NMDA Receptors in the Rat Orbital Prefrontal Cortex are Involved in Guidance of Instrumental Behaviour under Reversal Conditions

Ines Bohn, Christian Giertler and Wolfgang Hauber

Abteilung Tierphysiologie, Biologisches Institut, Universität Stuttgart, D-70550 Stuttgart, Germany

The orbital prefrontal cortex (OPFC) might be particularly involved in adapting instrumental responses to changes of stimulus–reward contingencies. We investigated whether signals in the OPFC transmitted via *N*-methyl-D-aspartate (NMDA) receptors are critical for learning a reversal of stimulus–reward contingencies. Rats were trained in a reaction time (RT) task demanding conditioned lever release with discriminative stimuli signalling in advance the upcoming reward magnitude (one or five pellets). After acquisition, RT of responses with expectancy of high reward magnitude were significantly shorter. Thereafter, stimulus–reward contingencies were reversed and rats received intra-OPFC infusions of saline or of the NMDA receptor antagonist AP5. Reversal learning was tested for 6 days, with microinfusions being given on days 1, 3 and 5. Intra-OPFC blockade of NMDA receptors impaired the learning of a reversal of previously acquired stimulus–reward magnitude contingencies: (i) latencies of correct responses were generally shortened, regardless of the response-associated reward magnitude; (ii) the proportion of premature responses was increased; and (iii) responses were not guided by the current significance of the reward-predicting stimuli. These findings provide novel evidence for NMDA-receptor-dependent plasticity in the OPFC in reversal learning.

Introduction

The orbital prefrontal cortex (OPFC) has been suggested to be part of the circuitry through which information on the incentive value of stimuli influences the selection and execution of reward-directed behavioural responses (Rolls, 1999; Schoenbaum and Setlow, 2001; Cardinal *et al.*, 2002). This view is based on findings in rats and primates that the acquired motivational value of stimuli is encoded in OPFC (Lipton *et al.*, 1999; Rogers *et al.*, 1999; Yonemori *et al.*, 2000; Schroeder *et al.*, 2001). Electrophysiological data further indicate that neuronal activity in the OPFC reflects the conjunction of the acquired incentive value of stimuli with the use of that information to guide behaviour (Schoenbaum and Eichenbaum, 1995; Schoenbaum *et al.*, 1999). Furthermore, cue-selective firing in OPFC was altered markedly when stimulus-reinforcement contingencies were reversed (Thorpe *et al.*, 1983; Kubota and Komatsu, 1985; Rolls *et al.*, 1996; Schoenbaum *et al.*, 1999, 2000). Behavioural studies in rats are consistent with these findings, as OPFC lesions impaired reversal learning in go, no-go olfactory discrimination tasks (Ferry *et al.*, 2000; Schoenbaum *et al.*, 2002) and a reaction time (RT) task (Bohn *et al.*, 2003). In line with these results, primates, including humans with OPFC lesions, exhibit impairments in a number of tasks after changes of stimulus–reward contingencies (Rolls *et al.*, 1994; Dias *et al.*, 1996; Meunier *et al.*, 1997; Bechara *et al.*, 2000; Elliott *et al.*, 2000).

These data clearly suggest an involvement of the OPFC in learning of reversed stimulus–reward contingencies; however, no information is yet available on underlying neurochemical processes. *N*-methyl-D-aspartate (NMDA) receptor dependent mechanisms in the OPFC might be one possible neuronal substrate involved in reversal learning. The OPFC receives glutamatergic input from numerous regions involved in processing of the motivational significance of stimuli, such as the basolateral amygdala (Reep *et al.*, 1996; Kalivas and Nakamura, 1999; Groenewegen and Uylings, 2000). In addition, NMDA receptor clustering in the rat OPFC was revealed by *in vitro* calcium macroimaging (Takita *et al.*, 1997). Furthermore, NMDA-receptor-dependent plasticity within a distributed corticostriatal network, comprising, for example, the medial prefrontal cortex and the amygdala, plays a major role in appetitive instrumental learning (Baldwin *et al.*, 2000, 2002). Therefore, in the present study we investigated whether signals in the OPFC transmitted via the NMDA subtype of glutamate receptors are critical for learning a reversal of previously acquired stimulus–reward magnitude contingencies. To this end, rats were trained in a reaction time (RT) task demanding conditioned lever release with instructive stimuli signalling in advance the upcoming reward magnitude (one or five pellets) (Hauber *et al.*, 2000, 2001; Bohn *et al.*, 2003). After acquisition, RTs of responses with expectancy of high reward magnitude were significantly shorter. Thereafter, stimulus–reward contingencies were reversed and rats received an intra-OPFC infusion of saline or of the competitive NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP5).

Material and Methods

Experiments were performed according to the German law on animal protection and approved by the proper authorities in Stuttgart, Germany.

Subjects

Forty-four male Sprague–Dawley rats (Charles-River, Sulzfeld, Germany) were maintained in a temperature- and humidity-controlled room on a 12 h light:12 h dark schedule (lights on 20.00–08.00 h) with testing in the dark phase. All rats were given *ad libitum* access to water. Standard laboratory maintenance chow (Altromin, Lage, Germany) was restricted to 12 g per animal and day. On days with behavioural tests, rats received 6–7 g food reward (45 mg pellets; Bioserv, Frenchtown, NJ) in the testing apparatus. On these days, the amount of standard laboratory chow was adapted in order to keep body wts constant. Rats weighed 230–260 g on arrival and 240–270 g at the time of surgery.

Surgery

For stereotaxic surgery, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) following pretreatment with atropine sulphate (0.05 mg/kg i.p.; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and secured in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Bilateral 14 mm stainless steel guide cannulae with an outer diameter of 0.8 mm were aimed at the OPFC and implanted using standard stereotaxic methods. Coordinates for the cannulae placement were as follows: 3.5 mm anterior bregma, 2.6 mm lateral to midline and 3.4 mm ventral from skull surface (Paxinos and Watson, 1986). Stainless steel stylets

(14 mm long) prevented occlusion of the guide cannulae. Each rat was given at least 3 days to recover from surgery before the post-operative habituation session was started.

Drug Microinfusion

On injection days, the stainless steel stylets were removed and bilateral injection cannulae with an outer diameter of 0.45 mm and protruding 2 mm beyond the guide cannulae were lowered at the final site of infusion and attached via polyethylene tubing to microlitre syringes controlled by a syringe pump (Med Associates, St Albans, USA). The competitive NMDA receptor antagonist AP5 (7 µg in 0.7 µl saline, n = 22; Research Biochemicals International, Koeln, Germany) and 0.7 µl saline as vehicle (n = 22) were delivered bilaterally over a 70 s interval. Injection cannulae were left in position for a further 1 min after infusion to allow for diffusion. Each rat remained in its home cage for an additional 6 min before being placed in the test chamber.

Apparatus

Six operant test chambers (24 × 21 × 30 cm; Med Associates, St Albans, USA) 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 on the right-hand side of the retractable lever and two stimulus lights, one above the retractable lever (providing the imperative stimulus) and one above the food receptacle (providing the instructive stimulus). The experiments were controlled online (SmartControl®-Interfaces; Med Associates, St Albans, USA) by a computer system (MedPC-Software; Med Associates).

