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ORIGINAL INVESTIGATION

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NMDA and dopamine D_2 receptors in the caudate-putamen are not involved in control of motor readiness in rats

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Abstract *Rationale*: In reaction time (RT) paradigms, in which a variable preparation interval preceded the imperative stimulus, RT become shorter as a function of increasing time from the start of a trial until presentation of the imperative stimulus. The shortening of RT as the preparatory foreperiod elapses reflects increasing motor readiness; however, the underlying neurochemical mechanisms are still poorly defined. *Objective*: The present study investigated in rats whether signals transmitted via the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors and via dopamine D_2 receptors in the caudate-putamen (CPu) are involved in motor readiness. *Methods*: A simple RT task demanding conditioned lever release was used, in which the upcoming reward magnitude (5 pellets or 1 pellet) was signalled in advance by discriminative stimuli and the imperative stimulus was subsequently presented after a variable foreperiod (200, 500 or 800 ms). *Results*: In intact rats, RT of conditioned responses was shortened with foreperiod lengthening and with expectancy of the high reward magnitude, but there was no interaction between both factors. Bilateral infusion of the competitive NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV) (2, 10 µg in 0.5 µl/side), of the preferential dopamine D_2 antagonist haloperidol (5, 12.5 µg in 0.5 µl/side) or infusion of vehicle (0.5 µl/side) into the central subregion of the CPu had no effect on progressive RT shortening with increasing foreperiod. *Conclusion*: The present data provide no clues to suggest that motor readiness relies on stimulation of dopamine D_2 and NMDA receptors in the central CPu.

Keywords Caudate-putamen · Reaction time · Dopamine · Glutamate · Rat

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Introduction

The caudate-putamen (CPu) as part of a motor basal ganglia-thalamocortical circuit (Joel and Weiner 1994) is generally supposed to play a major role in response selection, initiation and execution (Graybiel 1990; Robbins and Brown 1990; Marsden and Obeso 1994). The modulation of the CPu by nigrostriatal dopamine is known to be of predominant importance for theses processes (Dunnett and Robbins 1992; Hauber 1998). However, the specific role of dopamine in motor planning and control is still poorly defined.

In a recent study, striatal dopamine has been suggested to be involved in processes of response preparation termed as motor readiness (Brown and Robbins 1991). Motor readiness refers to observations in simple and choice reaction time (RT) paradigms, in which a variable preparation interval preceded the imperative stimulus. It has been found that RT became shorter as a function of increasing time from the start of a trial until presentation of the imperative stimulus in various hole box and lever press tasks in rats (Brown and Robbins 1991; Baunez et al. 1994; Brown and Bowman 1995; Brown et al. 1996; Brasted et al. 1997, 1998). A progressive shortening of RT with increasing foreperiod has also been observed in human RT studies (Frith and Done 1986). This decrease in RT as the preparatory foreperiod lengthens is supposed to reflect motor readiness (or temporal probability summation) (Brown and Robbins 1991; Brown et al. 1996): the longer the foreperiod, the greater the probability of stimulus occurrence and the greater motor readiness.

Motor readiness might be brought about by catecholamine-dependent mechanisms. Systemic administration of amphetamine to rats enhanced processes by which the conditional probability of stimulus occurrence is computed (Brown et al. 1996). Furthermore, unilateral dopamine depletion of the CPu abolished progressive shortening of RT with increasing foreperiod (Brown and Robbins 1991). Thus dopamine in the CPu might control aspects of motor preparation which rely on the use of en-

dogenous cues for increasing motor readiness and allow to respond across a foreperiod with increasing probability of imperative stimulus occurrence (Brown and Robbins 1991).

In the present study, we sought to determine in more detail neurochemical mechanisms in the CPu which contribute to motor readiness in rats. Furthermore, we examined whether motor readiness is modulated by motivational factors, i.e. the expected magnitude of the reward to be obtained after a response. There is consistent evidence that afferent input to the CPu provided both by the nigrostriatal dopaminergic pathway and the glutamatergic corticostriatal pathway is involved in RT performance (see Hauber 1998 for review). In particular, glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype and dopamine D_2 receptors have been implicated in response initiation (Hauber and Schmidt 1990; Amalric et al. 1993, 1994, 1995a; Baunez et al. 1994, 1995; Hauber 1996; Blokland and Honig 1999). Therefore, we examined the effects (1) of an intra-CPu dopamine D_2 receptor blockade by the preferential antagonist haloperidol and (2) of an intra-CPu NMDA receptor blockade by the selective antagonist APV on motor readiness. A simple RT task was used demanding conditioned lever release with instructive stimuli indicating the reward magnitude to be obtained after a subsequent imperative stimulus which was presented after a variable foreperiod.

Materials and methods

All animal experiments were conducted according to the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and were approved by the proper authorities according to the current version of the German Law on the Protection of Animals.

