINGER, R. J. AND HAHN, B. L. Pimozide blocks establishment but not expression of amphetamine-produced environment-specific conditioning. *Science Wash. DC* 220: 1304–1306, 1983.

BINDRA, D. Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. *Psychol. Rev.* 75: 1–22, 1968.

BINDRA, D. A motivational view of learning, performance, and behavior modification. *Psychol. Rev.* 81: 199–213, 1974.

BOLLES, R. C. Reinforcement, expectancy and learning. *Psychol. Rev.* 79: 394–409, 1972.

BUNNEY, B. S., WALTERS, J. R., ROTH, R. H., AND AGHAJANIAN, G. K. Dopaminergic neurons: effects of antipsychotic drugs and amphetamine on single cell activity. *J. Pharmacol. Exp. Ther.* 185: 560–571, 1973.

CADOR, M., ROBBINS, T. W., AND EVERITT, B. J. Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. *Neuroscience* 30: 77–86, 1989.

CARLI, M., JONES, G. H., AND ROBBINS, T. W. Effects of unilateral dorsal and ventral striatal dopamine depletion on visual neglect in the rat: a neural and behavioural analysis. *Neuroscience* 29: 309–327, 1989.

DELONG, M. R., CRUTCHER, M. D., AND GEORGOPOULOS, A. P. Relations between movement and single cell discharge in the substantia nigra of the behaving monkey. *J. Neurosci.* 3: 1599–1606, 1983.

DICKINSON, A. *Contemporary Animal Learning Theory.* Cambridge, UK: Cambridge Univ. Press, 1980.

FELTEN, D. L. AND SLADECK, J. R. Monoamine distribution in primate brain. V. Monoaminergic nuclei: anatomy, pathways and local organisation. *Brain Res. Bull.* 10: 171–284, 1983.

FIBIGER, H. C. AND PHILLIPS, A. G. Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. In: *Handbook of Physiology. The Nervous System. Intrinsic Regulatory Systems of the Brain.* Bethesda, MD: Am. Physiol. Soc., 1986, vol. IV, sect. 1, p. 647–675.

FREEMAN, A. S. AND BUNNEY, B. S. Activity of A9 and A10 dopaminergic neurons in unrestrained rats: further characterization and effects of cholecystokinin. *Brain Res.* 405: 46–55, 1987.

GAFFAN, E. A., GAFFAN, D., AND HARRISON, S. Disconnection of the amygdala from visual association cortex impairs visual reward association learning in monkeys. *J. Neurosci.* 8: 3144–3150, 1988.

GRACE, A. A. AND BUNNEY, B. S. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons. 1. Identification and characterization. *Neuroscience* 10: 301–315, 1983.

HEINDEL, W. C., BUTTERS, N., AND SALMON, D. P. Impaired learning of a motor skill in patients with Huntington's disease. *Behav. Neurosci.* 102: 141–147, 1988.

LJUNGBERG, T., APICELLA, P., AND SCHULTZ, W. Diminished responses of monkey dopamine neurons to behavioral trigger stimuli after extensive training. *Eur. J. Neurosci.* 3, *Suppl.*: 296, 1990.

LJUNGBERG, T., APICELLA, P., AND SCHULTZ, W. Responses of monkey dopamine neurons to external stimuli: changes with learning. In: *Basal Ganglia*, edited by G. Bernardi, M. B. Carpenter, and G. Di Chiara. New York: Plenum, 1991, vol. III, p. 469–476.

MILLER, J. D., SANGHERA, M. K., AND GERMAN, D. C. Mesencephalic dopaminergic unit activity in the behaviorally conditioned rat. *Life Sci.* 29: 1255–1263, 1981.

NISHINO, H., ONO, T., MURAMOTO, K. I., FUKUDA, M., AND SASAKI, K. Neuronal activity in the ventral tegmental area (VTA) during motivated bar press feeding behavior in the monkey. *Brain Res.* 413: 302–313, 1987.

PEARCE, J. M. AND HALL, G. A model for Pavlovian conditioning: varia-

tions in the effectiveness of conditioned but not of unconditioned stimuli. *Psychol. Rev.* 87: 532–552, 1980.