Reaction Time Task

A simple RT task (Hauber *et al.*, 2000, 2001) was used which demands conditioned lever release with instructive stimuli indicating the reward magnitude to be obtained after a subsequent imperative stimulus.

A trial was initiated by a spontaneous lever press. After a foreperiod of 0.3 s, the presentation of the imperative stimulus signalled to the rats to release the lever quickly and to respond to the food receptacle in which the food pellets were delivered (45 mg pellets; Bioserv, Frenchtown, USA). On each correct trial, rats received one or five food pellets. The reward magnitude for each trial was randomly determined in advance and signalled to the rats by two distinct brightness levels of the instructive stimulus light. The instructive stimulus light was turned on at the beginning of each trial before lever press and remained present until delivery of the food reward. To check for equal perception of the two different brightness levels of the instructive stimulus light, for 50% of the rats the bright stimulus was associated with delivery of five pellets and the dim stimulus was associated with delivery of one pellet. For the other 50% of the rats, the opposite design was used.

RTs, defined as latencies from the onset of the imperative stimulus to lever release, were recorded with an accuracy of 10 ms. For a correct trial, rats had to release the lever within 1.5 s of presentation of the imperative stimulus. Responses before onset of the imperative stimulus were defined as 'premature responses', responses with RTs >1.5 s were defined as 'late responses'. A daily individual session lasted ~15 min and demanded 50 correct trials, i.e. 25 correct trials for each reward magnitude (one and five pellets). All rats were trained in one daily session on 7 days per week during the complete experimental period. A schematic representation of the order of trial events is given in Figure 1.

Experimental Procedure

Preoperative Habituation

In the first two sessions, subjects were habituated to the operant chamber with access to food pellets placed into the food receptacle. In the following five sessions, a habituation programme with an FR-1 schedule commenced until a criterion of 20 consecutive lever responses was attained. Afterwards, rats were subjected to surgery.

Post-operative Habituation

The habituation programme with an FR-1 schedule was given for one session.

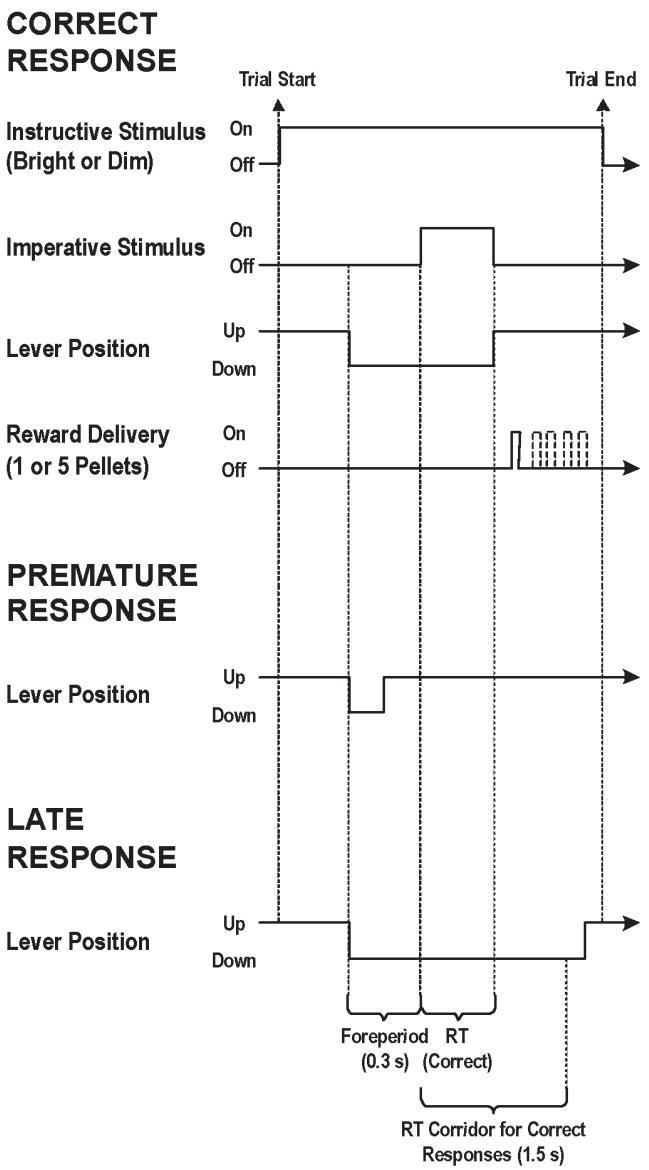

Figure 1. Schematic representation of the order of trial events. At the beginning of a trial, the instructive stimulus light was turned on at one of two brightness levels which were associated with different reward magnitudes (one or five pellets). Thereafter, the rat pressed the lever spontaneously. After the foreperiod of 0.3 s, the imperative stimulus light signalled the rat to release the lever in order to get the food reward. Responses with reaction times (RTs) < 1.5 s were considered as being correct and were rewarded as indicated by the instructive stimulus (top). Premature responses (before onset of the imperative stimulus; middle) or late responses (RT > 1.5 s; bottom) caused the trial to be repeated with the identical foreperiod and reward magnitude.

Acquisition

Subsequently, rats were trained in the RT task for 15 sessions. After the last acquisition session, the correct response rate was at least 70%, i.e. rats needed at maximum 71 trials to attain the 50 correct responses per session and responding was guided by the stimulus-associated reward magnitude, i.e. mean RTs of all responses of a session associated with the low reward magnitude were significantly longer than those associated with the high reward magnitude. To adapt animals to microinfusions, all animals received an intra-OPFC microinfusion of saline (0.7 µl) on day 13 of acquisition.

Reversal

Subsequently, learning of reversed stimulus–reward magnitude contingencies was tested for 6 sessions, i.e. rats had to learn that the

stimulus formerly predicting high reward magnitude was associated with low reward magnitude under reversal conditions (and vice versa). On days 1, 3 and 5 of reversal testing, each rat received a drug or vehicle microinfusion (AP5-treated group, n = 22; vehicle-treated group, n = 22) before the onset of behavioural testing. To exclude an impact of the handling procedure used for microinfusions, all animals were exposed to the handling procedure before each session during acquisition and reversal. As already pointed out, on days with behavioural tests rats received 6–7 g pellets as food reward in the testing apparatus. On these days, the amount of standard laboratory chow was adapted accordingly to keep body wts constant. No differences were observed in the body wts of animals treated with AP5 or saline.