Animals

Twenty-one male Sprague-Dawley rats (Charles-River, Sulzfeld, Germany) were housed in groups up to four animals in transparent plastic cages (type IV; 35×55×10 cm; Ebeco, Castrop-Rauxel, Germany). Temperature (20±2°C) and humidity (50±10%) were kept constant in the animal house and a 12:12-h light-dark schedule was used with lights on between 0600 and 1800 hours. Rats were given ad libitum access to water. On days without behavioural testing food was restricted to 12 g standard laboratory maintenance chow (Altromin, Lage, Germany) per animal. On days with behavioural tests rats received in the testing apparatus 2–8 g food reward (45 mg pellets; Bioserv, Frenchtown, USA) depending on their individual performance. On these days the amount of standard laboratory chow given was adapted individually to a total of 12 g per animal/day in order to keep body weights constant. Rats weighed 200–250 g on arrival and 270–350 g at the time of surgery.

Surgery

For stereotaxic surgery, animals were anaesthetized with sodium pentobarbital (50 mg/kg, IP) (Sigma-Aldrich, Steinheim, Germany) following pretreatment with atropine sulphate (0.05 mg/kg, IP) (Sigma-Aldrich) and secured in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, USA). Bilateral stainless steel cannulae with an outer diameter of 0.8 mm were aimed at the CPu and implanted using standard stereotaxic procedures. The co-ordinates with reference to the atlas of Pellegrino et al. (1986) (toothbar 5 mm above the interaural line) were anteroposterior: 1.8 mm anterior bregma, mediolateral: 3.0 mm; dorsoventral: –4.25 mm below dura. Each rat was given at least 7 days to recover from surgery before postoperative training was started.

Drug infusion

On injection days, the obturators were removed and bilateral injection cannulae with an outer diameter of 0.45 mm were lowered at the final site of infusion and attached via polyethylene tubing to microliter syringes controlled by a microdrive pump (Kopf Instruments). The preferential dopamine D_2 antagonist haloperidol (Sigma-Aldrich, Deisenhofen, Germany) (5 and 12.5 µg in 0.5 µl 1% lactate), the competitive NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV) (Research Biochemicals International, Köln, Germany) (2 and 10 µg in 0.5 µl saline) and respective vehicles (0.5 µl) were delivered bilaterally at a rate of 0.5 µl/min. Injection cannulae were left in position for an additional 1 min after each infusion to allow for diffusion. Each rat remained in its home cage for an additional 5 min before being placed in the test chamber.

Apparatus

Four operant test chambers (24×21×30 cm) (Med Associates, St. Albans, UK) were used. Test chambers were placed in separate sound attenuating cubicles with fans providing a constant low level of background noise. Each chamber was supplied with a retractable lever, a food dispenser with receptacle and two stimulus lights, one above the retractable lever, the other above the food receptacle. The experiments were controlled online by a computer system (SmartControl-Interfaces; MedPC-Software; Med Associates).

RT task

A simple RT task was used demanding conditioned lever release (Amalric and Koob 1987; Baunez et al. 1994) with instructive stimuli indicating the reward magnitude to be obtained after a subsequent imperative stimulus (Brown and Bowman 1995). According to the protocols of Amalric and Koob (1987) and Baunez et al. (1994), rats had to press the lever and to wait for the imperative stimulus which was provided by the stimulus light above the lever after a variable foreperiod of 200, 500 or 800 ms. The imperative stimulus signalled to the rats to release the lever quickly and to respond to the food receptacle in which the food pellets (45 mg pellets; Bioserv, Frenchtown, USA) were delivered as indicated. On each trial, the rat received either one or five food pellets. The number of pellets for each trial was randomly determined in advance and signalled to the rats by two distinct brightness levels of the cue lights which provide the instructive stimulus as described by Brown and Bowman (1995) for a hole box task. The instructive stimulus was turned on at the beginning of each trial before lever press and remained present until delivery of food reward. To control for equal perception of instructive stimuli of different brightness levels, for 50% of the rats, a bright stimulus was associated with delivery of 5 pellets and a dim stimulus was associated with delivery of 1 pellet. For the other 50% of the rats, the opposite pattern was used. As results revealed that rats were able to discriminate between bright and dim stimuli, RT data obtained with both stimulus patterns were pooled.