RANDRUP, A. AND MUNKVAD, I. Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamines. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven, 1970, p. 695–713.

ROBBINS, T. W. AND SAHAKIAN, B. J. Behavioral effects of psychomotor stimulant drugs: clinical and neuropsychological implications. In: *Stimulants: Neurochemical, Behavioral, and Clinical Perspectives,* edited by I. Creese. New York: Raven, 1983, p. 301–337.

ROMO, R. AND SCHULTZ, W. Dopamine neurons of the monkey midbrain: contingencies of responses to active touch during self-initiated arm movements. *J. Neurophysiol.* 63: 592–606, 1990.

SAINT-CYR, J. A., TAYLOR, A. E., AND LANG, A. E. Procedural learning and neostriatal function in man. *Brain* 111: 941–959, 1988.

SCHULTZ, W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. *J. Neurophysiol.* 56: 1439–1462, 1986.

SCHULTZ, W., LJUNGBERG, T., AND APICELLA, P. Responses of monkey dopamine neurons to external stimuli develop during learning and decrease after overtraining. *Soc. Neurosci.* Abstr. 16: 235, 1990.

SCHULTZ, W. AND ROMO, R. Responses of nigrostriatal dopamine neurons to high intensity somatosensory stimulation in the anesthetized monkey. *J. Neurophysiol.* 57: 201–217, 1987.

SCHULTZ, W. AND ROMO, R. Dopamine neurons of the monkey midbrain: contingencies of responses to stimuli eliciting immediate behavioral reactions. *J. Neurophysiol.* 63: 607–624, 1990.

SCHULTZ, W., RUFFIEUX, A., AND AEBISCHER, P. The activity of pars compacta neurons of the monkey substantia nigra in relation to motor activation. *Exp. Brain Res.* 51: 377–387, 1983.

SCHULTZ, W., STUDER, A., ROMO, R., SUNDSTRÖM, E., JONSSON, G., AND SCARNATI, E. Deficits in reaction times and movement times as correlates of hypokinesia in monkeys with MPTP-induced striatal dopamine depletion. *J. Neurophysiol.* 61: 651–668, 1989.

SHANTA, T. R., MANOCHA, S. L., AND BOURNE, G. H. *A Stereotaxic Atlas of the Java Monkey Brain (Macaca irus).* Basel: Karger, 1968.

SIEGEL, S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.

STEINFELS, G. F., HEYM, J., AND JACOBS, B. L. Single unit activity of dopaminergic neurons in freely moving animals. *Life Sci.* 29: 1435–1442, 1981.

STEINFELS, G. F., HEYM, J., STRECKER, R. E., AND JACOBS, B. L. Behavioral correlates of dopaminergic unit activity in freely moving cats. *Brain Res.* 258: 217–228, 1983.

STRECKER, R. E. AND JACOBS, B. L. Substantia nigra dopaminergic unit activity in behaving cats: effect of arousal on spontaneous discharge and sensory evoked activity. *Brain Res.* 361: 339–350, 1985.

TOATES, F. *Motivational Systems.* Cambridge, UK: Cambridge Univ. Press, 1986.

TOMBAUGH, T. N., TOMBAUGH, J., AND ANISMAN, H. Effects of dopamine receptor blockade on alimentary behaviors: home cage food consumption, magazine training, operant acquisition, and performance. *Psychopharmacology* 66: 219–225, 1979.

WATANABE, M. Prefrontal unit activity during associative learning in the monkey. *Exp. Brain Res.* 80: 296–309, 1990.

WISE, R. A. Neuroleptics and operant behavior: the anhedonia hypothesis. *Behav. Brain Sci.* 5: 39–87, 1982.

WISE, R. A. AND BOZARTH, M. A. A psychomotor stimulant theory of addiction. *Psychol. Rev.* 94: 469–492, 1987.

WISE, R. A. AND ROMPRE, P.-P. Brain dopamine and reward. *Annu. Rev. Psychol.* 40: 191–225, 1989.

WISE, R. A. AND SCHWARTZ, H. V. Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol. Biochem. Behav.* 15: 655–656, 1981.