Data Analysis

Results showed equal perception of the two different brightness levels of the instructive stimulus light, indicating that all rats discriminated stimulus–reward magnitude contingencies irrespective of the brightness–reward magnitude relationship. For example, in the last acquisition session, RT of responses of the subgroup of rats to be treated with vehicle with the dim stimulus indicating low reward magnitude (635 ± 39 ms) were significantly longer than RT of responses with the bright stimulus indicating the high reward magnitude (589 ± 36 ms) [$F(1,18) = 5.54$, $P = 0.0300$]. For the other subgroup of rats to be treated with vehicle, RT of responses with the bright stimulus indicating the low reward magnitude (687 ± 39 ms) were significantly longer than RT of responses with the dim stimulus indicating the high reward magnitude (647 ± 31 ms) [$F(1,18) = 5.26$, $P = 0.0340$]. Furthermore, RT for each reward magnitude level of both subgroups did not significantly differ [$F(1,18) = 1.25$, $P = 0.2780$]. Therefore, RT and accuracy data obtained with both stimulus–reward magnitude contingencies were pooled.

Accuracy of performance was determined by using (i) the mean number of trials necessary to reach the criterion of 25 correct responses for each stimulus–reward magnitude relationship (± SEM); (ii) the percentage means of correct responses from the number of trials per session (± SEM); (iii) the percentage means of premature responses from the number of trials per session (± SEM); and (iv) the percentage means of late responses from the number of trials per session (± SEM). Two-way analyses of variance (ANOVAs) for acquisition and reversal, respectively, were conducted with treatment groups as between factor and days as within (repeated measures) factor, followed by planned contrasts.

The calculations of RT performance were conducted with RT data from correct responses (RT < 1.5 s). When averaging RT data, a geometric mean was calculated for each rat for each session, as the geometric mean is less influenced by outlying data points than is the arithmetic mean (Brasted *et al.*, 1997; Lane, 2002). Overall RT means of responses associated with the high and low reward magnitudes, respectively, represent the arithmetic average of the geometric means of individual rats (Brasted *et al.*, 1997). After acquisition sessions, RTs of responses associated with the low reward magnitude were significantly longer than those associated with the high reward magnitude. Calculated RT differences (±SEM) were further analysed by ANOVAs for acquisition and reversal sessions, respectively, followed by planned contrasts. A calculated 'positive' difference between RT associated with low versus high reward magnitude was used as an indication of intact determination of instrumental responses by the reward magnitudes. Statistical computations were carried out with the Statistica ('99; StatSoft Inc., Hamburg, Germany) statistical package. The level of statistical significance (α-level) was set at $P < 0.05$.

Histology

After completion of behavioural testing, animals were killed with an overdose of sodium pentobarbital (150 mg/kg, i.p.) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) to control for correct placement of cannulae. Brains were rapidly removed, fixed in 10% formalin for 2.5 h and stored in 30% glucose. Brain sections (40 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 Paxinos and Watson (Paxinos and Watson, 1986).

Results

Histology

As shown in Figure 2, injection cannulae tips were located in the lateral and ventrolateral orbital cortex. Two rats, treated with AP5 and vehicle, respectively, were excluded from data analysis because of cannulae misplacement (cannulae tips > 0.5 mm from target coordinates). Thus, final sample sizes were n = 20 for each treatment group. No relationship between placements of

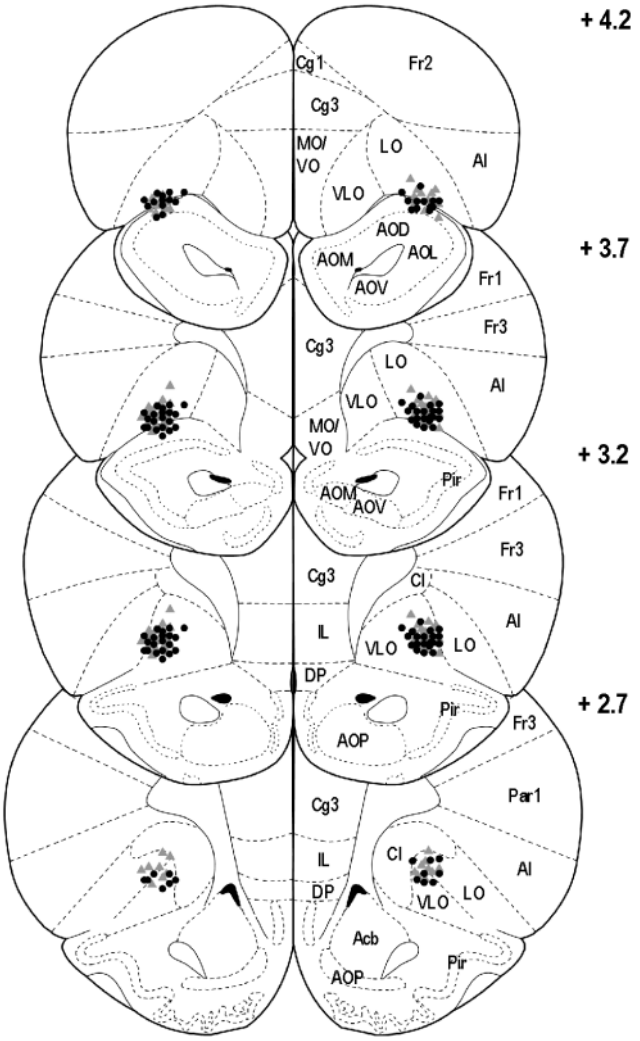

Figure 2. Location of injection sites in the orbital prefrontal cortex (OPFC). The schematic depicts the location of injection cannulae tips in the OPFC for all AP5-treated rats (n = 20) and vehicle-treated rats (n = 20) used for data analysis. For each animal, all those brain sections corresponding to the plates with visible damage caused by the cannulae tips were labelled with a symbol. Black circles represent damage caused by cannulae tips in rats with AP5 infusions. Triangles represent damage caused by a cannulae tips in rats with vehicle infusions. Plates are adaptations from the atlas of Paxinos and Watson (Paxinos and Watson, 1986). Numbers beside each plate correspond to millimetres anterior to bregma. Acb, nucleus accumbens; AI, agranular insular cortex; AOD, dorsal anterior olfactory nucleus; AOL, lateral anterior olfactory nucleus; AOM, medial anterior olfactory nucleus; AOP, posterior anterior olfactory nucleus; AOV, ventral anterior olfactory nucleus; Cl, claustrum; Fr1, frontal cortex (area 1); Fr2, frontal cortex (area 2); Fr3, frontal cortex (area 3); Cg1, cingulate cortex (area 1); Cg3, cingulate cortex (area 3); DP, dorsal peduncular nucleus; IL, infralimbic cortex; MO/VO, medial/ventral orbital cortex; LO, lateral orbital cortex; Par1, parietal cortex (area 1); Pir, piriform cortex; VLO, ventrolateral cortex.

cannulae tips and any behavioural measure was observed in animals treated with AP5 or vehicle.

Acquisition

Accuracy of Task Performance

Overall number of trials. Two-way ANOVAs on the overall number of trials (premature + correct + late responses) per session with treatment groups as between factor and days as within (repeated measures) factor, revealed no significant differences between groups to be treated with AP5 or vehicle during reversal learning. As shown in Figure 3, in both treatment groups the overall number of trials associated with low [$F(14,532) = 14.15$, $P < 0.0001$] and high reward magnitude [$F(14,532) = 13.27$, $P < 0.0001$] decreased significantly as a function of days. To achieve the criterion of 25 correct responses rewarded with 1/5 pellets, respectively, the overall number of trials was 28 ± 1/30 ± 1 in rats to be treated with vehicle and 30 ± 1/30 ± 1 in rats to be treated with AP5.