RT were defined as latency from the onset of the imperative stimulus to lever release and recorded with an accuracy of 10 ms. For a correct trial, animals had to release the lever within 100–1000 ms. Responses with RT <100 ms and responses during the foreperiod were defined as "early" responses, responses with

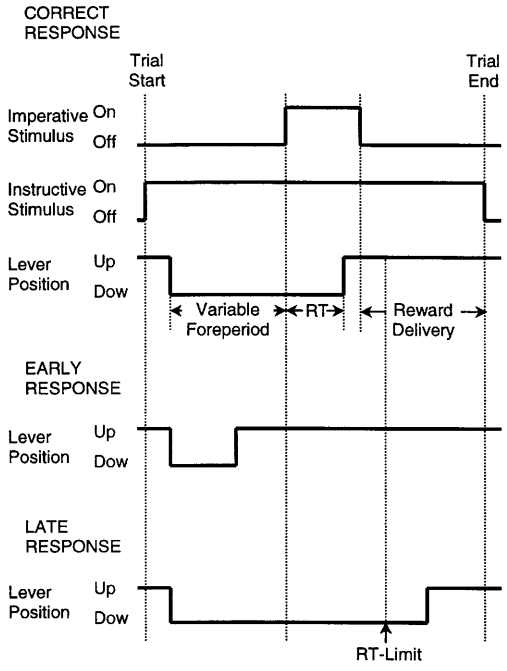


Fig. 1 Schematic representation of the order of events in a trial. At the beginning of a trial the instructive stimulus presented by a cue light above the food receptacle was turned on at one of two brightness levels which were associated with different reward magnitudes (1 or 5 pellets). Thereafter a rat spontaneously pressed the lever. After a variable foreperiod (200, 500 or 800 ms) the imperative stimulus provided by a cue light above the lever signalled the animal to release the lever in order to get the food reward in the receptacle. RT was defined as latency between presentation of the imperative stimulus and the lever release. Responses with RT within 100–1000 ms (*top*) were considered as being correct and were rewarded as indicated by the instructive stimulus. Early responses (RT<100 ms and responses during the foreperiod) (*middle*) or late responses (RT>1000 ms; *bottom*) caused the trial to be repeated with the identical foreperiod and reward magnitude

RT>1000 ms were defined as "late" responses. A daily individual session demanded 72 correct trials, i.e. 12 correct trials for each foreperiod (200, 500, and 800 ms) and reward magnitude (1 and 5 pellets) and lasted 15–25 min depending on the rat. A scheme on the order of trial events is given in Fig. 1.

Behavioural procedure

Training

Animals were preoperatively trained for 8 weeks until operant behaviour was reliably established with a mean accuracy was about 75%, i.e. on average a total of 96 trials was necessary to attain 72 correct responses. Then animals were subjected to surgery. After 7 days of recovery, postoperative training was given for 1 week to reach preoperative accuracy levels.

Experimental procedure

All animals were trained in one daily session on 5 days per week during the complete experiment. Effects of drug and vehicle infusions were investigated in one experimental session per week. In each experimental session, one single drug dose and the respective vehicle were tested. A series of four different experiments was performed to examine the effects of intra-CPu infusion of APV (2 µg), APV (10 µg), haloperidol (5 µg), haloperidol (12.5 µg) in the order as given. Before each experiment animals were assigned at random to two treatment groups receiving either vehicle or drug infusions to prevent order effects of drug administration. Random assignments were done until two criteria were met: (1) mean RT of both groups had to be significantly shorter with longer foreperiods, and, (2) mean RT of both treatment groups had to be significantly shorter with expectancy of a high reward magnitude (5 pellets) as compared to a low reward magnitude (1 pellet) (for calculation see Data analysis). Each animal was tested in all four experiments and thus received a total of four infusions. However, occasionally some rats showed pronounced irritation caused by the microinfusion procedure and were not tested in the subsequent experiment. Also, few animals developed permanent guide cannulae occlusion and were not used for further experiments. For these reasons, sample sizes were different in each experiment and became smaller at the last experiment. Prior to any experimental treatment, all animals were subjected to a test session preceded by a saline infusion in order to familiarize them with the experimental procedure.

Data analysis

Treatment effects were assessed by within-subjects comparisons of control and drug groups. Due to considerable inter-individual variability of baseline performance, a between-subjects design would be less powerful (Winer 1971). The performance in sessions with drug or vehicle infusions ("injection") with the preceding session (on the day before) without drug or vehicle infusion ("preinjection") was compared, respectively.

Drug effects on accuracy of task performance were determined by using two parameters: (1) The mean of the overall number of trials to achieve the criterion of 72 correct responses (±SEM), and, (2) percent means of early, correct and late responses from the total number of trials per session (±SEM) from each session with drug and vehicle injection and from respective preinjection sessions. These data were compared by means of a one-way analysis of variance (ANOVA) for repeated measures with treatment as factor.

The following calculations were conducted with RT data from correct responses (RT 100–1000 ms) of all preinjection and injection sessions. The decrease of RT as a function of foreperiod reflecting motor readiness was characterized by the slope of the regression straight lines. Mean slopes (±SEM) of (pre-)injection sessions were given. Treatment effects on motor readiness were calculated by comparing slopes of straight regression lines of drug and control groups on preinjection and injection days by means of a one-way analysis of covariance (ANCOVA) with the preinjection foreperiods as covariate followed by a test on parallelism. Furthermore, in control rats RT of responses with an expected high reward magnitude were significantly shorter than those with an expected low reward magnitude. This shortening of RT was used as an index of RT guidance by reward expectancy. Treatment-induced effects were determined by comparing RT shortening in drug and control groups on preinjection and injection days. Mean RT differences (±SEM) of responses with high and low reward magnitudes were given and analysed statistically by means of a one-way ANOVA for repeated measures with treatment as factor. The effect of the reward magnitude on motor readiness was calculated by means of a two-way ANOVA with reward magnitude and foreperiods as factors. The Statistica (Vers. 5.5A, Stat. Soft, Inc., Hamburg, Germany) statistical package was used for all statistical computations. The level of statistical significance (α-level) was set at $P<0.05$.