Correct response rate. Two-way ANOVAs on the rates of correct responses (responses with 0 < RT < 1.5 s after presentation of the imperative stimulus) with treatment groups as between factor and days as within (repeated measures) factor, revealed no significant differences between rats to be treated with AP5 or vehicle (data not shown). In both treatment groups, rates of correct responses associated with low [$F(14,532) = 13.96$, $P < 0.0001$] and high reward magnitude [$F(14,532) = 15.37$, $P < 0.0001$] increased significantly. In the last acquisition session, the rate of correct responses associated with low reward magnitude was 88.7 ± 1.4% in rats to be treated with vehicle and 85.3 ± 2.1% in rats to be treated with AP5. The rate of correct responses associated with high reward magnitude was 85.0 ± 2.4% in rats to be treated with vehicle and 85.3 ± 1.9% in rats to be treated with AP5.

Premature response rate. In addition, rates of premature responses (responses initiated before the onset of the imperative stimulus) associated with low [$F(14,532) = 15.85$, $P < 0.0001$] and high reward magnitude [$F(14,532) = 15.41$, $P < 0.0001$] decreased significantly, as depicted in Figure 4. In the last acquisition session, the rate of premature responses associated with low reward magnitude was (7.9 ± 1.3% in rats to be treated with vehicle and 10.4 ± 1.7% in rats to be treated with AP5; the rate of premature responses associated with high reward magnitude was 12.1 ± 2.4% in rats to be treated with vehicle and 13.4 ± 1.9% in rats to be treated with AP5. Differences between both treatment groups were not significant.

Late response rate. Rates of late responses (responses with RT > 1.5 s after presentation of the imperative stimulus) associated with low reward magnitude increased significantly [$F(14,532) = 2.29$, $P = 0.0048$], while those associated with high reward magnitude remained nearly constant. Differences between the treatment groups were not significant (data not shown).

RT Performance

RTs of correct responses associated with low reward magnitude increased significantly [$F(14,532) = 5.75$, $P < 0.0001$], while

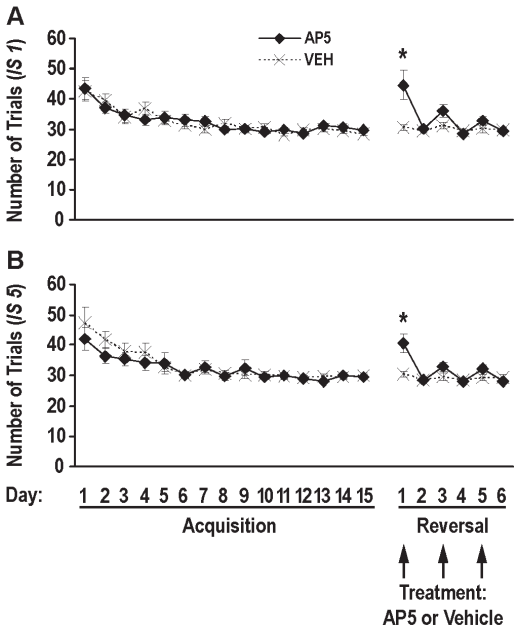

Figure 3. Overall number of trials per session (±SEM; premature + correct + late trials) to reach criterion (25 correct trials). During acquisition, the overall number of trials per session with the instructive stimulus predictive of (*A*) one pellet (*IS 1*), or (*B*) five pellets (*IS 5*) decreased, respectively, in animals to be treated with saline or AP5. During reversal learning, treatment groups received intra-OPFC microinfusion either of saline (0.7 µl per side) or AP5 (7 µg in 0.7 µl saline per side) on days 1, 3 and 5. On days with microinfusion of AP5, the overall number of trials with *IS 1* as well *IS 5* were increased. Two-way ANOVAs on the overall number of trials with *IS 1* and *IS 5*, respectively, revealed significant differences between treatment groups and a significant treatment × day interaction ($P < 0.05$). *$P < 0.05$, planned contrasts between treatment groups on single days.

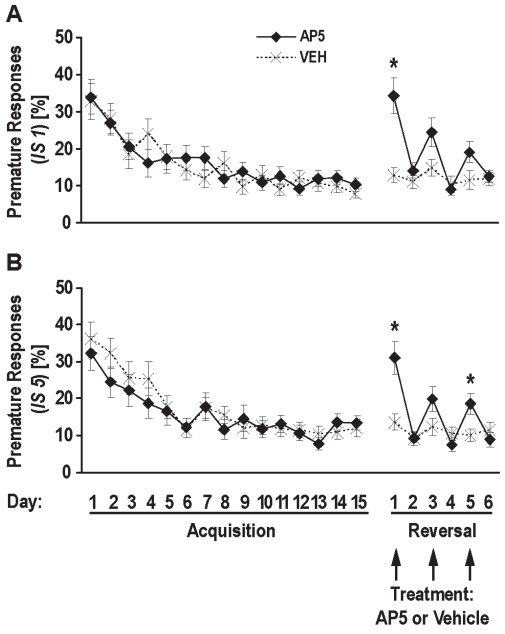

Figure 4. Proportion (±SEM) of premature responses (responses initiated before imperative stimulus onset) of the overall number of trials to reach criterion (25 correct trials). During acquisition, premature responses with the instructive stimulus predictive of (*A*) one pellet (*IS 1*) or (*B*) five pellets (*IS 5*) decreased, respectively, in animals to be treated with saline or AP5. During reversal learning, treatment groups received intra-OPFC microinfusion of saline (0.7 µl per side) or AP5 (7 µg in 0.7 µl saline per side) on days 1, 3 and 5. On days with microinfusion of AP5, premature response rates with *IS 1* and *IS 5*, respectively, were increased. Two-way ANOVAs on premature response rates with *IS 1* and *IS 5*, respectively, revealed significant differences between treatment groups and a significant treatment × day interaction ($P < 0.05$). *$P < 0.05$, planned contrasts between treatment groups on single days.

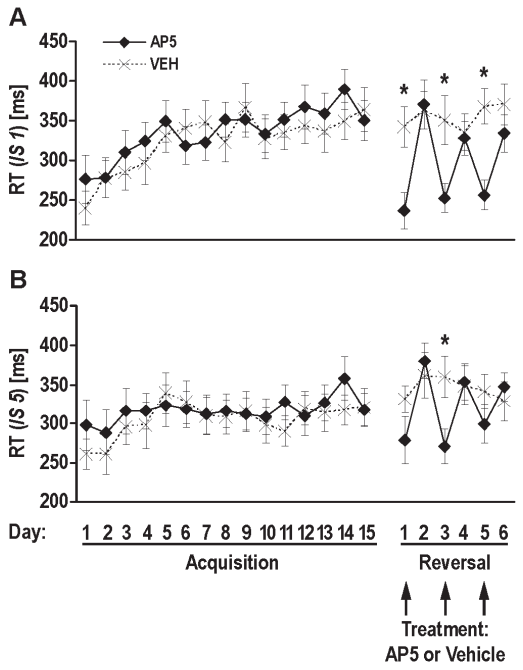

Figure 5. Reaction times (RTs; ±SEM) of correct responses (0 < RT < 1.5 s) during acquisition and reversal. During acquisition, RTs with the instructive stimulus predictive of one pellet (*IS 1*) increased significantly (*A*) and RTs with the instructive stimulus predictive of five pellets (*IS 5*) increased only slightly (*B*) in animals to be treated with saline or AP5. During reversal learning, treatment groups received intra-OPFC microinfusion of saline (0.7 μl per side) or AP5 (7 μg in 0.7 μl saline per side) on days 1, 3 and 5. On days with microinfusion of AP5, RTs with *IS 1* and *IS 5*, respectively, were shortened. A two-way ANOVA on premature response rates with *IS 1* revealed significant differences between treatment groups and a significant treatment × day interaction, the two-way ANOVA on premature response rates with *IS 5* revealed a significant treatment × day interaction ($P < 0.05$). *$P < 0.05$, planned contrasts between treatment groups on single days.

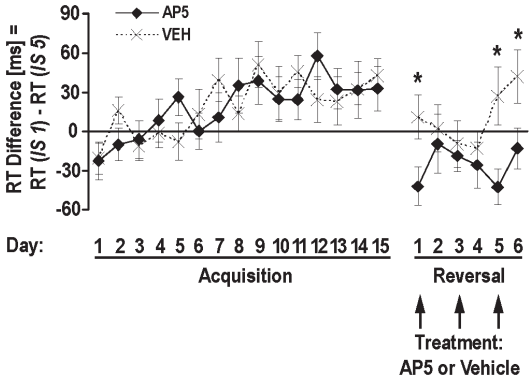

Figure 6. Reaction time (RT) differences (±SEM) of correct responses (0 < RT < 1.5 s) during acquisition and reversal. After acquisition, RT differences are positive in animals to be treated with saline or AP5, indicating intact stimulus–reward magnitude discrimination. During reversal learning, treatment groups received intra-OPFC microinfusion of saline (0.7 μl per side) or AP5 (7 μg in 0.7 μl saline per side) on days 1, 3 and 5. In vehicle-treated rats (VEH), RT differences were ~0 ms in the first reversal session and positive in the last reversal session. In AP5-treated rats (AP5), RT differences were negative in reversal sessions, indicating that responses were predominantly guided by the prior significance of the reward predicting stimuli. Two-way ANOVAs on RT differences revealed significant differences between treatment groups ($P < 0.05$). *$P < 0.05$, planned contrasts between treatment groups on single days. RT (*IS 1*), RTs of responses with the instructive stimulus predictive of one pellet; RT (*IS 5*), RTs of responses with the instructive stimulus predictive of five pellets.

those associated with high reward magnitude increased only slightly. There were no significant differences between rats to be treated with AP5 or vehicle, as shown in Figure 5. Direct comparison of RTs associated with low versus high reward of the last acquisition session revealed that mean RTs associated with low reward magnitude were significantly longer, resulting in positive RT differences (±SEM) of +33 ± 17 ms in rats to be treated with AP5 [$F(1,38) = 4.85$, $P = 0.0338$] and of +43 ± 13 ms in rats to be treated with vehicle [$F(1,38) = 8.27$, $P = 0.0066$]. A two-way ANOVA on RT differences with treatment groups as between factor and days as within (repeated measures) factor, revealed no significant differences between both treatment groups, but a significant increase of RT differences during acquisition [$F(14,532) = 3.50$, $P < 0.0001$] as shown in Figure 6.

Reversal

Reversal was tested on six subsequent days, treatment groups received microinfusion of saline or AP5 on days 1, 3 and 5.

Accuracy of Task Performance

Overall number of trials. A two-way ANOVA on the overall number of trials (premature + correct + late trials) per session associated with low reward magnitude with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences between treatment groups [$F(1,38) = 8.88$, $P = 0.0050$] and days [$F(5,190) = 8.07$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 5.81$, $P = 0.0001$]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 8.45$, $P = 0.0061$], as shown in Figure 3.

A two-way ANOVA on the number of trials per session associated with high reward magnitude revealed significant differences between treatment groups [$F(1,38) = 6.08$, $P = 0.0183$] and days [$F(5,190) = 10.53$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 5.91$, $P = 0.0001$]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 8.98$, $P = 0.0048$].

Correct response rate. A two-way ANOVA on rates of correct responses (0 < RT < 1.5 s after presentation of the imperative stimulus) associated with low reward magnitude, with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences between treatment groups [$F(1,38) = 6.65$, $P = 0.0139$] and days [$F(5,190) = 8.93$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 4.74$, $P = 0.0004$; data not shown]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 12.22$, $P = 0.0012$].

Likewise, a two-way ANOVA on rates of correct responses associated with high reward magnitude revealed significant differences between treatment groups [$F(1,38) = 4.11$, $P = 0.0497$] and days [$F(5,190) = 11.77$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 5.28$, $P = 0.0001$]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 11.86$, $P = 0.0014$]. The decrease of correct response rates was primarily due to the increase of premature response rates on injection days.

Premature response rate. A two-way ANOVA on rates of premature responses (responses initiated before the imperative stimulus) associated with low reward magnitude, with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences between

treatment groups [$F(1,38) = 11.01$, $P = 0.0020$] and days [$F(5,190) = 9.33$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 5.84$, $P < 0.0001$; Fig. 4]. Planned contrasts indicated significant differences between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 16.60$, $P = 0.0002$] and day 3 [$F(1,38) = 4.05$, $P = 0.0404$].

A two-way ANOVA on rates of premature responses associated with high reward magnitude revealed significant differences between treatment groups [$F(1,38) = 5.61$, $P = 0.0231$] and days [$F(5,190) = 10.89$, $P < 0.0001$] and a significant treatment × day interaction [$F(5,190) = 6.33$, $P < 0.0001$]. Planned contrasts indicated significant differences between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 12.91$, $P = 0.0009$] and day 5 [$F(1,38) = 6.24$, $P = 0.0169$; Fig. 4].

Late response rate. Two-way ANOVAs on the rates of late responses (responses with RT > 1.5 s after presentation of the imperative stimulus), with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences neither between treatment groups nor between days (data not shown).

RT Performance

A two-way ANOVA on RTs of correct responses associated with low reward magnitude, with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences between treatment groups [$F(1,38) = 6.51$, $P = 0.0148$] and between days [$F(5,190) = 4.88$, $P = 0.0003$] and a significant treatment × day interaction [$F(5,190) = 3.78$, $P = 0.0028$]. Planned contrasts indicated significant differences between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 9.74$, $P = 0.0034$), day 3 [$F(1,38) = 7.67$, $P = 0.0087$] and day 5 [$F(1,38) = 14.61$, $P = 0.0005$], as shown in Figure 5.