Histology

After completion of behavioural testing, animals were killed by an overdose of sodium pentobarbital (150 mg/kg, IP) (Sigma-Aldrich) to control for correct placement of cannulae. Brains were rapidly removed, fixed in 10% formalin for 2.5 h and stored in

Table 1 Mean number of trials (±SEM) to reach criterion (72 correct trials per session, RT: 100–1000 ms) of control and drug groups in sessions without intra-CPu infusions (preinjection) and in sessions with intra-CPu infusions (injection) of vehicle (0.5 µl) or drug. In four separate experiments, the following drugs/doses were tested: *HP-5*: 5 µg haloperidol in 0.5 µl; *HP-12.5*: 12.5 µg haloperidol in 0.5 µl; *APV-2*: 2 µg APV in 0.5 µl; *APV-10*: 10 µg APV in 0.5 µl

No. of experiments	Control group				Drug group			
	n	Preinjection		Injection Trials	*n*	Preinjection		Injection Trials
		Trials	Solution			Trials	Drug	
1	10	100.3±11.4	Vehicle	100.0±6.1	9	89.2±4.1	AP-2	116.7±19.7
2	6	88.7±3.7	Vehicle	102.2±5.0	5	113.6±29.0	AP-10	103.8±11.1
3	10	93.0±8.7	Vehicle	102.4±8.6	9	86.9±2.2	HP-5	99.4±4.8
4	5	85.6±2.9	Vehicle	88.8±2.1	3	90.7±11.2	HP-12.5	102.0±10.8*

*P<0.05; one-way ANOVA using within-subjects comparisons with injection day as factor

Table 2 Percent means (±SEM) of correct (RT: 100–1000 ms), early (RT: <100 ms) and late (RT: >1000 ms) responses from the total number of trials of control and drug groups in sessions without intra-CPu infusions (preinjection) and in sessions with intra-CPu (injection) of vehicle (0.5 µl) or drug. In four separate experiments the following drugs/doses were tested: *HP-5*: 5 µg haloperidol in 0.5 µl; *HP-12.5*: 12.5 µg haloperidol in 0.5 µl; *APV-2*: 2 µg APV in 0.5 µl; *APV-10*: 10 µg APV in 0.5 µl

No. of experiments	*n*	Infusion	Correct responses (%)		Early responses (%)		Late responses (%)	
			Preinjection	Injection	Preinjection	Injection	Preinjection	Injection
1	10	Vehicle	76.7±4.9	74.2±4.1	21.9±5.1	20.7±3.5	1.4±0.6	5.1±2.2
	9	APV-2	81.9±3.2	70.5±7.0	16.0±3.4	27.4±7.4	2.1±1.2	2.1±0.7
2	6	Vehicle	81.9±3.1	71.3±3.5*	17.0±3.4	25.0±3.6	1.2±0.8	3.7±3.3
	5	APV-10	74.7±11.3	72.2±6.7	24.8±11.4	22.7±7.2	0.5±0.5	5.2±2.4
3	10	Vehicle	81.5±4.9	73.5±4.4	16.7±5.0	24.1±4.5	1.8±0.8	2.4±1.2
	9	HP-5	83.3±2.1	73.7±3.4*	15.0±2.2	20.4±3.7	1.7±0.7	5.9±1.3*
4	5	Vehicle	84.5±2.9	81.3±2.0	14.6±3.0	15.8±3.0	0.9±0.5	3.0±1.9
	3	HP-12.5	81.8±9.6	71.2±7.1	18.2±9.6	24.2±4.5	0.0±0.0	3.7±2.7

*P<0.05; one-way ANOVA using within-subjects comparisons with injection day as factor

30% sucrose. Brain sections (20 µm) were cut with a cryostat (Reichert & Jung, Heidelberg, Germany), mounted on coated slides and stained with Cresyl Violet. Placements were verified with reference to the atlas of Pellegrino et al. (1986). Only those animals were evaluated in which cannulae tip placements deviated less than 0.5 mm from target co-ordinates in the CPu.

Results

Accuracy

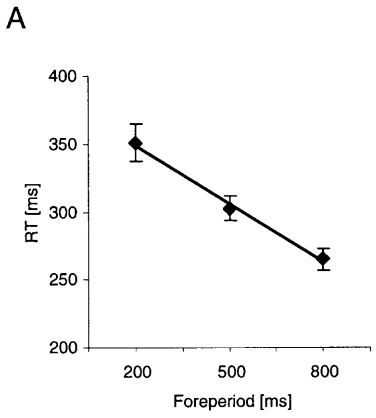
As shown in Table 1 intra-CPu infusion of vehicle to rats of the control groups did not significantly alter the number of trials to reach criterion as compared to the preinjection session (P>0.05, respectively). Thus the infusion procedure per se did not interfere with this aspect of task performance. Infusion of the lower dose of haloperidol (5 µg) significantly increased the number of trials [F(1,18)=5.71; P<0.03], while infusion of the higher dose of haloperidol (12.5 µg) had no significant effect on the number of trials to reach criterion compared with the respective preinjection sessions (P>0.05, respectively; Table 1). Infusion of APV (2 and 10 µg) had no significant effect on this parameter.

Analysis of the response distribution further showed that in rats receiving vehicle infusions the percent means of early, correct and late responses were altered only moderately as compared to the respective preinjection sessions. As shown in Table 2 there was a decrease in the percent means of correct responses after vehicle infusion in experiment 2 [F(1,10)=5.05; P<0.05].

Intra-CPu infusions of haloperidol moderately reduced the number of correct responses and increased the number of early and late responses. The decrease of correct [F(1,16)=5.92; P<0.03] and the increase of late responses [F(1,16)=8.05, P<0.012] after infusion of the low dose of haloperidol reached significance. The lower dose of APV had no effects on correct and late responses, but increased, albeit insignificantly, the early responses. The higher dose of APV had only minor to moderate effects on all three measures that were insignificant, respectively.

Reaction time

On completion of postoperative training, RT were significantly shorter with the expectancy of a higher reward magnitude (main effect of pellet: F=11.46; P<0.001) as shown in Fig. 2. RT were also faster as a function of lengthening of the foreperiod (main effect of the foreperiod: F=17.61; P<0.001; Fig. 2). No interaction between



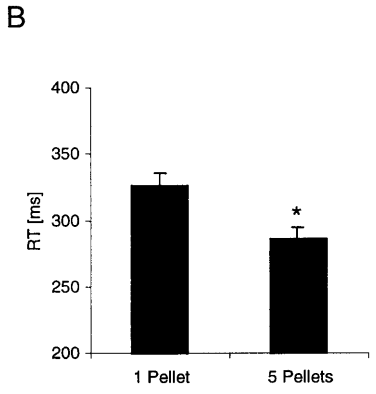
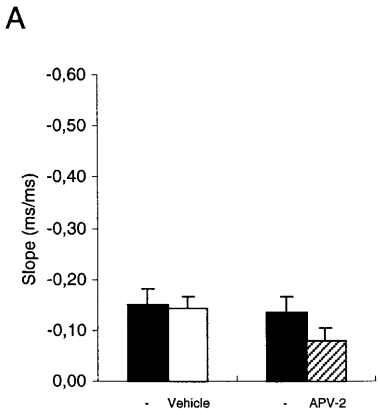


Fig. 2A,B The effect of the number of expected pellets and the lengthening of foreperiod on RT in the last postoperative training session (n=21, n=72 correct responses per animal) in animals without intra-CPu infusion. **A** RTs were significantly shorter with lengthening of the foreperiod. The mean slope of the regression straight line was m=–0.143 ms/ms. **B** Mean RT (±SEM) were significantly determined by the number of expected pellets. Expectancy of a high reward magnitude produced a significant RT shortening of 40 ms, *P<0.001, ANOVA with reward magnitude and foreperiod as factors



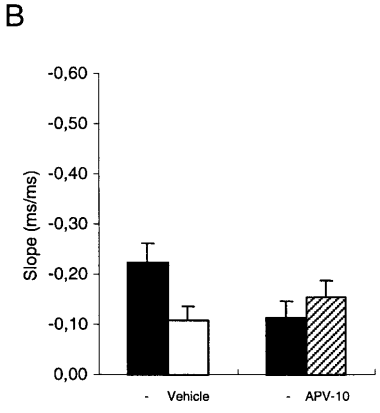


Fig. 3A,B Effects of intra-CPu infusion of APV or vehicle on motor readiness. Mean slopes (±SEM) of regression straight lines from RT as a function of the length of foreperiod in correct responses are given. Regression straight lines from drug and control groups in sessions with APV (**A** APV-2: 2 µg in 0.5 µl; n=9; **B** APV-10: 10 µg in 0.5 µl, n=5) or vehicle (0.5 µl, **A** n=10; **B** n=6) infusions and from preceding sessions without infusion were compared using an ANCOVA with foreperiods as covariate followed by a test on parallelism. APV and vehicle did not significantly affect shortening of RT as a function of foreperiod lengthening

number of pellets and foreperiod was found, suggesting that independent mechanisms account for shortening of RT by reward expectation and foreperiod (pellets×foreperiod; F=0.06; P>0.05).