Likewise, a two-way ANOVA on RTs associated with high reward magnitude revealed significant differences between days [$F(5,190) = 3.26$, $P = 0.0076$] and a significant treatment × day interaction [$F(5,190) = 2.65$, $P = 0.0234$]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 3 [$F(1,38) = 6.62$, $P = 0.0141$; Fig. 5].

A two-way ANOVA on RT differences, with treatment groups as between factor and days as within (repeated measures) factor, revealed significant differences between treatment groups [$F(1,38) = 6.94$, $P = 0.0121$]. Planned contrasts indicated a significant difference between AP5- and vehicle-treated rats on day 1 [$F(1,38) = 5.62$, $P = 0.0230$], day 5 [$F(1,38) = 7.12$, $P = 0.0111$] and day 6 [$F(1,38) = 4.54$, $P = 0.0397$], as depicted in Figure 6. Further analysis revealed that RTs associated with low reward magnitude were significantly longer, resulting in a positive RT difference (±SEM) of +42 ± 20 ms on day 6 [$F(1,38) = 5.30$, $P = 0.0268$] in vehicle-treated rats. By contrast, in AP5-treated rats, RTs associated with low reward magnitude were significantly shorter, resulting in negative RT differences (±SEM) of –42 ± 15 ms on day 1 [$F(1,38) = 7.10$, $P = 0.0112$] and of –43 ± 14 ms on day 5 [$F(1,38) = 5.36$, $P = 0.0261$].

Discussion

The present study demonstrates that a blockade of NMDA receptors in the OPFC impaired learning a reversal of previously acquired stimulus–reward magnitude contingencies. In AP5-treated rats (i) RTs of correct responses were generally shortened regardless of the response-associated reward magnitude, (ii) the proportion of premature responses was increased and (iii) responses were not guided by the current significance of the reward predicting stimuli. These findings provide evidence for an involvement of NMDA receptors in the rat OPFC in reversal learning.

Histology

Injection cannulae tips were located in the lateral and ventrolateral orbital cortex, with deviations <0.5 mm from target coordinates in all rats used for data analysis. The exact amount of spread of AP5 from the site of infusion in the OPFC is not known, as studies with radiolabelled AP5 would be necessary. The coordinates and the volume used here were derived from pilot studies with dye infusions, which revealed a spread within major parts of and largely restricted to the OPFC, with some minor diffusion in a dorsal direction. The volume (0.7 µl) and the concentration (7 µg/0.7 µl) were selected with reference to studies employing AP5 infusions into the medial PFC (Swerdlow *et al.*, 1992; Zhang *et al.*, 1997; Izquierdo *et al.*, 1998; Baldwin *et al.*, 2000). Furthermore, it has been shown that selective behavioural functions of the OPFC and medial PFC in rats can be dissociated by microinfusions of highly lipophilic compounds, such as lidocaine, into these adjacent cortical areas (DeBruin *et al.*, 2000). Most of the cannulae tips in vehicle- and AP5-treated animals were located between +3.2. and 3.5 mm anterior bregma. In a few animals, cannulae tips were positioned slightly more anterior or posterior, but we have no evidence that AP5 produced different effects on task performance in these animals. Thus, we assume that the behavioural effects determined here are brought about primarily by actions of AP5 within the OPFC.

Acquisition of the RT Task

After acquisition, accuracy of task performance was very similar in both treatment groups subjected to vehicle or AP5 infusion during subsequent reversal learning. The proportion (83–88%) of correct responses (0 < RT < 1.5 s), the proportion (9–12%) of premature responses (responses initiated before onset of the imperative stimulus) and the proportion (3–5%) of late responses (RT > 1.5 s) were comparable in both treatment groups and indicate a high level of instrumental performance, which exceeded those in comparable tasks (Brown and Bowman, 1995; Hauber *et al.*, 2000, 2001). Likewise, RTs associated with low reward magnitude were significantly longer than those associated with high reward magnitude in both treatment groups. The calculated 'positive' RT difference of responses associated with low versus high reward was ~+40 ms and corresponds to those determined in similar tasks (Brown and Bowman, 1995; Hauber *et al.*, 2000, 2001). Apparently, specific reward magnitude expectancies account for the RT difference. Hence, RT difference was used as a sensitive measure to characterize discriminative guidance of instrumental responses by reward expectancy.

Reversal Learning after NMDA Receptor Blockade in the OPFC

In vehicle-treated rats, the number of correct, premature and late responses per session remained unchanged on days with and without vehicle infusion, indicating that the infusion procedure *per se* did not affect accuracy of task performance during reversal. In addition, the level of RTs was similar in vehicle-treated rats on days with and without vehicle infusion, demonstrating that the infusion procedure *per se* did not affect RT performance. However, RT differences were ~0 ms or negative on days 1–4 of reversal learning. This finding suggests that in the initial phase of reversal learning, stimuli were not interpreted according to their current incentive significance. Eventually, RT differences increased on day 5 of reversal learning

and reached a significantly positive value on day 6, indicating that vehicle-treated rats were able to adapt instrumental responding to reversed stimulus–reward magnitude contingencies within this period.

By contrast, in AP5-treated rats, accuracy of task performance was significantly impaired on infusion days. The total number of trials (premature + correct + late responses) necessary to achieve the criterion of 50 correct responses per session was increased, largely due to an increased number of premature responses. Notably, the effects of AP5 on accuracy of task performance were always independent of the response-associated reward magnitude and particularly strong on day 1 of reversal. Furthermore, in AP5-treated animals, RTs with the expected low and high reward were both markedly shorter on infusion days compared to days without infusion, demonstrating that AP5 produced a general speeding of instrumental responding. Also, RT differences were negative during reversal, i.e. AP5-treated animals responded significantly faster to the stimulus originally associated with high reward on days 1 and 5. Though less pronounced, this was also observed on the other reversal days. Overall, during reversal, AP5 treated animals did not respond to the stimuli according to their current significance. Taken together, intra-OPFC blockade of NMDA receptors impaired several aspects of instrumental responding under reversal conditions: (i) RTs of correct responses were generally shortened, regardless of the response-associated reward magnitude; (ii) the proportion of premature responses was increased; and (iii) responses were not guided by the current significance of the reward predicting stimuli.

The general reduction of RTs and the increased proportion of premature responses are probably both manifestations of the tendency to respond quickly. These changes can not easily be explained in terms of a nonspecific performance deficit, as rats were still able to reach the criterion number of correct responses. Moreover, the number of late responses was unchanged and responses were guided by the instructive stimuli, even if not according to their present significance. In addition, no indications of nonspecific motor symptoms have been reported in studies with rats investigating the effects of transient inactivation or lesion of the OPFC in go/no-go discrimination (Eichenbaum *et al.*, 1983; Ferry *et al.*, 2000; Schoenbaum *et al.*, 2002), delayed-nonmatching-to-sample (Otto and Eichenbaum, 1992), T-maze (De Bruin, 1994) and lever pressing (De Bruin *et al.*, 2000, Mobini *et al.*, 2002) tasks, as well as in the RT task used here (Bohn *et al.*, 2003).