Motor readiness

ANCOVA revealed no significant effect of vehicle injection to rats of the control groups on the slopes of the regression straight lines indicating no change of motor readiness as shown in Fig. 3 (A: n=10, P>0.05; B: n=9, P>0.05) and Fig. 4 (A: n=10, P>0.05; B: n=6, P>0.05). This suggests that the infusion procedure per se had no effect on motor readiness. In addition, there was no effect on motor readiness after infusion of APV as shown in Fig. 3 (A: APV 2 µg/side: n=9, P>0.05; B: APV 10 µg/side: n=5, P>0.05) or haloperidol as depicted in Fig. 4 (A: haloperidol 5 µg/side, n=9, P>0.05; B: haloperidol 10 µg/side, n=3, P>0.05). Thus, infusion of APV or haloperidol did not affect motor readiness, i.e. the determination of RT by lengthening of the foreperiod.

Reward expectancy

ANOVA revealed no significant treatment effect on the shortening of RT with expectancy of the high reward magnitude in control groups by vehicle infusion (not shown) in three out of four experiments. In one control experiment there was a treatment effect (P=0.044) after vehicle treatment for unknown reasons. Together, these data suggest that the infusion procedure per se had no prominent effect on this parameter. Likewise, infusion of

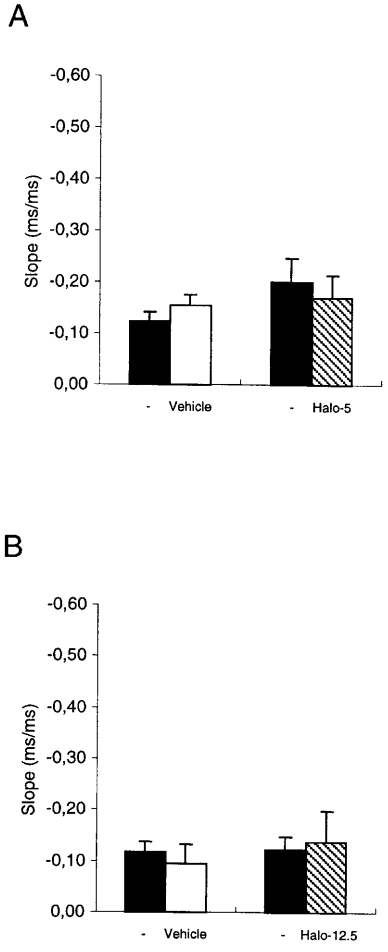


Fig. 4A,B Effects of intra-CPu infusion of haloperidol or vehicle on motor readiness. Mean slopes (±SEM) of regression straight lines from RT as a function of the length of foreperiod in correct responses are given. Slopes from drug and control groups in sessions with haloperidol (**A** HP-5: 5 µg in 0.5 µl, *n*=9; **B** HP-12.5: 12.5 µg in 0.5 µl, *n*=3) or vehicle (0.5 µl; **A** *n*=10, **B** *n*=5) infusion and from preceding sessions without infusion were compared using an ANCOVA with foreperiods as covariate followed by a test on parallelism. Haloperidol and vehicle did not significantly affect shortening of RT as a function of foreperiod lengthening

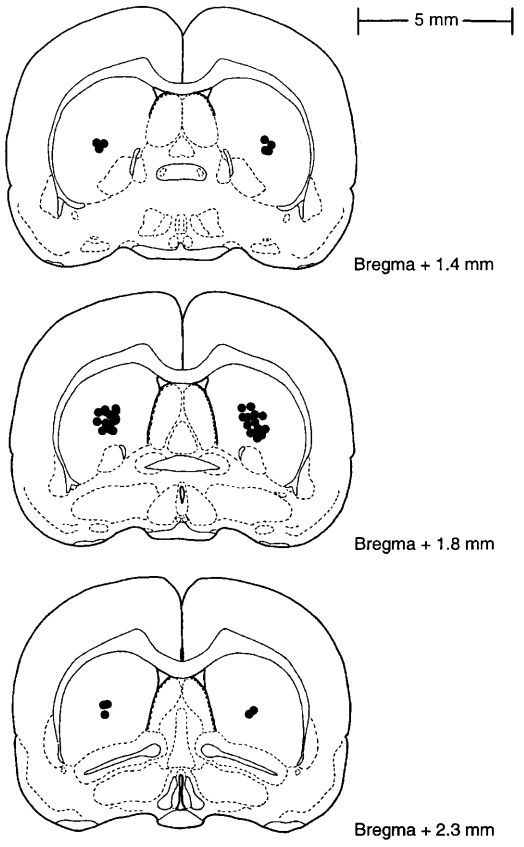


Fig. 5 Location of cannulae tips (*black circles*) for all rats used for data analysis receiving infusions into the CPu. Plates are adaptations from the atlas of Pellegrino et al. (1986). Numbers beside each plate correspond to mm anterior bregma

APV (2 µg, *n*=9; 10 µg, *n*=5; p>0.05, respectively) or haloperidol (5 µg, *n*=9; 12.5 µg, *n*=3; *P*>0.05, respectively) did not affect shortening of RT with expectancy of a high reward magnitude (not shown).

Histology

The locations of cannulae tips for all rats receiving infusions are represented in Fig. 5.

Discussion

In the simple RT task used here, intact rats showed a progressive shortening of RT with increasing foreperiod until presentation of the imperative stimulus, a relationship which has been suggested to reflect motor readiness (Brown and Robbins 1991). The present data reveal that motor readiness was not affected by a blockade of NMDA or dopamine D_2 receptors by bilateral microinfusions of APV (2, 10 µg) and haloperidol (5, 12.5 µg) into the central CPu.

The RT task used in this study represents an adaptation of a hole box task with different foreperiods and discriminative stimuli indicating the upcoming reward magnitude (Brown and Bowman 1995) to a lever release task originally described by Amalric and Koob (1987). Using this task, we measured a total RT decrease of about 100 ms across the complete foreperiod which is in keeping with data from various hole box and Skinner box tasks (Brown and Robbins 1991; Baunez et al. 1994; Brown and Bowman 1995; Brown et al. 1996; Brasted et al. 1997, 1998) although the length of foreperiods as well as the number of intervals are not identical in these studies. Furthermore, instructive stimuli signalling a high reward magnitude to be obtained after the subsequent imperative stimulus produced in the present study a RT shortening of about 50 ms which corresponds well with data from a nine-hole box task (Brown and Bowman 1995).

The two main questions addressed by the present study were (1) whether glutamatergic and dopaminergic

mechanisms in the CPu are involved in motor readiness and (2) whether an instructive stimulus predictive for a high reward magnitude increases motor readiness.

In intact rats, RTs were significantly decreased with the expectation of a high reward magnitude. However, we found no evidence to suggest that the expectation of different reward magnitudes influenced motor readiness thus confirming similar results in a nine-hole box task (Brown and Bowman 1995). Consequently, motor readiness which might depend on endogenous cues for increasing general readiness to respond across the foreperiod (Brown and Robbins 1991) seems not to be influenced by motivationally significant stimuli.

Furthermore, the shortening of the RT by expectation of a high reward magnitude was not altered by an NMDA or dopamine D_2 receptor blockade in the CPu. In keeping with this finding, lesions of the CPu did not alter effects of motivational factors, e.g. manipulations of the reward magnitude, on progressive ratio performance (Eagle et al. 1999).

A blockade of NMDA receptors in the CPu had only minor and variable effects on the number of trials necessary to reach criterion as well as on the distribution of early, correct and late responses. The lower dose of APV (2 µg) increased the number of trials to reach criterion and the proportion of early responses, but reduced correct responses. The higher dose of APV (10 µg) increased the fraction of late responses. Overall, these effects were moderate and did not reach significance. Previous studies revealed that 5 µg, but not 0.5 and 1 µg, APV produced a significant decrease of correct responses, a non-significant shortening of RT of correct responses and a significant increase of early responses (Amalric et al. 1994; Baunez et al. 1994). The results of the present study parallel these findings; however, the effects of APV were much less pronounced. Procedural differences might account for this discrepancy. In our task rats had to perform 72 correct trials per session, while in the task used by Amalric et al. (1994) and Baunez et al. (1994) a session ended after 100 (in-)correct trials. Furthermore, RT definitions for (in-)correct responses were disparate resulting in strikingly different distributions of correct, early and late responses in control animals. These factors might underlie the quantitatively different effects of APV on response distribution determined in these studies.

Most importantly, the progressive shortening of RT with foreperiod lengthening was not affected by an intra-CPu blockade of NMDA receptors suggesting that motor readiness might not to rely on NMDA receptor activation in the CPu. The CPu receives prominent glutamatergic projections from the cerebral cortex (see Gerfen and Wilson 1996 for review). Input of these structures converges on medium sized striatal projection neurons involving NMDA and non-NMDA receptors (Albin et al. 1992). In particular, NMDA receptors in the CPu play an important role in cortico-striatal information processing (Berretta et al. 1997) and have been implicated in response selection and performance (see Schmidt et al. 1992 Hauber 1998; for review). In view of its connectivity, the failure to detect an involvement of intra-CPu NMDA receptors in motor readiness might be surprising. On the other hand, there is consistent evidence that ibotenic acid-induced cell body lesions of the CPu did not affect the shortening of RT as a function of foreperiod in nine-hole and Skinner box tasks (Brasted et al. 1997, 1998; Dobrossy and Dunnett 1997). Thus, our finding that a pharmacological blockade of intra-CPu NMDA receptors did not affect motor readiness is consistent with these studies which fail to detect effects of CPu lesions on progressive shortening of RT with increasing foreperiod.