Thus, we can not rule out an AP5-induced motor impairment to account for the increase of premature responses and the decrease of RTs, but the available data give no evidence in favour of this possibility. Rather, the reduced accuracy of task performance might be related to an impairment of inhibitory control mechanisms, which has been postulated to account for behavioural deficits induced by prefrontal lesions in various paradigms (Fuster, 1997; Jentsch and Taylor, 1999). The increased number of premature responses and the shortened RTs, regardless of the stimuli-associated reward magnitude, might be tentatively interpreted as a reduced inhibition of reward directed responses or an increased impulsivity.

Our results further indicate that after intra-OPFC blockade of NMDA receptors, responses were not only initiated generally faster, but were not guided by the current significance of the stimuli, indicating impaired reversal learning. Such an impairment of reversal learning has also been observed in rats with OPFC lesions in odour-guided go/no-go discrimination tasks (Ferry *et al.*, 2000, Schoenbaum *et al.*, 2002), an attentional set-shifting task (Brown and Bowman, 2002) and a RT task as used here (Bohn *et al.*, 2003). Likewise, in primates with excitotoxic lesions it has been shown that the OPFC is a critical brain region in reversing stimulus–reward associations within a particular perceptual dimension (Dias *et al.*, 1996, 1997). Problems in reversal learning caused by OPFC lesions have been attributed to impairments in inhibitory response control. It has been suggested that processes for modulating stimulus–reward-response associations, allowing animals to shift between old and new contingencies and to inhibit inappropriate responses to conditioned stimuli, are impaired after OPFC dysfunction (Jentsch and Taylor, 1999). Accordingly, damage to the OPFC in monkeys affected inhibitory control in a visual discrimination task, probably reflecting a failure to suppress the influence of previously acquired stimulus–reward associations, rather than an impairment in learning new stimulus–reward associations (Dias *et al.*, 1996).

Recent data further suggest that the rat OPFC might not subserve response inhibition unless responses have to be adapted to changed stimulus–reward contingencies, as only reversal learning, but not acquisition of go/no-go discriminations was impaired (Schoenbaum *et al.*, 2002). Together, these data support the general notion that the OPFC is an important neural substrate for reversal learning (Rolls, 1996, 2000; Schoenbaum and Setlow, 2001; Brown and Bowman, 2002). Considering mechanisms underlying impaired reversal learning in the task used here in more detail, there are several possible explanations. Though not tested explicitly, different RT of responses associated with low versus high reward magnitude might reflect guidance of instrumental responding by stimulus reward magnitude associations. Thus, one possible interpretation of the impaired guidance of RT in AP5-treated animals is that an NMDA receptor blockade interferes with encoding of new stimulus–outcome associations in the OPFC. As a consequence, AP5-treated animals responded primarily according to the prior significance of the stimuli resulting in the observed negative RT differences on infusions days.

On the other hand, the tendency of RT differences in AP5-treated animals on non-infusion days 2 and 6 to become less negative might indicate some reversal learning. However, this was not seen on reversal day 4, thus bringing this possibility into question. Nevertheless, an alternative explanation could be that blockade of NMDA receptor processing in the OPFC blocks the utilization of original and new stimulus–reward magnitude associations to guide instrumental responding. As a consequence, animals might revert to a response pattern established during early acquisition.

Regardless of the precise mechanisms involved, our data extend previous results indicating that the OPFC is critical to reversal learning and reveal an involvement of intra-OPFC stimulation of NMDA receptors. The OPFC receives glutamatergic inputs from a number of cortical and limbic regions (Kalivas and Nakamura, 1999; Groenewegen and Uylings, 2000; Öngür and Price, 2000) involved in processing of reward related stimuli, such as the amygdala (Baxter and Murray, 2002). Furthermore, NMDA-receptor-dependent plasticity in related corticostriatal structures such as the amygdala, medial prefrontal cortex and nucleus accumbens has been shown to mediate appetitive instrumental learning (Kelley *et al.*, 1997; Baldwin *et al.*, 2000, 2002). A role for glutamate transmitted signals in the rat OPFC in reversal learning is also suggested by recent electrophysiological findings. After reversal of stimulus–reward contingencies, changes in the functional connectivity in OPFC and basolateral amygdala have been detected. In particular,

correlated firing increased in the OPFC, possibly indicating that the original associations were maintained within the OPFC while acquiring new stimulus–reward associations (Schoenbaum *et al.*, 2000). This type of encoding has been suggested to facilitate comparisons of original and reversed stimulus–reward contingencies, permitting fast behavioural adaptation during reversal learning observed in intact, but not in OPFC-lesioned animals (Schoenbaum *et al.*, 2002; Bohn *et al.*, 2003). A pivotal role of NMDA receptors in cortical plasticity is well established (Wittenberg and Tsien, 2002). The present finding that intra-OPFC infusion of AP5 impaired reversal learning, in particular during early stages, suggests that NMDA-receptor-mediated synaptic plasticity in the OPFC might play an important role in processes related to encoding of new stimulus–reward magnitude contingencies. Likewise, some of the functional changes in the OPFC observed during reversal learning in respective electrophysiological experiments (Schoenbaum *et al.*, 2000) might rely on NMDA-receptor-dependent plasticity.

Conclusions

The OPFC plays an important role in adapting behavioural responses to altered incentive information in several species (Rolls, 1996, 2000; Schoenbaum and Setlow, 2001; Brown and Bowman, 2002). Using a RT task highly sensitive to changes of behavioural guidance by the anticipated value of actions, the present data confirm a role of the OPFC in reversal learning. In addition, this study provides novel evidence for NMDA-receptor-mediated neural plasticity in the OPFC underlying learning a reversal of stimulus–reward magnitude contingencies.

Notes

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Address correspondence to Dr Wolfgang Hauber, Universität Stuttgart, Biologisches Institut, Abteilung Tierphysiologie, Pfaffenwaldring 57, D-70550 Stuttgart, Germany. Email: hauber@po.uni-stuttgart.de.

References

Baldwin AE, Holahan MR, Sadeghian K, Kelley AE (2000) *N*-methyl-D-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. Behav Neurosci 114:84–98.

Baldwin AE, Sadeghian K, Kelley AE (2002) Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. J Neurosci 22:1063–1071.

Baxter MG, Murray EA (2002) The amygdala and reward. Nat Rev Neurosci 3:563–573.

Bechara A, Damasio H, Damasio AR (2000) Emotion, decision making and the orbitofrontal cortex. Cereb Cortex 10:295–307.

Bohn I, Giertler C, Hauber W (2003) Orbital prefrontal cortex and guidance of instrumental behaviour in rats under reversal conditions. Behav Brain Res 143:49–56.

Brasted PJ, Humby T, Dunnett SB, Robbins TW (1997) Unilateral lesions of the dorsal striatum in rats disrupt responding in egocentric space. J Neurosci 17:8919–8926.

Brown VJ, Bowman EM (1995) Discriminative cues indicating reward magnitude continue to determine reaction time of rats following lesions of the nucleus accumbens. Eur J Neurosci 7:2479–2485.