In order to delineate the role of dopamine D_2 receptors in the CPu to mediate motor readiness we performed local microinfusions of the preferential dopamine D_2 antagonist haloperidol. The effects of halperidol on overall task performance were modest confirming similar observations with comparable doses in a completely different RT task (Blokland and Honig 1999). An additional reason for the moderate effects of haloperidol might be the use of instructive stimuli which predict upcoming reward already starting from trial onset in the present task. As food-predictive cues induce motor activation which is not attenuated by systemic haloperidol (McFarland and Ettenberg 1999), similar effects could contribute to the weak effects of haloperidol. A further possibility that cannot be excluded to account for the moderate effects of haloperidol is the microinfusion schedule. Haloperidol microinfusions were preceded by two APV microinfusions (one per week) which might interfere with the behavioural effects of haloperidol. In detail, infusion of haloperidol produced a moderate increase in the number of trials to reach criterion, which was significant with regard to the lower dose (5 µg). In line with these findings, Blokland and Honig (1999) reported that haloperidol (3 and 10 µg) had no pronounced effects on the number of trials in a 30-min session and did only moderately increase the number of invalid responses with the lower dose being more effective. Furthermore, the distribution of responses was shifted in that the proportion of early and late responses was increased, while the proportion of the correct responses was decreased. Correspondingly, intra-CPu infusion of haloperidol (2.5 and 5 µg) (Amalric and Koob 1989) or partial dopamine depletion of the CPu (Amalric et al. 1995b) increased the number of late responses, while larger dopamine depletions increased early and late responses (Amalric et al. 1995b). However, these effects were less profound in our study and reached only partly significance. The reduced number of correct responses measured here is also in accordance with earlier findings showing that haloperidol (2.5 and 5 µg) reduced the number of correct responses (Amalric and Koob 1989).

Another major finding is that intra-CPu blockade of dopamine D_2 receptors did not impair shortening of RT with progressive foreperiod lengthening. This result differs from a dopamine depletion in the CPu which abolished RT shortening as a function of foreperiod lengthening (Brown and Robbins 1991). Several reasons might

account for these deviating results. Firstly, as the CPu comprises functionally different subregions (see Robbins and Brown 1990, for review), microinfusion of haloperidol into the more lateral aspects of the CPu may have an effect on motor readiness. Secondly, as haloperidol preferentially blocks dopamine D_2 receptors, D_1 receptors might play an important role in motor readiness. However, the selectivity of haloperidol for dopamine D_2 receptors (D_2 receptors; K_i=1.2 nM; D_1 receptors: K_i=80 nM) (Seeman and Van Tol 1994) is poor and given the high concentration of haloperidol infused (5 and 12.5 µg in 0.5 µl) an almost complete blockade not only of dopamine D_2, but also of D_1, receptors is likely in this condition. Furthermore, preliminary data show that even higher doses of haloperidol had no effect on motor readiness (Giertler and Bohn, unpublished observations). Nevertheless, the role of dopamine D_1 receptors in motor readiness has to be tested in future experiments with selective antagonists. Another possibility is that dopamine in structures outside of the CPu could be involved in motor readiness. Accordingly, intra-CPu infusion of dopamine (Baunez et al. 1995) or relatively circumscribed uni- or bilateral intra-CPu lesions with 6-hydroxydopamine (4 µg) (Florio et al. 1999) did not affect motor readiness. In the study by Brown and Robbins (1991), higher doses of 6-hydroxydopamine (8 µg) were infused into the CPu producing additional dopamine depletion in the nucleus accumbens of about 50%. There is evidence that 6-hydroxydopamine-induced dopamine depletions in the nucleus accumbens can be associated with depletions of cortical dopamine (Roberts et al. 1980). Thus a compromised dopamine neurotransmission in areas such as the prefrontal cortex known to be involved in intact simple RT performance (Hauber et al. 1994) may contribute to the impairment of motor readiness after pronounced striatal 6-hydroxydopamine lesions. Moreover, experiments with systemic amphetamine administration (Brown et al. 1996) provided clear evidence for an involvement of catecholamine transmitters in motor readiness. Noradrenergic mechanisms shown to be involved in attentional processes (Cole and Robbins 1992) could therefore contribute to mediate motor readiness as well. Overall, our findings do not point to an involvement of intra-CPu dopamine D_2 receptors in motor readiness, at least in the central subregion of the CPu where the microinfusions have been made. Major support for this notion is also provided by clinical studies showing that slowness of movement initiation in patients with Parkinson's disease can not be attributed to a deficient motor readiness (Jahanshahi et al. 1992a, 1992b).

In conclusion, the present data provide no clues to suggest that motor readiness relies on stimulation of dopamine D_2 and NMDA receptor in the central CPu. These findings are in accordance with negative results from studies with cell body lesions of the CPu as well as clinical data.

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