Brown VJ, Bowman EM (2002) Rodent models of prefrontal cortical function. Trends Neurosci 25:340–343.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321–352.

De Bruin JPC (1994) Evolution of prefrontal cortex: comparative aspects of its behavioral functions. In: Flexibility and constraint in behavioral systems (Greenspan RJ, Kyriacou CP, eds), pp. 185–192. Chichester: John Wiley.

De Bruin JPC, Feenstra MG, Broersen LM, Van Leeuwen M, Arens C, De Vries S, Joosten RN (2000) Role of the prefrontal cortex of the rat in learning and decision making: effects of transient inactivation. Prog Brain Res 126:103–113.

Dias R, Robbins TW, Roberts AC (1996) Dissociation in prefrontal cortex of affective and attentional shifts. Nature 380:69–72.

Dias R, Robbins TW, Roberts AC (1997) Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from 'on-line' processing. J Neurosci 17:9285–9297.

Eichenbaum H, Clegg RA, Feeley A (1983) Reexamination of functional subdivisions of the rodent prefrontal cortex. Exp Neurol 79:434–451.

Elliott R, Dolan RJ, Frith CD (2000) Dissociable functions in the medial and lateral orbitofrontal cortex: evidence from human neuroimaging studies. Cereb Cortex 10:308–317.

Ferry AT, Lu XC, Price JL (2000) Effects of excitotoxic lesions in the ventral striatopallidal–thalamocortical pathway on odor reversal learning: inability to extinguish an incorrect response. Exp Brain Res 131:320–335.

Fuster JM (1997) The prefrontal cortex: anatomy, physiology, and neuropsychology of the frontal lobe. New York: Raven Press.

Groenewegen HJ, Uylings HB (2000) The prefrontal cortex and the integration of sensory, limbic and autonomic information. Prog Brain Res 126:3–28.

Hauber W, Bohn I, Giertler C (2000) NMDA, but not dopamine D(2), receptors in the rat nucleus accumbens are involved in guidance of instrumental behavior by stimuli predicting reward magnitude. J Neurosci 20:6282–6288.

Hauber W, Giertler C, Bohn I (2001) NMDA and dopamine D2 receptors in the caudate-putamen are not involved in control of motor readiness in rats. Psychopharmacology (Berl) 155:43–51.

Izquierdo I, Izquierdo LA, Barros DM, Mello e Souza T, de Souza MM, Quevedo J, Rodrigues C, Sant'Anna MK, Madruga M, Medina JH (1998) Differential involvement of cortical receptor mechanisms in working, short-term and long-term memory. Behav Pharmacol 9:421–427.

Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl) 146:373–390.

Kalivas PW, Nakamura M (1999) Neural systems for behavioral activation and reward. Curr Opin Neurobiol 9:223–227.

Kelley AE, Smith-Roe SL, Holahan MR (1997) Response-reinforcement learning is dependent on *N*-methyl-D-aspartate receptor activation in the nucleus accumbens core. Proc Natl Acad Sci USA 94:12174–12179.

Kubota K, Komatsu H (1985) Neuron activities of monkey prefrontal cortex during the learning of visual discrimination tasks with GO/NO-GO performances. Neurosci Res 3:106–129.

Lane D (2002) Hyperstat. pp. 10–16. Cincinnati, OH: Atomic Dog.

Lipton PA, Alvarez P, Eichenbaum H (1999) Crossmodal associative memory representations in rodent orbitofrontal cortex. Neuron 22:349–359.

Meunier M, Bachevalier J, Mishkin M (1997) Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. Neuropsychologia 35:999–1015.

Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF, Anderson IM (2002) Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology (Berl) 160:290–298.

Öngür D, Price JL (2000) The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10:206–219.

Otto T, Eichenbaum H (1992) Complementary roles of the orbital prefrontal cortex and the perirhinal–entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. Behav Neurosci 106:762–775.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. San Diego, CA: Academic Press.

Reep RL, Corwin JV, King V (1996) Neuronal connections of orbital cortex in rats: topography of cortical and thalamic afferents. Exp Brain Res 111:215–232.

Rogers RD, Owen AM, Middleton HC, Williams EJ, Pickard JD, Sahakian BJ, Robbins TW (1999) Choosing between small, likely rewards and

large, unlikely rewards activates inferior and orbital prefrontal cortex. J Neurosci 19:9029–9038.
Rolls ET (1996) The orbitofrontal cortex. Philos Trans R Soc Lond B Biol Sci 351:1433–1444.
Rolls ET (1999) The brain and emotion. London: Oxford University Press.
Rolls ET (2000) The orbitofrontal cortex and reward. Cereb Cortex 10:284–294.
Rolls ET, Hornak J, Wade D, McGrath J (1994) Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. J Neurol Neurosurg Psychiatry 57:1518–1524.
Rolls ET, Critchley HD, Mason R, Wakeman EA (1996) Orbitofrontal cortex neurons: role in olfactory and visual association learning. J Neurophysiol 75:1970–1981.
Schoenbaum G, Eichenbaum H (1995) Information coding in the rodent prefrontal cortex. II. Ensemble activity in orbitofrontal cortex. J Neurophysiol 74:751–762.
Schoenbaum G, Setlow B (2001) Integrating orbitofrontal cortex into prefrontal theory: common processing themes across species and subdivisions. Learn Mem 8:134–147.
Schoenbaum G, Chiba AA, Gallagher M (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci 19:1876–1884.
Schoenbaum G, Chiba AA, Gallagher M (2000) Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. J Neurosci 20:5179–5189.
Schoenbaum G, Nugent SL, Saddoris MP, Setlow B (2002) Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. Neuroreport 13:885–890.
Schroeder BE, Binzak JM, Kelley AE (2001) A common profile of prefrontal cortical activation following exposure to nicotine- or chocolate-associated contextual cues. Neuroscience 105:535–545.
Swerdlow NR, Caine SB, Geyer MA (1992) Regionally selective effects of intracerebral dopamine infusion on sensorimotor gating of the startle reflex in rats. Psychopharmacology 108:189–195.
Takita M, Yokoi H, Mizuno T (1997) NMDA receptor clustering in rat prefrontal cortex revealed by *in vitro* calcium macroimaging. Neuroreport 8:551–553.
Thorpe SJ, Rolls ET, Maddison S (1983) The orbitofrontal cortex: neuronal activity in the behaving monkey. Exp Brain Res 49:93–115.
Wittenberg G, Tsien J (2002) An emerging molecular and cellular framework for memory processing by the hippocampus. Trends Neurosci 25:501–505.
Yonemori M, Nishijo H, Uwano T, Tamura R, Furuta I, Kawasaki M, Takashima Y, Ono T (2000) Orbital cortex neuronal responses during an odor-based conditioned associative task in rats. Neuroscience 95:691–703.
Zhang S, Tang JS, Yuan B, Jia H (1997) Involvement of the frontal ventrolateral orbital cortex in descending inhibition of nociception mediated by the periaqueductal gray in rats. Neurosci Lett 224:142–146.